VIP *Very Important Paper*

An Advanced Apralog with Increased in vitro and in vivo Activity toward Gram-negative Pathogens and Reduced ex vivo Cochleotoxicity

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We describe the convergent synthesis of a 5-*O*-β-D-ribofuranosyl-based apramycin derivative (apralog) that displays significantly improved antibacterial activity over the parent apramycin against wild-type ESKAPE pathogens. In addition, the new apralog retains excellent antibacterial activity in the presence of the only aminoglycoside modifying enzyme (AAC(3)-IV) acting on the parent, without incurring susceptibility to the APH(3′) mechanism that disables other 5-*O*-β-D-ribofuranosyl 2-deoxystreptamine type aminoglycosides by phosphorylation at the ribose 5-position. Consistent with this antibacterial activity, the new apralog has excellent 30 nM activity (IC_{50}) for the inhibition of protein synthesis by the bacterial ribosome in a cell-free translation assay, while retaining the excellent across-the-board selectivity of the parent for inhibition of bacterial over eukaryotic ribosomes. Overall, these characteristics translate into excellent in vivo efficacy against *E. coli* in a mouse thigh infection model and reduced ototoxicity vis à vis the parent in mouse cochlear explants.

The ever-increasing spread of multidrug resistant infectious diseases demands the continued development of new and improved antibiotics. Next generation aminoglycoside antibiotics (AGAs) have much to offer in this regard in view of their ready availability, extensive and well-described chemistry and documented mechanism of action, broad spectrum activity, and lack of allergic response.[1]

Apramycin **1** is a rare example of a 2-deoxystreptamine-type (DOS) aminoglycoside that carries only a single substituent on the DOS ring in the form of a 4-aminoglucopyranosylated deoxyoctodiosyl moiety.[2] The unusual structure of apramycin prevents the action of all common aminoglycoside-modifying enzymes (AMEs),[3] by far the most common cause of AGA resistance,[4] except the rare aminoacetyltransferase isoform AAC(3)-IV.[5] The structure of apramycin also circumvents the action of the increasingly widespread G1405 16S ribosomal RNA methyltransferases,[3a,c,6] whose presence nullifies all AGAs in current clinic practice including the most recently introduced plazomicin.[7] These attributes, coupled with the ready availability of apramycin and its reduced ototoxicity compared to other common AGAs,[3a,8] have resulted in the development of apramycin as a promising therapeutic for treatment of life-threatening MDR infections, especially *Acinetobacter baumannii*, *Enterobacterales*, and other Gram-negative ESKAPE pathogens,[3c] culminating in a phase I clinical trial.[9]

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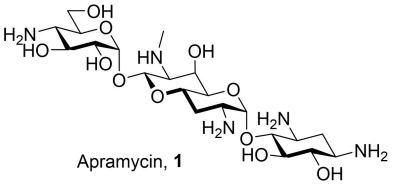
Table 1. Antiribosomal activities and selectivities.

Strain	IC_{50} [μM] wt	Mit13	A1555G	Cyt14	Selectivity Mit13	A1555G	Cyt14
Apramycin	0.15 ± 0.05	119 ± 30	98 ± 33	129 ± 26	793	653	860
2	0.071 ± 0.015	68 ± 20	13 ± 3	190 ± 105	957	183	2676
3	0.052 ± 0.026	118 ± 34	99 ± 22	97 ± 14	2269	1904	1865
4	0.032 ± 0.014	43 ± 7	21 ± 3	40 ± 7	1343	656	1250



In addition to its own inherent advantages, apramycin also provides an excellent starting point for the development of next-generation AGAs that retain its multiple advantages while exhibiting enhanced levels of antibacterial activity and reduced susceptibility to the remaining resistance determinant – the AAC(3)-IV AME that acts by acetylation of N3.[3e,5–6,10] With this in mind, and building on the early promise of 5-*O*-β-D-ribofuranosyl apramycin,[11] we reported earlier on the synthesis and evaluation of a series of 5-*O*-furanosylated apramycin derivatives exemplified by **2** and **3** (Figure 1). As anticipated on the basis of work by the Wong group in the ribostamycin series,[12] the β-D-ribofuranosyl derivative **2** carrying a 3-*O*-(2-aminoethyl) substituent in the ribose ring was more active than the earlier[11] 5-*O*-β-D-ribofuranosyl derivative and apramycin itself with regards to both the inhibition of the bacterial ribosome (Table 1) and antibacterial activity against wild-type ESKAPE pathogens (Table 2). However, **2** showed a marked reduction in selectivity for the bacterial over the eukaryotic hybrid ribosomes carrying the human mutant mitochondrial (A1555G) decoding A site[13] (Table 1) indicative of potentially increased ototoxicity.[4g,13] Additionally, the ribofuranosyl moiety of **2** conferred susceptibility to deactivation by the aminoglycoside phosphotransferases (APHs) acting on the ribofuranosyl primary hydroxyl group, the APH(3′,5″) isozymes.[14] Moving the pendant amino group from the ribofuranosyl 3-position of **2** to the primary ribofuranosyl 5-position, as was done in **3**, restored across-the-board selectivity for the bacterial over the eukaryotic hybrid ribosomes, and eliminated deactivation by the APH-(3′,5″)s. We now show that incorporation of aspects of both **2** and **3**, namely an aminoethyl ether at the 3-position and a deoxyamino modification at 5-position of the ribose ring, into a single compound results in an improved 5-*O*-β-D-ribofuranosyl apramycin derivative **4**.

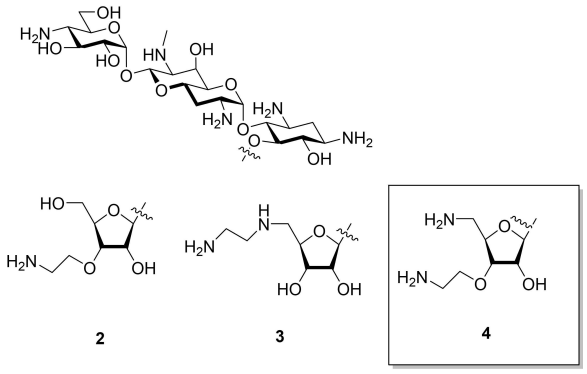


Figure 1. Apralogs **2**–**4**.

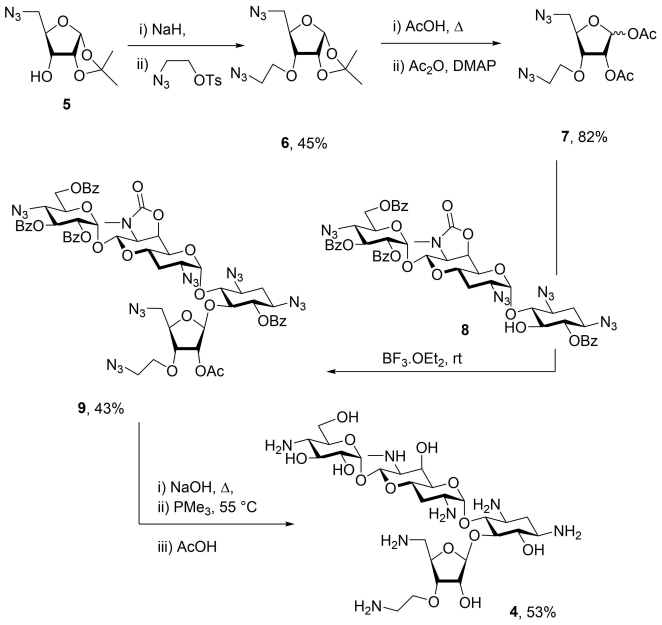
For the synthesis of **4**, a donor **7** was prepared in a simple two-pot sequence via **6** from the known ribose derivative **5**,[15] and coupled under robust Helferich conditions to the apramycin derivative **8** followed by a one pot deprotection sequence involving saponification and Staudinger reduction of the azido groups (Scheme 1). As **8** is available in four steps from apramycin,[10b] the synthesis of **4** requires only six steps from the parent aminoglycoside **1**.

Apralog **4** was an excellent inhibitor of the bacterial ribosome for which it showed good across-the-board selectivity over the hybrid ribosomes mimicking the various eukaryotic drug binding pockets, i.e., human mitochondrial (Mit13), mutant mitochondrial (A1555G), and cytoplasmic (Cyt14) decoding A sites (Table 1). We used dimethyl sulfate foot printing[16] of the *E. coli* 70S ribosome to demonstrate binding to A1408 in the decoding A site and estimate apparent K_D values of 5 and 0.5 μM for the parent **1** and apralog **4**, consistent with the relative levels of inhibition of the bacterial ribosome (Figures 2 and 3).

In addition to this high level of inhibition of the bacterial ribosome, **4** showed outstanding activity against wild-type isolates of ESKAPE pathogens (Table 2). Turning to the inhibition of *E. coli* strains characterized by the presence of AAC(3)

Table 2. Antibacterial activities against wild-type *E. coli* and ESKAPE pathogens (MIC, mg/L).

Species Strain	*E. coli* AG001	*K. pneu.* AG215	*Enterob.* AG290	*A. baum.* AG225	*P. aerug.* AG220	MRSA AG038
Apramycin	4	1–2	2–4	4	4	4
2	2	1–2	1–2	4–8	16–32	2-4
3	2	1	1–2	4–8	4–8	4
4	1–2	0.5–1	1	2	2	1



Scheme 1. Synthesis of **4**.

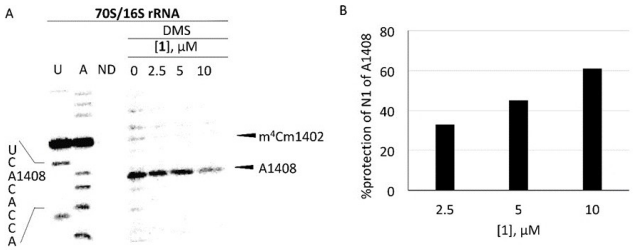


Figure 2. Dimethyl sulfate (DMS) foot printing of apramycin (1) binding to the A site (helix 44) region in 70S ribosomes. (**A**) The autoradiogram of reacted rRNA followed by primer extension using a radiolabeled primer is shown with the A1408 sequencing stop site, DMS methylation of A1408 stop site, and naturally methylated C1402 (m^4Cm1402) highlighted (U and A sequencing; ND, no DMS). Note that DMS modification produces a reverse-transcription stop site one nucleotide prior to the modified site (A1408) and dideoxy sequencing stop sites. (**B**) Calculated % protection of N1 of A1408 is shown with increasing concentration of **1** (single runs only).

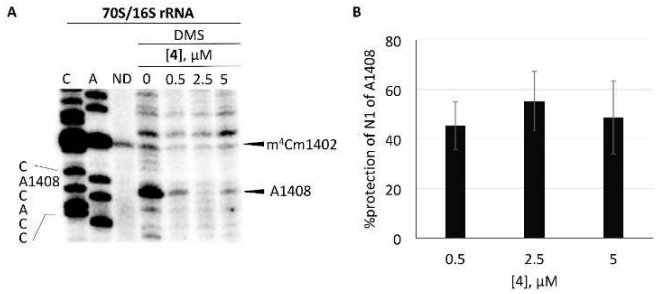


Figure 3. Dimethyl sulfate (DMS) foot printing of 4 binding to the A site (helix 44) region in 70S ribosomes. (**A**) The autoradiogram of reacted rRNA followed by primer extension using a radiolabeled primer is shown with the A1408 sequencing stop site, DMS methylation of A1408 stop site, and naturally methylated C1402 (m^4Cm1402) highlighted (C and A sequencing; ND, no DMS). Note that DMS modification produces a reverse-transcription stop site one nucleotide prior to the modified site (A1408) and dideoxy sequencing stop sites. (**B**) Calculated % protection of N1 of A1408 is shown with increasing concentration of **4** (standard error is for three independent experiments).

and APH(3′,5″) AMEs, we studied recombinant *E. coli* strains, which expressed the resistance determinants in an isogenic background. This revealed that of the various AAC(3) and APH (3′) isozymes tested only AAC(3)-IV and APH(3′)-Ia affect apralogs **2** and **3** (Table 3). AAC(3)-IV affects both **2** and **3**, but primarily the latter, while APH(3′)-Ia only affects **2**. These features were also evident when we screened a panel of *E. coli* clinical isolates with acquired AAC(3) and APH(3′) resistance (Table 4). Overall, the Achilles heel of apramycin and **3** is AAC (3)-IV, while that of **2** is APH(3′)-Ia. Apralog **4** on the other hand retained high levels of activity in the presence of AAC(3) isozymes including AAC(3)-IV, whether in recombinant or clinical strains. These findings are particularly significant not only as AAC(3)-IV is the only AME known to act on the parent apramycin,[5] but also because they demonstrate the capability of enhancing the activity of the parent by introduction of a modified ribosyl-type substituent without incurring the penalty of susceptibility to APH(3′)-Ia seen in the comparator lividomycin B.

Finally, we screened apralog **4** for activity against a small panel of clinical isolates of *E. coli* with two or more relevant resistance determinants (Table 5). It was found that **4** retains strong activity in the combined presence of both AAC(3)-IV and APH(3′)-I, as well as in the presence of combinations of AAC(3) and or APH(3′) isozymes with AAC(6′), the most prevalent resistance mechanism against the clinical AGA gentamicin, and the ribosomal methyltransferase RmtB, which completely abrogates the activity of all 4,6-type AGAs including gentamicin and plazomicin.

To assess potential ototoxicity, increasing concentrations of apralog **4** and the parent apramycin were incubated with cochlear explants from postnatal 3 day FVB/NJ mice for 72 h before staining and counting of outer hair cells (OHCs).[7c]

Table 3. Antibacterial activities against recombinant *E. coli* strains expressing single resistance determinants in an isogenic background (MIC, mg/L).

Resistance Determinant Strain	wt parental EC026	AAC(3)-IV EC118	AAC(3)-II EC200	APH(3′)-Ia EC122	APH(3′)-IIa EC123	APH(3′)-IIb EC125	APH(3′)-VI EC141	armA EC102	rmtB EC103
Apramycin	2	128	2–4	1–2	1	1	1	1	1
2	0.5–1	4	0.5–1	2–4	0.5	0.5	0.5	0.5	0.5
3	0.5–1	8–16	0.5	0.5	0.5	0.5	0.5	0.5	0.5–1
4	0.25–0.5	2	0.25	0.25	0.25	0.25	0.25	0.5	0.5

Table 4. Antibacterial activities (MIC, mg/L) against *E. coli* strains with acquired AAC(3) and APH(3′) resistance.

Resistance Determinant	wt	AAC(3)-II	AAC(3)-IV	APH(3′)-I	APH(3′)-II
Strain	AG001	AG170	AG173	AG163	AG166
Apramycin	4	4-8	256	4	4–8
Lividomycin B	4–8	4-8	16	>256	4–8
2	2	2	4	4–8	2
3	2	2	16	2–4	2–4
4	1–2	1–2	4	1–2	1–2

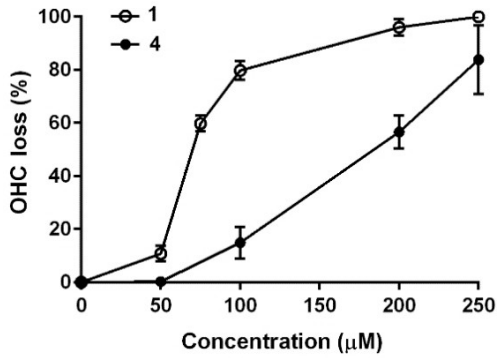


Figure 4. Dose-response plots of the percentage of outer hair cell loss (OHC) versus concentration of aminoglycoside. Data are presented as mean values $\pm\sigma$, $n=6-7$ per point.

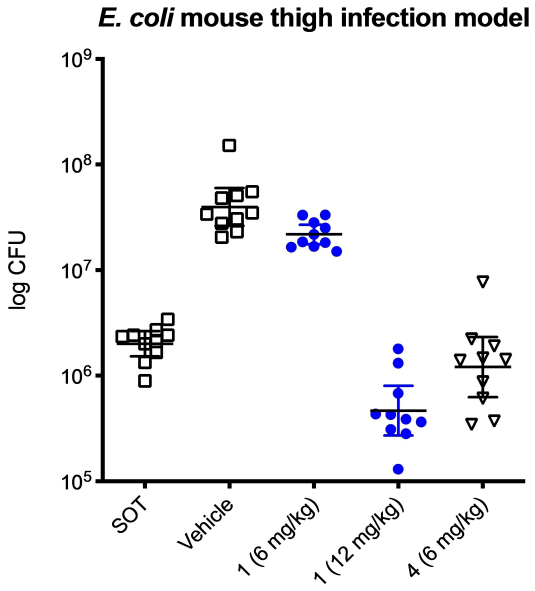


Figure 5. In vivo efficacy of apralog **4** in comparison to apramycin (**1**) in a neutropenic mouse thigh infection model.

Plotting the percentage of OHC loss against AGA concentration (Figure 4) then allowed the determination of the LD_{50} values. Apralog **4** showed an approximate of 2-fold reduction in cochleotoxicity ($LD_{50}=175\pm19.2$ μM) compared to apramcyin ($LD_{50}=71\pm1.8$ μM). This reduction of cochleotoxicity was achieved with only minor enhancement in mitoribosomal selectivity with respect to the parent (Table 1).

Finally, we turned to in vivo efficacy for which we employed an *E. coli* mouse thigh infection model. At a dose of 6 mg kg^{-1} apralog **4** reduced the bacterial burden in the blood by approximately 1 log unit, comparable to the parent at double the dose and significantly more than the parent at the same dose level (Figure 5).

In conclusion, apralog **4** carrying a doubly modified β-D-ribofuranosyl substituent at the apramycin 5-position is obtained by a robust, convergent synthesis in only six steps from readily available apramycin. It displays significantly improved affinity for the bacterial decoding A site and similarly improved inhibition of the bacterial ribosome over the parent apramycin. This increased affinity and activity at the target level is reflected in significantly improved activity against multiple wild-type ESKAPE pathogens and both recombinant and clinical isolates of *E. coli* carrying one or more relevant resistance determinants. Thus, apralog **4** is not affected by the APH(3′)-Ia class of AMEs despite the presence of the ribofuranosyl ring and shows excellent activity in the presence of the AAC(3)-IV resistance determinant that constitutes the only known[5] mechanism of resistance to the parent. The increased levels of antibacterial activity are reflected in the increased efficacy in the *E. coli* mouse thigh model. Albeit for reasons that are not yet clear, apralog **4** shows reduced levels of toxicity toward mouse cochlear explants compared to the parent with its already low ototoxicity. These multiple attributes combine to make apralog **4** an ideal candidate for further development as a next generation aminoglycoside for the treatment of MDR Gram-negative and other bacterial infections.

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Table 5. Antibacterial activities (MIC, mg/L) against *E. coli* strains with two or more acquired resistance determinants.

Resistance Determinant	AAC(3)-IV, APH(3′)-I	AAC(3)-IV, APH(3′)-I	AAC(6′)-Ib, AAC(3)-IId	APH(3′)-Ia, AAC(3)-IId	AAC(3)-IIa, AAC(6′)-Ib, RmtB	AAC(3)-II, APH(3′)-II, RmtB
Strain	AG182	AG183	AG157	AG180	AG341	AG153
Apramycin	>256	>256	8	4	2–4	8
2	16	16–32	4	4	1–2	4
3	32	32–64	4	4	1–2	4
4	8	8	2	1	2–4	4

assistance with the RNA foot printing, and Evotec for the in vivo efficacy study.

Conflict of Interest

S.N.H., A.V., E.C.B., and D.C. are cofounders of and equity holders in Juvabis AG, a biotech start-up developing aminoglycoside antibiotics.

Keywords: Antibiotics · Biological Activity · Drug Design · Glycosylation

[1] a) E. C. Böttger, D. Crich, *ACS Infect. Dis.* **2020**, *6*, 168–172; b) T. Brennan-Krohn, R. Manetsch, G. A. O'Doherty, J. E. Kirby, *Transl. Res.* **2020**; c) Y. Takahashi, M. Igarashi, *J. Antibiot.* **2018**, *71*, 4–14.
[2] S. O'Connor, L. K. T. Lam, N. D. Jones, M. O. Chaney, *J. Org. Chem.* **1976**, *41*, 2087–2092.
[3] a) T. Matt, C. L. Ng, K. Lang, S.-H. Sha, R. Akbergenov, D. Shcherbakov, M. Meyer, S. Duscha, J. Xie, S. R. Dubbaka, D. Perez-Fernandez, A. Vasella, V. Ramakrishnan, J. Schacht, E. C. Böttger, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 10984–10989; b) R. Ryden, B. J. Moore, *J. Antimicrob. Chemother.* **1977**, *3*, 609–613; c) M. Juhas, E. Widlake, J. Teo, D. L. Huseby, J. M. Tyrrell, Y. Polikanov, O. Ercan, A. Petersson, S. Cao, A. F. Aboklaish, A. Rominski, D. Crich, E. C. Böttger, T. R. Walsh, D. E. Hughes, S. N. Hobbie, *J. Antimicrob. Chemother.* **2019**, *74*, 944–952; d) I. Galani, K. Nafplioti, M. Chatzikonstantinou, H. Giamarellou, M. Souli, *ECCMID* **2018**, P 0096; e) K. P. Smith, J. E. Kirby, *Diagn. Microbiol. Infect. Dis.* **2016**, *86*, 439–441.
[4] a) S. Magnet, J. S. Blanchard, *Chem. Rev.* **2005**, *105*, 477–497; b) L. Yang, X. S. Ye, *Curr. Top. Med. Chem.* **2010**, *10*, 1898–1926; c) V. R. Bacot-Davis, A. V. Bassenden, A. M. Berghuis, *MedChemComm* **2016**, *7*, 103–113; d) S. Garneau-Tsodikova, K. J. Labby, *MedChemComm* **2016**, *7*, 11–27; e) S. G. Zárate, M. L. De la Cruz Claure, R. Benito-Arenas, R. Revuelta, A. G. Santana, A. Bastida, *Molecules* **2018**, *23*, 284, doi: 210.3390/molecules23020284; f) E. S. Armstrong, C. F. Kostrub, R. T. Cass, H. E. Moser, A. W. Serio, G. H. Miller, in *Antibiotic Discovery and Development* (Eds.: T. J. Dougherty, M. J. Pucci), Springer Science + Business Media, New York, **2012**, pp. 229–269; g) M. Jospe-Kaufman, L. Siomin, M. Fridman, *Bioorg. Med. Chem. Lett.* **2020**, *30*, 127218.
[5] M. Plattner, M. Gysin, K. Haldimann, K. Becker, S. N. Hobbie, *Int. J. Mol. Sci.* **2020**, *21*, 6133.
[6] a) T. Brennan-Krohn, J. E. Kirby, *Antimicrob. Agents Chemother.* **2019**, *63*, e01374–01319; b) Y. Doi, J. I. Wachino, Y. Arakawa, *Infect. Dis. Clin. North Am.* **2016**, *30*, 523–537.
[7] a) D. M. Livermore, S. Mushtaq, M. Warner, J.-C. Zhang, S. Maharjan, M. Doumith, N. Woodford, *J. Antimicrob. Chemother.* **2011**, *66*, 48–53; b) G. Cox, L. Ejim, P. J. Stogios, K. Koteva, E. Bordeleau, E. Evdokimova, A. O. Sieron, A. Savchenko, A. W. Serio, K. M. Krause, G. D. Wright, *ACS Infect. Dis.* **2018**, *4*, 980–987; c) A. Sonousi, V. A. Sarpe, M. Brilkova, J. Schacht, A. Vasella, E. C. Böttger, D. Crich, *ACS Infect. Dis.* **2018**, *4*, 1114–1120.
[8] M. Ishikawa, N. García-Mateo, A. Čusak, I. López-Hernández, M. Fernández-Martínez, M. Müller, L. Rüttiger, W. Singer, H. Löwenheim, G. Kosec, S. Fujs, I. Martínez-Martínez, T. Schimmang, H. Petković, M. Knipper, M. B. Durán-Alonso, *Sci. Rep.* **2019**, *9*, 2410.
[9] https://www.imi.europa.eu/projects-results/project-factsheets/enable; accessed Feb 12, 2019.
[10] a) A. R. Mandhapati, D. Shcherbakov, S. Duscha, A. Vasella, E. C. Böttger, D. Crich, *ChemMedChem* **2014**, *9*, 2074–2083; b) J. C. K. Quirke, P. Rajasekaran, V. A. Sarpe, A. Sonousi, I. Osinnii, M. Gysin, K. Haldimann, Q.-J. Fang, D. Shcherbakov, S. N. Hobbie, S.-H. Sha, J. Schacht, A. Vasella, E. C. Böttger, D. Crich, *J. Am. Chem. Soc.* **2020**, *142*, 530–544; c) Y. Hu, L. Liu, X. Zhang, Y. Feng, Z. Zong, *Front. Microbiol.* **2017**, *8*, 2275, doi: 2210.3389/fmicb.2017.02275; d) A. D. Kang, K. P. Smith, G. M. Eliopoulos, A. H. Berg, C. McCoy, J. E. Kirby, *Diagn. Microbiol. Infect. Dis.* **2017**, *88*, 188–191; e) S. L. Zada, B. B. Baruch, L. Simhaev, H. Engel, M. Fridman, *J. Am. Chem. Soc.* **2020**, *142*, 3077–3087.
[11] Y. Abe, S. Nakagawa, T. Naito, H. Kawaguchi, *J. Antibiot.* **1981**, *34*, 1434–1446.
[12] P. B. Alper, M. Hendrix, P. Sears, C.-H. Wong, *J. Am. Chem. Soc.* **1998**, *120*, 1965–1978.
[13] a) S. N. Hobbie, S. Akshay, S. K. Kalapala, C. Bruell, D. Shcherbakov, E. C. Böttger, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 20888–20893; b) S. N. Hobbie, C. M. Bruell, S. Akshay, S. K. Kalapala, D. Shcherbakov, E. C. Böttger, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 3244–3249; c) S. N. Hobbie, S. K. Kalapala, S. Akshay, C. Bruell, S. Schmidt, S. Dabow, A. Vasella, P. Sander, E. C. Böttger, *Nucl. Acids Res.* **2007**, *35*, 6086–6093; d) M. E. Huth, A. J. Ricci, A. G. Cheng, *Int. J. Otolaryngol.* **2011**, 937861; e) T. R. Prezant, J. V. Agapian, M. C. Bohlman, X. Bu, S. Öztas, W.-Q. Qiu, K. S. Arnos, G. A. Cortopassi, L. Jaber, J. l. Rotter, M. Shohat, N. Fischel-Ghodsian, *Nat. Genet.* **1993**, *4*, 289–294; f) E. C. Böttger, J. Schacht, *Hear. Res.* **2013**, *303*, 12–19.
[14] G. D. Wright, P. R. Thompson, *Front. Biosci.* **1999**, *4*, d9–21.
[15] E. Raluy, O. Pàmies, M. Diéguez, *Adv. Synth. Catal.* **2009**, *351*, 1648–1670.
[16] a) D. Moazed, H. F. Noller, *Nature* **1987**, *327*, 389–394; b) P. Waduge, Y. Sakakibara, C. S. Chow, *Methods* **2019**, *156*, 110–120.

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