

Total Synthesis and Activity of the Metallo- β -lactamase Inhibitor Aspergillomarasmine A

Kalinka Koteva, Andrew M. King, Alfredo Capretta,* and Gerard D. Wright*

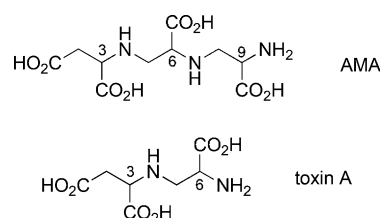
Abstract: Resistance to β -lactam antibiotics is mediated primarily by enzymes that hydrolytically inactivate the drugs by one of two mechanisms: serine nucleophilic attack or metal-dependent activation of a water molecule. Serine β -lactamases are countered in the clinic by several codrugs that inhibit these enzymes, thereby rescuing antibiotic action. There are no equivalent inhibitors of metallo- β -lactamases in clinical use, but the fungal secondary metabolite aspergillomarasmine A has recently been identified as a potential candidate for such a codrug. Herein we report the synthesis of aspergillomarasmine A. The synthesis enabled confirmation of the stereochemical configuration of the compound and offers a route for the synthesis of derivatives in the future.

β -Lactam antibiotics are the most widely used group of antimicrobial drugs in the clinic today. The penicillins, cephalosporins, carbapenems, and monobactams comprise a family of bactericidal antibiotics that share the β -lactam ring that is the reactive moiety of this class of drugs. Resistance to β -lactams can occur through altered antibiotic uptake or efflux, or the synthesis of insensitive target proteins (the cell-wall biosynthetic enzymes that cross-link peptidoglycans), but it is a large group of hydrolytic enzymes, the β -lactamases, that represent the principal mode of resistance and drug failure in the clinic. β -Lactamases are subdivided by their use of one of two general chemical mechanisms that result in hydrolytic ring-opening reactions. Serine β -lactamases (SBLs) use an active-site serine residue in a covalent capture mechanism that results in the formation of a transient acyl enzyme intermediate, followed by hydrolytic release of the inactive product. Metallo- β -lactamases (MBLs) on the other hand employ active-site metals (one or two Zn^{2+} centers) to activate an active-site water molecule that acts as a nucleophile in β -lactam ring opening.^[1]

To overcome the emergence of β -lactamases in pathogenic bacteria, new derivatives of β -lactam antibiotics have been regularly introduced to the market over the past

decades, thus resulting in several generations of drugs with improved pharmacological profiles and ability to evade resistance. This strategy, while highly successful, is proving increasingly challenging to pursue with effectiveness. A parallel approach has been the coformulation of β -lactam drugs with inhibitors of β -lactamases. This method has also proven to be highly effective. The introduction of β -lactamase inhibitors clavulanic acid, tazobactam, sulbactam, and recently avibactam^[2] in various coformulations with penicillins and cephalosporins has extended the lifetime and clinical efficacy of several drugs. This approach so far has focused on the SBLs, which have been the most prominent β -lactamases in pathogenic bacteria. Over the past several years, however, MBLs have increased in frequency and concern. MBLs, unlike most SBLs, can inactivate essentially all penicillins, cephalosporins, and carbapenems, thereby threatening the majority of clinically used antibiotics. In particular, the emergence and widespread global distribution of Gram-negative pathogens harboring the NDM-1 MBL has proven to be a grave cause of concern.^[3] There is a growing clinical need for inhibitors of MBLs that can be given as codrugs.

We have recently shown that the fungal natural product aspergillomarasmine A (AMA, Scheme 1) is a potent inacti-



Scheme 1. Aspergillomarasmine A (AMA) and toxin A.

vator of MBLs.^[4] AMA operates by a zinc-chelation mechanism resulting in an inactive enzyme. NDM and VIM MBLs are particularly sensitive to the action of AMA. Furthermore, AMA in combination with meropenem successfully cured mice infected with a lethal dose of *Klebsiella pneumoniae* harboring the NDM-1 MBL, thus demonstrating the potential of AMA as a candidate MBL inhibitor that could find use as a codrug with β -lactam antibiotics.

We obtained AMA through fermentation of a producing strain of *Aspergillus versicolor*. A study of AMA in the past revealed a lack of clarity on the absolute configuration of the natural product. In the original 1965 description of the discovery of AMA, the stereochemical assignment was L-aspartic acid, D-aminopropionic acid, and D-aminopropionic acid (LDD configuration at carbon atoms 3, 6, and 9,

[*] Dr. K. Koteva, A. M. King, Prof. A. Capretta, Prof. G. D. Wright
Michael G. DeGroote Institute for Infectious Disease Research
McMaster University
1280 Main Street West, Hamilton, ON (Canada)
E-mail: capretta@mcmaster.ca
wrightge@mcmaster.ca

Prof. A. Capretta
Department of Chemistry and Chemical Biology
McMaster University
1280 Main Street West, Hamilton, ON (Canada)

Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under <http://dx.doi.org/10.1002/anie.201510057>.

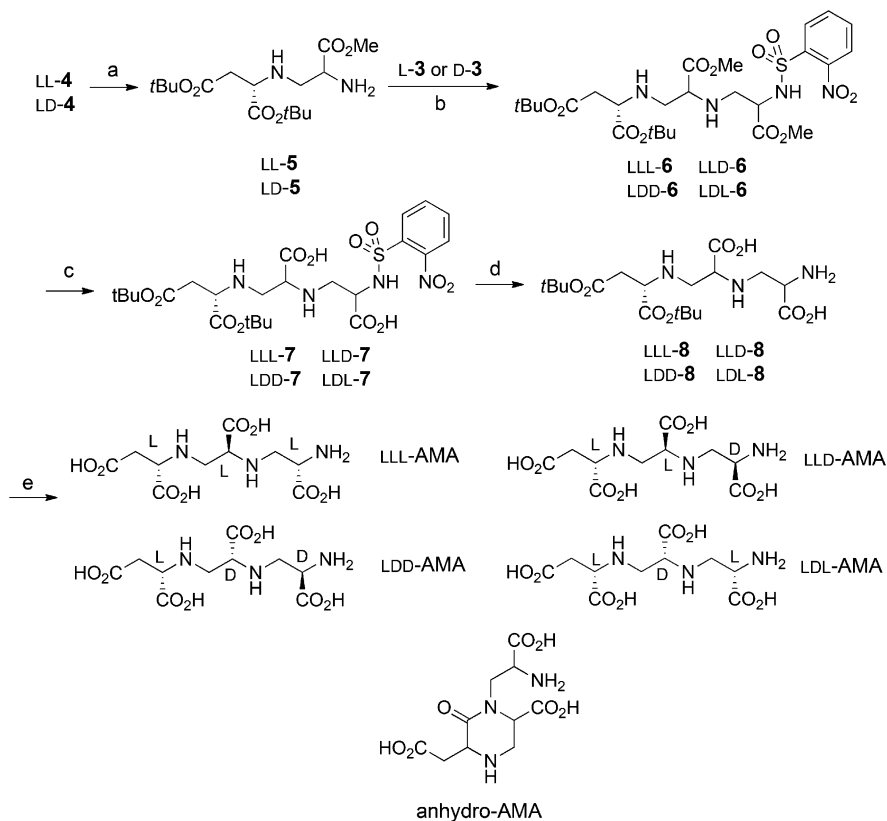
respectively, Scheme 1).^[5] In 1979, the structure of toxin A (equivalent to AMA lacking the N-terminal aminopropionic acid, Scheme 1) was assigned the LD configuration (at C3 and C6, respectively).^[6] Later, in 1991, this assignment was corrected to LL through chemical synthesis and feeding experiments.^[7] Access to analogues of AMA has also been limited owing to the reliance on available products of fermentation. We have developed a synthetic strategy towards AMA to enable the synthesis of derivatives possessing the AMA scaffold and to confirm the absolute configuration.

Retrosynthetic analysis of AMA suggests the compound is derived from an aspartic acid moiety coupled to two activated serine fragments. Experiments by Haenni et al.^[5] and Friis et al.^[7] demonstrated that the aspartic acid residue has the L configuration; therefore, we prepared four stereoisomers of AMA to definitively assign the absolute configuration of the natural product. The synthesis takes advantage of two successive reactions with the aziridine derived from either D- or L-serine. By the use of either antipode of the *N*-tritylated methyl ester of serine^[8] and the procedure described by Zwaneburg and co-workers,^[9] aziridines **L-2** and **D-2** were prepared (Scheme 2).

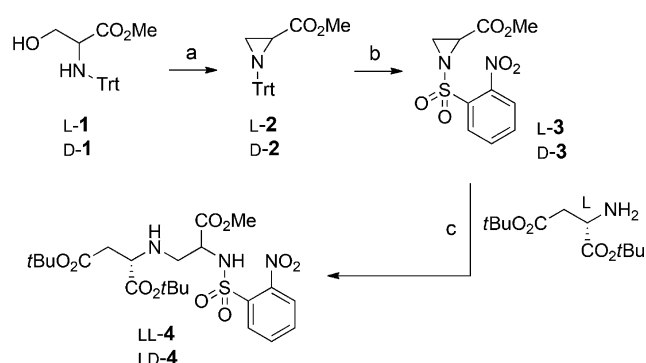
To facilitate efficient ring opening, it was necessary to replace the trityl group with a more electron withdrawing protective group. An *o*-nosyl group was introduced (to yield **L-3** or **D-3**) by an in situ “one-pot” strategy to avoid isolation of the intermediate free aziridine, which is reported to be very unstable. The nucleophilic ring-opening reaction of aziridines **L-3** and **D-3**

with the free base of L-aspartic acid di-*tert*-butyl ester in dry tetrahydrofuran gave the protected α,β -diamino derivatives **LL-4** and **LD-4**, respectively.^[10]

The *o*-nosyl group was removed by the use of a solution of thiophenol and diisopropylethylamine in acetonitrile to yield either **LL-5** or **LD-5** (Scheme 3).^[11] The second serine equivalent could then be introduced once again by treatment of the



Scheme 3. a) PhSH, DIPEA, acetonitrile, room temperature, 1 h, 80%; b) THF, 30%; c) Me₃SnOH (6 equiv), DCE, 80°C, 3 h, 82%; d) PhSH, DIPEA, acetonitrile, room temperature, 5 h, 50%; e) TFA/CH₂Cl₂ (1:1), 4°C, 24 h, 23%. DCE = dichloroethane, DIPEA = diisopropylethylamine.



Scheme 2. a) MsCl, triethylamine, THF, 65°C, 60 h, 90%; b) TFA, CH₂Cl₂/MeOH, 0°C, 30 min, then *o*-NsCl, room temperature, 16 h, 71%; c) L-aspartic acid di-*tert*-butyl ester, THF, RT, 16 h, 80%. Ms = mesyl, Ns = nosyl, THF = tetrahydrofuran, TFA = trifluoroacetic acid, Trt = trityl.

α,β -diamino derivative **LL-5** with the *o*-nosyl-protected aziridine **L-3** to yield **LLL-6**. Similarly, **LD-5** was treated with **D-3** to give yield **LDD-6**, **LL-5** was treated with **D-3** to give **LLD-6**, and **LD-5** was treated with **L-3** to give **LDL-6**. The deprotection sequence took advantage of the trimethyltin hydroxide protocol described by Nicolaou et al.^[12] for the hydrolysis of the methyl esters (to give **LLL-7**, **LDD-7**, **LLD-7**, and **LDL-7**), followed by removal of the *o*-nosyl group as described above (to give **LLL-8**, **LDD-8**, **LLD-8**, and **LDL-8**). Finally, cleavage of the *tert*-butyl ester groups with trifluoroacetic acid in dichloromethane yielded **LLL-AMA**, **LDD-AMA**, **LLD-AMA**, and **LDL-AMA**. Interestingly, under these conditions, small amounts of the cyclic anhydro-AMA were produced. Anhydro-**LLL-AMA** and anhydro-**LDD-AMA** were isolated and characterized.

The optical rotation of the natural AMA isolated from *Aspergillus versicolor* showed an $[\alpha]_D^{20}$ value of -48° . The synthetic samples showed values of -47° (**LLL-AMA**), $+7^\circ$

(LDD-AMA), -13° (LLD-AMA), and -19° (LDL-AMA). Examination of the NMR spectra of these compounds afforded further evidence that the natural product has the LLL configuration, as the ^1H NMR spectra of the synthetic and the natural sample were identical. Furthermore, a ^1H NMR spectrum obtained after the mixing of LLL-AMA with natural AMA (in a 1:1 ratio) showed only one set of peaks (see the Supporting Information).

The MBLs NDM-1, VIM-2, and IMP-7 as well as the SBL TEM-1 were assayed against the synthesized compounds (Table 1; see also Figures S64–S77 in the Supporting Infor-

Table 1: IC_{50} values from concentration-response assays against metallo- β -lactamases.

Compound	NDM-1 IC_{50} [μM]	VIM-2 IC_{50} [μM]
natural AMA	9.9 ± 0.3	10.8 ± 0.9
LLL-AMA	6.8 ± 0.5	8.3 ± 0.2
LDD-AMA	7.8 ± 0.3	9.7 ± 0.3
LDL-AMA	6.9 ± 0.7	7.8 ± 1.0
LLD-AMA	5.2 ± 0.1	6.1 ± 0.1
anhydro-LLL-AMA	> 256	> 256
anhydro-LDD-AMA	> 256	> 256

mation). As expected, the compounds displayed no inhibitory effect on TEM-1 or IMP-7 up to a concentration of $256 \mu\text{M}$ (data not shown), a result similar to that found in the original study of AMA as an MBL inactivator.^[4]

Assessment of the MBL-inactivation properties of the synthesized compounds with purified MBLs NDM-1 and VIM-2 revealed that all isomers of AMA had very similar properties to those of AMA derived from fermentation (Table 1). This result was initially surprising but is consistent with a chelation model of enzyme inactivation, for which model building shows that different configurations do not significantly impact a predicted octahedral coordination of Zn^{2+} . Further studies need to be carried out to explore the precise Zn^{2+} ligands in AMA. Our synthesis offers a route to the further exploration of AMA structure and function as well as an entry to the development of new MBL inhibitors.

Acknowledgements

This research was funded by a Collaborative Health Research Project Grant (446433-13), a Canadian Institutes of Health Research Grant (MT-13536), and a Canada Research Chair in Antibiotic Biochemistry (to G.D.W.).

Keywords: antibiotic resistance · configuration determination · inhibitors · metallo- β -lactamases · total synthesis

How to cite: *Angew. Chem. Int. Ed.* **2016**, 55, 2210–2212

Angew. Chem. **2016**, 128, 2250–2252

- [1] G. D. Wright, *Chem. Commun.* **2011**, 47, 4055–4061.
- [2] S. M. Drawz, K. M. Papp-Wallace, R. A. Bonomo, *Antimicrob. Agents Chemother.* **2014**, 58, 1835–1846.
- [3] P. Nordmann, L. Poirel, T. R. Walsh, D. M. Livermore, *Trends Microbiol.* **2011**, 19, 588–595.
- [4] A. M. King, S. A. Reid-Yu, W. Wang, D. T. King, G. De Pascale, N. C. Strynadka, T. R. Walsh, B. K. Coombes, G. D. Wright, *Nature* **2014**, 510, 503–506.
- [5] A. L. Haenni, M. Robert, W. Vetter, L. Roux, M. Barbier, E. Lederer, *Helv. Chim. Acta* **1965**, 48, 729–750.
- [6] E. Bach, S. Christensen, L. Dalgaard, P. O. Larsen, C. E. Olsen, V. Smedegård-Petersen, *Physiol. Plant Pathol.* **1979**, 14, 41–46.
- [7] P. Friis, C. E. Olsen, B. L. Møller, *J. Biol. Chem.* **1991**, 266, 13329–13335.
- [8] K. O'Brien, K. ó Proinsias, F. Kelleher, *Tetrahedron* **2014**, 70, 5082–5092.
- [9] J. G. H. Willems, M. C. Hersmis, R. de Gelder, J. M. M. Smits, J. B. Hammink, F. J. Dommerholt, L. Thijs, B. Zwanenburg, *J. Chem. Soc. Perkin Trans. 1* **1997**, 963–968.
- [10] C. A. Olsen, C. Christensen, B. Nielsen, M. M. Farah, M. Witt, R. P. Clausen, J. L. Kristensen, H. Franzyk, J. W. Jaroszewski, *Org. Lett.* **2006**, 8, 3371–3374.
- [11] J. J. Turner, F. D. Sikkema, D. V. Filippov, G. A. van der Marel, J. H. van Boom, *Synlett* **2001**, 1727–1730; K.-I. Nihei, M. J. Kato, T. Yamane, M. S. Palma, K. Konno, *Synlett* **2001**, 1167–1169.
- [12] K. C. Nicolaou, A. A. Estrada, M. Zak, S. H. Lee, B. S. Safina, *Angew. Chem. Int. Ed.* **2005**, 44, 1378–1382; *Angew. Chem.* **2005**, 117, 1402–1406.

Received: October 28, 2015

Published online: December 28, 2015