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Bisphosphonated fluoroquinolone esters as osteotropic prodrugs for the prevention of osteomyelitis

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ABSTRACT

Osteomyelitis is a difficult to treat bacterial infection of the bone. Delivering antibacterial agents to the bone may overcome the difficulties in treating this illness by effectively concentrating the antibiotic at the site of infection and by limiting the toxicity that may result from systemic exposure to the large doses conventionally used. Using bisphosphonates as osteophilic functional groups, different forms of fluoroquinolone esters were synthesized and evaluated for their ability to bind bone and to release the parent antibacterial agent. Bisphosphonated glycolamide fluoroquinolone esters were found to present a profile consistent with effective and rapid bone binding and efficient release of the active drug moiety. They were assessed for their ability to prevent bone infection in vivo and were found to be effective when the free fluoroquinolones were not.

1. Introduction

Osteomyelitis refers to an inflammatory process often accompanied by bone necrosis which results from an underlying microbial infection,[1] primarily caused by *Staphylococcus aureus*.[2] In general, it is established as a result of trauma, bone surgery, joint replacement or in cases of reduced vascularization, such as in diabetic and elderly patients. This is a notoriously challenging illness to treat with a need for surgical intervention and sometimes amputation,[3] as well as frequent relapses.[4] There are no marketed antibiotics approved with a Gram positive osteomyelitis indication, and treatment is often a result of the preferences of the attending physicians.

It is generally believed that the difficulties in treating osteomyelitis stem from the sheltered physiological environment offered to the bacteria, poorly accessible to immune responsive elements and to therapeutic agents. In such settings, bacterial growth is likely very slow or even halted, and thus more resistant to antibacterial treatment. These are difficult conditions for effective treatment requiring a large concentration of the antibiotic to be maintained in the infected bone over a long period of time, and therefore frequent intravenous administration of high doses of antibiotics is typically required. The ability to deliver an antibacterial agent to the bone where it would be released over time could conceivably circumvent these difficulties. The use of beads impregnated with antibiotics is being examined for such a purpose.[5] The inconvenience of this approach stems from the need to implant these materials surgically which severely impedes the repeat applications that are often necessary in the treatment of the disease.

Delivery of agents to bone has been achieved after systemic administration by the use of bisphosphonates.[6] These pyrophosphate analogs demonstrate strong affinity for hydroxyapatite, the calcium phosphate bone mineral, and rapidly diffuse to osseous tissues in vivo. Bisphosphonates have been used to deliver small molecule therapeutics,[7] ligands for radioisotope imaging[8] and even proteins[9] to osseous tissues. Recently, a report has described the use of bisphosphonates for the delivery of ciprofloxacin to bone.[10] Although the ciprofloxacin-bisphosphonate conjugate described appeared to bind efficiently to bone, it was unable to release of the ciprofloxacin moiety (unpublished results). This would limit the potential of this agent, as it is reasonable to propose that because the target of ciprofloxacin is located within the cell and because the hydrophilic bisphosphonate moiety is likely unable to cross the hydrophobic cell membrane, the release of the bioactive moiety is necessary to achieve a positive therapeutic outcome. Bacteria are not very likely to be impacted by an antibacterial agent which is essentially irreversibly bound to the bone surface. Likewise, bacteria are not known to possess bone-resorptive activity which is required to absorb bisphosphonates once they are bound on bone. The relationship between resorptive activity and efficacy of therapeutic bisphosphonates has been demonstrated in the case

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of osteoporosis treatments.[11] Although the local acidity[12] brought on by the infecting organism might be associated with a measure of bisphosphonate release from bone, the efficiency of such a process in providing a sufficient concentration of the antimicrobial agent is doubtful.

Bisphosphonate prodrugs have been described for the delivery of small molecules, such as diclofenac,[7a,7b] prostaglandins,[7c] steroids,[7d] carboxyfluorescein[7e,7f], tryptophan[7g] and camptothecin[7g] to bone. We have therefore opted for a similar approach. Unlike the strategy that was reported for ciprofloxacin,[10] the antibiotic-bisphosphonate conjugates reported here were designed to release the drug moiety and thus to exploit localized activity of free antibiotic.

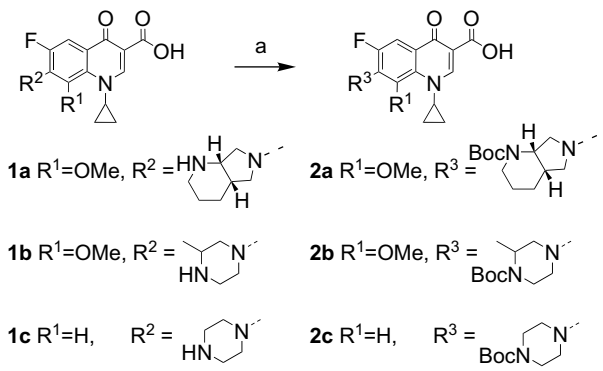
Fluoroquinolones are a well-defined class of wholly synthetic antibacterial agents that have proven to be successful clinically.[13] They possess a set of desirable attributes, being generally safe and efficacious following oral and parenteral administration, with a broad antimicrobial spectrum that includes the bacterial pathogens that are frequently encountered in osteomyelitis. They are bactericidal at clinically achievable doses, a feature which is most desirable in treating osteomyelitis given the sheltered environment of the microorganism and the high levels of recurrence associated with the disease. While drug efflux and mutations in the drug target have been associated with resistance to fluoroquinolones, such resistance occurs at a rate low enough that these agents continue to provide clinical utility when used in monotherapy. Fluoroquinolones have demonstrated some efficacy in animal models of osteomyelitis.[14] Not unimportantly, they are relatively easy to synthesize and modify.

Therefore, the preparation and evaluation of bisphosphonated fluoroquinolone prodrugs appeared to be of significant interest for the treatment and the prevention of osteomyelitis. This report describes our efforts in preparing such prodrugs using the carboxy group of the fluoroquinolones as a synthetic handle.

2. Chemistry

In this process, moxifloxacin **1a**, gatifloxacin **1b** and ciprofloxacin **1c** were selected as starting materials for the prodrugs. These can be easily protected as the Boc carbamates (**2a–c**) under standard conditions to leave the carboxylate as a readily modifiable functionality (Scheme 1).

Simple bisphosphonated aliphatic esters can be prepared from iodides **6a–b** which are obtained through the alkylation of tetraethyl methylenebisphosphonate with protected ω-bromoalcohols **4a–b** followed by deprotection and iodination (Scheme 2). These iodides **6a–b** undergo a displacement reaction with the carboxylate of **2a**, followed by the simultaneous deprotection of the phosphonates and removal of the Boc protecting group on the amine



Scheme 1. Reagents: (a) Boc_2O, NaOH, THF, H_2O.

with trimethylsilylbromide, to provide fluoroquinolone–bisphosphonate conjugates **9a–b**.

The typically more labile thioesters **12a–b** were prepared by coupling the protected fluoroquinolones with thiol **10** obtained from the similar iodide **7c** (Scheme 3), while coupling with phenol **13**[15] gave, after deprotection, aryl ester **15** (Scheme 4).

Given the potential of glycolamide esters as prodrugs,[16] a set of such compounds were prepared for evaluation. Alkylation of the carboxylates of **2a–c** with bromoacetamide **16**[7c] followed by deprotection provides glycolamide esters **18a–c** (Scheme 5). Alkylation of tetramethyl methylenebisphosphonate with *p*-nitrobenzyl bromide followed by reduction of the nitro group and acylation of the resulting aniline provided bromoacetamide **21** which was used similarly to prepare glycolamide esters **23a–b** (Scheme 6). Glycolamide esters **28a–b** were likewise obtained from bromoacetamide **26** which was synthesized from *N*-methyl benzylamine (Scheme 7).

The moxifloxacin thioglycolamide ester **32** was produced by coupling **2a** to thiol **30** followed by deprotection (Scheme 8). This thiol was itself prepared by the direct condensation of mercaptoacetic acid with amine **29**.[8c]

The preparation of prodrug **38** started from protected lysine **33**, through coupling with **29**, deprotection to the diamine **35**, and acylation to afford the bis(bromoacetamide) **36** (Scheme 9). This later compound was condensed with **2b** and deprotected to provide **38**.

3. In vitro evaluation of prodrugs

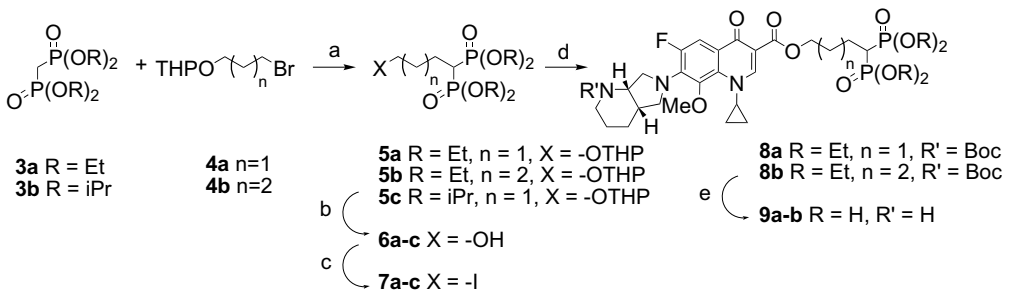
The antibacterial activities of the prodrugs against *S. aureus* ATCC13709 were measured by broth microdilution as per guidelines of the Clinical and Laboratory Standards Institute (results not shown). The minimum inhibitory concentrations (MICs) were >4 μg/mL, significantly higher than those of moxifloxacin (0.03 μg/mL), gatifloxacin (0.06 μg/mL) and ciprofloxacin (0.12 μg/mL). Given that at least a portion of the antibacterial activity of the prodrugs is likely to come from parent drug that is released from the bisphosphonate conjugate during the course of the 24 h MIC assay, it is clear that the prodrugs lack significant antibacterial activity of their own.

To demonstrate in vivo activity, the bisphosphonated prodrugs likely need not only to bind to bone but also to release the active fluoroquinolone. These two requirements were evaluated in vitro to predict the therapeutic potential of these compounds.

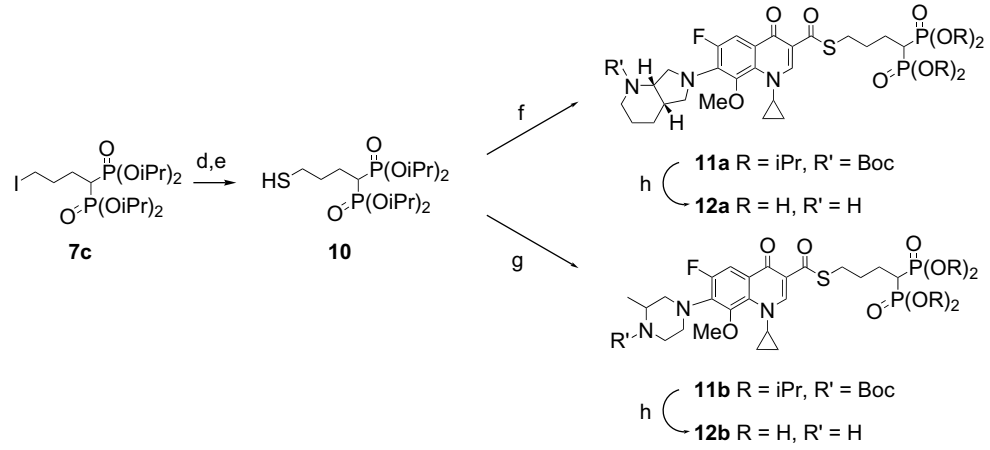
Bone-bound prodrugs are immobilized on a insoluble matrix, and therefore the affinity of fluoroquinolone prodrugs for osseous tissues can be estimated by measuring the amount of unbound prodrug in supernatant by fluorescence spectroscopy relative to the total amount of input prodrug following exposure to bone powder in phosphate buffered saline (PBS) at 37 °C over 1 h (Table 1).

The release of the parent fluoroquinolone from these prodrugs immobilized on bone powder can similarly be determined by measuring the appearance of fluorescence in the supernatant over time. This was done in PBS and 50% rat or human serum, to evaluate the potential for enzymatic cleavage (Table 1). In addition to fluorescence measurements, the nature of the released entity was also confirmed by determining its antibacterial activity, and in all cases, this activity was identical to that of the parent fluoroquinolone (data not shown).

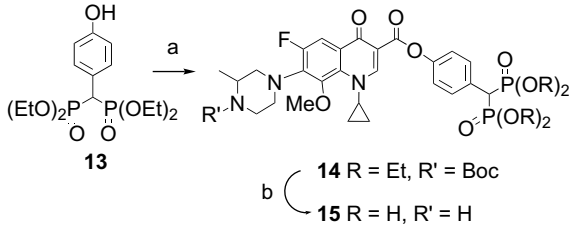
The results from these in vitro assays suggest several trends. First, the fluoroquinolone–bisphosphonate prodrugs are very efficient at binding bone powder, being taken up at >80% and generally >90% over 1 h, while the parent drugs are at best negligibly bound. In fact, it is reasonable to assume that at least part of the unbound fraction results from cleavage of the prodrug during the



Scheme 2. Reagents and condition: (a) NaH, THF, Δ. (b) *p*TsOH, MeOH. (c) I_2, PPh_3, imidazole, CH_2Cl_2. (d) Compound **2a**, K_2CO_3, DMF. (e) TMSBr, CH_2Cl_2.



Scheme 3. Reagents and conditions: (a) NaH, THF, Δ. (b) Amberlyst, MeOH. (c) I_2, PPh_3, imidazole, CH_2Cl_2. (d) Thiourea, EtOH, Δ. (e) NaOH, H_2O, Δ. (f) Compound **2a**, 2-fluoro-1-methylpyridinium tosylate, Et_3N, CH_2Cl_2. (g) Compound **2b**, 2-fluoro-1-methylpyridinium tosylate, Et_3N, CH_2Cl_2. (h) TMSBr, CH_2Cl_2.



Scheme 4. Reagents: (a) Compound **2b**, 2-fluoro-1-methylpyridinium tosylate, Et_3N, CH_2Cl_2. (b) TMSBr, CH_2Cl_2.

time-course of the assay, thereby under-representing the true affinity of the prodrugs for bone powder. Second, it is clear that simple bisphosphonated esters are not able to release the parent drugs. Compounds **9a–b** are not cleaved at all, while the thioesters 12a–**b** and aryl ester **15** release the parent fluoroquinolones only very slowly. With all of these compounds, the rates of cleavage are not impacted by the presence of serum, suggesting that they are poor serum esterase substrates.

In contrast, the glycolamide esters are able to appreciably release their antibacterial moieties. Compounds **18a–b** display similar rates of cleavage in PBS and in sera, suggesting that the process at least does not require the participation of enzymes. Compound **18c** differs in the sense that it is not hydrolyzed in simple buffer but only in sera. This result does not however preclude the possibility that this is a result of medium effects rather than enzymatic cleavage.

Lengthening the distance between the fluoroquinolone glycolamide ester and the bisphosphonate functionality decreases the rate of hydrolysis, as can be seen with **23a–b** and **38**. The use of a tertiary glycolamide, which in most instances of glycolamide prodrugs accelerates cleavage,[16] also results in a reduction, as can be seen with **28a–b**. Unlike the situation with the simple fluoroquinolone–bisphosphonate esters, the use of a thioglycolamide ester, for compound **32**, results in a decrease in the rate of cleavage.

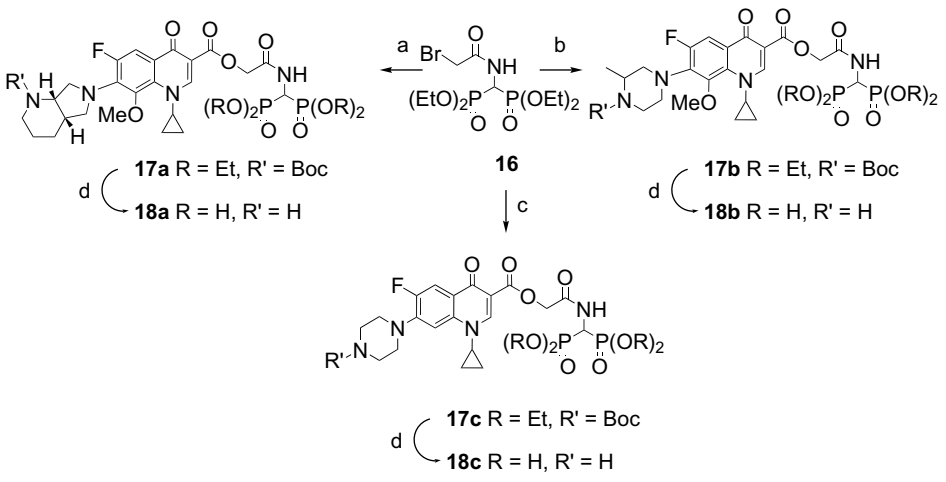
Interestingly, prodrug **38** exemplifies the versatility of the bisphosphonate osteotropic moiety, with a single bisphosphonate being able to target two fluoroquinolones to bone.

The trends observed with the glycolamide esters would suggest that the bisphosphonate functionality can participate in the cleavage, possibly by serving as an intramolecular acid catalyst. The more electron rich structures of moxifloxacin **1a** and gatifloxacin **1b** present carbonyl groups which are more readily protonated or are more apt to coordinate a Lewis acidic cation in the medium, and their prodrugs are more readily cleaved than the more electron poor derivatives of ciprofloxacin **1c**. These two factors tend to suggest an intramolecular protonation by the bisphosphonate as a rate determining event.

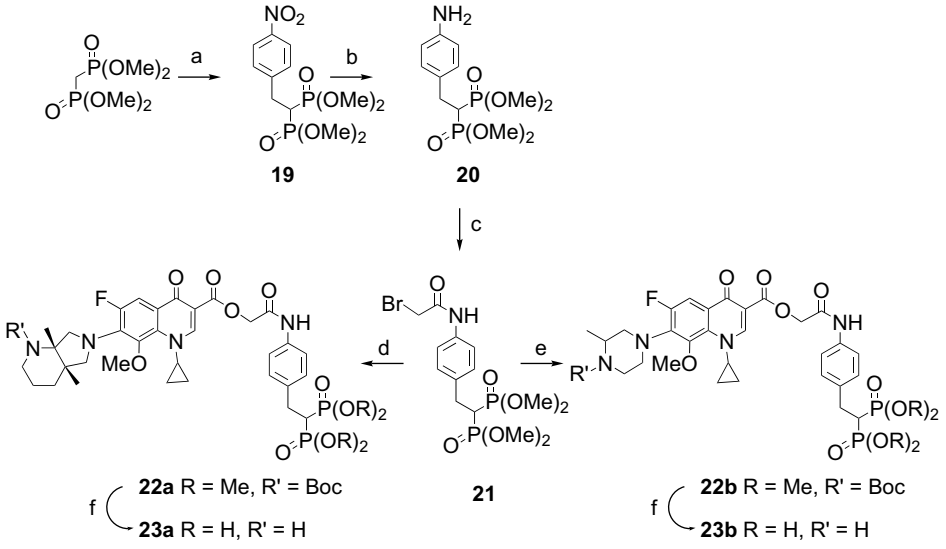
4. In vivo evaluation of prodrugs

Prodrugs **18a** and **18b** appreciably release their parent fluoroquinolones in vitro in a process which does not require the participation of an enzyme. Such a feature is attractive since it is predicted to minimize potential discrepancies during translation of animal efficacy results to humans. As such, these compounds were selected for evaluation in vivo.

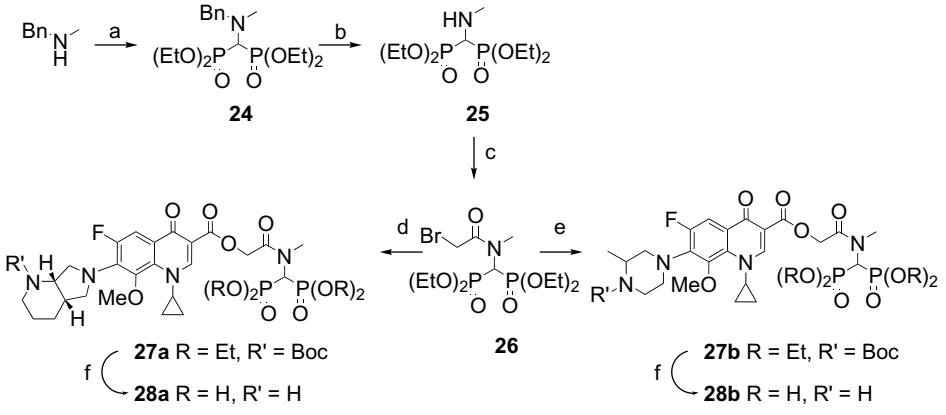
Compounds **18a–b** were tested in a rat model for the prevention of osteomyelitis, which was adapted from the known parent treatment models.[17] Briefly, the animals were injected with a single bo-



Scheme 5. Reagents: (a) Compound **2a**, Cs_2CO_3, DMF. (b) Compound **2b**, Cs_2CO_3, DMF. (c) Compound **2c**, Cs_2CO_3, DMF. (d) TMSBr, CH_2Cl_2.

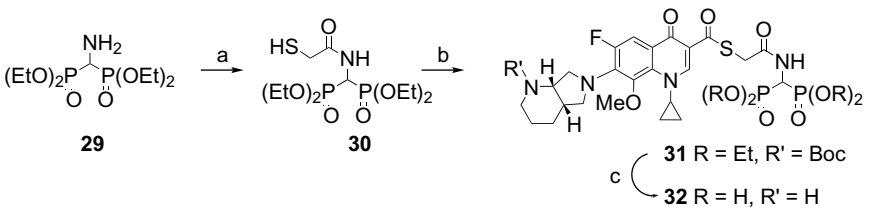


Scheme 6. Reagents: (a) NaH, THF, DMF. (b) H_2, PtO_2. (c) Bromoacetyl bromide, pyridine, CH_2Cl_2. (d) Compound **2a**, Cs_2CO_3, DMF. (e) Compound **2b**, Cs_2CO_3, DMF. (f) TMSBr, CH_2Cl_2.

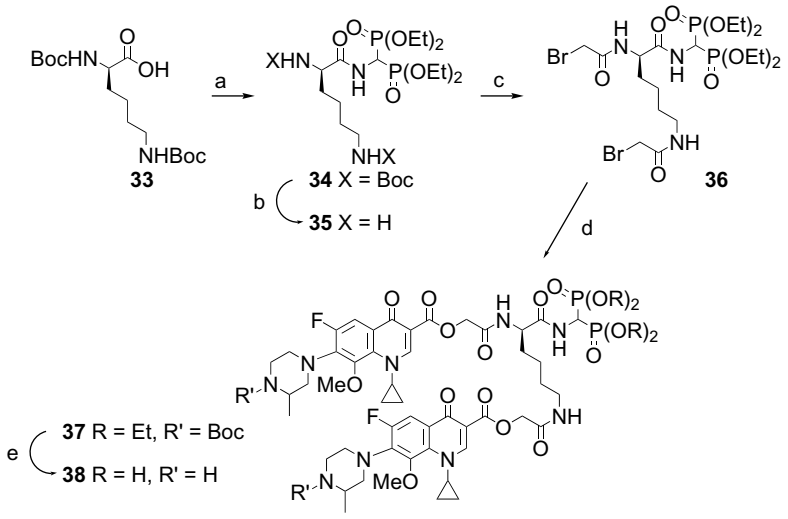


Scheme 7. Reagents and conditions: (a) Diethylphosphite, $HC(OEt)_3$, Δ. (b) Cyclohexene, Pd/C, EtOH, Δ. (c) Bromoacetyl bromide, pyridine, CH_2Cl_2. (d) Compound **2a**, Cs_2CO_3, DMF. (e) Compound **2b**, Cs_2CO_3, DMF. (f) TMSBr, CH_2Cl_2.

lus intravenous dose of the prodrug two days before an infection of the bone was established by surgically instilling *S. aureus* bacteria (ATCC 13709) into the tibiae. The animals were sacrificed 24 h after challenge and the bacterial titer in the tibiae was determined. Moxifloxacin was administered 1 h after infection as the positive control. It should be noted that moxifloxacin is ineffective when



Scheme 8. Reagents and conditions: (a) Mercaptoacetic acid, Δ. (b) Compounds **2a**–**b**, 2-fluoro-1-methylpyridinium tosylate, Et_3N, CH_2Cl_2. (c) TMSBr, CH_2Cl_2.



Scheme 9. Reagents: (a) Compound **29**, *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride, DMAP, CH_2Cl_2. (b) TFA, CH_2Cl_2. (c) Bromoacetyl bromide, pyridine, CH_2Cl_2. (d) Compound **2b**, Cs_2CO_3, DMF. (e) TMSBr, CH_2Cl_2.

Table 1
Bone binding and regeneration of parent fluoroquinolone from prodrugs

Compound	Amount bound to bone (%)	Amount of parent drug released over 24 h (%)		
		PBS	50% rat serum	50% human serum
1a	<l.o.d.[a]	—	—	—
1b	<l.o.d.	—	—	—
1c	<l.o.d.	—	—	—
9a	93.8	<l.o.d.	<l.o.d.	<l.o.d.
9b	92.7	0.01	<l.o.d.	<l.o.d.
12a	99.9	0.04	0.1	0.1
12b	98.7	0.1	0.1	0.1
15	79.2	0.2	0.1	0.1
18a	98.7	1.2	1.4	1.4
18b	91.0	2.7	1.3	1.6
18c	97.2	0.01	0.5	0.4
23a	97.2	0.6	1.3	1.2
23b	88.9	0.5	0.6	0.7
28a	97.3	0.3	0.7	0.8
28b	90.4	1.1	0.9	0.6
32	99.0	0.3	0.4	0.5
38	92.8	0.2	0.4	0.4

[a] Below the limit of detection.

used 24 h after the infection at that dose, likely a result of its relatively rapid clearance in vivo.

This experiment was performed with **18a** at 15.8 mg/kg of body weight, and compared with the equivalent dose of moxifloxacin (10 mg/kg), both administered two days prior to the establishment of infection (Fig. 1). Whereas the osteotropic prodrug clearly yielded an efficacious outcome, the parent drug was unable to prevent the infection under these conditions. The same experiment was performed with **18b** at 17.3 mg/kg and compared with gatifloxacin at 10 mg/kg, with the same conclusions (Fig. 2).

These experiments and the *in vitro* evaluations of the prodrugs are consistent with the ability of the bisphosphonate moiety to deliver the fluoroquinolone to the bone and release it over time to sustain a concentration sufficient to significantly reduce the bacterial titer in bone beyond a point in time by which the parent drug is already cleared, and is completely ineffective.

5. Conclusion

A number of methods were presented to tether a bisphosphonate group to the carboxylic acid functionality of moxifloxacin, gatifloxacin and ciprofloxacin. The resulting fluoroquinolone bisphosphonate conjugates were evaluated for their affinity to bone and their ability to release their parent drug once bound to bone. In vitro investigations highlighted the strong affinity of methylenebisphosphonate derivatives for bone, as opposed to the negligible bone affinities of the parent drugs.

This study presents the successful combination of bisphosphonate-based drug delivery and the known glycolamide ester class of prodrugs. These bisphosphonated esters were shown to release their parent drugs once they were bound to bone, under circumstances where simple bisphosphonated esters and even thioesters only produced negligible rates of regeneration.

Bisphosphonate fluoroquinolone conjugates **18a–b** were further evaluated in vivo for their ability to prevent infection in a rat model of osteomyelitis. They were shown to prevent the establishment of infection while moxifloxacin and gatifloxacin, their respective parent drugs, were unable to do so.

These prodrugs are being further evaluated in well-established animal models for their ability to treat chronic osteomyelitis, and the results of these investigations will be presented in due course.

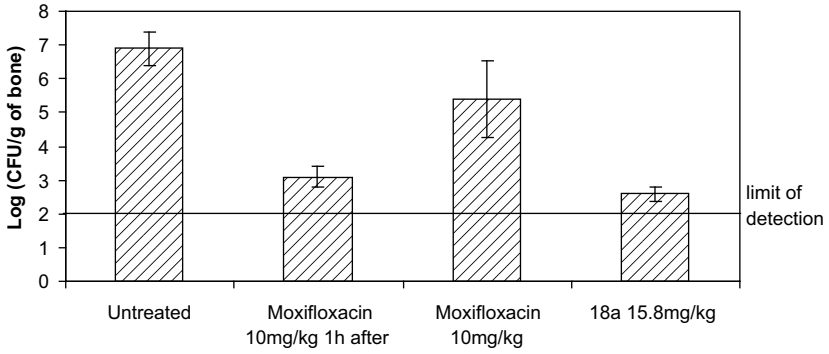


Figure 1. Prophylactic effect by prodrug **18a** and moxifloxacin in the rat bone infection model when injected at 15.8 mg/kg and 10 mg/kg, respectively, 2 days prior to the establishment of infection.

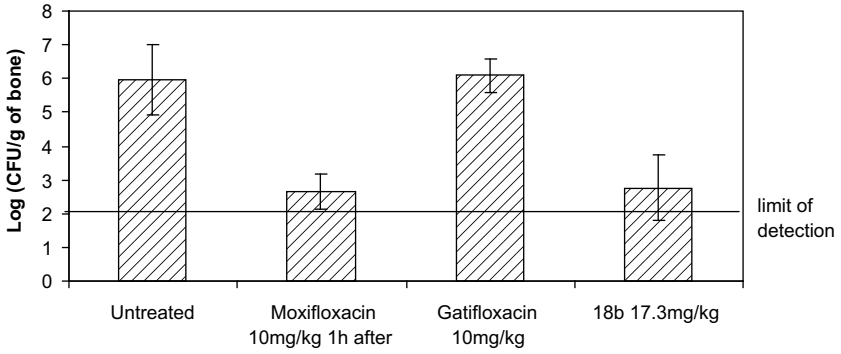


Figure 2. Prophylactic effect by prodrug **18b** and gatifloxacin in the rat bone infection model when injected at 17.3 mg/kg and 10 mg/kg, respectively, 2 days prior to the establishment of infection.

6. Experimental

6.1. General

^{1}H NMR spectra were recorded on a Varian Mercury™ 400 spectrometer. The reported chemical shifts (in parts per million) are referenced using the signals assigned to the residual non-deuterated solvents or to tetramethylsilane as the internal standard. Mass spectral analyses were performed on an Agilent mass spectrometer under electron spray ionization (ESI). Reactions were monitored by TLC on Silica gel Gel 60 F254 (0.25 mm, Merck). Column chromatography was performed on silica gel (70–230 mesh). All reactions were carried out under an argon atmosphere with anhydrous solvents, unless otherwise noted. All chemicals, unless otherwise stated, were obtained from commercial sources.

Compound purities were evaluated by LC/MS on an Agilent 1100 HPLC system using a Waters XTerra RP18 (3.5 μ, 2.1 mm × 50 mm) column at three different wavelengths using the following methods:

- Method A. Eluents: A (0.1% HCO_2H in H_2O) and B (0.1% HCO_2H in acetonitrile). Method: 0–50% B in A over 6 min, then 50–100% B in A over 2 min then 100% B for 1 min at a flow rate of 0.3 mL/min.
- Method B. Eluents: A (10 mM NH_4OAc) and B (MeOH). Method: 0–50% B in A over 6 min, then 50–100% B in A over 2 min at a flow rate of 0.3 mL/min.
- Method C. Eluents: A (10 mM NH_4OAc) to B (MeOH). Method: 20–70% B in A over 6 min, then 70–100% B in A over 2 min at a flow rate of 0.3 mL/min.

All animal experiments followed the Canadian Council of Animal guidelines in an accredited animal facility, and protocols were approved by the institutional animal care and use committee.

6.2. Chemistry

6.2.1. N-Boc protection of fluoroquinolones

General procedure. A mixture of the fluoroquinolone (2 mmol), Boc_2O (460 mg, 2.1 mmol) and 4.2 mL of 1 M NaOH aqueous solution in 20 mL of THF was stirred at room temperature overnight. For **2a–b**, after the removal of the organic solvent, the residue was neutralized with saturated ammonium chloride aqueous solution. The mixture was extracted with ethyl acetate (3×) and dried over anhydrous sodium sulfate. The combined organics were concentrated in vacuo to provide the product. For **2c**, the product precipitates from the reaction mixture and was collected by filtration.

6.2.1.1. 7-((4a*S*,7a*S*)-1-(*tert*-Butoxycarbonyl)-octahydropyrrolo-[3,4-*b*]pyridin-6-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid (2a). Yield: 91%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79–0.86 (m, 1H), 1.03–1.18 (m, 2H), 1.23–1.34 (m, 2H), 1.44–1.54 (m, 1H), 1.49 (s, 9H), 1.76–1.84 (m, 2H), 2.25–2.29 (m, 1H), 2.89 (t, J = 11.8, 1H), 3.22–3.30 (m, 1H), 3.38 (br s, 1H), 3.57 (s, 3H), 3.88 (dt, J = 2.7, 10.0, 1H), 3.96–4.01 (m, 1H), 4.07–4.12 (m, 2H), 4.79 (br s, 1H), 7.82 (d, J = 13.7, 1H), 8.79 (s, 1H) ppm.

6.2.1.2. 7-(4-(*tert*-Butoxycarbonyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid (2b). Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.94–1.04 (m, 2H), 1.19–1.26 (m, 2H), 1.33 (d, J = 6.9, 3H), 1.50 (s, 9H), 3.23–3.37 (m, 3H), 3.44–3.51 (m, 2H), 3.73 (s, 3H), 3.95–4.03 (m, 2H), 4.36 (br s, 1H), 7.89 (d, J = 11.4, 1H), 8.83 (s, 1H).

6.2.1.3. 7-(4-(*tert*-Butoxycarbonyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (2c). Yield: 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ *1.89–1.23* (m, 2H), 1.37–1.43 (m, 2H), 1.50 (s, 9H), 3.28 (bd, J = 5.0, 4H), 3.51–3.56 (m, 1H), 3.67 (bt, J = 5.0, 4H), 7.37 (d, J = 7.3, 1H), 8.05 (d, J = 13.0, 1H), 8.78 (s, 1H).

6.2.2. Iodoalkylene-1,1-bisphosphonate esters

General procedure for the preparation of ω-(2-tetrahydro-2H-pyranyloxy)alkylene-1,1-bisphosphonates. To a suspension of NaH (60% suspension in mineral oil, 900 mg, 22.0 mmol) in dry THF (20 mL) was added dropwise the methylenebisphosphonate tetraester **3a** or **3b** (22.4 mmol). The resulting clear solution was stirred 15–45 min at room temperature, and 2-(ω-bromoalkoxy)tetrahydro-2*H*-pyran **4a** or **4b** (22.6 mmol) was added dropwise. The reaction mixture was heated to reflux for 6 h and worked up as indicated.

6.2.2.1. Tetraethyl 4-(2-tetrahydro-2*H*-pyranyloxy)butylene-1,1-bisphosphonate (5a)[19]. From **3a** and **4a**. After reflux, the reaction mixture was diluted with CH_2Cl_2 (75 mL) washed with brine (2× 50 mL), dried ($MgSO_4$) and evaporated. The crude product was used as such in the following step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (t, J = 7.1, 12H), 1.44–2.14 (m, 10H), 2.35 (tt, J = 24.1, 6.1, 1H), 3.52 (m, 2H), 3.86 (m, 2H), 4.17 (m, 8H), 4.58 (m, 1H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 24.98 (s, 2P).

6.2.2.2. Tetraethyl 5-(2-tetrahydro-2*H*-pyranyloxy)pentylene-1,1-bisphosphonate (5b). From **3a** and **4b**. After reflux, the reaction mixture was quenched with saturated aqueous solution of ammonium chloride and a small amount of water to dissolve all solids. The mixture was extracted with ethyl acetate (3×), dried over anhydrous sodium sulfate and concentrated in vacuo. Flash chromatography on silica gel with 20:1 (v/v) dichloromethane/methanol as the eluent afforded impure product **5b** as a slightly yellow oil. The material was used directly in the next step without further purification. Selected ^{1}H NMR signals (400 MHz, $CDCl_3$): δ 1.32 (t, J = 7.1,12H), 1.45–2.00 (m, 12H), 2.28 (tt, J = 6.1, 24.3, 1H), 3.44 (m, 2H), 3.80 (m, 2H), 4.17 (m, 8H), 4.57 (m, 1H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 25.08 (s, 2P).

6.2.2.3. Tetraisopropyl 4-(2-tetrahydro-2H-pyranyloxy)butylene-1,1-bisphosphonate (5c). From **3b** and **4a**. After reflux, the reaction mixture was concentrated, the residue taken up in ethyl acetate and washed with semi-saturated brine. The aqueous layer was extracted with ethyl acetate and the combined organics washed with brine, dried over $MgSO_4$ and concentrated to dryness. It was used as such in the following step. ^{1}H NMR (400 MHz, $CDCl_3$) δ *1.33–1.36* (m, 24H), 1.45–2.06 (m, 8H), 2.13 (m, 2H), 2.19 (tt, J = 24.3, 5.9, 1H), 3.51 (m, 2H), 3.86 (m, 2H), 4.59 (m, 1H), 4.67 (m, 4H).^{31}P NMR (162 MHz, $CDCl_3$) δ 23.00 (s, 2P).

General procedure for the preparation of ω-hydroxyalkylene-1,1-bisphosphonates. To a solution of the crude ω-(2-tetrahydro-2*H*-pyranyloxy)alkylene-1,1-bisphosphonate **5a–c** (0.5 M) in methanol was added a catalytic amount of the indicated acid and the mixture was heated to reflux until consumption of the starting material as indicated by TLC (4–24 h). If an acidic resin was used, it was filtered off, and the solution was concentrated in vacuo and the residue was purified by silica gel chromatography.

6.2.2.4. Tetraethyl 4-hydroxybutylene-1,1-bisphosphonate (6a). From **5a** using amberlite IR-120 as the acidic catalyst. The eluent for chromatography was 5–10% methanol/ethyl acetate. Yield: 34% from tetraethyl methylenebisphosphonate (2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (t, J = 7.1, 12H), 1.81 (quint, J = 6.5, 2H), 1.99–2.13 (m, 2H), 2.37 (tt, J = 24.4, 5.6, 1H), 3.66 (q, J = 5.9, 2H), 4.13–4.22 (m, 8H).

6.2.2.5. Tetraethyl 5-hydroxypentylene-1,1-bisphosphonate (6b). From **5b** using *p*-toluenesulfonic acid monohydrate as the acidic catalyst. The eluent for chromatography was 1/15:1/6 methanol/ethyl acetate. Yield: 41% from tetraethyl methylenebisphosphonate (2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ *1.24–1.36* (m, 12H), 1.55–1.72 (m, 4H), 1.89–2.03 (m, 2H), 2.16 (br s, 1H), 2.29 (tt, J = 6.1, 24.3, 1H), 3.66 (br s, 2H), 4.11–4.22 (m, 8H).

6.2.2.6. Tetraisopropyl 4-hydroxybutylene-1,1-bisphosphonate (6c). From **5b** using amberlyst 15 as the acidic catalyst. The eluent for chromatography was 0–10% methanol/ethyl acetate. Yield: 48% from tetraisopropyl methylenebisphosphonate (2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ *1.33–1.36* (m, 24H), 1.77–1.83 (m, 1H), 1.96–2.10 (m, 2H), 2.21 (tt, J = 24.8, 5.4, 1H), 2.31–2.42 (m, 2H), 3.66 (t, J = 5.9 2H), 4.70–4.83 (m, 4H).

General procedure for the preparation of ω-iodoalkylene-1,1-bisphosphonates. To a solution of ω-hydroxyalkylene-1,1-bisphosphonate **6a–c** (4.39 mmol) in CH_2Cl_2 (50 mL) were added triphenylphosphine (1.32 g, 5.033 mmol) and imidazole (0.45 g, 6.61 mmol). The reaction mixture was cooled to 0 °C, before the addition of iodine (1.22 g, 4.81 mmol). The mixture was then removed from the cooling bath, stirred for 2 h and worked up as indicated.

6.2.2.7. Tetraethyl 4-iodobutylene-1,1-bisphosphonate (7a). From **6a**. The reaction mixture was diluted with hexanes (100 mL) and filtered washing the precipitate with further hexanes (2× 30 mL). The filtrate was concentrated and purified by flash chromatography on silica gel using 0–10% methanol/ethyl acetate as the eluent to give pure **7a**. Yield: 80%. ^{1}H NMR (400 MHz, $CDCl_3$) δ *1.32–1.38* (m, 12H), 1.95–2.15 (m, 4H), 2.28 (tt, J = 24.1, 6.1, 1H), 3.18 (t, J = 6.6, 2H), 4.12–4.24 (m, 8H).

6.2.2.8. Tetraethyl 5-iodopentylene-1,1-bisphosphonate (7b). From **6b**. The reaction mixture was concentrated in vacuo and the residue was taken up in a mixture of ethyl acetate and saturated $Na_2S_2O_3$ aqueous solution. The mixture was stirred until the organic layer turned pale yellow and the two phases were separated. The organic phase was dried over anhydrous sodium sulfate and concentrated. Flash chromatography on silica gel with 15:1 ethyl acetate/methanol as the eluent afforded the product **7b** as a yellow oil. Yield: 68%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.36 (t, J = 7.0, 12H), 1.66–1.72 (m, 2H), 1.81–1.99 (m, 4H), 2.35 (tt, J = 5.9, 24.1, 1H), 3.20 (t, J = 6.9, 2H), 4.17–4.23 (m, 8H).

6.2.2.9. Tetraisopropyl 4-iodobutylene-1,1-bisphosphonate (7c). From **6c**. The reaction mixture was added to hexanes (300 mL) and filtered washing the precipitate with further hexanes (2× 50 mL). The filtrate was evaporated and purified by flash chromatography on silica gel eluting with ethyl acetate to give pure **7c**. Yield: 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ *1.33–1.37* (m, 24H), 1.92–2.23 (m, 5H), 3.18 (t, J = 6.7, 2H), 4.74–4.83 (m, 4H).

6.2.3. Simple bisphosphonated fluoroquinolone esters

6.2.3.1. 4,4-Bis(diethylphosphono)butyl 7-((4a*S*,7a*S*)-1-(*tert*-butoxycarbonyl)-octahydropyrrolo[3,4-*b*]pyridin-6-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (8a). A mixture of **2a** (576 mg, 1.15 mmol), iodo bisphosphonate **7a** (497 mg, 1.09 mmol) and potassium carbonate (151 mg, 1.09 mmol) in 10 mL anhydrous DMF was stirred at room temperature for 21 h. Ethyl acetate (100 mL) was added, and the organics extracted with water (3× 20 mL) and brine (20 mL), and dried over $MgSO_4$. Flash chromatography on silica gel with gradient elution from 5 to 10% methanol/ethyl acetate afforded the pure

product (518 mg, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ *0.72–0.80* (m, 1H), 0.92–1.10 (m, 1H), 1.16–1.30 (m, 2H), 1.33 (t, J = 7.0, 12H), 1.47 (s, 9H), 1.71–1.83 (m, 4H), 2.05–2.16 (m, 4H), 2.18–2.28 (m, 1H), 2.32–2.50 (m, 1H), 2.81–2.94 (m, 1H), 3.13–3.26 (m, 1H), 3.28–3.42 (m, 1H), 3.55 (s, 3H), 3.78–3.90 (m, 2H), 3.98–4.08 (m, 2H), 4.12–4.23 (m, 8H), 4.25–4.36 (m, 2H), 4.76 (br s, 1H), 7.79 (d, J = 4.3, 1H), 8.51 (s, 1H).

6.2.3.2. 5,5-Bis(diethylphosphono)pentyl 7-((4a*S*,7a*S*)-1-(*tert*-butoxycarbonyl)-octahydropyrrolo[3,4-*b*]pyridin-6-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (8b). The mixture containing compound **2a** (464.7 mg, 0.9265 mmol), iodo bisphosphonate 7 b (435.5 mg, 0.9262 mmol) and potassium carbonate (129.3 mg, 0.9355 mmol) in 15 mL of anhydrous DMF was heated at 65 °C for 2d. After cooling to room temperature, the reaction was diluted with water, extracted with ethyl acetate (3×), dried over anhydrous sodium sulfate and concentrated. Flash chromatography on silica gel with gradient elution from 15:1 ethyl acetate/methanol to 8:1 then 5:1 afforded 425.6 mg (54%) product **8b** as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.72–0.80 (m, 1H), 1.00–1.08 (m, 2H), 1.24–1.36 (m, 13H), 1.48 (s, 9H), 1.63–1.81 (m, 8H), 1.92–2.04 (m, 2H), 2.20–2.28 (m, 1H), 2.36 (tt, J = 5.8, 24.1, 1H), 2.82–2.92 (m, 1H), 3.16–3.24 (m, 1H), 3.30–3.38 (m, 1H), 3.56 (s, 3H), 3.80–3.85 (m, 1H), 3.89–3.94 (m, 1H), 4.01–4.08 (m, 2H), 4.12–4.21 (m, 8H), 4.29–4.36 (m, 2H), 4.77 (br s, 1H), 7.78 (d, J = 14.4, 1H), 8.55 (s, 1H).

General procedure for bisphosphonated deprotection. TMSBr (0.82 mL, 6.21 mmol) was added in one portion to a stirring solution of the protected bisphosphonate (518 mg, 0.624 mmol) in CH_2Cl_2 (50 mL) and the resulting mixture was stirred at room temperature for 1 d. The solvent was removed under reduced pressure and the solid was dried under high vacuum for 1 h.

6.2.3.3. 4,4-Bisphosphonobutyl 7-((4a*S*,7a*S*)-octahydropyrrolo[3,4-*b*]pyridin-6-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (9a). The solid was then resuspended in H_2O and the pH was immediately adjusted to pH 7 by the addition of 1 M NaOH, with concomitant dissolution of the product. The product solution was washed with $CHCl_3$, filtered and concentrated to dryness to give the product in quantitative yield. ^{1}H NMR (400 MHz, D_2O) δ 0.83 (br s, 1H), 0.98 (br s, 1H), 1.08 (br s, 1H), 1.21 (br s, 1H), 1.65–2.15 (m, 9H), 2.61 (br s, 1H), 2.93 (br s, 1H), 3.20–3.35 (m, 1H), 3.43–3.64 (m, 2H), 3.52 (s, 3H), 3.75 (br s, 2H), 3.84–4.17 (m, 2H), 4.31 (br s, 2H), 7.41 (d, J = 12.1, 1H), 8.80 (s, 1H). ESI-MS: calculated for ($C_{25}H_{34}FN_3O_{10}P_2$+H$^+$): 618; found: 618 (M+H$^+$).

6.2.3.4. 5,5-Bisphosphonopentyl 7-((4a*S*,7a*S*)-octahydropyrrolo[3,4-*b*]pyridin-6-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (9b). The solid was dissolved in water. The resulting solution was brought to pH 7.4 with 1 N aqueous sodium hydroxide and the solvent was removed. The solid was twice dissolved in water and the solvent removed. The solid obtained was subjected to a Waters® C18 Sep-Pak™ cartridge (20 cc) with gradient elution from neat water to 10:1 water/methanol to 5:1 to afford **9b** as an off-white solid (211 mg, 70%). ^{1}H NMR (400 MHz, D_2O): δ 0.78–0.84 (m, 1H), 0.97–1.10 (m, 2H), 1.17–1.24 (m, 1H), 1.62–2.00 (m, 11H), 2.81 (br s, 1H), 3.04–3.10 (m, 1H), 3.39–3.43 (m, 1H), 3.54 (s, 3H), 3.63–3.68 (m, 2H), 3.80 (dt, J = 3.3, 9.6, 1H), 3.92–3.94 (m, 1H), 4.05–4.08 (m, 2H), 4.25–4.28 (m, 2H), 7.47 (d, J = 14.1, 1H), 8.76 (s, 1H); ^{31}P NMR (162 MHz, D_2O): δ 21.45; ^{19}F NMR (376 MHz, D_2O): δ −121.64 (d, J = 13.8). ESI-MS: calculated for ($C_{26}H_{36}FN_3O_{10}P_2$-H$^+$): 630; found: 630 (M−H)$^-$. Purity (method A): 97.2% (254 nm), 98.5% (220 nm), 97.8% (320 nm).

6.2.4. Preparation of bisphosphonated fluoroquinolone thioesters

6.2.4.1. Tetraisopropyl 5-thiapentylene-1,1-bisphosphonate (10). To a solution of **7c** (3.8 g, 7.4 mmol) in ethanol (20 mL) was added thiourea (0.59 g, 7.75 mmol). The reaction mixture was refluxed for 18 h, concentrated to dryness and used as such in the following step. ^{1}H NMR (400 MHz, D_2O) δ *1.35–1.38* (m, 24 H), 1.94–2.09 (m, 4 H), 2.50–2.67 (m, 1 H), 3.17 (t, J = 6.1, 2 H), 4.70–4.85 (m, 4 H). To this residue in water (30 mL) was added sodium hydroxide (0.396 g, 9.90 mmol). The reaction mixture was refluxed for 1.5 h, cooled to 0 °C and acidified with 1 M HCl (10 mL). The product was extracted with $CHCl_3$ (3× 50 mL), the organics washed with brine (70 mL), dried ($MgSO_4$) and concentrated to give a quantitative yield of crude **10** used as such in the following steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ *1.33–1.36* (m, 24 H), 1.88–2.19 (m, 5 H), 2.50–2.56 (m, 2 H), 4.74–4.83 (m, 4 H).

General procedure for coupling of thiol 10 to fluoroquinolones. To a solution of N-Boc protected fluoroquinolone (0.40 mmol) in CH_2Cl_2 (3 mL) was added 2-fluoro-1-methylpyridinium tosylate (0.136 g, 0.48 mmol). The reaction mixture was cooled to 0 °C, and triethylamine (0.20 mL, 1.43 mmol) was added via syringe. After stirring 1 h at 0 °C a solution of thiol **10** (0.208 g, 0.497 mmol) in CH_2Cl_2 (3 mL) was added. After a further 1 h at 0 °C the reaction was allowed to warm to room temperature overnight. The reaction mixture was diluted with ethyl acetate and washed with ice cold saturated NH_4Cl solution, 5% $NaHCO_3$, and water. After drying ($MgSO_4$) and concentration under vacuum, the residue was purified by flash chromatography on silica gel with gradient elution from 2.5 to 5% methanol/CH_2Cl_2 to give the product.

6.2.4.2. *S*-4,4-Bis(diisopropylphosphono)butyl 7-((4a*S*,7a*S*)-1-(*tert*-butoxycarbonyl)-octahydropyrrolo[3,4-*b*]pyridin-6-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carbothioate (11a). Yield: 67%. ^{1}H NMR (400 MHz, $CDCl_3$) δ *0.73–0.82* (m, 1H), 0.97–1.11 (m, 2H), 1.24–1.27 (m, 1H), 1.32–1.36 (m, 24H), 1.48 (s, 9H), 1.59–2.28 (m, 10H), 2.82–2.93 (m, 1H), 2.97 (t, J = 7.2, 2H), 3.17–3.26 (m, 1H), 3.30–3.43 (m, 1H), 3.55 (s, 3H), 3.78–3.95 (m, 2H), 4.05–4.12 (m, 2H), 4.72–4.85 (m, 5H), 7.84 (d, J = 13.9, 1H), 8.54 (s, 1H).

6.2.4.3. S-4,4-Bis(diisopropylphosphono)butyl 7-(4-(*tert*-butoxycarbonyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carbothioate (11b). Yield: 60% contaminated with a small amount of **2b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ *0.88–1.01* (m, 2H), 1.10–1.27 (m, 2H), 1.31–1.39 (m, 27H), 1.49 (s, 9H), 1.85–2.28 (m, 5H), 2.97 (t, J = 7.4, 2H), 3.18–3.52 (m, 5H), 3.71 (s, 3H), 3.80–4.05 (m, 2H), 4.34 (br s, 1H), 4.72–4.87 (m, 4H), 7.91 (d, J = 12.5, 1H), 8.57 (s, 1H).

General procedure for deprotection of bisphosphonates. To a solution of bisphosphonated fluoroquinolone thioester (0.70 mmol) in CH_2Cl_2 (50 mL) was added TMSBr (0.93 mL, 7.05 mmol). The reaction mixture was stirred for 65 h, the volatiles removed under reduced pressure and the solid dried under high vacuum for 1 h. The solid was suspended in H_2O (200 mL) and the pH was immediately adjusted to pH 7.5–8 by the addition of 1 M NaOH, with concomitant dissolution of the product. The product solution was filtered washing the insoluble material with water and $CHCl_3$. The aqueous solution was concentrated to dryness and purified by reverse-phase chromatography (gradient elution, 100% water–33% methanol/water) to provide the product.

6.2.4.4. *S*-4,4-Bisphosphonobutyl 7-((4a*S*,7a*S*)-octahydropyrrolo[3,4-*b*]pyridin-6-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carbothioate (12a). Yield: 47% based on tetrasodium salt of product. ^{1}H NMR (400 MHz, D_2O) δ *1.02–1.11* (m, 1H), 1.13–1.22 (m, 2H), 1.27–1.36 (m, 1H), 1.64–1.98 (m,

9H), 2.42–2.52 (m, 1H), 2.59–2.69 (m, 1H), 2.74–2.84 (m, 1H), 2.95–3.25 (m, 1H), 3.34–3.44 (m, 1H), 3.56–3.70 (m, 2H), 3.61 (s, 3H), 3.83–3.96 (m, 2H), 4.08–4.18 (m, 2H), 7.53 (d, J = 13.7, 1H), 8.59 (s, 1H).^{19}F (376 MHz, D_2O) δ *−121.38* (d, J = 13.7, 1F).^{31}P (162 MHz, D_2O) δ 20.74 (s, 2P). ESI-MS calculated for ($C_{25}H_{34}FN_3O_9P_2S$+H^+): 634; found: 634 (M+H^+). Purity (method A): 99.7% (254 nm), 99.7% (220 nm), 99.4% (320 nm).

6.2.4.5. *S*-4,4-Bisphosphonobutyl 7-(3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carbothioate (12b). Yield: 20% based on tetrasodium salt of product. ^{1}H NMR (400 MHz, D_2O) δ *0.96–1.04* (m, 2H), 1.16–1.25 (m, 2H), 1.33 (d, J = 6.3, 3H), 1.76–2.02 (m, 5H), 2.99–3.08 (m, 2H), 3.16–3.59 (m, 7H), 3.76 (s, 3H), 4.06–4.14 (m, 1H), 7.34 (d, J = 12.0, 1H), 8.66 (s, 1H). ^{19}F (376 MHz, D_2O) δ *−121.26* (d, J = 12.0, 1F). ^{31}P (162 MHz, D_2O) δ 20.80 (s, 2P). MS: (MH^+) 608.1. ESI-MS calculated for ($C_{23}H_{32}FN_3O_9P_2S$+H^+): 608; found: 608 (M+H^+). Purity (method B): 99.2% (254 nm), 100% (220 nm), 100% (290 nm).

6.2.5. Bisphosphonated gatifloxacin aryl ester

6.2.5.1. 4-(Bis(diethylphosphono)methyl)phenyl 7-(4-(*tert*-butoxycarbonyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (14). 2-Fluoro-1-methylpyridinium tosylate (0.360 g, 1.26 mmol) was added to a stirring solution of **2b** (500 mg, 1.05 mmol) in CH_2Cl_2 that was cooled in an ice-bath. Triethylamine (0.586 g, 4.2 mmol) was then added dropwise and the resulting mixture was stirred at that temperature for 70 min. A solution of **13** (0.40 g, 1.05 mmol) in CH_2Cl_2 (1 mL) was then added and the resulting solution was stirred while warming to room temperature over 18 h. After diluting with EtOAc, the organic layer was washed with 10% aqueous HCl, brine, 5% aqueous bicarbonate, brine then dried over Na_2SO_4. The crude product was purified by silica gel chromatography (0–30% MeOH in EtOAc) resulting in **14** as a colourless solid (0.318 g, 36%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (t, J = 3.9, 2H), 1.14–1.20 (m, 2H), 1.17 (t, J = 6.8, 6H), 1.29 (t, J = 6.8, 6H), 1.34 (d, J = 6.7, 3H), 1.50 (s, 9H), 3.20–3.25 (m, 3H), 3.40–3.47 (m, 2H), 3.74 (s, 3H), 3.91–4.00 (m, 3H), 4.02–4.16 (m, 8H), 4.35 (br s, 1H), 7.20 (d, J = 8.5, 2H), 7.40 (d, J = 8.5, 2H), 7.93 (d, J = 12.4, 1H), 8.71 (s, 1H). ^{19}F (376 MHz, D_2O) δ *−121.16* (d, J = 12.4, 1F). ^{31}P (162 MHz, $CDCl_3$) δ 19.35 (s, 2P).

6.2.5.2. 4-(Bisphosphonomethyl)phenyl 7-(3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (15). TMSBr (370 μL, 2.84 mmol) was added in one portion to a stirring solution of **14** (155 mg, 0.185 mmol) in CH_2Cl_2. After 18 h the solvent was removed at reduced pressure and the yellow solid was re-suspended in H_2O and the pH was adjusted to 7.4 by the addition of NaOH. The crude product was purified by Waters C18 Sep-Pak™ column (40 mL H_2O) to give **15** as a colourless solid (80 mg, 61%). ^{1}H NMR (400 MHz, D_2O) δ 1.24 (d, J = 6.2, 3H), 1.21–1.30 (m, 2H), 1.36–1.45 (m, 2H), 2.51–2.59 (m, 1H), 2.86 (br s, 1H), 3.04–3.14 (m, 2H), 3.20–3.26 (m, 1H), 3.42 (t, J = 23.2, 1H), 3.46–3.56 (m, 2H), 3.65 (s, 3H), 4.02 (br s, 1H), 7.27 (d, J = 7.7, 2H), 7.37 (d, J = 12.1, 1H), 7.67 (d, J = 7.0, 2H), 8.96 (s, 1H). ^{19}F (376 MHz, D_2O) δ *−121.76* (d, J = 12.1, 1F). ^{31}P (162 MHz, D_2O) δ 16.74 (s, 1P). ESI-MS calculated for ($C_{26}H_{30}FN_3O_{10}P_2$+$H^+$): 626; found: 626.1 (M+$H^+$). Purity (method B): 100% (254 nm), 100% (220 nm), 100% (290 nm).

6.2.6. Bisphosphonated fluoroquinolone glycolamide esters

6.2.6.1. Bisphosphonated bromoacetamides

*6.2.6.1.1. Dimethyl 1-(dimethoxyphosphoryl)-2-(4-nitrophenyl)ethylphosphonate (**19**).* Sodium hydride (1.02 g, 25.4 mmol) was added in portions to a stirring solution of tetramethyl methylenediphosphonate (5.8 g, 25 mmol) in DMF (40 mL). After 30 min a solution of 4-nitrobenzylbromide (5.00 g, 23.1 mmol) in THF (5 mL) was added and the resulting mixture was stirred at room temperature for 4.5 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl (20 mL). After the addition of water (100 mL) the product was extracted with EtOAc and the combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated at reduced pressure. The crude product was purified by silica gel chromatography (0% to 10% MeOH in EtOAc) resulting in **19** as a colorless solid (2.55 g, 30%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.65 (tt, J = 6.5, 23.8, 1H), 3.31 (dt, J = 6.5, 16.5, 2H), 3.73 (d, J = 7.0, 6H), 3.75 (d, J = 7.0, 6H), 7.42 (d, J = 8.9, 2H), 8.15 (d, J = 8.9, 2H).

*6.2.6.1.2. Dimethyl 2-(4-aminophenyl)-1-(dimethoxyphosphoryl)ethylphosphonate (**20**).* A mixture of **19** (1.01 g, 2.75 mmol) and PtO_2 (0.035 g, 0.15 mmol) in EtOH (40 mL, 95%) was shaken in a Parr apparatus under 3.8 hPa of H_2 for 14 h. The catalyst was removed by filtration through glass fiber filter paper and the solvent was removed under reduced pressure to give **20** as a pale yellow solid (0.959 g, 103%) that was used without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.62 (tt, J = 6.3, 23.9, 1H), 3.12 (dt, J = 6.3, 16.2, 2H), 3.70 (d, J = 1.9, 6H), 3.73 (d, J = 1.9, 6H), 6.61 (d, J = 8.5, 2H), 7.04 (d, J = 8.5, 2H).

*6.2.6.1.3. Dimethyl 2-{4-[(bromoacetyl)amino]phenyl}-1-(dimethoxyphosphoryl)ethylphosphonate (**21**).* A solution of **22** (0.959 g, 2.87 mmol) and pyridine (349 μL, 4.31 mmol) in 20 mL of CH_2Cl_2 was cooled in an ice-bath while stirring. A solution of bromoacetylbromide (250 μL, 2.87 mmol) in CH_2Cl_2 (5 mL) was added drop-wise and the resulting mixture was stirred for 4 h at that temperature. The reaction was quenched by the addition of water and the product was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude yellow solid was purified by silica gel chromatography resulting in **21** as a colorless solid (0.897 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.65 (tt, J = 6.2, 24.4, 1H), 3.22 (dt, J = 6.2, 17.4, 2H), 3.72 (d, J = 3.7, 6H), 3.75 (d, J = 3.7, 6H), 4.01 (s, 2H), 7.26 (d, J = 8.6, 2H), 7.47 (d, J = 8.6, 2H), 8.15 (br s, 1H). ^{31}P (162 MHz, $CDCl_3$) δ 26.33 (s, 2P).

*6.2.6.1.4. Tetraethyl N-benzyl-N-methyl-1-aminomethylenebisphosphonate (**24**).* Triethyl orthoformate (13.8 g, 93.3 mmol), diethyl phosphite (32.2 g, 233 mmol) and *N*-benzylmethyl amine (9.42 g, 77.7 mmol) were heated in a 100 mL round bottom flask fitted with a distillation apparatus. The reaction was heated to a temperature of 180–190 °C for 3 h under Ar at which time the evolution of EtOH was complete. The reaction mixture was cooled to room temperature, diluted with $CHCl_3$ (400 mL), washed with aqueous NaOH (1 M) and brine then dried over Na_2SO_4. The solvent was removed at aspirator pressure resulting in the colourless oil **24** (31.7 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (t, J = 7.1, 6H), 1.35 (t, J = 7.1, 6H), 2.66 (s, 3H), 3.48 (t, J = 24.9, 1H), 3.99 (s, 2H), 4.07–4.24 (m, 8H), 7.24–7.39 (m, 5H).

*6.2.6.1.5. Tetraethyl N-methyl-1-aminomethylenebisphosphonate (**25**).* Compound **24** (12.4 g, 30.4 mmol) was dissolved in EtOH (150 mL) followed by the addition of palladium on carbon (10%, 5 g) and cyclohexene (9.0 mL, 88.7 mmol). The resulting mixture was heated to reflux under argon for 16 h. The cooled solution was filtered through glassfiber filter paper and concentrated at reduced pressure to give **25** as a pale yellow oil (8.7 g, 90%), which was used directly in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (t, J = 7.4, 12H), 2.69 (s, 3H), 3.41 (m, 1H), 4.20–4.31 (m, 8H). ^{31}P (162 MHz, $CDCl_3$) δ 19.44 (s, 2P).

*6.2.6.1.6. Tetraethyl N-(bromoacetyl)-N-methyl-1-aminomethylenebisphosphonate (**26**).* A solution of bromoacetyl bromide (1.48 mL, 17.0 mmol) in CH_2Cl_2 (1 mL) was added dropwise to a stirred, cooled (ice-bath) solution of **25** (4.5 g, 14 mmol) and pyri-

dine (1.78 mL, 21.3 mmol) in CH_2Cl_2 (25 mL). The reaction was stirred for 18 h while slowly warming to room temperature. After quenching with water the product was extracted with CH_2Cl_2 and the combined organics were washed with 10% aqueous HCl, brine, dried over sodium sulfate and concentrated at reduced pressure. The crude yellow oil was purified by silica gel chromatography on an automated flash chromatography system (0–10% MeOH in EtOAc) resulting in **26** as a pale yellow liquid (2.93 g, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (t, J = 7.1, 12H), 3.38 (s, 3H), 3.92 (s, 2H), 4.15–4.25 (m, 8H), 5.69 (t, J = 24.5, 1H). ^{31}P (162 MHz, $CDCl_3$) δ 16.93 (s, 2P).

*6.2.6.1.7. Tetraethyl 1-(N-(N-α,ε-di-(tert-butoxycarbonyl)lysinoyl)amino)methylenebisphosphonate (**34**).* To a solution of Boc-Lys(Boc)-OH dicyclohexylamine salt (1.57 g, 2.97 mmol) in CH_2Cl_2 (12 mL) was added amine **29** (900 mg, 2.97 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (626 mg, 3.26 mmol) and DMAP (36 mg, 0.30 mmol). The mixture was stirred for 18 h, after which the precipitate was removed by filtration and washed with a portion of CH_2Cl_2. The combined filtrates were washed with 1 M HCl, saturated $NaHCO_3$ solution and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness. The residue was purified by flash chromatography on silica gel using a gradient of 0–15% MeOH/EtOAc. Amide **34** was obtained as a white foam (1.35 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ *1.30–1.34* (m, 12H), 1.43 (s, 18H), 1.38–1.53 (m, 4H), 1.59–1.69 (m, 1H), 1.79–1.87 (m, 1H), (3.07–3.12 (m, 2H), 4.11–4.24 (m, 8H), 4.71 (br s, 1H), 4.97 (dt, J = 21.4, 10.0, 1H), 5.11 (br s, 1H), 6.76 (d, J = 10.0, 1H).

*6.2.6.1.8. Tetraethyl 1-(N-lysinoylamino)methylenebisphosphonate, trifluoroacetate salt (**35**).* To carbamate **34** (1.35 g, 2.14 mmol) was added a solution of TFA/CH_2Cl_2 (11 mL, 40% v/v). After stirring for 18 h, the reaction mixture was concentrated to dryness and co-evaporated several times with Et_2O. The resulting deprotected material, a yellowish oil (2.1 g, >quant), was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ *1.22–1.28* (m, 12H), 1.34–1.40 (m, 2H), 1.48–1.54 (m, 2H), 1.68–1.74 (m, 2H), 2.71–2.76 (m, 2H), 3.93–3.97 (m, 1H), 4.03–4.15 (m, 8H), 4.82 (dt, J = 22.4, 9.8, 1H), 7.77 (br s, 3H), 8.25 (bd, J = 4.2, 3H), 9.27 (d, J = 9.8, 1H).

*6.2.6.1.9. Tetraethyl 1-(N-(N-α,ε-di-(bromoacetyl)lysinoyl)amino)-methylenebisphosphonate (**36**).* To TFA salt **35** (max 2.14 mmol) in CH_2Cl_2 (27 mL) at 0 °C was added pyridine (1.73 mL, 21.4 mmol) and bromoacetyl bromide (390 μL, 4.49 mmol). The mixture was stirred for 1.5 h at 0 °C after which it was diluted with CH_2Cl_2 and washed with 5% HCl, saturated $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated to dryness. Purification by flash chromatography on silica gel, using a gradient of 0–20% MeOH/EtOAc provided **36** as a white foam (574 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ *1.30–1.37* (m, 12 H), 1.38–1.46 (m, 2H), 1.53–1.61 (m, 2H), 1.72–1.81 (m, 1H), 1.86–1.93 (m, 1H), 3.25–3.34 (m, 2H), 3.87 (s, 2H), 3.88 (s, 2H), 4.13–4.25 (m, 8H), 4.53 (q, J = 6.2, 1H), 4.97 (dt, J = 21.7, 9.8, 1H), 6.96 (br s, 1H), 7.01 (bd, J = 9.8, 1H), 7.17 (d, J = 7.8, 1H).

6.2.6.2. Preparation of glycolamide esters. *General procedure for coupling of bromoacetamides with N-Boc fluoroquinolones.* A solution of N-Boc fluoroquinolone (2.1 mmol), bromoacetamide (2.1 mmol) and Cs_2CO_3 (0.76 g, 2.3 mmol) in 10 mL of DMF was stirred at room temperature for 12 h. The mixture was then diluted with H_2O and extracted with CH_2Cl_2. The organics were washed with brine, dried over sodium sulfate, filtered and concentrated at reduced pressure resulting in a brown oil that was purified by silica gel chromatography.

*6.2.6.2.1. (1,1-Bis(diethylphosphono)methylcarbamoyl)methyl 7-((4aS,7aS)-1-(tert-butoxycarbonyl)-octahydropyrrolo[3,4-b]pyridin-6-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (**17a**).* From **2a** and **16**. Chromatography eluent: 0–8% MeOH in CH_2Cl_2. Yield: 72%. ^{1}H NMR (400 MHz, $CDCl_3$) δ *0.75–0.80* (m, 1H), 0.99–1.09 (m, 2H), 1.22–1.29 (m, 1H), 1.31–1.37 (m, 12H), 1.46–1.51 (m, 1H), 1.48 (s, 9H), 1.75–1.83 (m, 3H), 2.22–2.27 (m, 1H), 2.86–2.91 (m, 1H), 3.19–3.23 (m, 1H), 3.34–3.38 (m, 1H), 3.56 (s, 3H), 3.83 (dt, J = 1.9, 10.0, 1H), 3.87–3.92 (m, 1H), 4.02–4.09 (m, 2H), 4.20–4.38 (m, 8H), 4.73–4.82 (br s, 1H), 4.83 (d, J = 15.8, 1H), 4.91 (d, J = 15.8, 1H), 5.22 (dt, J = 10.0, 23.0, 1H), 7.75 (d, J = 14.1, 1H), 8.49 (s, 1H), 9.24 (d, J = 9.2, 1H).

*6.2.6.2.2. (1,1-Bis(diethylphosphono)methylcarbamoyl)methyl 7-(4-(tert-butoxycarbonyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (**17b**).* From **2b** and **16**. Chromatography eluent: 0–5% MeOH in CH_2Cl_2. Yield: 62%. ^{1}H NMR (400 MHz, $CDCl_3$) δ *0.90–0.95* (m, 2H), 1.13–1.19 (m, 2H), 1.32–1.36 (m, 15H), 1.50 (s, 9H), 3.19–3.46 (m, 5H), 3.72 (s, 3H), 3.89–3.97 (m, 2H), 4.21–4.37 (m, 9H), 4.87 (s, 2H), 5.21 (dt, J = 9.9, 22.9, 1H), 7.81 (d, J = 12.4, 1H), 8.53 (s, 1H), 9.10 (d, J = 9.9, 1H). MS: (MH^+) 819.3.

*6.2.6.2.3. (1,1-Bis(diethylphosphono)methylcarbamoyl)methyl 7-(4-(tert-butoxycarbonyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**19c**).* From **2c** and **16**. Chromatography eluent: 0–7% MeOH in CH_2Cl_2. Yield: 90%. ^{1}H NMR (400 MHz, $CDCl_3$) δ *1.12–1.17* (m, 2H), 1.31–1.36 (m, 14H), 1.50 (s, 9H), 3.26 (bt, J = 4.9, 4H), 3.41–3.47 (m, 1H), 3.66 (bt, J = 4.9, 4H), 4.20–4.38 (m, 8H), 4.88 (s, 2H), 5.22 (dt, J = 10.3, 22.6, 1H), 7.31 (d, J = 7.2, 1H), 7.99 (d, J = 13.2, 1H), 8.49 (s, 1H), 9.21 (d, J = 9.8, 1H). ^{19}F (376 MHz, $CDCl_3$) δ *−123.57* (dd, J = 7.2, 13.2, 1F). ^{31}P (162 MHz, $CDCl_3$) δ 17.35 (s, 2P).

*6.2.6.2.4. (4-(2,2-Bis(dimethylphosphono)ethyl)phenylcarbamoyl)-methyl 7-((4aS,7aS)-1-(tert-butoxycarbonyl)-octahydropyrrolo[3,4-b]pyridin-6-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (**24a**).* From **2a** and **21.** Chromatography eluent: 0–8% MeOH in CH_2Cl_2. Yield: 65%. ^{1}H NMR (400 MHz, $CDCl_3$) δ *0.73–0.84* (m, 1H), 0.97–1.11 (m, 2H), 1.20–1.28 (m, 2H), 1.41–1.52 (m, 1H), 1.46 (s, 9H), 1.70–1.85 (m, 2H), 2.19–2.29 (m, 1H), 2.67 (tt, J = 6.3, 23.7, 1H), 2.82–2.93 (m, 1H), 3.20 (dt, J = 6.2, 16.0, 3H), 3.36 (br s, 1H), 3.55 (s, 3H), 3.69 (d, J = 3.1, 6H), 3.72 (d, J = 3.1, 6H), 3.74–3.95 (m, 2H), 4.01–4.13 (m, 2H), 4.77 (br s, 1H), 4.89 (AB q, J = 14.9, 2H), 7.24 (d, J = 8.4, 2H), 7.88 (d, J = 8.4, 2H), 7.89 (d, J = 14.1, 1H), 8.49 (s, 1H), 11.0 (s, 1H).

*6.2.6.2.5. (4-(2,2-Bis(dimethylphosphono)ethyl)phenylcarbamoyl)-methyl 7-(4-(tert-butoxycarbonyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (**24b**).* From **2b** and **21**. Chromatography eluent: 0–8% MeOH in CH_2Cl_2. Yield: 70%. ^{1}H NMR (400 MHz, $CDCl_3$) δ *0.92–1.00* (m, 2H), 1.16–1.25 (m, 2H), 1.35 (d, J = 6.8, 3H), 1.50 (s, 9H), 2.69 (tt, J = 6.4, 24.3, 1H), 3.17–3.50 (m, 7H), 3.71 (d, J = 2.5, 6H), 3.74 (s, 3H), 3.75 (d, J = 2.5, 6H), 3.93–3.99 (m, 2H), 4.36 (br s, 1H), 4.92 (s, 2H), 7.26 (d, J = 8.8, 2H), 7.89 (d, J = 8.8, 2H), 7.98 (d, J = 12.1, 1H), 8.57 (s, 1H), 10.90 (s, 1H). ^{19}F (376 MHz, $CDCl_3$) δ *−120.65* (d, J = 12.1, 1F). ^{31}P (162 MHz, $CDCl_3$) δ 26.5 (s, 2P).

*6.2.6.2.6. (N-Methyl-1,1-bis(diethylphosphono)methylcarbamoyl)-methyl 7-((4aS,7aS)-1-(tert-butoxycarbonyl) -octahydropyrrolo[3,4-b]pyridin-6-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (**29a**).* From **2a** and **26**. Chromatography eluent: 0% to 10% MeOH in CH_2Cl_2. Yield: 25%. ^{1}H NMR (400 MHz, $CDCl_3$) δ *0.74–0.83* (m, 1H), 0.96–1.15 (m, 2H), 1.18–1.25 (m, 1H), 1.31–1.39 (m, 14H), 1.48 (s, 9H), 1.75–1.81 (m, 2H), 2.20–2.27 (m, 1H), 2.88 (bt, J = 8.7, 1H), 3.21 (br s, 1H), 3.31 (s, 3H), 3.36 (br s, 1H), 3.54 (s, 3H), 3.80–3.91 (m, 2H), 4.01–4.08 (m, 2H), 4.13–4.26 (m, 8H), 4.77 (br s, 1H), 4.99 (AB q, J = 15.8, 2H), 5.70 (t, J = 24.8, 1H), 7.81 (d, J = 7.8, 1H), 8.61 (s, 1H). ^{31}P (162 MHz, $CDCl_3$) δ 17.10 (s, 2P).

*6.2.6.2.7. (N-Methyl-1,1-bis(diethylphosphono)methylcarbamoyl)methyl 7-(4-(tert-butoxycarbonyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (**29b**).* From **2b** and **26**. Chromatography eluent: 0–10% MeOH in CH_2Cl_2. Yield: 30%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (bt,

J = 3.8, 2H), 1.13 (d, *J* = 7.5, 2H), 1.30–1.39 (m, 15H), 1.50 (s, 9H), 3.19–3.27 (m, 3H), 3.31 (s, 3H), 3.42 (bt, *J* = 13.4, 2H), 3.70 (s, 3H), 3.84–3.90 (m, 1H),3.94 (d, *J* = 12.2, 1H), 4.11–4.26 (m, 8H), 4.33 (br s, 1H), 4.97–5.01 (m, 2H), 5.70 (t, *J* = 24.8, 1H), 7.88 (d, *J* = 12.8, 1H), 8.63 (s, 1H). ^{19}F (376 MHz, $CDCl_3$) δ *−121.61* (d, *J* = 12.8, 1F). ^{31}P (162 MHz, $CDCl_3$) δ 17.11 (s, 2P).

*6.2.6.2.8. Bis(gatifloxacin ester) conjugate **39**.* From **2b** and **36** (½ mole equivalent). The crude product was purified by reverse phase flash chromatography on a C18 column, using a gradient of 20–100% MeCN/H_2O, followed by flash chromatography on silica gel using a gradient of 0–10% MeOH/CH_2Cl_2. Yield: 47%. ^{1}H NMR (400 MHz, $CDCl_3$) δ *0.93–0.99* (m, 4H), 1.14–1.19 (m, 4H), 1.25–1.34 (m, 18H), 1.49 (s, 9H), 1.50 (s, 9H), 1.55–1.61 (m, 1H), 1.65–1.77 (m, 3H), 1.99–2.06 (m, 2H), 3.20–3.35 (m, 9H), 3.39–3.47 (m, 4H), 3.74 (s, 6H), 3.91–3.98 (m, 4H), 4.11–4.18 (m, 8H), 4.34 (br s, 2H), 4.49–4.68 (m, 5H), 5.03 (dt, *J* = 21.9, 10.2, 1H), 7.12 (d, *J* = 10.2, 1H), 7.86 (d, *J* = 12.3, 2H), 8.47–8.50 (m, 2H), 8.72 (t, *J* = 5.6, 1H), 9.51 (d, *J* = 8.2, 1H). ESI-MS calculated for $C_{67}H_{95}F_2N_9O_{21}P_2$ 1461, found 1460 $(M\text{-}H)^-$.

General procedure for deprotection of bisphosphonates. TMSBr (3.0 mL, 23 mmol) was added in one portion to a stirring solution of protected bisphosphonated fluoroquinolone (1.50 mmol) in CH_2Cl_2. After 18 h the solvent was removed at reduced pressure. The yellow solid resuspended in H_2O and the pH adjusted to 7.4 by the addition of NaOH. The resulting solution was the subjected to chromatography on a Waters C18 Sep-Pak™ to furnish the product.

*6.2.6.2.9. (1,1-Bisphosphonomethylcarbamoyl)methyl 1-cyclopropyl-6-fluoro-1,4-dihydro-7-((4aS,7aS)-octahydropyrrolo[3,4-b]pyridin-6-yl)-8-methoxy-4-oxoquinoline-3-carboxylate (**18a**).* Compound **18a** eluted in H_2O then 5% MeOH/H_2O. Yield: 69%. ^{1}H NMR (400 MHz, D_2O) δ *0.70–0.78* (m, 1H), 0.86–1.00 (m, 2H), 1.03–1.10 (m, 1H), 1.67–1.78 (m, 4H), 2.61 (br s, 1H), 2.90–2.95 (m, 1H), 3.23–3.26 (m, 1H), 3.40 (s, 3H), 3.51–3.65 (m, 3H), 3.75 (br s, 1H), 3.83–3.87 (m, 1H), 3.92–3.97 (m, 1H), 4.19 (t, *J* = 18.0, 1H), 4.74 (s, 2H), 7.24 (d, *J* = 15.0, 1H), 8.77 (s, 1H). ^{19}F (376 MHz, D_2O) δ *−121.76* (d, *J* = 15.0, 1F). ^{31}P (162 MHz, D_2O) δ 13.90 (s, 2P). ESI-MS calculated for $(C_{24}H_{31}FN_4O_{11}P_2{+}H^+)$: 633; found: 633.0 $(M{+}H^+)$. Purity (method B): 99.4% (254 nm), 99.8% (220 nm), 99.7% (290 nm).

*6.2.6.2.10. (1,1-Bisphosphonomethylcarbamoyl)methyl 7-(3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (**18b**).* Compound **18b** eluted in H_2O then 5% MeOH/H_2O. Yield: 75%. ^{1}H NMR (400 MHz, D_2O) δ *1.02–1.06* (m, 2H), 1.18–1.23 (m, 2H), 1.38 (d, *J* = 6.7, 3H), 3.31–3.43 (m, 2H), 3.49–3.69 (m, 5H), 3.81 (s, 3H), 4.14–4.20 (m, 1H), 4.49 (t, *J* = 20.1, 1H), 4.94 (s, 2H), 7.68 (d, *J* = 12.2, 1H), 9.00 (s, 1H). ^{19}F (376 MHz, D_2O) δ *−119.20* (d, *J* = 12.2, 1F). ^{31}P (162 MHz, D_2O) δ 16.41 (s, 2P). ESI-MS calculated for $(C_{22}H_{29}FN_4O_{11}P_2{+}H^+)$: 606; found: 606.0 $(M{+}H^+)$. Purity (method B): 99.5% (254 nm), 99.5% (220 nm), 99.7% (290 nm).

*6.2.6.2.11. (1,1-Bisphosphonomethylcarbamoyl)methyl 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid (**18c**).* Compound **18c** eluted with H_2O then 5% MeOH/H_2O. Yield: 66%. ^{1}H NMR (400 MHz, D_2O) δ *1.18–1.22* (m, 2H), 1.36–1.41 (m, 2H), 3.15 (m, 4H), 3.25 (m, 4H), 3.52 (br s, 1H), 4.22 (t, *J* = 18.7, 1H), 4.79 (s, 2H), 7.36 (d, *J* = 7.0, 1H), 7.60 (d, *J* = 12.2, 1H), 8.80 (s, 1H). ^{19}F (376 MHz, D_2O) δ *−123.72* (dd, *J* = 7.0, 12.2, 1F). ^{31}P (162 MHz, D_2O) δ 13.91 (s, 2P). ESI-MS calculated for $(C_{20}H_{25}FN_4O_{10}P_2{+}H^+)$: 563; found: 563.1 $(M{+}H^+)$. Purity (method A): 97.9% (254 nm), 97.4% (220 nm), 98.2% (290 nm).

*6.2.6.2.12. (4-(2,2-Bisphosphonoethyl)phenylcarbamoyl)methyl 1-cyclopropyl-6-fluoro-1,4-dihydro-7-((4aS,7aS)-octahydropyrrolo[3,4-b]pyridin-6-yl)-8-methoxy-4-oxoquinoline-3-carboxylate (**23a**).* Compound **23a** eluted with 0% to 10% MeOH in H_2O. Yield: 9%. ^{1}H NMR (400 MHz, D_2O) δ *0.60–0.69* (m, 1H), 0.93–1.07 (m, 2H), 1.12–1.21 (m, 1H), 1.72–1.96 (m, 4H), 2.16 (tt, *J* = 6.9, 20.6, 1H), 2.62–2.72 (m, 1H), 2.90–3.17 (m, 3H), 3.31–3.40 (m, 1H), 3.44–3.63 (m, 5H), 3.65–3.72 (m, 1H), 3.75–3.99 (m, 3H), 4.80 (s, 2H), 7.25–7.39 (m, 5H), 8.57 (s, 1H). ^{19}F (376 MHz, D_2O) δ *−121.42* (d, *J* = 14.0, 1F). ^{31}P (162 MHz, D_2O) δ 20.25 (d, *J* = 22.4, 2P). ESI-MS calculated for $(C_{31}H_{37}FN_4O_{11}P_2{+}H^+)$: 723; found: 723.2 $(M{+}H^+)$. Purity (method A): 95.7% (254 nm), 95.4% (220 nm), 96% (290 nm).

*6.2.6.2.13. (4-(2,2-Bisphosphonoethyl)phenylcarbamoyl)methyl 7-(3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (**23b**).* Compound **23a** eluted with 0% to 10% MeOH in H_2O. Yield: 43%. ^{1}H NMR (400 MHz, D_2O) δ *0.98–1.01* (m, 2H), 1.15–1.18 (m, 2H), 1.22 (d, *J* = 6.3, 3H), 2.20 (tt, *J* = 6.7, 21.0, 1H), 3.07–3.49 (m, 9H), 3.80 (s, 3H), 4.05–4.11 (m, 1H), 4.92 (s, 2H), 7.46 (AB q, *J* = 8.4, 4H), 7.55 (d, *J* = 12.3, 1H), 8.80 (s, 1H). ^{19}F (376 MHz, D_2O) δ *−121.16* (d, *J* = 12.3, 1F). ^{31}P (162 MHz, D_2O) δ 20.23 (s, 2P). ESI-MS calculated for $(C_{29}H_{35}FN_4O_{11}P_2{+}H^+)$: 697; found: 697.2 $(M{+}H^+)$. Purity (method B): 89.3% (254 nm), 91.4% (220 nm), 94.0% (290 nm).

*6.2.6.2.14. (N-Methyl-1,1-bisphosphonomethylcarbamoyl)methyl 1-cyclopropyl-6-fluoro-1,4-dihydro-7-((4aS,7aS)-octahydropyrrolo[3,4-b]pyridin-6-yl)-8-methoxy-4-oxoquinoline-3-carboxylate (**28a**).* Compound **28a** eluted with 0% to 10% MeOH in H_2O. Yield: 27%. ^{1}H NMR (400 MHz, D_2O) δ *0.89–0.96* (m, 1H), 1.06–1.17 (m, 2H), 1.20–1.26 (m, 1H), 1.83–1.93 (m, 4H), 2.78 (br s, 1H), 3.10 (br s, 1H), 3.16 (s, 1/3-3H), 3.27 (s, 2/3-3H), 3.39 (br s, 1H), 3.55 (s, 1/3-3H), 3.57 (s, 2/3-3H), 3.67–3.83 (m, 3H), 3.88–4.14 (m, 3H), 4.92 (t, *J* = 21.9, 1H), 5.12 (AB q, *J* = 15.7, 2/3-2H), 5.16 (AB q, *J* = 15.7, 1/3-2H), 7.37 (d, *J* = 14.0, 2/3-1H), 7.44 (d, J = 14.0, 1/3-1H), 8.96 (s, 1H). ^{19}F (376 MHz, D_2O) δ *−121.92* (d, *J* = 14.0, 2/3-1F), −121.84 (d, *J* = 14.0, 1/3-1F). ^{31}P (162 MHz, D_2O) δ 12.31 (s, 1/3-2P), 13.08 (s, 2/3-2P). ESI-MS calculated for $(C_{25}H_{33}FN_4O_{11}P_2{+}H^+)$: 647; found: 647.1 $(M{+}H^+)$. Purity (method B): 98.4% (254 nm), 99.2% (220 nm), 98.9% (320 nm).

*6.2.6.2.15. (N-Methyl-1,1-bisphosphonomethylcarbamoyl)methyl 7-(3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (**28b**).* Compound **28b** eluted with 0–5% MeOH in H_2O. Yield: 54%. ^{1}H NMR (400 MHz, D_2O) δ *1.06–1.11* (m, 2H), 1.19–1.23 (m, 2H), 1.33–1.36 (m, 3H), 3.16 (s, 1/3-3H), 3.27 (s, 2/3-3H), 3.21–3.30 (m, 1H), 3.32–3.36 (m, 1H), 3.40–3.48 (m, 1H), 3.53–3.64 (m, 3H), 3.74 (s, 2/3-3H), 3.79 (s, 1/3-3H), 3.89 (t, *J* = 21.0, 1/3-1H), 4.13–4.17 (m, 1H), 4.92 (t, *J* = 21.0, 2/3-1H), 5.13 (s, 2/3-2H), 5.19 (s, 1/3-2H), 7.45 (bd, *J* = 12.0, 2/3-1H), 7.59 (bd, *J* = 12.0, 1/3-1H), 9.02 (s, 2/3-1H), 9.03 (s, 1/3-1H). ^{19}F (376 MHz, D_2O) δ *−121.83* (d, *J* = 12.0, 1/3-1F), -122.02 (d, *J* = 12.0, 2/3-1F). ^{31}P (162 MHz, D_2O) δ 12.32 (s, 1/3-2P), 13.11 (s, 2/3-2P). ESI-MS calculated for $(C_{23}H_{31}FN_4O_{11}P_2{+}H^+)$: 621; found: 621.1 $(M{+}H^+)$. Purity (method B): 98.0% (254 nm), 97.3% (220 nm), 97.4% (290 nm).

*6.2.6.2.16. Bis(gatifloxacin ester) conjugate **38**.* To a solution of protected conjugate **39** (391 mg, 0.27 mmol) in CH_2Cl_2 (5 mL) was added 2,6-lutidine (1.55 mL, 13.4 mmol). The mixture was cooled to -78 °C and trimethylsilylbromide (882 μL, 6.68 mmol) was added slowly. The mixture was brought to room temperature and stirred for 18 h, then was concentrated to dryness. Crude product was purified by 2 consecutive reverse phase flash chromatographies on a C18 column, using a gradient of 5–60% MeCN/50 mM $Et_3NH_2CO_3$ buffer, pH 7 for the first column, then a gradient of 5–50% MeCN/50 mM $Et_3NH_2CO_3$ buffer, pH 7 for the second column. Lyophilization of the combined pure fractions provided conjugate **40** as a white solid (16 mg, 5%). ^{1}H NMR (400 MHz, DMSO-d_6+TFA) δ 0.97 (br s, 4H), 1.07–1.10 (m, 4H), 1.17 (t, *J* = 7.4, 9H), 1.26 (d, *J* = 6.4, 6H), 1.34–1.49 (m, 4H), 1.61–1.64 (m, 1H), 1.72–1.76 (m, 1H), 3.10 (q, *J* = 7.4, 6H), 3.17–3.26 (m, 4H), 3.38–3.52 (m, 10H), 3.81 (s, 6H), 4.01, 4.06 (m, 2H), 4.45–4.64 (m, 5H), 7.67 (d, *J* = 12.1, 1H), 7.68 (d, *J* = 12.1, 1H), 8.52 (s, 1H), 8.53 (s, 1H). ESI-MS calculated for $(C_{49}H_{63}F_2N_9O_{17}P_2{-}H^+)$: 1148; found: 1148.2 $(M\text{-}H)^-$. Purity (method C): 98.1% (254 nm), 97.6% (220 nm), 99.1% (320 nm).

6.2.7. Preparation of bisphosphonated moxifloxacin thioglycolamide ester

6.2.7.1. Tetraethyl 1-(*N*-3-thiapropionylamino)methylenebisphosphonate (30). A mixture of amine **31** (691 mg, 2.28 mmol) and mercaptoacetic acid (200 μL, 2.89 mmol) was heated to 140–150 °C under a continuous argon purge. When steam evolution appeared complete, the residue was purified by flash chromatography on silica gel eluting with 5% methanol/CH_2Cl_2 to give **30** (0.321 g, 37%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.339 (t, J = 7.0, 6H), 1.344 (t, J = 7.0, 6H), 1.99 (t, J = 8.8, 1H), 3.25–3.35 (m, 2H), 4.11–4.30 (m, 8H), 4.97 (td, J = 21.4, J = 10.1, 1H).

6.2.7.2. S-(1,1-Bis(diethylphosphono)methylcarbamoyl)methyl 7-((4a*S*,7a*S*)-1-(*tert*-butoxycarbonyl) -octahydropyrrolo[3,4-*b*]pyridin-6-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carbothioate (31). To a solution of **2a** (427 mg, 0.851 mmol) in CH_2Cl_2 (6.5 mL) was added 2-fluoro-1-methylpyridinium tosylate (0.292 g, 1.03 mmol). The reaction mixture was cooled to 0 °C, and triethylamine (0.43 mL, 3.09 mmol) was added via syringe. After stirring 1 h at 0 °C a solution of thiol **30** (0.32 g, 0.85 mmol) in CH_2Cl_2 (10 mL) was added. After a further 10 min at 0 °C the reaction was allowed to warm to room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with ice cold saturated NH_4Cl solution, ice cold 5% $NaHCO_3$, water and brine. After drying ($MgSO_4$) and concentration to dryness, the residue was purified by flash chromatography on silica gel eluting with 4% methanol/CH_2Cl_2 to give almost pure **31** (0.418 g, 57%) as a yellow foam, which was used without additional purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ *0.75–0.85* (m, 1H), 0.99–1.17 (m, 2H), 1.21–1.39 (m, 13H), 1.48 (s, 9H), 1.61 (s, 2H), 1.75–1.83 (m, 2H), 2.21–2.30 (m, 1H), 2.82–2.94 (m, 1 H), 3.17–3.28 (m, 1H), 3.30–3.44 (m, 1H), 3.57 (s, 3H), 3.72 (s, 2H), 3.81–3.88 (m, 1H), 3.91–3.98 (m, 1H), 4.01–4.28 (m, 10H), 4.70–4.86 (br s, 1H), 4.98 (td, J = 21.6, 9.9, 1H), 7.10 (d, J = 10.3, 1H), 8.59 (s, 1H).

6.2.7.3. *S*-(1,1-Bisphosphonomethylcarbamoyl)methyl 1-cyclopropyl-6-fluoro-1,4-dihydro-7-((4a*S*,7a*S*)-octahydropyrrolo[3,4-*b*]pyridin-6-yl)-8-methoxy-4-oxoquinoline-3-carbothioate (32). To a solution of **31** (418 mg, 0.486 mmol) in CH_2Cl_2 (30 mL) was added TMSBr (0.64 mL, 4.8 mmol). The reaction mixture was stirred for 41 h, the solvent removed under reduced pressure and the solid dried under high vacuum for 1 h. The solid was suspended in H_2O (100 mL) and the pH was immediately adjusted to pH 7 by the addition of 1 M NaOH, with concomitant dissolution of the product. The product solution was washed with $CHCl_3$ (2× 50 mL), filtered, concentrated to dryness and purified by reverse-phase chromatography (gradient elution, 0–15% methanol in water) to furnish **32** as a slightly yellowish solid (90 mg, 25% recovery based on tetrasodium salt of product). ^{1}H NMR (400 MHz, D_2O) δ *0.93–1.03* (m, 1H), 1.03–1.19 (m, 2H), 1.19–1.29 (m, 1H), 1.78–2.01 (m, 4H), 2.79 (br s, 1H), 3.02–3.12 (m, 1H), 3.36–3.44 (m, 1H), 3.61 (s, 3H), 3.57–3.85 (m, 3H), 3.78 (br s, 2H), 3.89–3.95 (m, 1H), 4.02–4.16 (m, 2H), 4.27 (t, J = 18.7, 1H), 7.40 (d, J = 13.7, 1H), 8.59 (s, 1H). ^{19}F (376 MHz, D_2O) δ *−94.67* (d, J = 13.7, 1F). ^{31}P (162 MHz, D_2O) δ 14.08 (d, J = 22.0, 1P), 13.95 (d, J = 22.0, 1P). ESI-MS calculated for ($C_{24}H_{31}FN_4O_{10}P_2S+H^+$): 649; found: 649 ($M+H^+$). Purity (method A): 99.1% (254 nm), 99.6% (220 nm), 99.0% (290 nm).

6.3. Biology

6.3.1. Determination of minimum inhibitory concentrations against *S. aureus* ATCC 13709

The antimicrobial activities of the compounds were tested against *S. aureus* strain ATCC 13709. Minimum inhibitory concentration (MIC) testing was performed by the microdilution method according to M7–A7 guidelines set by the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards).[18]

6.3.2. Determination of levels of bone binding in vitro

Individual compounds were dissolved in PBS and added, at a final concentration of 1 mg/mL, to a slurry of bone meal powder (Now Foods, Bloomingdale, Illinois, USA) in PBS at 10 mg/mL. The suspension of drug/prodrug in bone meal powder was incubated at 37 °C for 1 h to allow for binding, and centrifuged at 13,000 rpm for 2 min, before recovering the supernatant. The bone meal powder pellet was then washed three times with 1 ml of PBS. All supernatants were saved and assessed for fluoroquinolone content by fluorescence measurements at excitation/emission wavelengths of 280/465 nm. The amount of fluoroquinolone was determined from standard curves generated for each experiment. The amount of drug/prodrug bound to bone powder was deduced from the difference between the input amount (typically 1 mg) and the amount recovered in the supernatants after binding. In all binding experiments, >99% of input drug was recovered in the supernatant for the parent drugs.

6.3.3. Regeneration of parent drug from bone-bound prodrug

Washed bone powder-bound prodrugs from the protocol described in Section 6.3.2 were resuspended in 400 μL PBS or in 400 μL 50% (v/v in PBS) human or rat serum. The suspension was incubated for 24 h at 37 °C, centrifuged at 13,000 rpm for 2 min and the supernatant was recovered. Methanol (5× volume relative to supernatant) was added to each supernatant and the mixture was vortexed on a floor model vortex for 15 min to extract freed fluoroquinolone. The mixture was then centrifuged at 10,000 rpm for 15 min to pellet the insoluble material. The supernatant containing the extracted fluoroquinolone was recovered and evaporated to dryness in a speed vac. The dried pellets were resuspended in PBS and the amount of fluoroquinolone was determined by fluorescence measurements as described previously. The percentage of drug regenerated was deduced from the difference between amount of bound prodrug and the amount of regenerated drug. The identity of regenerated drug was deduced by determination of the minimum inhibitory concentration (as per the guidelines of the Clinical and Laboratory Standards Institute, guideline M7–A7), which always matched those of parent drugs but not those of prodrugs.

6.3.4. Prophylactic use of prodrugs in a rat model of bone infection

Staphylococcus aureus ATCC 13709 cells were grown overnight at 37 °C in brain heart infusion broth (BHIB). Cells were subcultured into fresh BHIB and incubated for 4–5 h at 37 °C. The cells were washed twice with PBS and resuspended in BHIB supplemented with 10% (vol./vol.) fetal bovine serum at a density of approximately 10^{10} colony forming units (CFU)/ml (based upon turbidimetry). The suspension was aliquoted and a portion was plated to check the CFU count. The culture was stored frozen (−80 °C) and was used without subculture. For use as an inoculum the culture was thawed, diluted in PBS and kept in an ice bath until it was used.

Animals were injected with the drugs intravenously and, after the indicated duration, infected as described[17b] to generate the bone infection. Female CD rats (age 57–70 days; n = 5/group; Charles River, St-Constant, Canada) were anaesthetized by isofluorane before and during the surgery. Following complete induction of anesthesia, the rat was placed ventral side up and hair was shaved from the surgical site. The skin over the leg was cleaned and disinfected (proviodine-ethanol 70%). A longitudinal incision below the knee joint was made in the sagital plane. The incision was made over the bone below the "knee joint" (tibia head or con-

dyle) but not completely extending to the ankle. A high speed drill fitted with a 2-mm bulb bit was used to drill a hole into the medullar cavity of the tibia. Rats were injected intra-tibially with 0.05 ml 5% sodium morrhuate (sclerosing agent) and then with 0.05 ml of *S. aureus* suspension (ca. 5×10^5 CFU/rat). The hole was sealed by applying a small amount of dry dental cement which immediately absorbs fluids and adheres to the site. The wound was closed using three metal skin clips. Moxifloxacin **1a** (as a positive control) was injected once at 10 mg/kg intravenously 1 h postinfection in saline, while the test compounds (prepared in 0.9% saline) were injected as a single intravenous bolus dose at the indicated time points prior to the infection.

Infected rats were sacrificed by CO_2 asphyxiation 24 h postinfection to monitor the bacterial CFU count. Infected tibiae were removed, dissected free of soft tissue, and weighed. The bones were ground using a metal ball mill, resuspended in 5 ml 0.9% NaCl, serially diluted and processed for quantitative cultures. Treatment efficacy was measured in terms of Log viable bacteria (Log CFU per gram of bone). The results obtained for each group of rats were evaluated by calculating the mean Log CFU and standard deviation. The limit of detection is 2 Log CFU/g of bone. Statistical comparisons of viable bacterial counts for the different treated and untreated groups were performed with Dunnett's multiple-comparison test or Kruskal–Wallis analysis. Differences were considered significant when the P value was <0.05 when comparing treated infected animals to the untreated infected ones.

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