Late-Stage Modification of Aminoglycoside Antibiotics Overcomes Bacterial Resistance Mediated by APH(3') Kinases

Andreas A. Bastian, Maria Bastian, Manuel Jäger, Mark Loznik, Eliza M. Warszawik, Xintong Yang, Nabil Tahiri, Peter Fodran, Martin D. Witte, Anne Thoma, Jens Köhler, Adriaan J. Minnaard, and Andreas Herrmann

Abstract: The continuous emergence of antimicrobial resistance is causing a threat to patients infected by multidrug-resistant pathogens. In particular, the clinical use of aminoglycoside antibiotics, broad-spectrum antibacterials of last resort, is limited due to rising bacterial resistance. One of the major resistance mechanisms in Gram-positive and Gram-negative bacteria is phosphorylation of these amino sugars at the 3'-position by O-phosphotransferases [APH(3')s]. Structural alteration of these antibiotics at the 3'-position would be an obvious strategy to tackle this resistance mechanism. However, the access to such derivatives requires cumbersome multi-step synthesis, which is not appealing for pharma industry in this low-return-on-investment market. To overcome this obstacle and combat bacterial resistance mediated by APH(3')s, we introduce a novel regioselective modification of aminoglycosides in the 3'-position via palladium-catalyzed oxidation. To underline the effectiveness of our method for structural modification of aminoglycosides, we have developed two novel antibiotic candidates overcoming APH(3')s-mediated resistance employing only four synthetic steps.

According to the latest reports of the World Health Organization and the Infectious Disease Society of America, we are entering the post-antibiotic era, due to insufficient discovery and development of new antibiotics.[1] With approximately 15 million deaths annually, infectious diseases remain the second leading cause of death worldwide.[2] With only four new antibiotic classes introduced since the 1980s, the world is running out of antibacterial treatment options against multidrug-resistant (MDR) pathogens.[3-5] Failure of high-throughput screening methods in discovery of novel antibacterials is among others one reason for this alarming situation.[6] Development of novel antibiotics based on ‘classic’ natural product-based scaffolds, for example beta-lactams, glycopeptides, macrolides and aminoglycosides, is still the most promising approach to tackle MDR infections.[7] The ‘next’ generation of drugs, however, is often more expensive in development and manufacturing. This holds especially true for structurally complex antibiotic classes, such as macrolides and aminoglycosides. Their structural alterations involve very challenging and time-consuming processes,[8] making them economically less attractive for the antibiotic market. Therefore, securing low-cost, high-yielding and short-synthesis routes is one of the key challenges for novel antibiotics development based on these pharmacophores.

Aminoglycosides (AGs) (Figure 1) are one of the most efficacious and safest antibiotics for the single-drug treatment of infections caused by Gram-negative pathogens.[9] Physicians prescribe AGs regularly for treatment of patients due to their broad-spectrum activity against Gram-positive and Gram-negative bacteria,[10] including Mycobacterium tuberculosis,[11] and non-tuberculosis Mycobacteria (NTM) strains.[12] Their therapeutic effectiveness, the low treatment price, convenient administration and low propensity for resistance development,[13] are reasons why there is a regained interest in this antibiotic class. For instance, Arikayce, an amikacin (2) liposome inhalation suspension, was approved in 2018 for treating adults with a lung infection caused by NTM bacterial strains.[14] Apramycin, a

[1] Dr. A. A. Bastian, Dr. M. Jäger, Dr. N. Tahiri, Dr. P. Fodran, Prof. Dr. M. D. Witte, Prof. Dr. A. J. Minnaard
Department of Chemical Biology, Stratingh Institute for Chemistry
Nijenborgh 7, 9747 AG Groningen (The Netherlands)
E-mail: a.j.minnaard@rug.nl
[b] Dr. A. A. Bastian, Dr. M. Bastian
AGILEBiots B.V.
De Mudden 14, 9747 AV Groningen (The Netherlands)
[c] M. Loznik, Dr. E. M. Warszawik, X. Yang, Prof. Dr. A. Herrmann
Department of Polymer Chemistry, Zernike Institute for Advanced Materials
Nijenborgh 4, 9747 AG Groningen (The Netherlands)
E-mail: herrmann@dwi.rwth-aachen.de
[d] Dr. E. M. Warszawik
Department of Biomedical Engineering-FB80, W. J. Kolf Institute-FB41
Antonius Deusinglaan 1, 9713 AV Groningen (The Netherlands)
[e] M. Loznik, X. Yang, A. Thoma, Dr. J. Köhler, Prof. Dr. A. Herrmann
DWI – Leibniz Institute for Interactive Materials
Forckenbeckstr. 50, 52056 Aachen (Germany)
[f] Dr. A. A. Bastian, M. Loznik, A. Thoma, Prof. Dr. A. Herrmann
Institute for Technical and Macromolecular Chemistry, RWTH Aachen University
Worringerweg 2, 52074 Aachen (Germany)
Supporting information for this article is available on the WWW under https://doi.org/10.1002/chem.202200883
© 2022 The Authors. Chemistry - A European Journal published by Wiley-VCH GmbH. This is an open access article published under the terms of the Creative Commons Attribution Non-Commercial NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
**vet**e**rine aminoglycoside antibiotic**, which is currently in development against MDR infections in humans,\[11\] has just entered the clinical stage.\[12\] However, both programs focus on “old” generic AGs failing to tackle the most relevant bacterial resistance mechanism, which is based on the structural alteration by aminoglycoside-modifying enzymes (AMEs). AMEs modify AGs at specific sites and are categorized in three classes: As shown in Figure 1 for kanamycin A (1a), amino groups undergo acetylation by N-acetyltransferases (AACs), O-phosphotransferases (APHs) and O-nucleotidyltransferases (ANTs) leading to a sharp decrease in the drug’s affinity for its biological target, the 16S ribosomal RNA. Therefore, site-specific modification of aminoglycosides is a promising tool to protect the neamine moiety (ring I and II), which is the structural key element of AGs, from modifications by AMEs and tackle bacterial resistance.\[13a–d\] For instance, amikacin (2), an AG antibiotic used in intensive care units against MDR infection,\[14d\] is obtained from kanamycin A (1a) via introduction of a 4-amino-2-hydroxybutanoyl (AHB) residue (Figure 1). Nevertheless, access to modified AGs remains a challenge due to similar reactivity of the functional groups.\[13e,15\] It has been shown before that modifications of aminoglycoside antibiotics in particular at ring I are highly desired to overcome bacterial resistance caused by AMEs.\[13e,14a\] Mobashery and coworkers developed a self-regenerating antibiotic candidate based on kanamycin A by introducing a keto-group in the C3′-position, which is in equilibrium with the hydrated variant (gem-diol). Although being phosphorylated by APH(3′), the phosphate moiety is eliminated spontaneously in a nonenzym-
motic fashion under physiological conditions, thereby regenerating the C3'-keto form. This derivative showed increased activity against pathogens expressing the kinase APH(3').\[^{1,6}\] However, the synthesis relies on more than 10 synthetic steps (see Supporting Information Figure S6), which limits the access to this lead compound and its further development.

A synthetic strategy for 4,5-disubstituted AGs to cope with AG-modifying enzymes including APH(3') was reported by Baasov and coworkers. Synthetic analogues of neomycin B were prepared in complex multistep synthesis, where the C5''-position was modified systematically with a variety of mono- and disaccharides and the effects of these additional moieties on antibacterial activity and substrate conversion with APH(3')-III were demonstrated.\[^{17}\]

In our study, we show that elaborate multi-step synthesis can be overcome by employing a catalytic oxidation of the hydroxyl group in C3'-position of amino-protected AGs in a single synthetic step.

In previous work, we introduced the palladium-catalyzed oxidation of unprotected mono-, di- and oligoglycosides at the C3'-position.\[^{19}\] Similar findings have been reported by K. Chung and M. Waymouth in 2016, where unprotected carbohydrates have been oxidized selectively thereby demonstrating the tolerability of the palladium catalyst [neocuproine]Pd-\((OAc)\)\(_2\)OTf\(_2\), 4 to functional groups.\[^{19}\] As shown in Figure 2(a), we succeeded in selectively converting methyl glucopyranoside 5, which bears vicinal hydroxyl groups in equatorial orientation, into the corresponding 3'-keto-derivative 6 using catalyst 4 and benzoquinone as the terminal oxidant.\[^{19}\] Most AGs, although considerably more complex than monosaccharide 5, possess a similar glucopyranoside motive, that is, ring I of the neamine structure (Figure 1). Therefore, we hypothesized that the same catalytic system could discriminate between the various rings in AGs and would result in selective oxidation of the C3'-hydroxyl group. As proof of concept, we chose one of the most complex aminoglycoside antibiotics, neomycin B (3), which is a 4,5-disubstituted pseudo-tetrasaccharide consisting of three carbohydrate rings and the 2-desoxystreptamine (2-DOS, ring II) moiety. The amino groups in 3 wereCbz-protected, as the palladium catalyst 4 is incompatible with free amines, and the resulting (Cbz)\(_n\)-neomycin B 7 was exposed to three equivalents of benzoquinone and 2.5 mol% of catalyst 4. Despite incomplete conversion, the reaction resulted in a single product, the desired 3'-keto-(Cbz)\(_n\)-neomycin B 8 (Figure 2b). Remarkably, just one hydroxyl group reacted despite the fact that all seven hydroxyl groups of tetrasaccharide 7 were unprotected. NMR, in particular heteronuclear single quantum coherence (HSQC) analysis, revealed that the oxidation occurred exclusively at the C3'-position (Figure 2c).

Disappearance of the J(C3'-H) coupling signal and a shift of the J(C1'-H)- (purple arrow), J(C2'-H)- (green arrow) and J(C4'-H) (blue arrow) coupling signals to lower field compared to the parental structure 7 underpin this regioselective oxidation. As shown in Table 1 (entry 1), the isolated yield after one hour was 29%. An additional 2.5 mol% of catalyst 4 improved the isolated yield of 8 to 41% (Table 1, entry 2). The reaction, however, could not be driven to completion; a possible explanation is that both the product and the substrate act as chelators for the palladium, thus removing catalyst 4 from the reaction.

Next, we investigated whether the regioselective oxidation could be expanded to 4,6-disubstituted AGs, that is, kanamycin A (1a), and kanamycin B (1b). For this purpose, we used the amino-protected derivatives (Cbz)\(_n\)-kanamycin A (9) and (Cbz)\(_n\)-kanamycin B (10). As shown in Table 1 (entries 3 and 4), both 9 and 10 were oxidized selectively to their corresponding 3'-oxo-derivatives 11 and 12, respectively (see also Figures S4 and S5 in Supporting Information). In contrast to substrate 7, full conversion was achieved for derivatives 9 and 10, reaching a reaction yield of 59% and 65%, respectively. At this point, it needs to be emphasized that the reported synthesis of 11 (though with the amino group at C6' unprotected) from kanamycin A took 10 synthetic steps (see Supporting Information Figure S6).\[^{11,9}\]

In addition, we have investigated the scalability of the palladium-catalyzed selective oxidation to demonstrate the high potential of this late-stage modification for industrial application. Therefore, we have used approx. 12 g and 15 g of substrates 9 and 10, respectively, for the regioselective oxidation. As shown in Table 1 (entries 5 and 6), both reactions yielded the corresponding products 11 and 12 reaching a yield of 61% and 76%, respectively.

Next, we employed this synthetic shortcut to obtain novel antibacterial candidates overcoming bacterial resistance caused

| Entry | Sub-strate | Pro-duct | PG | R | t [h] | Catalysis load [%] | Yield [%] |
|-------|------------|----------|----|----|-------|-------------------|-----------|
| 1     | 7[^{3}]    | 8        | Cbz | NHBCbz | 1 | 2.5 | 29 |
| 2     | 7[^{3}]    | 8        | Cbz | NHBCbz | 20 | 2.5 × 2 | 41 |
| 3     | 9[^{2}]    | 11       | Boc | OH  | 1 | 2.5 | 59 |
| 4     | 10[^{2}]   | 12       | Boc | NHBoc | 1 | 6 | 65 |
| 5     | 9[^{2}]    | 11       | Boc | OH  | 2 | 2.5 | 61 |
| 6     | 10[^{2}]   | 12       | Boc | NHBoc | 2 | 6 | 76 |

PG = Protecting group, t = reaction time, BQ = benzoquinone. [a] Isolated yield. [b] Reaction scale = 0.42 mmol, 0.6 g. [c] Reaction scale = 0.34 mmol, 0.3 g. [d] Reaction scale = 12.43 mmol, 11 g. [e] Reaction scale = 15.26 mmol, 15 g.
by APH(3')s-type kinases. In this regard, we used our late-stage modification first on kanamycin A (1a), since the clinical use of this AGs is limited due to bacterial resistance caused by these AMEs. We envisioned that epimerization of the 3'-hydroxyl group in the pristine antibiotic 1a resulting in derivative 13 might result in a superior drug candidate: while the change of the orientation of this hydroxyl group would resist the phosphorylation reaction catalyzed by APH(3'), at the same time keeping a hydroxyl group at this position would maintain affinity for the bacterial ribosome. As shown in Scheme 1, 3'-epi-kanamycin A (13) was obtained starting from derivative 11 in only two synthetic steps, i.e., diastereoselective reduction of ketone 11 to 14 (step c) and subsequent removal of the protecting groups (step d). In a similar fashion, we have also produced the corresponding neomycin B derivative 15 carrying an axial oriented hydroxyl group in 3'-position (see Supporting Information).

To investigate the antibacterial performance of both derivatives, i.e., 3'-epi-kanamycin A (13) and 3'-epi-neomycin B (15), we determined their minimal inhibitory concentration (MIC) against Escherichia coli and compared them with the antibacterial activity of the corresponding pristine AGs 1a and 7a.

Figure 2. Catalytic regioselective oxidation of monosaccharide 5 (a) and aminoglycoside 7 (b) at C3'-position, as well as structure confirmation of product 8 by 2D NMR spectroscopy (c). a) Oxidation of methyl alpha-D-glucopyranoside 5 with benzoquinone and catalyst 4. b) Selective oxidation of (Cbz)3-neomycin B 7 to the corresponding 3'-keto-derivative 8. c) Confirmation of the selective oxidation of the hydroxyl group at the C3'-position of 7 employing HSQC NMR spectroscopy.
3 (Table 2). All compounds were tested against non-resistant wild type (WT) E. coli (ATCC 25922) strains and bacterial strains expressing the AMEs APH(3')Ia and APH(3')IIIa, which cause resistance in Gram-negative and Gram-positive bacilli, respectively.\[20\]

From the outcome of the antibacterial evaluation shown in Table 2, we can conclude that epimerization at the 3'-position significantly improves the antibacterial performance against E. coli strains harboring APH(3') enzymes. Epimer 13 has a 16-fold higher activity against the resistant bacterial strains harboring kinases APH(3')Ia and APH(3')IIIa. However, in comparison to 1a, an 8-fold lower activity of 13 against wild type E. coli was observed. We assume that this decrease in activity is a consequence of the changed conformation of the 3'-OH group. It’s equatorial conformation in AGs is important for A-site recognition and binding. 3'-OH forms a direct hydrogen bond with the anionic phosphate oxygen of adenine A[α\(\gamma\)]\[14\], which helps to stabilize the bulging conformation.\[21\] This could explain the lower efficacy of the epimerized derivatives towards E. coli wild type.

Similar, 3'-epi-neomycin B (15) has a lower activity when compared to the pristine AG 3. However, in contrast to the 3'-epi-kanamycin A (13), it appears to tackle the resistance mediated by both AMEs completely. As shown in Table 2, antibiotic derivative 15 shows very similar activity against the wild type and both resistance E. coli strains. This biological evaluation clearly demonstrates the potential of the regioselective modification of AGs at the C3'-position to overcome bacterial resistance mediated by APH(3') kinases in a straightforward manner. Interestingly, modifications at ring I of aminoglycosides have been introduced previously to reduce the drug class-related toxicity.\[22\]

From our antibacterial studies, we can conclude that a straightforward epimerization of the 3'-hydroxyl group in kanamycin A (1a) and neomycin B (3), as in 13 and 15, seems to significantly reduce the ability of kinases APH(3')Ia and APH(3')IIIa to modify the antibiotic at the C3'-position. To understand the impact of this modification on the activity of this class of enzymes towards 13, we conducted in silico docking studies with the co-crystal structure of APH(3')IIIa (see Figures 3 and S1–S3).

In Figure 3(a and b) in silico docking of compounds 1a and 13 is shown. In these crystal structures, a non-hydrolysable adenosine tri-phosphate (ATP) analogue, i.e., adenosine 5'-trimido(triphosphate (AMP-PNP), is present.\[23\] This allows to compare the distances between the docked compound and the terminal phosphate group of the ATP analogue while occupying the active site. As shown in Figure 3(a and b), docking predicts a slightly different binding mode of 1a and 13, respectively.

While for antibiotic 1a a hydrogen bond between its 3'-hydroxyl group and aspartate 190 (Asp190) is shown. In these crystal structures, a non-hydrolysable adenosine tri-phosphate (ATP) analogue, i.e., adenosine 5'-trimido(triphosphate (AMP-PNP), is present.\[23\] This allows to compare the distances between the docked compound and the terminal phosphate group of the ATP analogue while occupying the active site. As shown in Figure 3(a and b), docking predicts a slightly different binding mode of 1a and 13, respectively.

While for antibiotic 1a a hydrogen bond between its 3'-hydroxyl group and aspartate 190 (Asp190) is observed (Figure 3a), this key interaction is missing with 13 (Figure 3b). In the latter case, the 3'-hydroxyl group of 13 participates in an intramolecular hydrogen bond with the anomic oxygen of ring I (see inset in Figure 3b). Extensive studies by Wright and coworkers on the molecular mechanism of phosphorylation have revealed that Asp190 plays a critical role in the activity of the antibiotic kinase APH(3')IIIa.\[24\] Besides acting as a general base, Asp190 participates in orienting the 3'-hydroxyl group of
the aminoglycoside by non-covalent interaction for the nucleophilic attack on the terminal phosphate of ATP. Additionally, due to the axial orientation of the 3‘-hydroxyl group of 13, its distance to the γ-phosphate group is slightly increased (Figure 3b). We assume, that the lack of interaction with Asp190 and the increased distance of the 3‘-hydroxyl group of 13 to the γ-phosphate of ATP accounts for the increased performance of 3‘-epi kanamycin A 13 against bacterial strains expressing O-phosphotransferases APH(3’)Ia and APH(3’)IIIa (see Table 2). To underline the credibility of the docking program, we compared the predicted binding mode of 1a (Figure 3a) with the actual binding conformation of 1a in APH(3’)IIIa observed in a previously published co-crystal structure (PDB-code: 1L8T) in the presence of the cofactor adenosine diphosphate (ADP) (PDB-code: 1L8T) (Figure 3c). The binding mode of 1a is virtually identical. This study revealed that the epimerization of the hydroxyl group at the 3‘-position erases an interaction with the enzyme’s key residue Asp190 in the active site, a key hydrogen bond interaction that is crucial for phosphorylation, thus preventing the deactivation of AGs by the resistance enzyme.

In conclusion, we have established a scalable catalytic alcohol oxidation of structurally complex 4,5-substituted and 4,6-substituted aminoglycoside antibiotics in a single position. NMR spectroscopy studies have proven a selective oxidation in 3‘-position in ring I of aminoglycosides kanamycin A (1a), kanamycin B (1b) and neomycin B (3), employing palladium-catalyst [(neocuproine)PdOAc]2(OTf)2 and benzoquinone as terminal oxidant. In only four synthetic steps, we have also obtained novel antibacterial candidates, 3‘-epi kanamycin A (13) and 3‘-epi-neomycin B (15), with superior antibacterial performance against resistant bacteria harboring resistance-causing AMEs APH(3’)Ia and APH(3’)IIIa. In vitro activity and in silico docking studies have revealed that epimerization at the C3‘-position of aminoglycoside antibiotics is sufficient to tackle bacterial resistance by O-phosphotransferases.

In summary, we have succeeded to improve the activity of aminoglycoside antibiotics against resistant bacteria employing a novel regioselective catalytic oxidation that minimizes the synthetic effort to access novel derivatives. The epimerization in 3‘-position of aminoglycosides is a great starting point for the development of more potent antibiotic candidates against resistant bacteria. Undoubtedly, this novel late-stage modification of aminoglycoside antibiotics represents a new synthetic strategy to tackle multidrug-resistant bacteria in both an academic and industrial setting.

Acknowledgements

We would like to sincerely acknowledge Prof. P. Courvalin from the Pasteur Institute, Paris, France for providing the APH(3’)III genes. This work was financially supported by the European Research Council Advanced Grant SUPRABIOTICS (No. 694610). Open Access funding enabled and organized by Projekt DEAL.

Figure 3. Complexes of APH(3’)IIIa with docked antibiotics 1a and 13, and co-crystallized with kanamycin A 1a. a) In silico docking of kanamycin A 1a in kinase APH(3’)IIIa. b) In silico docking of 3‘-epi kanamycin A 13 in APH(3’)IIIa. c) Co-crystal structure of APH(3’)IIIa with kanamycin A 1a.[23] The coordinates of APH(3’)IIIa in panels a and b are taken from PDB entry 3TM0,[23] in panel c from PDB entry 1L8T.[23] In silico docking was performed with LeadIT[25] and scoring by SeeSAR[26] (a and b). All images were generated with PyMol.[27] The protein backbone is shown in green, magnesium ions as grey spheres, and Asp190 side chain is shown as sticks colored by atom: C, grey; O, red. a and b) AMP-PNP and c) ADP are shown as sticks colored by atom: C, yellow; O, red; N, blue; P, orange. Antibiotics 1a and 13 in a and b are shown as sticks, hydrogens are indicated as lines. Compounds 1a and 13 are colored by atom: C, light blue; O, red; N, blue, H, grey. White dashed lines show distances (in Å) between atoms and yellow dashed lines indicate hydrogen bond interactions (length is given in Å).
Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: aminoglycoside antibiotics · antimicrobial resistance · catalytic oxidation · epimerization · regioselective modification

[1] a) World Health Organization. Antimicrobial resistance: global report on surveillance. 2014, http://www.who.int/drugresistance/documents/surveillancereport/en/; b) M. McKenna, Nature 2013, 499, 394–396.

[2] A. S. Fauci, N. A. Toccelli, G. K. Folkers, Emerging Infect. Dis. 2005, 11, 519–525.

[3] a) M. Bassetti, F. Ginocchio, M. Mikulska, A. S. Fauci, N. A. Touchette, G. K. Folkers, J. Am. Chem. Soc. 2012, 134, 5743–5745; b) B. Spellberg, R. Guidos, D. Gilbert, J. Bradley, H. W. Boucher, W. M. Scheld, J. G. Bartlett, J. Edwards Jr., Clin. Infect. Dis. 2008, 46, 153–164; c) J. Conly, B. Johnston, Can. J. Infect. Dis. Med. Microbiol. 2005, 16, 159–166; d) M. A. Cooper, D. Shlaes, Nature 2011, 472, 32–32; e) D. M. Shlaes, D. Sahm, C. Oppila, B. Spellberg, Antimicrob. Agents Chemother. 2013, 57, 4605–4607.

[4] D. G. Brown, T. L. May-Dracka, M. M. Gagnon, R. Tommasi, J. Med. Chem. 2014, 57, 10144–10161.

[5] Center for Disease Dynamics, Economics & Policy. State of the World’s Antibiotics, 2015. CDDEP: Washington, D. C., 2015, https://cddep.org/publications/state worlds antibiotics 2015/.

[6] a) R. E. Procópio, I. R. Silva, M. K. Martins, J. L. Azevedo, J. M. Araújo, J. Am. Chem. Soc. 2012, 134, 4666–4671; b) D. A. Evans, H. P. Ng, D. L. Rieger, J. Am. Chem. Soc. 1993, 115, 11446–11459; c) E. Kaufmann, H. Hattori, H. Miyatake-Ondozabal, K. Gademann, Org. Lett. 2015, 17, 3514–3517.

[7] L. Leibovici, L. Vidal, M. Paul, J. Antimicrob. Chemother. 2008, 63, 246–251.

[8] a) K. D. Green, S. Garneau-Tsodikova, Front. Microbiol. 2013, 4, 208; b) X. Y. Zhang, L. J. Ding, M. Z. Fan, Res. Vet. Sci. 2009, 87, 449–454.

[9] O. Khan, N. Chaudary, Drug Des. Dev. Ther. 2020, 14, 2287–2294.

[10] S. D. Rajendran, Y. M. Rao, V. P. Karthikeyan, V. P. Karthikeyan, S. D. Rajendran, Y. M. Rao, Antimicrob. Agents Chemother. 2019, 74, 944–952.

[11] M. Juhás, E. Widilake, J. Teo, D. L. Huseby, J. M. Tyrrell, Y. S. Polikanov, O. Ercan, A. Petersson, S. Cao, A. F. Aboklaish, A. Rominski, D. Crich, E. C. Böttger, T. R. Walsh, D. Hughes, S. N. Hobbie, J. Antimicrob. Chemother. 2019, 74, 944–952.

[12] clinicaltrials.gov, First-In-Human Study of Apramycin, 2021, https://clinicaltrials.gov/ct2/show/NCT04105205.

[13] a) S. Garneau-Tsodikova, K. J. Labby, MedChemComm 2016, 7, 11–27; b) M. P. Mingnot-Leclercq, Y. Glupczynski, P. M. Tulkens, Antimicrob. Agents Chemother. 1999, 43, 737–737; c) M. S. Ramirez, M. E. Tolmasy, Drug Resist. Updates 2010, 13, 151–171; d) J. Zhang, F.-l. Chiang, L. Wu, P. G. Czyzyczka, D. Li, C.-W. T. Chang, J. Med. Chem. 2008, 51, 7563–7573; e) J. Haddad, S. Vakulenko, S. Mobashery, J. Am. Chem. Soc. 1999, 121, 11922–11923.

[14] a) J. Li, F.-l. Chiang, H.-N. Chen, C.-W. T. Chang, J. Org. Chem. 2007, 72, 4055–4066; b) N. Thamban Chandrika, K. D. Green, J. L. Houghton, S. Garneau-Tsodikova, ACS Med. Chem. Lett. 2015, 6, 1134–1139; c) R. J. Fair, L. S. McCoy, M. E. Hensler, A. Aguilar, V. Nizet, Y. Tor, ChemMedChem 2019, 14, 2164–2171; d) M. S. Ramirez, M. E. Tolmasy, Molecules 2017, 22, 2267.

[15] P. B. Alper, M. Hendrix, P. Sears, C.-H. Wong, J. Am. Chem. Soc. 1998, 120, 1965–1978.

[16] K. Eljaedy, A. Alharbi, S. Alshehri, J. K. Ortwine, J. M. Pogue, Drugs 2019, 79, 243–269.

[17] a) M. Fridman, V. Belakhov, S. Yaron, T. Baasov, Org. Lett. 2003, 5, 3575–3578; b) M. Hainrichson, V. Pokrovskaya, D. Shallom-Sheziff, M. Fridman, V. Belakhov, D. Shachar, S. Yaron, T. Baasov, Biogog. Med. Chem. 2005, 13, 5797–5807; c) M. Fridman, V. Belakhov, L.V. Lee, F. Liang, C.-H. Wong, T. Baasov, Angew. Chem. Int. Ed. 2005, 44, 447–452; Angew. Chem. 2005, 117, 451–456.

[18] a) M. Jäger, M. Hartmann, J. G. de Vries, A. J. Minnaard, Angew. Chem. Int. Ed. 2013, 52, 7809–7912; Angew. Chem. 2013, 125, 7963–7966; b) N. N. H. M. Eisink, J. Lohse, M. D. Witte, A. J. Minnaard, Org. Biomol. Chem. 2016, 14, 4859–4864.

[19] K. Chung, R. M. Waymouth, ACS Catal. 2016, 6, 4653–4659.

[20] K. J. Shaw, P. N. Rather, R. S. Hare, G. H. Miller, Microbiol. Rev. 1993, 57, 138–163.

[21] a) G. Vicens, E. Westhof, Chem. Biol. 2002, 9, 747–755; b) S. Sallan, T. Matt, R. Akbergenov, S. Harish, M. Meyer, S. Duscha, D. Scherbakov, B. B. Bernet, A. Vasella, E. Westhof, E. C. Böttger, Antimicrob. Agents Chemother. 2012, 56, 6104–6108.

[22] D. Perez-Fernandez, D. Scherbakov, T. Matt, N. C. Leong, I. Kudyba, S. Duscha, H. Boukari, R. Patak, S. R. Dubbaka, K. Lang, M. Meyer, R. Akbergenov, P. Freihofer, S. Vaddi, P. Thommes, V. Ramakrishnan, A. Vasella, E. C. Böttger, Nat. Commun. 2014, 5, 3112.

[23] D. H. Fong, A. M. Berghuis, EMBIO J. 2001, 21, 2323–2323.

[24] D. H. Fong, A. M. Berghuis, Antimicrob. Agents Chemother. 2009, 53, 3049–3055.

[25] LeadIT 2.1.8, BioSolveIT GmbH, An Der Ziegelei 79, 53757 Sankt Augustin, Germany (2014).

[26] SeeSAR 3.3, BioSolveIT GmbH, An Der Ziegelei 79, 53757 Sankt Augustin, Germany (2015).

[27] L. Schrödinger, The PyMOL Molecular Graphics System, Version 1.6.x (2013).

[28] D. D. Boehr, P. R. Thompson, G. D. Wright, J. Org. Chem. 2001, 276, 23929–23936.