Antibiotics

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Gold-Catalyzed Cyclization Leads to a Bridged Tetracyclic Indolenine that Represses β-Lactam Resistance**

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Abstract: *A gold-catalyzed desilylative cyclization was developed for facile synthesis of bridged tetracyclic indolenines, a common motif in many natural indole alkaloids. An antimicrobial screen of the cyclization products identified one compound which selectively potentiates β-lactam antibiotics in methicillin-resistant S. aureus (MRSA), and resensitizes a variety of MRSA strains to β-lactams.*

Antibiotic resistance has become a public health threat worldwide. The World Health Organization (WHO) has recognized antimicrobial resistance as a global problem which has needed urgent attention since 2001.[1] The recent data obtained for the three most common bacterial pathogens (i.e., *E. coli*, *K. pneumoniae* and *S. aureus*) showed that the proportion those resistant to commonly used antibacterials exceeded 50% in many settings.[2] The US Centers for Disease Control and Prevention (CDC) also released a report on "antibiotic-resistance threats" in 2013, and estimated that MRSA alone was responsible for over 11000 deaths in the United States every year.[3] In recent years, although the incidence of healthcare-acquired methicillin-resistant *S. aureus* (HA-MRSA) decreased, the incidence of community-acquired MRSA (CA-MRSA) increased significantly and has become more prevalent.[4] Vancomycin used to be effective for treating MRSA, however, resistance against vancomycin has also emerged and vancomycin-resistant *S. aureus* (VRSA) has become a problem of its own.[5] While new classes of antibacterials have proven difficult to develop,[6] antibiotic adjuvants are favored because they can increase the life span of antibiotics which are currently used in the clinic.[7] While these agents pose no or little selective pressure on bacteria, the chance of developing resistance to the compounds discovered by this strategy is smaller than conventional antibiotics.[8]

Inspired by the diverse family of bioactive natural indole alkaloids, we initiated an integrated chemical biology approach to discover novel reagents to fight against resistant bacteria,[9] and it entails rapid synthesis and screening of a variety of polycyclic indole alkaloids. Indole alkaloids represent a large and highly diverse family of complex natural products. Most of these were discovered as plant secondary metabolites and were reported to possess a wide range of biological activity.[10] Bridged polycyclic indolenines are a prevalent structural motif in natural indole alkaloids.[11] Many of these share a common tetracyclic core as highlighted in bold in Figure 1, and were reported to have anti-inflammatory and anti-infective activity. For example, strictamine was reported to inhibit herpes simplex virus and adenovirus, as well as the NF-κB pathway.[10d,e] Several methods have been developed for the synthesis of the indolenine functional group, such as oxidation of indole, palladium-catalyzed decarboxylative allylation/benzylation, and interrupted Fischer indolization.[12] However, the bridged tetracyclic indolenine framework has proven difficult to construct.[13] Until now, only a few examples of the preparation of bridged polycyclic indolenines (e.g., picrinine→strictamine and synthesis of quebrachidine; Figure 1) have been reported.[14] Herein, we report a facile synthesis of bridged tetracyclic indolenines using a gold-catalyzed desilylative cyclization reaction.

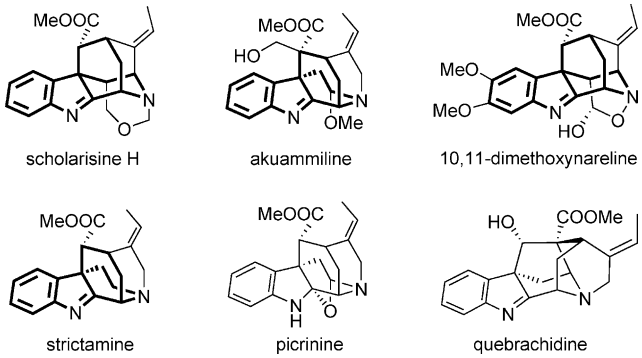

Figure 1. Representative bridged polycyclic indolenines and related natural products.

Gold/platinum catalysis has become an increasingly popular approach for carbon–carbon bond-formation reactions and has been used to synthesize a variety of complex natural products.[15] We previously reported a series of gold-catalyzed tandem cyclizations of alkynyl indoles (e.g., **1**→**3**; Figure 2).[9,16] Computational studies using density functional theory supported our proposed key intermediate, **2**, which reacts with a nucleophile, typically in an intramolecular fashion, to provide the indoline product **3**.[17] Based on these studies, we envisioned that the gold-catalyzed cyclization of the substrate **4** (Figure 2) may first form the intermediate **5**, and then may be used to produce the bridged tetracyclic indolenine **6** in the absence of a nucleophile.

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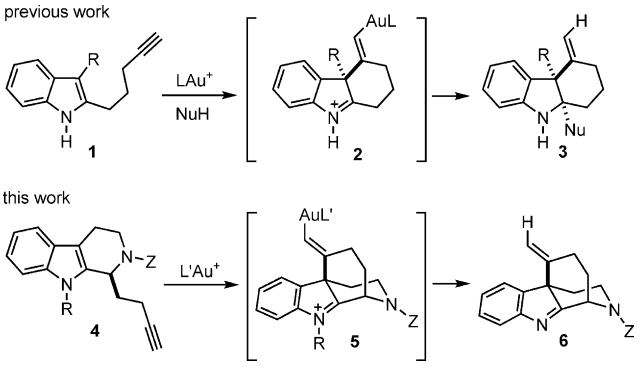
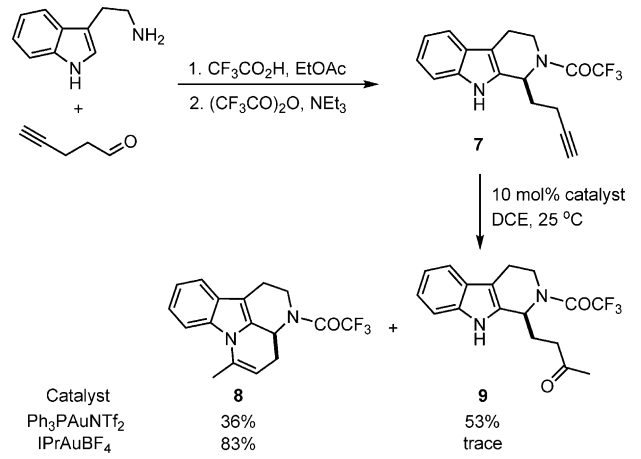


Figure 2. Gold-catalyzed cyclizations of alkynyl indoles.



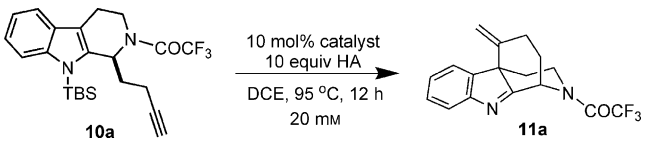
Scheme 1. Synthesis of the cyclization precursor and initial attempts on gold-catalyzed cyclization. Tf = trifluoromethanesulfonyl.

To test this hypothesis, we constructed the cyclization precursor **7** (Scheme 1) in racemic form using the Pictet–Spengler reaction of tryptamine and 4-pentynal, and subsequent acylation of the resulting secondary amine with trifluoroacetic anhydride. To our surprise, under our standard gold catalysis conditions ($Ph_3PAuNTf_2$, 25 °C), the C–N cyclization product **8** and ketone **9** were isolated as the major products. It appeared that upon activation of the gold catalyst, the alkyne of **7** reacted with either the indole nitrogen atom or a water molecule. Screening of a series of gold and platinum catalysts led only to diminishing the formation of the ketone. The C–N cyclization remained the dominant reaction pathway and no desired bridged tetracyclic indolenine was identified. We attributed the inability of **7** to undergo the desired C–C cyclization to its disfavored conformation, a result of the third ring formed by the Pictet–Spengler reaction. Hence, we decided to block the indole nitrogen atom using a silyl group and investigate a desilylative cyclization to form the bridged tetracyclic indolenine. The silylated cyclization precursor **10a** (for structure see Table 1) was prepared from **7** by sequential treatment with NaH and *tert*-butyldimethylsilyl (TBS) chloride.

Silyl enol ethers have been demonstrated as suitable substrates for gold catalysis to construct polycyclic frameworks, including bridged bicyclic systems.[18] However, gold-catalyzed cyclizations of N-silylated substrates have never been reported. With the **10a** in hand, we first surveyed a panel of gold and platinum catalysts in the presence of methanol as the silyl scavenger and proton source (Table 1). The reaction using $AuCl_3$ afforded small amounts of the desired indolenine **11a** (entry 1), while no desired product was formed when either $Ph_3PAuNTf_2$ or $PtCl_2$ was used (entries 2 and 3). Because carbene ligands were reported to show unique steric and electronic attributes,[19] we next investigated 1,3-bis(2,4,6-trimethylphenyl)-imidazol-2-ylidene- (IMes-) and 1,3-bis(2,6-diisopropylphenyl)-imidazol-2-ylidene- (IPr-) ligated gold(I) catalysts. The results showed that, compared with IMesAuBF$_4$, IPrAuBF$_4$ is superior in terms of both conversion of **10a** and yield of **11a** (entries 4 and 5). Alternative counterions of IPrAu$^+$ (entries 6 and 7) and silyl scavengers (entries 8–10) were evaluated, and all gave much lower yields. Further studies showed that neither IPrAuCl, $AgBF_4$, nor HBF_4 was not able to promote the desired cyclization.[20] The substrate that contains a triethylsilyl (TES) group instead of the TBS group was consumed rapidly. However, the reaction gave only a poor yield accompanied by a significant amount of the proto-desilylation product **7**.[20] Overall, IPrAuBF$_4$-catalyzed desilylative cyclization of **10a** using MeOH as the silyl scavenger and proton source was optimal.

We next investigated the scope of this reaction. Substrates bearing a variety of functional groups on the tryptamine nitrogen atom (e.g., amide, carbamate, and sulfonamides) and/or on the 5-position of the indole (e.g., MeO, H, and Cl) were prepared in the racemic forms using the aforementioned approach. All substrates were converted into the tetracyclic indolenines **10b–k** (Table 2) using the optimized reaction conditions. A variety of functional groups (entries 1–5) are tolerated on the amine nitrogen atom. However, they showed slightly different reactivity. Compared with **10a**, the sulfona-

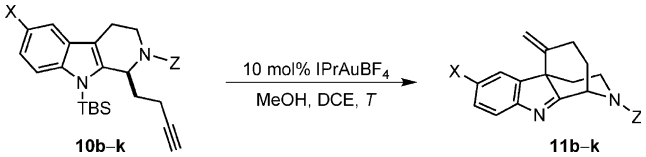
Table 1: Optimization of reaction conditions.



Entry	Catalyst	HA	Conv. [%][a]	Yield [%][a]
1	$AuCl_3$	MeOH	51	6
2	$Ph_3PAuNTf_2$	MeOH	100	0
3	$PtCl_2$	MeOH	37	0
4	IMesAuBF$_4$	MeOH	52	27
5	IPrAuBF$_4$	MeOH	82	67 (66)[b]
6	IPrAuOTf	MeOH	100	0
7	IPrAuSbF$_6$	MeOH	43	7
8	IPrAuBF$_4$	*i*PrOH	64	20
9	IPrAuBF$_4$	H_2O	63	27
10	IPrAuBF$_4$	*p*-$NO_2C_6H_4OH$	60	22

[a] 1 equivalent of 4-(dimethylamino)pyridine was added as an internal standard before the analysis by ^{1}H NMR spectroscopy. Conversion of **10a** and yield of the product were calculated based on ^{1}H NMR integrations. [b] The number within parentheses is the yield of the isolated product (80% based on recovered starting material). DCE = 1,2-dichloroethane, HA = silyl scavenger and proton source.

Table 2: Substrate scope of gold-catalyzed desilylative cyclization.



10b–k **11b–k**

Entry	**10**	X	Z	MeOH (equiv)	*T* [°C]	Yield [%]
1	**10b**	H	*p*-ClC_6H_4CO	20	90	66
2	**10c**	H	Ns	20	70	86
3	**10d**	H	*p*-$ClC_6H_4SO_2$	10	55	64
4[a]	**10e**	H	Ts	15	85	65
5	**10f**	H	Cbz	20	100	53[b]
6[c]	**10g**	MeO	$COCF_3$	20	95	66
7[a]	**10h**	MeO	Ns	5	55	78
8[d]	**10i**	Cl	$COCF_3$	20	105	44
9[e]	**10j**	Cl	Ns	5	70	66
10[e]	**10k**	Cl	*p*-$FC_6H_4SO_2$	15	105	51

[a] 50 mM. [b] 78% yield brsm. [c] 15 mol% $IPrAuBF_4$, 100 mM. [d] 20 mol% $IPrAuBF_4$, 100 mM. [e] 15 mol% $IPrAuBF_4$, 50 mM. Cbz = carboxybenzyl, Ns = 4-nitrobenzenesulfonyl, Ts = 4-toluenesulfonyl.

mides are more reactive and the carbamate is less reactive. Hence, minor modifications of the reaction conditions were applied to further improve the yields. When the indole is substituted with an electron-donating group (i.e., MeO), cyclizations of the substrates **10g** and **10h** went smoothly and furnished the desired products in 66 and 78% yields, respectively (entries 6 and 7). When an electron-withdrawing group (entries 8–10) was present on the indole of the substrates, the cyclizations were significantly slower. Higher catalyst loading and high reaction concentrations were required, and less MeOH was also favored to minimize the formation of the undesired C–N cyclization product **8**.

Encouraged by our previous success on the discovery of polycyclic indolines as novel antibacterial agents and ones that re-sensitize MRSA to β-lactam antibiotics,[9] we evaluated the bridged tetracyclic indolenines in a series of bacterial whole-cell assays. Gratifyingly, the compound **11b** was found to potentiate the activity of methicillin in a multidrug-resistant MRSA strain, ATCC BAA-44. All other indolenines were inactive in the original screen.[20] To assess the degree of synergy between **11b** and methicillin, a checkerboard minimum inhibitory concentration (MIC) study was next performed. The fractional inhibitory concentration index (FICI) of these two compounds was determined to be 0.039 (≤ 0.5) in BAA-44, thus indicating a strong synergistic effect.[21]

To evaluate the potentiation scope of **11b**, we next determined the MICs of a variety of antibiotics in both the presence and absence of **11b**. In addition to methicillin, BAA-44 is also resistant to a wide range of antibiotics, such as tetracycline, erythromycin, and daptomycin. As shown in Table 3, **11b** potentiates the activity of all β-lactams tested, such as oxacillin, amox/clav, cefazolin, and meropenem, but not antibiotics from other structural classes.[20] We thus concluded that **11b** is a selective potentiator of β-lactams.

We next tested the minimum re-sensitizing concentrations (MRCs) of **11b** to re-sensitize a variety of MRSA strains to two representative β-lactam antibiotics, amox/clav and cefazolin.[9] In addition to BAA-44, we also tested two CA-MRSA strains: NRS-100 (a.k.a., COL) and NRS-384 (a representative strain of the highly prevalent CA-MRSA type USA 300), and two VRSA strains, NR-46414 and NR-46421. The MRCs of **11b** were found to be in the range of 0.25–2 $\mu g\,mL^{-1}$ in all strains tested (Table 4). Furthermore, **11b** showed low antibacterial activity on its own with MICs of 32–64 $\mu g\,mL^{-1}$ in all strains tested (Table 4), and low mammalian toxicity with a half growth inhibitory concentration (GI_{50}) of 41 $\mu g\,mL^{-1}$ in human cervical carcinoma (HeLa) cells.[20]

Table 3: Selected potentiation profile of **11b**.

Antibiotic	MIC [$\mu g\,mL^{-1}$]	MIC (+**11b**)[a] [$\mu g\,mL^{-1}$]	Fold of potentiation
Methicillin	128	8	16
Oxacillin	32	1	32
Amox/clav	16	2	8
Cefazolin	128	4	32
Meropenem	4	1	4

[a] MIC value in the presence of 10 μM **11b**.

Table 4: Minimum inhibitory and re-sensitizing concentrations of **11b** in MRSA strains.

Strain	Subcategory	MIC[a]	MRC[a] amox/clav	MRC[a] cefazolin
BAA-44	HA-MRSA	64	2	2
NRS100	CA-MRSA	64	0.5	1
NRS384	CA-MRSA	64	1	n.r.
NR-46414	VRSA	32	0.25	n.r.
NR-46421	VRSA	32	2	0.25

[a] All MIC and MRC numbers are in $\mu g\,mL^{-1}$. n.r. = not resistant.

In summary, we have developed a gold-catalyzed desilylative cyclization to construct bridged tetracyclic indolenines, a common motif in many natural indole alkaloids. This work is the first report of gold-catalyzed cyclizations using indole-nitrogen-silylated substrates. The silyl group is critical and completely changed the selectivity of the reaction from C–N to C–C cyclization. A variety of substitutions on the indole and functional groups on the amine nitrogen atom are well tolerated in this reaction. Antibacterial screens identified one cyclized compound which selectively represses β-lactam resistance in MRSA, and may be further developed as an adjuvant therapy to treat resistant bacterial infections. Further studies such as asymmetric synthesis and structure–activity relationships of these bridged indolenines are ongoing and will be reported in due course.

Keywords: antibiotics · drug discovery · gold · heterocycles · synthetic methods

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