Stable Analogues of Aminoacyl−tRNA for Inhibition of an Essential Step of Bacterial Cell-Wall Synthesis

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · NOVEMBER 2007

Impact Factor: 12.11 · DOI: 10.1021/ja0749946 · Source: PubMed

6 AUTHORS, INCLUDING:

Matthieu Fonvielle
French Institute of Health and Medical Resea…
24 PUBLICATIONS **248** CITATIONS
SEE PROFILE

Mélanie Etheve-Quelquejeu
Université René Descartes - Paris 5
48 PUBLICATIONS **729** CITATIONS
SEE PROFILE

J|A|C|S
COMMUNICATIONS

Published on Web 10/02/2007

Stable Analogues of Aminoacyl-tRNA for Inhibition of an Essential Step of Bacterial Cell-Wall Synthesis

Maryline Chemama, Matthieu Fonvielle, Régis Villet, Michel Arthur, Jean-Marc Valéry, and Mélanie Etheve-Quelquejeu*

Université Pierre et Marie Curie, UMR CNRS 7613, Synthèse, Structure et Fonction de Molécules Bioactives 4 place Jussieu, case 179, Paris, France, Centre de Recherche des Cordeliers, LRMA, Equipe 12, INSERM, U872, Paris, F-75006 France, Université Pierre et Marie Curie-Paris6, UMR S 872, Paris, F-75006 France, Université Paris Descartes, UMR S 872, Paris, F-75006 France

Received July 19, 2007; E-mail: quelque@ccr.jussieu.fr

Peptidoglycan, the major component of the bacterial cell wall (figure in Supporting Information), is a macromolecule in which glycan chains made of alternating β-1,4-linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) units are cross-linked by short peptides of variable composition and complexity.[1] In most Gram-positive bacteria, including major pathogens such as *Staphylococcus aureus* and *Streptococcus pneumoniae*, the peptides contain a side chain linked to the third position of a stem pentapeptide. The transferases for sequential amino acid addition, originally identified as factors essential for methicillin resistance (Fem) in *S. aureus*,[2] form a unique family of non-ribosomal peptide bond-forming enzymes. The Fem transferases have the particularity to use aminoacyl-tRNAs as a substrate[3] (**A** in Figure 1), in contrast to other peptidoglycan biosynthesis enzymes that function with a more conventional ATP-dependent activation of the substrate.[1] The side chain supplies the branching point to cross-link peptides from adjacent glycan chains, an essential reaction catalyzed by the D,D-transpeptidase catalytic domain of penicillin-binding proteins (PBPs).[4] Fem transferases are considered as attractive targets for the development of novel antibiotics active against multiresistant bacteria[2] since these enzymes have a unique catalytic mechanism[3] and are essential for the synthesis of the appropriate substrate for the D,D-transpeptidases,[5] including low-affinity PBPs responsible for resistance to β-lactam antibiotics.[6]

The oxadiazoles have been extensively used in medicinal chemistry as stable analogues of esters,[7] including the 1,2,4-oxadiazole, known to be the best mimic owing to its geometry and electronic properties.[8]

We have developed the synthesis of an analogue of the aminoacyl-tRNA substrate containing an 1,2,4-oxadiazole ring (**B** in Figure 1). This application is novel since oxadiazoles have never been used as bioisosters of esters in the field of nucleoside or nucleotide chemistry.[9] We show here that the aminoacyl-tRNA analogue **B** acts as a Fem inhibitor revealing a novel area of application of the oxadiazole ring in medicinal chemistry.

Synthesis of nucleosides **6** and **7** (Scheme 1) starts with the synthesis of cyanide deoxyadenosine **1** from adenosine using a known method.[10] Compound **1** was converted quantitatively into the amidoxime **2** by addition of hydroxylamine in methanol. **2** was then condensed with activated L-Boc-alanine following two different approaches. In the first approach leading to **6**, L-Boc-alanine was activated with EDCI and DMAP which reacted with the amidoxime **2**. Cyclodehydration was obtained by heating the oxime ester intermediate **3**, leading to compound **4** in 52% yield.

The O-acylated intermediate **3** was isolated in 60% yield for structural confirmation by NMR and mass spectroscopy. Compound **4** afforded compound **6** (48% yield) after benzoylation and selective

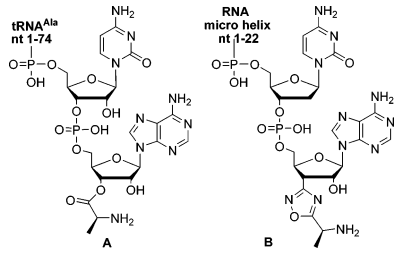
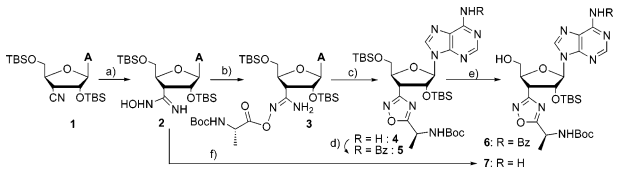


Figure 1. (A) Ala-tRNA^{Ala} substrate of FemX$_{Wv}$; (B) stable analogue containing an RNA microhelix.

Scheme 1. Synthesis of Compounds **6** and **7**[a]



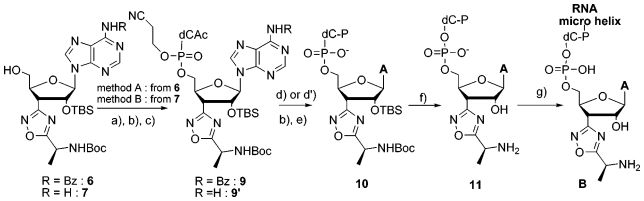
[a] Reagents and conditions: (a) NH_2OH, MeOH, 0 °C, 48 h, 98%; (b) Boc-L-Ala, EDCI, DMAP, DMF, 36 h; (c) 110 °C, 24 h, 52% over two steps; (d) BzCl, pyridine, followed by NH_4OH, 72%; (e) TFA/H_2O, 6 h, 0 °C, THF, 67%; (f) (*S*)-tert-butyl-1-fluoro-1-oxopropan-2-ylcarbamate, pyridine, 110 °C, 48 h, 33%; (g) HCl 6 N/THF/CH_3OH, 98%.

monodeprotection of the *tert*-butyl-dimethylsilyl (TBS) group. The second method to **7** involved activation of the L-Boc-alanine by formation of the corresponding acyl fluoride. The coupling with **2** in pyridine afforded after cyclodehydration the monodeprotected compound **7** in 33% yield. In comparison to the other approach, the yield was lower (52 vs 33%) but the number of steps to the target compound **B** was smaller (see below). Compounds **6** and **7** were coupled with the commercially available deoxycytidine phosphoramidite (Scheme 2). From the N-protected compound **6**, the classical phosphoramidite approach in the presence of tetrazole, followed by oxidation with iodine and treatment with trichloroacetic acid (method A), afforded compound **9** in 67% yield. Phosphorylation of **9** with the bis(2-cyanoethyl)diisopropyl phosphoramidite in tetrazole, followed by oxidation and the deprotection of cyanoethyl, acetyl, and benzoyl groups with methylamine afforded **10** in 65% yield. The *N*-Boc and TBS protecting groups were removed by stirring with HCl for 24 h producing **11** in quantitative yield.

For the N-unprotected compound **7**, the same methodology (method A) gave mainly the undesired trinucleotide **12** (45% yield, see structure in Supporting Information) and the expected compound **9′** only in 13% yield. To avoid formation of the P−N bond, we used the "proton-block" strategy described recently[11] that consists in using 5-nitrobenzimidazolium triflate (NBT) instead of tetrazole. In these conditions (method B, Scheme 2), the dinucleotide **9′** was

10.1021/ja0749946 CCC: $37.00

Scheme 2. Synthesis of the Target Compound **B**[a]



[a] Method A: (a) Ac-dC-CE phosphoramidite, tetrazole, DCM, room temp, 1 h; (b) I_2, 30 min, room temp; (c) TCA, DCM, 30 min, room temp. Method B: Method A using NBT instead of tetrazole. (d) From **9**, bis(2-cyanoethyl)diisopropylphosphoramidite, tetrazole, DCM, 1 h, room temp. (d′) From **9′**, bis(2-cyanoethyl)diisopropylphosphoramidite, NBT, DCM, 1 h, room temp; (e) CH_3NH_2, 24 h, room temp; (f) HCl 6 N/THF/CH_3OH, 24 h, room temp, 97%; (g) RNA microhelix 22-nt, T4 RNA ligase in Hepes buffer, DMSO, ATP, $MgCl_2$, 37 °C, 120 min.

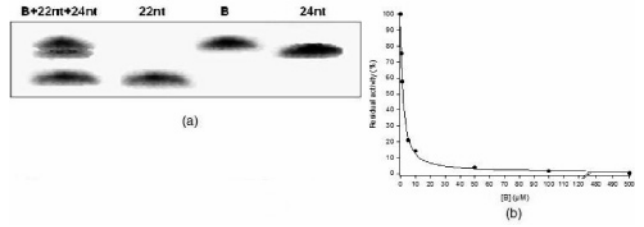


Figure 2. (a) Analysis of **B** by polyacrylamide gel electrophoresis; (b) inhibition of $FemX_{Wv}$ by compound **B**.

obtained in 54% yield. Phosphorylation of **9′** with the bis(2-cyanoethyl)diisopropylphosphoramidite in presence of NBT, followed by oxidation and deprotection of cyanoethyl and acetyl groups with methylamine afforded **10** in 49% yield. The overall yield to obtain **10** from **2** is similar in the two pathways (ca 10%) but the best route involves nucleoside **7** since the complete synthesis (Schemes 1 and 2) can be performed without selective 5′-deprotection. Compound **B** (Scheme 2) was obtained based on enzymatic ligation between **11** and a RNA microhelix. This approach, originally developed to introduce non-natural amino acids in proteins,[12] involves chemical synthesis of an aminoacylated dinucleotide and its enzymatic coupling to an incomplete RNA molecule lacking the 3′OH nucleotides C^{75} and A^{76}. In this study, we used a synthetic RNA molecule of 22 nucleotides that mimics the acceptor arm of $tRNA^{Ala}$. **B** was purified by size exclusion chromatography to remove excess of **11**. Characterization of **B** by polyacrylamide gel electrophoresis (Figure 2a) indicated that the microhelix was quantitatively converted to **B**.

Inhibition tests were performed with $FemX_{Wv}$ from *Weissella viridescens* which has been used as a model enzyme for kinetics and structural analyses of transferases of the Fem family (Figure 2b).[3] The enzyme catalyzes the transfer of L-Ala from Ala-$tRNA^{Ala}$ to the peptidoglycan precursor UDP-MurNAc-pentapeptide to introduce the first residue of an L-Ala-L-Ser-L-Ala side chain. Inhibition of Fem_{Wv} was tested in a radioactive coupled assay as previously described.[13] Inhibition of the formation of the hexapeptide revealed an IC_{50} value of 1.4 ± 0.1 μM similar to the K_m of $FemX_{Wv}$ for the Ala-$tRNA^{Ala}$ substrate (1.7 μM).[13] Compound **11** did not inhibit $FemX_{Wv}$ (IC_{50} > 1 mM; data not shown) indicating that the microhelix was required. This portion of the tRNA was also sufficient for inhibition since ligation of **11** to a 74-nt RNA resulted in an analogue of the full length substrate that inhibited $FemX_{Wv}$ with an IC_{50} of 0.17 ± 0.03 μM.

In conclusion, we have developed an efficient route for synthesis of modified nucleotides containing an 1,2,4-oxadiazole ring as mimes of esters in the 3′ position. A first demonstration of the inhibitory activity of this type of compounds was obtained for a transferase of the Fem family that catalyzes an essential step of peptidoglycan synthesis. This is the first report of an inhibitor of this class of enzymes which has been only recently investigated at the mechanistic and structural levels.[3] The inhibitor described here will be important to further develop structural studies of Fem enzymes, in particular by allowing cocrystallization with a stable analogue of the aminoacyl-tRNA substrate. Finally, the modified nucleotides obtained in this study mimic substrates or products of important targets including the amino acyl-tRNA synthetases and the peptidyl-transferase center of the ribosome. The IC_{50} of **B** for $FemX_{Wv}$ indicates that the use of five-membered heterocyclic ring[7,8] as ester surrogates can be extended to the field of aminoacyl-tRNA biology.

Acknowledgment. This work was supported by the European Community (EUR-INTAFAR, Project No. LSHM-CT-2004-512138, 6th PCRD).

Supporting Information Available: Details on the experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Van Heijenoort, J. *Nat. Prod. Rep.* **2001**, *18*, 503−519.
(2) Kopp, U.; Roos, M.; Wecke, J.; Labischinski, H. *Microb. Drug. Resist.* **1996**, *2*, 29−41.
(3) (a) Biarrotte-Sorin, S.; Maillard, A.; Delettré, J.; Sougakoff, W.; Arthur, M.; Mayer, C. *Structure* **2004**, *12*, 257−267. (b) Hegde, S. S.; Shrader, T. E. *J. Biol. Chem.* **2001**, *276*, 6998−7003.
(4) Goffin, C.; Ghuysen, J.-M. *Microbiol. Mol. Biol. Rev.* **2002**, *66*, 702−738.
(5) Rohrer, S.; Ehlert, K.; Tschierske, M.; Labischinski, H.; Berger-Bachi, B. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9351−9356.
(6) Magnet, S.; Arbeloa, A.; Mainardi, J. L.; Hugonnet, J. E.; Fourgeaud, M.; Dubost, L.; Marie, A.; Delfosse, V.; Mayer, C.; Rice, L. B.; Arthur, M. *J. Biol. Chem.* **2007**, *282*, 13151−13159.
(7) (a) Rudolph, J.; Theis, H.; Hanke, R.; Endermann, L.; Geschke, F.-U. *J. Med. Chem.* **2001**, *44*, 619−626. (b) Tully, W. R.; Gardner, C. R.; Gillespie, R. J.; Westwood, R. *J. Med. Chem.* **1991**, *34*, 2060−2067.
(8) (a) Moreira, L.; Barreiro, E. J. *Curr. Med. Chem.* **2005**, *12*, 23−49. (b) Orlek, S. B.; Blaney, F. E.; Brown, F.; Clark, M. S. G.; Hadley, M. S.; Hatcher, J.; Riley, G. J.; Rosenberg, H. E.; Wadworth, H. J.; Wyman, P. *J. Med. Chem.* **1991**, *34*, 2726−2735.
(9) For aminoacyl-tRNA mimics already described see: (a) Schmeing, T. M.; Huang, K. S.; Strobel, S. A.; Steitz, T. A. *Nature* **2005**, *438*, 520−524. (b) Cressina, E.; Lloyd, A. J.; De Pascale, G.; Roper, D. I.; Dowson, C. G.; Bugg, T. D. H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4654−4656. (c) Huang, K. S.; Weinger, J. S.; Butler, E. B.; Strobel, S. A. *J. Am. Chem. Soc.* **2006**, *128*, 3108−3109.
(10) Camarasa, M.-J.; Diaz-Ortiz, A.; Calvo-Mateo, A.; De las Heras, F. G.; Balzarini, J. *J. Med. Chem.* **1989**, *32*, 1732−1738.
(11) Mitsuo, S.; Ohkubo, A.; Seio, K. *J. Org. Chem.* **2003**, *68*, 5478−5492.
(12) (a) Robertson, S. A.; Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schultz, P. G. *Nucleic Acids Res.* **1989**, *17*, 9649−9660. (b) Heckler, T. G.; Chang, L. H.; Zama, Y.; Naka, T.; Chorghade, M. S.; Hecht, S. M. *Biochemistry* **1984**, *23*, 1468−1473.
(13) Maillard, A.; Biarrotte-Sorin, S.; Villet, R.; Mesnage, S.; Bouhss, A.; Sougakoff, W.; Mayer, C.; Arthur, M. *J. Bacteriol.* **2005**, *187*, 3833−3838.

JA0749946