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Lectin-Targeted Prodrugs Activated by *Pseudomonas aeruginosa* for Self-Destructive Antibiotic Release

Joscha Meiers, Katharina Rox, and Alexander Titz*
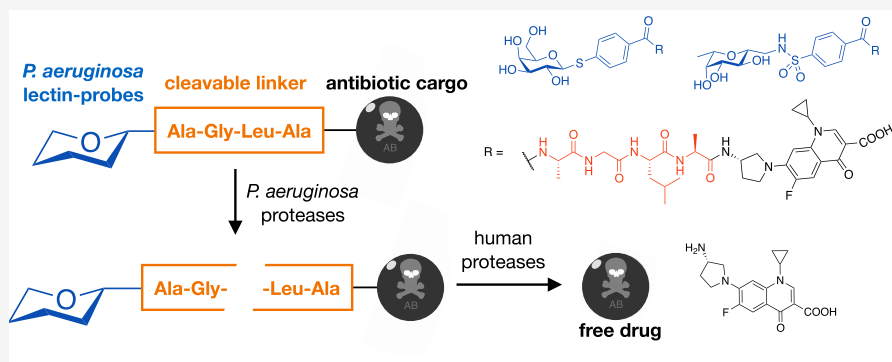
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ABSTRACT: Chronic *Pseudomonas aeruginosa* infections are characterized by biofilm formation, a major virulence factor of *P. aeruginosa* and cause of extensive drug resistance. Fluoroquinolones are effective antibiotics but are linked to severe side effects. The two extracellular *P. aeruginosa*-specific lectins LecA and LecB are key structural biofilm components and can be exploited for targeted drug delivery. In this work, several fluoroquinolones were conjugated to lectin probes by cleavable peptide linkers to yield lectin-targeted prodrugs. Mechanistically, these conjugates therefore remain non-toxic in the systemic distribution and will be activated to kill only once they have accumulated at the infection site. The synthesized prodrugs proved stable in the presence of host blood plasma and liver metabolism but rapidly released the antibiotic cargo in the presence of *P. aeruginosa* in a self-destructive manner in vitro. Furthermore, the prodrugs showed good absorption, distribution, metabolism, and elimination (ADME) properties and reduced toxicity in vitro, thus establishing the first lectin-targeted antibiotic prodrugs against *P. aeruginosa*.

■ INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative, opportunistic pathogen and has become a serious threat for our health care system.[1−3] Chronic infections—especially in immuno-compromised patients (e.g., hospitalized patients, geriatrics) and people suffering from cystic fibrosis (CF) or chronic obstructive pulmonary disease—can lead to recurrent pneumonia, lung injuries, sepsis, and other life-threatening conditions.[4] In fact, 20% of intensive care unit-acquired pneumonia is associated with *P. aeruginosa* in Europe.[5] Its high pathogenicity is driven by various virulence factors together with intrinsic and acquired resistance against multiple antibiotic classes.

Almost any part of the human body can be infected by *P. aeruginosa*, leading to wound infections, urinary tract infections, sepsis, or pneumonia.[6−8] Thus, it is vital to tailor the antibiotic treatment according to suitable pharmacokinetic properties. Further, targeted drug delivery is a powerful but as-yet underrepresented field in antibiotic therapy.[9,10] In order to focus research activities on this perilous pathogen, carbapenem-resistant *P. aeruginosa* was declared critical priority I pathogen by the WHO.[11]

The antibiotic resistance of *P. aeruginosa* is intrinsically caused by the additional outer membrane of Gram-negative bacteria and highly efficient efflux pumps.[12] Further resistances like antibiotic-modifying enzymes or reduced membrane permeability can be acquired by horizontal gene transfer or can result from spontaneous mutations. In 2019, 17.6% of all invasive *P. aeruginosa* isolates were resistant to at least two antibiotic classes in Europe.[13] During chronic infections, *P. aeruginosa* forms biofilms, which are described as complex hydrogels stabilized by extracellular polymeric substances like extracellular DNA, polysaccharides, and a plethora of proteins.[14] These biofilms further enhance antimicrobial resistance up to 1000-fold and also provide an additional barrier for the host immune system.[15]

Fluoroquinolone antibiotics, for example, ciprofloaxcin, are standard-of-care drugs in the treatment of *P. aeruginosa* infections. Despite their overall drug safety, they are linked to rare but severe side effects like tendon rupture, neuropathy, or heart valve regurgitation. As a result, regulatory agencies like FDA and BfArM have alerted about the inappropriate use of fluoroquinolone antibiotics.[16−18] Although the molecular principles underlying these side effects are not yet fully understood, there is evidence for inhibition of the human mitochondrial topoisomerase II[19,20] intracellular production of reactive oxygen species (ROS)[21] combined with an unspecific accumulation in sensitive tissues like muscle tissues.[22]

Bifunctional hybrid antibiotics[23−26] (intensively reviewed by Klahn and Brönstrup[27]) are currently trending. Targeted delivery of antibiotics[10] or diagnostic dyes[28] by conjugation to targeting probes can focus the drug's distribution and thereby reduce systemic side effects and improve therapeutic efficacy by increasing the drug's concentration at the site of infection (reviewed by Devarajan et al.[9]). In addition, unfavorable pharmacokinetic parameters of a drug can be overcome by a prodrug approach, that is, the use of chemically masked analogues of the parent drug molecule with no or only minor pharmacological effects. These prodrugs are then activated to liberate their active components by metabolizing enzymes or under specific chemical conditions.[29] In the context of antibacterial research, the rational design of selectively cleavable prodrugs is a powerful[10,30] but yet underrepresented approach. At present, a promising antibody−antibiotic conjugate prodrug targeting the Gram-positive pathogen *Staphylococcus aureus* is under clinical investigation.[31,32]

Promising structures for drug targeting in *P. aeruginosa* infections are two abundant carbohydrate-binding proteins in the *P. aeruginosa* biofilm, the quorum-sensing regulated lectins LecA (PA-IL) and LecB (PA-IIL).[33−35] These homotetrameric, Ca^{2+}-dependent lectins are virulence factors and essential for infection and biofilm formation. Due to their multivalency, they can cross-link carbohydrate epitopes present on bacterial cells, in the biofilm matrix, and the host cells and thereby strengthen the biofilm structure. A genetic or functional knockout of either of the two proteins leads to a significantly reduced biofilm formation.[36,37] It has recently been shown that the D-mannose- and L-fucose-binding LecB acts as a spatial organizer of the exopolysaccharide Psl within the biofilm matrix.[38]

LecA and LecB are also involved in the direct infection process. The D-galactose-binding LecA mediates cell invasion as a lipid zipper and triggers host cell signaling upon binding to its glycosphingolipid receptor Gb3 on the eukaryotic cell surface.[39] On the other hand, LecB facilitates and sustains an infection by adhesion to glycoconjugates on host tissue and inhibition of both airway ciliary beating[40] and wound tissue healing.[41,42] Interestingly, LecB can also activate murine B cells in a carbohydrate-dependent manner in murine infection models.[43]

The high genetic diversity and adaptability of *P. aeruginosa* results in a wide range of clinical isolates with varying characteristics.[44] While the protein sequence of LecA is highly conserved among clinical isolates, LecB can be clustered into PAO1-like and PA14-like homologues representing these major clinical strain clades. Independent of the type, both variants bind to the same glycosides, providing a basis for universal LecB inhibitors.

The intrinsically low affinity of lectins toward their natural carbohydrate ligands is often compensated by multivalent presentation of receptors or receptor-binding domains.[45] Thus far, many multivalent inhibitors of LecA and LecB have been studied by various groups (reviewed by Meiers et al.[46] Cecioni et al.,[47] and Bernardi et al.[48]). However, highly potent bivalent and non-carbohydrate-based inhibitors of LecA have also been recently disclosed.[49,50] The *P. aeruginosa* biofilm-related lectins LecA and LecB both show a flat SAR beyond the carbohydrate core, due to their shallow receptor binding sites on their surfaces. Thus, conjugation of lectin probes to larger molecules like fluorescent dyes[51] or antibiotics[52] without reduction of the binding affinity is possible.

In our previous work[52] we presented lectin-targeted conjugates of ciprofloxacin that are connected to lectin probes via a stable triazole linker. These conjugates showed antibiotic activity and accumulation in *P. aeruginosa* biofilm in vitro. Their antibiotic activity was, however, significantly reduced compared to free ciprofloxacin, likely due to the higher molecular weight of the antibiotic conjugates, which resulted in low bacterial bioavailability.[53,54]

In this work, the stable linker of the above-mentioned conjugates was exchanged with a cleavable peptide linker for in situ activation. The Zn(II)-dependent metalloprotease LasB is an important virulence factor secreted by *P. aeruginosa* and well recognized as a target in antimicrobial research.[55−58] LasB is involved in tissue damage and host immune system evasion by degradation of immunoglobulins, complement factors,[59] and the host-derived antimicrobial peptide LL-37[60] We therefore hypothesized that LasB can be exploited for an infection-focused prodrug activation. After targeting to the lectins present at the infection site, LasB-mediated proteolysis of these prodrugs shall release their antibiotic cargo and therefore overcome the cellular uptake issues of the uncleavable conjugates. Here, we report the design, synthesis, and characterization of the first *P. aeruginosa* lectin-targeted antibiotic prodrugs.

■ RESULTS AND DISCUSSION

Following the strategy of our previous work[52] an aromatic *β*-linked thiogalactoside and a *C*-glycosidic hybrid structure based on L-fucose and D-mannose were used as LecA- and LecB-targeting probes, respectively. In contrast to natural *O*-glycosides, which are prone to unwanted degradation, these lectin probes should be chemically and metabolically stable. To overcome low antimicrobial activities of our previous triazole-linked conjugates, a peptidic linker was introduced instead that is cleaved in the presence of *P. aeruginosa* to release a potent antibiotic cargo.

The peptide sequence H_2N-Ala-Gly-Leu-Ala-COOH is an established substrate for the bacterial endopeptidase LasB, which specifically cuts between Gly and Leu.[57,61] This peptide motif can thus be exploited as a cleavable linker between the carbohydrate lectin probe and the antibiotic drug, which are conjugated to the *N*- and *C*-termini, respectively. This capping of the peptide protects it from unspecific cleavage by other host-derived exopeptidases, resulting in sufficient metabolic stability. Cleavage of the conjugate by LasB would then release a lectin−probe−dipeptide and a dipeptidyl−antibiotic fragment. Due to its lower molecular weight, this antibiotic dipeptide fragment could be taken up by the bacterium and show antibiotic activity. Eventually, these terminal dipeptides would get further cleaved in biological matrices, either by *P.*
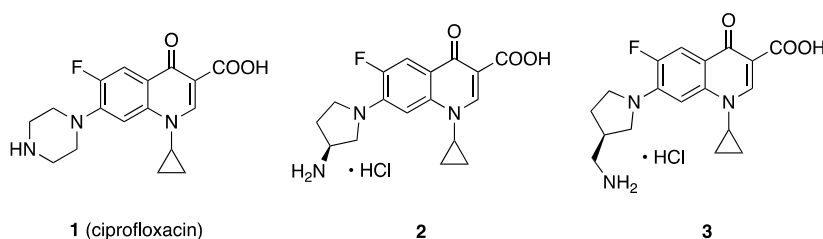
Figure 1. Selected fluoroquinolone antibiotic cargo for our lectin-targeted prodrug conjugates. Ciprofloxacin (**1**) is a very potent approved drug, while the investigational molecules aminopyrrolidine **2** and aminomethylpyrrolidine **3** show high antibiotic activity and carry a primary amine, which serves as a handle for conjugation to the peptide linker in this work.

Scheme 1. Chemical Synthesis of the Lectin-Targeted Carbohydrate–Peptide Conjugates: LecA-Targeted 10 and LecB-Targeted 16[a]
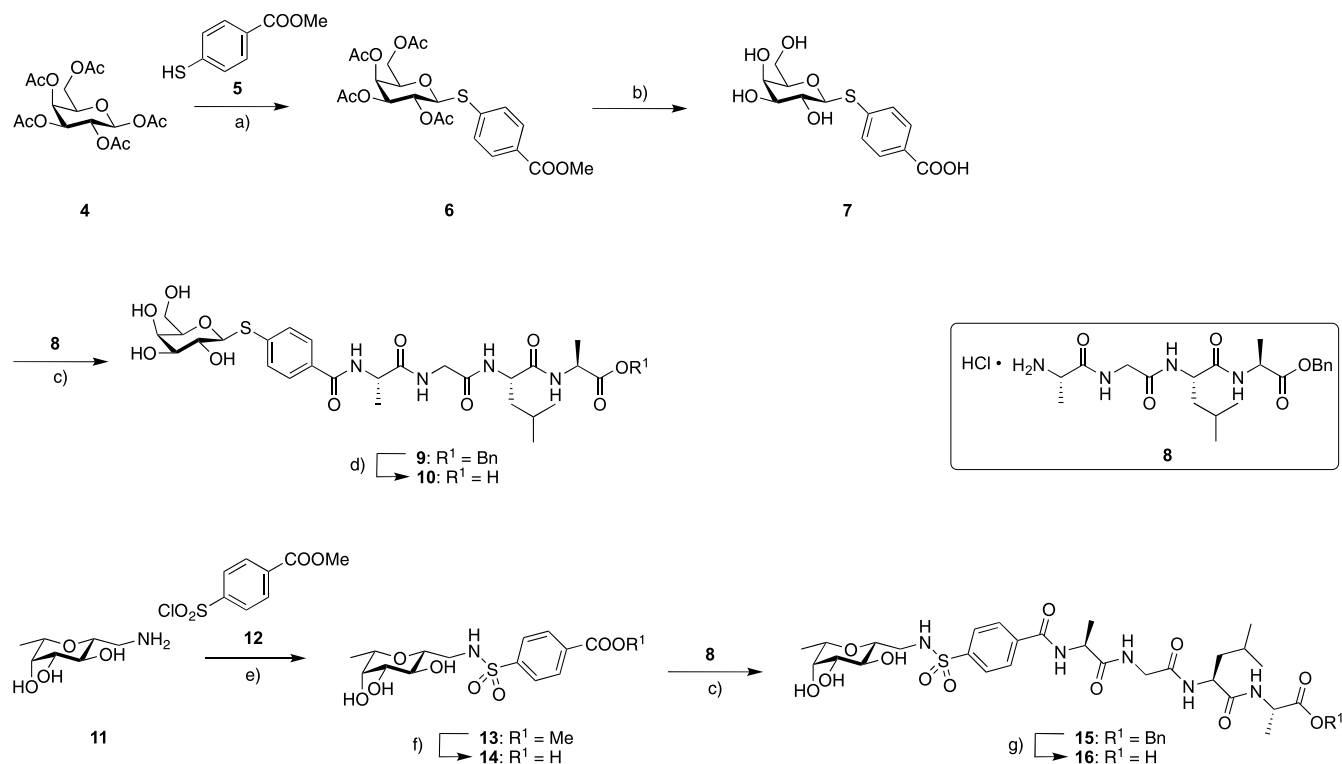
[a]Reagents and conditions: (a) BF$_3$·Et$_2$O, 0 °C to 22 °C, 16 h, 58%; (b) 1. NaOMe, MeOH, 22 °C, 30 min; 2. LiOH, MeOH/H$_2$O (20:3), 1 h, quant.; (c) TBTU, DIPEA, DMF, 22 °C, 1 h, 58% for **9**, 80% for **15**; (d) LiOH, DMF/H$_2$O, 50 °C, 49%; (e) K$_2$CO$_3$, DMF, 0 to 22 °C, 16 h, 27%; (f) LiOH, THF/MeOH/H$_2$O (3/1/1), 22 °C, 16 h, 95%; (g) H$_2$, cat. Pd/C, MeOH, 22 °C, 16 h, 95%.

aeruginosa-associated proteases or by host-derived enzymes and thus release the free cargo with full antimicrobial activity.

The structure−activity relationship of fluoroquinolone antibiotics allows the conjugation to larger moieties only at two positions: (i) the carboxylic acid at the quinolone core, only as a prodrug, however, or (ii) the heterocycle attached to quinolone C-7 that is present in almost all fluoroquinolone drugs (SAR was reviewed by Gootz and Brighty[62] Chu and Fernandes[63]). For ciprofloxacin, conjugation of a peptide linker to its piperazine amino function would result in a secondary peptide, a motif considered to have higher stability against proteases.[64] Thus, conjugation of fluoroquinolones carrying a primary amine was also envisaged as a strategy to circumvent a potentially slower and inefficient drug release for the ciprofloxacin-based prodrugs. Compounds **2** and **3** share very similar structures and antibiotic profiles when compared to ciprofloxacin, but both contain a primary amine that might be crucial for proteolysis of the residual dipeptide product after

LasB cleavage.[65−67] The 3-aminomethylpyrrolidine residue in **3** further improves steric accessibility to the corresponding amide and thus potentially improves proteolysis (Figure 1).

Synthesis of Lectin-Targeted Carbohydrate-Peptide Conjugates. The LecA-targeted galactoside precursor **7** was synthesized in analogy to Novoa et al. (Scheme 1).[68] In brief, Lewis acid-mediated glycosylation of methyl 4-mercaptobenzoate (**5**) with β-D-galactose pentaacetate (**4**) resulted in thiogalactoside **6**. After subsequent global deprotection in two steps, LecA probe **7** was obtained quantitatively.

The tetrapeptide benzyl ester H$_2$N-Ala-Gly-Leu-Ala-COOBn (**8**) was synthesized in 5 chemical steps by solution-phase peptide synthesis (see the Supporting Information for details). Coupling of peptide **8** to the LecA-probe **7** was performed with 2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU) as a peptide-coupling reagent, yielding ester protected conjugate **9**. The following debenzylation proved to be problematic, and only small

Scheme 2. Synthesis of the Lectin-Targeted Ciprofloxacin Prodrugs: LecA-Targeted 24, LecB-Targeted 25, and Dipeptidyl Ciprofloxacin 19 as a Control[a]
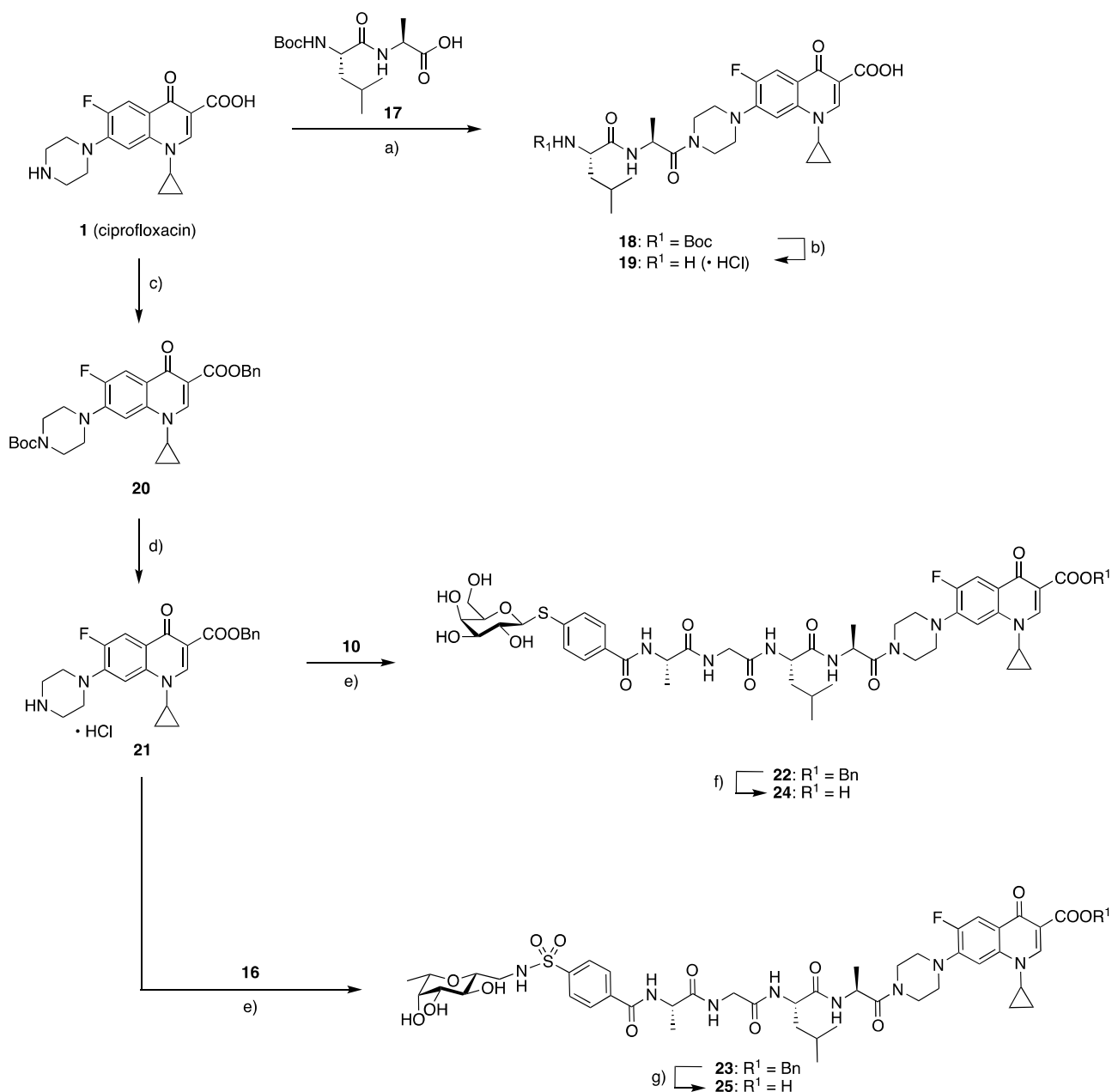
[a]Reagents and conditions: (a) 1. **17**, Ibcf, NMM, THF, −15 °C, 20 min, 2. **1**, THF, 22 °C, 3 h, 25%; (b) HCl, dioxane, 22 °C 1 h, 54%; (c) 1. Boc₂O, KHCO₃, DMF, 40 °C, 90 min, 2. BnBr, 115 °C, 90 min, 86% over 2 steps; (d) HCl, dioxane, 22 °C, 1 h, quant.; (e) TBTU, DIPEA, 22 °C, 1 h, 84% for **23**; (f) H₂, cat. Pd black, MeOH, 22 °C, 6 d, 22% over two steps; (g) H₂, cat. Pd/C, MeOH, 22 °C, 24 h, 74%.

amounts of acid **10** could be isolated after hydrogenolysis, even under an elevated H₂ pressure of 3.5 bar and catalysis with Pd black. Eventually, saponification with LiOH gave the desired acid **10** in 49% yield (Scheme 1).

LecB-targeted β-C-glycoside **11** was synthesized as reported.[69,70] The reaction with sulfonylchloride **12** resulted in sulfonamide **13**, which was subsequently saponified with LiOH to yield the corresponding carboxylic acid **14**. Conjugation to the tetrapeptide **8** was again performed with TBTU to give benzyl-protected **15**, which in contrast to **9** was smoothly debenzylated to **16** by hydrogenolysis under standard conditions of 1 atm hydrogen and 22 °C using palladium on charcoal (Scheme 1). Lectin-targeted peptide building blocks

10 and **16** were then conjugated to the antibiotic cargo, compounds **1–3** (Schemes 2–4).

Ciprofloxacin Series. Ciprofloxacin-based prodrugs were synthesized starting from ciprofloxacin (**1**, Scheme 2). For the synthesis of controls, Boc-Leu-Ala (**17**, see the Supporting Information for synthesis) was also coupled to ciprofloxacin (**1**) after activation with isobutyl chloroformate (Ibcf) to give Boc-protected intermediate **18**. The latter molecule was subsequently deprotected with HCl to give dipeptidyl-ciprofloxacin **19** as a reference compound for analytical and antibiotic susceptibility studies (see below). Conjugation of the lectin-targeted peptide precursors, LecA-targeted **10** and LecB-targeted **16**, with benzyl-protected ciprofloxacin (**21**) was

Scheme 3. Synthesis of Lectin-Targeted Aminopyrrolidine Prodrugs: LecA-Targeted 36 and LecB-Targeted 37 and Controls 2 and Dipeptidyl 35[a]
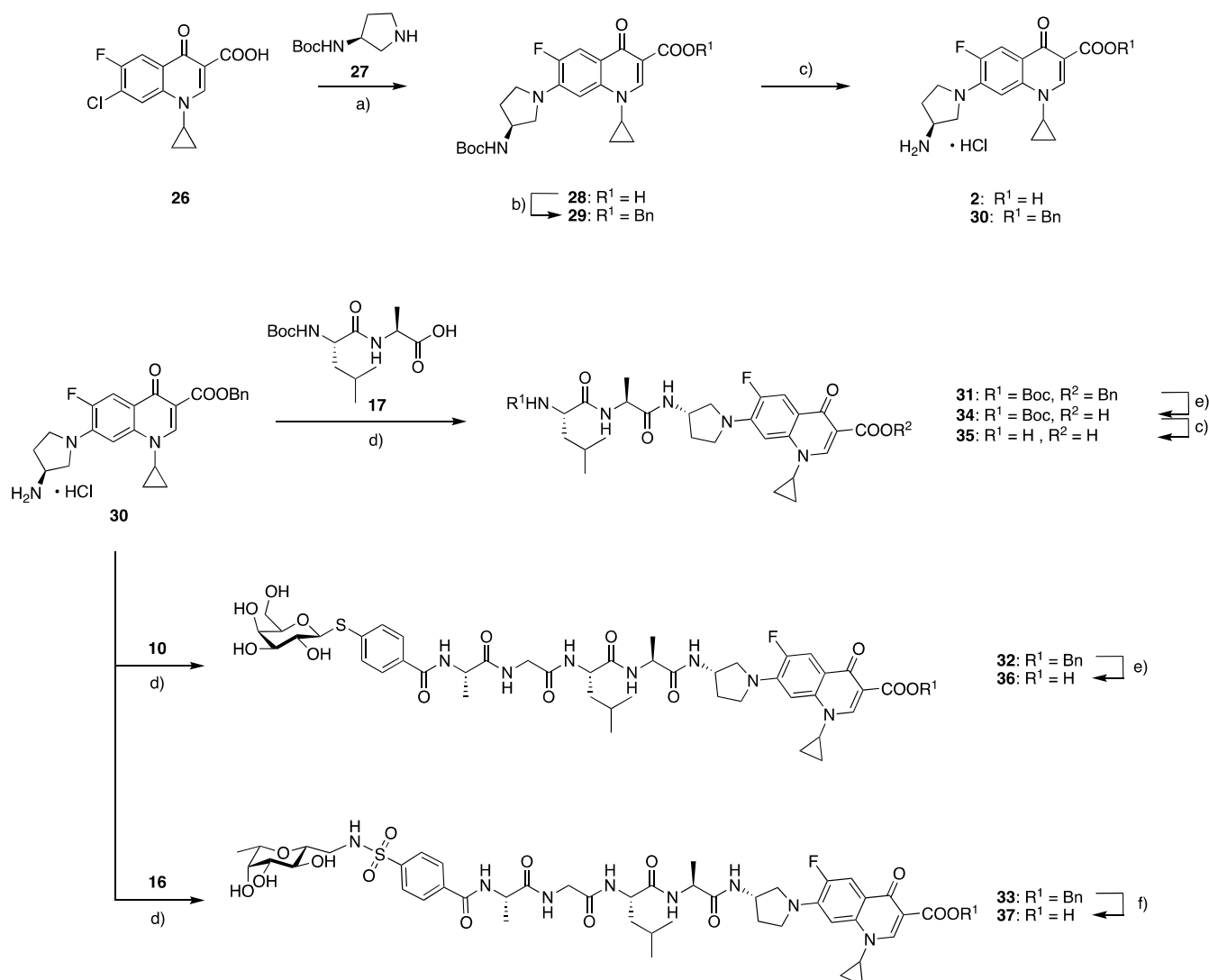
[a]Reagents and conditions: (a) pyridine, reflux, 16 h, 50%; (b) BnBr, K$_2$CO$_3$, DMF, 110 °C, 60 min, 86%; (c) HCl, dioxane, 22 °C, 1 h, 97% for 2, quant. for 30, 61% over two steps for 35; (d) TBTU, DIPEA, 22 °C, 1 h, 74% for 31, 49% for 33; (e) H$_2$, cat. Pd black, MeOH, 22 °C, 16−24 h, 48% over two steps for 36; (f) H$_2$, cat. Pd/C, MeOH, 22 °C, 41%.

performed with TBTU and resulted in the protected prodrugs **22** and **23**. Hydrogenolytic deprotection with Pd on activated charcoal finally gave lectin-targeted ciprofloxacin prodrugs **24** and **25** (Scheme 2).

Aminopyrrolidine Series. For the aminopyrrolidine series (Scheme 3), chlorinated fluoroquinolone (FQ) **26** was reacted with aminopyrrolidine **27** in a nucleophilic aromatic substitution in refluxing dry pyridine, according to Sanchez et al.[65] After chromatographic separation of the 6- and 7-regioisomers, the carboxylic acid in **28** was reacted with benzylbromide to give fully protected ester **29** in good yield. A small amount of **28** was directly Boc-deprotected under acidic conditions to obtain aminopyrrolidine **2** as a control for biological studies. In parallel, Boc-protected **29** was cleaved to give amine **30**, which was subsequently conjugated to Boc-Leu-Ala (**17**), LecA-targeted tetrapeptide **10**, and LecB-targeted tetrapeptide **16** after activation with TBTU to give protected conjugates **31**, **32**, and **33**, respectively. Dipeptidyl-FQ **31** was first deprotected hydrogenolytically to acid **34**, which was then

Boc-deprotected using HCl in dioxane to yield dipeptidyl aminopyrrolidine-FQ **35**. The protected lectin-targeted conjugates **32** and **33** were hydrogenolytically deprotected with Pd-black or Pd/C to yield the two aminopyrrolidine-based prodrugs LecA-targeted **36** and LecB-targeted **37**.

Aminomethylpyrrolidine Series. For the aminomethylpyrrolidine series (Scheme 4), fluoroquinolone chloride **26** was reacted with (S)-β-prolinol (**38**) in a nucleophilic substitution in refluxing dry pyridine. The desired regioisomer **39** precipitated from the reaction at room temperature. After acid-catalyzed esterification, its methylester **40** was obtained in excellent yield.

Substitution of the hydroxy group in **40** into azide **41** had its pitfalls: transformation of the primary alcohol into a leaving group, for example, with PBr$_3$ or TsCl, led to decomposition of the starting material. Eventually, Bose−Mitsunobu conditions using diphenylphosphoryl azide (DPPA), diisopropyl azodicarboxylate (DIAD), and PPh$_3$ gave azide **41** in one step.[71] After hydrogenation, amine **42** was trapped as its HCl salt to

Scheme 4. Synthesis of Aminomethylpyrrolidine-Based Lectin-Targeted Prodrugs: LecA-Targeted 48 and LecB-Targeted 49 and the Controls 3 and 47[a]
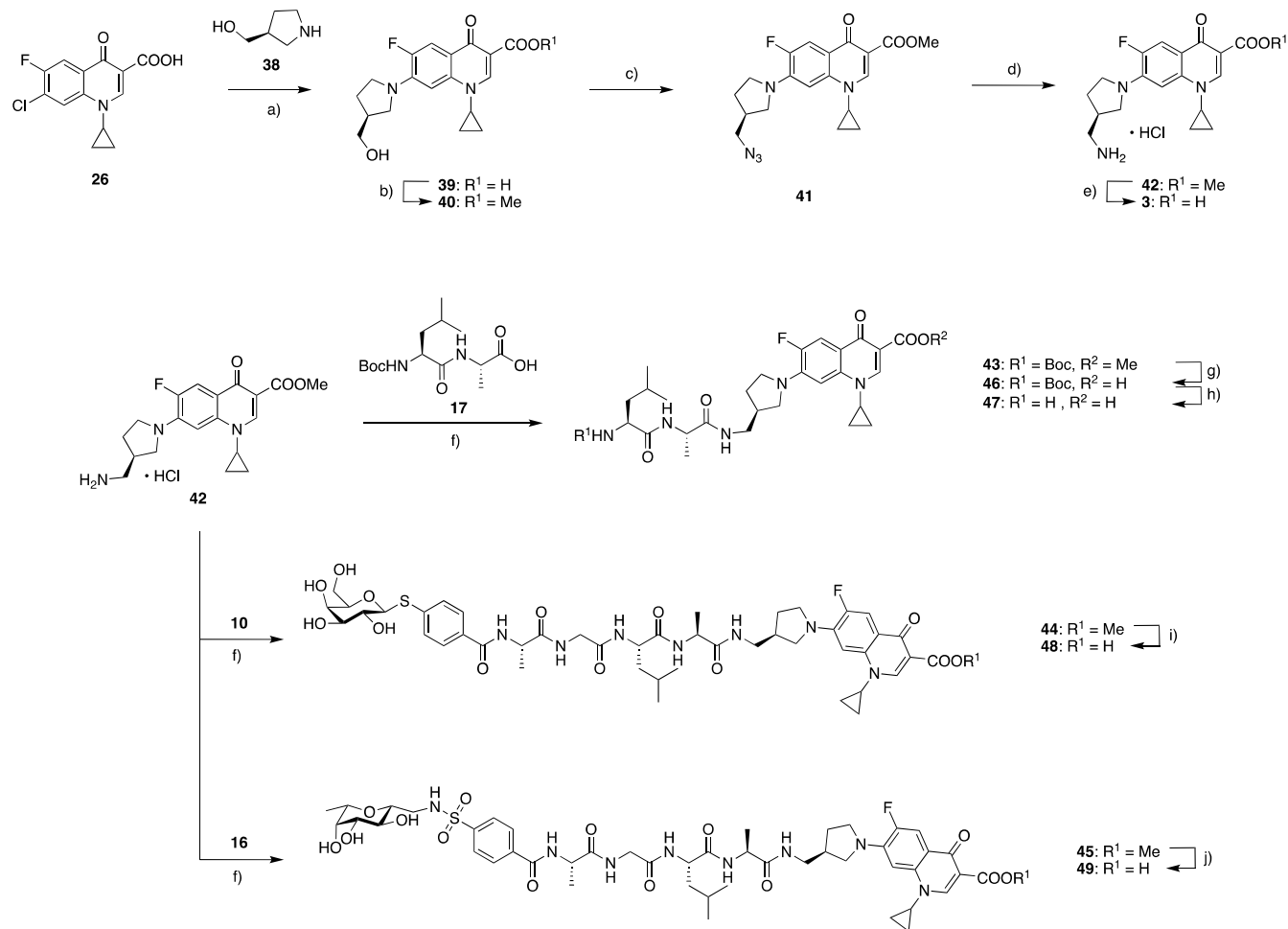
[a]Reagents and conditions: (a) pyridine, reflux, 16 h, 62%; (b) rac-CSA, MeOH, reflux, 72 h, 96%; (c) DIAD, PPh$_3$, DPPA, THF, 22 °C, 1 h, 65%; (d) H$_2$, cat. Pd/C, MeOH, 22 °C, 16 h, then HCl, dioxane/Et$_2$O, 0 °C, 5 min, 78%; (e) LiOH, THF/MeOH/H$_2$O (3:1:1), 22 °C, 2 d, 43%; (f) TBTU, DIPEA, DMF, 22 °C, 1 h, 50% for 43, 72% for 45; (g) LiOH, THF/H$_2$O/MeOH (5:5:1), 22 °C, 3 h, 86%; (h) HCl, dioxane, 22 °C, 1 h, 54%; (i) LiOH, H$_2$O/THF (5:1), 22 °C, 3 h, 70% over two steps; (j) LiOH, THF/H$_2$O/MeOH (3:1:1), 22 °C, 12 h, 96%.

prevent it from reacting with its methylester during workup. Saponification with LiOH yielded the corresponding reference antibiotic **3** in 43% yield. As for the aminopyrrolidine series, methyl-protected fluoroquinolone **42** was coupled to the peptides Boc-Leu-Ala (**17**), LecA-targeted tetrapeptide **10**, and LecB-targeted tetrapeptide **16** after activation with TBTU to yield the protected conjugates **43**, **44**, and **45**, respectively. Dipeptide **43** was then saponified with LiOH to give carboxylic acid **46**. After Boc-deprotection under acidic conditions, the reference compound dipeptidyl aminomethylpyrrolidine-FQ **47** was obtained. The methyl-protected lectin-targeted conjugates **44** and **45** were deprotected by saponification to give both aminomethylpyrrolidine-based prodrugs, LecA-targeted **48** and LecB-targeted **49**, in good yields.

Biophysical and Microbiological Evaluation. Biofilm accumulation was demonstrated before for the previous uncleavable conjugates.[52] Due to their lability toward LasB, this experiment is conceptually not possible for the herein reported prodrug conjugates. Therefore, we analyzed their target binding in a biophysical competitive binding assay to quantify their binding affinity for LecA and LecB from the two strains PAO1 and PA14 (Figure 2).[44,72]

In the case of LecA (Figure 2, top), the lectin-targeted prodrugs i.e., ciprofloxacin-based **24**, aminopyrrolidine-based **36**, and aminomethylpyrrolidine-based **48** showed very similar binding affinities around 30 μM. Methyl α-D-galactoside (Me-α-D-Gal, IC$_{50}$ = 113 ± 5 μM) and p-nitrophenyl β-D-galactoside (pNP-β-D-Gal, IC$_{50}$ = 61.9 ± 0.6 μM) were used as positive controls. The two *P. aeruginosa* strains PAO1 and PA14 and their respective LecB sequence variants represent a broad range of clinical isolates.[44] LecB$_{PAO1}$ bound the LecB-targeted prodrugs ciprofloxacin-based **25**, aminopyrrolidine-based **37**, and aminomethylpyrrolidine-based **49** with high affinity in the one-digit micromolar range (Figure 2, middle), comparable to L-fucose (IC$_{50}$ = 2.63 ± 1.7 μM[52]). As observed for LecA, the different prodrugs possessed comparable affinity independent of their cargo. Because terminal mannosides and fucosides are the natural ligands of LecB, methyl α-D-mannoside (Me-α-D-Man, IC$_{50}$ = 104 ± 15 μM) and methyl α-L-fucoside (Me-α-L-Fuc, IC$_{50}$ = 0.60 ± 0.08 μM) were used as controls. We further tested the LecB homologue from *P. aeruginosa* PA14. As observed before[44] for mannose- and fucose-based carbohydrates, LecB$_{PA14}$ bound all conjugates and the control compound Me-α-D-Man with higher affinity
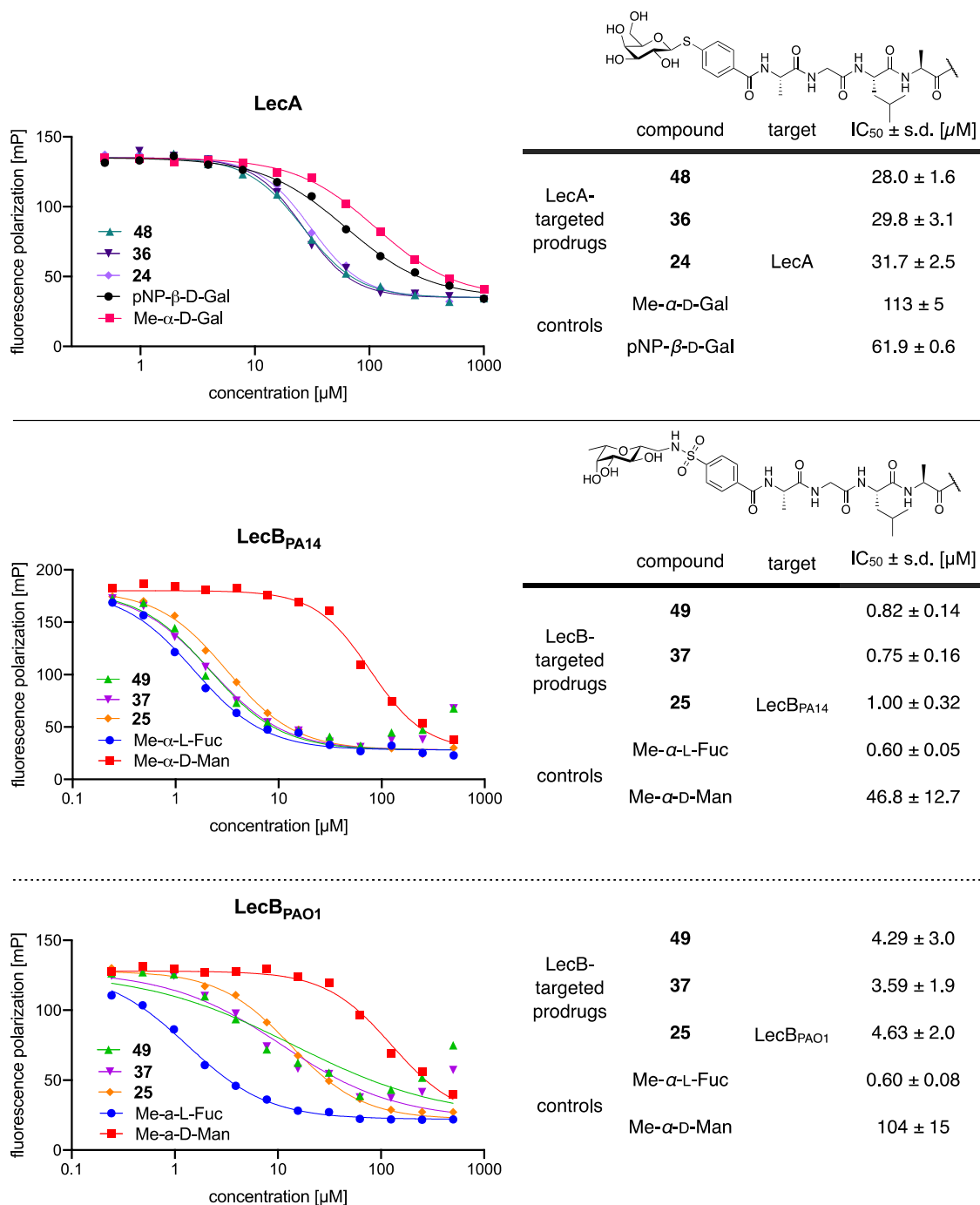
compound	target	$IC_{50} \pm$ s.d. [μM]
48		28.0 ± 1.6
36		29.8 ± 3.1
24	LecA	31.7 ± 2.5
Me-α-D-Gal		113 ± 5
pNP-β-D-Gal		61.9 ± 0.6

LecA-targeted prodrugs: **48**, **36**, **24**

controls: Me-α-D-Gal, pNP-β-D-Gal

compound	target	$IC_{50} \pm$ s.d. [μM]
49		0.82 ± 0.14
37		0.75 ± 0.16
25	LecB$_{PA14}$	1.00 ± 0.32
Me-α-L-Fuc		0.60 ± 0.05
Me-α-D-Man		46.8 ± 12.7

LecB-targeted prodrugs: **49**, **37**, **25**

controls: Me-α-L-Fuc, Me-α-D-Man

compound	target	$IC_{50} \pm$ s.d. [μM]
49		4.29 ± 3.0
37		3.59 ± 1.9
25	LecB$_{PAO1}$	4.63 ± 2.0
Me-α-L-Fuc		0.60 ± 0.08
Me-α-D-Man		104 ± 15

LecB-targeted prodrugs: **49**, **37**, **25**

controls: Me-α-L-Fuc, Me-α-D-Man

Figure 2. Competitive binding assay of the lectin-targeted prodrugs and reference carbohydrates with LecA, LecB$_{PA14}$, and LecB$_{PAO1}$. One representative titration of triplicates on plate is shown for each compound. IC_{50} values were determined from at least three independent experiments of technical triplicates each and are given as mean and standard deviation (K$_i$ in Table S1).

compared to LecB$_{PAO1}$, reaching IC_{50} values in the low micromolar to high nanomolar range (e.g., 3.59 ± 1.92 vs 0.75 ± 0.16 μM for **37**; Figure 2, bottom). Interestingly, Me-α-L-Fuc showed equal IC_{50} values on both LecB homologues in these experiments. In conclusion, the lectin-targeted prodrugs have the potential to target a broad range of *P. aeruginosa* strains.

Prodrugs Release Antibiotic Cargo in the Presence of Bacterial Proteins and Human Blood Plasma. The peptide linker of the reported prodrugs was designed to be cleaved in the presence of LasB, an endopeptidase expressed by

P. aeruginosa. In order to better resemble the complex variety of bacterial products present during an infection, a culture supernatant from *P. aeruginosa* PA14 was used instead of only LasB. This matrix contains a plethora of enzymes, some of them being potentially able to further process the resulting dipeptides from the first LasB-mediated cleavage, and should finally release the native antibiotic cargo.

Cleavage of the tetrapeptide prodrugs in the presence of this matrix derived from *P. aeruginosa* was generally very fast and occurred within minutes. In contrast, experiments showed that dipeptides **19** and **35** only slowly released their antibiotic cargo
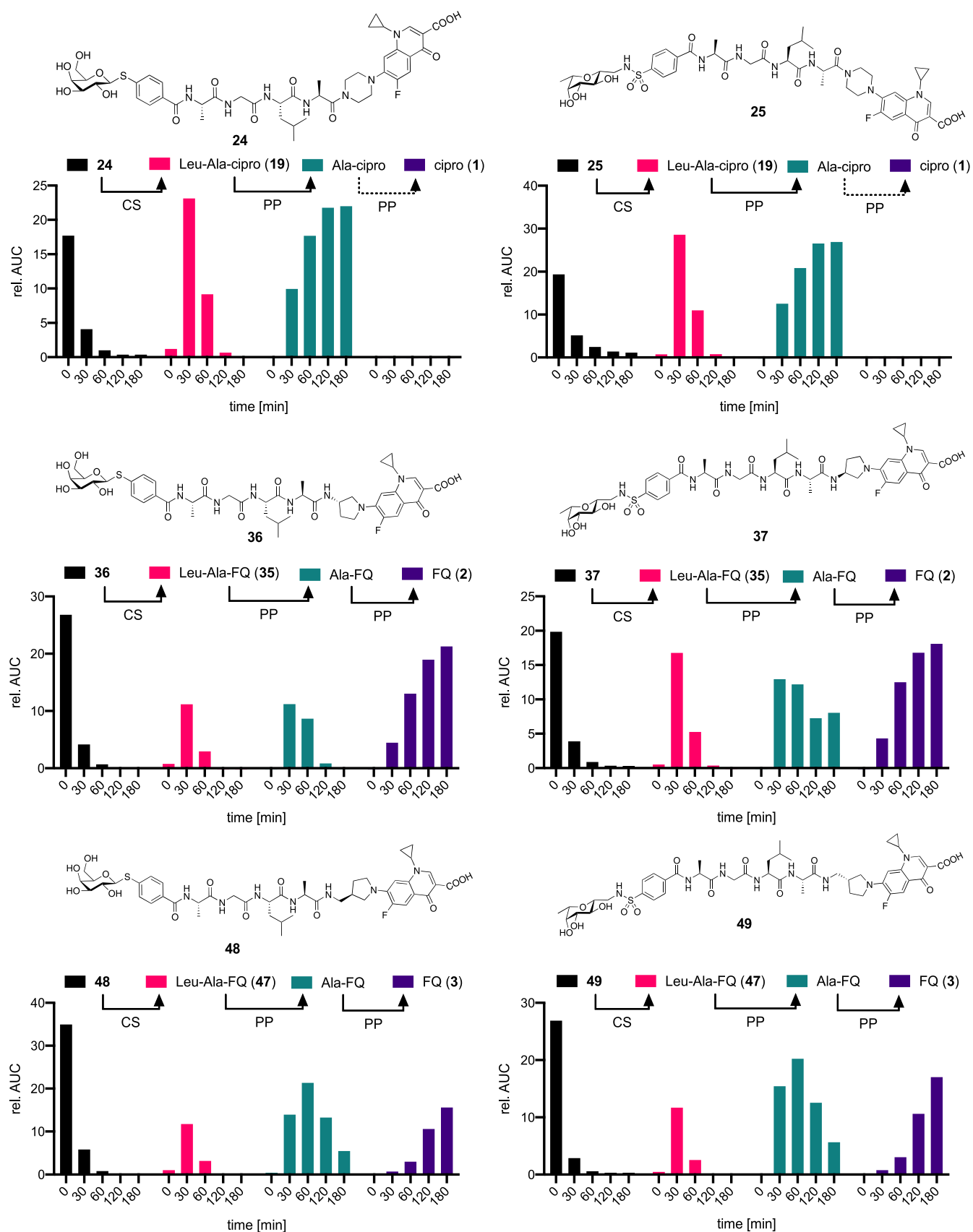
Figure 3. Activation of lectin-targeted prodrugs in human blood plasma spiked with *P. aeruginosa* culture supernatant: the ciprofloxacin-based prodrugs **24** and **25** were not completely processed to release free ciprofloxacin, while the antibiotic cargo from primary amide-based prodrugs **36**, **37**, **48**, and **49** was efficiently released. CS = culture supernatant, PP = plasma proteins.
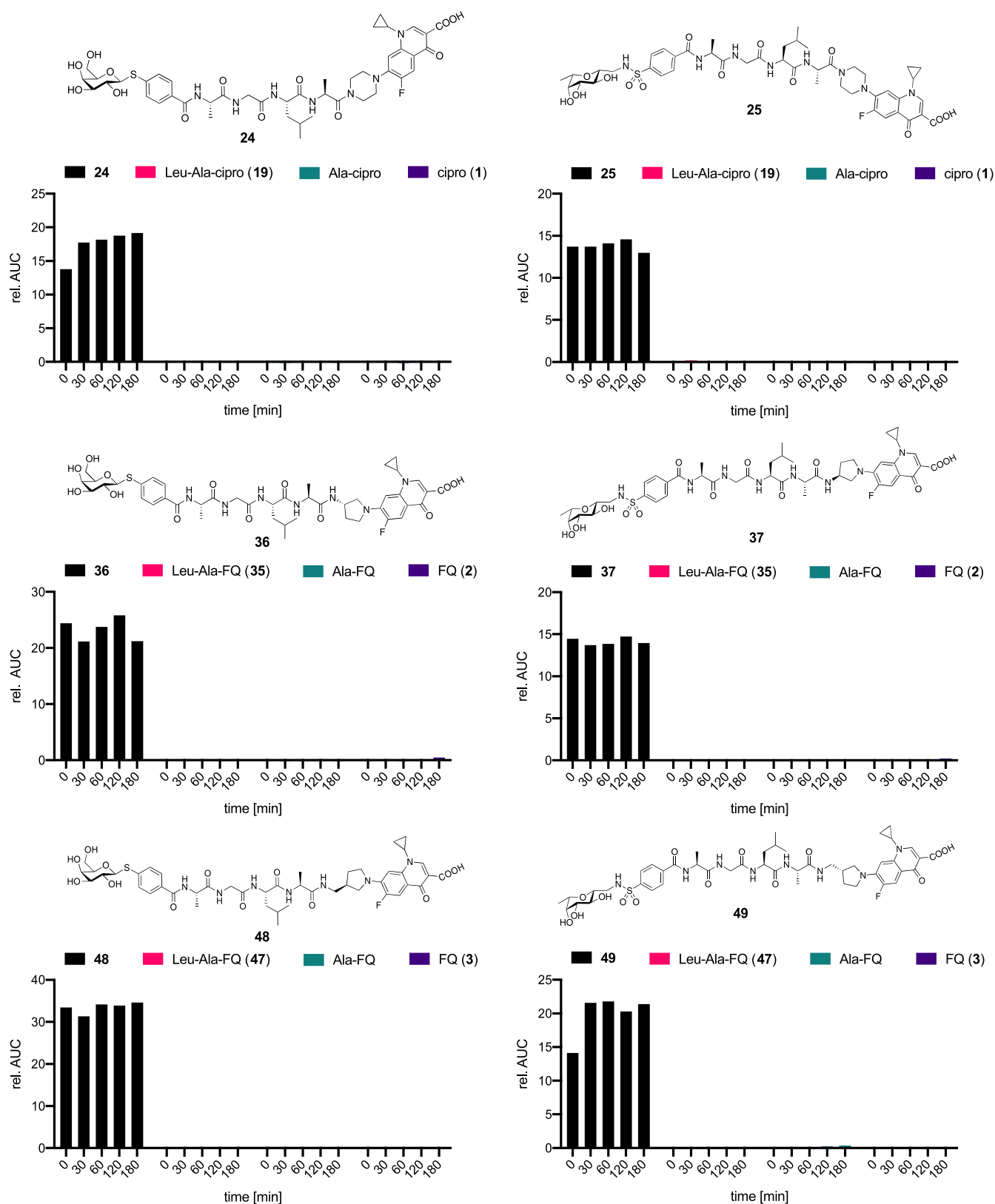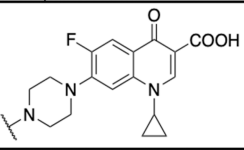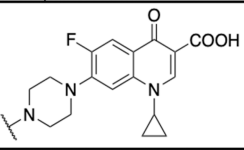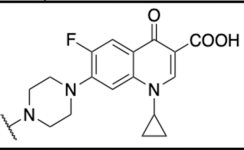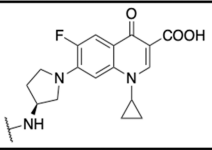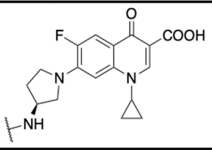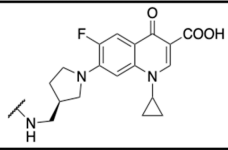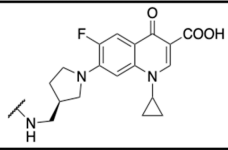
Figure 4. Stability of lectin-targeted prodrugs in human blood plasma spiked with fresh bacterial growth medium as a control: no significant release of the antibiotic cargo was observed for prodrugs **24**, **25**, **36**, **37**, **48**, and **49**.

in the same PA14 culture filtrate, and even after 24 h full release was not achieved (Figure S1).

To better mimic the infection scenario, we increased the complexity of the biological matrix, and human blood plasma

was added (Figure 3). Indeed, in the presence of the PA14 filtrate and human blood plasma, the lectin-targeted prodrugs containing primary amides, **36**/**37** and **48**/**49**, released a significant amount of their native antibiotic cargo within 3 h.

Table 1. Antibacterial Activity of the Lectin-Targeted LasB-Cleavable Prodrugs 24, 25, 36, 37, 48, and 49 and the Controls 1, 2, 3, 19, 35, and 47 against *P. aeruginosa* PA14[a,b]

	1	24	25	36	37	48	49
	control	LecA-targeted	LecB-targeted	LecA-targeted	LecB-targeted	LecA-targeted	LecB-targeted
antibiotic cargo		*(ciprofloxacin structure)*		*(aminopyrrolidine structure)*		*(aminomethylpyrrolidine structure)*	
Matrix 1: PBS							
< 10 min	0.125 - 0.156	> 25	> 25	> 25	> 25	> 25	> 25
3 h	0.125 - 0.25	> 25	> 25	> 25	> 25	> 25	> 25
Matrix 2: 50% human blood plasma + 10% *P. aeruginosa* culture supernatant in PBS							
< 10 min	0.25 - 0.313	≥ 25	≥ 25	0.195 - 0.39	0.78 - 1.56	3.13 - 12.5	3.13 - 6.25
3 h	0.156 - 0.25	≥ 25	≥ 25	0.1	0.1 - 0.195	1.56	1.56 - 3.13
Matrix 3: 10% *P. aeruginosa* culture supernatant in PBS							
< 10 min	0.125 - 0.313	≥ 25	≥ 25	0.78 - 3.13	1.56 - 12.5	1.56 - 3.13	3.13 - 6.25
3 h	0.125 - 0.313	≥ 25	≥ 25	0.195 - 3.13	0.39 - 3.13	1.56 - 3.13	1.56 - 6.25
Matrix 4: 50% human blood plasma + 10% LB in PBS							
< 10 min	0.156 - 0.313	> 25	≥ 25	≥ 25	≥ 25	> 25	≥ 25
3 h	0.156 - 0.313	≥ 25	≥ 25	0.78 - 6.25	0.78 - 6.25	12.5 - >25	12.5 - >25
MIC* (parent drug)		1	0.125 - 0.25	2	0.027 - 0.054	3	0.29 - 1.45
MIC* (dipeptide-FQ)		19	7.25 - 14.5	35	58	47	28 - 56

[a]Prodrugs were tested by varying pre-incubation time (<10 min vs 3 h) in the different biological matrices 1−4 before addition of the inoculum. [b]Minimal inhibitory concentration (MIC in μM) ranges from at least three independent experiments (exception: $N = 2$ for matrix 1, < 10 min). *Antibiotic activity determined without preincubation in biological matrix.

While the ciprofloxacin-based secondary amide containing prodrugs **24** and **25** were also cleaved by LasB and the resulting dipeptide **19** was further metabolized, degradation stopped at the stage of the secondary amide, that is, *N*-alanyl-ciprofloxacin. In conclusion, ciprofloxacin-based prodrugs **24** and **25** were not fully metabolized to release their native antibiotic cargo, in this case ciprofloxacin.

When comparing the aminopyrrolidine with the amino-methylpyrrolidine series, only minor differences in cleavage kinetics were observed. All primary amide-based prodrugs were quickly metabolized and efficiently released their parent fluoroquinolones in the biological matrix. Release of antibiotic cargo **2** from aminopyrrolidine-based prodrugs **36** and **37** occurred faster than the formation of aminomethylpyrrolidine **3** from prodrugs **48** and **49**. This observation was unexpected, as the aminomethylpyrrolidine series **36** and **37** was designed to have an additional CH$_2$ spacer in order to increase accessibility for proteolytic enzymes to the peptide bond for cleavage.

Furthermore, prodrug stability was assessed in human blood plasma and in the absence of bacterial matrix as a control

experiment (Figure 4). Indeed, release of fluoroquinolone or peptide-conjugated intermediates was not observed for any of the tested compounds within 3 h. In conclusion, activation of the prodrugs requires the presence of proteases expressed by *P. aeruginosa*, while the conjugates remain stable in plasma, indicating their potential for specific activation at the infection site.

Lectin-Targeted Prodrugs Reach High Antibiotic Activity after Activation by *P. aeruginosa*. The antibiotic activity of our conjugates and the controls was subsequently analyzed against *P. aeruginosa* PA14 in a broth microdilution assay, and minimal inhibitory concentrations (MIC) were determined (Table 1).[74]

In agreement with a previous report by Sanchez et al.,[67] free fluoroquinolone **2** (MIC = 0.027−0.054 μM) was more active than ciprofloxacin (**1**, MIC = 0.125−0.25 μM). The antibacterial activity of aminomethylpyrrolidine **3** (MIC = 0.29−1.45 μM) was slightly weaker than that of the latter two.[65] The synthetic dipeptidyl-fluoroquinolone conjugates **19** (ciprofloxacin), **35** (aminopyrrolidine), and **47** (amino-methylpyrrolidine) are the products of the initial LasB

Table 2. In Vitro ADMET Data of Aminopyrrolidine-Based Prodrugs 36 and 37 and Their Fluoroquinolone Cargo 2[a,b]

| Compound | Metabolic stability | | | | Plasma stability, $t_{1/2}$ [min] | | Plasma protein binding [%] | | Cytotoxicity |
| | $t_{1/2}$ [min] | | CL_{int} [μL/min/mg protein] | | | | | | |
	MLM	HLM	MLM	HLM	Mouse	Human	Mouse	Human	A549 cells [μM]
36	100	93	14	15	>240	>240	74.0 ± 3.7	97.2 ± 4.8	21.7 ± 1.9
37	216	178	6.4	7.8	74	135	30.1 ± 9.2	51.1 ± 13.3	>50
2	>60	41	<23	33.49	>240	135	77.7 ± 10.9	93.5 ± 1.3	20.8 ± 1.4

[a]All compounds showed good metabolic stability in blood plasma and in the presence of liver cell microsomal fractions. Cytotoxicity against A549-cells was assessed. [b]Means and standard deviations from at least three independent experiments. MLM, mouse liver microsomes; HLM, human liver microsomes; CL_{int}, intrinsic clearance.

cleavage, and they were also tested and showed only reduced antibiotic activity in a higher micromolar range.

Because our proteolytic cleavage experiments showed that a majority of free antibiotic drugs were released from the prodrugs within 3 h in the presence of bacterial culture supernatant and human blood plasma (Figure 3), the prodrugs were incubated for 3 h or <10 min as a control in different matrices before assessment in the antibiotic susceptibility assay (Table 1). The different matrices tested were buffer (matrix 1), human blood plasma spiked with bacterial culture supernatant in buffer (matrix 2), bacterial culture supernatant in buffer (matrix 3), or human blood plasma spiked with fresh medium as a control in buffer (matrix 4).

As expected for prodrugs, the lectin-targeted conjugates 24, 25, 35, 36, 48, and 49 (Table 1, matrix 1) did not show any antibiotic activity up to 25 μM after preincubation in buffer (MIC > 25 μM). In contrast, even a brief pre-incubation of <10 min in a mixture of human blood plasma and bacterial culture supernatant in buffer (matrix 2) activated all primary amide-based prodrugs 36/37 (MIC = 0.195−0.39 and 0.78−0.156 μM, respectively) and 48/49 (MIC = 3.13−12.5 and 3.13−6.25 μM, respectively), while the ciprofloxacin-based prodrugs 24 and 25 remained inactive (MIC ≥ 25 μM). After 3 h of pre-incubation, the ciprofloxacin series still remained inactive, while especially the aminopyrrolidine-based prodrugs 36 and 37 were highly potent (MIC = 0.098−0.195 μM) and nearly reached the antibiotic activity of their parent fluoroquinolone 2 (MIC = 0.027−0.054 μM), indicating a very efficient drug release during the experiment. Under the same conditions (Matrix 2), the aminomethylpyrrolidines 48 and 49 reached low micromolar antibacterial activities around 1.56−3.13 μM, which was close to the activity of their parent fluoroquinolone 3 (MIC = 0.29−1.45 μM).

It has to be noted that MICs determined for the parent drugs 1−3 and the dipeptide-conjugates 19, 35, and 47 were measured under conventional conditions in the absence of blood plasma or bacterial culture supernatant. Thus, biological matrix effects like metabolism or plasma protein binding that potentially decrease antibiotic activity were absent, and the MIC values under these conditions might be lower than in the presence of these matrices.

The antibiotic activity differences within the different fluoroquinolone series matched well with the different drug-release kinetics of the prodrugs (Figure 3, Table 1) and to the intrinsically lower activity of aminomethylpyrrolidine 3 compared to aminopyrrolidine 2 (MIC = 0.29−1.45 μM and MIC = 0.027−0.054 μM, respectively).

Interestingly, primary amide-containing prodrugs 36, 37, 48, and 49 were presumably also processed in the presence of *P. aeruginosa* culture filtrate only to release a significant amount of

active drug (Table 1, matrix 3), as deduced from their antibiotic activities in the low micromolar range (e.g., MIC = 0.195−0.78 μM for 36 after 3 h pre-incubation). We reason that the increased duration of the antibiotic susceptibility experiment (18 h, 37 °C) was responsible for the release of a significant amount of drug, despite the slower metabolism observed in bacterial culture filtrate (Figure S1). This assumption is further supported by the fact that even with a pre-incubation time of 3 h, the antibiotic activity increased only mildly [e.g., for 36: MIC (<10 min pre-incubation) = 0.78−1.56 μM versus MIC (3 h pre-incubation) = 0.195−0.78 μM, for 49: MIC (<10 min pre-incubation) = 3.13 μM versus MIC (3 h pre-incubation) = 1.56−3.13 μM].

In all primary amide conjugates, the antibiotic activity after pre-incubation in human blood plasma alone (Table 1, matrix 4) was significantly lower than that after incubation with the other biological matrices containing bacterial culture supernatants in the presence or absence of plasma (matrices 2 and 3). However, the aminopyrrolidine-based prodrugs 36 and 37 also reached moderate antibacterial potency in plasma alone, but the MIC values varied extensively between replicates (MIC = 0.78−6.25 μM).

Overall, the antibiotic activity of lectin-targeted prodrugs correlated well with their metabolic activation in the presence of human blood plasma proteins and bacterial culture supernatant. Both ciprofloxacin-based prodrugs could not be fully activated due to the stable secondary amide. Thus, they showed only weak antibiotic activity, despite their otherwise potent antibiotic cargo. In contrast, the primary amide-based prodrugs showed efficient release of their antibiotic cargo, resulting in highly potent antimicrobial activity.

Prodrugs: Stability against Plasma and Liver Metabolism and Absence of Acute Cytotoxicity. Aminopyrrolidine 2 and its corresponding lectin-targeted prodrugs 36 and 37 were chosen for further early in vitro ADMET studies due to their excellent antibiotic activity profile against *P. aeruginosa* PA14 (Table 2).

Metabolic stability of these molecules was quantified using human and mouse liver microsomes. A high metabolic stability in mouse and human liver microsomes was observed for the prodrugs 36 ($t_{1/2, MLM}$ = 100 min, $t_{1/2, HLM}$ = 93 min) and 37 ($t_{1/2, MLM}$ = 216 min, $t_{1/2,}$ = 178 min). In contrast, metabolism of the parent fluoroquinolone 2 was twofold faster in human liver microsomes ($t_{1/2, HLM}$ = 41 min), which could be a result of the free primary amine in 2, which is masked in the prodrugs.

Because very high plasma protein binding can mask the prodrugs and prevent binding to their target lectins and prevent metabolic activation, plasma protein binding was assessed in mouse and human blood plasma. LecA-targeted
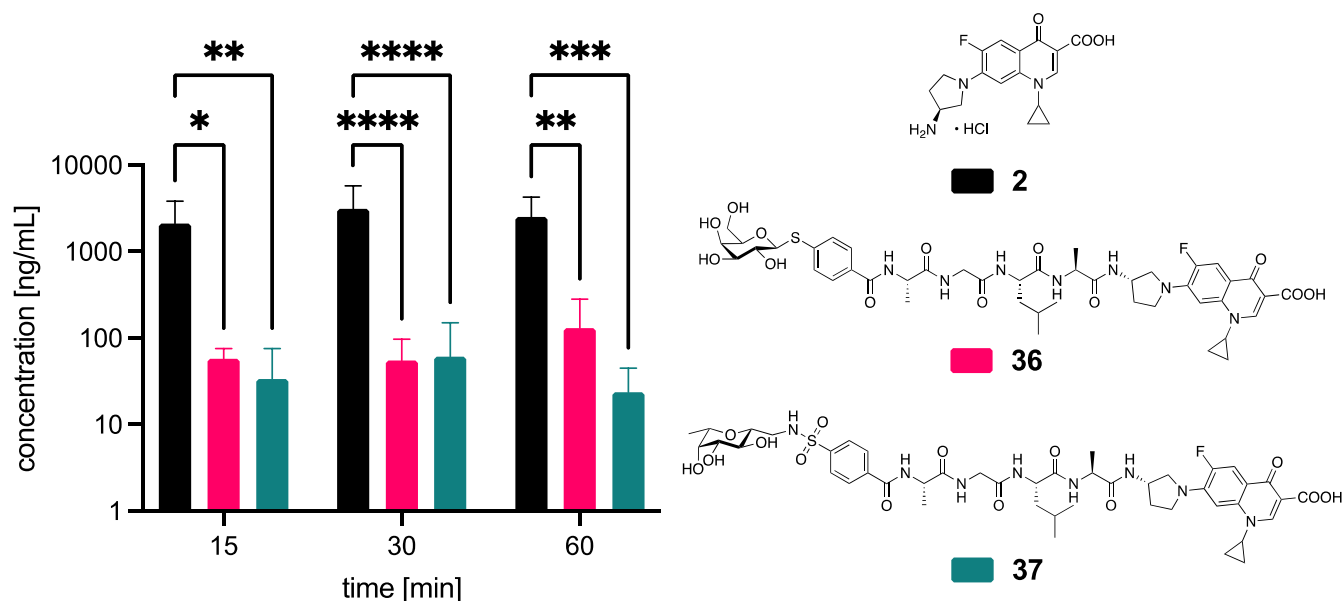
Figure 5. Cellular accumulation assay of the lectin-targeted prodrugs **36**/**37** and their antibiotic cargo **2** on A549-cells at 10 μg/mL. While fluoroquinolone **2** easily permeated and was found in high intracellular concentrations, both prodrugs were significantly worse permeators and are therefore unable to reach intracellular off-targets. Means and standard deviations from three biological replicates with 2−3 technical replicates each. Statistical analysis was calculated with two-way ANOVA and Dunnett's multiple comparisons test. Incubation time had no statistical influence on concentration. ($p > 0.05$, ns; $p \leq 0.05$, *; $p \leq 0.01$, **; $p \leq 0.001$, ***; $p \leq 0.0001$, ****).

prodrug **36** showed comparable protein binding (74% for mouse blood plasma, 97% for human blood plasma) to its parent fluoroquinolone **2** (78% for mouse blood plasma, 94% for human blood plasma). Interestingly, *C*-glycoside-based prodrug **37** showed significantly reduced plasma protein binding (30% for mouse blood plasma, 51% for human blood plasma).

In mouse and human blood plasma, LecA-targeted **36** was fully stable ($t_{1/2} > 240$ min), while LecB-targeted **37** was somewhat less stable under these conditions ($t_{1/2, \text{MBP}} = 74$ min, $t_{1/2, \text{HBP}} = 135$ min). Parent compound **2** showed full stability in murine blood plasma, while it was as stable as **37** in human plasma ($t_{1/2, \text{MBP}} > 240$ min, $t_{1/2, \text{HBP}} = 135$ min).

Cytotoxicity of those molecules was then assessed against human alveolar A549 cells. Whereas **37** showed no detectable cytotoxicity up to 50 μM, **36** and **2** showed weak toxicity with IC$_{50}$ values of 21.7 and 20.8 μM, respectively. Because these toxicities were observed at much higher concentrations compared to their more potent antimicrobial activity, the respective selectivity indices are greater than 20 for **36** and **2** (Table 1).

Prodrugs Cannot Reach Human Intracellular Off-Targets. The specific mechanisms leading to the wide side effects of fluoroquinolones in patients are not yet fully understood. There is evidence for oxidative stress[21,73] and for impairment of the mitochondrial DNA replication system[19,74,75] induced by ciprofloxacin. Combined with unspecific drug accumulation in sensitive tissues, the effects described above could lead to severe tissue damage.[20,22,76] These intracellular side effects therefore partially result from the excellent permeation properties of fluoroquinolone drugs across biological membranes. We therefore reasoned that these side effects could be reduced by lowering the fluoroquinolones' intracellular availability. To test this hypothesis, cell accumulation experiments were performed with prodrugs **36**/**37** and with their parent fluoroquinolone **2** in human alveolar cells

(Figure 5). While compound **2** efficiently penetrated into the cells and was highly abundant intracellularly (2000−8000 ng/mL), both prodrugs **36** and **37** showed very low intracellular concentrations (55−130 ng/mL after 15, 30, and 60 min for **36** and between 20 and 60 ng/mL for **37** after 15, 30, and 60 min). It is interesting to note that **2** showed high intracellular levels after only 15 min incubation, suggesting that it is rapidly taken up, whereas only low concentrations of **36** and **37** were found. Moreover, to assess if **36** and **37** are cleaved intracellularly, we searched for compound **2**, which was not detected in the cells after lysis.

Therefore, the chemical modifications of **2** into the prodrugs **36** and **37** resulted in an up to 100-fold decreased ability to permeate into human cells and reach the possible intracellular off-targets. In combination with the targeted drug delivery approach, this could synergistically lead to a drastic reduction of severe side effects observed for the free fluoroquinolones in current clinical use.

CONCLUSIONS AND OUTLOOK

Chronic infections with *P. aeruginosa* can lead to life-threatening conditions, especially in vulnerable patients, and their bacterial biofilms are a major contributor to pathogenicity and antibiotic resistance. The large discovery void of antibiotics with new mode of actions for the past 30 years culminated in the current antibiotic resistance crisis.[77]

In this work, we present the first *P. aeruginosa* biofilm-targeted prodrugs in which carbohydrate probes targeting the two soluble bacterial lectins LecA and LecB were linked via a cleavable peptide linker to an antibiotic cargo. The introduced linker was designed as a substrate for the endopeptidase LasB, the major secreted virulence factor of *P. aeruginosa*. Taken together, these features therefore allow drug accumulation by lectin binding followed by specific release of the antibiotic at the infection site. This mechanism of action was designed to increase the antibiotic's concentration at the infection site in

order to break antimicrobial resistance and to reduce systemic side effects of the otherwise toxic fluoroquinolones. Furthermore, the lectin-inhibiting moiety could weaken the biofilm architecture and thereby further contribute to the antimicrobial efficacy of the conjugates.

Three types of fluoroquinolones differing in the attached amide linkage were conjugated to two biofilm-targeted lectin probes and analyzed in various assays. All prodrugs showed effective target binding to LecA and both homologues of LecB from *P. aeruginosa* PA14 and PAO1, thus covering a broad range of clinical isolates. Further, their stability and activation were characterized in various biological matrices. While unspecific activation in human blood plasma was not observed, their initial cleavage in the presence of *P. aeruginosa* culture supernatant containing LasB was very fast for all prodrugs. In combination, when bacterial enzymes and human blood plasma were present, the primary amide-based prodrugs **36**, **37**, **48**, and **49** efficiently released their antibiotic cargo within 3 h. In contrast, proteolysis of ciprofloxacin-based prodrugs was halted at the stage of the secondary amide, preventing the release of free ciprofloxacin.

In antimicrobial activity assays, it was demonstrated that the unactivated prodrugs were inactive, while proteolytic activation leads to the release of very potent antibiotic drugs. Especially the aminopyrrolidine-based prodrugs **36** and **37** reached high antibiotic activities (0.098−0.195 μM), comparable to their parent fluoroquinolone cargo **2** (0.027−0.054 μM). Ciprofloxacin-based prodrugs did not show significant antibiotic activity, independent of the biological activating matrix, which was consistent with the high stability of the secondary amide bond seen in our kinetic cleavage analysis (Figure 3).

In vitro ADMET analysis of the most active aminopyrrolidine-based compounds **2**, **36**, and **37** proved the conjugates' enhanced metabolic stability in microsomal liver fractions and in plasma from human and mouse. Furthermore, both prodrugs showed strongly reduced cellular uptake in alveolar A549 cells compared to the free quinolone **2** in cell accumulation experiments, which suggests a reduced uptake and dissemination after inhalative administration in lung infections. This is a major improvement compared to the parent drugs and reduces the high risk of intracellular off-target inhibition and formation of ROS, which has been suggested to be responsible for some of the severe fluoroquinolone-induced pathologies. The observed 100-fold reduction in cellular uptake in A549 cells suggests that the conjugates' permeation will potentially also be reduced in other tissue types more relevant for the fluoroquinolone-associated side effects.

Oral administration is potentially problematic due to the peptide linker and the low cell permeability of the prodrugs. However, this can be circumvented by administration via inhalation or *i.v.* infusion, especially in the context of CF or urinary tract infections, respectively. In conclusion, this work defines the starting point for the first *P. aeruginosa* biofilm-targeted antibiotic prodrugs aimed at breaking antimicrobial resistance and overcoming the major drawbacks of the parent fluoroquinolones, that is, severe systemic side effects.

■ EXPERIMENTAL SECTION

General Experimental Details. Commercial chemicals and solvents were used without further purification. Deuterated solvents were purchased from EurisoTop (Saarbrücken, Germany). Ciprofloxacin was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany), and ciprofloxacin · HCl was purchased from

Cayman Chemical (Ann Arbor, Michigan, USA). Thin layer chromatography (TLC) was performed on Silica Gel 60-coated aluminum sheets containing a fluorescence indicator (Merck KGaA, Darmstadt, Germany) and was developed under UV light (254 nm) and with an aqueous permanganate solution (4 g $KMnO_4$, 26.7 g K_2CO_3, and 8 mL 1 M NaOH in 400 mL H_2O), a molybdate solution (0.02 M ammonium cerium sulfate and 0.02 M ammonium molybdate in aqueous 10% H_2SO_4), or a ninhydrin solution (0.6 g ninhydrin in 200 mL *n*-BuOH and 6 mL AcOH). Self-packed silica gel 60 columns (60 Å, 400 mesh particle size, Fluka, for normal-phase liquid chromatography) or Chromabond Flash RS15 C18 ec columns (Macherey-Nagel, Düren, Germany, for reversed-phase liquid chromatography) were used on a Teledyne Isco Combiflash Rf200 system for preparative medium pressure liquid chromatography (MPLC). Nuclear magnetic resonance (NMR) spectroscopy was performed on a Bruker AVANCE III 500 UltraShield spectrometer at 500 MHz (1H) or 126 MHz (13C). Chemical shifts were given in parts per million (ppm) and were calibrated on residual solvent peaks as an internal standard. Multiplicities were specified as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). The signals were assigned with the help of 1H,1H COSY and DEPT-135-edited 1H,13C HSQC experiments. Assignment numbering of the C-glycoside atoms and groups corresponds to the numbering in fucose. Assignment numbering of the galactoside atoms and groups corresponds to the numbering in galactose. Assignment numbering of the fluoroquinolone atoms and groups corresponds to the numbering in ciprofloxacin (cipro).[78] If not stated otherwise, the purity of the final compounds was further analyzed by HPLC-UV, and all UV active compounds had a purity of ≥ 95%. Chromatographic separation was performed on a Dionex Ultimate 3000 HPLC (Thermo Scientific, Germany) with UV detection at 254 nm using an RP-18 column (Nucleoshell RP18plus, 2.7 μm, 100 × 2 mm, from Macherey-Nagel, Germany) as a stationary phase. LCMS-grade distilled MeCN and double distilled H_2O were used as mobile phases containing formic acid (0.1% v/v). In a gradient run, an initial concentration of 5% MeCN in H_2O was increased to 95% during 7 min at a flow rate 600 μL/min. The injection volume was 4 μL of 1 mM compound in H_2O/DMSO = 100:1. Mass spectra were obtained on a Bruker amaZon SL spectrometer, and data were analyzed using DataAnalysis from Bruker (Bruker Daltonics, Bremen, Germany). HPLC-HRMS for key compounds was obtained after chromatographic separation using an RP-18 column (EC 150/2 Nucleodur C18 Pyramid, 3 μm, from Macherey-Nagel, Germany) and a Q Exactive Focus Orbitrap spectrometer (Thermo Scientific, Germany). The data were analyzed using Xcalibur data acquisition and interpretation software (Thermo Scientific, Germany).

Chemical Synthesis. Methyl 4-mercaptobenzoate (**5**) was synthesized according to the protocol from Novoa et al.:[68] 4-mercaptobenzoic acid (5.2 g, 34 mmol, 1 equiv) was dissolved in 50 mL dry MeOH under argon atmosphere, and 6 drops of conc. H_2SO_4 were added. The mixture was heated to reflux for 3 days. After cooling to room temperature, the pH was adjusted to 5 using NaOMe (1 M in MeOH), and the solution was loaded on silica in vacuo. The product was eluted with CH_2Cl_2. After evaporation of the solvent, the product was obtained as a white amorphous solid (4.8 g, 80%, 93% purity determined by ^{1}H NMR) and was used without further purification. ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.89 (d, J = 8.4 Hz, 2H, Ar−H), 7.29 (d, J = 8.4 Hz, 2H, Ar−H), 3.90 (s, 3H, COOMe), 3.62 (s, 1H, SH).

4-Methylbenzoyl 2,3,4,6-Tetra-O-acetyl-β-d-thiogalactopyranoside (6). **6** was synthesized according to a previously reported protocol from Novoa et al.:[68] β-D-galactose pentaacetate (3 g, 7.7 mmol, 1 equiv) was dissolved in 20 mL dry CH_2Cl_2 in a heat-dried flask under a N_2 atmosphere. The solution was cooled on ice, and BF$_3$·Et$_2$O (3.8 mL, 30.7 mmol, 4 equiv) was added dropwise under vigorous stirring. A solution of **5** (3.88 g, 23.07 mmol, 3 equiv, 0.4 M) in CH_2Cl_2 was added dropwise to the reaction. The reaction was allowed to warm to 22 °C and then stirred overnight. After the reaction was quenched with ice water, the organic phase was subsequently washed with satd. aqueous NaHCO$_3$ (2x), water (2x), and brine (2x). The organic layer was dried over Na$_2$SO$_4$, and after

filtration, the solvent was evaporated in vacuo. After purification by MPLC (SiO$_2$, EtOAc/toluene, 5−30% EtOAc), the title compound was recrystallized from EtOAc-hexane (1:1) and obtained as white crystals (2.2 g, 58%). ^{1}H NMR (500 MHz, MeOH-$d4$): δ 7.96 (d, J = 8.5 Hz, 2H, Ar−H), 7.58 (d, J = 8.5 Hz, 2H, Ar−H), 5.46 (dd, J = 2.9, 1.1 Hz, 1H, glyco-H-4), 5.26−5.17 (m, 2H, glyco-H2, glyco-H-3), 5.15 (dd, J = 8.7, 1.4 Hz, 1H, glyco-H-1), 4.25 (td, J = 6.4, 1.2 Hz, 1H, glyco-H-5), 4.16 (d, J = 6.0 Hz, 2H, glyco-H6), 3.90 (s, 3H, COOC<u>H</u>$_3$), 2.14 (s, 3H, Ac-CH$_3$), 2.05 (s, 3H, Ac-CH$_3$), 2.03 (s, 3H, Ac-CH$_3$), 1.94 (s, 3H, Ac-CH$_3$). ^{13}C NMR (126 MHz, MeOH-$d4$): δ 172.03 (C=O), 171.88 (C=O), 171.36 (C=O), 171.18 (C=O), 168.01(C=OOMe), 141.30 (Ar−C), 131.07 (Ar−C), 130.85 (Ar−C), 129.99 (Ar−C), 85.58 (glyco-C-1), 75.60 (glyco-C-5), 73.25 (glyco-C-2), 69.02 (glyco-C-2), 68.41 (glyco-C-4), 63.01 (glyco-C-3), 52.71 (COOC<u>H</u>$_3$), 20.63 (Ac-CH$_3$), 20.60 (Ac-CH$_3$), 20.48 (2x Ac-CH$_3$). LR-MS: C$_{22}$H$_{26}$NaO$_{11}$S$^+$ [M + Na]$^+$, calcd 521.11;; found, , 521.1.

4-Carboxyphenyl β-d-thiogalactopyranoside (7). **6** (1 g, 2.0 mmol, 1 equiv) was dispersed in 20 mL dry MeOH. A solution of NaOMe (300 μL, 1 M, 1.5 equiv) in MeOH was added dropwise while cooling on ice. The reaction was allowed to warm to 22 °C and stirred for 30 min. A solution of LiOH (50 mg, 2 mmol, 1 equiv) in 3 mL water was then added to the reaction. After 1 h, the pH was adjusted to 4 with Amberlite IR-120 H$^+$ ion exchange resin while cooling on ice. The resin was removed by filtration, and the solvent was evaporated in vacuo. The title compound was obtained as a white amorphous solid (630 mg, quant.) containing approximately 17% NaOAc as an impurity, as determined by ^{1}H NMR. ^{1}H NMR (500 MHz, MeOH-$d4$): δ 7.88 (d, J = 8.5 Hz, 2H, Ar−H), 7.51 (d, J = 8.4 Hz, 2H, Ar−H), 4.70 (d, J = 9.8 Hz, 1H, glyco-H-1), 3.94 (dd, J = 3.3, 1.0 Hz, 1H, glyco-H-4), 3.77 (dd, J = 11.5, 6.8 Hz, 1H, glyco-H-6), 3.72 (dd, J = 11.5, 5.3 Hz, 1H, glyco-H-6), 3.68−3.58 (m, 2H, glyco-H-2, glyco-H-5), 3.54 (dd, J = 9.2, 3.4 Hz, 1H, glyco-H3). ^{13}C NMR (126 MHz, MeOH)-$d4$ δ 174.26 (COOH), 139.62 (Ar−C), 136.33 (Ar−C), 130.78 (Ar-CH), 130.10 (Ar-CH), 89.51 (glyco-C-1), 80.55 (glyco-C-5), 76.27 (glyco-C-3), 70.95 (glyco-C-2), 70.42 (glyco-C-4), 62.56 (glyco-C-6). LR-MS: C$_{13}$H$_{15}$O$_7$S$^-$ [M − H]$^-$, calcd 315.05; found, 315.1.

Bn-Protected LecA-Targeted Peptide Linker 9. Glycoside **7** (316 mg, 1 mmol, 1 equiv), Ala-Gly-Leu-Ala benzyl ester **8** (590 mg, 1.3 mmol, 1.3 equiv), and TBTU (414 mg, 1.3 mmol, 1.3 equiv) were dissolved in 10 mL dry DMF. DIPEA (360 μL, 2 mmol, 2 equiv) was added dropwise, and the reaction was stirred for 1 h at room temperature. The solvent was evaporated in vacuo, and the reaction was purified by MPLC (MeCN in EtOH/H$_2$O (1:1) 5−15%). The title compound was obtained as a white amorphous solid (420 mg, 58%), approximately 15% contaminated with coupling reagent side products, determined by ^{1}H NMR. ^{1}H NMR (500 MHz, MeOH-$d4$): δ 8.33 (d, J = 7.1 Hz, 1H, NH), 7.98 (d, J = 8.1 Hz, 1H, NH), 7.88−7.81 (m, 2H, glyco-Ar-H), 7.65−7.55 (m, 2H, glyco-Ar-H), 7.39−7.25 (m, 5H, Bn), 5.16 (d, J = 12.3 Hz, 2H, Bn), 5.12 (d, J = 12.3 Hz, 1H, Bn), 4.72 (d, J = 9.7 Hz, 1H, glyco-H-1), 4.49−4.37 (m, 2H, Ala-C$_\alpha$-H, Ala'-C$_\alpha$-H, Leu-Cα-H), 3.95 (d, J = 16.9 Hz, 1H, Gly-C$_\alpha$-H), 3.92 (dd, J = 3.4, 0.9 Hz, 1H, glyco-H-4), 3.79 (d, J = 17.0 Hz, 1H, Gly-C$_\alpha$-H), 3.79−3.68 (m, 2H, glyco-H-6), 3.66 (t, J = 9.4 Hz, 1H, glyco-H-2), 3.62 (ddd, J = 6.7, 5.1, 1.1 Hz, 1H, glyco-H-5), 3.52 (dd, J = 9.1, 3.3 Hz, 1H, glyco-H-3), 1.74−1.50 (m, 3H, Leu-CH, Leu-CH$_2$), 1.48 (d, J = 7.2 Hz, 3H, Ala-CH$_3$), 1.41 (d, J = 7.3 Hz, 3H, Ala'-CH$_3$), 0.84 (d, J = 6.0 Hz, 3H, Leu-CH$_3$), 0.80 (d, J = 5.9 Hz, 3H, Leu-CH$_3$). ^{13}C NMR (126 MHz, MeOH-$d4$): δ 176.17 (CONH), 174.63 (CONH), 173.76 (CONH), 171.59 (CONH), 169.71 (COOBn), 142.01 (Ar−C), 137.26 (Ar−C), 132.53 (Ar−C), 130.03 (Ar−C), 129.59 (Ar−C), 129.30 (Ar−C), 129.24 (Ar−C), 129.15 (Ar−C), 89.04 (glyco-C-1), 80.76 (glyco-C-5), 76.31 (glyco-C-3), 70.90 (glyco-C-2), 70.41 (glyco-C-4), 67.93, 62.65 (glyco-C-6), 52.93 (Leu-C$_\alpha$), 52.00 (Ala-C$_\alpha$), 49.70 (Leu-C$_\alpha$), 43.75 (Gly-C$_\alpha$), 41.73 (Leu-CH), 25.64 (Leu-CH$_2$), 23.44 (Leu-CH$_3$), 21.77 (Leu-CH$_3$), 17.27 (Ala'-CH$_3$), 17.22 (Ala-CH$_3$). LR-MS: C$_{34}$H$_{47}$N$_4$O$_{11}$S$^+$ [M + H]$^+$, calcd 719.30;; found, 719.3.

LecA-Targeted Peptide Linker (10). Benzyl ester **9** (116 mg, 0.16 mmol, 1 equiv) was dissolved in DMF (2 mL) at 50 °C. LiOH (35 mg, 9 equiv) was dissolved in 1 mL H$_2$O and added stepwise over three days until a full turnover was observed. The reaction was neutralized with Amberlite IR-120 H$^+$ exchange resin. After filtration, the solvent was removed via lyophilization. The product was purified by preparative HPLC (MeCN/H$_2$O, 5−30%, 0.1% formic acid) and was obtained as a white amorphous solid (49 mg, 49%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.44 (br s, 1H, COOH), 8.60 (d, J = 6.6 Hz, 1H, NH), 8.25 (t, J = 5.8 Hz, 1H, NH), 8.16 (d, J = 7.1 Hz, 1H, NH), 7.84 (d, J = 8.5 Hz, 2H, Ar−H), 7.76 (d, J = 8.5 Hz, 1H, NH), 7.50 (d, J = 8.5 Hz, 2H, Ar−H), 5.21 (d, J = 6.1 Hz, 1H, OH), 4.91 (br s, 1H, OH), 4.71 (d, J = 9.6 Hz, 1H, glyco-H-1), 4.66 (br s, 1H, OH), 4.52 (d, J = 4.4 Hz, 1H, OH), 4.45−4.31 (m, 2H, Ala-C$_\alpha$-H), 4.16 (dq, J = 7.3, 7.3 Hz, 1H, Ala-C$_\alpha$-H), 3.76−3.60 (m, 3H, Gly-CH$_2$, glyco-H-4), 3.57−3.42 (m, 4H, glyco-H-6, glyco-H-2, glyco-H-5), 3.38 (dd, J = 9.5, 3.1 Hz, 1H, glyco-H-3), 1.56 (dh, J = 13.3, 6.5 Hz, 1H, Leu-CH), 1.51−1.41 (m, 2H, Leu-CH$_2$), 1.34 (d, J = 7.2 Hz, 3H, Ala-CH$_3$), 1.27 (d, J = 7.3 Hz, 3H, Ala-CH$_3$), 0.82 (d, J = 2.5 Hz, 3H, Leu-CH$_3$), 0.80 (d, J = 2.6 Hz, 3H, Leu-CH$_3$). ^{13}C NMR (126 MHz, DMSO-d_6): δ 174.04 (NHC = O), 173.03 (NHC = O), 171.73 (NHC = O), 168.51 (NHC = O), 165.95 (COOH), 140.30 (Ar−C), 130.82 (Ar−C), 128.06 (Ar−C), 127.53 (Ar−C), 86.63 (glyco-C-1), 79.32 (glyco-C-5), 74.68 (glyco-C-3), 69.16 (glyco-C-2), 68.41 (glyco-C-4), 60.62 (glyco-C-6), 50.43 (Leu-C$_\alpha$), 49.55 (Ala-C$_\alpha$), 47.50 (Ala-C$_\alpha$), 42.17 (Gly-C$_\alpha$), 40.91 (Leu-CH$_2$), 23.94 (Leu-CH), 23.14 (Leu-CH$_3$), 21.62 (Leu-CH$_3$), 17.52 (Ala-CH$_3$), 17.00 (Ala-CH$_3$). LR-MS: C$_{27}$H$_{41}$N$_4$O$_{11}$S$^+$ [M + H]$^+$, calcd 629.25; found, 629.2.

N-β-L-Fucopyranosylmethyl-2-(p-carboxybenzyl-methyl)-sulfonamide (13). β-L-Fucopyranosylmethylamine (**11**, 400 mg, 2.26 mmol, 1 equiv) was dissolved in dry DMF (15 mL), and K$_2$CO$_3$ (625 mg, 4.52 mmol, 2 equiv) was added while cooling on ice. Sulfonylchloride **12** (970 mg, 4.13 mmol, 1.8 equiv) was dissolved in dry DMF (5 mL) and added dropwise to the starting material. The ice bath was removed, and the reaction was stirred at room temperature overnight. The reaction was quenched with MeOH (1 mL) and neutralized to pH 7 with HCl (1 M) while cooling on ice. The solvent was removed in vacuo, and the reaction was purified by MPLC (SiO$_2$, MeOH in CH$_2$Cl$_2$, 1−10%). The title compound was obtained as a white amorphous solid (228 mg, 27%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.13 (d, J = 8.4 Hz, 2H, Ar−H), 7.94 (d, J = 8.5 Hz, 2H, Ar−H), 7.81 (br s, 1H, -N<u>H</u>SO$_2$-), 4.78 (d, J = 5.3 Hz, 1H, OH), 4.59 (d, J = 5.7 Hz, 1H, OH), 4.26 (d, J = 5.4 Hz, 1H, OH), 3.89 (s, 3H, COOC<u>H</u>$_3$), 3.39−3.35 (m, 1H, glyco-H-4), 3.29 (q, J = 6.2 Hz, 1H, glyco-H-5), 3.24 (d, J = 13.4 Hz, 1H, linker-C<u>H</u>$_2$-), 3.18 (ddd, J = 9.0, 5.7, 3.2 Hz, 1H, glyco-H-3), 3.11 (td, J = 9.2, 4.7 Hz, 1H, glyco-H-1), 2.96 (td, J = 8.8, 2.2 Hz, 1H, glyco-H-2), 2.78−2.68 (m, 1H, linker-C<u>H</u>$_2$-), 1.01 (d, J = 6.4 Hz, 6H, glyco-H-6). ^{13}C NMR (126 MHz, DMSO-d_6): δ 165.28 (C=O), 145.10 (Ar−C), 132.63 (Ar−C), 129.88 (Ar−C), 126.89 (Ar−C), 78.33 (glyco-C-2), 74.58 (glyco-C-3), 73.57 (glyco-C-5), 71.51 (glyco-C-4), 68.22 (glyco-C-1), 52.61 (COOC<u>H</u>$_3$), 44.56 (linker-C<u>H</u>$_2$-), 16.86 (glyco-C-6). LR-MS: C$_{15}$H$_{22}$NO$_8$S$^+$[M + H]$^+$, calcd 376.11; found, 376.1.

N-β-L-Fucopyranosylmethyl-2-(p-carboxybenzyl)-sulfonamide (14). Methyl ester **13** (224 mg, 0.60 mmol, 1 equiv) was dissolved in a mixture of THF, MeOH, and H$_2$O (3:1:1, 7 mL), and LiOH (72 mg, 3 mmol, 5 equiv) was added. The reaction was stirred overnight at room temperature until the disappearance of the starting material. After neutralization with Amberlite IR-120 H$^+$ to pH 7, the solvents were removed in vacuo. The title compound was obtained after lyophilization as white powder (206 mg, 95%). ^{1}H NMR (500 MHz, D$_2$O): δ 8.19 (d, J = 8.6 Hz, 1H, Ar−H), 7.99 (d, J = 8.5 Hz, 1H, Ar−H), 3.69 (d, J = 3.3 Hz, 1H, glyco-H-2), 3.49 (dd, J = 9.6, 3.4 Hz, 1H, glyco-H-1), 3.44 (q, J = 6.5 Hz, 1H, glyco-H-5), 3.42−3.37 (m, 2H, glyco-linker-C<u>H</u>$_2$-, glyco-H-4), 3.20−3.05 (m, 2H, glyco-linker-C<u>H</u>$_2$-, glyco-H-3), 1.10 (d, J = 6.5 Hz, 3H, glyco-H-6). ^{13}C NMR (126 MHz, D$_2$O): δ 169.46 (COOH), 142.73 (Ar−C), 134.86 (s, Ar−C), 130.49 (Ar−C), 126.84 (Ar−C), 77.43 (glyco-C-3), 74.11 (glyco-C-1), 73.92 (glyco-C-5), 71.66 (glyco-C-2), 68.03 (glyco-C-4), 43.92

(linker-CH$_2$-), 15.65 (glyco-C-6). LR-MS: C$_{14}$H$_{20}$NO$_8$S$^+$ [M + H]$^+$, calcd 362.09; found, 362.1.

Bn-Protected LecB-Targeted Peptide Linker 15. N-β-L-Fucopyranosylmethyl-2-(p-carboxybenzyl)-sulfonamide (**14**, 200 mg, 0.55 mmol, 1 equiv), Ala-Gly-Leu-Ala benzyl ester (**8**, 302 mg, 0.66 mg, 1.2 equiv), and TBTU (267 mg, 0.83 mmol, 1.5 equiv) were dissolved in dry DMF (10 mL). DIPEA (288 μL, 1.65 mmol, 3 equiv) was added dropwise, and the reaction was stirred for 1 h. The solvent was evaporated in vacuo, and the reaction was purified by RP-MPLC (C18, MeCN in Water, 10−35%, 0.1% formic acid). After lyophilization, the title compound was isolated as a white powder (336 mg, 80%). ^{1}H NMR (500 MHz, MeOH-*d4*): δ 8.06 (d, *J* = 8.5 Hz, 2H, ArH), 7.95 (d, *J* = 8.4 Hz, 2H, ArH), 7.41−7.27 (m, 5H, Bn), 5.17 (d, *J* = 12.3 Hz, 1H, Bn-CH$_2$-), 5.12 (d, *J* = 12.3 Hz, 1H, Bn-CH$_2$-), 4.49−4.40 (m, 3H, Ala-Cα-H, Ala′-Cα-H, Leu-Cα-H), 3.97 (d, *J* = 16.8 Hz, 1H, Gly-Cα-H), 3.79 (d, *J* = 16.9 Hz, 1H, Gly-Cα-H), 3.58 (dd, *J* = 2.9, 0.7 Hz, 1H, glyco-H-4), 3.44 (qd, *J* = 6.4, 1.1 Hz, 1H, glyco-H-5), 3.41−3.36 (m, 2H, glyco-H-3, glyco-H-1), 3.34 (dd, *J* = 8.3, 2.9 Hz, 1H, linker-CH$_2$-), 3.11 (ddd, *J* = 9.0, 7.1, 2.4 Hz, 1H, glyco-H-2), 3.04 (dd, *J* = 12.9, 7.1 Hz, 1H, linker-CH$_2$-), 1.68−1.52 (m, 3H, Leu-CH, Leu-CH$_2$), 1.50 (d, *J* = 7.2 Hz, 3H, Ala′-CH$_3$), 1.41 (d, *J* = 7.4 Hz, 3H, Ala′-CH$_3$), 1.17 (d, *J* = 6.4 Hz, 3H, glyco-C6-H), 0.84 (d, *J* = 5.9 Hz, 3H, Leu-CH$_3$), 0.78 (d, *J* = 6.0 Hz, 3H, Leu-CH$_3$). ^{13}C NMR (126 MHz, MeOH-*d4*): δ 175.85 (CONH), 174.62 (COOBn), 173.76 (CONH), 171.53 (CONH), 168.83 (CONH), 145.04 (Ar−C), 138.56 (Ar−C), 137.25 (Bn-C), 129.59 (Bn-C), 129.51 (Ar−C), 129.32 (Bn-C), 129.25 (Bn-C), 128.10 (Ar−C), 79.61 (glyco-C-2), 76.33 (glyco-C-3), 75.52 (glyco-C-5), 73.56 (glyco-C-4), 69.62 (glyco-C-1), 67.93 (Bn-CH$_2$), 52.88 (Ala-Cα), 52.04 (Ala′-Cα), 49.67 (Leu-Cα), 45.55 (linker-CH$_2$-), 43.72 (Gly-Cα), 41.75 (Leu-CH$_2$), 25.63 (Leu-CH), 23.44 (Leu-CH$_3$), 21.78 (Leu-CH$_3$), 17.24 (Ala-CH$_3$), 17.21 (Ala-CH$_3$), 17.10 (glyco-C6-H). LR-MS: C$_{35}$H$_{50}$N$_5$O$_{12}$S$^+$ [M + H]$^+$, calcd 764.32; found, 764.2.

LecB-Targeted Peptide Linker 16. Benzyl ester **15** (300 mg, 0.39 mmol, 1 equiv) was dissolved in MeOH (4 mL). Pd/C (10 wt-%, 41 mg) was added, and the atmosphere was changed to H$_2$ (1 atm). The reaction was stirred at room temperature for 16 h until full transformation of the starting material. Pd/C was removed by centrifugation (17600 rcf, 10 min), and the solvent was removed in vacuo. The title compound was obtained as a white amorphous solid (250 mg, 95%). ^{1}H NMR (500 MHz, MeOH-*d4*): δ 8.87 (d, *J* = 5.5 Hz, 1H, NH), 8.56 (t, *J* = 5.9 Hz, 1H, NH), 8.22 (d, *J* = 7.2 Hz, 1H, NH), 8.07 (d, *J* = 8.5 Hz, 2H, Ar−H), 7.98 (s, 1H, NH), 7.96 (d, *J* = 8.6 Hz, 2H, Ar−H), 4.56−4.43 (m, 2H, Leu-Cα-H, Ala-Cα-H), 4.37 (qd, *J* = 7.3, 2.3 Hz, 1H, Ala-Cα-H), 3.99 (d, *J* = 17.0 Hz, 1H, Gly-Cα-H), 3.81 (d, *J* = 16.8 Hz, 1H, Gly-Cα-H), 3.59 (d, *J* = 2.9 Hz, 1H, glyco-H-4), 3.44 (q, *J* = 6.6 Hz, 1H, glyco-H-5), 3.41−3.32 (m, 3H, glyco-H-3, glyco-H-1, linker-CH$_2$-), 3.11 (ddd, *J* = 9.0, 7.2, 2.4 Hz, 1H, glyco-H-2), 3.03 (dd, *J* = 12.9, 7.2 Hz, 1H, linker-CH$_2$-), 1.74−1.58 (m, 3H, Leu-CH, Leu-CH$_2$), 1.51 (d, *J* = 7.2 Hz, 3H, Ala-CH$_3$), 1.41 (d, *J* = 7.3 Hz, 3H, Ala′-CH$_3$), 1.17 (d, *J* = 6.6 Hz, 3H, glyco-H-6), 0.88 (d, *J* = 6.3 Hz, 3H, Leu-CH$_3$), 0.82 (d, *J* = 6.3 Hz, 3H, Leu-CH$_3$). ^{13}C NMR (126 MHz, MeOH-*d4*): δ 175.85 (CONH), 175.80 (COOH), 174.44 (CONH), 171.55 (CONH), 168.85 (CONH), 145.04 (Ar−C), 138.56 (Ar−C), 129.51 (Ar−C), 128.11 (Ar−C), 79.62 (glyco-C-2), 76.32 (glyco-C-3), 75.52 (glyco-C-5), 73.56 (glyco-C-4), 69.63 (glyco-C-1), 52.96 (Ala-Cα), 52.03 (Ala′-Cα), 49.85 (Leu-Cα), 45.55 (linker-CH$_2$-), 43.72 (Gly-Cα), 41.73 (Leu-CH$_2$), 25.65 (Leu-CH), 23.46 (Leu-CH$_3$), 21.81 (Leu-CH$_3$), 17.61 (Ala-CH$_3$), 17.23 (Ala′-CH$_3$), 17.09 (glyco-C6-H). LR-MS: C$_{28}$H$_{44}$N$_5$O$_{12}$S$^+$ [M + H]$^+$, calcd 674.27; found, 674.2.

Boc-Protected Leu-Ala-Ciprofloxacin-conjugate 18. N-Boc-protected Leu−Ala **17** (100 mg, 0.33 mmol, 1 equiv) and NMM (36 μL, 0.33 mmol, 1 equiv) were dissolved in THF (3 mL). The solution was cooled to −20 °C, and Ibcf (43 μL, 0.33 mmol, 1 equiv) was added dropwise under vigorous stirring. The reaction was stirred for 20 min. This solution was then added dropwise to a dispersion of ciprofloxacin (119 mg, 0.36 mmol, 1.1 equiv) and NMM (51 μL, 0.46 mmol, 1.4 equiv) in dry THF (4 mL) via a transfer channel. The reaction was allowed to warm to 22 °C and stirred for 2.5 h. The

reaction was poured on ice water (20 mL) and acidified with aqueous HCl (1 M) to pH = 4. The aqueous phase was extracted with CH$_2$Cl$_2$ (3 × 20 mL). The combined organic layers were washed with satd. aqueous NH$_4$Cl and dried over Na$_2$SO$_4$. After filtration, the solvent was removed in vacuo and the product was purified by MPLC (CHCl$_3$/PE (9:1): MeOH, 1−10%), yielding the product as a beige amorphous solid (50 mg, 25%). ^{1}H NMR (500 MHz, CHCl$_3$-*d*CDCl$_3$): δ 8.78 (s, 1H, cipro-C2-H), 8.05 (d, *J* = 12.6 Hz, 1H, cipro-C5-H), 7.45 (d, *J* = 5.3 Hz, 1H, cipro-C8-H), 7.01 (d, *J* = 7.0 Hz, 1H, Ala-NH), 5.03−4.89 (m, 1H, Ala-C$_\alpha$-H), 4.88−4.83 (m, 1H, Leu-NH), 4.14 (s, 1H, Leu-C$_\alpha$-H), 4.09−3.98 (m, 1H, piperazine-C-H), 3.95−3.80 (m, 1H, piperazine-C-H), 3.74−3.67 (m, 2H, 2x piperazine-C-H), 3.56 (s, 1H, cPr-H), 3.39 (s, 3H, 3x piperazine-C-H), 3.30−3.15 (m, 1H, piperazine-C-H), 1.75−1.59 (m, 2H, Leu-CH$_2$ + Leu-CH-CH$_3$CH$_3$), 1.44 (s, 12H, cPr-CH$_2$ + Boc-CH$_3$ + Leu-CH$_2$), 1.37 (d, *J* = 6.6 Hz, 3H, Ala-CH$_3$), 1.21 (s, 2H, cPr-CH$_2$), 1.00−0.81 (m, 6H, Leu-CH$_3$). ^{13}C NMR (126 MHz, CDCl$_3$): δ 177.21 (cipro-C4 = O), 172.07 (C=O), 170.77 (C=O), 166.99 (cipro-COOH), 155.69 (carbamate-C=O), 153.77 (d, *J* = 251.8 Hz, cipro-C-6), 147.85 (cipro-C-2), 145.13 (cipro-C-7), 139.09 (cipro-C-8a), 120.85 (d, *J* = 7.8 Hz, cipro-C-4a), 112.92 (d, *J* = 23.2 Hz, cipro-C-5), 108.45 (cipro-C-3), 105.71 (cipro-C-8), 80.34 (Boc), 53.40 (Leu-C$_\alpha$), 50.16 (piperazine-C), 49.84 (piperazine-C), 45.27 (piperazine-C), 45.07 (Leu-C$_\alpha$), 41.91 (piperazine-C), 41.57 (Leu-CH$_2$), 35.54 (cPr-CH), 28.42 (Boc-CH$_3$), 24.92 (Leu-CH-CH$_3$CH$_3$), 23.20 (Leu-CH$_3$), 21.89 (Leu-CH$_3$), 18.96 (Ala-CH$_3$), 8.46 (cPr-CH$_2$). LR-MS: C$_{26}$H$_{35}$FN$_5$O$_5$$^+$ [M + H]$^+$, calcd 516.26; found, 516.3.

Leu-Ala-Ciprofloxacin Conjugate 19. Boc-protected conjugate **18** (43 mg, 0.07 mmol, 1 equiv) was dissolved in 3 mL HCl in dioxane (4 M) while cooling on ice. The ice bath was removed, and the reaction was stirred at 22 °C for 4 h. The solvent was evaporated in vacuo, and the residue was dissolved in 1 mL MeOH. The product was precipitated with ice-cold Et$_2$O (20 mL), and the resulting precipitate was washed three times with ice-cold Et$_2$O. The precipitate was dried in vacuo and was obtained as a yellow solid (30 mg, 78%). ^{1}H NMR (500 MHz, DMSO-*d$_6$*): δ 15.17 (s, 1H, cipro-COOH), 8.85 (d, *J* = 7.5 Hz, 1H, Ala-NH), 8.67 (s, 1H, cipro-C2-H), 8.27 (s, 1H, Leu-NH$_3$$^+$), 7.93 (d, *J* = 13.1 Hz, 1H, cipro-C5-H), 7.58 (d, *J* = 7.2 Hz, 1H, cipro-C8-H), 4.92−4.82 (m, 1H, Ala-C$_\alpha$-H), 3.82 (s, 2H, Leu-C$_\alpha$-H + cPr-CH), 3.78−3.73 (m, 2H, 2x piperazine-C-H), 3.73−3.69 (m, 2H, 2x piperazine-C-H), 1.68 (dp, *J* = 13.2, 6.6 Hz, 1H, Leu-CH-CH$_3$CH$_3$), 1.59−1.49 (m, 2H, Leu-CH$_2$), 1.32 (d, *J* = 6.3 Hz, 2H, cPr-CH$_2$), 1.28 (d, *J* = 6.9 Hz, 2H, Ala-CH$_3$), 1.19 (s, 2H, cPr-CH$_2$), 0.91 (d, *J* = 6.5 Hz, 3H, Ala-CH$_3$), 0.89 (d, *J* = 6.5 Hz, 3H, Ala-CH$_3$). ^{13}C NMR (126 MHz, DMSO-*d$_6$*): δ 176.39 (cipro-C4), 169.95 (C=O), 168.29 (C=O), 165.92 (cipro-COOH), 152.96 (d, *J* = 249.3 Hz, cipro-C6), 148.15 (cipro-C2), 144.80 (d, *J* = 10.1 Hz, cipro-C7), 139.15 (cipro-C8a), 118.92 (d, *J* = 7.6 Hz, cipro-C4a), 111.08 (d, *J* = 23.0 Hz, cipro-C5), 106.79 (cipro-C3), 106.71 (d, *J* = 2.7 Hz, cipro-C8), 50.62 (Leu-C$_\alpha$), 49.70 (piperazine-C), 49.19 (piperazine-C), 44.65 (piperazine-C), 44.57 (Ala-C$_\alpha$), 41.21 (piperazine-C), 40.23 (Leu-CH$_2$), 35.94 cPr-CH, 23.53 (Leu-CH-CH$_3$CH$_3$), 22.68 (Leu-CH$_3$), 22.04 (Leu-CH$_3$), 17.75 (Ala-CH$_3$), 7.64 (cPr-CH$_2$). HR-MS: calcd [C$_{26}$H$_{35}$FN$_5$O$_5$]$^+$, 516.2617; found, 516.2610.

N-Boc-Ciprofloxacin Benzyl Ester (20). Ciprofloxacin (**1**) (1000 mg, 3.02 mmol, 1 equiv), KHCO$_3$ (1511 mg, 15.1 mmol, 5 equiv), and Boc$_2$O (775 μL, 3.62 mmol, 1.2 equiv) were dispersed in 12 mL dry DMF. The reaction was heated to 40 °C and stirred for 2 h. Then, BnBr (430 μL, 3.62 mmol, 1.2 equiv) was added, and the reaction was heated to 120 °C and stirred for 90 min. The reaction was allowed to cool to 22 °C and poured on 100 mL ice-cold water. The precipitate was filtered out and dried in vacuo. The product was obtained as a beige amorphous solid (1.36 g, 86%). No NMR was measured due to solubility issues: the sample degraded in CDCl$_3$ and was not soluble in other common solvents. LR-MS: C$_{29}$H$_{33}$FN$_3$O$_5$$^+$ [M + H]$^+$, calcd 522.24; found, 522.3.

Ciprofloxacin Benzyl Ester·HCl (21). Protected ciprofloxacin **20** (500 mg, 0.96 mmol, 1 equiv) was suspended in 2 mL CH$_2$Cl$_2$ and cooled with an ice bath. 10 mL HCl in dioxane (4 N) was added slowly under vigorous stirring, and the reaction was allowed to warm

to 22 °C and stirred for 1 h. The solvent was evaporated in vacuo, and the product was obtained as a yellow solid (448 mg, quant). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.54 (s, 2H, piperazine-NH$_2^+$), 8.48 (s, 1H, cipro-C2-H), 7.81 (d, J = 13.2 Hz, 1H, cipro-C5-H), 7.51−7.46 (m, 3H, cipro-C8-H + Bn-Ar), 7.42−7.37 (m, 2H, Bn-Ar), 7.35−7.30 (m, 1H, Bn-Ar), 5.27 (s, 2H, Bn-CH$_2$-), 3.73−3.64 (m, 1H, cPr-CH), 3.52−3.45 (m, 4H, 4x piperazine-C-H), 3.30 (s, 4H, 4x piperazine-C-H), 1.25 (dd, J = 6.9 Hz, 2H, cPr-CH$_2$), 1.15−1.04 (m, 2H, cPr-CH$_2$). ^{13}C NMR (126 MHz, DMSO-d_6): δ 172.05 (C=O), 164.96 (COOBn), 152.94 (d, J = 246.5 Hz, cipro-C6), 149.06 (cipro-C2), 143.28 (d, J = 10.9 Hz, cipro-C7), 138.50 (cipro-C8a), 137.10 (Bn), 128.86 (Bn), 128.25 (Bn), 128.09 (Bn), 123.03 (d, J = 6.3 Hz, cipro-C4a), 112.30 (d, J = 22.7 Hz, cipro-C5), 109.42 (cipro-C3), 107.19 (cipro-C8), 65.73 (piperazine-C), 46.93 (piperazine-C), 46.90 (piperazine-C), 43.01 (piperazine-C), 35.39 (cPr-CH), 8.05 (cPr-CH$_2$). LR-MS: C$_{24}$H$_{25}$FN$_3$O$_3^+$ [M + H]$^+$, calcd 422.19; found, 422.1.

LecA-targeted ciprofloxacin-prodrug **24**: The title compound was synthesized in two chemical steps: First, LecA-targeted peptide linker **10** (31 mg, 0.049 mmol, 1 equiv), ciprofloxacin benzyl ester **21** (34 mg, 0.074 mmol, 1.5 equiv), and TBTU (24 mg, 0.074 mmol, 1.5 equiv) was dissolved in 1 mL dry DMF. DIPEA (17 μL, 0.098 mmol, 2 equiv) was added dropwise, and the reaction was stirred for 1 h. After evaporation of the solvent, the residue was taken up in 1.5 mL MeOH/DMF (2:1). Pd black (10 mg, 0.05 mmol, 1 equiv) was added, and the reaction was stirred under H$_2$ atmosphere for 6 d. Afterward, the reaction was filtered over celite and further purified by preparative HPLC (MeCN/H$_2$O, 20−33%, 0.1% formic acid). The title compound was obtained as a beige amorphous solid (10 mg, 22% over 2 chemical steps). ^{1}H NMR (500 MHz, MeOH-d_4): δ 8.79 (s, 1H, cipro-H-2), 7.91 (d, J = 13.0 Hz, 1H, cipro-H-5), 7.81 (d, J = 8.1 Hz, 2H, Ar−H), 7.61 (d, J = 6.8 Hz, 1H, cipro-C8-H), 7.54 (d, J = 8.0 Hz, 2H, Ar−H), 4.92−4.87 (m, 1H, Ala-C$_\alpha$-H), 4.72 (d, J = 9.7 Hz, 1H, glyco-H-1), 4.48 (dd, J = 10.7, 3.8 Hz, 1H, Leu-C$_\alpha$-H), 4.40 (q, J = 6.0 Hz, 1H, Ala-C$_\alpha$-H), 4.06−3.88 (m, 4H, 2x piperazine-CH, Gly-CH, glyco-H-4), 3.83−3.59 (m, 8H, 2x piperazine-CH, Gly-CH, cPr-CH, glyco-H6, glyco-H-2, glyco-H-5), 3.53 (dd, J = 9.2, 3.4 Hz, 1H, glyco-H-3), 3.43 (s, 3H, piperazine-CH$_2$, piperazine-CH), 1.77−1.66 (m, 1H, Leu-CH$_2$), 1.67−1.57 (m, 2H, Leu-CH$_2$, Leu-CH), 1.48 (d, J = 7.1 Hz, 3H, Ala-CH$_3$), 1.42 (d, J = 6.3 Hz, 2H, cPr-CH$_2$), 1.36 (d, J = 6.8 Hz, 3H, Ala-CH$_3$), 1.27−1.18 (m, 2H, cPr-CH$_2$), 0.87 (d, J = 5.7 Hz, 3H, Leu-CH$_3$), 0.82 (d, J = 5.8 Hz, 3H, Leu-CH$_3$). ^{13}C NMR (126 MHz, MeOH-d_4): δ 177.03 (C=O), 174.85 (C=O), 172.80 (C=O), 171.14 (C=O), 170.36 (C=O), 168.31 (C=O), 168.26 (C=O), 153.70 (d, J = 250.0 Hz, cipro-C6), 148.03 (cipro-C), 145.50 (d, J = 10.1 Hz, cipro-C7), 140.79 (cipro-C), 139.38 (Ar−C), 130.87 (Ar−C), 128.35 (Ar−C), 127.77 (Ar−C), 119.59 (d, J = 8.1 Hz, cipro-C4a), 111.15 (d, J = 23.5 Hz, cipro-C5), 106.80 (cipro-C), 106.31 (d, J = 2.7 Hz, cipro-C8), 87.53 (glyco-C-1), 79.38 (glyco-C-5), 74.86 (glyco-C-3), 69.47 (glyco-C-2), 68.99 (glyco-C-4), 61.26 (glyco-C-6), 51.70 (Leu-C$_\alpha$), 50.77 (Ala-C$_\alpha$), 49.66 (d, J = 3.7 Hz, piperazine-C), 49.28 (d, J = 2.6 Hz, piperazine-C), 45.25 (Ala-C$_\alpha$), 45.08 (Gly-C$_\alpha$), 42.49 (piperazine-C), 41.80 (piperazine-C), 40.17 (Leu-CH$_2$), 35.67 (cPr-CH), 24.31 (Leu-CH), 22.16 (Leu-CH$_3$), 20.18 (Leu-CH$_3$), 16.40 (Ala-CH$_3$), 15.87 (Leu-CH$_3$), 7.24 (cPr-CH$_2$), 7.17 (cPr-CH$_2$). HR-MS: calcd [C$_{44}$H$_{57}$FN$_7$O$_{13}$S]$^+$, 942.3714; found, 942.3689.

LecB-targeted ciprofloxacin-prodrug-benzylester (**23**): LecB-targeted peptide linker **16** (70 mg, 0.10 mmol, 1 equiv), ciprofloxacin benzyl ester **21** (55 mg, 0.12 mg, 1.2 equiv), and TBTU (48 mg, 0.15 mmol, 1.5 equiv) were dissolved in dry DMF (2 mL). DIPEA (52 μL, 0.3 mmol, 3 equiv) was added dropwise, and the reaction was stirred for 1 h. The solvent was evaporated in vacuo, and the reaction was purified by preparative HPLC (MeCN in water, 25−40%, 0.1% formic acid). After lyophilization, the title compound was isolated as an off-white powder (90 mg, 84%). ^{1}H NMR (500 MHz, MeOH-d4): δ 8.65 (s, 1H, cipro-H-2), 8.04 (d, J = 8.5 Hz, 2H, glyco-Ar-H), 7.93 (d, J = 8.5 Hz, 2H, glyco-Ar-H), 7.88 (d, J = 13.3 Hz, 1H, cipro-H-5), 7.52 (d, J = 7.2 Hz, 1H, cipro-H-8), 7.48 (d, J = 7.4 Hz, 2H, Bn-Ar), 7.42−7.35 (m, 2H, Bn-Ar), 7.34−7.29 (m, 1H, Bn-Ar), 5.33 (s, 2H, Bn-CH$_2$), 4.92−4.88 (m, 1H, Ala-C$_\alpha$-H), 4.49−4.42 (m, 2H, Leu-C$_\alpha$-H,

Ala′-C$_\alpha$-H), 4.00 (d, J = 16.8 Hz, 1H, Gly-C$_\alpha$-H), 3.94−3.84 (m, 2H, 2x piperazine-H), 3.78 (d, J = 16.8 Hz, 1H, glycin-C$_\alpha$-H), 3.77−3.70 (m, 1H, piperazine-H), 3.70−3.61 (m, 2H, cPr-CH, piperazine-H), 3.58 (d, J = 2.9 Hz, 1H, glyco-H-4), 3.43 (q, J = 6.2 Hz, 1H, glyco-H-5), 3.40−3.34 (m, 4H, 3x piperazine-H, glyco-H-3, glyco-H-1), 3.34−3.27 (m, 1H, glyco-linker-CH$_2$), 3.28−3.22 (m, 1H, piperazine-H), 3.10 (ddd, J = 9.0, 7.1, 2.4 Hz, 1H, glyco-H-2), 3.02 (dd, J = 12.9, 7.1 Hz, 1H, glyco-linker-CH$_2$-), 1.76−1.56 (m, 3H, Leu-CH, Leu-CH$_2$), 1.50 (d, J = 7.2 Hz, 3H, Ala-CH$_3$), 1.35 (d, J = 6.9 Hz, 3H, Ala-CH$_3$), 1.34−1.25 (m, 2H, cPr-CH$_2$), 1.16 (d, J = 6.5 Hz, 3H, glyco-H-6), 1.14−1.06 (m, 2H, cPr-CH$_2$), 0.87 (d, J = 5.8 Hz, 3H, Leu-CH$_3$), 0.82 (d, J = 5.7 Hz, 3H, Leu-CH$_3$). ^{13}C NMR (126 MHz, MeOH-$d4$): δ 175.83 (CONH), 175.43 (cipro-C=O), 174.11 (CONH), 172.55 (CONH), 171.68 (CONH), 165.93 (COOBn), 154.88 (d, J = 248.4 Hz, cipro-C-6), 150.13 (cipro-Ar-C), 145.93 (d, J = 10.6 Hz, cipro-C-7), 145.06 (Ar−C), 139.87 (Ar−C), 138.52 (Ar−C), 137.93 (Ar−C), 129.56 (glyco-Ar-C), 129.53 (Bn-Ar), 129.24 (Bn-Ar), 129.15 (Bn-Ar), 128.08 (glyco-Ar-C), 123.70 (d, J = 6.5 Hz, cipro-C-4a), 113.18 (d, J = 24.3 Hz, cipro-C-5), 110.28 (Ar−C), 107.58 (d, J = 2.9 Hz, cipro-C-8), 79.62 (glyco-C-2), 76.33 (glyco-C-3), 75.51 (glyco-C-5), 73.55 (glyco-C-4), 69.62 (glyco-C-1), 67.16 (Bn), 53.11 (Leu-Cα), 52.13 (Ala-Cα), 51.25 (piperazine-C), 50.78 (piperazine-C), 46.58 (piperazine-C), 45.55 (glyco-linker-CH$_2$), 43.89 (Gly-Cα), 43.22 (piperazine-C), 41.60 (Leu-CH$_2$), 36.38 (cPr-CH), 25.74 (Leu-CH), 23.53 (Leu-CH$_3$), 21.63 (Leu-CH$_3$), 17.87 (Ala-CH$_3$), 17.23 (Ala-CH$_3$), 17.11 (glyco-C-6), 8.60 (cPr-CH$_2$), 8.54 (cPr-CH$_2$). LR-MS: C$_{52}$H$_{67}$FN$_8$O$_{14}$S^{2+} [M+2H]$^{2+}$, calcd 539.22; found, 539.2.

LecB-Targeted Ciprofloxacin-Prodrug **25**. Protected LecB-targeted ciprofloxacin-prodrug **23** (57 mg, 0.052 mmol, 1 equiv) was dissolved in MeOH (1 mL). Pd/C (10 wt %, 5 mg) was added, and the atmosphere was changed to H$_2$ (1 atm). The reaction was stirred at room temperature for 24 h until full consumption of the starting material. Pd/C was removed by centrifugation (17600 rcf, 10 min), and the solvent was removed in vacuo. After purification by preparative HPLC (MeCN in water, 22−35%, 0.1% formic acid), the title compound was obtained as an off-white amorphous solid (38 mg, 74%). ^{1}H NMR (500 MHz, MeOH-$d4$): δ 8.60 (s, 1H, FQ-H-2), 8.05 (d, J = 8.0 Hz, 2H, glyco-Ar-H), 7.94 (d, J = 8.0 Hz, 2H, glyco-Ar-H), 7.72 (d, J = 14.1 Hz, 1H, FQ-H-5), 7.05 (s, 1H, FQ-H-8), 4.47 (s, 1H, piperazine-H), 4.41 (q, J = 7.3 Hz, 1H, Ala-Cα-H), 4.36−4.23 (m, 2H, Ala′-Cα-H), 3.97−3.77 (m, 3H, 2x piperazine-H, Gly-Cα-H), 3.74−3.61 (m, 4H, 2x piperazine-H, Gly-Cα-H, cPr-CH), 3.58 (d, J = 1.9 Hz, 1H, glyco-H-4), 3.44 (q, J = 6.4 Hz, 1H, glyco-H-5), 3.41−3.27 (m, 4H, glyco-H-3, glyco-H-1, piperazine-H, glyco-linker-CH$_2$-), 3.10 (ddd, J = 8.9, 7.5, 2.4 Hz, 1H, glyco-H-2), 3.02 (dd, J = 12.9, 7.1 Hz, 1H, glyco-linker-CH$_2$-), 2.34−2.22 (m, 1H, piperazine-H), 2.21−2.10 (m, 1H, piperazine-H), 1.78−1.69 (m, 1H, Leu-CH), 1.69−1.56 (m, 2H, Leu-CH$_2$), 1.51 (d, J = 7.1 Hz, 3H, Ala-CH$_3$), 1.44−1.35 (m, 5H, Ala′-CH$_3$, cPr-CH$_2$), 1.18 (s, 2H, cPr-CH$_2$), 1.16 (d, J = 6.5 Hz, 3H, glyco-C-6), 0.86 (d, J = 4.6 Hz, 3H, Leu-CH$_3$), 0.85 (d, J = 4.6 Hz, 3H, Leu-CH$_3$). ^{13}C NMR (126 MHz, MeOH-$d4$): δ 177.54 (d, J = 2.3 Hz, FQ-C=O), 176.27 (CONH), 175.08 (CONH), 174.75 (CONH), 172.35 (CONH), 170.05 (CONH), 169.06 (COOH), 152.04 (d, J = 247.5 Hz, cipro-C6), 148.59 (cipro-C2), 145.13 (Ar−C), 143.54 (d, J = 12.1 Hz, cipro-C7), 141.38 (Ar−C), 138.36 (Ar−C), 129.54 (glyco-Ar-C), 128.11 (glyco-Ar-C), 111.96 (d, J = 23.9 Hz, cipro-C-5), 101.66 (cipro-C-8), 79.62 (glyco-C-2), 76.33 (glyco-C-3), 75.52 (glyco-C-5), 73.55 (glyco-C-4), 69.61 (glyco-C-1), 56.00 (d, J = 6.4 Hz, piperazine-C), 53.88 (Leu-Cα), 52.48 (Ala-Cα), 50.93 (piperazine-C), 50.84 (Ala′-CH$_3$), 49.24 (extracted from HSQC, piperazine-C), 45.55 (glyco-linker-CH$_2$), 44.20 (Gly-Cα), 41.19 (Leu-CH$_2$), 36.80 (cPr-CH), 31.80 (piperazine-C), 25.81 (Leu-CH), 23.53 (Leu-CH$_3$), 21.56 (Leu-CH$_3$), 17.85 (Ala-CH$_3$), 17.27 (Ala′-CH$_3$), 17.11 (glyco-C-6), 8.49 (cPr-CH$_2$), 8.46 (cPr-CH$_2$). HR-MS: calcd [C$_{45}$H$_{60}$FN$_8$O$_{14}$S]$^+$, 987.3928; found, 987.3908.

(S)-7-(3-Tertbutoxycarbonylamino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid **(28)**. 7-chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**26**, 500 mg, 1.78 mmol, 1 equiv) and (*S*)-3-(Boc-amino)-pyrrolidine (**27**, 995 mg, 5.34 mmol, 3 equiv) were dispersed

in 10 mL dry pyridine. The mixture was heated with an oil bath to 160 °C and refluxed overnight. After cooling to room temperature, the solvent was evaporated in vacuo and purified by normal phase MPLC (CH$_2$Cl$_2$: *n*-Hex (70:28): MeOH, 2–5%). The product was obtained as a beige amorphous solid (383 mg, 50%). ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 15.54 (s, 1H, COO**H**), 8.56 (s, 1H, Ar4H-2), 7.78 (d, *J* = 14.1 Hz, 1H, ArH-5), 7.30 (d, *J* = 6.7 Hz, 1H, BocN**H**), 7.03 (d, *J* = 7.6 Hz, 1H. ArH-8), 4.17 (q, *J* = 6.3 Hz, 1H, aminopyrrolidine-H), 3.86–3.77 (m, 1H, aminopyrrolidine-H), 3.77–3.67 (m, 2H, aminopyrrolidine-H, *c*Pr-CH), 3.64–3.56 (m, 1H, aminopyrrolidine-H), 3.44 (dt, *J* = 10.7, 3.9 Hz, 1H, amino-pyrrolidine-H), 2.15 (dq, *J* = 13.3, 7.1 Hz, 1H, aminopyrrolidine-H), 1.93 (dq, *J* = 12.6, 6.0 Hz, 1H, aminopyrrolidine-H), 1.39 (s, 9H, Boc-CH$_3$), 1.34–1.25 (m, 2H, *c*Pr-CH$_2$), 1.18–1.10 (m, 2H, *c*Pr-CH$_2$). ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ 175.87 (d, *J* = 3.3 Hz, C-4), 166.32 (COOH), 155.29 (Boc-C=O), 149.96 (d, *J* = 246.3 Hz, C-6), 147.44 (C), 141.65 (d, *J* = 11.6 Hz, C-7), 139.83 (C), 114.42 (d, *J* = 7.0 Hz, C-4a), 110.71 (d, *J* = 22.6 Hz, C-5), 106.17 (C), 100.43 (d, *J* = 5.7 Hz, C-8), 78.04 (Boc-C), 55.18 (d, *J* = 6.8 Hz, aminopyrrolidine-C), 49.84 (aminopyrrolidine-C), 48.15 (d, *J* = 3.8 Hz, aminopyrrolidine-C), 35.74 (*c*Pr-C), 30.43 (aminopyrrolidine-C), 28.28 (Boc-CH$_3$), 7.59 (*c*Pr-CH$_2$), 7.53 (*c*Pr-CH$_2$). LR-MS: C$_{22}$H$_{27}$FN$_3$O$_5$$^+$ [M + H]$^+$, calcd 432.19; found, 432.2.

(S)-7-(3-Amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihy-dro-4-oxo-1,8-naphthyridine-3-carboxylic Acid ● HCl (2). (*S*)-7-(3-Tertbutoxycarbonylamino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**28**, 59 mg, 0.14 mmol, 1 equiv) was dissolved in 3 mL HCl in dioxane (4 N) while cooling on ice. The reaction was allowed to warm to 22 °C and stirred for 90 min until full consumption of the starting material. After the solvent was evaporated in vacuo, the remaining solid was taken up in 2 mL MeOH and the product was precipitated with Et$_2$O. The precipitate was first washed three times with Et$_2$O, then dried in vacuo. The product was obtained as a yellow amorphous solid (m = 45 mg, 86%). ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 15.46 (s, 1H, COOH), 8.60 (s, 1H, H-2), 8.33 (s, 3H, NH3$^+$), 7.86 (d, *J* = 14.2 Hz, 1H, H-5), 7.10 (d, *J* = 7.6 Hz, 1H, H-8), 4.10–3.88 (m, 2H, aminopyrrolidine-CH + *c*Pr-CH), 3.88–3.69 (m, 3H, amino-pyrrolidine-CH$_2$, aminopyrrolidine-CH), 3.69–3.61 (m, 1H, amino-pyrrolidine-CH), 2.34 (ddt, *J* = 14.0, 8.0, 7.5 Hz, 1H, amino-pyrrolidine-CH), 2.15 (ddt, *J* = 12.2, 7.8, 4.5 Hz, 1H, amino-pyrrolidine-CH), 1.34–1.27 (m, 2H, *c*Pr-CH$_2$), 1.23–1.11 (m, 2H, *c*Pr-CH$_2$). ^{13}C NMR (126 MHz, D$_2$O): δ 173.95 (d, C-4), 169.31 (C), 150.05 (d, *J* = 249.4 Hz, C-6), 147.02 (C), 141.31 (d, *J* = 11.0 Hz, C-7), 139.14 (C), 113.20 (d, *J* = 7.1 Hz, C-4a), 109.51 (d, *J* = 23.2 Hz, C-5), 104.62 (C), 100.57 (C-8), 52.62 (d, *J* = 7.8 Hz, aminopyrrolidine-C), 50.11 (aminopyrrolidine-C), 47.27 (d, *J* = 3.2 Hz, aminopyrrolidine-C), 35.79 (*c*Pr-C), 28.36 (aminopyrrolidine-C), 7.18 (2x *c*Pr-CH$_2$). HR-MS: calcd [C$_{17}$H$_{19}$FN$_3$O$_3$]$^+$, 332.1405; found, 332.1397.

(S)-7-(3-Tertbutoxycarbonylamino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid Benzyl Ester (29). **28** (364 mg, 0.84 mmol, 1 equiv) and freshly ground KHCO$_3$ were dried under high vacuum for 15 min. After dispersion in 10 mL dry DMF, BnBr (150 µL, 1.26 mmol, 1.5 equiv) was added and the reaction was heated to 110 °C. Full conversion was achieved after 60 min, and the reaction was allowed to cool to 22 °C. The solvent was reduced in vacuo and diluted with CH$_2$Cl$_2$. The organic phase was washed with water, KHSO$_4$ (1 M), and brine. The combined organic layers were dried over Na$_2$SO$_4$, and after filtration, the solvent was evaporated in vacuo, giving the title compound as a white amorphous solid (217 mg, 50%). ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 8.40 (s, 1H, H-2), 7.68 (d, *J* = 14.5 Hz, 1H, H-5), 7.51–7.46 (m, 2H, Bn-Ar), 7.42–7.36 (m, 2H, Bn-Ar), 7.36–7.29 (m, 1H, Bn-Ar), 7.28 (d, *J* = 6.7 Hz, 1H, NH), 6.94 (d, *J* = 7.6 Hz, 1H, H-8), 5.25 (s, 2H, Bn-C**H**$_2$−), 4.23–4.08 (m, 1H, aminopyrrolidine-CH), 3.78–3.71 (m, 1H, aminopyrrolidine-CH), 3.69–3.62 (m, 1H, amino-pyrrolidine-CH), 3.59 (tt, *J* = 7.2, 4.0 Hz, 1H, *c*Pr-CH), 3.56–3.49 (m, 1H, aminopyrrolidine-CH), 3.43–3.36 (m, 1H, aminopyrroli-dine-CH), 2.13 (dddd, *J* = 13.5, 6.9, 6.9, 6.9 Hz, 1H, amino-

pyrrolidine-CH), 1.91 (dddd, *J* = 12.5, 6.2, 6.2, 6.2 Hz, 1H, aminopyrrolidine-CH), 1.39 (s, 9H, Boc-CH$_3$), 1.25–1.20 (m, 2H, *c*Pr-CH$_2$), 1.09–1.03 (m, 2H, *c*Pr-CH$_2$). ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ 171.51 (d, *J* = 2.0 Hz, C-4), 164.77 (COOBn), 155.29 (Boc-C=O), 149.47 (d, *J* = 242.8 Hz, C-6), 148.15 (C), 140.37 (d, *J* = 11.8 Hz, C-7), 138.64 (C), 136.79 (Bn-C), 128.42 (Bn-C), 127.77 (Bn-C), 127.62 (Bn-C), 117.82 (d, *J* = 5.9 Hz, C-4a), 111.54 (d, *J* = 22.8 Hz, C-5), 108.53 (C), 100.53 (d, *J* = 5.3 Hz, C-8), 77.98 (Boc-C), 65.14 (Bn-CH$_2$), 55.09 (d, *J* = 5.0 Hz, aminopyrrolidine-C), 49.85 (aminopyrrolidine-C), 47.98 (d, *J* = 4.7 Hz, aminopyrrolidine-C), 34.73 (*c*Pr-CH), 30.47 (aminopyrrolidine-C), 28.28 (Boc-CH$_3$), 7.57 (*c*Pr-CH$_2$), 7.52 (*c*Pr-CH$_2$). LR-MS: C$_{29}$H$_{33}$FN$_3$O$_5$$^+$ [M + H]$^+$, calcd 522.24; found, 522.2.

(S)-7-(3-Amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihy-dro-4-oxo-1,8-naphthyridine-3-carboxylic Acid Benzyl Ester ● HCl (30). Boc-**29** (187 mg, 0.36 mmol, 1 equiv) was dissolved in 4 mL HCl in dioxane (4 N) while cooling on ice. The reaction was allowed to warm to 22 °C and stirred for 1 h until full consumption of the starting material. After the solvent was evaporated in vacuo, the remaining solid was taken up in 2 mL MeOH and the product was precipitated with Et$_2$O. The precipitate was first washed three times with Et$_2$O, then dried in vacuo. The product was obtained as a yellow amorphous solid (160 mg, 97%). ^{1}H NMR (500 MHz, MeOH-*d*$_4$): δ 8.85 (s, 1H, H-2), 7.81 (d, *J* = 14.1 Hz, 1H, H-5), 7.53 (d, *J* = 7.0 Hz, 2H, Bn-H), 7.47–7.32 (m, 3H, Bn-H), 6.96 (d, *J* = 7.3 Hz, 1H, H-8), 5.49 (d, *J* = 8.4 Hz, 1H, Bn-CH$_2$), 5.39 (d, *J* = 12.1 Hz, 1H, Bn-CH$_2$), 4.18–4.07 (m, 2H, aminopyrrolidine-CH + aminopyrrolidine-CH), 4.05–3.98 (m, 1H, aminopyrrolidine-CH), 3.81–3.67 (m, 3H, aminopyrrolidine-CH$_2$ + *c*Pr-CH), 2.50 (dddd, 1H, aminopyrroli-dine-CH), 2.27 (dddd, *J* = 16.0, 5.8, 3.0 Hz, 1H, aminopyrrolidine-CH), 1.49–1.30 (m, 2H, *c*Pr-CH$_2$), 1.23–1.07 (m, 2H, *c*Pr-CH$_2$). ^{13}C NMR (126 MHz, MeOH-*d*$_4$): δ 171.63 (d, *J* = 3.6 Hz, C-4), 167.13 (COOBn), 152.61 (d, *J* = 250.1 Hz, C-6), 149.77 (C), 144.39 (d, *J* = 12.0 Hz, C-7), 141.62 (C), 136.83 (Bn-C), 129.92 (Bn-C), 129.84 (Bn-C), 129.79 (Bn-C), 115.09 (d, *J* = 8.2 Hz, C-4a), 112.27 (d, *J* = 24.7 Hz, C-5), 106.16 (C), 101.91 (d, *J* = 5.9 Hz, C-8), 68.76 (Bn-CH$_2$), 54.92 (d, *J* = 9.2 Hz, aminopyrrolidine-C), 51.82 (d, *J* = 3.0 Hz, aminopyrrolidine-C), 48.68 (aminopyrrolidine-C, extracted from ^{1}H−^{13}C-HSQC) 38.04 (*c*Pr-CH), 29.90 (aminopyrrolidine-C), 8.69 (2x *c*Pr-CH$_2$). LR-MS: C$_{24}$H$_{25}$FN$_3$O$_3$$^+$ [M + H]$^+$, calcd 422.19; found, 422.2.

Benzyl-7-((S)-3-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-4-methylpentanamido)propanamido)- Pyrrolidin-1-yl) -1-Cycloprop-yl-6-fluoro-4-oxo-1,4 -Dihydroquinoline-3-carboxylate (31). Amine **30** (55 mg, 0.12 mg, 1 equiv), *N*-Boc protected Leu−Ala **17** (54 mg, 0.18 mmol, 1.5 equiv), and DIPEA (100 µL, 0.6 mmol, 5 equiv) were dissolved in 900 µL dry DMF. TBTU (77 mg, 0.24 mmol, 2 equiv) was added and the reaction was heated to 40 °C. Reaction progress was monitored by TLC (CH$_2$Cl$_2$: MeOH, 95:5), and full turnover was achieved after 1 h. The reaction was concentrated in vacuo and diluted with CH$_2$Cl$_2$. The organic phase was washed with KHSO$_4$ (1 M), aqueous satd. NaHCO$_3$, and brine. The combined organic layers were dried over Na$_2$SO$_4$, and the solvent was evaporated in vacuo. After purification via normal phase MPLC (CH$_2$Cl$_2$: MeOH, 1–5%), the title compound was obtained as a beige amorphous solid (63 mg, 74%). ^{1}H NMR (500 MHz, Acetone-*d*$_6$): δ 8.47 (s, 1H, FQ-H-2), 8.21 (d, *J* = 7.0 Hz, 1H, NH), 7.62–7.51 (m, 3H, Bn-H, FQ-H-5), 7.47–7.38 (m, 2H, Bn-H), 7.37–7.31 (m, 1H, Bn-H), 6.73 (d, *J* = 7.5 Hz, 1H, FQ-H-8), 6.25 (d, *J* = 8.1 Hz, 1H, NH), 5.35 (d, *J* = 12.6 Hz, 1H, Bn-C**H**$_2$-), 5.26 (d, *J* = 12.6 Hz, 1H, Bn-C**H**$_2$-), 4.57–4.52 (m, 1H, aminopyrrolidine-H), 4.48 (dq, *J* = 7.2, 7.1 Hz, 1H, Ala-C$_\alpha$-H), 4.09–4.00 (m, 1H, Leu-C$_\alpha$-H), 3.92 (ddd, *J* = 10.3, 6.0, 3.8 Hz, 1H, aminopyrrolidine-H), 3.62 (dt, *J* = 9.7, 3.1 Hz, 1H, aminopyrrolidine-H), 3.56–3.46 (m, 1H, aminopyrrolidine-H), 3.45–3.37 (m, 2H, aminopyrrolidine-H, *c*Pr-CH), 2.24–2.11 (m, 1H, aminopyrrolidine-H), 2.03–1.96 (m, 1H, aminopyrrolidine-H), 1.65 (ddd, *J* = 13.1, 13.1, 6.6 Hz, 1H, Leu-CH$_2$), 1.57–1.49 (m, 2H, Leu-CH$_2$, Leu-CH), 1.40 (s, 9H, Boc-CH$_3$), 1.33 (d, *J* = 7.1 Hz, 3H, Ala-CH$_3$), 1.26–1.20 (m, 1H, *c*Pr-CH$_2$), 1.20–1.09 (m, 2H, *c*Pr-CH$_2$), 1.00−0.93 (m, 1H, *c*Pr-CH$_2$), 0.85 (d, *J* = 6.6 Hz, 3H, Leu-CH$_3$), 0.80 (d, *J* = 6.5 Hz, 3H,

Leu-CH$_3$). ^{13}C NMR (126 MHz, Acetone-d_6): δ 172.11 (d, J = 4.6 Hz, FQ-C=O), 171.85 (C=O), 171.78 (C=O) 164.70 (COOBn), 155.69 (Boc-C=O), 149.71 (d, J = 240.1 Hz, FQ-C-6), 147.77 (FQ-C), 140.87 (d, J = 11.6 Hz, (FQ-C-7), 138.53 (FQ-C), 137.13 (Bn-C), 128.42 (Bn-C), 128.18 (Bn-C), 127.87 (Bn-C), 118.04 (d, J = 9.4 Hz, FQ-C-4a), 111.94 (d, J = 22.3 Hz, FQ-C-5), 109.01 (FQ-C), 100.34 (FQ-C-8), 78.46 (Boc-C), 65.43 (Bn-CH$_2$), 55.12 (amino-pyrrolidine-C), 53.35 (Leu-C$_\alpha$), 49.65 (aminopyrrolidine-C), 48.77 (Ala-C$_\alpha$), 47.44 (Leu-C$_\alpha$), 40.69 (Leu-CH$_2$), 34.43 (cPr-CH), 31.18 (aminopyrrolidine-C), 27.68 (Boc-CH$_3$), 24.50 (Leu-CH), 22.58 (Leu-CH$_3$), 20.91 (Leu-CH$_3$), 18.24 (Ala-CH$_3$), 7.51 (cPr-CH$_2$), 7.43 (cPr-CH$_2$). LR-MS: C$_{38}$H$_{49}$FN$_5$O$_7^+$ [M + H]$^+$, calcd 706.36; found, 706.4.

7-((S)-3-((S)-2-((S)-2-Amino-4-methylpentanamido)-propanamido)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-di-hydroquinoline-3-carboxylic Acid (35). The title compound was synthesized from *N*-Boc protected dipeptidyl fluoroquinolone **31** over two chemical steps. *N*-Boc protected dipeptidyl fluoroquinolone **31** (60 mg, 0.09 mmol, 1 equiv) was dissolved in MeOH (1 mL), and Pd black (5 mg, 0.05 mmol, 0.5 equiv) was added. The reaction was stirred under H$_2$ atmosphere (1 atm) overnight at room temperature. Palladium was removed via centrifugation (17,600 rcf, 5 min), and the solvent was evaporated in vacuo. Residual solid was dissolved in HCl in dioxane (4 N) while cooling on ice. The reaction was allowed to warm to room temperature. After disappearance of the starting material (1 h), the solvent was evaporated in vacuo. The remaining solid was taken up in 2 mL MeOH, and the product was precipitated with Et$_2$O. The precipitate was isolated by decantation and further purified by preparative HPLC (H$_2$O: MeCN, 15−30%, 0.1% formic acid). After lyophilization, the product was obtained as an off-white solid (28 mg, 61%). ^{1}H NMR (500 MHz, MeOH-d_4): δ 8.53 (s, 0.5 H, HCOOH), 8.49 (s, 1H, FQ-H-2), 7.64 (d, J = 14.1 Hz, 1H, FQ-H-5), 6.99 (d, J = 7.3 Hz, 1H, FQ-H-8), 4.52 (s, 1H, Leu-C$_\alpha$-H), 4.40 (q, J = 6.2 Hz, 1H, Ala-C$_\alpha$-H), 4.00−3.84 (m, 1H, aminopyrrolidine-H), 3.84−3.74 (m, 1H, aminopyrrolidine-H), 3.74−3.55 (m, 4H, cPr-CH, 3x aminopyrrolidine-H), 2.38−2.25 (m, 1H, aminopyrrolidine-H), 2.20−2.07 (m, 1H, aminopyrrolidine-H), 1.82−1.71 (m, 1H, Leu-CH), 1.68 (ddd, J = 13.9, 8.1, 5.8 Hz, 1H, Leu-CH$_2$), 1.53 (ddd, J = 14.0, 8.3, 6.1 Hz, 1H, Leu-CH$_2$), 1.40 (d, J = 7.0 Hz, 3H, Ala-CH$_3$), 1.37 (s, 2H, cPr-CH$_2$), 1.20 (s, 2H, cPr-CH$_2$), 1.00 (d, J = 6.5 Hz, 3H, Leu-CH$_3$), 0.98 (d, J = 6.5 Hz, 3H, Leu-CH$_3$). ^{13}C NMR (126 MHz, MeOH-d_4): δ 177.28 (d, J = 2.8 Hz, FQ-C-4), 174.73 (FQ-COOH), 173.91 (HCOOH), 170.09 (C=O), 169.91 (C=O), 151.97 (d, J = 247.4 Hz, FQ-C-6), 148.41 (C), 143.39 (d, J = 11.8 Hz, FQ-C-7), 141.24 (C), 116.00 (d, J = 7.3 Hz, FQ-C-4a), 111.77 (d, J = 23.5 Hz, FQ-C-5), 107.33 (C), 101.55 (d, J = 5.9 Hz, FQ-C-8), 55.98 (d, J = 7.1 Hz, aminopyrrolidine-C), 53.56 (aminopyrrolidine-C), 50.93 (d, J = 1.8 Hz, aminopyrrolidine-C), 50.49 (cPr-CH), 43.46 (Leu-CH$_2$), 36.72 (cPr-CH), 31.88 (aminopyrrolidine-C), 25.56 (Leu-CH), 23.32 (Leu-CH$_3$), 22.27 (Leu-CH$_3$), 18.27 (Ala-CH$_3$), 8.39 (2x cPr-CH$_2$). HR-MS: calcd [C$_{26}$H$_{35}$FN$_5$O$_5$]$^+$, 516.2617; found, 516.2610.

LecA-Targeted Aminopyrrolidine-fluoroquinolone Prodrug 36. The title compound was synthesized in two chemical steps: First, LecA-targeted peptide linker **10** (31 mg, 0.049 mmol, 1 equiv), benzyl protected fluoroquinolone **30** (34 mg, 0.074 mmol, 1.5 equiv), and TBTU (24 mg, 0.074 mmol, 1.5 equiv) were dissolved in 1 mL dry DMF. DIPEA (27 μL, 0.16 mmol, 3.2 equiv) was added dropwise, and the reaction was stirred for 1 h. After evaporation of the solvent, the residue was taken up in MeOH (2 mL). Pd black (10 mg, 0.05 mmol, 1 equiv) was added, and the reaction was stirred under H$_2$ atmosphere for 2 d. Afterward, the reaction was filtered over celite and further purified by preparative HPLC (MeCN: H$_2$O, 20−33%, 0.1% formic acid). The title compound was obtained as a beige amorphous solid (22 mg, 48% over 2 chemical steps). ^{1}H NMR (500 MHz, MeOH-d_4): δ 8.60 (s, 1H, FQ-H-2), 7.82 (d, J = 8.2 Hz, 2H, Ar−H), 7.71 (d, J = 14.2 Hz, 1H, FQ-H-5), 7.56 (d, J = 8.3 Hz, 2H, Ar−H), 7.04 (d, J = 7.4 Hz, 1H, FQ-H-8), 4.71 (d, J = 9.8 Hz, 1H, glyco-H-1), 4.54−4.44 (m, 1H, aminopyrrolidine-CH), 4.36 (q, J = 7.2 Hz, 1H, Ala-C$_\alpha$-H), 4.33−4.25 (m, 2H, Ala-C$_\alpha$-H, Leu-C$_\alpha$-H), 3.91 (d, J = 3.5 Hz, 1H, glyco-H-4), 3.90−3.59 (m, 11H, cPr-CH, 2x amino-

pyrrolidine-CH$_2$, Gly-CH$_2$, glyco-H-2, glyco-H-5, glyco-H-6), 3.52 (dd, J = 9.2, 3.3 Hz, 1H, glyco-H-3), 2.37−2.20 (m, 1H, aminopyrrolidine-H), 2.20−2.10 (m, 1H, aminopyrrolidine-H), 1.75 (ddd, J = 14.6, 11.2, 3.6 Hz, 1H, Leu-CH$_2$), 1.70−1.56 (m, 2H, Leu-CH$_2$ + Leu-CH), 1.49 (d, J = 7.2 Hz, 3H, Ala-CH$_3$), 1.41 (d, J = 7.2 Hz, 3H, Ala-CH$_3$), 1.38 (d, J = 7.1 Hz, 2H, cPr-CH$_2$), 1.30−1.14 (m, 2H, cPr-CH$_2$), 0.87 (d, J = 6.2 Hz, 3H, Leu-CH$_3$), 0.85 (d, J = 6.2 Hz, 3H, Leu-CH$_3$). ^{13}C NMR (126 MHz, MeOH-d_4): δ 177.52 (d, J = 2.8 Hz, FQ-C=O), 176.71 (C=O), 175.15 (C=O), 174.86 (C=O), 172.46 (C=O), 170.07 (C=O), 169.97 (C=O), 152.04 (d, J = 247.4 Hz, FQ-C-6), 148.62 (FQ-C), 143.56 (d, J = 11.9 Hz, FQ-C-7), 142.33 (FQ-C), 141.38 (Ar−C), 132.15 (Ar−C), 129.81 (Ar−C), 129.18 (Ar−C), 116.06 (d, J = 6.5 Hz, FQ-C-4a), 111.92 (d, J = 23.6 Hz, FQ-C-5), 107.34 (FQ-C), 101.64 (d, J = 5.9 Hz, FQ-C-8), 88.88 (glyco-C-1), 80.79 (glyco-C-5), 76.26 (glyco-C-3), 70.85 (glyco-C-2), 70.39 (glyco-C-4), 62.67 (glyco-C-6), 55.95 (d, J = 5.3 Hz, aminopyrrolidine-C), 53.91 (Leu-C$_\alpha$), 52.60 (Ala-C$_\alpha$), 50.94 (aminopyrrolidine-C, Ala-C$_\alpha$), 44.21 (Gly-C$_\alpha$), 41.12 (Leu-CH$_2$), 36.82 (cPr-CH), 31.81 (aminopyrrolidine-C), 25.79 (Leu-CH), 23.55 (Leu-CH$_3$), 21.50 (Leu-CH$_3$), 17.78 (Ala-CH$_3$), 17.25 (Ala-CH$_3$), 8.49 (cPr-CH$_2$), 8.47 (cPr-CH$_2$). HR-MS: calcd [C$_{44}$H$_{57}$FN$_7$O$_{13}$S]$^+$, 942.3714; found, 942.3694.

LecB-Targeted Aminopyrrolidine-fluoroquinolone Prodrug Benzyl Ester 33. LecB-targeted peptide linker **16** (70 mg, 0.10 mmol, 1 equiv), benzyl protected fluoroquinolone **30** (55 mg, 0.12 mg, 1.2 equiv), and TBTU (48 mg, 0.15 mmol, 1.5 equiv) were dissolved in dry DMF (2 mL). DIPEA (52 μL, 0.3 mmol, 3 equiv) was added dropwise, and the reaction was stirred for 1 h. The solvent was evaporated in vacuo, and the reaction was purified by preparative HPLC (MeCN: H$_2$O, 25−40%, 0.1% formic acid). After lyophilization, the title compound was isolated as an off-white powder (53 mg, 49%). ^{1}H NMR (500 MHz, MeOH-$d4$): δ 8.56 (s, 1H, FQ-H-2), 8.04 (d, J = 8.2 Hz, 2H, glyco-Ar-H), 7.93 (d, J = 8.3 Hz, 2H, glyco-Ar-H), 7.75 (d, J = 14.5 Hz, 1H, FQ-H-5), 7.47 (d, J = 7.2 Hz, 2H, Bn), 7.39−7.34 (m, 2H, Bn), 7.34−7.29 (m, 1H, Bn), 6.98 (d, J = 7.5 Hz, 1H, FQ-H-8), 5.31 (s, 2H, Bn), 4.47−4.38 (m, 2H, Ala-Cα-H, aminopyrrolidine-H), 4.36−4.26 (m, 2H, Ala-Cα-H, Leu-Cα-H), 3.91−3.82 (m, 1H, aminopyrrolidine-H), 3.79 (d, J = 16.7 Hz, 1H, Gly-Cα-H), 3.80−3.74 (m, 1H, aminopyrrolidine-H), 3.66 (d, J = 16.7 Hz, 1H, Gly-Cα-H), 3.64−3.59 (m, 1H, aminopyrrolidine-H), 3.58 (d, J = 2.8 Hz, 1H, glyco-H-4), 3.58−3.51 (m, 2H, cPr-CH, aminopyrrolidine-H), 3.43 (dq, J = 6.5, 0.5 Hz, 1H, glyco-H-5), 3.40−3.35 (m, 2H, glyco-H-3, glyco-H-1), 3.34−3.27 (m, 1H, linker-$\underline{CH_2}$-), 3.10 (ddd, J = 9.0, 7.1, 2.4 Hz, 1H, glyco-H-2), 3.02 (dd, J = 12.9, 7.1 Hz, 1H, linker-$\underline{CH_2}$-), 2.35−2.20 (m, 1H, aminopyrrolidine-H), 2.15−2.07 (m, 1H, aminopyrrolidine-H), 1.81−1.69 (m, 1H, Leu-CH), 1.69−1.55 (m, 2H, Leu-CH$_2$), 1.50 (d, J = 7.2 Hz, 3H, Leu-CH$_3$), 1.39 (d, J = 7.2 Hz, 3H, Leu-CH$_3$), 1.33−1.28 (m, 1H, cPr-CH$_2$), 1.16 (d, J = 6.4 Hz, 3H, glyco-H-6), 1.12−1.05 (m, 2H, cPr-CH$_2$), 0.85 (d, J = 6.4 Hz, 3H, Leu-CH$_3$), 0.84 (d, J = 6.3 Hz, 3H, Leu-CH$_3$). ^{13}C NMR (126 MHz, MeOH-$d4$): δ 176.30 (CONH), 175.32 (d, J = 2.7 Hz, FQ-C=O), 175.05 (CONH), 174.75 (CONH), 172.38 (CONH), 169.09 (CONH), 166.10 (COOBn), 151.73 (d, J = 244.9 Hz, FQ-C-6), 149.61 (FQ-Ar-C), 145.13 (Ar−C), 142.67 (d, J = 12.0 Hz, FQ-C-7), 140.44 (Ar−C), 138.34 (Ar−C), 138.00 (Ar−C), 129.56 (glyco-Ar-C), 129.53 (Bn-Ar-C), 129.25 (Bn-Ar-C), 129.13 (Bn-Ar-C), 128.10 (glyco-Ar-C), 119.19 (d, J = 6.7 Hz, FQ-C-4a), 112.91 (d, J = 23.1 Hz, FQ-C-5), 109.81 (Ar−C), 101.62 (d, J = 5.1 Hz, FQ-C-8), 79.62 (glyco-C-2), 76.32 (glyco-C-3), 75.51 (glyco-C-5), 73.54 (glyco-C-4), 69.60 (glyco-C-1), 67.07 (Bn), 55.94 (d, J = 6.7 Hz, aminopyrrolidine-C), 53.94 (Leu-Cα), 52.54 (Ala-Cα), 50.87 (d, J = 1.8 Hz, aminopyrrolidine-C), 50.76 (Ala-Cα), 48.84 (aminopyrrolidine-C, extracted from ^{1}H−^{13}C-HSQC) 45.55 (glyco-linker-CH$_2$-), 44.19 (Gly-Cα), 41.16 (Leu-CH$_2$), 36.18 (cPr-CH), 31.88 (aminopyrrolidine-C), 25.80 (Leu-CH), 23.52 (Leu-CH$_3$), 21.53 (Leu-CH$_3$), 17.88 (Ala-CH$_3$), 17.26 (Ala-CH$_3$), 17.11 (glyco-C-6), 8.51 (cPr-CH$_2$), 8.49 (cPr-CH$_2$). LR-MS: C$_{52}$H$_{67}$FN$_8$O$_{14}$S^{2+} [M +2H]$^{2+}$, calcd 539.22; found, 539.2 [M+2H]$^{2+}$.

LecB-Targeted Aminopyrrolidine-FQ-Prodrug 37. Benzyl protected LecB-targeted aminopyrrolidine-FQ-prodrug **33** (40 mg, 0.037

mmol, 1 equiv) was dissolved in MeOH (1 mL). Pd/C (10 wt-%, 4 mg) was added, and the atmosphere was changed to H_2 (1 atm). The reaction was stirred at room temperature for 24 h until full consumption of the starting material. Pd/C was removed by centrifugation (17600 rcf, 10 min), and the solvent was removed in vacuo. After purification by preparative HPLC (MeCN in water, 22−35%, 0.1% formic acid), the title compound was obtained as an off-white amorphous solid (15 mg, 41%). ^{1}H NMR (500 MHz, MeOH-d4): δ 8.78 (s, 1H, FQ-H-2), 8.05 (d, J = 8.1 Hz, 2H, glyco-Ar-H), 7.93 (d, J = 8.3 Hz, 2H, glyco-Ar-H), 7.90 (d, J = 13.1 Hz, 1H, FQ-H-5), 7.61 (d, J = 7.2 Hz, 1H, FQ-H-8), 4.90 (q, J = 6.9 Hz, 1H, Ala-C$_\alpha$-H), 4.54−4.37 (m, 2H, Ala′-Cα-H, Leu-C$_\alpha$-H), 4.00 (d, J = 16.9 Hz, 1H, Gly-C$_\alpha$-H), 3.96−3.84 (m, 2H, 2x aminopyrrolidine-H), 3.83−3.72 (m, 2H, aminopyrrolidine-H, Gly-C$_\alpha$-H), 3.72−3.63 (m, 1H, aminopyrrolidine-H), 3.58 (d, J = 2.9 Hz, 1H, glyco-H-4), 3.50−3.27 (m, 7H, 3x aminopyrrolidine-H, glyco-H-5, glyco-H-1, glyco-H-3, glyco-linker-C$\underline{H}_2$-), 3.10 (ddd, J = 9.0, 7.1, 2.4 Hz, 1H, glyco-H-2), 3.02 (dd, J = 12.9, 7.2 Hz, 1H, glyco-linker-C$\underline{H}_2$-), 1.78−1.57 (m, 3H, Leu-CH, Leu-CH$_2$), 1.50 (d, J = 7.1 Hz, 3H, Ala-CH$_3$), 1.42 (d, J = 6.9 Hz, 2H, cPr-CH$_2$), 1.36 (d, J = 6.8 Hz, 3H, Ala′-CH$_3$), 1.26−1.20 (m, 2H, cPr-CH$_2$), 1.16 (d, J = 6.4 Hz, 3H, glyco-H-6), 0.87 (d, J = 5.6 Hz, 3H, Leu-CH$_3$), 0.83 (d, J = 5.6 Hz, 3H, Leu-CH$_3$). ^{13}C NMR (126 MHz, MeOH-d4): δ 178.37 (d, J = 2.0 Hz, FQ-C=O), 175.82 (CONH), 174.14 (CONH), 172.57 (CONH), 171.68 (CONH), 169.67 (CONH), 168.86 (COOH), 155.08 (d, J = 250.0 Hz, FQ-C-6), 149.36 (FQ-C-2), 146.84 (d, J = 9.9 Hz, FQ-C-7), 145.06 (Ar−C), 140.78 (Ar−C), 138.51 (Ar−C), 129.53 (glyco-Ar-C), 128.08 (glyco-Ar-C), 121.07 (d, J = 7.0 Hz, FQ-C-4a), 112.56 (d, J = 23.4 Hz, FQ-C-5), 107.68 (Ar−C), 79.62 (glyco-C-2), 76.33 (glyco-C-3), 75.52 (glyco-C-5), 73.55 (glyco-C-4), 69.61 (glyco-C-1), 53.12 (Leu-Cα), 52.12 (Ala-Cα), 51.06 (aminopyrrolidine-C), 50.67 (d, J = 3.8 Hz, aminopyrrolidine-C), 46.60 (Ala′-Cα), 46.46 (aminopyrrolidine-C), 45.55 (glyco-linker-C$\underline{H}_2$-), 43.91 (Gly-Cα), 43.17 (aminopyrrolidine-C), 41.61 (Leu-CH$_2$), 37.02 (cPr-CH), 25.74 (Leu-CH), 23.54 (Leu-CH$_3$), 21.63 (Leu-CH$_3$), 17.85 (Ala-CH$_3$), 17.26 (Ala′-CH$_3$), 17.10 (glyco-CH$_3$), 8.61 (cPr-CH$_2$), 8.56 (cPr-CH$_2$). HR-MS: calcd $[C_{45}H_{60}FN_8O_{14}S]^+$, 987.3928; found, 987.3903.

(S)-7-(3-(hydroxymethyl)-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (39). 7-chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**26**, 1000 mg, 3.55 mmol, 1 equiv) was dispersed in dry pyridine (10 mL) and heated to 80 °C to fully dissolve. L-β-prolinol (**38**, 732 μL, 7.1 mmol, 2 equiv) was added, and the temperature was increased to 140 °C. The reaction was refluxed overnight. After cooling to room temperature, the product precipitated from the reaction and was isolated by filtration. The precipitate was washed with ice-cold MeOH and obtained as a yellow solid (750 mg, 62%). ^{1}H NMR (500 MHz, MeOH-d_4 + CDCl$_3$, 1:1): δ 8.78 (s, 1H, FQ-H-2), 7.87 (d, J = 13.9 Hz, 1H, FQ-H-5), 7.03 (d, J = 7.3 Hz, 1H, FQ-H-8), 3.88−3.76 (m, 2H, 2× pyrrolidinyl-methanol-H), 3.76−3.67 (m, 3H, 2x pyrrolidinyl-methanol-H, cPr-CH), 3.60 (dd, J = 10.8, 7.2 Hz, 1H, pyrrolidinyl-methanol-H), 3.51 (ddd, J = 10.1, 7.4, 2.1 Hz, 2H, 2× pyrrolidinyl-methanol-H), 2.59 (hept, J = 7.2 Hz, 1H, pyrrolidinyl-methanol-H), 2.18 (dtd, J = 11.9, 7.1, 4.5 Hz, 1H, pyrrolidinyl-methanol-H), 1.88 (dq, J = 12.6, 8.2 Hz, 1H, pyrrolidinyl-methanol-H), 1.45 (q, J = 6.8 Hz, 2H, cPr-CH$_2$), 1.21 (q, J = 6.6 Hz, 2H, cPr-CH$_2$). ^{13}C NMR (126 MHz, MeOH-d_4 + CDCl$_3$, 1:1): δ 173.16 (FQ-C=O), 169.65 (COOH), 152.06 (d, J = 252.5 Hz, FQ-C-6), 148.01 (FQ-C-2), 144.44 (d, J = 15.8 Hz, FQ-C-7), 141.51 (FQ-C), 128.36 (FQ-C), 111.48 (d, J = 24.1 Hz, FQ-C-5), 105.36 (d, J = 2.6 Hz, FQ-C-8), 100.04 (d, J = 6.3 Hz, FQ-C-4a), 63.69 (pyrrolidinyl-methanol-C), 53.78 (d, J = 5.7 Hz, pyrrolidinyl-methanol-C), 50.71 (d, J = 6.6 Hz, pyrrolidinyl-methanol-C), 41.38 (d, J = 1.9 Hz, pyrrolidinyl-methanol-C), 37.18 (s, cPr-CH), 28.25 (pyrrolidinyl-methanol-C), 8.47 (cPr-CH$_2$). LR-MS: $C_{18}H_{20}FN_2O_4^+$ $[M + H]^+$, calcd 347.14; found, 347.2.

(S)-7-(3-(hydroxymethyl)-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid methyl Ester (40). (S)-7-(3-(hydroxymethyl)-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**39,**

500 mg, 1.44 mmol, 1 equiv) and *rac*-camphor-10-sulfonic acid (668 mg, 2.88 mmol, 2 equiv) were dried under high vacuum and subsequently dissolved in dry MeOH (15 mL). The reaction was refluxed until a clear solution was obtained (72 h). After cooling to room temperature, the solvent was evaporated in vacuo. The residual solid was dissolved in CH$_2$Cl$_2$ (10 mL), and the organic phase was washed with satd. aqueous NaHCO$_3$ (3 x) and brine (2 x) and dried over Na$_2$SO$_4$. After evaporation of the solvent, the product was obtained as a yellow solid (510 mg 96%), containing 4% of the starting material as an impurity, determined by ^{1}H NMR. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.35 (s, 1H, FQ-H-2), 7.64 (d, J = 14.6 Hz, 1H, FQ-H-5), 6.94 (d, J = 7.6 Hz, 1H, FQ-H-8), 4.80 (t, J = 5.2 Hz, 1H, OH), 3.72 (s, 3H, COOMe), 3.66−3.52 (m, 4H, 3x pyrrolidinyl-methanol-H, cPr-CH), 3.49 (td, J = 10.8, 5.0 Hz, 1H, pyrrolidinyl-methanol-H), 3.46−3.39 (m, 1H, pyrrolidinyl-methanol-H), 3.35−3.30 (m, 1H, pyrrolidinyl-methanol-H), 2.44 (p, J = 7.0 Hz, 1H, pyrrolidinyl-methanol-H), 2.04 (dtd, J = 11.8, 7.1, 4.8 Hz, 1H, pyrrolidinyl-methanol-H), 1.76 (dq, J = 11.8, 7.7 Hz, 1H, pyrrolidinyl-methanol-H), 1.27−1.20 (m, 1H, cPr-CH$_2$), 1.11−1.02 (m, 1H, cPr-CH$_2$). ^{13}C NMR (126 MHz, DMSO-d_6): δ 171.46 (FQ-C=O), 165.20 (COOMe), 149.43 (d, J = 242.8 Hz, FQ-C-6), 147.93 (FQ-C-2), 140.48 (d, J = 11.3 Hz, FQ-C-7), 138.63 (FQ-C), 117.62 (d, J = 5.5 Hz, FQ-C-4a), 111.44 (d, J = 22.5 Hz, FQ-C-5), 108.57 (FQ-C), 100.47 (d, J = 5.3 Hz, FQ-C-8), 62.55 (pyrrolidinyl-methanol-C), 52.50 (d, J = 5.4 Hz, pyrrolidinyl-methanol-C), 51.24 (COO$\underline{CH}_3$), 49.23 (d, J = 5.3 Hz, pyrrolidinyl-methanol-C), 40.68 (d, J = 1.7 Hz, pyrrolidinyl-methanol-C), 34.63 (cPr-CH), 27.46 (pyrrolidinyl-methanol-C), 7.53 (cPr-CH$_2$). LR-MS: $C_{19}H_{22}FN_2O_4^+$ $[M + H]^+$, calcd 361.16; found, 361.2.

(S)-7-(3-(azidomethyl)-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid methyl Ester (41). (S)-7-(3-(hydroxymethyl)-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid methyl ester (**40**,400 mg, 1.1 mmol, 1 equiv) and PPh$_3$ (577 mg, 2.2 mmol, 2 equiv) were dispersed in dry THF (10 mL) at room temperature. Diisopropyl azodicarboxylate (DIAD, 473 μL, 2.2 mmol, 2 equiv) was added dropwise under vigorous stirring, resulting in a clear solution. Afterward, DPPA (432 μL, 2.2 mmol, 2 equiv) was added dropwise, which resulted in precipitation after 10 min. The reaction was stirred for 1 h and subsequently quenched with MeOH. After evaporation of the solvent in vacuo, the product was purified via normal phase MPLC (CH$_2$Cl$_2$/PE (9/5): EtOH, 1−5%), yielding the title compound as an off-white solid (277 mg, 65%). ^{1}H NMR (500 MHz, MeOH-d_4): δ 8.46 (s, 1H, FQ-H-2), 7.64 (d, J = 14.7 Hz, 1H, FQ-H-5), 6.89 (d, J = 7.6 Hz, 1H, FQ-H-8), 3.82 (s, 3H, COOCH$_3$) 3.71 (ddd, J = 10.3, 7.3, 3.1 Hz, 1H, azidomethylpyrrolidine-H), 3.65 (ddq, J = 10.8, 7.6, 3.5 Hz, 1H, azidomethylpyrrolidine-H), 3.62−3.55 (m, 1H, azidomethylpyrrolidine-H), 3.55−3.50 (m, 2H, azidomethylpyrrolidine-H, cPr-CH), 3.47 (dd, J = 12.3, 7.3 Hz, 1H, azidomethylpyrrolidine-H), 3.35 (ddd, J = 10.2, 7.4, 2.7 Hz, 1H, azidomethylpyrrolidine-H), 2.61 (hept, J = 7.2 Hz, 1H, azidomethylpyrrolidine-H), 2.20 (dtd, J = 11.6, 7.0, 4.1 Hz, 1H, azidomethylpyrrolidine-H), 1.84 (dq, J = 12.4, 8.2 Hz, 1H, azidomethylpyrrolidine-H), 1.40−1.24 (m, 2H, cPr-CH$_2$), 1.20−1.06 (m, 2H, cPr-CH$_2$). ^{13}C NMR (126 MHz, MeOH-d_4): δ 175.19 (FQ-C=O), 166.90 (COOMe), 151.68 (d, J = 244.4 Hz, FQ-C-6), 149.64 (FQ-C-2), 142.60 (d, J = 11.8 Hz, FQ-C-7), 140.45 (FQ-C), 119.15 (d, J = 6.4 Hz, FQ-C-4a), 112.85 (d, J = 23.1 Hz, FQ-C-5), 109.77 (FQ-C), 101.46 (d, J = 5.4 Hz, FQ-C-8), 54.73 (azidomethylpyrrolidine-C), 54.24 (d, J = 6.2 Hz, azidomethylpyrrolidine-C), 52.01 (COO$\underline{CH}_3$), 50.46 (d, J = 5.6 Hz, azidomethylpyrrolidine-C), 39.86 (azidomethylpyrrolidine-C), 36.11 (cPr-CH), 29.77 (azidomethylpyrrolidine-C), 8.44 (cPr-CH$_2$). LR-MS: $C_{19}H_{21}FN_5O_3^+$ $[M + H]^+$, calcd 386.16; found, 386.2.

(S)-7-(3-(azidomethyl)-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid • HCl (42). (S)-7-(3-(azidomethyl)-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid methyl ester (**41**, 150 mg, 0.39 mmol, 1 equiv) and Pd/C (10% m/m, 42 mg, 10 mol %) were stirred in MeOH (10 mL) under H$_2$ atmosphere (1 atm)

overnight. The reaction was filtered over celite and concentrated in vacuo. HCl in dioxane (4 N, 100 μL) was mixed with 40 mL Et$_2$O and carefully added to the solution of product in MeOH while stirring on ice, yielding the title compound as a yellow solid (120 mg, 78%) ^{1}H NMR (500 MHz, D$_2$O): δ 8.34 (s, 1H, FQ-H-2), 7.27 (d, J = 14.6 Hz, 1H, FQ-H-5), 6.54 (d, J = 7.4 Hz, 1H, FQ-H-8), 3.82 (s, 3H, COOC<u>H</u>$_3$), 3.69–3.60 (m, 1H, aminomethylpyrrolidine-H), 3.55–3.40 (m, 2H, aminomethylpyrrolidine-H, cPr-CH), 3.30 (s, 1H), 3.25–3.08 (m, 3H, 3x aminomethylpyrrolidine-H), 2.72–2.58 (m, 1H, aminomethylpyrrolidine-H), 2.35–2.20 (m, 1H, aminomethyl-pyrrolidine-H), 1.94–1.64 (m, 1H, aminomethylpyrrolidine-H), 1.25 (d, J = 5.6 Hz, 2H, cPr-CH$_2$), 0.98 (s, 2H, cPr-CH$_2$). ^{13}C NMR (126 MHz, D$_2$O): δ 173.63 (FQ-C=O), 166.68 (<u>C</u>OOCH$_3$), 149.62 (d, J = 245.1 Hz, FQ-C-6), 148.66 (FQ-C-2), 140.59 (d, J = 11.7 Hz, FQ-C-7), 138.43 (FQ-C), 116.50 (d, J = 6.5 Hz, FQ-C-4a), 110.83 (d, J = 23.6 Hz, FQ-C-5), 107.09 (FQ-C), 99.97 (d, J = 5.4 Hz, FQ-C-8), 52.65 (d, J = 5.8 Hz, aminomethylpyrrolidine-C), 51.90 (COO<u>C</u>H$_3$), 48.98 (d, J = 6.0 Hz, aminomethylpyrrolidine-C), 41.69 (aminomethylpyrrolidine-C), 36.30 (cPr-CH), 35.01 (aminomethylpyrrolidine-C), 28.55 (aminomethylpyrrolidine-C), 7.13 (cPr-CH$_2$). LR-MS: C$_{19}$H$_{23}$FN$_3$O$_3$$^+$ [M + H]$^+$, calcd 360.17; found, 360.2.

(S)-7-(3-(aminomethyl)-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (3). (S)-7-(3-(azidomethyl)-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid ● HCl (**42**, 39 mg, 0.1 mmol, 1 equiv) was dissolved in a mixture of THF, MeOH, and H$_2$O (3/1/1) at room temperature. LiOH (13 mg, 0.5 mmol, 5 equiv) was added, and the reaction was stirred overnight until full transformation. After evaporation of the solvent in vacuo, the product was purified by preparative HPLC (MeCN in water, 10–25%, 0.1% formic acid), yielding the title compound as an off-white amorphous solid (15 mg, 43%). ^{1}H NMR (500 MHz, D$_2$O): δ 8.31 (s, 1H, FQ-H-2), 6.93 (d, J = 13.8 Hz, 1H, FQ-H-5), 6.56 (d, J = 7.4 Hz, 1H, FQ-H-8), 3.75–3.67 (m, 1, aminomethylpyrrolidine-H), 3.63–3.51 (m, 1H, aminomethylpyrrolidine-H), 3.54–3.48 (m, 1H, aminomethylpyrrolidine-H), 3.42 (s, 1H, aminomethylpyrrolidine-H), 3.29–3.12 (m, 3H, cPr-CH, aminomethylpyrrolidine-CH$_2$), 2.69 (dt, J = 14.2, 5.8 Hz, 1H, aminomethylpyrrolidine-H), 2.35–2.23 (m, 1H, aminomethylpyrrolidine-H), 1.89–1.75 (m, 1H, aminomethylpyrrolidine-H), 1.35 (d, J = 6.2 Hz, 2H, cPr-CH$_2$), 1.08 (s, 2H, cPr-CH$_2$). ^{13}C NMR (126 MHz, D$_2$O): δ 173.86 (d, J = 3.5 Hz, FQ-C=O), 169.38 (COOH), 149.71 (d, J = 249.2 Hz, FQ-C-6), 146.83 (FQ-C), 141.48 (d, J = 11.1 Hz, FQ-C-7), 139.22 (FQ-C), 112.55 (d, J = 7.3 Hz, FQ-C-4a), 109.34 (d, J = 23.5 Hz, FQ-C-5), 104.99 (d, J = 3.5 Hz, FQ-C-8a), 99.64 (d, J = 5.6 Hz, FQ-C-8), 52.70 (d, J = 6.8 Hz, aminomethylpyrrolidine-C), 49.19 (d, J = 4.9 Hz, aminomethylpyrrolidine-C), 41.51 (aminomethylpyrrolidine-C), 36.32 (aminomethylpyrrolidine-C), 35.72 (cPr-CH), 28.39 (aminomethylpyrrolidine-C), 7.22 (cPr-CH$_2$). HR-MS: calcd [C$_{18}$H$_{21}$FN$_3$O$_3$]$^+$, 346.1561; found, 346.1555.

1-Cyclopropyl-6-fluoro-7-((R)-3-((4S,7S)-7-isobutyl-4,11,11-tri-methyl-3,6,9-trioxo-10-oxa-2,5,8-triazadodecyl)pyrrolidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid methyl Ester (43). (S)-7-(3-(azidomethyl)-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihy-dro-4-oxo-1,8-naphthyridine-3-carboxylic acid ● HCl (**42**, 50 mg, 0.13 mmol, 1 equiv), **17** (48 mg, 0.16 mmol, 1.2 equiv), and TBTU (51 mg, 0.16 mmol, 1.2 equiv) were dissolved in dry DMF (1.5 mL) and cooled on ice. DIPEA (45 μL, 0.26 mmol, 2 equiv) was added dropwise, and the reaction was allowed to warm to room temperature. After 16 h, the solvent was concentrated in vacuo and diluted with CH$_2$Cl$_2$ (20 mL). The organic phase was washed with aqueous KHSO$_4$ (1 M, 2x), neutralized with aqueous satd. NaHCO$_3$ (1x), washed with brine (2x), and dried over Na$_2$SO$_4$. After purification via normal phase MPLC (CH$_2$Cl$_2$/MeOH, 1–10%), the title compound was obtained as a white solid (42 mg, 50%). The compound was directly used for global deprotection. LR-MS: C$_{33}$H$_{47}$FN$_5$O$_7$$^+$ [M + H]$^+$, calcd 644.35; found, 644.4.

1-Cyclopropyl-6-fluoro-7-((R)-3-((4S,7S)-7-isobutyl-4,11,11-tri-methyl-3,6,9-trioxo-10-oxa-2,5,8-triazadodecyl)pyrrolidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (47). The title com-pound was synthesized over two chemical steps: 1-cyclopropyl-6-fluoro-7-((R)-3-((4S,7S)-7-isobutyl-4,11,11-trimethyl-3,6,9-trioxo-10-oxa-2,5,8-triazadodecyl)pyrrolidin-1-yl)-4-oxo-1,4-dihydroquino-line-3-carboxylic acid methyl ester (**43**, 42 mg, 0.065 mmol, 1 equiv) and LiOH (7.8 mg, 0.325 mmol, 5 equiv) were dissolved in a mixture of THF/H$_2$O/MeOH (5/5/1, 1.5 mL) and stirred at room temperature until full consumption of the starting material (3 h). The reaction was diluted with MeOH (5 mL) and neutralized with Amberlite IR-120 H$^+$ exchange resin. After evaporation of the solvent, the residue (35 mg, 86%) was dissolved in a mixture of dioxane/MeOH (8/2, 1 mL). While cooling on ice, HCl in dioxane (4 N, 2 mL) was added dropwise. The reaction was allowed to warm to 22 °C and stirred until full consumption of the starting material (1 h). After removal of the solvent in vacuo, the product was purified by preparative HPLC (H$_2$O/MeCN, 18–30%, 0.1% formic acid). The title compound was obtained as a white solid (16 mg, 46% over two steps). ^{1}H NMR (500 MHz, MeOH-d_4): δ 9.01 (s, 1H, FQ-H-2), 7.96 (d, J = 13.9 Hz, 1H, FQ-H-5), 7.23 (d, J = 7.4 Hz, 1H, FQ-H-8), 4.40 (q, J = 7.1 Hz, 1H, Ala-C$_α$-H), 4.04–3.83 (m, 4H, cPr-CH, Ala-C$_α$-H, Leu-C$_α$-H, aminomethylpyrrolidine-H), 3.81–3.70 (m, 1H, aminomethylpyrrolidine-H), 3.61–3.53 (m, 1H, aminomethylpyrrolidine-H), 3.36 (d, J = 6.9 Hz, 2H, aminomethylpyrrolidine-CH$_2$), 2.63 (tt, J = 7.1, 7.1 Hz, 1H, aminomethylpyrrolidine-H), 2.38–2.09 (m, 1H, aminomethylpyrrolidine-H), 1.97–1.84 (m, 1H, amino-methylpyrrolidine-H), 1.83–1.72 (m, 2H, Leu-CH$_2$, Leu-CH), 1.72–1.63 (m, 1H, Leu-CH$_2$), 1.53–1.48 (m, 2H, cPr-CH$_2$), 1.42 (d, J = 7.1 Hz, 3H, Ala-CH$_3$), 1.34–1.24 (m, 2H, cPr-CH$_2$), 1.02 (d, J = 6.2 Hz, 3H, Leu-CH$_3$), 1.00 (d, J = 6.1 Hz, 3H, Leu-CH$_3$). ^{13}C NMR (126 MHz, MeOH-d_4): δ 174.81 (C=O), 171.62 (d, J = 4.2 Hz, FQ-C=O), 170.69 (C=O), 170.36 (COOH), 153.15 (d, J = 252.6 Hz, FQ-C-6), 149.44 (FQ-C-2), 145.64 (d, J = 12.1 Hz, FQ-C-7), 142.75 (FQ-C), 112.62 (d, J = 8.4 Hz, FQ-C-4a), 111.28 (d, J = 24.9 Hz, FQ-C-5), 104.76 (FQ-C), 101.14 (d, J = 6.3 Hz, FQ-C-8), 55.03 (d, J = 6.5 Hz, aminomethylpyrrolidine-C), 52.84 (Ala-C$_α$), 51.17 (d, J = 5.9 Hz, aminomethylpyrrolidine-C), 50.74 (Ala-C$_α$), 42.15 (aminomethylpyr-rolidine-C), 41.66 (Leu-CH2), 40.25 (aminomethylpyrrolidine-C), 38.49 (cPr-CH), 29.67 (aminomethylpyrrolidine-C), 25.35 (Leu-CH), 23.18 (Leu-CH$_3$), 22.07 (Leu-CH$_3$), 18.30 (Ala-CH$_3$), 8.64 (cPr-CH$_2$). HR-MS: calcd [C$_{27}$H$_{37}$FN$_5$O$_5$]$^+$, 530.2773,; found, 530.2766.

LecA-Targeted Aminomethylpyrrolidine-FQ-Prodrug 48. The title compound was synthesized in two chemical steps: First, LecA-targeted peptide linker **10** (27 mg, 0.049 mmol, 1 equiv), (S)-7-(3-(azidomethyl)-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid·HCl (**42**, 30 mg, 0.076 mmol, 1.8 equiv), and TBTU (21 mg, 0.065 mmol, 1.8 equiv) were dissolved in 2 mL dry DMF. DIPEA (15 μL, 0.086 mmol, 2 equiv) was added dropwise, and the reaction was stirred for 1 h. After evaporation of the solvent, the residue was taken up in 1 mL H$_2$O/THF (1:1). LiOH (10 mg, 0.4 mmol, 10 equiv) was dissolved in 1 mL water and added stepwise to the reaction until a full transformation was observed (3 h). Afterward, the reaction was neutralized with Amberlite IR-120 H$^+$ exchange resin and further purified by preparative HPLC (MeCN/H$_2$O, 20–33%, 0.1% formic acid). The title compound was obtained as a beige amorphous solid (33 mg, 70% over 2 chemical steps). ^{1}H NMR (500 MHz, MeOH-d_4): δ 8.65 (s, 1H, FQ-H-2), 7.79 (d, J = 8.0 Hz, 2H, Ar–H), 7.72 (d, J = 14.2 Hz, 1H, FQ-H-5), 7.52 (d, J = 8.0 Hz, 2H, Ar–H), 7.02 (d, J = 7.2 Hz, 1H, FQ-H-8), 4.71 (d, J = 9.8 Hz, 1H, glyco-H-1), 4.43–4.32 (m, 2H, Ala-C$_α$-H, Leu-C$_α$-H), 4.29 (q, J = 7.3 Hz, 1H, Ala-C$_α$-H), 3.97–3.89 (m, 2H, glyco-H-4, Gly-CH), 3.82–3.58 (m, 9H, Gly-CH, 3x aminomethylpyrrolidine-H, cPr-CH, glyco-H-6, glyco-H-2, glyco-H-5), 3.52 (dd, J = 9.2, 3.3 Hz, 1H, glyco-H-3), 3.47–3.33 (m, 2H, 2× aminomethylpyrrolidine-H), 3.29–3.22 (m, 1H, aminomethylpyrrolidine-H), 2.60 (tt, J = 13.4, 6.6 Hz, 1H, aminomethylpyrrolidine-H), 2.18 (td, J = 12.0, 6.2 Hz, 1H, aminomethylpyrrolidine-H), 1.86–1.71 (m, 1H, aminomethyl-pyrrolidine-H, Leu-CH$_2$), 1.71–1.58 (m, 2H, Leu-CH$_2$, Leu-CH), 1.49 (d, J = 7.3 Hz, 3H, Ala-CH$_3$), 1.42 (d, J = 7.2 Hz, 3H, Ala-CH$_3$), 1.39–1.36 (m, 2H, cPr-CH$_2$), 1.18 (s, 2H, cPr-CH$_2$), 0.88 (d, J = 5.5 Hz, 6H, 2× Leu-CH$_3$). ^{13}C NMR (126 MHz, MeOH-d_4): δ 177.62 (C=O), 176.64 (C=O), 175.27 (C=O), 174.88 (C=O), 172.68

(C=O), 170.26 (C=O), 169.79 (C=O), 152.02 (d, J = 248.5 Hz, FQ-C-6), 148.66 (FQ-C), 142.31 (FQ-C), 141.49 (Ar−C), 132.09 (Ar−C), 129.69 (Ar−C), 129.13 (Ar−C), 111.98 (d, J = 23.3 Hz, FQ-C-5), 101.44 (d, J = 6.1 Hz, FQ-C-8), 88.86 (glyco-C-1), 80.79 (glyco-C-5), 76.26 (glyco-C-3), 70.85 (glyco-C-2), 70.40 (glyco-C-4), 62.68 (glyco-C-6), 54.49 (d, J = 5.9 Hz, aminomethylpyrrolidine-C), 53.94 (Leu-C$_\alpha$), 52.42 (Ala-C$_\alpha$), 51.07 (Ala-C$_\alpha$), 50.67 (d, J = 6.2 Hz, aminomethylpyrrolidine-C), 44.22 (Gly-C$_\alpha$), 42.42 (aminomethyl-pyrrolidine-C), 41.16 (Leu-CH$_2$), 40.00 (aminomethylpyrrolidine-C), 36.85 (cPr-CH), 29.90 (aminomethylpyrrolidine-C), 25.80 (Leu-CH), 23.57 (Leu-CH$_3$), 21.49 (Leu-CH$_3$), 17.66 (Ala-CH$_3$), 17.31 (Ala-CH$_3$), 8.53 (cPr-CH$_2$), 8.49 (cPr-CH$_2$). HR-MS: calcd [C$_{45}$H$_{59}$FN$_7$O$_{13}$S]$^+$, 956.3870; found, 956.3852.

LecB-Targeted Aminomethylpyrrolidine-FQ-Prodrug methyl Ester 45. LecB-targeted peptide linker **16** (70 mg, 0.10 mmol, 1 equiv), (*S*)-7-(3-(azidomethyl)-1-pyrrolidinyl)-1-cyclopropyl-6-fluo-ro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid ● HCl (**42**, 48 mg, 0.12 mg, 1.2 equiv), and TBTU (48 mg, 0.15 mmol, 1.5 equiv) were dissolved in dry DMF (2 mL). DIPEA (64 μL, 0.36 mmol, 3.6 equiv) was added dropwise and the reaction was stirred for 1 h. The solvent was evaporated in vacuo, and the reaction was purified by preparative HPLC (MeCN/water, 25−40%, 0.1% formic acid). After lyophilization, the title compound was isolated as an off-white powder (73 mg, 72%). ^{1}H NMR (500 MHz, MeOH-*d4*): δ 8.55 (s, 1H, FQ-H-2), 8.02 (d, J = 8.5 Hz, 2H, glyco-Ar-H), 7.91 (d, J = 8.5 Hz, 2H, glyco-Ar-H), 7.75 (d, J = 14.6 Hz, 1H, FQ-H-5), 6.98 (d, J = 7.5 Hz, 1H, FQ-H-8), 4.43 (q, J = 7.1 Hz, 1H, Ala-Cα-H), 4.36−4.26 (m, 2H, Ala′-Cα-H, Leu-Cα), 3.95 (d, J = 16.6 Hz, 1H, Gly-Cα-H), 3.83 (s, 3H, COOC$\underline{H}_3$), 3.79 (d, J = 16.6 Hz, 1H, Gly-Cα-H), 3.74−3.65 (m, 2H, 2× aminomethylpyrrolidine-H), 3.64−3.54 (m, 3H, cPr-CH, glyco-H-4, aminomethylpyrrolidine-H), 3.44 (q, J = 6.5 Hz, 1H, glyco-H-5), 3.41−3.35 (m, 4H, glyco-H-1, glyco-H-3, 2× amino-methylpyrrolidine-H), 3.35−3.28 (m, 1H, glyco-linker-CH$_2$), 3.25 (dd, J = 13.6, 7.5 Hz, 1H, aminomethylpyrrolidine-H), 3.10 (ddd, J = 9.0, 7.1, 2.5 Hz, 1H, glyco-H-2), 3.02 (dd, J = 12.9, 7.1 Hz, 1H, glyco-linker-C$\underline{H}_2$-), 2.68−2.52 (m, 1H, aminomethylpyrrolidine-H), 2.32−2.10 (m, 1H, aminomethylpyrrolidine-H), 1.85−1.76 (m, 1H, aminomethylpyrrolidine-H), 1.76−1.58 (m, 3H, Leu-CH, Leu-CH$_2$), 1.50 (d, J = 7.2 Hz, 3H, Ala-CH$_3$), 1.41 (d, J = 7.2 Hz, 3H, Ala′-CH$_3$), 1.37−1.29 (m, 2H, cPr-CH$_2$), 1.16 (d, J = 6.4 Hz, 3H, glyco-H-6), 1.14−1.04 (m, 2H, cPr-CH$_2$), 0.88 (d, J = 4.0 Hz, 3H, Leu-CH$_3$), 0.87 (d, J = 4.1 Hz, 3H, Leu-CH$_3$). ^{13}C NMR (126 MHz, MeOH-*d4*): δ 176.22 (CONH), 175.30 (d, J = 2.7 Hz, FQ-C=O), 175.17 (CONH), 174.76 (CONH), 172.58 (CONH), 168.94 (CONH), 166.97 (COOMe), 151.72 (d, J = 244.6 Hz, FQ-C-6), 149.61 (Ar−C), 145.07 (Ar−C), 142.80 (d, J = 12.0 Hz, FQ-C-7), 140.51 (Ar−C), 138.35 (Ar−C), 129.50 (glyco-Ar-C), 128.07 (glyco-Ar-C), 118.97 (d, J = 6.4 Hz, FQ-C-4a), 112.82 (d, J = 23.3 Hz, FQ-C-5), 109.75 (Ar−C), 101.40 (d, J = 5.4 Hz, FQ-C-8), 79.63 (glyco-C-2), 76.33 (glyco-C-3), 75.51 (glyco-C-5), 73.54 (glyco-C-4), 69.61 (glyco-C-1), 54.47 (d, J = 5.9 Hz, aminopyrrolidine-C), 53.93 (Leu-Cα), 52.35 (Ala-Cα), 52.04 (aminopyrrolidine-C), 50.96 (Ala′-Cα), 50.53 (d, J = 6.2 Hz, aminopyrrolidine-C), 45.55 (glyco-linker-CH$_2$), 44.23 (Gly-Cα), 42.61 (aminopyrrolidine-C), 41.20 (Leu-CH$_2$), 40.01 (aminopyrrolidine-C), 36.17 (cPr-CH), 29.93 (RHNCH$_2$-aminopyrrolidine), 25.81 (Leu-CH), 23.56 (Leu-CH$_3$), 21.54 (Leu-CH$_3$), 17.77 (Ala-CH3), 17.31 (Ala-CH$_3$), 17.11 (glyco-C-6), 8.53 (cPr-CH$_2$), 8.50 (cPr-CH$_2$). LR-MS: C$_{47}$H$_{65}$FN$_8$O$_{14}$S^{2+} [M+2H]$^{2+}$, calcd 508.22; found, 508.2.

LecB-Targeted Aminomethylpyrrolidine-FQ-Prodrug 49. LecB-targeted aminomethylpyrrolidine-FQ-Prodrug methyl ester **45** (50 mg, 0.049 mmol, 1 equiv) was dissolved in a mixture of THF, MeOH, and H$_2$O (3:1:1, 1 mL), and LiOH (9 mg, 0.368 mmol, 7.5 equiv) was added. The reaction was stirred overnight at room temperature until disappearance of the starting material. After neutralization with Amberlite IR-120 H$^+$ to pH 7 and filtration, the solvents were removed in vacuo. The title compound was obtained after lyophilization as an off-white powder (47 mg, 96%). ^{1}H NMR (500 MHz, MeOH-*d4*): δ 8.64 (s, 1H, FQ-H-2), 8.03 (d, J = 8.1 Hz, 2H, glyco-Ar-H), 7.91 (d, J = 8.1 Hz, 2H, glyco-Ar-H), 7.69 (d, J = 14.2

Hz, 1H, FQ-H-5), 7.03 (d, J = 7.4 Hz, 1H, FQ-H-8), 4.43 (q, J = 7.1 Hz, 1H, Ala-Cα-H), 4.34 (dd, J = 10.6, 4.2 Hz, 1H, Leu-Cα-H), 4.30 (q, J = 7.2 Hz, 1H, Ala-Cα-H), 3.95 (d, J = 16.6 Hz, 1H, Gly-Cα-H), 3.79 (d, J = 16.6 Hz, 1H, Gly-Cα-H), 3.77−3.60 (m, 4H, 3× aminomethylpyrrolidine-H, cPr-CH), 3.58 (d, J = 1.9 Hz, 1H, glyco-H-4), 3.49−3.22 (m, 7H, 3× aminomethylpyrrolidine-H, glyco-H-3, glyco-H-5, glyco-H-1, glyco-linker-C$\underline{H}_2$-), 3.10 (ddd, J = 9.0, 7.0, 2.6 Hz, 1H, glyco-H-2), 3.02 (dd, J = 12.9, 7.1 Hz, 1H, glyco-linker-CH$_2$), 2.67−2.55 (m, 1H, aminomethylpyrrolidine-H), 2.23−2.13 (m, 1H, aminopyrrolidine-H), 1.82 (ddd, J = 12.2, 7.8 Hz, 1H, aminopyrrolidine-H), 1.77−1.58 (m, 3H, Leu-CH, Leu-CH$_2$), 1.50 (d, J = 7.1 Hz, 3H, Ala-CH$_3$), 1.41 (d, J = 7.2 Hz, 3H, Ala′-CH$_3$), 1.39−1.37 (m, 2H, cPr-CH$_2$), 1.20−1.18 (m, 2H, cPr-CH$_2$), 1.16 (d, J = 6.4 Hz, 3H, glyco-H-6), 0.88 (d, J = 5.0 Hz, 3H, Leu-CH$_3$), 0.87 (d, J = 5.2 Hz, 3H, Leu-CH$_3$). ^{13}C NMR (126 MHz, MeOH-*d4*): δ 177.58 (d, J = 2.8 Hz, FQ-C=O), 176.19 (CONH), 175.21 (CONH), 174.78 (CONH), 172.56 (CONH), 170.13 (CONH), 168.92 (COOH), 151.98 (d, J = 247.3 Hz, FQ-C-6), 148.61 (FQ-C-2), 145.07 (Ar−C), 143.64 (d, J = 11.2 Hz FQ-C-7), 141.45 (Ar−C), 138.34 (Ar−C), 129.50 (glyco-Ar-C), 128.08 (glyco-Ar-C), 111.95 (d, J = 23.5 Hz, FQ-C-5), 101.44 (d, J = 6.0 Hz, FQ-C-8), 79.63 (glyco-C-2), 76.33 (glyco-C-3), 75.52 (glyco-C-5), 73.55 (glyco-C-4), 69.61 (glyco-C-1), 54.51 (d, J = 5.6 Hz, aminomethylpyrrolidine-C), 53.92 (Leu-Cα), 52.33 (Ala-Cα), 50.98 (Ala-Cα), 50.64 (d, J = 6.3 Hz, aminopyrrolidine-C), 45.55 (glyco-linker-CH$_2$), 44.22 (Gly-Cα), 42.46 (aminomethylpyrrolidine-C), 41.22 (Leu-CH$_2$), 40.00 (amino-methylpyrrolidine-C), 36.80 (cPr-CH), 29.89 (aminomethylpyrroli-dine-C), 25.81 (Leu-CH), 23.56 (Leu-CH$_3$), 21.56 (Leu-CH$_3$), 17.76 (Ala-CH$_3$), 17.31 (Ala′-CH$_3$), 17.11 (glyco-C-6), 8.51 (cPr-CH$_2$), 8.48 (cPr-CH$_2$). HR-MS: calcd [C$_{46}$H$_{62}$FN$_8$O$_{14}$S]$^+$, 1001.4085; found, 1001.4063.

Recombinant Expression and Purification of Lectins. LecA and LecB$_{PAO1}$ or LecB$_{PA14}$ were recombinantly expressed using the previously described protocols from Blanchard et al.,[79] Sommer et al.,[44] Mitchell et al.,[80] respectively. *Escherichia coli* BL21(DE3) carrying the plasmid for LecA or LecB was cultured at 37 °C and 180 rpm in 1 L LB-medium supplemented with ampicillin (100 μg/mL). When an OD$_{600}$ of 0.5 to 0.6 was reached, expression was induced with IPTG (0.25 mM final concentration) and bacteria were cultured for 4 h at 30 °C and 180 rpm. The cells were then harvested by centrifugation (9000 rcf, 10 min, 4 °C), and the pellet was washed with TBS/Ca (8.0 g/L NaCl, 2.4 g/L Tris, 0.19 g/L KCl, 0.15 g/L CaCl$_2$·2H$_2$O, pH 7.4). The cells were resuspended in 25 mL TBS/Ca supplemented with PMSF (1 mM) and lysozyme (0.4 mg/mL) and subsequently disrupted using a microfluidizer (Microfluidics). Cell debris was removed by centrifugation (60 min, 10 000 rcf), and the supernatant was loaded on galactosylated (for LecA) or mannosylated (for LecB) sepharose CL-6B.[81] The column was washed with TBS/Ca, and LecA was eluted by addition of 100 mM D-galactose (for LecA) or D-mannose (for LecB) to the buffer. The eluted fractions were extensively dialyzed against TBS/Ca. Between 20 and 35 mg LecA per liter bacterial culture was obtained. The protein concentration was determined by UV spectroscopy at 280 nm using a molar extinction coefficient of 27 385 mol L^{-1} cm^{-1} (for LecA) or 6990 mol L^{-1} cm^{-1} (for LecB).[82]

Competitive Lectin Binding Assays. For **LecA**, the procedure published by Joachim et al.[72] was employed: A serial dilution of the test compounds was prepared in TBS/Ca (8.0 g/L NaCl, 2.4 g/L Tris, 0.19 g/L KCl, 0.15 g/L CaCl$_2$·2H$_2$O, pH 7.4), with 30% dimethyl sulfoxide (DMSO) as co-solvent. A concentrated solution of LecA was diluted in TBS/Ca together with the fluorescent reporter ligand (*N*-(fluorescein-5-yl)-*N*′-(*m*-aminophenyl β-D-galactopyrano-side)-thiocarbamide) to yield concentrations of 40 μM and 20 nM, respectively. 10 μL of this mix was added to 10 μL serial dilutions of the test compounds in black 384-well microtiter plates (Greiner Bio-One, Germany, cat. no. 781900) in triplicates. After centrifugation (2680 rcf, 1 min, 22 °C), the reactions were incubated for 30−60 min at 22 °C in a humidity chamber. Fluorescence (excitation 485 nm, emission 535 nm) was measured parallel and perpendicular to the excitation plane on a PheraStar FS plate reader (BMG Labtech

GmbH, Germany). The measured intensities were reduced by the values of only LecA in TBS/Ca and fluorescence polarization was calculated. The data were analyzed with the MARS Data Analysis Software (BMG Labtech GmbH, Germany) and fitted according to the four-parameter variable slope model. Bottom and top plateaus were fixed according to the control compounds in each assay (*p*-nitrophenyl β-D-galactoside), and the data were reanalyzed with these values fixed. A minimum of three independent experiments on three plates were performed for each inhibitor.

For the LecB homologues, the procedures of LecB$_{PAO1}$ according to Hauck et al.[83] and of LecB$_{PA14}$ according to Sommer et al.[44] were employed: A serial dilution of the test compounds was prepared in TBS/Ca, with 20% DMSO as co-solvent. A concentrated solution of LecB from PAO1 or PA14 was diluted in TBS/Ca together with the fluorescent reporter ligand [*N*-(fluorescein-5-yl)-*N*′-(α-L-fucopyrano-syl ethyl)-thiocarbamide] to yield concentrations of 300 nM and 20 nM, respectively. 10 μL of this mix was added to 10 μL serial dilutions of the test compounds in black 384-well microtiter plates (Greiner Bio-One, Germany, cat. no. 781900) in triplicates. After centrifuga-tion (2680 rcf, 1 min, 22 °C), the reactions were incubated for 4−8 h at 22 °C in a humidity chamber. Fluorescence was measured and analyzed as described for LecA. Bottom and top plateaus were fixed according to the control compound present in each assay (Me-α-L-Fuc), and the data were reanalyzed with these values fixed. A minimum of three independent experiments on three plates were performed for each inhibitor.

Bacterial Strains. *P. aeruginosa* PA14 (DSM 19882) was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ Braunschweig, Germany).

Prodrug activation Assay. *P. aeruginosa* PA14 was streaked on LB-agar plates (1% agar) from glycerol stocks and incubated at 37 °C overnight. 2−5 colonies were picked and dispersed in 10 mL LB (10 g/L trypton, 10 g/L NaCl, 5 g/L yeast extracts). This culture was grown overnight at 37 °C, 180 rpm under high humidity. The culture was centrifuged (4000 rcf, 22 °C, 10 min), and the supernatant was filtered (0.22 μm pore size). For the cleavage experiments, 1 mL filtrate was mixed with 9 mL human plasma (BioIVT—West Sussex, United Kingdom, LiHep-treated, pooled, mixed gender) to result in the matrix for this experiment.

A 1 mM solution of the studied compound was prepared in phosphate-buffered saline (PBS) (150 mM NaCl, 2.6 mM KCl, 1.4 mM KH$_2$PO$_4$, 10 mM Na$_2$HPO$_4$, pH 7.4) with 20% DMSO. 50 μL of this solution was diluted in 950 μL human plasma (spiked with 10% culture supernatant or LB) on ice. After brief vortexing, 100 μL was immediately treated with 100 μL ice-cold MeCN (spiked with 1.5 μM diphenhydramine as an internal standard). The rest of the solution was incubated at 37 °C and 500 rpm in an Eppendorf thermomixer. At various time points (30, 60, 120, and 180 min), 100 μL samples were taken and treated as described above. After extensive vortexing, the samples were centrifuged (17600 rcf, 10 min, 10 °C), and the supernatant was analyzed by HPLC-MS. The AUC of the parent drug, its cleavage products, and the internal standard were quantified using Compass QuantAnalysis quantification software. The relative AUC was calculated by AUC(compound)/AUC(ISTD). Procaine was used as a positive control as it is readily degraded in human plasma.

Antibiotic Susceptibility (MIC Assay). The antibiotic activity of the reference compounds **1**, **2**, **3**, **19**, **35**, and **47** was determined by broth microdilution assay based on the EUCAST guidelines, according to Wiegand, Hilpert, and Hancock.[14] Serial dilutions in sterile Müller-Hinton broth II (Fluka analytical, cat. no. 90922: 17.5 g/L casein acid hydrolysate, 3 g/L beef extract, 1.5 g/L starch, supplemented with 20−25 mg/L Ca^{2+} and 10−15 mg/L Mg^{2+}, pH 7.3) of the test compounds were prepared from 100 mM DMSO stocks [for ciprofloxacin (**1**), a 10 mM aqueous stock of ciprofloxacin·HCl was used] in sterile 96-well plates, yielding a concentration range from 128 μg/mL to 0.125 μg/mL (12.8−0.0125 for ciprofloxacin (**1**) and fluoroquinolones **2** and **3**). *P. aeruginosa* PA14 was streaked on LB-agar plates (1% agar) from glycerol stocks and incubated at 37 °C overnight. Colonies were picked from plate and dispersed in fresh Müller-Hinton broth II (MHB II) to yield an OD600 of 0.08−0.13.

This dispersion was diluted 1:100 in fresh MHB II, which was then used for the assay to achieve a final inoculum of 5×10^5 cfu/mL. 50 μL inoculum was mixed with 50 μL of the serial dilution in the corresponding well of the 96-well plate.

For the measurement of the time- and matrix-dependent antibiotic activity of prodrugs **24**, **25**, **36**, **37**, **48**, and **49**, a serial dilution in PBS with 20% DMSO was prepared in sterile 96-well plates, yielding a concentration range of 1 mM to 1.9 μM (100−0.19 μM for ciprofloxacin). The different matrices were prepared under sterile conditions: (i) 5 mL human plasma and 1 mL sterile filtrate from a *P. aeruginosa* PA14 overnight culture in LB were mixed with 4 mL PBS, (ii) 1 mL sterile filtrate from an *P. aeruginosa* PA14 overnight culture was diluted in 9 mL PBS, (iii) only PBS, and (iv) 5 mL human plasma and 1 mL LB were mixed with 4 mL PBS. 6 μL of each compound dilution series was diluted in 115 μL matrix (≈1:20 dilution) in a 96-well format at the time point $t = -3$ h or $t = -10$ min. The plates prepared at $t = -3$ h were sealed with gas-permeable foil and incubated at 37 °C in a humid incubator. The other plates were kept at room temperature. At $t = 0$ h, 50 μL of each well was mixed with 50 μL inoculum (as described above) in double-concentrated MHBII in a sterile 96-well plate.

The plates were sealed with gas-permeable foil and incubated at 37 °C for 18−20 h in a humid incubator. Growth inhibition was assessed by visual inspection, and the given MIC values are the lowest concentration of antibiotic at which there was no visible growth.

In Vitro ADMET. *Cytotoxicity.* The epithelial cell line A549 (ATCC(R) CCL-185) was cultivated in Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated fetal calf serum (FCS) and 20 mM L-glutamine at 37 °C and 5% CO$_2$. A549 cells were seeded into a 96-well plate (Nunc, Roskilde, Denmark) and grown to 75% confluence. The compounds **36**, **37**, and **2** were tested in the cell assay. Every compound was dissolved in DMSO and diluted in PBS (final DMSO concentration in the cell assay: 1%). Cells were incubated with the respective compound in concentrations ranging from 0.001 to 50 μM for 24 h at 37 °C and 5% CO$_2$. Cells treated with only vehicle (DMSO diluted in PBS, final DMSO concentration in the cell assay: 1%) served as a negative control. Furthermore, pure medium (DMEM + 10% FCS) and completely damaged cells served as positive controls. To damage cells, cells were treated with 0.5% Triton X-100 1 h prior to the addition of MTT (Sigma). After 24 h, cells were washed twice with the respective medium. MTT diluted in PBS (stock solution 5 mg/mL) was added to the wells at a final concentration of 1 mg/mL. The cells were again incubated for 4 h at 37 °C and 5% CO$_2$. Medium was removed, and 0.04 M HCl in 2-propanol was added. The cells were incubated at room temperature for 15 min. Then, the supernatant was transferred to a 96-well plate. UV absorbance of the samples was measured at 560 nm and 670 nm as a reference wavelength on a Tecan Sunrise ELISA Reader using Magellan software. Data were normalized using the following formula: $(A-B)/(C-B)$, with "A' as the respective data point, "B" as the value of the Triton X-100-treated control, and "C" as the vehicle control. The experiment was repeated at least three times, and the IC$_{50}$ was given as mean ± standard deviation.

Plasma Stability Assays. Each compound dissolved in DMSO was added to prewarmed (37 °C) mouse (pH 7.4) or human plasma (pH 7.4) to yield a final concentration of 1 μM. In addition, procaine and procainamide (dissolved in DMSO) were added to mouse or human plasma (pH 7.4) to yield a final concentration of 1 μM. Procaine served as a positive control as it is unstable in mouse plasma. Procainamide served as a negative control as it is stable in mouse plasma. The samples were incubated for 0, 15, 30, 60, 90, 120, and 240 min at 37 °C. At each time point, 10 μL of the respective sample was extracted, with 90 μL acetonitrile containing 12.5 ng/mL caffeine as the internal standard for 5 min at 2000 rpm on a MixMate vortex mixer (Eppendorf). Acetonitrile and caffeine were dispensed using a Mantis Formulatrix. The samples were centrifuged for 20 min at 2270 rcf at 4 °C, and the supernatants were transferred to 96-well Greiner V-bottom plates. Samples were analyzed using HPLC-MS/MS analysis as described in the respective section. Peak areas of each compound and of the internal standard were analyzed using the

Table 3. HPLC-MS/MS Analysis

ID	Q1 mass [Da]	Q3 mass [Da]	time[msec]	DP [volts]	CE [volts]	CXP [volts]
2	332.917	314.9	30	1	29	34
	332.917	272.2	30	1	27	14
36	940.206	778.2	30	−300	−48	−37
	940.206	734.3	30	−300	−62	−29
37	985.228	941.3	30	−300	−58	−43
	985.228	527.2	30	−300	−70	−23
naproxene	231.106	185.1	50	80	19	10
	231.106	170.2	50	80	33	12
caffeine	195.024	138.0	50	80	25	14
	195.024	110.0	50	80	31	18
procaine	236.773	100.0	30	80	21	12
	236.773	120.0	30	80	31	14
procainamide	235.744	163.0	30	80	21	18
	235.744	120.0	30	80	39	12

MultiQuant 3.0 software (AB Sciex). Peak areas of the respective compound were normalized to the internal standard peak area and to the respective peak areas at time point 0 min: $(C/D)/(A/B)$ with A: peak area of the compound at time point 0 min, B: peak area of the internal standard at time point 0 min, C: peak area of the compound at the respective time point, and D: peak area of the internal standard at the respective time point. Every experiment was repeated independently at least three times.

Microsomal Stability Assay. Liver microsomes (mouse or human, ThermoFisher) were slowly thawed on ice. 5 μL 20 mg/mL of microsomes, 2 μL of a 100 μM solution of every compound, and 183 μL of 100 mM phosphate buffer were incubated for 5 min at 37 °C in a water bath. Reactions were initiated using 10 μL of 20 mM NADPH (Carl Roth, Germany). Samples were incubated in three replicates at 37 °C under gentle agitation at 150 rpm. At 0, 5, 15, 30, and 60 min, reactions were terminated by the addition of 180 μL acetonitrile using a Mantis Formulatrix dispenser. Samples were vortexed for 5 min using an Eppendorf MixMate vortex mixer and centrifuged at 2270 rcf for 20 min at 4 °C. The supernatants were transferred to 96-well Greiner V-bottom plates, sealed, and analyzed according to the section HPLC-MS/MS analysis. Peak areas of the respective time point of the compounds were normalized to the peak area at time point 0 min. Then, half-life was calculated using linear regression (Microsoft Excel). Cl_{int} [μL/min/mg protein] was calculated using the following formula

$$Cl_{int} = 0.693/(0.0005 \text{ mg}/\mu L \times t_{1/2})$$

Cell Accumulation Assay. A549 cells were seeded into 96-well plates as described for the cytotoxicity assay. Cells were cultivated at 37 °C and 5% CO_2 until they reached 95% confluence. Cells were treated with compounds **2**, **36**, or **37** at a final concentration of 10 μg/mL or left untreated. Each condition was assayed in technical duplicates with two biological replicates. Cells were treated for 15, 30, and 60 min. After incubation for the respective time point, cells were washed twice with pre-warmed PBS and were lysed in MeOH and scratched from the surface. Supernatants from medium, wash fluids as well as the cell extracts were subjected to mass spectrometric analysis. For wash fluid and medium samples, calibration and QC samples were prepared using PBS as matrix and spiking the respective compounds into the matrix. For cell extract samples, calibration and QC samples were prepared using MeOH as matrix. For calibration and QC samples, compounds were dispensed using a Mantis Formulatrix. Medium, wash fluid, cell extract samples as well as both calibration and QC samples were extracted using MeOH containing 12.5 ng/mL caffeine as internal standard for 10 min at 800 rpm on an Eppendorf VortexMixMate and then centrifuged at 4000 rpm for 20 min at 4 °C. Supernatants were transferred to a Greiner V-bottom plate, sealed, and subjected to HPLC-MS/MS analysis.

Plasma Protein Binding Assay. Plasma protein binding was assessed using the rapid equilibrium device (RED) system from ThermoFisher. Compounds **2**, **36**, and **37** were dissolved in DMSO. Naproxene served as a control as it shows high plasma protein binding. Compounds were diluted in murine plasma (from CD-1 mice, pooled) or in human plasma (human donors, both genders, pooled) to a final concentration of 1 μM. Dialysis buffer and plasma samples were added to the respective chambers according to the manufacturer's protocol. The RED plate was sealed with a tape and incubated at 37 °C for 2 h at 800 rpm on an Eppendorf MixMate vortex mixer. Then, samples were withdrawn from the respective chambers. To 25 μL of each dialysis sample, 25 μL of plasma was added, and to 25 μL of plasma sample, 25 μL of dialysis buffer was added. Then, 150 μL ice-cold extraction solvent [MeCN/H_2O (90:10) containing 12.5 ng/mL caffeine as the internal standard] was added. Samples were incubated for 30 min on ice. Then, samples were centrifuged at 4 °C at 2270 rcf for 10 min. Supernatants were transferred to Greiner V-bottom 96-well plates and sealed with a tape. Then, samples were subjected to HPLC-MS/MS analysis as described in the section "HPLC-MS/MS Analysis". The percentage of bound compound was calculated as follows

$$f_{bound} = 1 - (c_{buffer\ chamber}/c_{plasma\ chamber}) \times 100$$

Cell samples as well as plasma stability, plasma protein binding, and metabolic stability samples were analyzed using an Agilent 1290 Infinity II coupled to an AB Sciex 6500plus mass spectrometer (Table 3). LC conditions were as follows: Column: Agilent Zorbax Eclipse Plus C18, 50 × 2.1 mm, 1.8 μm; temperature: 30 °C; injection volume: 5 μL per sample; flow rate: 700 μL/min. Solvents: A: H_2O + 0.1% formic acid, B: 95% MeCN/5% H_2O + 0.1% formic acid. Gradient for **2**, **36**, and **37**: 99% A from 0 min until 1 min, 99−0% A from 1.0 until 2.2 min, 0% A until 3.2 min. Gradient for naproxene and caffeine: 99% A from 0 min until 1 min, 99−0% A from 1.0 until 5.5 min, 0% A until 6.0 min. Gradient for procaine and procainamide: 99% A from 0 min until 1.0 min, 99−0% A from 1.0 until 3.5 min, 0% A until 3.7 min. Mass transitions for controls and compounds are depicted in Table 3.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.2c01214.

> ^{1}H and ^{13}C NMR spectra of new compounds; replicates of the cleavage assays in human blood plasma + PA14-filtrate and stability assays in human blood plasma; lectin inhibition K_i values calculated from IC_{50}; key compounds and intermediates as SMILES; and purity of key compounds **2**, **3**, **19**, **24**, **25**, **35−37**, and **47−49** by HPLC-UV (PDF)
>
> Molecular formula strings (CSV)

■ AUTHOR INFORMATION

Corresponding Author

    Alexander Titz − *Chemical Biology of Carbohydrates (CBCH), Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research, D-66123 Saarbrücken, Germany; Deutsches Zentrum für Infektionsforschung (DZIF), Germany; Department of Chemistry, Saarland University, D-66123 Saarbrücken, Germany;* orcid.org/0000-0001-7408-5084; Email: alexander.titz@helmholz-hzi.de

Authors

    Joscha Meiers − *Chemical Biology of Carbohydrates (CBCH), Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research, D-66123 Saarbrücken, Germany; Deutsches Zentrum für Infektionsforschung (DZIF), Germany; Department of Chemistry, Saarland University, D-66123 Saarbrücken, Germany;* orcid.org/0000-0003-0869-0088

    Katharina Rox − *Deutsches Zentrum für Infektionsforschung (DZIF), Germany; Chemical Biology (CBIO), Helmholtz Centre for Infection Research (HZI), Helmholtz Centre for Infection Research, D-38124 Braunschweig, Germany*

Complete contact information is available at:
https://pubs.acs.org/10.1021/acs.jmedchem.2c01214

Author Contributions

J.M. synthesized prodrugs and individual building blocks. J.M. performed lectin inhibition assays, cleavage assays, and antibiotic susceptibility assays. K.R. analyzed ADME and cell accumulation data. J.M. and A.T. conceived the study and wrote the paper with input from all co-authors.

Notes

The authors declare the following competing financial interest(s): J.M. and A.T. are inventors on a pending patent application covering this work. The authors declare no further competing financial interest.

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■ LIST OF ABBREVIATIONS

BfArM, Bundesinstitut für Arzneimittel und Medizinprodukte; CL_{MIC}, microsomal clearance; *c*Pr, cyclopropyl; EPS, extracellular polymeric substance; FQ, fluoroquinolone; HBP, human blood plasma; HLM, human liver microsomes; HR-MS, high-resolution mass spectrometry; ibcf, isobutyl chloroformate; LR-MS, low-resolution mass spectrometry; MBP, mouse blood plasma; MIC, minimum inhibitory concentration; MLM, mouse liver microsomes; NMM, *N*-methylmorpholine; PE, petroleum ether; RBD, receptor binding domain; rcf, relative centrifugation force; ROS, reactive oxygen species; TBTU, 2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate

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