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Synthesis, Structural Reassignment, and Antibacterial Evaluation of 2,18-*Seco*-Lankacidinol B

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Abstract

Lankacidins are a group of polyketide natural products with activity against several strains of Gram-positive bacteria. We developed a route to stereochemically diverse variants of 2,18-seco-lankacidinol B and found that the stereochemical assignment at C4 requires revision. This has interesting implications for the biosynthesis of natural products of the lankacidin class, all of which possessed uniform stereochemistry prior to this finding. We have evaluated 2,18-seco-lankacidinol B and three stereochemical derivatives against a panel of pathogenic Gram-positive and Gram-negative bacteria.

Keywords

antibiotics; biosynthesis; natural products; structural reassignment; total synthesis

The lankacidin group of natural products was first discovered in the extracts of *Streptomyces rochei* over fifty years ago and contain an unusual 17-membered macrocyclic ring and a highly substituted β-keto-δ-lactone function (Figure 1A).[1] Lankacidin C (**1**) has inhibitory activity equal to or better than erythromycin against many species of Gram-positive bacteria, but is not susceptible to common forms of erythromycin resistance, making it a promising candidate for further development.[2] In an effort to improve the therapeutic potential of lankacidins, McFarland and colleagues at Pfizer pursued structural modifications by semisynthesis, but their efforts were limited by the chemical instability of the β-keto-δ-lactone core, which is required for potent antibiotic activity (Figure 1B).[2b] Fully synthetic methods to generate lankacidin derivatives could circumvent many of the limitations of semisynthetic approaches and enable systematic investigation of structure–activity effects in the class.[3] Three fully synthetic routes to lankacidin natural products have been reported,[4] including an elegant recent biomimetic approach by Hong and colleagues.[4d] To the best of our knowledge, the effects of modifications of the β-keto-δ-lactone on antibiotic activity have not yet been systematically investigated.[5]

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Conflict of interest

The authors declare no conflict of interest.

Earlier this year, Lu et al. reported the isolation and structural elucidation of 2,18-*seco*-lankacidinols A (**3**) and B (**4**), two new members of the lankacidin class that lack the C2–C18 bond that is common to other family members.[6] 2,18-*Seco*-lankacidinol B (**4**) is the first acyclic member of the lankacidin class to be isolated and likely arises from a modified biosynthetic pathway.[7] During the course of our efforts to develop a modular platform for the synthesis of lankacidin antibiotics, we synthesized acyclic intermediates that closely resembled **4**, including several stereochemical variants. Herein we report the initial results from those efforts, which have provided material for microbiological evaluation and have resulted in a structural reassignment of 2,18-*seco*-lankacidinol B (**4**).

In the design of our route to lankacidins, we prioritized convergency and the use of reactions that we anticipated would have high functional group tolerance so as to streamline the synthesis of derivatives. Thus, our first efforts were aimed at the synthesis of two halves of approximately equal complexity, right half **11** and left halves **17**–**20** (Scheme 1). The synthesis of right half **11** commences with exposure of oxirane **5**[8] to lithium acetylide (generated from 1-bromopropene and butyllithium)[9] followed by addition of *tert*-butyldimethylsilyl chloride to generate protected diol **7** in 78% yield (Scheme 1A). Oxidative deprotection of the PMB group, Mitsunobu displacement of the resulting primary alcohol with benzo[d]thiazole-2-thiol, and in situ oxidation of the resulting thioether delivers sulfone **8** in 77% yield over 2 steps. Julia-Kociensky olefination with commercially available aldehyde **9** provides dienoate **10** in 80% yield as a single stereoisomer.[10] Reduction of the ethyl ester to the primary alcohol (DIBAL-H) followed by palladium-mediated hydro-stannylation[11] delivers right half **11** in 84% yield. The route to **11** requires 6 steps from **5** and **6**, proceeds in 40% overall yield, and has enabled the production of multi-gram quantities of this key intermediate.

We designed our route to the β-keto-δ-lactone functionality to enable stereochemical control within the ring. By leveraging the Evans aldol reaction with common intermediate **15** as an electrophile, it would be possible to generate all four possible diastereomeric combinations at C4 and C5 in a predictable fashion. As shown in Scheme 1B, common intermediate **15** is prepared by an aldol reaction between β-iodoacrolein (**12**)[12] and acetyl thiazolidinethione **13** (70% yield) followed by silyl protection of the resulting secondary alcohol and reductive cleavage of the auxiliary (91%, 2 steps). To access the 4*R*,5*R* and 4*S*,5*S* diastereomers, an *anti*-selective aldol reaction is required, and our efforts were informed by elegant work by Hong and co-workers in their synthesis of lankacidinol (**2**).[4d] Thus, exposure of the boron enolate of bketo imide **16**[13] to aldehyde **15** in the presence of excess chlorodicyclohexylborane provides two *anti*-aldol products, which lactonize upon exposure to sodium methoxide to provide 4*R*,5*R* left half **17** (61% isolated yield) and 4*S*,5*S* left half **18** (21% isolated yield). Stereochemical assignments of the two diastereomers were made by comparison to similar intermediates in Hong‚s lankacidin synthesis[4d] and by ^{1}H-^{1}H NOESY NMR analysis of the final compounds **4** and **23** (see Scheme 2 and Supporting Information).

To access the 4*R*,5*S* and 4*S*,5*R* stereochemical variants, *syn*-aldol reactions are required. Under conditions reported by Evans and co-workers,[13] *syn*-aldol reaction of β-keto imide **21** with aldehyde **15** followed by lactonization provides 4*R*,5*S* left half **19** in 98% yield over

2 steps. Similarly, aldolization of β-keto imide **16** followed by lactonization provides 4*S*,5*R* left half **20** in 90% yield over 2 steps. Left half **20** was transformed into its ferrocene carboxylate **22**, which readily crystallized from ethyl ether. X-ray crystallographic analysis confirmed its relative and absolute stereochemistry.

To complete the synthesis of 2,18-*seco*-lankacidinols B bearing all four C4,C5-diastereomeric variations, we attached left half **11** to each right half **17**–**20** by means of Stille cross coupling reactions (Scheme 2). The use of a tin scavenger ($Ph_2PO_2NBu_4$)[14] and a highly polar solvent (DMSO) greatly accelerates the reaction and results in increased isolated yields (see supporting information for optimization details).[15] These convergent coupling reactions proceed efficiently (73–78% yield) without the need for protection of the free enol ether at C2/C3 or the primary alcohol at C18. Desilylation of each of the Stille products with hydrogen fluoride provides 2,18-*seco*-lankacidinols B (**4**, **23**–**25**). Each of the final products **4** and **23**–**25** were oils, and we were unable to obtain X-ray-quality crystals of derivatives thereof. However, the structural assignments are strongly supported by the Evans' aldol stereochemical models, nuclear Over-hauser effect data (not included in the isolation report, see supporting information), and the X-ray crystallographic data of ferrocene carboxylate **22**.

Much to our surprise, the ^{1}H- and ^{13}C-NMR spectra of **4** (Figure 2, teal traces) did not match the reported spectra[6] of 2,18-*seco*-lankacidinol B (Figure 2, black traces). Most notably, the methine proton off of C5 and the C20 methyl protons were significantly shifted, as were the ^{13}C signals corresponding to C3, C5, C6, and C20. Taken together, these discrepancies suggested to us that the structural differences resided on the β-keto-δ-lactone ring. We then compared the spectra of remaining three diastereomers to the reported spectra, we found that the spectra of the 4*R*,5*S* diastereomer **25** (Figure 2, red traces), which has the opposite C4 stereochemistry as the originally reported structure, matched very well, necessitating a structural reassignment of 2,18-*seco*-lankacidinol B from structure **4** to structure **25**.

We measured the inhibitory activity of **4** and **23**–**25** against a panel of Gram-positive and Gram-negative bacteria using levofloxacin as a positive control (Table 1). All four compounds exhibited no activity up to a concentration of 64 $mgmL^{-1}$ against most strains, although **4**, **23**, and **25** exhibited weak activity against *H. influenzae* ATCC 49247 (32 μgmL^{-1}). These data are consistent with the previously reported weak inhibitory activity of 2,18-*seco*-lankacidinol B against *B. subtilis* and *S. aureus* (obtained with disk diffusion tests).[6] It is interesting to note that all four stereochemical derivatives of the β-keto-δ-lactone ring exhibit poor inhibitory activity, suggesting that the C18 pyruvamide function or the macrocyclic ring (or both) are required for the antimicrobial properties of the class.

The reassignment of **4** to **25** makes it stereochemically unique among reported lankacidin-class natural products, all of which have been assigned the opposite stereochemistry at C4. This raises interesting questions about the biosynthesis of **25**. The C20 methyl groups of lankacidin C (**1**) and lankacidinol (**2**) are proposed to be installed by a methyltransferase in the LkcF module of the lankacidin polyketide synthase, prior to macrocyclization.[7] It is possible that the analogous methyl transfer in the biosynthesis of **25** proceeds with the

opposite stereochemical preference, or that epimerization occurs during the biosynthesis. Alternatively, it is possible that epimerization of the C20 methyl group occurs independent of the biosynthetic machinery, and may be driven by macrocyclic conformational stability. The biosynthetic origin of the stereochemical inconsistencies at C4 remains an interesting unsolved question.

In conclusion, we have developed the first fully synthetic route to acyclic members of the lankacidin class in a linear sequence of ≤8 steps from simple precursors (≤10 steps from commercially available compounds). This route enabled investigation of stereochemical variability on the β-keto-δ-lactone core, resulting in the first chemical synthesis of 2,18-*seco*-lankacidinol B and evidence that the original stereochemical assignment of C4 requires revision from *R* to *S*. Additionally, we conducted the first evaluation of stereochemically diverse, acyclic lankacidin derivatives against a panel of Gram-positive and Gram-negative bacteria, the results of which suggest that the macrocyclic ring or the C18 pyruvamide (or both) are required for substantial antibacterial activity. Efforts to generate cyclic lankacidin derivatives that grant insight into origin of C4 stereochemical diversity and have improved chemical stability are underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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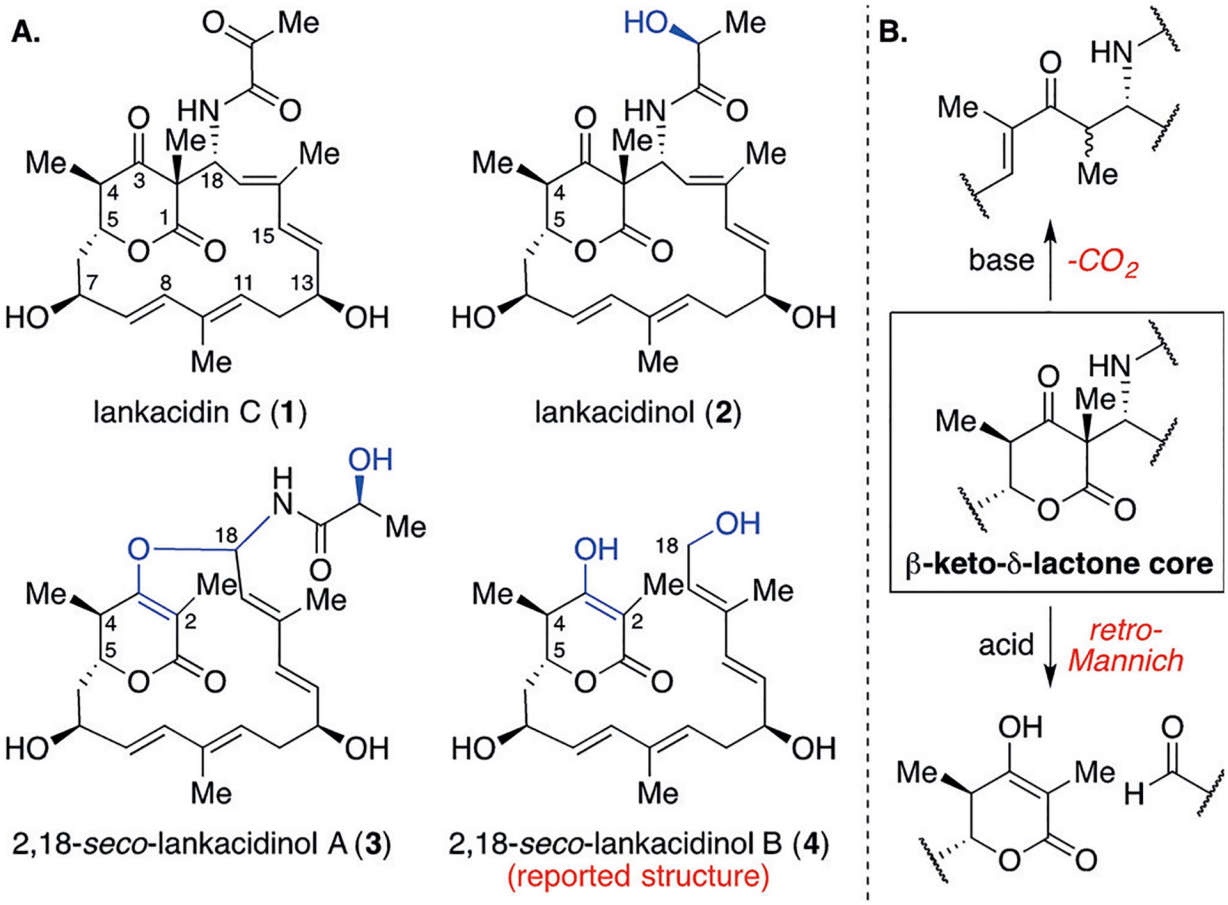


Figure 1.

A) Selected members of the lankacidin class of natural products, with structural differences compared to lankacidin C (**1**) highlighted in blue. B) Chemical instability of the β-keto-δ-lactone core structure.

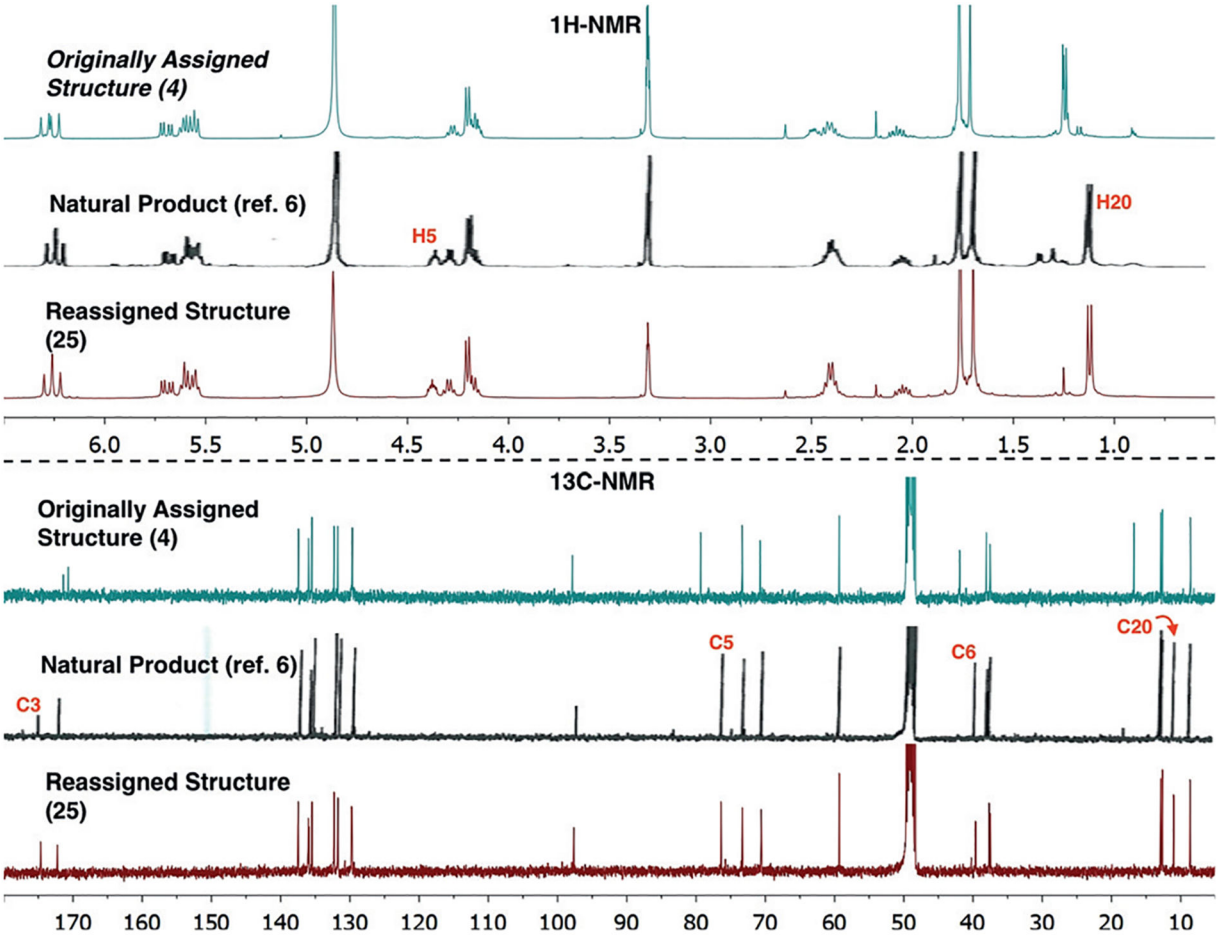
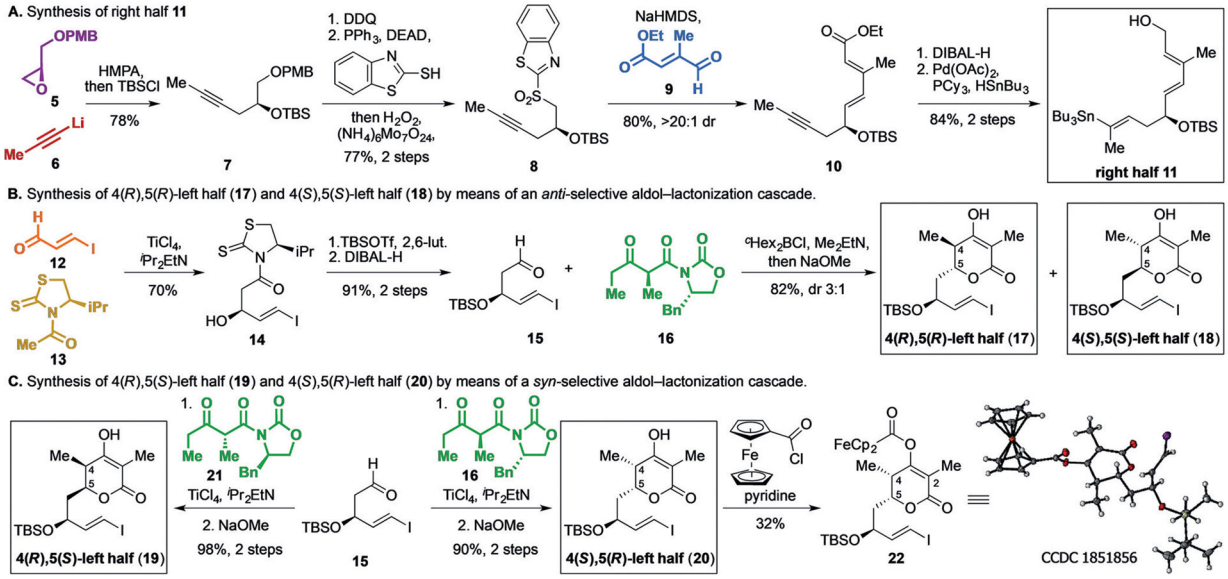


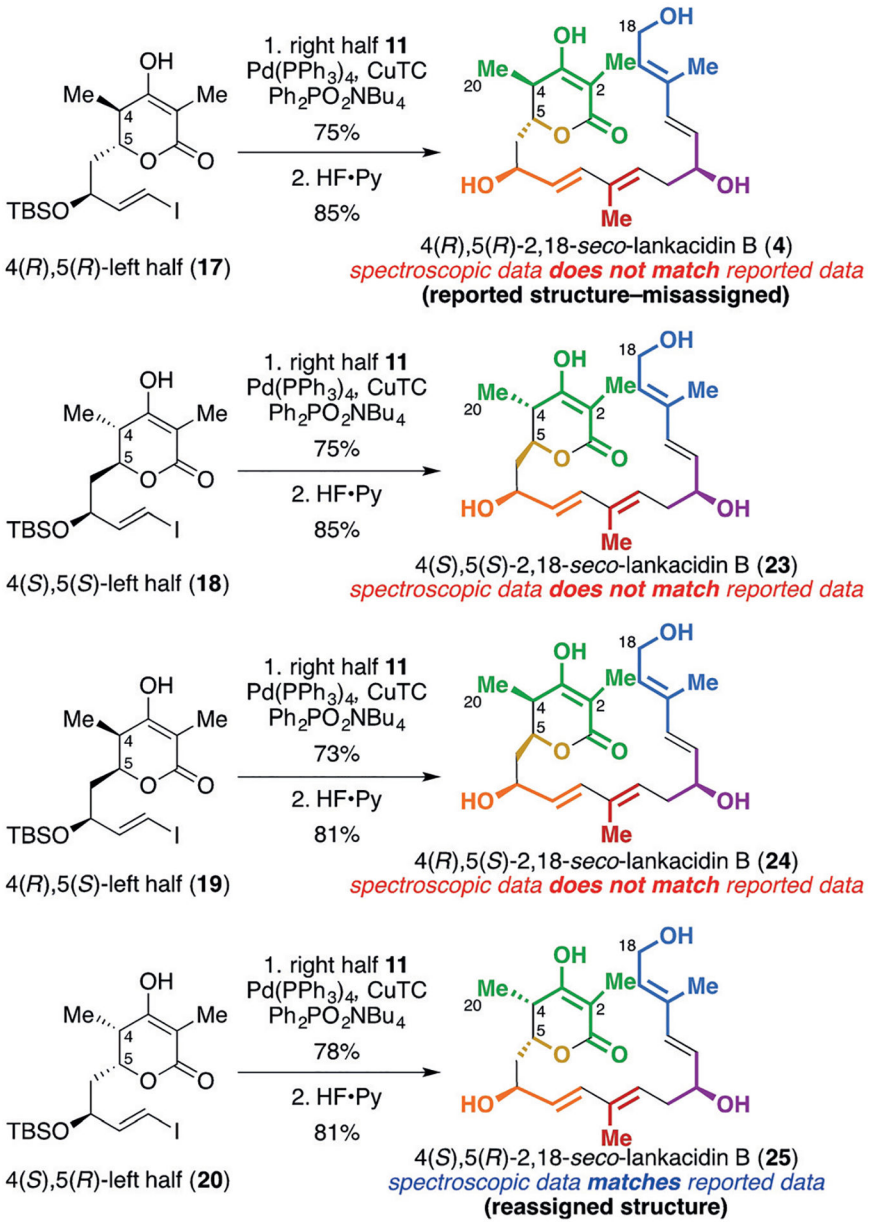
Figure 2.

^{1}H- and ^{13}C-NMR spectral comparison of **4** (teal) and **25** (red) to the spectra of the natural product (black).[6] Horizontal axes are expressed in parts per million (ppm).



Scheme 1.

Synthesis of coupling partners en route to 2,18-*seco*-lankacidinol B. A) Synthesis of right half **11**. B) Synthesis of left halves **17** and **18** by means of an *anti*-selective aldol-lactonization cascade. C) Synthesis of left halves **19** and **20** by means of a *syn*-selective aldol followed by lactonization.



Scheme 2.

Convergent coupling of right half **11** to left halves **17**–**20** and structural reassignment of 2,18-*seco*-lankacidinol B.

Table 1:

Inhibitory activities of 2,18-*seco*-lankacidinols against selected strains of Gram-positive and Gram-negative bacteria.[a,b]

	***A. baumannii* ATCC 19606**	***E. coli* ATCC 25922**	***K. pneumoniae* ATCC 43816**	***P. aeruginosa* ATCC 27853**	***H. influenzae* ATCC 49247**	***E. faecalis* ATCC 29212**	***E. faecium* ATCC 35667**	***S. aureus* ATCC 29213**	***S. aureus (MRSA)* ATCC 33591**	***S. pneumoniae* ATCC 49619**
Compound										
4	>64	>64	>64	>64	32	>64	>64	>64	>64	>64
23	>64	>64	>64	>64	32	>64	>64	>64	>64	>64
24	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
25	>64	>64	>64	>64	32	>64	>64	>64	>64	>64
levofloxacin	0.25	0.015	0.03	2	0.03	1	4	0.25	0.12	0.5

[a] Values given in $\mu g mL^{-1}$,

[b] ATCC= American Type Culture Collection.