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COMMUNICATION

Synthesis of lipid-carbohydrate-peptidyl-RNA conjugates to explore the limits imposed by the substrate specificity of cell wall enzymes on the acquisition of drug resistance

Matthieu Fonvielle[a]*, Ahmed Bouhss[b], Coralie Hoareau[a], Delphine Patin[b], Dominique Mengin-Lecreulx[b], Laura Iannazzo[c], Nicolas Sakkas[c], Affaf el Sagheer, [d,e] Tom Brown[e], Mélanie Ethève-Quelquejeu[c]*+ and Michel Arthur[a]*+

Abstract: Conjugation of RNA with multiple partners to obtain mimics of complex biomolecules is limited by the identification of orthogonal reactions. Here, lipid-carbohydrate-peptidyl-RNA conjugates were obtained by post-functionalization reactions, solid-phase synthesis, and enzymatic steps, to generate molecules mimicking the substrates of FmhB, an essential peptidoglycan synthesis enzyme of *Staphylococcus aureus*. Mimics of Gly-tRNA^{Gly} and lipid intermediate II (undecaprenyl-diphospho-disaccharide-pentapeptide) were combined in a single "bi-substrate" inhibitor (IC_{50} = 56 nM). The synthetic route was exploited to generate substrates and inhibitors containing D-Lac instead of D-Ala at the C-terminus of the pentapeptide stem, a modification responsible for vancomycin resistance in the enterococci. The substitution impaired recognition of peptidoglycan precursors by FmhB. The associated fitness cost may account for limited dissemination of vancomycin resistance genes in *S. aureus*.

Various RNA conjugates have been synthesized to develop new therapeutic strategies and to investigate basic biological processes.[1] These molecules combine an oligonucleotide with carbohydrates, peptides, small molecules, aptamers, or lipids.[1a] Carbohydrate-oligonucleotide and small molecule-oligonucleotide conjugates have been mostly used to target specific receptors or to increase uptake in target organs.[2] Aptamer-RNAs were developed to improve the combined delivery of drugs and siRNAs in multidrug-resistant cancer cells.[3] Peptidyl-RNAs have played pivotal roles in mechanistic and structural studies of ribosomal and non-ribosomal peptide synthesis.[4] Peptidyl-RNA conjugates were also developed to increase the cellular uptake of siRNA.[5] Lipid-RNA conjugates were designed to increase the lipophilicity of negatively charged oligonucleotides and to leverage lipoprotein-mediated endocytosis.[2] All of these conjugates offer interesting properties and access to novel chemical space.[1a] Various types of linkers have been used to conjugate RNAs to the various partners, including amide, amine, oxyamine, oxyimine, carbamate, and triazoles, which are chemically and biologically stable.[6] Bioreversible linkers rely on disulfide bridges.[7] The conjugation is mostly performed at the 5' and 3' ends of the RNA, rather than internally, with the molecules attached to the nucleobase or to the ribose.

There are limited examples of the conjugation of a single RNA molecule with multiple partners. This type of adduct is required to investigate the biosynthesis of bacterial cell walls since its major component, the peptidoglycan, is assembled from a precursor combining a disaccharide [β-1,4-linked *N*-acetyl-glucosamine and *N*-acetyl-muramic acid, (GlcNAc-MurNAc)], a phospholipid carrier (undecaprenyl-diphosphate), and a branched pentapeptide, such as [L-Ala-D-iGlu-L-Lys(Gly_5)-D-Ala-D-Ala] in *Staphylococcus aureus* (Figure 1a and b). Since the pentaglycine side-chain is assembled from glycyl-tRNAs by amino acid transferases of the Fem family there is interest in developing lipid-carbohydrate-peptidyl-RNA conjugates (Figure 1c) to investigate the interaction of the enzymes with their substrates. The biological question addressed in our study concerns the acquisition of resistance to the glycopeptide antibiotic vancomycin in *S. aureus* by replacement of the terminal D-Ala residue by D-Lac. The substitution reduces the affinity of the drugs for peptidoglycan by 1,000 fold leading to high-level resistance (Figure 1d). Since the resistance mechanism is widespread in the enterococci but only sporadically detected in *S. aureus* in spite of easy horizontal gene transfer between these bacteria[8] our specific aim is to evaluate whether the D-Ala to D-Lac substitution is tolerated by the *S. aureus* transferase FmhB responsible for incorporation of the first residue of the penta-glycine side chain.[9] We focus on FmhB since this enzyme is essential presumably because the side-chain of peptidoglycan precursors directly participates in the peptidoglycan cross-linking reaction (Figure 1e).[9-10] To investigate the specificity of FmhB, we have synthesized D-Ala- and D-Lac-containing substrates analogues (Figure 2) and lipid-carbohydrate-peptidyl-RNA conjugates acting as inhibitors (Figure 1c). This strategy was designed to assess the impact of the D-Ala to D-Lac substitution both on the catalytic efficacy of the transfer of Gly from Gly-tRNA^{Gly} to the peptidoglycan precursors and on the affinity of FmhB for inhibitors that mimic both substrates of the enzyme (referred to as "bi-substrates"). The target inhibitor molecules (Figure 1c) comprise soluble analogues of the peptidoglycan precursors (Lipid II) covalently-linked by a triazole to the acceptor arm of tRNA^{Gly}. Our synthesis strategy enables to modulate the length of the RNA and lipid moieties in order to obtain ligands with suitable size and solubility for investigating enzyme activity and affinity. It also enables to selectively modify the terminal residue of the peptide (D-Ala *versus* D-Lac). One of the major difficulty for obtaining the desired

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lipid-carbohydrate-peptidyl-RNA conjugates is the identification of orthogonal reactions compatible with the various functional groups specifically present in each component of the multi-RNA conjugates. In our synthetic strategy, we overcome these problems by developing post-functionalization of RNA and peptides moieties as well as a combination of enzymatic and chemical reactions (Figure 1c).

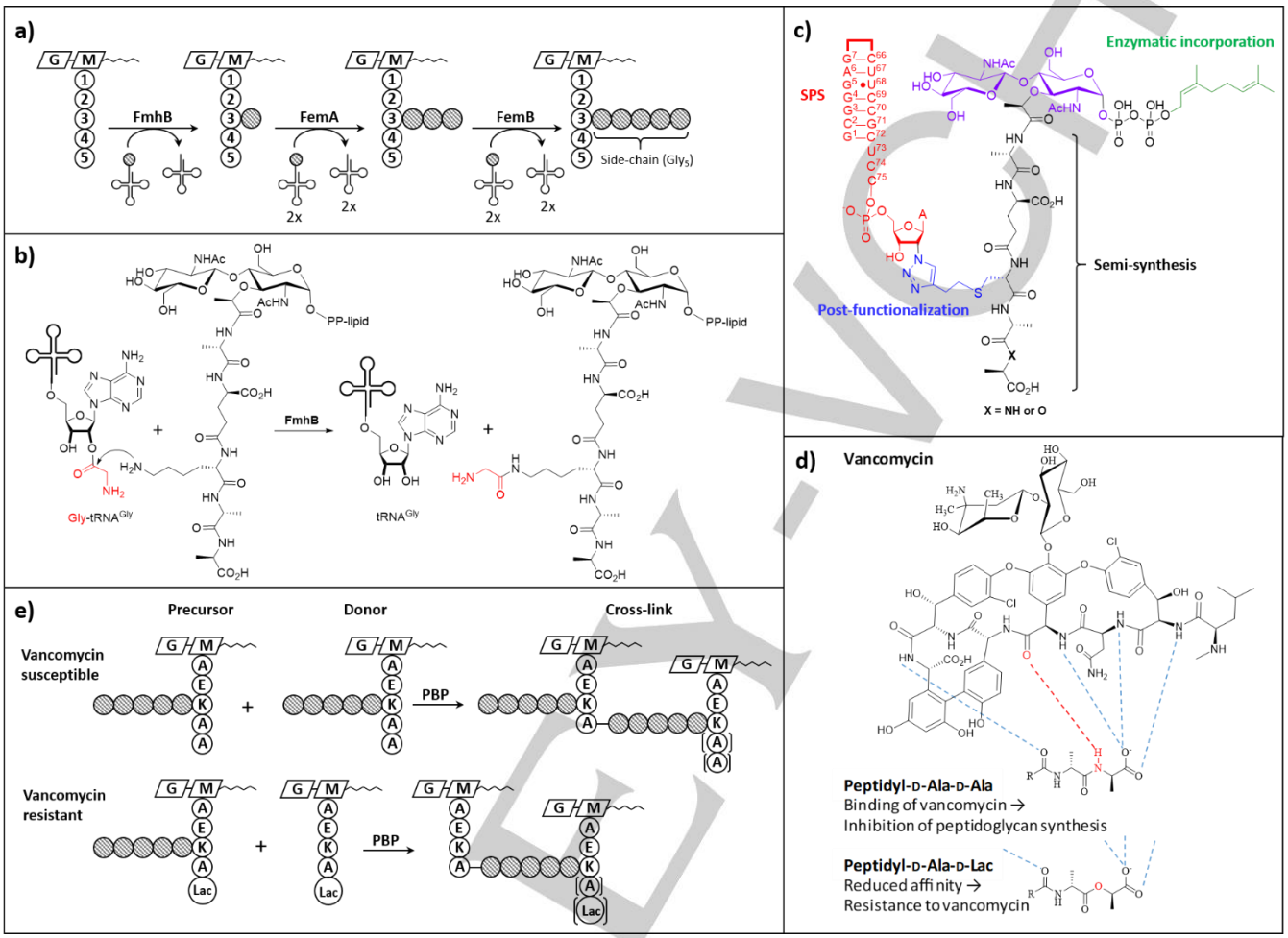

Figure 1. a) Assembly of the pentaglycine side-chain of peptidoglycan precursors by amino-acyl transferases of the Fem family. (b) Reaction catalyzed by FmhB. (c) Target lipid-carbohydrate-peptidyl-RNA conjugates developed as bi-substrate inhibitors. (d) Hydrogen bonding interaction between vancomycin and the peptidyl-D-Ala-D-Ala extremity of peptidoglycan precursors. Substitution of the C-terminal D-Ala by D-Lac prevents formation of the hydrogen bond indicated in red. (e) Penicillin Binding Protein (PBP) mediated cross-linking for vancomycin-susceptible and -resistant strains of *Staphyloccocus aureus*.[11]

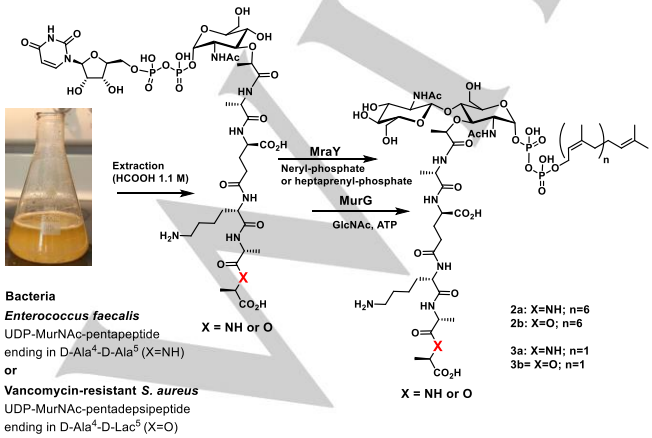

Figure 2. Semi-synthesis of lipid II analogues. MraY, Phospho-*N*-acetylmuramoyl-pentapeptide-transferase. MurG, *N*-acetylglucosamine-transferase.

To generate the D-Ala- and D-Lac-ending substrates of FmhB, the soluble nucleotide precursors were extracted from vancomycin-susceptible (D-Ala^5) and vancomycin-resistant (D-Lac^5) bacteria (Figure 2). Transfer of the phospho-MurNAc-peptide moiety to the lipid and addition of GlcNAc were obtained enzymatically.[12] For these substrates, we used commercially available heptaprenyl-phosphate instead of the natural undecaprenyl-phosphate lipid carrier (bactoprenyl). Transfer of [^{14}C]Gly from [^{14}C]Gly-tRNA^{Gly} to the heptaprenyl-containing lipid II analogue was tested by a coupled assay involving acylation of tRNA^{Gly} by purified glycyl-tRNA synthetase (GlyRS). The product was identified by thin layer chromatography (Supplementary information, Figure S5). This

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analysis revealed that substitution of D-Ala by D-Lac at the C-terminus of the lipid II analogues reduces the catalytic efficacy of FmhB by 4.6 fold (Figure 3a).

There are several limitations resulting from the insolubility of the heptaprenyl-containing substrates including uncertainty regarding the concentrations of lipid II analogues actually accessible to FmhB and time consuming solvent extractions required for purification. In order to gain access to soluble lipid II analogues, we replaced heptaprenyl-phosphate by neryl-phosphate in the synthesis procedure. The resulting neryl-containing lipid II analogues were effectively used as substrates by FmhB revealing again a reduced catalytic efficacy for D-Lac-ending substrates (7 fold; Figure 3b and Table 1).

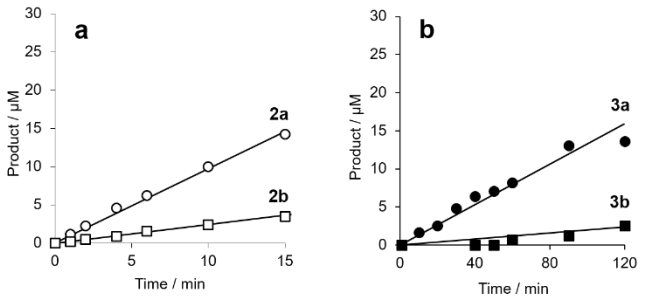

Figure 3. Catalytic efficacy of FmhB for the transfer of Gly from Gly-tRNA^{Gly} to lipid II analogues ending in D-Ala or D-Lac. (a) and (b), transfer of Gly from heptaprenyl- and neryl-containing substrates (30 µM) was tested with FmhB concentrations of 50 nM and 170 nM, respectively. Circles, D-Ala-ending substrate; squares, D-Lac-ending substrate.

Lipid-carbohydrate-peptidyl-RNA conjugates mimicking both the Gly-tRNA^{Gly} and lipid II (lipid-carbohydrate-peptide) substrates were synthesized in order to explore the impact of the D-Ala to D-Lac substitution on the affinity of FmhB for peptidoglycan precursors (Figure 2). The presence of the carbohydrate and lipid parts have been shown to be essential for the activity of the FmhB transferase.[13] The synthesis of the Lipid II moiety started by four enzymatic reactions for sequential addition of L-Ala, D-Glu, *meso*-cystine, and D-Ala-D-Ala or D-Ala-D-Lac to UDP-MurNAc by Mur synthetases (Figure 4). *Meso*-cystine is a structure analogue of *meso*-2,6-diaminopimelic acid (DAP), which is recognized by the enzyme MurE and enables post-functionalization of the peptide via formation of a dehydroalanine.[14] For this purpose, we have developed a new one-pot reaction based on reduction of *meso*-cystine into cysteine by a soluble phosphine followed by its conversion to dehydroalanine by 2,5-dibromohexanediamide. By this approach, a dehydroalanine residue was introduced at the 3rd position of the stem peptide. In the following step, 1,4-addition of 1-thio butynyl to the dehydroalanine afforded two stereoisomers containing an alkyne function at the extremity of the side-chain of the 3rd residue. The (*R*) diastereoisomer was purified by *rp*HPLC as previously described.[15] MraY and MurG were permissive for the transfer of the phospho-MurNAc-peptide moieties to heptaprenyl-phosphate and neryl-phosphate and the subsequent addition of GlcNAc, respectively, generating the corresponding alkyne-containing lipid II analogues.[12] For the RNA moiety, 2'-azido-5'-dimethoxytrityl-benzoyl adenosine was coupled to a resin for solid phase synthesis of an 18-mer mimicking the acceptor arm of tRNAGly.[4a] During solid phase synthesis, a hexaethylene glycol linker was incorporated to stabilize the hairpin duplex formed by the RNA strand. The alkyne-containing lipid II analogues were coupled to the azido-containing RNA by the Cu^{I}-catalyzed Huisgen-Sharpless cycloaddition reaction in the presence of a metal ligand (THPTA).[15] The resulting triazole-containing lipid-carbohydrate-peptidyl-RNA conjugates were purified by denaturing polyacrylamide gel electrophoresis and by *rp*HPLC for heptaprenyl- and neryl-containing molecules, respectively (Supplementary Information).

Our next objective was to assess the impact of the substitution of the C-terminal D-Ala by D-Lac on the inhibition of FmhB by lipid-carbohydrate-peptidyl-RNA conjugates ("bi-substrates"). The conjugates were tested as FmhB inhibitors in the GlyRS-FmhB coupled assay (Figure 5). Substitution of D-Ala by D-Lac at the C-terminus of the peptide stems of the conjugates led to a 15-fold increase in the IC_{50} values (from 56 ± 6 nM to 820

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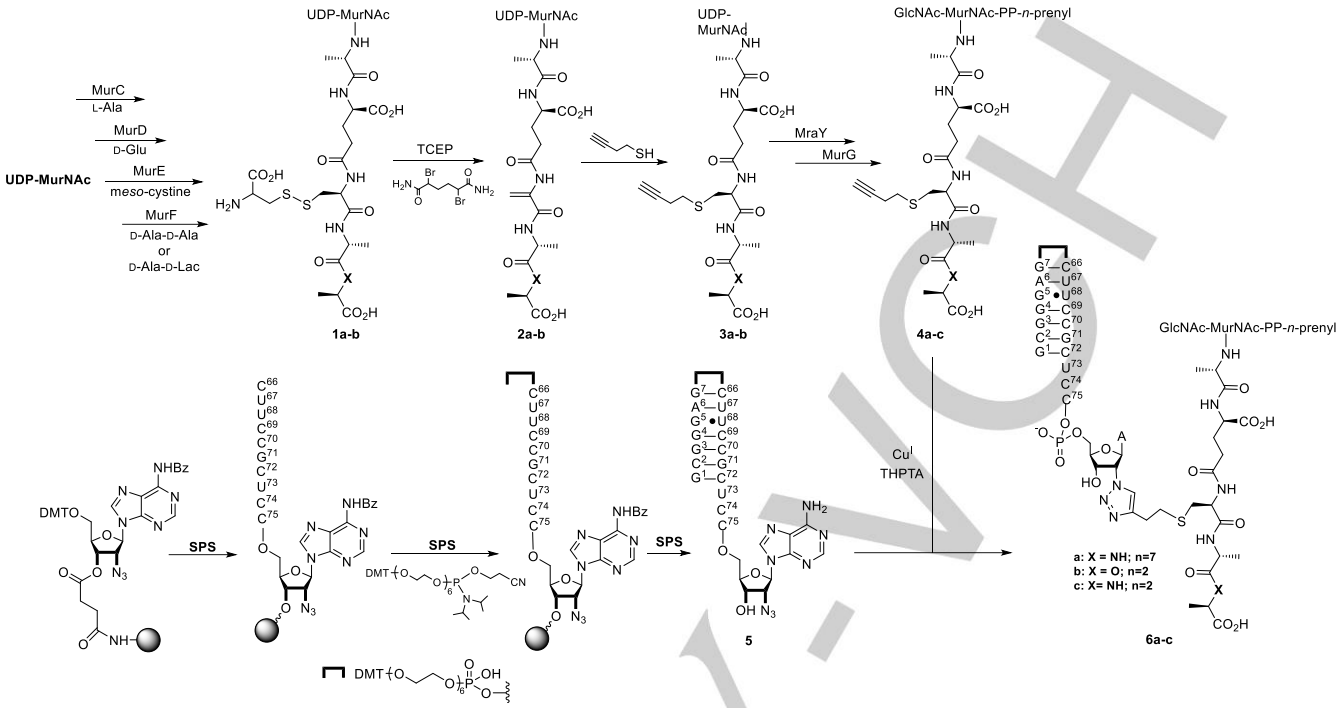

Figure 4. Synthesis of the *n*-prenyl-carbohydrate-peptidyl-RNA conjugates. X = NH (D-Ala) or X = O (D-Lac); n = 2 (neryl); n = 7 (heptaprenyl). THPTA, tris(3-hydroxypropyltriazolylmethyl)amine. TCEP, tris(2-carboxyethyl)phosphine..

± 50 nM for neryl-containing analogues; Table 1). This difference highlights the contribution of the D-Ala-D-Ala amide bond to the binding energy to the enzyme. IC_{50}s of 56 ± 6 nM and 5 ± 3 nM were determined for D-Ala-ending heptaprenyl- and neryl-PP-disaccharide-pentapeptide-RNA conjugates indicating that shortening of the lipid moiety (7 *versus* 2 prenyl groups) leads to a *ca*. 11-fold reduction in affinity (Supplementary Information and Table 1). This mirrors the 7- and 10-fold reduction observed with the substrates (Table 1).

Table 1. Impact of replacement of D-Ala by D-Lac on the catalytic activity of FmhB and its inhibition by bi-substrates

	C-terminal residue	
Substrate or inhibitor	**D-Ala**	**D-Lac**
Heptaprenyl-containing substrate (turnover, min^{-1})	**0.95 ± 0.02**	**0.20 ± 0.01**
Neryl-containing substrate (turnover, min^{-1})	**0.14 ± 0.01**	**0.020 ± 0.005**
Heptaprenyl-containing inhibitor (IC_{50}, nM)	**5 ± 3**	**ND**
Neryl-containing inhibitor (IC_{50}, nM)	**56 ± 6**	**820 ± 50**

Total[10a,16] and enzymatic[12] synthesis of lipid II have been described previously and were mostly used to incorporate fluorescent tags. Here, we report a hybrid strategy using dehydroalanine-containing lipid analogues for post-functionalization. This approach was used to generate lipid-carbohydrate-peptidyl-RNA conjugates mimicking the lipid II and Gly-$tRNA^{Gly}$ substrates of the FmhB transferase from *S. aureus*. Variability was introduced in the sequence of the peptide and in the number of isoprenyl repeats in the lipid moiety. Solid-phase synthesis of the RNA moiety offers the possibility to modulate the size and the sequence of the RNA moiety. The 1,4 addition of the thiol to dehydroalanine also offers a versatile access to bi-substrates containing linkers with various lengths and structures. Our lipid-carbohydrate-peptidyl-RNA conjugates provide a versatile tool to study the tRNA-dependent amino acyltransferases involved in peptidoglycan synthesis.

In this study, we investigated the specificity of the FmhB aminoacyl-transferase by comparing substrates and inhibitors differing by the presence of D-Ala or D-Lac at the C-terminus of the stem peptide. Testing the substrates indicated that the D-Ala to D-Lac substitution impairs the activity of FmhB. Testing the inhibitors indicated that the substitution reduced the affinity of FmhB for the peptidoglycan precursor analogues. Structural analyses of peptidoglycan purified from vancomycin-resistant *S. aureus* showed that a high portion of the stem peptides were devoid of the pentaglycine side-chain.[9,11] Our data indicate that impaired activity of FmhB accounts for the presence of incomplete stem peptides lacking the pentaglycine side-chain. Such incomplete precursors are only used as acyl donors by the transpeptidases responsible for the formation of peptidoglycan cross-links (Figure 1e). Our data also indicate that the production of D-Lac-ending precursors may have a fitness cost by impairing the assembly of peptidoglycan precursors. Such a fitness cost could account for the lack of dissemination of vancomycin-resistance staphylococci in spite of the sporadic detection of this type of strain in patients treated with vancomycin. The origin of this fitness cost is not known. The presence of incomplete side-chain only in the "donor" stem of dimers suggests that impaired activity of the transpeptidases might be involved.

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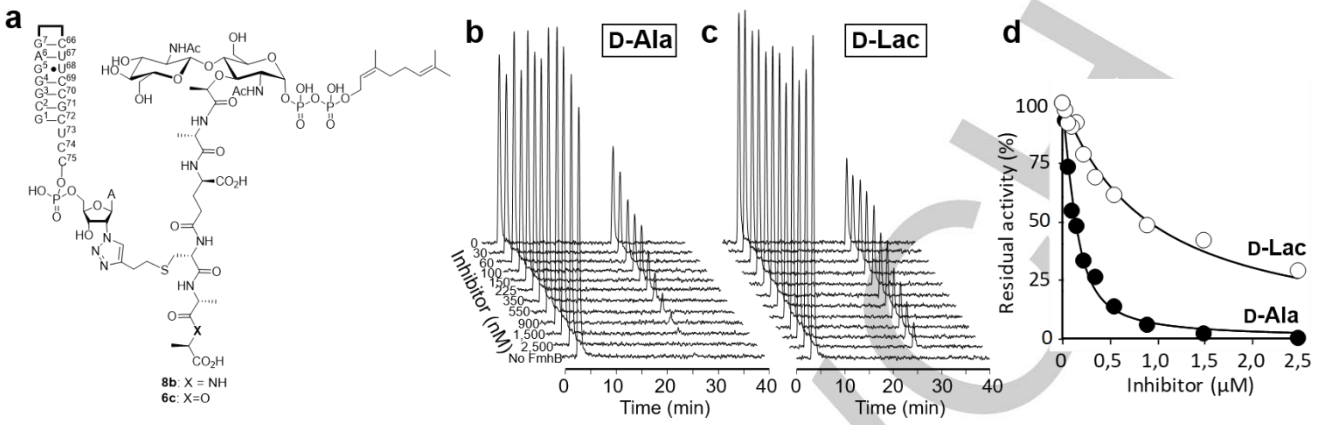

Figure 5. Inhibition of FmhB by lipid-carbohydrate-peptidyl-RNA conjugates. (a) Structure of the inhibitors. X = NH (D-Ala) or X = O (D-Lac). (b and c) Separation of [^{14}C]Gly from [^{14}C]Gly-lipid II analogues by *rp*HPLC coupled to a radioflow detector to assess the extent of inhibition of FmhB by increasing concentrations of inhibitors **8b** and **8c** ending in D-Ala and D-Lac, respectively. (d) % inhibition of FmhB by increasing concentrations of **8b** and **8c** (close and open circle, respectively). The IC_{50} values (56 ± 6 nM and 820 ± 50 nM for **8b** and **8c**, respectively) were deduced by fitting the Morrison equation to the data.

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Keywords: Fem • RNA conjugates • *Staphylococcus aureus* • VanA • vancomycin

[1] a) E. Valeur, S. M. Gueret, H. Adihou, R. Gopalakrishnan, M. Lemurell, H. Waldmann, T. N. Grossmann, A. T. Plowright, Angew. Chem. Int. Ed. Engl. **2017**, 56, 10294-10323; b) J. Winkler, Therapeutic delivery **2013**, 4, 791-809.

[2] a) M. Manoharan, Antisense Nucleic Acid Drug Dev. **2002**, 12, 103-128; b) N. S. Kruglova, M. I. Meschaninova, A. G. Venyaminova, M. A. Zenkova, V. V. Vlassov, E. L. Chernolovskaya, Molecular Biology **2010**, 44, 254-261; c) J. Baillet, V. Desvergnes, A. Hamoud, L. Latxague, P. Barthélémy, **2018,** Advanced Materials, 1705078.

[3] a) G. Zhu, G. Niu, X. Chen, Bioconjug. Chem. **2015**, 26, 2186-2197; b) H. Jeong, S. H. Lee, Y. Hwang, H. Yoo, H. Jung, S. H. Kim, H. Mok, Macromolecular bioscience **2017**, 17, 1600343

[4] a) M. Fonvielle, I. Li de La Sierra-Gallay, A. H. El-Sagheer, M. Lecerf, D. Patin, D. Mellal, C. Mayer, D. Blanot, N. Gale, T. Brown, H. van Tilbeurgh, M. Etheve-Quelquejeu, M. Arthur, Angew. Chem. Int. Ed. Engl. **2013**, 52, 7278-7281; b) S. Terenzi, E. Biala, N. Q. Nguyen-Trung, P. Strazewski, Angew. Chem. Int. Ed. Engl. **2003**, 42, 2909-2912; c) H. Moroder, J. Steger, D. Graber, K. Fauster, K. Trappl, V. Marquez, N. Polacek, D. N. Wilson, R. Micura, Angew. Chem. Int. Ed. Engl. **2009**, 48, 4056-4060.

[5] a) M. R. Alam, X. Ming, M. Fisher, J. G. Lackey, K. G. Rajeev, M. Manoharan, R. L. Juliano, Bioconjug. Chem. **2011**, 22, 1673-1681; b) M. R. Alam, V. Dixit, H. Kang, Z. B. Li, X. Chen, J. Trejo, M. Fisher, R. L. Juliano, Nucleic Acids Res. **2008**, 36, 2764-2776.

[6] a) M. Arthur, M. Ethève-Quelquejeu, Chemistry of organo-hybrids: synthesis and characterization of functional nano-objects. (Eds.: E. Lacôte, B. Charleux, C. Coperet), Wiley-VCH, **2015**; b) E. Paredes, M. Evans, S. R. Das, Methods **2011**, 54, 251-259.

[7] A. Ardana, A. K. Whittaker, K. J. Thurecht, Macromolecular Research **2017**, 25, 599-614.

[8] P. C. Appelbaum, International journal of antimicrobial agents **2007**, 30, 398-408.

[9] A. Severin, K. Tabei, F. Tenover, M. Chung, N. Clarke, A. Tomasz, J. Biol. Chem. **2004**, 279, 3398-3407.

[10] a) J. Nakamura, H. Yamashiro, H. Miya, K. Nishiguchi, H. Maki, H. Arimoto, Chem. Eur. J. **2013**, 19, 12104-12112; b) V. Srisuknimit, Y. Qiao, K. Schaefer, D. Kahne, S. Walker, J. Am. Chem. Soc. **2017**, 139, 9791-9794.

[11] D. Panesso, P. J. Planet, L. Diaz, J.-E. Hugonnet, T. T. Tran, A. Narechania, J. M. Munita, S. Rincon, L. P. Carvajal, J. Reyes, A. Londoño, H. Smith, R. Sebra, G. Deikus, G. M. Weinstock, B. E. Murray, F. Rossi, M. Arthur, C. A. Arias, Emerging infectious diseases **2015**, 21, 1844-1848.

[12] L. Y. Huang, S. H. Huang, Y. C. Chang, W. C. Cheng, T. J. Cheng, C. H. Wong, Angew. Chem. Int. Ed. Engl. **2014**, 53, 8060-8065.

[13] T. Schneider, M. M. Senn, B. Berger-Bachi, A. Tossi, H. G. Sahl, I. Wiedemann, Mol. Microbiol. **2004**, 53, 675-685.

[14] J. M. Chalker, S. B. Gunnoo, O. Boutureira, S. C. Gerstberger, M. Fernadez-Gonzalez, J. G. L. Bernardes, L. Griffin, A. Hailu, C. J. Schofield, B. G. Davis, Chem. Sci. **2011**, 2, 1666-1676.

[15] M. Fonvielle, D. Mellal, D. Patin, M. Lecerf, D. Blanot, A. Bouhss, M. Santarem, D. Mengin-Lecreulx, M. Sollogoub, M. Arthur, M. Etheve-Quelquejeu, Chem. Eur. J. **2013**, 19, 1357-1363.

[16] a) M. S. VanNieuwenhze, S. C. Mauldin, M. Zia-Ebrahimi, B. E. Winger, W. J. Hornback, S. L. Saha, J. A. Aikins, L. C. Blaszczak, J. Am. Chem. Soc. **2002**, 124, 3656-3660; b) Y. Zhang, E. J. Fechter, T. S. Wang, D. Barrett, S. Walker, D. E. Kahne, J. Am. Chem. Soc. **2007**, 129, 3080-3081.

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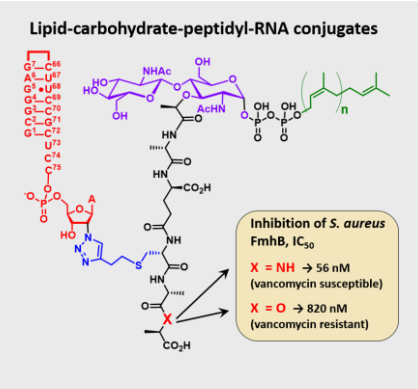
Entry for the Table of Contents (Please choose one layout)

Layout 1:

COMMUNICATION

Lipid-carbohydrate-peptidyl-RNA conjugates were synthesized to generate inhibitors of the essential peptidoglycan biosynthesis enzyme FmhB from pathogenic *Staphylococcus aureus.* A combination of solid phase synthesis, post-functionalization, and enzymatic steps provided access to selective modifications of the RNA, peptide, and lipid moieties of the precursors. Acquisition of vancomycin resistance by *S. aureus* was found to be limited by the specificity of FmhB for D-Ala-ending precursors.



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