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Design and Synthesis of Mimics of the T7-loop of FtsZ

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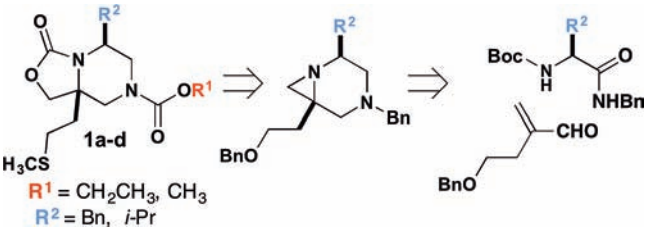
ABSTRACT



Mimics of the T7-loop of the bacterial cell division protein FtsZ have been designed and synthesized. The design is based on the X-ray cocrystal structure of *P. aeruginosa* FtsZ:SulA. Fast Rigid Exhaustive Docking (FRED) was employed to select compounds that can mimic the key interacting residues. Bicyclic oxazolidinones 1a−d were selected for further study and synthesized from a key bicyclic aziridine intermediate, which is synthesized from a readily available unsaturated aldehyde and amides derived from α-amino acids.

Inhibition of cell division by the SOS response protects bacterial organisms against antibiotics that target cell wall synthesis, including β-lactams.[1] Although these antibiotics are among the most commonly used for the treatment of infections, bacterial resistance has rendered them ineffective in many cases.[2] For this reason, it is imperative to find new pathways to battle bacterial infections. While developing new antibiotics is vital, restoring the lethality of current antibiotics is a strategy that could also be effective. Researchers at Merck recently reported that certain β-lactams regain their lethality against MRSA when used in combination with compound PC190723.[3] This compound is known to disrupt the normal function of the bacterial cell division protein FtsZ. This fascinating result can be adapted to other pathways related to the cell division machinery such as the bacterial SOS response, which triggers DNA repair in bacteria.[4] This event activates the overexpression of SulA, an endogenous inhibitor of FtsZ. SulA inhibits the polymerization of FtsZ monomers, an essential process leading to bacterial cell division, by binding to the T7-loop of FtsZ.[5] The crucial role that the T7-loop plays during the SOS response has led us to hypothesize that mimics of this loop can act as SulA modulators. The SOS response represents an attractive target that can be utilized to develop compounds that can operate in synergy with antibiotics to restore their lethality.[1] Mimics of the T7-loop of FtsZ represent a great starting point in the search for such compounds. Aided by computational methods and inspired by the X-ray cocrystal structure of FtsZ and SulA,[6] we report the design and the synthesis of mimics of the T7-loop of FtsZ.

(1) Miller, C.; Thomsen, L. E.; Gaggero, C.; Mosseri, R.; Ingmer, H.; Cohen, S. N. *Science (Washington, DC, U.S.)* **2004**, *305*, 1629–1632.
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(3) (a) Elsen, N. L.; Lu, J.; Parthasarathy, G.; Reid, J. C.; Sharma, S.; Soisson, S. M.; Lumb, K. J. *J. Am. Chem. Soc.* **2012**, *134*, 12342–12345. (b) Tan, C. M.; Therien, A. G.; Lu, J.; Lee, S. H.; Caron, A.; Gill, C. J.; Lebeau-Jacob, C.; Benton-Perdomo, L.; Monteiro, J. M.; Pereira, P. M.; Elsen, N. L.; Wu, J.; Deschamps, K.; Petcu, M.; Wong, S.; Daigneault, E.; Kramer, S.; Liang, L.; Maxwell, E.; Claveau, D.; Vaillancourt, J.; Skorey, K.; Tam, J.; Wang, H.; Meredith, T. C.; Sillaots, S.; Wang-Jarantow, L.; Ramtohul, Y.; Langlois, E.; Landry, F.; Reid, J. C.; Parthasarathy, G.; Sharma, S.; Baryshnikova, A.; Lumb, K. J.; Pinho, M. G.; Soisson, S. M.; Roemer, T. *Sci. Transl. Med.* **2012**, *4*, 126ra35. (c) Haydon, D. J.; Stokes, N. R.; Ure, R.; Galbraith, G.; Bennett, J. M.; Brown, D. R.; Baker, P. J.; Barynin, V. V.; Rice, D. W.; Sedelnikova, S. E.; Heal, J. R.; Sheridan, J. M.; Aiwale, S. T.; Chauhan, P. K.; Srivastava, A.; Taneja, A.; Collins, I.; Errington, J.; Czaplewski, L. G. *Science* **2008**, *321*, 1673–1675.
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(5) Cordell, S. C.; Robinson, E. J. H.; Loewe, J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 7889–7894.
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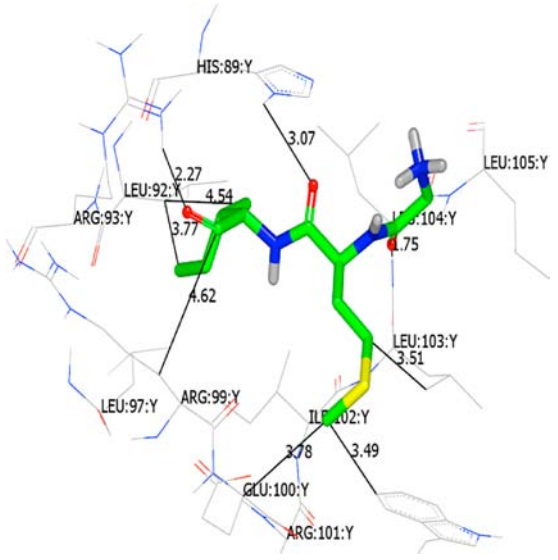


Figure 1. PDB ID 1OFU. Truncated T7-loop (green). Left to right; Ile, Met, and Gly showing the interactions and distances (in Å) between SulA and FtsZ.

As revealed by the cocrystal structure,[6] the interaction between the T7-loop of FtsZ and SulA is limited to only a few residues, specifically Pro-204, Gly-205, Met-206, Ile-207, and Asn-208.[5] Upon closer inspection of the crystal structure, we observed that Gly-205, Met-206, and Ile-207 were participating in key hydrogen bonding and hydrophobic interactions with the SulA surface (Figure 1). These key interactions prompted us to utilize these amino acid residues as a template for the design of T7-loop mimics.

The search for T7-loop mimics began by screening potential ligands using fast rigid exhaustive docking (FRED).[7,8] Initially, we searched for molecules that fit into the SulA pocket and placed the functional groups in the same location as the T7-loop tripeptide shown above. Although this process led to the identification of some molecules that were synthetically unfeasible, it provided us with a starting point for the design of a core structure that was more tractable. After an extensive search of an in-house library, we were pleased to find that core structures **1** and **2** mimicked the positioning of the three amino acid side chains (Figure 2). These scaffolds showed tolerance of functionalization at R^1 and R^2. Bulky substituents, such as *tert*-butyl at R^1, adopted an undesired orientation (not shown). Less sterically demanding groups on the other hand, such as methyl and ethyl, adopted the desired orientation. Substitution at R^2 was also explored, and we found that a simple methyl group again resulted in an unwanted binding orientation, while longer hydrophobic chains such as isopropyl and benzyl were well-tolerated.

(7) McGaughey, G. B.; Sheridan, R. P.; Bayly, C. I.; Culberson, J. C.; Kreatsoulas, C.; Lindsley, S.; Maiorov, V.; Truchon, J.-F.; Cornell, W. D. *J. Chem. Inf. Model.* **2007**, *47*, 1504–1519.

(8) Inquiries about the details of virtual screening should be directed to Prof. Dean J. Tantillo (djtantillo@ucdavis.edu). See SI for computational details, including FRED results for diastereomers of **1a**–**d**.

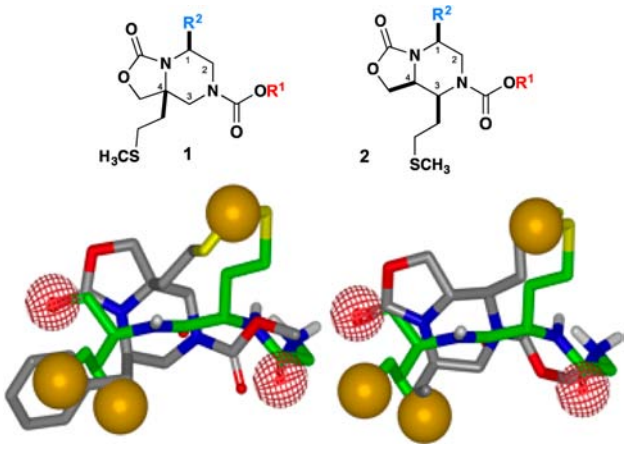
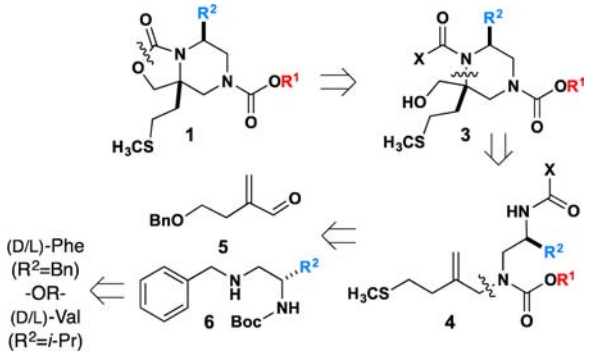


Figure 2. (Top) Core structures **1** (R^1 = CH_3, R^2 = Bn) and **2** (R^1 = CH_3, R^2 = *i*-Pr). (Bottom) Overlay of the truncated T7-loop (green) and mimics (gray).

Despite the fact that scaffolds **1** and **2** differ at C_3 and C_4, docking experiments showed that both of these compounds adopted very similar orientations. Retrosynthetic analysis of **1** revealed that it was an ostensibly easier target, given that it has fewer stereogenic centers and more tractable intermediates.[9] We envisioned that the oxazolidinone ring would be installed last from piperazine **3** or possibly by an oxidative cyclization from acyclic alkene precursor **4** (Scheme 1). Alkene **4** could come from unsaturated aldehyde **5** and diamines **6**, derived from α-amino acids. We reasoned that racemic amino acid precursors would double the number of compounds that were assessed, once we established an assay for the disruption of the FtsZ-SulA complex.

Scheme 1. Retrosynthesis of Compound **1**, (R^1 = CH_2CH_3, CH_3, R^2 = Bn, *i*-Pr)



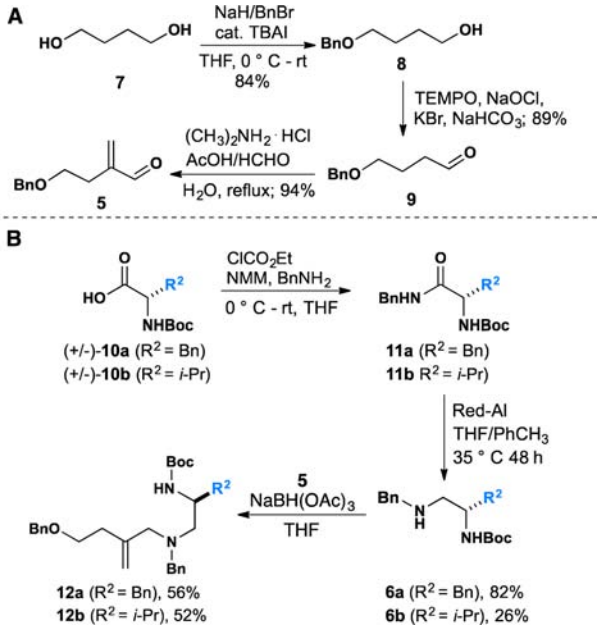
Synthesis of fragments **5** and **6** was accomplished in good yield. The α,*β* unsaturated aldehyde **5** was synthesized by the monoprotection of 1,4-butanediol (**7**) with benzyl bromide in the presence of TBAI.[10] TEMPO

(9) Preliminary synthetic studies (not shown) toward **2** revealed this target to be less tractable than **1**.

(10) Davis, J. M.; Truong, A.; Hamilton, A. D. *Org. Lett.* **2005**, *7*, 5405–5408.

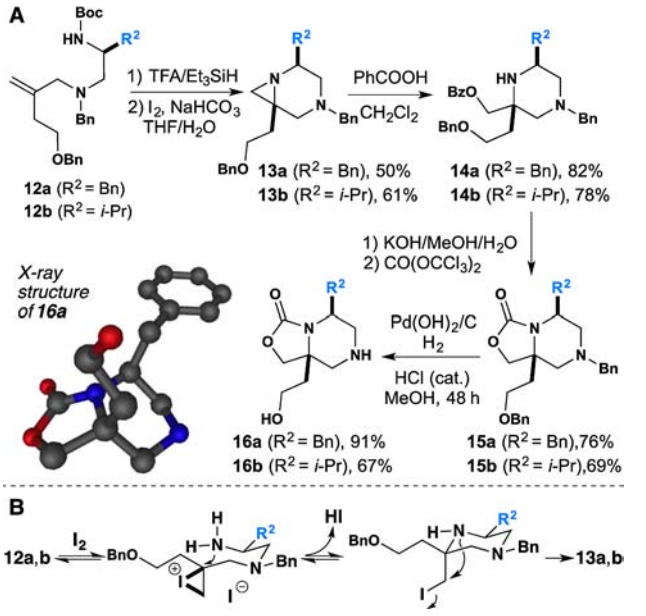
oxidation[11] followed by a Mannich reaction to install the exomethylene moiety[12] provided the desired aldehyde in high yield (Scheme 2A). The synthesis of **6** was achieved by the reaction between *N*-Boc protected amino acid **10** and ethyl chloroformate to form a mixed anhydride intermediate, which upon treatment with benzyl amine yielded **11**.[13] Although various reducing agents such as borane (THF and DMS) and $LiAlH_4$ were used for the reduction of **11**, we obtained the best results with Red-Al. This method of reduction yielded **6** in modest to high yield (Scheme 2B).[14] Aldehyde **5** and amine **6** underwent reductive amination in the presence of $NaBH(OAc)_3$ to produce **12** in moderate yield.[15]

Scheme 2. (A) Synthesis of α,β-Unsaturated Aldehyde **5**; (B) Synthesis of Amine **6**



The synthesis of compounds **1a**–**d** was achieved in nine steps from intermediate **12**. We first attempted a cascade aminocarboxylation of olefin **12** to oxazolidinone **15** by employing conditions that were recently reported for the direct aminocarboxylation of monosubstituted olefins.[16] Unfortunately, this method failed for our disubstituted substrate **12**, resulting in no reaction or decomposition of the starting material. Although a one-pot oxazolidinone formation was not feasible, we were pleased to observe

Scheme 3. (A) Synthesis of **16a**–**b** and X-ray Crystal Structure of **16a**, R^2 = Bn; (B) Conformational Model to Explain Diastereoselective Formation of **13** from **12**



that, upon *N*-Boc removal, treatment of the intermediate amine with I_2 resulted in rapid and diastereoselective formation of aziridines **13a** and **13b** from **12a** and **12b**, respectively. The diastereoselectivity of this process is estimated to be >85:15.[17] The selective formation of the cis diastereomer can be explained by a six-membered ring transition state for an exocyclization in which the two substituents are held in pseudoequatorial positions (Scheme 3b). Although related reactions of amine-appended alkenes have been reported[18a–d] this is one of the few examples in which high diastereoselectivity for the formation of a fused aziridine is observed. Treatment of **13** with benzoic acid yielded **14**, which, upon saponification and addition of triphosgene, yielded compound **15** in good yield.[19,20] Removal of both benzyl-protecting groups with $Pd(OH)/H_2$ and catalytic HCl yielded **16** in 60–90% yield (Scheme 3).

The target bicyclic lactams were completed in a short sequence from piperazines **16a** and **16b**. Amine acylation[21]

(11) Packard, G. K.; Hu, Y.; Vescovi, A.; Rychnovsky, S. D. *Angew. Chem., Int. Ed.* **2004**, *43*, 2822–2826.
(12) Pichlmair, S.; de, L. R. M.; Vilotijevic, I.; Paquette, L. A. *Tetrahedron* **2006**, *62*, 5791–5802.
(13) Vaughan, J. R., Jr.; Osato, R. L. *J. Am. Chem. Soc.* **1951**, *73*, 5553–5.
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(17) Only one peak with the correct mass is observed by LCMS under several conditions, suggesting high diastereoselectivity. Although the complexity of the splitting patterns in the ^1H NMR spectrum of the unpurified reaction mixture prevents unequivocal confirmation of the LCMS results, the selectivity appears to be at least 85:15.
(18) (a) Tranchant, M.-J.; Dalla, V.; Jabin, I.; Decroix, B. *Tetrahedron* **2002**, *58*, 8425–8432. (b) Black, D. S. C.; Doyle, J. E. *Aust. J. Chem.* **1978**, *31*, 2247–57. (c) Yoo, S.-E.; Kim, J.-H.; Yi, K. Y. *Bull. Korean Chem. Soc.* **1999**, *20*, 139–140. (d) Zhou, Y.; Murphy, P. V. *Org. Lett.* **2008**, *10*, 3777–3780. (d) McCormick, J. L.; Osterman, R.; Chan, T.-M.; Das, P. R.; Pramanik, B. N.; Ganguly, A. K.; Girijavallabhan, V. M.; McPhail, A. T.; Saksena, A. K. *Tetrahedron Lett.* **2003**, *44*, 7997–8000.
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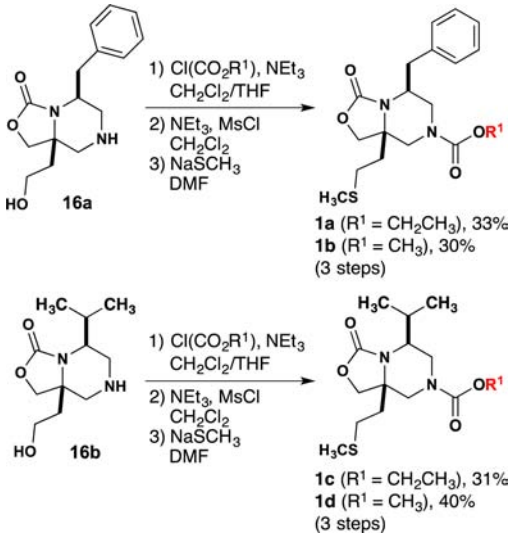
(20) Goujon, J.-Y.; Gueyrard, D.; Compain, P.; Martin, O. R.; Ikeda, K.; Kato, A.; Asano, N. *Bioorg. Med. Chem.* **2005**, *13*, 2313–2324.
(21) Deyine, A.; Poirier, J.-M.; Duhamel, P. *Synlett* **2008**, 260–262.

Scheme 4. Completion of Bicyclic Lactams **1a**–**d**



followed by mesylation of the alcohol moiety and displacement with sodium methanethiolate yielded the desired final products **1a**–**d** in 30–40% over three steps (Scheme 4).[22]

(22) Ekegren, J. K.; Roth, P.; Kallstrom, K.; Tarnai, T.; Andersson, P. G. *Org. Biomol. Chem.* **2003**, *1*, 358–366.

The late introduction of the two side chains in this synthesis will allow for straightforward variation of these two groups in subsequent SAR studies.

In summary, we have designed and synthesized heterocyclic small molecules that will serve as mimics for the T7-loop of FtsZ. Successful mimicry of this crucial binding region should provide the basis for disruption of the interaction of this essential cell division protein with its natural peptide inhibitor SulA. Current studies are focused on the development of biochemical and phenotypic assays to assess the propensity of **1a**–**d** to disrupt the interaction of these two bacterial proteins.

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Supporting Information Available. Characterization of all new compounds, ^{1}H and ^{13}C NMR spectra, and docking results. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.