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Letter

NMR Backbone Assignment of VIM-2 and Identification of the Active Enantiomer of a Potential Inhibitor

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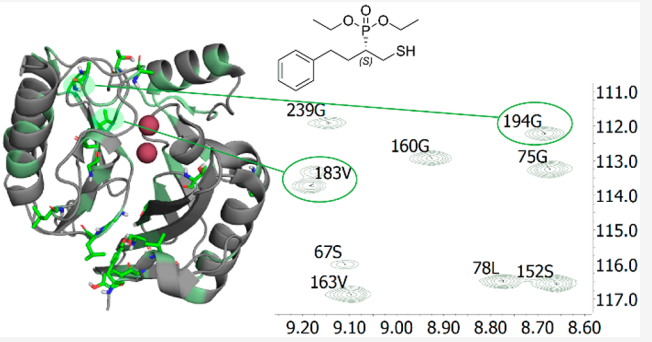
ABSTRACT: Carbapenem resistance caused by metallo-β-lactamases is a serious global challenge that, if not tackled efficiently, is expected to lead to millions of deaths in the coming decades. Verona-integron encoded metallo-β-lactamase 2 (VIM-2) is a bacterial enzyme that has been reported from multidrug-resistant nosocomial isolates of *Pseudomonas aeruginosa* and other Gram-negative pathogens. As it hydrolyzes most β-lactams efficiently, including carbapenems, it is a major threat to current antimicrobial chemotherapies. So far, there is no clinically applicable inhibitor for this enzyme. In this work, the backbone NMR resonance assignment of VIM-2 is disclosed, opening up NMR investigations of this clinically important enzyme and its potential inhibitors for solutions, enabling a rational improvement of inhibitor candidates. Making use of the assignment, we identified the active enantiomer of a VIM-2 inhibitor candidate as well as its possible binding site and K_d, utilizing NMR chemical shift titration experiments.



KEYWORDS: *metallo-β-lactamase, antibiotic resistance, NMR, VIM-2*

Bacterial infections have had a major impact on public health for as long as humans have existed. The discovery of antibiotic agents initially provided relief by allowing the control of infectious diseases that were previously the foremost cause of mortality for most of human existence. However, antibiotics gave only temporary relief, leading humanity into an era where bacterial resistance toward existing antibiotics is rapidly increasing[1,2] whereas the development of new antimicrobials is ever more challenging.[3,4] Bacteria have developed an arsenal of resistance mechanisms, of which metallo-β-lactamases are among the most devastating. These enzymes dismantle β-lactams, the cheapest and most widely used group of antibiotics, and deactivate carbapenems, our last-resort antibiotics that are reserved for the treatment of multidrug resistant infections.[5] Metallo-β-lactamases are increasingly recurring in the clinically most relevant multidrug resistant strains, including Gram-negative pathogens.[6]

Inhibiting metallo-β-lactamase activity is expected to provide a potential solution for the emerging crisis by allowing a renewed use of broad-spectrum β-lactam antibiotics, such as penicillins, cephalosporins, and carbapenems.[5−8] Metallo-β-lactamases have been divided into three (Ambler) subcategories: B1, B2, and B3.[6−9] Subclass B1 is the most prevalent among clinically relevant strains, with the New Delhi metallo-β-lactamases (NDMs), Verona-integron encoded metallo-β-lactamases (VIMs), imipenemases (IMPs), and German imipenemases (GIMs) belonging to this subclass and the common structural element being two zinc residues in their active site.[6] Despite their identical αβ/βα-folding motif, it has proven difficult to find a common strong binder to these enzymes.[4,6] On top of that, no clinically applicable selective inhibitors have been developed for any of these enzymes.

Understanding the binding event is key for inhibitor development. Although the protein residues involved in inhibitor binding may be identified by X-ray crystallography when a cocrystal structure is available, the binding dynamics are detectable only in solution. Solution NMR spectroscopy is the method of choice to study both the binding site and the binding dynamics of protein−ligand interactions.[10,11] NDM-1 and VIM-2 are two of the clinically most distressing metallo-β-lactamases. Although NDM-1 has been studied by NMR,[12,13] the NMR backbone assignment of VIM-2, which is a

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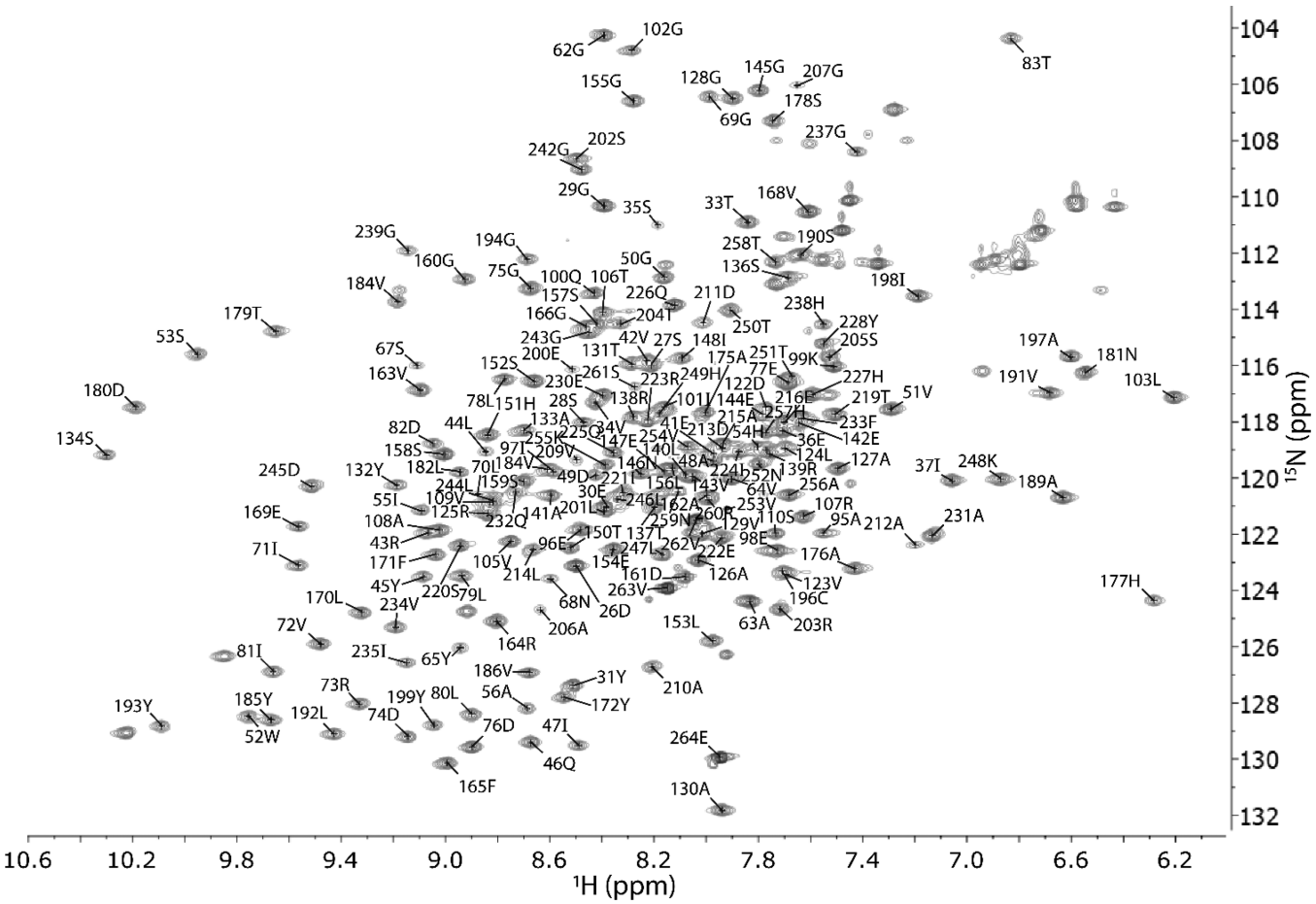


Figure 1. ^{1}H,^{15}N-HSQC spectrum (700 MHz, pH 7, 37 °C) of VIM-2 with the backbone amide proton assignment.

prerequisite for NMR binding studies, has not yet been reported.

Herein, we report the first NMR backbone assignment (Figure 1) of VIM-2 as well as that of its complex with L-captopril, a previously identified binder (IC_{50} 4.4 μM).[14] We further identify the active enantiomer of **1** (Figure 2), a

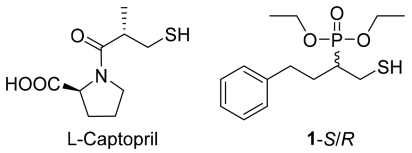
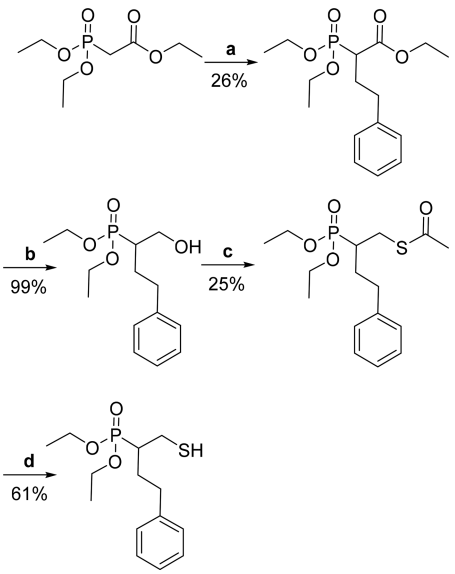


Figure 2. Structure of L-captopril and of the VIM-2 inhibitor **1**, whose enantiomers were investigated.

compound that has previously been reported to inhibit VIM-2 (IC_{50} 0.89 μM, 78% inhibition in a whole cell experiment), yet has so far only been studied as a racemate,[15] and demonstrate it to bind to the same cleft as L-captopril, providing useful information for drug development.

To support the further development of **1** into a clinically applicable VIM-2 inhibitor, we resynthesized it (Scheme 1), isolated its enantiomers by chiral HPLC, and evaluated their cytotoxicity (**1**-*S*, 99.8 μM; **1**-*R*, 82.3 μM on HeLa cells) and VIM-2 binding (for details on synthesis, separation, determination of stereochemistry, and cytotoxicity, see the Supporting Information). As the cocrystallization of **1** with VIM-2 has been unsuccessful, thus preventing X-ray diffraction studies, we

Scheme 1. Synthetic Pathway for Compound 1[a]



[a]Conditions used for the synthesis: **a** R-Br, KO*t*Bu, DMF, 60 °C; **b** $LiBH_4$, THF, r.t.; **c** (1) MsCl, Et_3N, DMAP, CH_2Cl_2, r.t., (2) KSAc, DMF, r.t.; **d** NaOMe/MeOH, r.t.

expressed ^{13}C,^{15}N uniformly labeled VIM-2 and performed its backbone resonance assignment to study the binding event by solution NMR.

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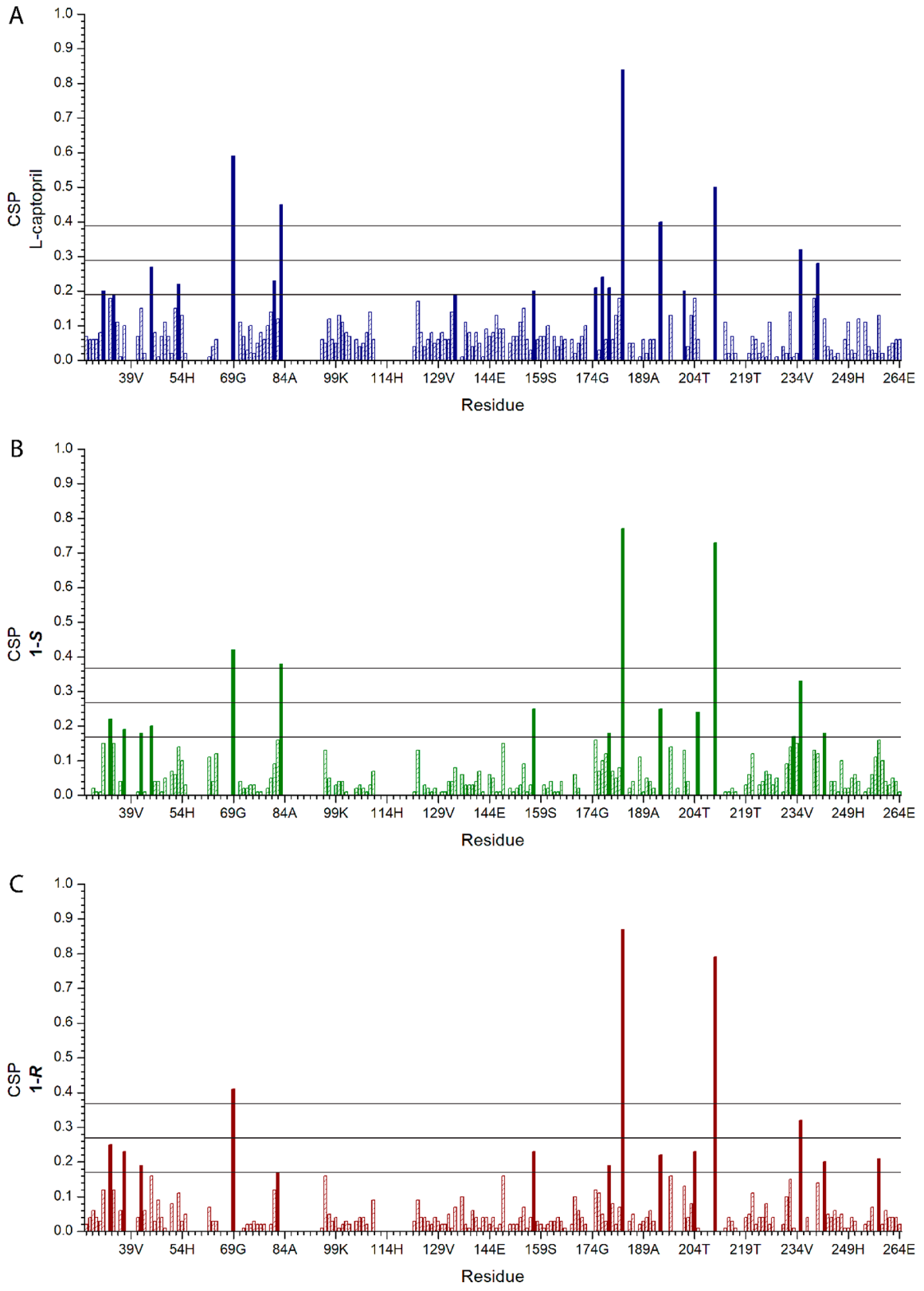

Figure 3. Chemical shift perturbations (CSPs) observed in the titration experiments of (A) L-captopril, (B) **1**-*S*, and (C) **1**-*R*. Residues with a CSP of 1 standard deviation or more above the mean are indicated by the filled bars. The black lines indicate 1, 2, and 3 std. dev. above the mean CSP. The residues involved in ligand binding to VIM-2 (PDB: 4C1D) are shown in Figure S8.

Protein Expression and Purification. Unlabeled VIM-2 was expressed from *E. coli* giving 37 mg/L yield; U-[^{15}N]-labeled VIM-2 gave 17 mg/L, whereas U-[^{15}N−^{13}C]-labeled VIM-2 gave 4 mg/L of cell culture. The purity of the samples was ≥95%, as estimated by SDS-PAGE. For details, see the Supporting Information.

NMR Experiments. With a ^{1}H,^{15}N-HSQC,[16−18] HNCO,[18−20] HNcaCO,[21] HNCA,[18−20] HNcoCA,[19,20]

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HNCACB,[22,23] and HNcoCACB[24] data set recorded at 37 °C, 700 MHz, 84% of the backbone resonances were assigned, resulting in 193 expected NH cross-peaks that could be used for titration experiments (Figure 1). A total of 178 residues for the sample without L-captopril and 190 residues for the sample with captopril were confirmed for spectra recorded at 25 °C with addition of 4.8% EtOH. The backbone resonance assignments are given in Tables S3−S5 and have been deposited to the BMRBI with code 51165. NMR titration experiments were performed with L-captopril and the inhibitors **1**-*S* and **1**-*R* (Figure 2) and revealed similar behavior of L-captopril with **1**-*S* and **1**-*R* (Figure 3). In the titrations with L-captopril **1**-*S* and **1**-*R*, nearly all residues underwent measurable chemical shift perturbations (CSPs), with the amino acids 69G, 83T, 183V, and 210A having a comparably large CSP for L-captopril and **1**-*S*. The signals of the free and the bound VIM-2 species were observable throughout the titration with all three ligands, indicating a slow exchange process and allowing the estimation of the K_d values from the signal intensities[10] of the residues that shifted at least 1 std. dev. above the mean CSP (Table S9).

The dissociation constants found for the various residues for L-captopril titration ranged between 0.63 and 6.8 μM, which is in the same order of magnitude as the K_d reported for metallo-β-lactamases,[25,26] including VIM-2 (0.6 μM).[14,25,26] The K_d values obtained for **1**-*S* displayed a slightly wider range, typically between 0.23 and 3.9 μM, and were in the same order of magnitude as those of L-captopril (Table S9). The dissociation constants obtained for **1**-*R* are, in contrast, in the millimolar range, revealing the far lower affinity of this enantiomer.

This observation corroborates the conclusions drawn from the chemical shift perturbation analyses (Figure 3), which identified the **1**-*S* isomer to be responsible for the bioactivity of **1**. Comparable chemical shift changes for the same $^{1}H,^{15}N$ HSQC cross peaks of VIM-2 upon addition of L-captopril and both enantiomers of **1** (Figure 4) indicate that the latter likely binds to the previously identified L-captopril binding site.[27]

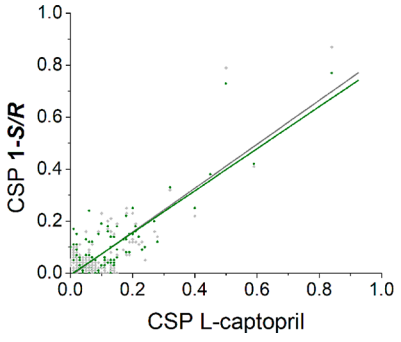

Figure 4. Residue-specific chemical shift perturbations (CSPs) of VIM-2 upon titration with L-captopril and the two enantiomers of **1** (**1**-*S*, green; **1**-*R*, gray) show a linear correlation. The linear fit for the respective correlations are given as a solid line in green for **1**-*S* (R^2 = 0.82) and in a dashed gray line for **1**-*R* (R^2 = 0.80).

The NMR backbone resonance assignment for the metallo-β-lactamase VIM-2 (84%) is disclosed, providing the basis for rational development of a clinically applicable inhibitor, which will be a long sought tool in fighting antibiotic resistance. We synthesized and resolved the enantiomers of the inhibitor compound **1**, and revealed by HSQC titration that its *S*-enantiomer has a comparable VIM-2 affinity (0.23−3.9 μM) to that of L-captopril. It likely binds to the same cleft on VIM-2, which has previously been identified by crystallographic analysis.[14] L-Captopril's dissociation constant estimated from the NMR titration experiments (0.63−6.8 μM) is in excellent agreement with the literature (0.6 μM).[14,28] The *R*-enantiomer of **1** binds weakly (mM) to VIM-2. In our hands, the *S*-isomer did not show significant cytotoxicity on HeLa cells. Our observations may be important in the development of potent inhibitors to VIM-2.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.1c00635.

Details for the general procedures, HPLC separation, the determination of the stereochemistry, the NMR backbone resonance assignment, the NMR titration experiments and the cytotoxicity assay (PDF)

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Notes

The authors declare no competing financial interest.

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zenodo.5676156. The backbone resonance assignments have been deposited to the BMRBI with code 51165.

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■ ABBREVIATIONS

CSP, chemical shift perturbation
GIM, German imipenemase
HPLC, high-pressure liquid chromatography
IMP, imipenemase
NDM, New Delhi metallo-β-lactamase
NMR, nuclear magnetic resonance
SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis
VIM, Verona-integron encoded metallo-β-lactamase

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