

## Inhibitors

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## Total Synthesis of Aspergillomarasmine A and Related Compounds: A Sulfamidate Approach Enables Exploration of Structure–Activity Relationships

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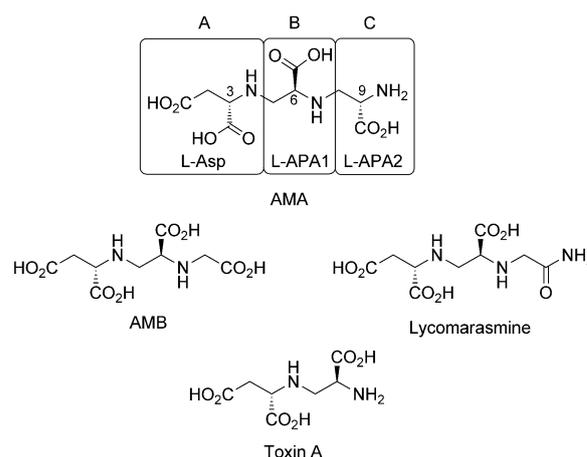
**Abstract:** The fungal secondary metabolite aspergillomarasmine A (AMA) has recently been identified as an inhibitor of metallo- $\beta$ -lactamases NDM-1 and VIM-2. Described herein is an efficient and practical route to AMA and its related compounds by a sulfamidate approach. In addition, a series of derivatives has been prepared and tested for biological activity in an effort to explore preliminary structure activity relationships. While it was determined that natural LLL isomer of AMA remains the most effective inactivator of NDM-1 enzyme activity both *in vitro* and in cells, the structure is highly tolerant of the changes in the stereochemistry at positions 3, 6, and 9.

Antibiotic resistance is a growing health crisis.<sup>[1]</sup> Especially concerning is the resistance to the most commonly prescribed class of antibiotics, the  $\beta$ -lactams. Resistance to  $\beta$ -lactam antibiotics is mediated primarily by enzymes which hydrolytically inactivate the  $\beta$ -lactam warhead characteristic of the drugs by one of two mechanisms: serine  $\beta$ -lactamases (SBLs) cleave the lactam ring by nucleophilic attack of a serine residue; and metallo- $\beta$ -lactamases (MBLs) act by using a zinc-stabilized hydroxy anion. Compounds which inactivate serine- $\beta$ -lactamases are approved as co-drugs and are administered together with  $\beta$ -lactam antibiotics,<sup>[2]</sup> yet inhibitors of MBLs are not yet clinically available. The importance of discovering such molecules is even more urgent since the recent identification of New Delhi metallo- $\beta$ -lactamase-1 (NDM-1), which is now widespread across the globe.<sup>[3]</sup> Organisms capable of harboring the associated *ndm* gene include most species of Enterobacteriaceae, *Acinetobacter*, and *Pseudomonas*. Typically, these strains are simultaneously resistant to all antibiotics except tigecycline and colistin. Alarming, colistin resistance in such multidrug resistant pathogens has recently been reported.<sup>[4]</sup> Bacteria-producing

NDM-1 and other MBLs therefore pose a pressing public health threat, thus making the discovery of an MBL inhibitor a high priority.

Recently we identified the fungal natural product aspergillomarasmine A (AMA) as a potent inhibitor of two key types of MBLs, NDM-1 and VIM-2.<sup>[5]</sup> AMA inactivates MBLs by sequestration of a catalytic zinc ion and successfully rescued the activity of meropenem in a mouse infected with NDM-1 expressing *Klebsiella pneumoniae*.<sup>[5]</sup>

AMA can be retrosynthetically deconstructed into three units: Asp and two aminopropionic acid (APA) moieties (Figure 1). Each unit has one chiral center at carbon atoms 3,



**Figure 1.** AMA and naturally occurring analogues. AMA comprises three units: A: Asp, B: APA1, and C: APA2.

6, and 9. In the original 1965 description of the discovery of AMA,<sup>[6]</sup> the stereochemical assignment was *L*-Asp, *D*-APA1, and *D*-APA2 (*LDD* configuration). We<sup>[7]</sup> and others<sup>[8]</sup> have recently shown, by total synthesis, that the configuration of natural AMA is actually *LLL* (*SSS*). In 1979, the structure of toxin A (AMA lacking unit C, the N-terminal APA2; Figure 1), was assigned *LD* stereochemistry (at positions 3 and 6, respectively).<sup>[9]</sup> Later, in 1991, this assignment was corrected to *LL* through chemical synthesis and isotope feeding experiments.<sup>[10]</sup> Replacement of the terminal APA2 moiety with glycine gives the natural product aspergillomarasmine B (AMB, Figure 1) whose stereochemical assignment has yet to be confirmed by total synthesis, but is expected to that of mirror AMA.

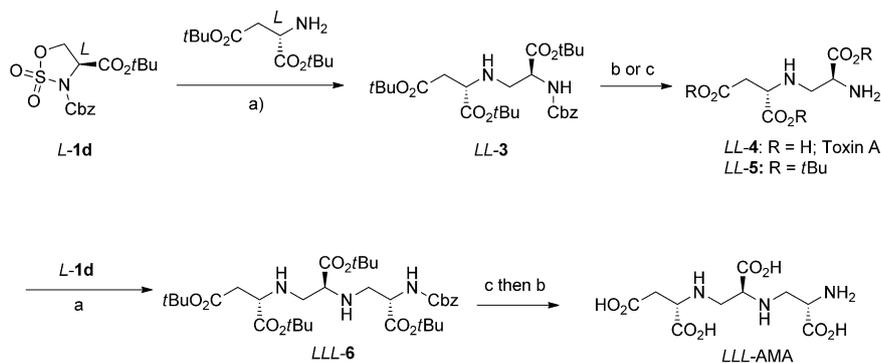
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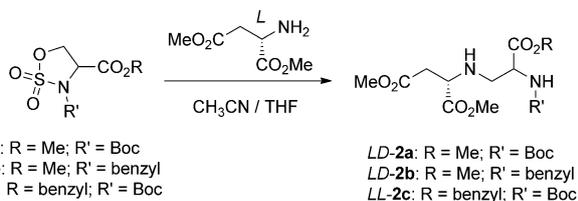
Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under:  
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This report describes an improved, efficient, and practical route to AMA and its related compounds, and utilizes a sulfamidate approach. Furthermore, using this strategy and our previously published aziridine method,<sup>[7]</sup> a series of derivatives has been prepared and tested for biological activity in an effort to explore preliminary structure–activity relationships (SAR) along with gaining insight into the mechanism of action of AMA against MBLs.

AMA can be derived from the coupling of aspartic acid and two



**Scheme 2.** Synthetic route to toxin A and aspergillomarasmine A. Reagents: a)  $CH_3CN/THF$  (1:1); b)  $CF_3SO_3H$ , anisole, DCM, 1 h at  $0^\circ C \rightarrow RT$ ; c)  $H_2$ , Pd/C. Cbz = benzyloxycarbonyl, DCM = dichloromethane.



**Scheme 1.** Aspartic acid ring opening of sulfamidates. Boc = *tert*-butoxycarbonyl, THF = tetrahydrofuran.

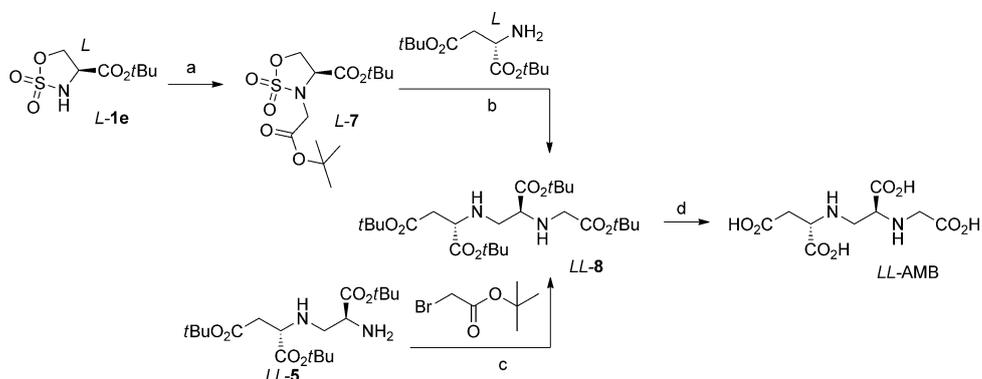
activated serine fragments. While our original synthesis of AMA took advantage of an aziridine approach,<sup>[7]</sup> the present work utilizes a sulfamidate<sup>[11]</sup> as the active serine moiety. In our initial exploratory experiments, we prepared a series of sulfamidates from either a protected *D*-serine (to give **D-1a** and **D-1b**; Scheme 1) or *L*-serine (to give **L-1c**) using a modification of the procedure described by Chandrasekaran and co-workers.<sup>[11f]</sup> Treatment of sulfamidates with the dimethyl ester of *L*-aspartic acid in acetonitrile allowed smooth coupling and the isolation of **LD-2a**, **LD-2b**, and **LL-2c** in good yields. In our hands, we found the reaction tolerated a number of protecting groups. Selective and total deprotection, however, proved to be cumbersome with the standard reaction conditions for methyl and benzyl ester hydrolysis, as well as removal of the Boc or benzyl amino protecting groups, and resulted in poor yields of the isolated products.

In their synthesis of AMA, Lei and co-workers<sup>[8]</sup> utilized a deprotection protocol involving  $CF_3SO_3H$ <sup>[12]</sup> and anisole in dichloromethane for the one-pot removal of both Boc and Cbz groups. With this mind, we developed a sulfamidate route to toxin A and AMA (Scheme 2). Treatment of commercially available *N*-(benzyloxycarbonyl)-*L*-serine

*tert*-butyl ester with  $SOCl_2$  followed by oxidation with ruthenium(III) chloride and sodium periodate allowed preparation of **L-1d**. Treatment of the sulfamidate with the di-*tert*-butyl ester of *L*-aspartic acid gave **LL-3**, which could be either completely deprotected using Lei's method to give toxin A (**LL-4**) or hydrogenated in the presence of a palladium catalyst to give the intermediate **LL-5**. Reaction with additional **L-1d** allowed introduction of a second APA moiety to yield **LLL-6**. Deprotection using  $CF_3SO_3H$  and anisole in dichloromethane gave **LLL-AMA**. The spectroscopic and physical properties of synthesized material and natural AMA were identical.

The versatility of the approach was demonstrated in the synthesis of aspergillomarasmine B (**LL-AMB**). Two routes were followed and are shown in Scheme 3. In the first, the Cbz group of **L-1d** was hydrogenated to give **L-1e**, then alkylated with *tert*-butyl bromoacetate to give **L-7**. Treatment of the sulfamidate with the di-*tert*-butyl ester of *L*-aspartic acid gave **LL-8**, which was deprotected to give **LL-AMB**. Alternatively, alkylation of **LL-5** with *tert*-butyl bromoacetate also yielded **LL-8** and subsequently **LL-AMB**.

The sulfamidate route above offers a number of advantages over the aziridine approach previously developed. Issues surrounding the regioselectivity of aziridine ring opening are well documented<sup>[11f,13]</sup> and while these factors



**Scheme 3.** Synthetic route to aspergillomarasmine B. Reagents: a)  $CsCO_3$  then *tert*-butyl bromoacetate; b)  $CH_3CN/THF$  (1:1); c) TEA in EtOH/DMF (1:1); d)  $CF_3SO_3H$ , anisole, DCM, 1 h at  $0^\circ C \rightarrow RT$ . TEA = trimethylamine.

played a very minor role in our original synthesis (with yields of the unwanted regioisomer never greater than 5% and easily separable by chromatography), the sulfamidate approach completely avoids these. Furthermore, the streamlining of protecting groups and the reduction in the number of overall steps allows a much higher yielding synthesis with an overall yield of *LLL*-AMA of 19%, starting from the protected serine.

A series of compounds related to AMA were synthesized (by routes either described previously<sup>[7]</sup> or as presented in the Supporting Information) and the biological activity of these analogues was explored through dose-response assays using purified, recombinant NDM-1 as well as cell-based assays to assess in vivo synergy with meropenem in an NDM-1 expressing strain of *E. coli* (Table 1). We focused in particular on units A and C (Figure 1) in this study as these are sites of known natural variants (unit C) or are readily sampled using our synthetic strategies.

As we reported previously,<sup>[7]</sup> altering the stereochemistry at positions 6 (*LDL*-AMA, *LDD*-AMA) and 9 (*LDD*-AMA, *LLD*-AMA) had minimal effect on in vitro NDM-1 inactivation and isomers retained synergy with meropenem, though with reduced efficiency. The stereochemistry of the  $\alpha$ -carbon atom of Asp at position 3 (*DLL*-AMA) is also flexible, with no significant influence on activity. Therefore there is tolerance in the stereochemistry at positions 3, 6, and 9, thus reflecting significant plasticity in biological activity.

NDM-1  $IC_{50}$  curves for the known natural products *LL*-AMB (AMA structure in which APA2 is replaced by Gly) and lycomarasmine (AMA where APA2 is replaced by Glyamide, *LL*-26) exhibited complete enzyme inactivation, although the latter had only marginal activity in cells expressing NDM-1. The N-terminal amine of unit C is therefore dispensable but retention of this group confers optimal activity with pure enzyme and in cells. Protection of the amine with the bulky nosyl group (*LLL*-14) significantly weakens enzyme inhibition activity and abrogates in-cell activity. Alternate spacing of the C-terminal carboxy group (C2 of AMB vs. C3 AMA) is accommodated with retention of activity, nevertheless, a free carboxy rather than an amide is preferred for activity in the cell, perhaps reflecting access and transport to the periplasm.

Complete removal of APA2 (toxin A; *LL*-4) has a significant impact on both in vitro enzyme activity and synergy with meropenem in cells. Unlike compounds with either a Gly or APA equivalent unit in the APA2 position, toxin A showed unusual behavior in enzyme inhibition studies. Rather than precipitous dose dependence from full activity to inactivation, toxin A  $IC_{50}$  curves showed a shallow inhibition gradient and did not result in complete loss of enzyme action (see Figure S90 in the Supporting Information), thus indicating complex, and possibly nonspecific inhibition. Furthermore, toxin A had no ability to rescue meropenem activity in cell potentiation assays. The APA2 unit is therefore essential for AMA activity, but is tolerant of some modification. Replacement of the N-terminal Asp of toxin A with Glu (*LL*-17) abolished both enzyme inhibition and in-cell activity.

We explored the role of the unit A Asp by change to Glu (*LLL*-23). This analogue retained good in vitro enzyme

**Table 1:** Concentration response and FICI values for select AMA compounds.<sup>[a]</sup>

Compound	Structure	$IC_{50}$ [ $\mu$ M]	RC [ $\mu$ M] <sup>[b]</sup>
<i>LLL</i> -AMA		9.3 ± 0.3	12.5
<i>LDL</i> -AMA		12.5 ± 0.2	50
<i>LLD</i> -AMA		11.9 ± 1.5	50
<i>LDD</i> -AMA		10.2 ± 0.4	50
<i>DLL</i> -AMA		11.6 ± 0.1	25
<i>LL</i> -AMB		18.3 ± 1.3	50
<i>LL</i> -4 (toxin A)		NA <sup>[c]</sup>	NA
<i>LL</i> -10		NA	NT
<i>LL</i> -11		NA	NT
<i>LLL</i> -12		NA <sup>[c]</sup>	NT
<i>LLL</i> -13		NA <sup>[c]</sup>	NA
<i>LLL</i> -14		36.4 ± 1.0	NA
<i>LL</i> -17		NA <sup>[c]</sup>	NA
<i>LLL</i> -21		NA <sup>[c]</sup>	NT
<i>LLL</i> -22		17.0 ± 0.2	NT
<i>LLL</i> -23		12.1 ± 0.4	NA
<i>LL</i> -26 (lycomarasmine)		13.5 ± 0.6	100

[a]  $IC_{50}$  measurements required < 20% residual activity. [b] RC = Rescue concentration is the concentration of compound needed to fully regain the activity of meropenem at the clinical breakpoint (2.5  $\mu$ M).

[c] Incomplete and non-ideal inhibition (see figure in Supporting Information). NA = not active and indicates that either no inhibition or synergy observed at given concentrations; NT = not tested (see Supporting Information for full data sets); Ns = 2-nitrobenzenesulfonyl.

inhibition activity, but lacked the ability to potentiate meropenem in cells.



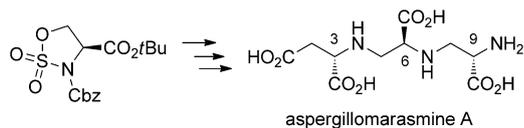
## Communications



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Total Synthesis of Aspergillomarasmine A and Related Compounds: A Sulfamidate Approach Enables Exploration of Structure–Activity Relationships



The **total synthesis** of aspergillomarasmine A and related natural products using a sulfamidate approach is described, and preliminary structure–activity relationships are explored. While the natural *LLL* isomer of AMA remains the

most effective inactivator of NDM-1 enzyme activity both in vitro and in cells, the structure is highly tolerant of changes in the stereochemistry at positions 3, 6, and 9. Cbz = benzyloxycarbonyl.