Linking Bisphosphonates to the Free Amino Groups in Fluoroquinolones: Preparation of Osteotropic Prodrugs for the Prevention of Osteomyelitis

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Osteomyelitis is an infection located in bone and a notoriously difficult disease to manage, requiring frequent and heavy doses of systemically administered antibiotics. Targeting antibiotics to the bone after systemic administration may provide both greater efficacy of treatment and less frequent administration. By taking advantage of the affinity of the bisphosphonate group for bone mineral, we have prepared a set of 13 bisphosphonated antibacterial prodrugs based on eight different linkers tethered to the free amino functionality on fluoroquinolone antibiotics. While all but one of the prodrugs were shown in vitro to be effective and rapid bone binders (over 90% in 1 h), only eight of them demonstrated the capacity to significantly regenerate the parent drug. In a rat model of the disease, a selected group of agents demonstrated their ability to prevent osteomyelitis when used in circumstances under which the parent drug had already been cleared and is thus inactive.

Introduction

Osteomyelitis is defined as an inflammatory process localized to the bone and accompanied by necrosis resulting from an underlying microbial infection,[1] primarily caused by *Staphylococcus aureus*.[2] In general, it is established as a result of damage to the osseous matrix by trauma or surgery or may appear in loci affected by reduced vascularization, as is the case with diabetic and elderly patients. This is an extremely difficult form of infection to treat with a need for surgical intervention and amputation[3] and is characteristically associated with frequent relapses.[4,5] There are no marketed antibiotics with an osteomyelitis label, and treatment is often a result of the preferences and experience of the attending physicians.

A combination of factors may contribute to the difficulties in treating the disease, such as a sheltered physiological environment that is poorly accessible to the immune system and to antibacterial agents and conditions favoring bacterial cells in a quiescent state and thus more resistant to treatment. These are challenging conditions for an antibacterial agent to overcome, requiring high drug concentrations to be maintained in bone over a long period of time and therefore frequent and heavy intravenous dosing. Delivery of the antibacterial agent to the bone where it would be released over time has been proposed to circumvent these difficulties. In particular, the use of beads loaded with antibiotics is being examined for such a purpose.[6−9] An inconvenience of this approach is that these materials have to be surgically implanted, a true hurdle with a disease for which recurrence is common and repeat applications are often necessary.

Bisphosphonates have been useful functionalities for the delivery of agents to bone after systemic administration.[10−12] These pyrophosphate analogues possess strong affinity to the calcium phosphate bone mineral (hydroxyapatite) and rapidly accumulate in osseous tissues in vivo. While they do possess therapeutic activity of their own as inhibitors of bone resorption,[13,14] bisphosphonates have been used to deliver small molecule therapeutics,[15−20] ligands for radioisotope imaging,[21−23] and even proteins[24−26] to the bone. Recently, a report has described the use of bisphosphonates for the delivery of ciprofloxacin to bone.[27] Although the ciprofloxacin−bisphosphonate conjugate described appeared to bind efficiently to bone, it is predictably unable to release the ciprofloxacin moiety. This would limit the potential of this agent for treatment of bone infections, as it is reasonable to propose that the release of the bioactive moiety is necessary to achieve a therapeutic outcome and bacteria are not likely to be inhibited by an antibacterial agent that is essentially irreversibly bound to the bone surface. They are not known to possess the bone-resorptive activity which would be 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 of osteoporosis treatments.[28] Although the local acidity[29] brought on by the infecting organism might be associated with some bisphosphonate release from bone, the efficiency of such a process in providing a sufficient concentration of the antimicrobial agent is doubtful.

An approach based on bisphosphonate prodrugs would present a greater potential from this respect. Such prodrugs have been described for the delivery of small molecules to bone, such as diclofenac,[15,16] prostaglandins,[17] steroids,[18] and carboxyfluorescein.[19,20] We have therefore opted for this approach.

Fluoroquinolones are a well defined class of wholly synthetic antibacterial agents with a very successful clinical track record.[30,31] They possess a set of desirable attributes, being generally safe agents, active orally and parenterally, with a broad antimicrobial spectrum that includes many frequently encountered pathogens. They are bactericidal in clinically achievable doses, a feature that is most desirable in treating osteomyelitis, given the sheltered environment of the microorganism, the high levels of recurrence associated with the disease, and the potential for regrowth. Spontaneous resistance levels to these agents are reasonably low. They have demonstrated some efficacy in

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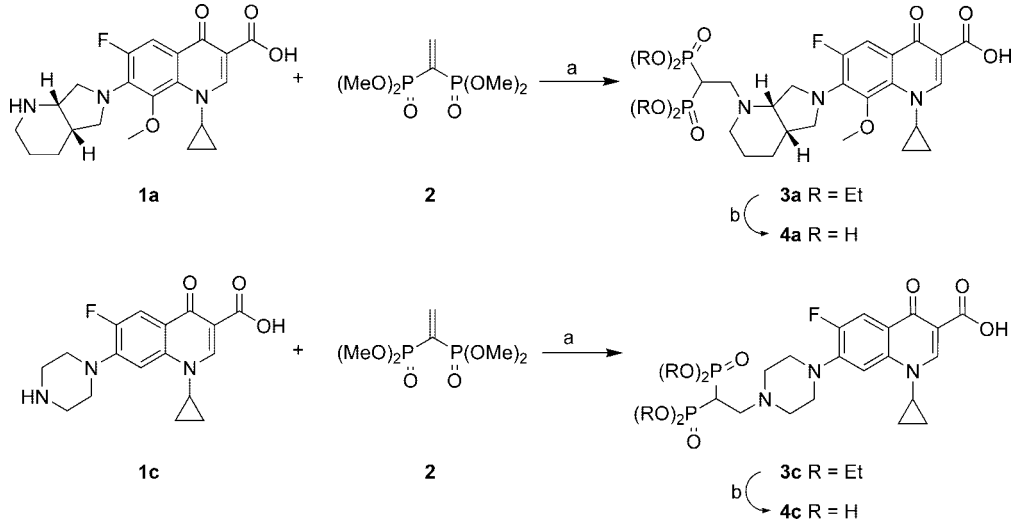
Scheme 1[a]



[a] Reagents and conditions: (a) DMAP, $CHCl_3$; (b) TMSBr, CH_2Cl_2.

animal models of osteomyelitis.[32−34] From a practical perspective, they are comparatively easily synthesized and modified.

Therefore, the preparation and evaluation of bisphosphonated fluoroquinolone prodrugs appeared to be advantageous for the treatment and the prevention of osteomyelitis. Past reports of prodrug strategies focusing on the release of bioactive amine compounds provided a catalogue of functional groups tethered to amino groups and labile under physiological conditions. The strategy presented in this report focused on the preparation of bisphosphonated version of these known functional groups to provide linkers tethering the bisphosphonate group to the secondary amino group present in many of the clinically used fluoroquinolones and therefore representing a useful synthetic handle.

Chemistry

In this process, moxifloxacin **1a**, gatifloxacin **1b**, and ciprofloxacin **1c** were selected as starting materials for the prodrugs. The previously described[27] ciprofloxacin−bisphosphonate conjugate **4c** was prepared by the addition of **1c** to 1,1-ethenylbisphosphonate **2**,[35] followed by deprotection of the phosphonate esters with TMSBr (Scheme 1). The parent moxifloxacin conjugate **4a** was assembled in the same fashion.

Fluoroquinolone allyl esters **9a**,**b** were produced via Boc protection of the amino groups (Scheme 2). These esters were acylated with acyl chloride **7**[17] to supply, after deprotection, simple bisphosphonated amides **13a**,**b**.

The well documented use of dioxolonylmethylamines and carbamates for the preparation of prodrugs[36,37] from amine functionalities directed our attention to the preparation of a bisphosphonated version for the fluoroquinolones (Scheme 3). In this case, the adequately substituted precursor **19** was prepared from 2-bromo-1-(4-bromophenyl)propan-1-one via palladium catalyzed coupling of a phosphinyl phosphonate[38] on cyclic carbonate **17** followed by radical bromination. Condensation of gatifloxacin **1b** with **19** and deprotection of the prodrug provided the bisphosphonated prodrug **21**.

Prodrugs based on favorable cyclizations to 4,4-dimethyldihydrocoumarins have been used successfully with drugs possessing a primary or a secondary amine functionality.[39,40] Bisphosphonated versions of such prodrugs (**29a**−**c**) were prepared for evaluation as osteotropic delivery agents for gatifloxacin (Scheme 4). Palladium catalyzed coupling of diethyl(ethoxyphosphinyl)methylphosphonate to 6-bromo-3,4-dihydro-4,4-dimethylchromen-2-one furnished the parent bisphosphonated dihydrocoumarin **23**. The ring was opened and converted to the benzyl ester **25**, which was acylated or phosphorylated to provide, after debenzylation, acids **27a**−**c**. These were coupled with gatifloxacin **1b** to furnish, after deprotection of the phosphonate groups, bisphosphonated prodrugs **29a**−**c**.

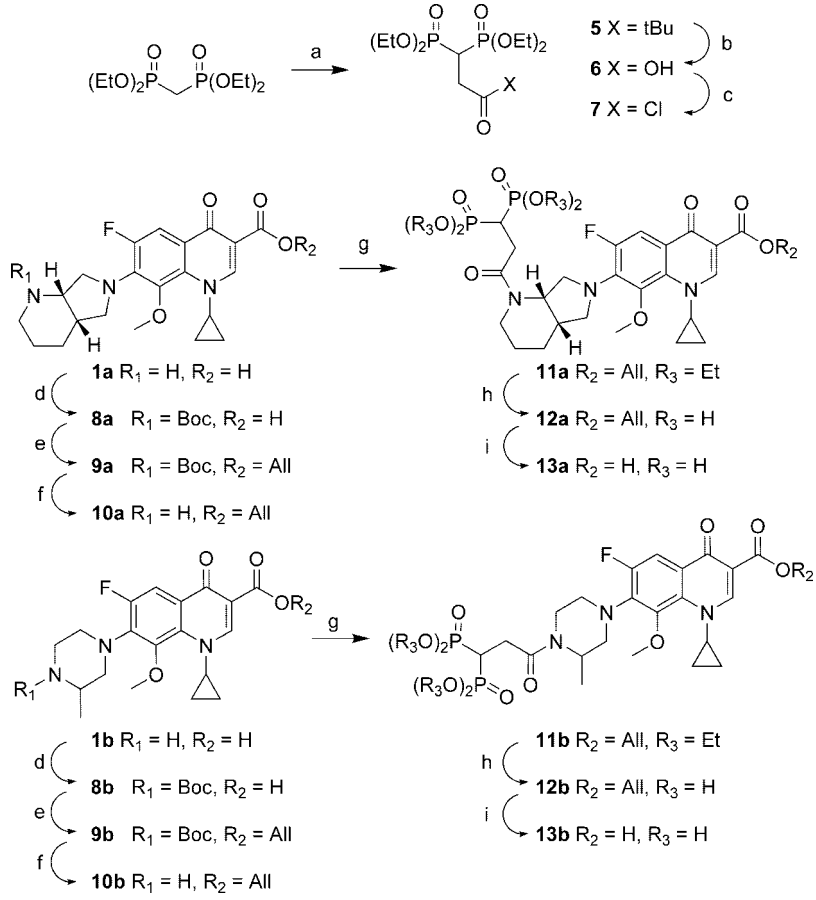
Recently, a β-aminoketone prodrug of ciprofloxacin has been proposed, whereby the drug is freed by elimination.[41] A similar approach was envisaged for gatifloxacin **1b** by the preparation of prodrug **36** (Scheme 5). A sequence of alkylation of the sodium salt of tetraethyl methylenebisphosphonate with the protected bromopropanol **30**, deprotection, and iodination furnished iodide **33**. Displacement with hydroxyphenylpropenone provided vinyl ketone **34**. The conjugate addition of gatifloxacin onto the enone, followed by deprotection of the phosphonate esters, provided the desired prodrug **36**.

Substituted acyloxyalkyl esters and carbamates are frequently used as prodrugs.[42−44] The bisphosphonated versions of such prodrugs were prepared using acids **37**, **41**, and **42**[45] (Scheme 6). Acids **37** and **41** were obtained through oxidation of the parent alcohols **32** and the similarly prepared alcohol **40**. Treatment of moxifloxacin **1a** and gatifloxacin **1b** with chloroethyl chloroformate in the presence of Proton Sponge gave the chloroethyl carbamates **43a**,**b** (Scheme 7). Substitution of the chlorines by acid **42** gave, after deprotection, prodrugs **45a**,**b**. Treatment of **43b** with acid **41** gave, after deprotection, the gatifloxacin prodrug **47**. However, treatment of **43b** with acid **37** failed to give the desired product **48**. Reasoning that the weak nucleophilicity of the carboxylate and the increased steric bulk in **37**, as opposed to **41**, may have favored an elimination reaction in this case, producing the labile vinyl carbamate rather than the expected substitution product, it was decided to attempt the same reaction with the unsubstituted chloromethyl carbamate **49** instead. In this case, the chloride was displaced as expected providing, after deprotection of the bisphosphonates, the gatifloxacin prodrug **51**.

Results and Discussion

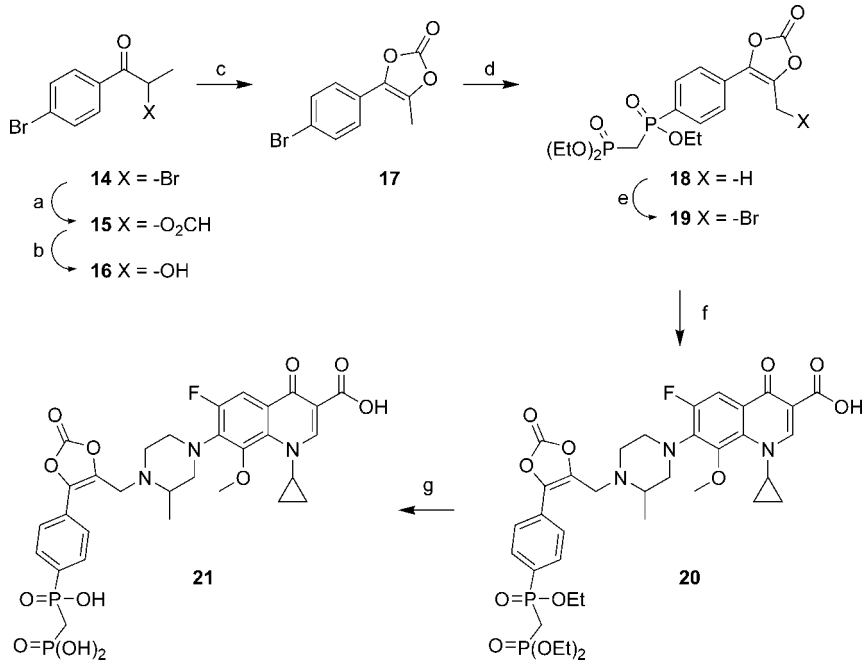
In Vitro Evaluation of Prodrugs. The antibacterial activities of the prodrugs against *S. aureus* ATCC13709 were measured as per CLSI guidelines (results not shown).[46] The minimum

Scheme 2[a]



[a] Reagents and conditions: (a) *tert*-butyl bromoacetate, NaH, DMF; (b) TFA; (c) $SOCl_2$, CH_2Cl_2; (d) Boc_2O, NaOH, H_2O, THF; (e): allyl bromide, K_2CO_3, DMF; (f) AcCl, MeOH; (g) **7**, Et_3N, DMAP, CH_2Cl_2; (h) sodium *p*-toluenesulfinate, $Pd(PPh_3)_4$, THF, H_2O; (i) TMSBr, CH_2Cl_2.

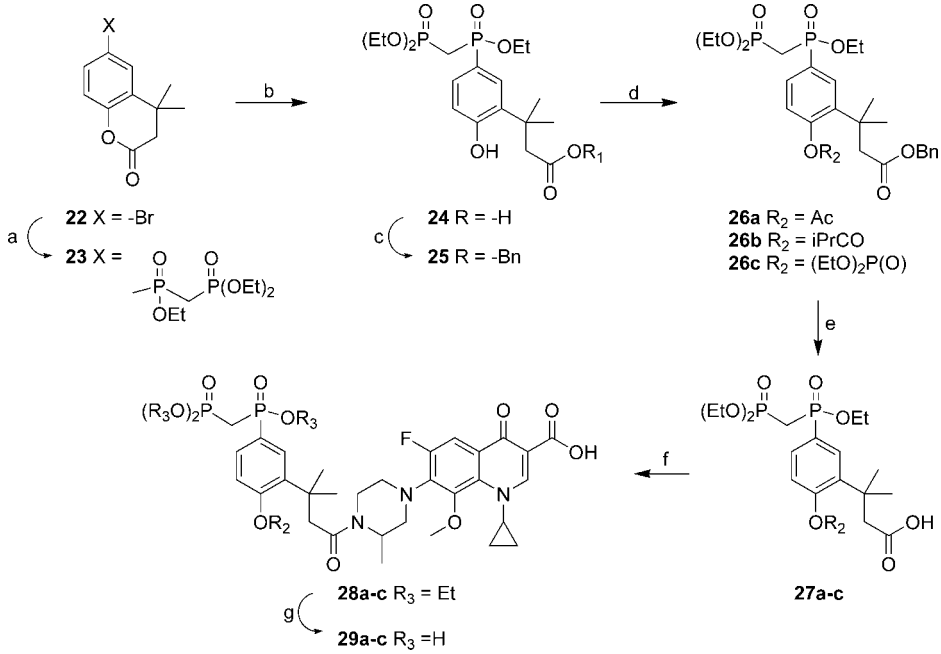
Scheme 3[a]



[a] Reagents and conditions: (a) formic acid, TEA, MeCN; (b) NaOH, MeOH; (c) $COCl_2$, $PhNMe_2$, $(CH_2Cl)_2$; (d) diethyl(ethoxyphosphinyl)methylphosphonate, $Pd(PPh_3)_4$, THF, H_2O; (e) NBS, 1,1′-azobis(cyclohexanecarbonitrile), CCl_4, Δ; (f) **1b**, DMF; (g) TMSBr, CH_2Cl_2.

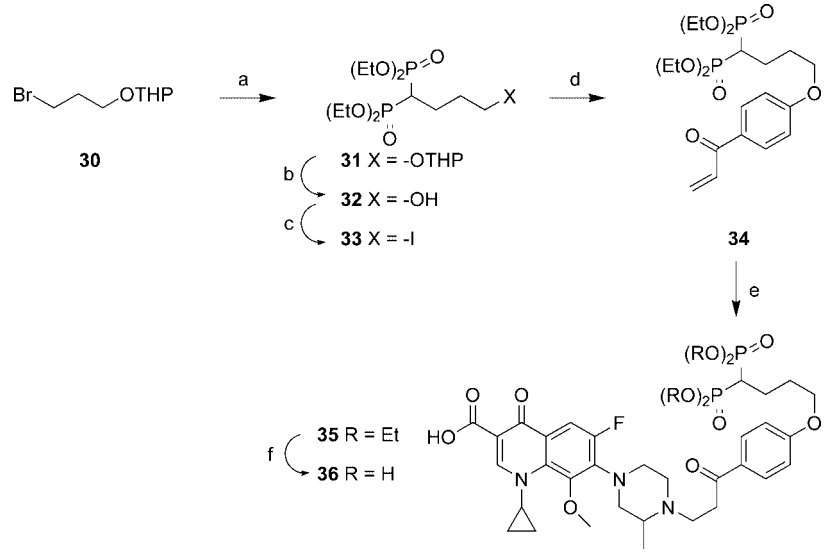
inhibitory concentrations were >4 μg/mL except for **21** (<0.12 μg/mL), **36** (0.12 μg/mL), and **51** (0.5 μg/mL). These values are generally significantly higher than those of moxifloxacin (0.03 μg/mL), gatifloxacin (0.06 μg/mL), and ciprofloxacin (0.12 μg/mL). Given that at least a portion of the antibacterial activity of the prodrugs may come from the release of the parent drug

Scheme 4[a]



[a] Reagents and conditions: (a) diethyl(ethoxyphosphinyl)methylphosphonate, $Pd(PPh_3)_4$, THF, H_2O; (b) KOH, MeOH; (c) KOH, MeCN, then BnBr, DMF; (d) acid chloride, DMAP, pyridine or diethyl chlorophosphate, Et_3N, THF; (e) H_2, Pd/C, MeOH; (f) **1b**, HBTU, DIPEA, DMF; (g) TMSBr, 2,6-lutidine, CH_2Cl_2.

Scheme 5[a]



[a] Reagents and conditions: (a) NaH, THF, Δ; (b) *p*TsOH, MeOH; (c) I_2, PPh_3, imidazole, CH_2Cl_2; (d) 1-(4-hydroxyphenyl)prop-2-en-1-one, K_2CO_3, acetone; (e) **1b**, Et_3N, DMAP, CH_2Cl_2; (f) TMSBr, CH_2Cl_2.

during the course of the assay, it is reasonable to assume that the prodrugs lack significant antibacterial activity of their own.

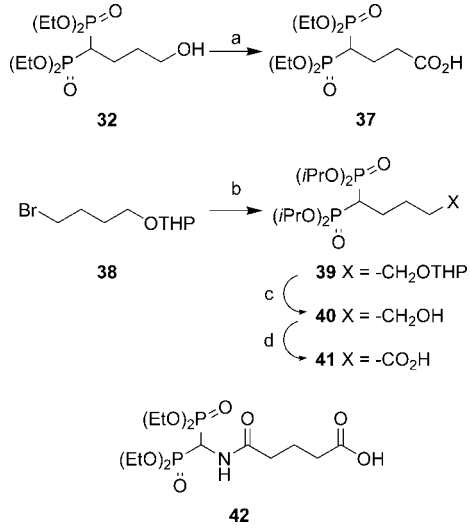
Therefore, in vivo activity would require the bisphosphonated prodrugs to bind to bone and release the active fluoroquinolones. These two requirements can be evaluated in vitro to predict the therapeutic potential of these compounds.

Since bone is essentially insoluble in aqueous media, bisphosphonates bound to bone powder are for all practical purposes immobilized on a solid matrix, and bound and free bisphosphonates can therefore be easily separated by centrifugation. As such, a sense of the affinity of the prodrug for osseous tissues can be obtained by determining the amount of prodrug bound to bone powder in phosphate buffered saline (PBS) at 37 °C over 1 h. This was ascertained by fluorescence measurements on the supernatant to measure the unbound fraction (Table 1).

The release of the parent fluoroquinolone from these prodrugs immobilized on bone powder was determined by measuring the appearance of fluorescence in the supernatant over time. This was done in PBS and 50% rat or human sera 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 indicate several trends. First, these prodrugs are very efficient at binding bone powder, being taken up at >80% and generally >90% over 1 h for the

Scheme 6[a]



[a] Reagents and conditions: (a) TEMPO, NaOCl, $NaOCl_2$, H_2O; (b) NaH, THF, Δ; (c) *p*TsOH, MeOH; (d) PDC, DMF.

methylenebisphosphonates (**4a**−**c**, **13a**,**b**, **36**, **45a**,**b**, **47**, and **51**) and 35−76% for the phosphinylmethylphosphonates (**21** and **29a**,**b**), when the parent drugs are at best negligibly bound. In fact, it is reasonable to assume the unbound fraction to be at least partially the result of cleavage of the prodrug during the time course of the assay, thereby under-representing the true efficiency of the process. The exception is compound **29c** for which there was surprisingly no binding in vitro. It is possible that the presence of the additional phosphate group, by increasing the number of potential protonation sites, might result in a phosphinylmethylphosphonate with a different degree of ionization at physiological pH, and this may reduce its affinity for hydroxyapatite.

Second, it is clear that the previously published bisphosphonated ciprofloxacin **4c**, its moxifloxacin analogue **4a**, and the simple amides **13a**,**b** are not able to release the parent drugs.

The dioxolone based prodrug **21** released its parent extremely efficiently. The rate accelerations observed in sera suggest that the cyclic carbonate moiety may be subject to the action of esterases or that its decomposition may be favored by the presence of nucleophilic substances in plasma.

Prodrugs **29a** and **29b** also appeared to efficiently release gatifloxacin. These prodrugs are expected to undergo a stepwise hydrolysis in which the phenolic ester is saponified, releasing a phenolate that rapidly cyclizes to the dihydrocoumarin. In PBS, the rates of cleavage of **29a** and **29b** differ by nearly an order of magnitude, reflecting the higher lability of the acetate ester in the former over the isobutyrate ester in the latter. On the other hand, the levels of regeneration of gatifloxacin are comparable in sera, a result that suggests similar rates of saponification, perhaps as a consequence of esterase action. This would be consistent with the ester hydrolysis being the rate determining event, followed by a very rapid cyclization.

Compound **36** demonstrates good rates for regenerating gatifloxacin, and other than through medium effects on the β-elimination, the process clearly does not require the participation of biocatalysts.

Acyloxymethyl carbamate based prodrugs are also shown to be able to regenerate gatifloxacin. Compounds **45a** and **45b** were designed to release the parent drug via cyclization of the glutaramic ester to the glutarimide, an intramolecular process not requiring biocatalysis. It is therefore not surprising that both of these prodrugs display relatively medium independent rates of cleavage. From this perspective, compounds **47** and **51** are quite interesting, as simple esters were not predictably hydrolyzed in the absence of esterases. It is plausible that the bisphosphonate functionality provides an intramolecular acid that catalyzes this process. The large rate acceleration in going from **47** to **51** likely reflects the shortened distance between the bisphosphonate group and the acetal and supports this hypothesis.

The very high bone affinity of these bisphosphonates and the wide range of different rates of regenerating the parent fluoroquinolones provide a valuable tool for investigating the ability of bisphosphonates to deliver fluoroquinolones to osseous tissues, with clear potential in the prevention and the treatment of osteomyelitis.

In Vivo Evaluation of Prodrugs. Prodrugs **36** and **51** appreciably release their parent fluoroquinolones in vitro in a process that does not require the participation of an enzyme. They also demonstrate a very strong affinity for bone. Given this attractive profile, these compounds were selected for evaluation in vivo.

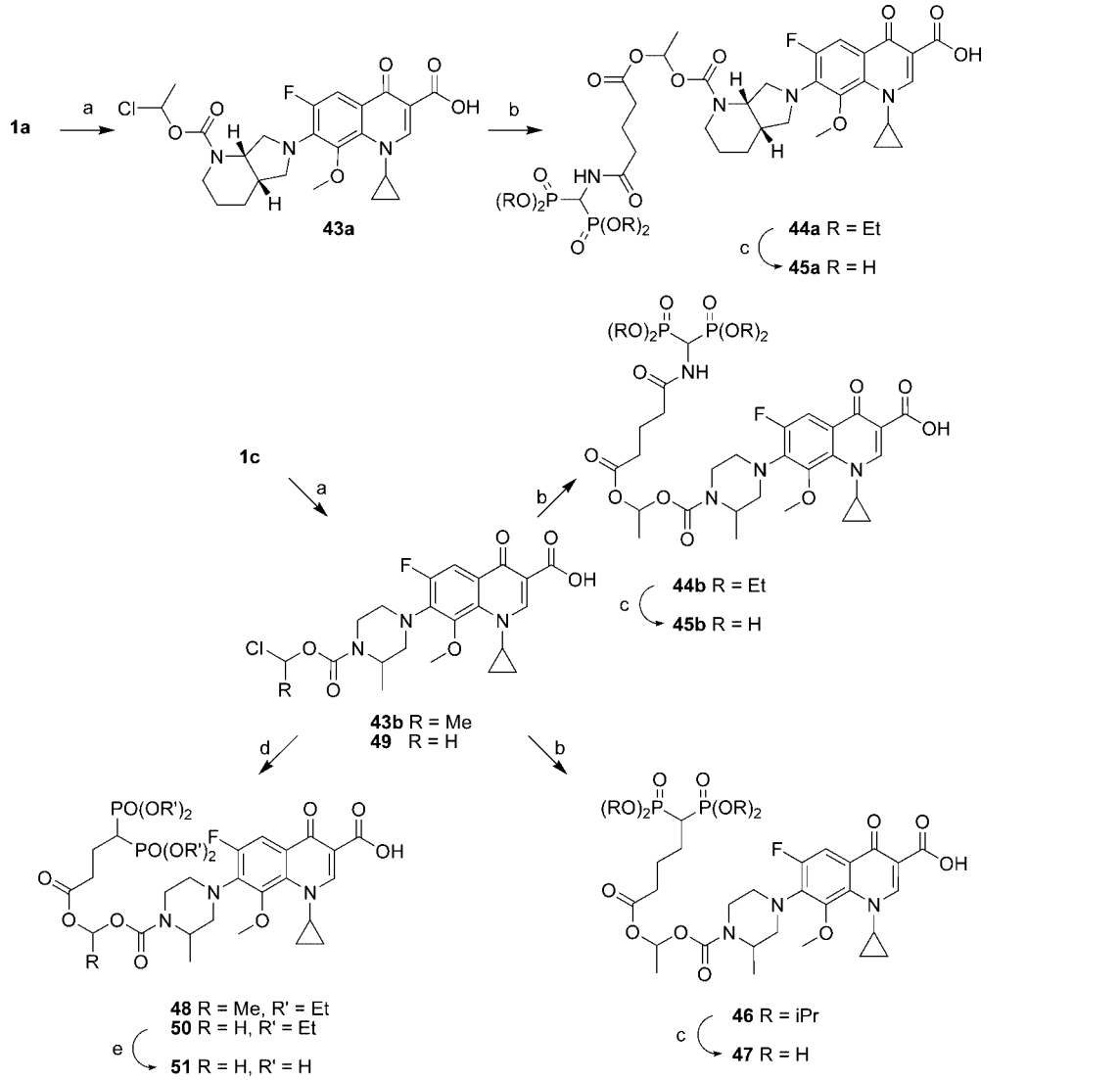
These bisphosphonated fluoroquinolone prodrugs were tested in a rat model for the prevention of osteomyelitis, which was adapted from the known parent treatment models.[47,48] Given the affinity of bisphosphonates for bone as demonstrated in vitro and given the precedents for clinically used bisphosphonates, it can be presumed that the ability to prevent infection over time would suggest the prodrug to be binding to osseous tissues and releasing the parent antibacterial agent over time. Inactivity in this model on the other hand would suggest either that the prodrug is not binding to bone in vivo or that it is not releasing the parent drug. In other words, it provides a useful if only indirect means to evaluate the pharmacokinetics of the prodrugs. Briefly, a single bolus intravenous dose of the prodrug was administered 1−2 days before an infection of the bone is simulated by surgically inserting a bacterial load into the tibiae, using *S. aureus* (ATCC 13709) as the infectious agent. The animals were sacrificed 24 h after the infection, and the bacterial titer in the tibiae was determined. Moxifloxacin injected at 10 mg/kg 1 h after the establishment of infection was used as a positive control. Indeed, at that time point, moxifloxacin clears the bacterial load, as the bacterial infection is not yet established and therefore represents a good means to evaluate the validity of the experiment. At this dose, moxifloxacin is not efficacious when used 24 h after the infection is established. It is therefore not a comparator.

This experiment was performed with the bisphosphonated prodrug **51** at 42 mg/kg of body weight 2 days prior to infection, and this was compared with the equivalent dose of gatifloxacin (20 mg/kg of body weight) (Figure 1).

The data provided demonstrate the ability of **51** to prevent the growth of bacteria when used 2 days prior to infection, under conditions in which the parent drug gatifloxacin **1b**, at the equivalent dose, is unable to do so. This is in agreement with the prodrug going to osseous tissues and slowly releasing the active drug but at concentrations that are sufficiently superior to its minimum inhibitory concentration to significantly reduce the bacterial load in bone. In fact, in a test group of five animals, two had completely sterile bones (data not shown).

Since **51** was very rapidly regenerating in vitro, a comparison with prodrugs regenerating their parent drugs at slower rates is warranted. Compounds **36**, which did release gatifloxacin at an appreciable rate, and **13a**, which was not able to regenerate its

Scheme 7[a]



[a] Reagents and conditions: (a) ClCH(R)OCOCl, Proton Sponge, $CHCl_3$ or CH_2Cl_2; (b) **41** or **42**, NaOH, THF, H_2O, then **43a** or **43b**, MeCN, 60 °C; (c) TMSBr, CH_2Cl_2; (d) **37**, KOH, MeCN, then **46** or **47**, DMF; (e) TMSBr, 2,6-lutidine, CH_2Cl_2.

Table 1. Bone Binding and Regeneration of Parent Fluoroquinolone from Prodrugs

		% parent drug released over 24 h		
compd	% bound to bone[a]	PBS	50% rat serum	50% human serum
1a	<lod[b]			
1b	<lod[b]			
1c	<lod[b]			
4a	92.2	<lod[b]	<lod[b]	<lod[b]
4c	99.6	<lod[b]	<lod[b]	<lod[b]
13a	99.9	<lod[b]	<lod[b]	<lod[b]
13b	98.2	<lod[b]	<lod[b]	<lod[b]
21	63.7	7.5	30.0	22.8
29a	36.6	5.4	6.2	3.7
29b	76.0	0.8	6.1	3.6
29c	<lod[b]	nd[c]	nd[c]	nd[c]
36	94.7	2.3	4.2	4.3
45a	93.9	1.2	1.4	1.8
45b	94.3	1.5	1.8	2.4
47	88.4	0.7	0.7	0.9
51	86.4	13.4	25.4	21.2

[a] At 37 °C for 1 h. [b] <lod: under the limit of detection (0.01%). [c] nd: not determined.

parent drug moxifloxacin, were assayed at 20.8 and 24 mg/kg of body weight a day prior to infection (Figure 2).

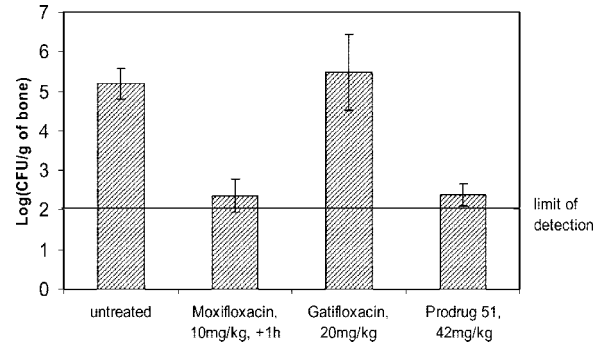


Figure 1. Prophylactic effect of prodrug **51** and gatifloxacin when injected at 42 and 20 mg/kg, respectively (mole equivalent doses), 2 days prior to the establishment of a bone infection in rats.

Prodrug **36** was able to significantly reduce the bacterial titer in the bone under this exposure. In a test group of five rats, one displayed sterile bones. This is in stark contrast with compound **13a**, which did not impact the establishment of the infection. This supports the notion that not only must the prodrug reach

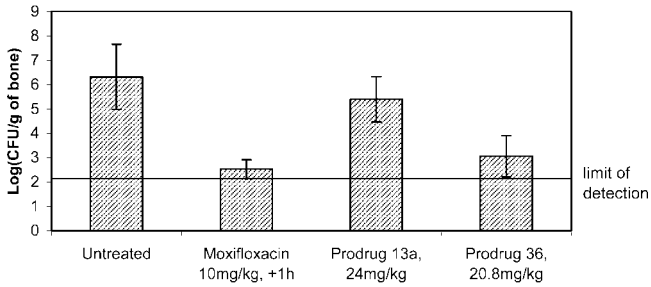


Figure 2. Prophylactic effect of prodrugs **36** and **13a** when injected at 20.8 and 24 mg/kg (mole equivalent doses), respectively, a day prior to the establishment of a bone infection in rats.

the bone, it must release its parent drug to be efficacious and emphasizes the importance of a prodrug strategy over simple conjugates.

This experiment demonstrates the ability of these prodrugs to produce a sufficient concentration of the parent drug to maintain antibacterial activity when the activity of the parent drug has already faded.

Conclusions

The free amino position of moxifloxacin, gatifloxacin, and ciprofloxacin represents a useful handle for tethering a bisphosphonate group. By assessment of their affinity for bone and their ability to release their parent drug once bound to bone, it is possible to predict a sense for the therapeutic potential of these fluoroquinolone bisphosphonate conjugates. These investigations highlighted the greater affinity of methylenebisphosphonate derivatives for bone over the parent phosphinomethylphosphonates lacking one of the acidic hydroxyls but also emphasized the far greater affinity of these two forms of conjugates over the negligible bone affinities of the parent drugs. With the lack of bone affinity of compound **29c**, they also exemplified the dramatic influence the overall structure can have over bone affinity.

Whereas simple conjugates, such as bisphosphonoethyl (**4a**−**c**) and bisphosphonopropionyl (**13a**,**b**) derivatives, were unable to release their parent drugs once bound to bone, this study presents the successful adaptation of the bisphosphonate approach to known forms of prodrugs. Thus, bisphosphonated versions of the dioxolenone (**21**), 2-acyloxyhydrocinnamate (**29a**−**c**), phenylpropanone (**36**), and acyloxyalkyl carbamate (**45a**,**b, 47**, **51**) prodrug strategies were presented here and shown to efficiently regenerate their parent drugs once they are bound to bone. The comparison between **47** and **51** also shows that, beyond its role in delivery to bone, the bisphosphonate moiety can itself markedly impact the ability of the prodrug to regenerate.

The abilities of bisphosphonate fluoroquinolone conjugates **13a**, **36**, and **51** to prevent infection in a rat model of osteomyelitis were further evaluated. Compounds **36** and **51** were shown to efficaciously and significantly prevent the establishment of infection while the parent drug gatifloxacin was unable to do so. The inability of conjugate **13a**, which was shown in vitro to be unable to regenerate its parent drug, to prevent infection is significant in that it highlights the fact that these forms of stable conjugates in practical terms irreversibly bind the drug to bone and are therefore not therapeutically useful in tackling osteomyelitis. This finding stresses the value of the prodrug strategy.

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

Experimental Section

General. ^{1}H NMR spectra were recorded on a Varian Unity 400 spectrometer. The reported chemical shifts (in parts per million) are referenced using the signals assigned to the residual nondeuterated solvents. 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). Silica gel column chromatography was performed on silica gel gel 60 (70−230 mesh). THF was dried by passage through a column of activated alumina. 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. All compounds were ≥95% pure as judged by ^{1}H NMR and LC/MS analyses.

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

2,2-Bisphosphonoethyl Derivatives of Fluoroquinolones. General Procedure for Condensation with Tetramethyl Ethenylidenebisphosphonate 2. The fluoroquinolone (2 mmol) was dissolved in dry $CHCl_3$ (30 mL). To this solution was added tetramethyl ethenylidenebisphosphonate **2** (0.515 g, 2.11 mmol) and a catalytic quantity of DMAP. The reaction mixture was stirred at room temperature for 3.5 h and then evaporated at 40 °C.

3a. The residue was taken up in EtOAc and filtered, and the product was precipitated by the addition of hexanes. Yield: 45%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86−0.87 (m, 1H), 0.94−1.05 (m, 2H), 1.10−1.35 (m, 4H), 1.55−1.85 (m, 5H), 2.25−2.40 (m, 2H), 2.64 (tt, J = 23.9, 6.0, 1H), 2.75−2.95 (m, 2H), 3.05−3.25 (m, 2H), 3.54 (s, 3H), 3.56−3.72 (m, 6H), 3.74−4.01 (m, 8H), 7.77 (d, J = 14.1, 1H), 8.76 (s, 1H).

3c. The residue was triturated in boiling toluene, and the insoluble product was collected. Yield: 44%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.17−1.21 (m, 2H), 1.36−1.43 (m, 2H), 1.63 (bs, 1H), 2.67−2.84 (m, 5H), 2.95−3.07 (m, 2H), 3.35 (bs, 4H), 3.48−3.56 (m, 1H), 3.80−3.88 (m, 12 H), 7.34 (d, J = 7.0, 1H), 8.02 (d, J = 12.9, 1H), 8.77 (s, 1H).

General Procedure for Deprotection. TMSBr (0.58 mL, 4.39 mmol) was added in one portion to a stirring solution of the protected bisphosphonated prodrug (0.44 mmol) in dry CH_2Cl_2 (10 mL), and the resulting mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure, and solid was dried under high vacuum for 1 h. The solid was suspended in H_2O (30 mL), and the pH was immediately adjusted to pH 7.6 by the addition of 1 M NaOH, with concomitant dissolution of the product.

4a. The solution was concentrated in vacuo, and the solid residue portion was purified on a C18 Sep-Pak (H_2O). Yield: 59%. ^{1}H NMR (400 MHz, D_2O) δ.0.78−1.36 (m, 4H), 1.56−2.15 (m, 4H), 2.36−2.53 (m,1H), 2.60−2.85 (m, 1H), 3.32−3.85 (m, 7H), 3.62 (s, 3H), 4.05−4.38 (m, 4H), 7.59 (d, J = 13.7, 1H), 8.59 (s, 1H).

4c. The product solution was washed with $CHCl_3$ (2 × 25 mL), filtered, and evaporated to give the product in quantitative yield. ^{1}H NMR (400 MHz, D_2O) δ 1.14 (bs, 2H), 1.32−1.38 (m, 2H), 2.35−2.50 (m, 1H), 3.36−3.90 (m, 11H), 7.66 (d, J = 7.0, 1H), 7.93 (d, J = 13.1, 1H), 8.51 (s, 1H).

3,3-Bisphosphonoproprionamides of Fluoroquinolones. Tetraethyl 2-*tert*-Butoxycarbonylethylene-1,1-bisphosphonate (5). To a solution of tetraethyl methylenebisphosphonate (3.00 g, 10.4 mmol) in dry DMF (9 mL) was added 0.46 g of NaH (60% suspension in mineral oil, 11.5 mmol) portionwise. The resulting slurry was stirred for 30 min at room temperature, after which *tert*-butyl bromoacetate (1.7 mL, 11.5 mmol) was added neat and at a rapid pace. The reaction mixture was stirred for 1 h and quenched by adding 2 mL of a saturated solution of NH_4Cl. The reaction mixture was

evaporated and purified by flash chromatography on silica gel, eluting with 5% methanol/ethyl acetate to give tetraethyl 2-*tert*-butoxycarbonylethylene-1,1-bisphosphonate (**6**, 2.1 g, 50%) as a clear, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (bt, J = 7.0, 12H), 1.46 (s, 9H), 2H), 2.76 (td, J = 16.0, 6.1, 2H), 3.07 (tt, J = 24.0, 6.1, 1H), 4.10−4.25 (m, 8H).

Tetraethyl 2-Carboxyethylene-1,1-bisphosphonate (6). *tert*-Butyl ester **7** (2.1 g, 5.2 mmol) was stirred in TFA (12 mL) for 2.5 min and concentrated under reduced pressure. The residue was purified by flash chromatography (gradient elution 100% ethyl acetate to 10% methanol/ethyl acetate) to furnish tetraethyl 2-carboxyethylene-1,1-bisphosphonate (**6**) as a white solid (1.35 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28−1.39 (m, 12H), 2.86 (td, J = 16.1, 6.3, 2H), 3.12 (tt, J = 24.0, 6.3, 1H), 4.13−4.26 (m, 8H).

Tetraethyl 2-Chlorocarbonylethylene-1,1-bisphosphonate (7). To acid **6** (1.02 g, 2.95 mmol) in CH_2Cl_2 (15 mL) was added freshly distilled $SOCl_2$ (0.84 mL, 11.6 mmol). The mixture was stirred at reflux for 3 h and concentrated to dryness to give crude tetraethyl 2-chlorocarbonylethylene-1,1-bisphosphonate (**7**) as a colorless oil (quantitative), which was used immediately for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30−1.40 (m, 12H), 3.05 (tt, J = 23.5, 6.2, 1H), 3.40 (td, J = 14.8, 6.2, 2H), 3.12 (tt, J = 24.0, 6.3, 1H), 4.13−4.27 (m, 8H).

General Procedure for Boc Protection of Fluoroquinolones. A mixture of the fluoroquinolone (2 mmol), Boc_2O (460 mg, 2.082 mmol), and 4.2 mL of 1 M NaOH aqueous solution in 20 mL of THF was stirred at room temperature overnight. After removal of the organic solvent, the residue was neutralized with a saturated aqueous ammonium chloride solution. The mixture was extracted with ethyl acetate (3×) and dried over anhydrous sodium sulfate. Removal of the solvent yielded the product in sufficient purity for the next step.

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 (8a). 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 (bs, 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 (bs, 1H), 7.82 (d, J = 13.7, 1H), 8.79 (s, 1H) ppm.

7-(4-(*tert*-Butoxycarbonyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (8b). 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 (bs, 1H), 7.89 (d, J = 11.4, 1H), 8.83 (s, 1H) ppm.

General Procedure for Allylation of *N*-Boc Fluoroquinolones. To a solution of *N*-Boc fluoroquinolone **8a**,**b** (2.0 mmol) in dry DMF (20 mL) was added K_2CO_3 (332 mg, 2.4 mmol) and allyl bromide (210 μL, 2.4 mmol). The reaction mixture was heated at 75−80 °C for 24 h and concentrated in vacuo, and the residue was partitioned between water and ethyl acetate. The organic layer was collected, and the aqueous layer was extracted once more with ethyl acetate. The combined organics were washed with brine, dried ($MgSO_4$), and concentrated to dryness to furnish material of sufficient purity for the subsequent step.

Allyl 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 (9a). Yield: 74%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.73−0.83 (m, 1H), 0.88−1.12 (m, 2H), 1.18−1.29 (m, 1H), 1.48 (s, 9H), 1.58−1.85 (m, 4H), 2.19−2.28 (m, 1H), 2.82−2.93 (m, 1H), 3.15−3.26 (m, 1H), 3.30−3.40 (m, 1H), 3.56 (s, 3H), 3.78−3.92 (m, 2H), 3.99−4.11 (m, 2H), 4.70−4.90 (m, 3H), 5.27 (dd, J = 10.4, 1.3, 1H), 5.48 (dd, J = 17.2, 1.5, 1H), 6.00−6.11 (m, 1H), 7.84 (d, J = 14.3, 1H), 8.56 (s, 1H).

Allyl 7-(4-(*tert*-Butoxycarbonyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (9b). Yield: 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83−0.98 (m, 2H), 1.07−1.20 (m, 2H), 1.33 (d, J = 6.6, 1H), 1.49 (s, 9H), 3.15−3.49 (m, 5H), 3.72 (s, 3H), 3.84−3.99 (m, 2H), 4.34 (bs, 1H), 4.83 (d, J = 5.9, 2H), 5.28 (dd, J = 10.4, 1.3, 1H), 5.48 (dd, J = 17.2, 1.5, 1H), 6.00−6.11 (m, 1H), 7.90 (d, J = 12.5, 1H), 8.59 (s, 1H).

General Procedure for Preparation of Fluoroquinolone Allyl Esters. To a solution of *N*-Boc fluroquinolone allyl ester **9a**,**b** (1.5 mmol) in dry methanol (25 mL) cooled to 0 °C was added acetyl chloride (5.33 mL, 74.6 mmol). The resulting solution was allowed to warm to room temperature over 1.5 h and concentrated, and the residue was partitioned between ice cold saturated $NaHCO_3$ and CH_2Cl_2. The organics were collected, dried over Na_2SO_4, and concentrated in vacuo to provide the fluoroquinolone allyl ester of sufficient purity for the next step.

Allyl 1-Cyclopropyl-6-fluoro-1,4-dihydro-7-((4a*S*,7a*S*)-octahydropyrrolo[3,4-*b*]pyridin-6-yl)-8-methoxy-4-oxoquinoline-3-carboxylate (10a). Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75−0.85 (m, 1H), 0.95−1.10 (m, 2H), 1.14−1.24 (m, 1H), 1.53−1.68 (m, 1H), 1.72−1.90 (m, 3H), 2.38 (bs, 1H), 2.73−2.87 (m, 1H), 3.12−3.24 (m, 1H), 3.35−3.64 (m, 3H), 3.55 (s, 3H), 3.82−4.02 (m, 3H), 4.74−4.88 (m, 2H), 5.26 (dd, J = 10.6, 1.1, 1H), 5.46 (dd, J = 17.2, 1.5, 1H), 5.98−6.11 (m, 1H), 7.61 (d, J = 13.9, 1H), 8.53 (s, 1H).

Allyl 7-(3-Methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (10b). Yield: 92%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86−1.00 (m, 2H), 1.08−1.18 (m, 5H), 2.87−2.97 (m, 1H), 3.00−3.14 (m, 3H), 3.21−3.39 (m, 3H), 3.77 (s, 3H), 3.86−3.95 (m, 1H), 4.80−4.86 (m, 2H), 5.25−5.30 (m, 1H), 5.45−5.51 (m, 1H), 6.00−6.10 (m, 1H), 7.88 (d, J = 12.5, 1H), 8.58 (s, 1H).

General Procedure for Acylation of Fluoroquinolone Allyl Esters. To a solution of the crude fluroquinolone allyl ester **10a**,**b** (1.4 mmol), triethylamine (0.24 mL, 1.69 mmol), and DMAP (17 mg, 0.14 mmol) in CH_2Cl_2 (20 mL) cooled to 0 °C was added dropwise a CH_2Cl_2 solution of crude acyl chloride **7** (1.76 mmol in 12.5 mL). The resulting mixture was allowed to warm to room temperature overnight, diluted with CH_2Cl_2, and washed with saturated $NaHCO_3$. The aqueous washes were back-extracted with CH_2Cl_2, and the combined organics were washed with brine, dried over $MgSO_4$, and concentrated. The product was purified by flash chromatography on SiO_2 (5% methanol/CH_2Cl_2).

Allyl 7-((4a*S*,7a*S*)-1-(3,3-Bis(diethylphosphono)propionyl)octahydropyrrolo[3,4-*b*]pyridin-6-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (11a). Yield: 74%. ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 0.72−0.81 (m, 1H), 0.93−1.10 (m, 2H), 1.15−1.26 (m, 1H), 1.28−1.38 (m, 12H), 1.43−1.64 (m, 2H), 1.78−1.90 (m, 2H), 2.18−2.36 (m, 1H), 2.75−3.10 (m, 3H), 3.13−3.29 (m, 2H), 3.34−3.66 (m, 2H), 3.55 (s, 3H, major rotamer), 3.59 (s, 3H, minor rotamer), 3.74−4.26 (m, 12H), 4.53−4.66 (m, 1H), 4.76−4.88 (m, 2H), 5.17−5.35 (overlapping doublets of doublets, 1H), 5.42−5.54 (overlapping doublets of doublets, 1H), 5.98−6.10 (m, 1H), 7.82 (d, J = 13.9, 1H, major rotamer), 7.84 (d, J = 14.3, 1H, minor rotamer), 8.54 (s, 1H, major rotamer), 8.55 (s, 1H, minor rotamer).

Allyl 7-(4-(3,3-Bis(diethylphosphono)propionyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (11b). Yield: 81%. ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 0.87−0.99 (m, 2H), 1.10−1.21 (m, 2H), 1.29−1.43 (m, 15H), 2.78−3.05 (m, 2H), 3.16−3.82 (m, 6H), 3.72 (s, 3H), 3.85−3.95 (m, 1H), 4.12−4.30 (m, 9H), 4.47−4.59 (m, 0.5H), 4.80−4.92 (m, 2.5H), 5.28 (dd, J = 10.4, 1.3, 1H), 5.45−5.55 (m, 1H), 6.00−6.12 (m, 1H), 7.91 (d, J = 12.5, 1H), 8.59 (s, 1H).

Procedure for Allyl Ester Deprotection. To a solution of allyl ester **11a**,**b** (1.04 mmol) in THF (20 mL) was added $Pd(PPh_3)_4$ (24 mg, 0.02 mmol) and an aqueous solution of sodium *p*-toluenesulfinate (204 mg in 2 mL, 1.14 mmol). The mixture was stirred at room temperature for 1.25 h, concentrated, and purified by flash chromatography on SiO_2.

7-((4a*S*,7a*S*)-1-(3,3-Bis(diethylphosphono)propionyl)octahydropyrrolo[3,4-*b*]pyridin-6-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (12a). 12a was purified by flash chromatography with gradient elution of 5% methanol/CH_2Cl_2 to 10% methanol/CH_2Cl_2. Yield: 79%. ^{1}H NMR

(400 MHz, $CDCl_3$, mixture of rotamers) δ 0.76−0.85 (m, 1H), 1.02−1.19 (m, 2H), 1.25−1.40 (m, 13H), 1.46−1.67 (m, 2H), 1.80−1.93 (m, 2H), 2.33−2.40 (m, 1H), 2.72−3.10 (m, 3H), 3.12−3.36 (m, 2H), 3.40−3.65 (m, 1H), 3.56 (s, 3H, major rotamer), 3.60 (s, 3H, minor rotamer), 3.78−4.28 (m, 11H), 4.57−4.70 (m, 1H), 5.20−5.30 (m, 1H), 7.81 (d, J = 13.9, 1H, major rotamer), 7.84 (d, J = 13.6, 1H, minor rotamer), 8.78 (s, 1H, major rotamer), 8.79 (s, 1H, minor rotamer).

7-(4-(3,3-Bis(diethylphosphono)propionyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (12b). 12b was purified by flash chromatography with 5% methanol/$CHCl_3$. Yield: 67%. ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 0.93−1.06 (m, 2H), 1.16−1.28 (m, 2H), 1.30−1.40 (m, 15H), 2.81−3.05 (m, 2H), 3.18−3.84 (m, 6H), 3.73 (s, 3H), 3.97−4.05 (m, 1H), 4.12−4.28 (m, 9H), 4.49−4.61 (m, 0.5H), 4.84−4.94 (m, 0.5H), 7.91 (d, J = 12.5, 1H), 8.83 (s, 1H).

General Procedure for Deprotection to Amides 13a,b. To a solution of protected fluoroquinolone bisphosphonate amide **12a**,**b** (0.82 mmol) in CH_2Cl_2 (40 mL) was added TMSBr (1.1 mL, 8.2 mmol). The reaction mixture was stirred for 38 h, the volatiles were removed under reduced pressure, and the solid was dried under high vacuum for 1 h. The solid was suspended in H_2O (80 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 concentrated and purified by reverse-phase chromatography on a Waters C18 Sep-Pak cartridge.

7-((4a*S*,7a*S*)-1-(3,3-Bisphosphonopropionyl)octahydropyrrolo[3,4-*b*]pyridin-6-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (13a). 13a was purified by reverse-phase chromatography with gradient elution, 100% water to 25% methanol/water. Yield: 32% based on tetrasodium salt of product. ^{1}H NMR (400 MHz, D_2O, mixture of rotamers) δ 0.73−0.83 (m, 1H), 0.95−1.16 (m, 2H), 1.17−1.28 (m, 1H), 1.46−1.73 (m, 2H), 1.76−1.89 (m, 2H), 2.30−2.62 (m, 2H), 2.67−4.48 (m, 8H), 3.60 (s, 3H), 4.87−4.98 (m, 0.43H), 5.08−5.18 (m, 0.57H), 7.64 (d, J = 14.3, 1H), 8.47 (s, 1H). ^{19}F (376 MHz, D_2O) δ −96.88 to 96.73 (m, 1F). ^{31}P (162 MHz, D_2O) δ 19.94−20.26 (m, 2P). MS: (MH^+) 618.1.

7-(4-(3,3-Bisphosphonopropionyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (13b). 13b was purified by two reverse-phase chromatographies, eluting with water. Yield: 34% based on tetrasodium salt of product. ^{1}H NMR (400 MHz, D_2O, mixture of rotamers) δ 0.88−1.04 (m, 2H), 1.06−1.22 (m, 2H), 1.38 (d, J = 7.0, 1.7H), 1.51 (d, J = 6.6, 1.3H), 2.39−2.62 (m, 1H), 2.78−3.08 (m, 2H), 3.27−3.48 (m, 4H), 3.77 (s, 3H), 3.98−4.78 (m, 3H), 7.75 (d, J = 12.8, 1H), 8.53 (s, 1H). ^{19}F (376 MHz, D_2O) δ −95.77 to 95.63 (m, 1F). ^{31}P (162 MHz, D_2O) δ 19.76−20.16 (m, 2P). MS: (M − H) 590.0.

Bisphosphonated Dioxolenone Prodrug of Gatifloxacin. 1-(4-Bromophenyl)-1-oxopropan-2-yl Formate (15). A solution of formic acid (1.6 mL, 43 mmol) in acetonitrile (20 mL) was cooled in an ice bath followed by the sequential dropwise addition of TEA (6.0 mL, 43 mmol) and then 2,4′-dibromopropriophenone (10.0 g, 34.2 mmol) in 10 mL of THF/acetonitrile (1:1). The resulting solution was stirred while warming to room temperature over 18 h. The resulting colorless precipitate was filtered off, and the organics were removed at reduced pressure. The residue was redissolved in EtOAc, refiltered, and concentrated to give **15** as a yellow oil that was used without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (d, J = 7.0, 3H), 6.02 (q, J = 7.0, 1H), 7.63 (d, J = 8.7, 2H), 7.80 (d, J = 8.7, 2H), 8.11 (s, 1H).

1-(4-Bromophenyl)-2-hydroxypropan-1-one (16). Crude **15** was dissolved in MeOH (100 mL). Then 1 M NaOH (1.5 mL) was added and the resulting solution was stirred for 18 h. Approximately half of the methanol was removed at reduced pressure. The reaction was quenched by the addition of saturated aqueous NH_4Cl, and the product was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to a yellow residue that was purified by flash silica gel chromatography (10−50% EtOAc in hexanes), resulting in **16** as a yellow oil (5.27 g, 68% over two steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (d, J = 7.0, 3H), 3.7 (bs, 1H), 5.11 (bq, J = 6.9, 1H), 7.65 (d, J = 8.5, 2H), 7.79 (d, J = 8.5, 2H).

4-(4-Bromophenyl)-5-methyl-1,3-dioxol-2-one (17). A solution of **16** in 1,2-dichloroethane (DCE, 60 mL) was cooled in an ice bath followed by the addition of 20% phosgene (23.5 mL, 40.1 mmol) in toluene. After the mixture was stirred for 15 min, a solution of *N*,*N*-dimethylaniline (4.0 mL, 79 mmol) in DCE (10 mL) was added dropwise over a period of 1 h at the same temperature. The ice bath was removed, and the mixture was heated to 70 °C for 20 h. The solution was diluted with CH_2Cl_2, washed with water, 10% aqueous HCl, water, brine, and then dried over Na_2SO_4 and filtered. After removal of the solvent the product was recrystallized from EtOAc/hexanes to furnish **17** (6.07 g, 63%) as a pale-green solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.36 (s, 3H), 7.33 (d, J = 8.7, 2H), 7.58 (d, J = 8.7, 2H).

Ethyl (Diethylphosphonomethyl)(4-(5-methyl-2-oxo-1,3-dioxol-4-yl)phenyl)phosphinate (18). A mixture of **17** (0.323 g, 1.27 mmol), diethyl(ethoxyphosphinyl)methylphosphonate (0.325 g, 1.33 mmol), TEA (0.530 mL, 3.80 mmol), and $Pd(PPh_3)_4$ (0.146 g, 0.127 mmol) in acetonitrile (3 mL) was heated 90 °C for 3 h. The solvent was removed at reduced pressure and the product purified by silica gel flash column chromatography (0−6% MeOH in CH_2Cl_2), resulting in **18** (0.368 g, 70%) as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15−1.23 (m, 3H), 1.27−1.36 (m, 6H), 2.42 (s, 3H), 2.54−2.72 (m, 2H), 3.93−4.21 (m, 6H), 7.58 (dd, J = 2.8, 8.5, 2H), 7.94 (dd, J = 8.3, 12.2, 2H). ^{31}P (162 MHz, $CDCl_3$) δ 17.41−17.54 (m, 1P), 30.86−31.08 (m, 1P).

Ethyl (Diethylphosphonomethyl)(4-(5-(bromomethyl)-2-oxo-1,3-dioxol-4-yl)phenyl)phosphinate (19). A mixture of **18** (1.79 g, 4.28 mmol), NBS (0.761 g, 4.28 mmol), and 1,1′-azobis(cyclohexanecarbonitrile) (0.11 g, 0.43 mmol) in CCl_4 was heated to reflux under a strong visible light for 4 h, at which time all the starting material had been consumed as was evident by ^{1}H NMR. The solvent was removed at aspirator pressure and the crude product was purified by silica gel flash column chromatography (0−5% MeOH in CH_2Cl_2) to furnish **19** (1.26 g, 60%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, J = 7.1, 3H), 1.31 (t, J = 7.1, 3H), 1.35 (t, J = 7.1, 3H), 2.64 (ddd, J = 2.8, 18.0, 20.4, 2H), 3.95−4.23 (m, 6H), 4.44 (s, 2H), 7.66 (dd, J = 2.9, 8.4, 2H), 8.02 (dd, J = 8.4, 12.2, 2H). ^{31}P (162 MHz, $CDCl_3$) δ 19.63 (d, J = 5.6, 1P), 32.93 (d, J = 5.6, 1P).

1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-4-((2-oxo-5-(4-(*O*-ethyl(diethylphosphonomethyl)phosphonoyl)phenyl)-1,3-dioxol-4-yl)methyl)piperazin-1-yl)-4-oxoquinoline-3-carboxylic Acid (20). A solution of gatifloxacin **1b** and **19** in DMF was stirred at room temperature for 16 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl, and the product was extracted with ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4, filtered, and concentrated at reduced pressure. The crude product was purified by silica gel HPFC (10% MeOH in EtOAc and then 5% MeOH in CH_2Cl_2) to give **20** (44 mg, 28%) as a pale-yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97−1.03 (m, 2H), 1.17−1.38 (m, 14H), 2.61−2.70 (m, 3H), 2.79 (bs, 1H), 2.97 (bd, J = 3.0, 1H), 3.16 (bt, J = 9.2, 1H), 3.39−3.47 (m, 3H), 3.58 (d, J = 14.7, 1H), 3.77 (s, 3H), 3.99−4.23 (m, 8H), 7.79 (dd, J = 2.1, 8.3, 2H), 7.89 (d, J = 7.9, 1H), 8.00 (dd, J = 8.3, 11.4, 2H), 8.82 (s, 1H).

1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-4-((2-oxo-5-(4-(phosphonomethylphosphinoyl)phenyl)-1,3-dioxol-4-yl)methyl)piperazin-1-yl)-4-oxoquinoline-3-carboxylic Acid (21). TMSBr (0.175 mL, 1.33 mmol) was added to a stirring solution of **20** (70 mg, 0.088 mmol) in CH_2Cl_2 (4 mL). The resulting solution was stirred at room temperature for 15 h, and then the solvent was removed at reduced pressure. The solid was suspended in 30 mM triethylammonium bicarbonate buffer (2 mL), and then the pH was adjusted to approximately 6 by the addition of triethylamine. The solution was then subjected to two consecutive C18 silica gel chromatographies (5−50% CH_3CN in 30 mM triethylammonium bicarbonate, then 5−50% CH_3CN in water) to give **21** (20 mg, 32%) as the monotriethylammonium salt: ^{1}H NMR (400 MHz, D_2O) δ 1.03−1.08 (m, 2H), 1.23 (d, J = 7.6, 2H), 1.36 (d, J = 5.8, 2H), 2.33 (d, J = 18.2, 2H),

3.05−3.25 (m, 2H), 3.32−3.44 (m, 2H), 3.55−3.70 (m, 3H), 3.81 (s, 3H), 4.23−4.33 (m, 2H), 4.58 (d, J = 14.8, 1H), 7.74−7.77 (m, 3H), 7.93 (dd, J = 8.3, 11.1, 2H), 8.92 (s, 1H). ^{31}P (162 MHz, D_2O) δ 12.82 (s, 1P), 29.37 (s, 1P). LCMS: 87.4% (254 nm), 87.1% (220 nm), 88.5% (290 nm). MS: (MH^+) 708.2.

Preparation of 2′-Acyloxyhydrocinnamylamides of Gatifloxacin 14a−d. 6-(Ethoxy(diethylphosphonomethyl)phosphinoyl)-3,4-dihydro-4,4-dimethylchromen-2-one (23). A mixture of 6-bromo-4,4-dimethylchroman-2-one (3.5 g, 9.7 mmol), diethyl(ethoxyphosphinyl)methylphosphonate (1.7 g, 9.7 mmol), triethylamine (4.1 mL, 29 mmol), and $Pd(PPh_3)_4$ (0.56 g, 0.48 mmol) in acetonitrile (20 mL) was heated to 100 °C for 18 h. The reaction mixture was cooled and diluted with acetonitrile (50 mL) followed by washing with aqueous HCl (10%), water, and saturated aqueous NaCl. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel chromatography (0−10% MeOH in CH_2Cl_2) on a Biotage flash chromatography system, resulting in **23** as pale-yellow oil (3.0 g, 73%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (t, J = 7.2, 3H), 1.30−1.37 (m, 6H), 1.40 (s, 6H), 2.61 (dd, J = 1.7, 17.2, 20.7, 2H), 2.66 (s, 2H), 3.95−4.08 (m, 2H), 4.11−4.21 (m, 4H), 7.16 (dd, J = 3.1, 8.3, 2H), 7.73 (dd, J = 3.1, 8.3, 2H). ^{31}P (162 MHz, $CDCl_3$) δ 20.07 (d, J = 7.7, 1P), 33.74 (d, J = 7.7, 1P).

3-(2-Hydroxy-5-(ethoxy(diethylphosphonomethyl)phosphinoyl)-phenyl)-3-methylbutanoic Acid (24). A solution of **23** (0.99 g, 2.4 mmol) and KOH (0.095 g, 2.4 mmol) in MeOH was stirred at room temperature for 2 h. The solvent was removed under reduced pressure. The product was resuspended in water, the pH was adjusted to 4 by the addition of HCl, and the product was extracted with CH_2Cl_2. The organics were dried over Na_2SO_4, filtered, and concentrated, resulting in **24** as a pale-yellow oil (1.1 g, 105%), which was used without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (t, J = 7.2, 3H), 1.29−1.37 (m, 6H), 1.45 (s, 3H), 1.48 (s, 3H), 2.63 (dd, J = 17.7, 20.9, 2H), 2.93 (AB q, J = 14.2, 2H), 4.00−4.20 (m, 6H), 6.74 (bs, 1H), 7.56 (ddd, J = 1.6, 8.5, 12.2, 2H), 7.63 (d, J = 13.3, 1H).

Benzyl 3-(2-Hydroxy-5-(ethoxy(diethylphosphonomethyl)phosphinoyl)phenyl)-3-methylbutanoate (25). An aqueous KOH solution (0.14 g, 2.5 mmol) was added to a stirring solution of **24** (1.1 g, 2.5 mmol) in acetonitrile (5 mL). After 10 min the solvent was evaporated under reduced pressure and the residue was dried under vacuum for 1 h. The pale-yellow solid was resuspended in DMF (10 mL) followed by the addition of benzyl bromide (330 μL, 2.8 equiv). The resulting solution was stirred at room temperature for 2 h. The mixture was diluted with EtOAC (80 mL) and washed with H_2O and saturated aqueous NaCl, followed by drying over Na_2SO_4. The crude product was purified by silica gel chromatography (0−10% MeOH in CH_2Cl_2) on a Biotage flash chromatography system, resulting in **25** as a pale-yellow liquid (0.64 g, 48%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (t, J = 7.1, 3H), 1.26 (t, J = 7.1, 3H), 1.30 (t, J = 7.1, 3H), 1.45 (s, 3H), 1.49 (s, 3H), 2.60 (dd, J = 17.4, 20.9, 2H), 3.00 (AB q, J = 14.0, 2H), 3.78−3.88 (m, 2H), 3.99−4.15 (m, 4H), 4.93 (s, 2H), 6.75−6.78 (m, 1H), 7.14 (dd, J = 2.0, 7.5, 2H), 7.25−7.31 (m, 3H), 7.58 (ddd, J = 1.4, 8.0, 11.9, 1H), 7.64 (d, J = 13.4, 1H). ^{31}P (162 MHz, $CDCl_3$) δ 21.04 (d, J = 4.6, 1P), 36.00 (d, J = 4.6, 1P).

General Procedure for the Acylation of 25. The acyl chloride (1.56 mmol) was added dropwise to a stirred solution of **25** (825 mg, 1.56 mmol) and DMAP (catalyst) in pyridine (10 mL) in an ice bath (**26a**) or at room temperature (**26b**,**c**). The resulting solution was stirred for 2 h at the same temperature followed by dilution with EtOAc (80 mL). The organics were washed with aqueous HCl (5%), water, and saturated aqueous NaCl, then dried over Na_2SO_4. The crude product was purified by silica gel chromatography on a Biotage flash chromatography system.

Benzyl 3-(2-Acetoxy-5-(ethoxy(diethylphosphonomethyl)phosphinoyl)phenyl)-3-methylbutanoate (26a). 26awas purified by silica gel chromatography using 0−10% MeOH in CH_2Cl_2 as the eluent. Yield: 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (t, J = 7.1, 3H), 1.30 (t, J = 7.1, 6H), 1.49 (s, 3H), 2.33 (s, 3H), 2.56 (ddd, J = 6.8, 17.3, 23.4, 2H), 2.84 (AB q, J = 14.4, 2H), 3.95−4.04 (m, 2H), 4.06−4.18 (m, 4H), 4.95 (s, 2H), 7.14−7.20 (m, 3H), 7.28−7.34 (m, 3H), 7.73 (ddd, J = 1.9, 8.2,11.8, 1H), 7.89 (dd, J = 1.9, 13.3, 1H). ^{31}P (162 MHz, $CDCl_3$) δ 20.20 (d, J = 9.9, 1P), 33.88 (d, J = 9.9, 1P).

Benzyl 3-(2-(2,2-Dimethylacetoxy)-5-(ethoxy(diethylphosphonomethyl)phosphinoyl)phenyl)-3-methylbutanoate (26b). 26bwas purified by silica gel chromatography using 0−10% MeOH in CH_2Cl_2 as the eluent. Yield: 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18−1.22 (m, 6H), 1.29−1.33 (m, 9H), 1.46 (s, 3H), 1.49 (s, 3H), 2.58 (ddd, J = 6.9, 17.5, 23.5, 2H), 2.79−2.90 (m, 3H), 3.96−4.19 (m, 6H), 4.96 (s, 2H), 7.09 (dd, J = 3.4, 8.3, 1H), 7.18−7.21 (m, 2H), 7.28−7.32 (m, 3H), 7.72 (ddd, J = 1.9, 8.3, 11.8, 1H), 7.88 (dd, J = 1.9, 13.3, 1H). ^{31}P (162 MHz, $CDCl_3$) δ 20.28 (d, J = 9.8, 1P), 34.04 (d, J = 9.8, 1P).

Benzyl 3-(2-(Diethylphosphoryloxy)-5-(ethoxy(diethylphosphonomethyl)phosphinoyl)phenyl)-3-methylbutanoate (26c). Diethyl chlorophosphate (283 μL, 1.98 mmol) was added dropwise to a stirring solution of **25** (695 mg, 1.32 mmol) and triethylamine (368 μL, 2.64 mmol) in THF. The resulting mixture was stirred for 24 h at room temperature followed by dilution with EtOAc (80 mL). The organics were washed with aqueous HCl (5%), water, and saturated aqueous NaCl, followed by drying over Na_2SO_4. The crude product was purified by silica gel chromatography (0−20% MeOH in EtOAc) on a Biotage flash chromatography system, resulting in **26d** as a pale-yellow liquid (390 mg, 45%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (t, J = 7.0, 3H), 1.24−1.36 (m, 12H), 1.51 (s, 3H), 1.53 (s, 3H), 2.50−2.61 (m, 2H), 2.94 (AB q, J = 14.1, 2H), 3.86−4.24 (m, 10H), 4.93 (s, 2H), 7.13−7.17 (m, 2H), 7.26−7.29 (m, 3H), 7.59 (dd, J = 2.9, 8.3, 1H), 7.71 (t, J = 9.8, 1H), 7.83 (d, J = 13.1, 1H).

General Procedure for Debenzylation of Esters 26a−c. Compounds **26a−c** (0.87 mmol) were dissolved in MeOH (20 mL) and hydrogenated over Pd/C (10%, 250 mg) under H_2 (1 atm) for 2 h. The catalyst was filtered off and the volatiles were removed in vacuo, resulting in the acid product.

3-(2-Acetoxy-5-(ethoxy(diethylphosphonomethyl)phosphinoyl)-phenyl)-3-methylbutanoic Acid (27a). Yield: 98%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (dt, J = 0.4, 7.1, 3H), 1.28 (dt, J = 0.4, 7.1, 3H), 1.31 (t, J = 7.1, 3H), 1.50 (s, 3H), 1.53 (s, 3H), 2.37 (s, 3H), 2.62 (ddd, J = 5.4, 18.4, 22.4, 2H), 2.74 (AB q, J = 13.9, 2H), 3.89−4.16 (m, 6H), 7.17 (dd, J = 3.6, 8.2, 1H), 7.69 (ddd, J = 1.9, 8.2, 11.9, 1H), 7.86 (dd, J = 1.9, 13.7, 1H). ^{31}P (162 MHz, $CDCl_3$) δ 20.17 (d, J = 5.0, 1P), 34.51 (d, J = 5.0, 1P).

3-(2-(2,2-Dimethylacetoxy)-5-(ethoxy(diethylphosphonomethyl)phosphinoyl)phenyl)-3-methylbutanoic Acid (27b). Yield: 88%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (t, J = 7.1, 3H), 1.29 (t, J = 7.3, 3H), 1.31 (t, J = 7.0, 3H), 1.36 (d, J = 7.0, 6H), 1.50 (s, 3H), 1.52 (s, 3H), 2.63 (ddd, J = 3.9, 17.2, 22.2, 2H), 2.76 (AB q, J = 14.0, 2H), 2.86 (septet, J = 7.1, 1H), 3.91−4.16 (m, 6H), 7.10 (dd, J = 3.4, 8.2, 1H), 7.69 (ddd, J = 1.8, 8.2, 11.7, 1H), 7.86 (dd, J = 1.8, 13.6, 1H).

3-(2-(Diethylphosphoryloxy)-5-(ethoxy(diethylphosphonomethyl)phosphinoyl)phenyl)-3-methylbutanoic Acid (27c). Yield: 98%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19−1.39 (m, 15H), 1.57 (s, 3H), 1.59 (s, 3H), 2.62 (bt, J = 19.4, 2H), 2.80 (AB q, J = 13.9, 2H), 3.90−4.17 (m, 6H), 4.22−4.32 (m, 4H), 7.57 (dd, J = 3.4, 8.4, 1H), 7.69 (ddd, J = 1.7, 8.4, 11.8, 1H), 7.81 (bd, J = 13.5, 1H).

General Procedure for Acylation of Gatifloxacin with 27a−c. HBTU (317 mg, 0.836 mmol) was added in one portion to a solution of **27a−c** (0.836 mmol), gatifloxacin **1b** (310 mg, 0.84 mmol), and diisopropylethylamine (291 μL, 1.67 mmol) in DMF (5 mL) cooled in an ice bath. The resulting mixture was stirred and left to come to room temperature on its own overnight. The reaction mixture was diluted with EtOAc (100 mL) and washed with aqueous HCl (10%), water, and saturated aqueous NaCl. The organics were dried over Na_2SO_4 and concentrated in vacuo.

7-(4-(3-(2-Acetoxy-5-(ethoxy(diethylphosphonomethyl)phosphinoyl)phenyl)-3-methylbutanoyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (28a). The residue was purified by silica gel chromatography (0−10% MeOH in CH_2Cl_2) on a Biotage flash chromatography

system. Yield: 34%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92−0.94 (m, 2H), 1.12−1.27 (m, 14H), 1.43 (s, 3H), 1.46 (s, 3H), 2.31 (s, 3H), 2.53−5.63 (m, 1H), 2.74−2.85 (m, 3H), 3.00−3.40 (m, 5H), 3.65 (s, 3H), 3.88−4.08 (m, 6H), 4.27 (bs, 1H), 4.66 (bs, 1H), 7.07 (dd, J = 3.2, 8.1, 1H), 7.60−7.65 (m, 1H), 7.75 (d, J = 12.0, 1H), 7.82 (dd, J = 5.2, 8.1, 1H), 8.72 (s, 1H). ^{31}P (162 MHz, $CDCl_3$) δ 20.07 (d, J = 8.7, 1P), 33.90 (d, J = 8.7, 1P).

7-(4-(3-(2-(2,2-Dimethylacetoxy)-5-(ethoxy(diethylphosphonomethyl)phosphinoyl)phenyl)-3-methylbutanoyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (28b). The residue was purified by silica gel chromatography (0−5% MeOH in EtOAc) on a Biotage flash chromatography system. Yield: 34%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97−1.00 (m, 2H), 1.18−1.36 (m, 14H), 1.38 (d, J = 7.1, 6H), 1.54 (s, 3H), 1.56 (s, 3H), 2.63 (ddd, J = 5.6, 17.2, 22.0, 2H), 2.85−2.92 (m, 3H), 3.13 (bs, 1H), 3.27−3.51 (m, 5H), 3.71 (s, 3H), 3.98−4.19 (m, 6H), 4.43 (bs, 1H), 4.83 (bs, 1H), 7.08 (dd, J = 3.5, 8.1, 1H), 7.67−7.74 (m, 1H), 7.89 (d, J = 12.1, 1H), 7.95 (dd, J = 1.7, 13.7, 1H), 8.83 (s, 1H). ^{31}P (162 MHz, $CDCl_3$) δ 20.1. (d, J = 9.0, 1P), 34.32 (d, J = 9.0, 1P).

7-(4-(3-(2-(Diethylphosphoryloxy)-5-(ethoxy(diethylphosphonomethyl)phosphinoyl)phenyl)-3-methylbutanoyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (28c). The material was used in the next step without further purification.

General Procedure for Deprotection of Phosphonate Esters 28a−c. TMSBr (355 μL, 2.69 mmol) was added to a stirring solution of **28a−c** (150 mg, 0.179 mmol) and 2,6-lutidine (416 μL, 3.59 mmol) in CH_2Cl_2 (5 mL). The resulting yellow solution was stirred at room temperature for 24 h, and then the solvent was removed at reduced pressure. The yellow solid was resuspended in water (30 mM triethylamine/carbonate buffer for **28d**), and the solution was adjusted to approximately pH 6.5 by the addition of 1 M NaOH. The solution was then subjected to reverse-phase chromatography (0−60% CH_3CN in water) on a Biotage flash chromatography system to give the product.

7-(4-(3-(2-Acetoxy-5-((phosphonomethyl)phosphonoyl)phenyl)-3-methylbutanoyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (29a). Yield: 60% as the mono 2,6-lutidine salt. ^{1}H NMR (400 MHz, D_2O) δ 0.99−1.09 (m, 2H), 1.16−1.29 (m, 5H), 1.51 (s, 6H), 2.46 (s, 3H), 2.70 (s, 6H), 2.98−3.10 (m,2H), 3.16−3.54 (m, 4H), 3.71 (s, 3H), 3.83 (d, J = 12.7, 1H), 4.17 (d, J = 12.9, 1H), 4.21−4.27 (m, 1H), 4.56 (bs, 1H), 7.20 (dd, J = 2.9, 8.0, 1H), 7.56 (dd, J = 3.7, 12.2, 1H), 7.62 (d, J = 8.0, 2H), 7.71 (bd, J = 9.2, 1H), 7.93 (dd, J = 3.8, 12.2, 1H), 8.26 (t, J = 8.0, 1H), 8.89 (s, 1H). MS: (MH^-) 750.1.

7-(4-(3-(2-(2,2-Dimethylacetoxy)-5-((phosphonomethyl)phosphonoyl)phenyl)-3-methylbutanoyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (29b). Yield: 16% as the disodium salt. ^{1}H NMR (400 MHz, D_2O) δ 0.99−1.09 (m, 2H), 1.21 (d, J = 7.3, 3H), 1.33−1.44 (m, 2H), 1.38 (d, J = 7.0, 6H), 1.51 (s, 6H), 2.29 (AB q, J = 16.4, 2H), 2.93−3.10 (m, 3H), 3.14−3.41 (m, 2H), 3.48−3.56 (m, 2H), 3.71 (s, 3H), 3.85 (d, J = 13.1, 1H), 4.18 (d, J = 13.1, 1H), 4.25 (septet,, J = 3.6, 1H), 4.58 (bs, 1H), 7.10−7.15 (m, 1H), 7.65 (d, J = 12.1, 1H), 7.70 (t, J = 9.4, 1H), 7.93 (bd, J = 12.5, 1H), 8.89 (s, 1H). MS: (MH^-) 778.1.

7-(4-(3-(2-Phosphoryloxy-5-((phosphonomethyl)phosphonoyl)phenyl)-3-methylbutanoyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (29c). Yield: 31% as the di-2,6-lutidine salt. ^{1}H NMR (400 MHz, D_2O) δ 0.97−1.09 (m, 2H), 1.26−1.36 (m, 5H), 1.56 (s, 6H), 2.34 (t, J = 16.7, 2H), 2.69 (s, 12H), 2.91−3.10 (m, 2H), 3.16−3.55 (m, 3H), 3.68 (s, 3H), 3.90 (d, J = 13.5, 1H), 4.14 (d, J = 13.5, 1H), 4.22−4.27 (m, 1H), 4.32 (bs, 1H), 4.57 (bs, 1H), 7.49 (bd, J = 8.5, 1H), 7.61 (d, J = 8.1, 4H), 7.64−7.66 (m, 1H), 7.73−7.81 (m, 2H), 8.25 (t, J = 8.1, 2H), 8.94 (s, 1H). ^{19}F NMR (376 MHz, D_2O): δ −119.15 (d, J = 12.8). MS: (MH^+) 790.2.

Preparation of the Bisphosphonated β-Aminoketone Prodrug of Gatifloxacin.

Tetraethyl 4-(2-Tetrahydro-2*H*-pyranyloxy)butylene-1,1-bisphosphonate (31). To a suspension of NaH (60% suspension in mineral oil, 900 mg, 22.0 mmol) in dry THF (20 mL) was added dropwise tetraethyl methylenebisphosphonate (6.46 g, 22.4 mmol). The resulting clear solution was stirred 15 min at room temperature, after which 2-(3-bromopropoxy)tetrahydro-2*H*-pyran (5.05 g, 22.6 mmol) was added dropwise. The reaction mixture was heated to reflux for 6 h, diluted with CH_2Cl_2 (75 mL), washed with brine (2 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo. The product was used as such in the following step.

Tetraethyl 4-Hydroxybutylene-1,1-bisphosphonate (32). To a stirred solution of the crude product **31** (max 22.4 mmol) in MeOH (40 mL) was added Amberlite IR-120 (0.6 g). The reaction mixture was heated to 50 °C for 4 h, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel with gradient elution from 5−10% methanol/ethyl acetate to give **32** (2.67 g, 34% from tetraethyl methylenebisphosphonate). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (t, J = 7.1 Hz, 12H), 1.81 (quint, J = 6.5 Hz, 2H), 1.99−2.13 (m, 2H), 2.37 (tt, J = 24.4, 5.6 Hz, 1H), 2.51 (t, J = 5.9 Hz, 2H), 3.66 (q, J = 5.9 Hz, 2H), 4.13−4.22 (m, 8H).

Tetraethyl 4-Iodobutylene-1,1-bisphosphonate (33). To a solution of **32** (1.52 g, 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) in small portions. The mixture was then removed from the cooling bath, stirred for 2 h, diluted with hexanes (100 mL), and filtered. The filter cake was washed with further hexanes (2 × 30 mL), and the combined filtrates were concentrated in vacuo. The residue was purified by flash chromatography on silica gel with gradient elution from 0 to 10% methanol/ethyl acetate to give pure **33** (1.6 g, 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).

1-(4-(4,4-Bis(diethylphosphono)butoxy)phenyl)prop-2-en-1-one (34). A mixture of 4′-hydroxyacetophenone (2.70 g, 19.9 mmol), paraformaldehyde (2.68 g, 89.3 mmol), and *N*-methylanilinium trifluoroacetate (6.51 g, 29.4 mmol) in THF (20 mL) was refluxed for 3 h. The mixture was cooled and added to diethyl ether (200 mL), rinsing the flask with further diethyl ether (100 mL). The product solution was decanted from the red gum and filtered. Evaporation gave crude 1-(4-hydroxyphenyl)prop-2-en-1-one (2.0 g, 68%), which was used immediately in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.69 (bs, 1H), 5.92 (dd, J = 10.4, 1.7, 1H), 6.44 (dd, J = 17.0, 1.7, 1H), 6.93 (d, J = 8.8, 2H), 7.18 (dd, J = 17.2, 10.6, 1H), 7.93 (d, J = 8.8, 2H). A mixture of iodide **33** (3.1 g, 6.8 mmol), 1-(4-hydroxyphenyl)prop-2-en-1-one (1.21 g, 8.17 mmol), and K_2CO_3 (1.033 g, 7.47 mmol) in acetone (75 mL) was heated to reflux for 6.5 h. The mixture was cooled, filtered, and concentrated in vacuo. The residue was redissolved in CH_2Cl_2 (170 mL), filtered through Celite, and concentrated to give crude **34** (3.2 g, 99%), which was used directly in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28−1.39 (m, 12H), 1.89−2.25 (m, 4H), 2.26−2.48 (m, 1H), 4.05 (t, J = 5.7, 2H), 4.12−4.26 (m, 8H), 5.87 (dd, J = 10.6, 1.8, 1H), 6.42 (dd, J = 16.9, 1.8, 1H), 6.93 (d, J = 8.8, 2H), 7.17 (dd, J = 17.0, 10.4, 1H), 7.95 (d, J = 8.8, 2H),

1-Cyclopropyl-6-fluoro-1,4-dihydro-7-(4-(3-(4-(4,4-bis(diethylphosphono)butoxy)phenyl)-3-oxopropyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxoquinoline-3-carboxylic Acid (35). A mixture of crude enone **34** (3.2 g, 6.7 mmol), gatifloxacin **1b** (3.07 g, 8.18 mmol), DMAP (200 mg, 1.64 mmol), and triethylamine (1.4 mL, 10.0 mmol) in CH_2Cl_2 (200 mL) was stirred at room temperature for 20 h. The mixture was concentrated in vacuo, followed by flash chromatography (gradient elution, 5% methanol/CH_2Cl_2 to 10% methanol/CH_2Cl_2) to give **35** (3.6 g, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94−1.04 (m, 2H), 1.12−1.28 (m, 5H), 1.30−1.37 (m, 12H), 2.06−2.24 (m, 4H), 2.27−2.45 (m, 1H), 2.55−3.50 (m, 10H), 3.74 (s, 3H), 3.97−4.08 (m, 3H), 4.13−4.24 (m, 8H), 6.92 (d, J = 8.8, 2H), 7.86 (d, J = 12.1,1H), 7.94 (d, J = 8.8, 2H), 8.80 (s, 1H).

1-Cyclopropyl-6-fluoro-1,4-dihydro-7-(4-(3-(4-(4,4-bisphosphonobutoxy)phenyl)-3-oxopropyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxoquinoline-3-carboxylic Acid (36). To a solution of **35** (3.6 g, 4.3 mmol) in CH_2Cl_2 (150 mL) was added TMSBr (5.6 mL, 42 mmol). The reaction mixture was stirred for 27 h, the volatiles were removed under reduced pressure, and the solid was dried under high vacuum for 1 h. The solid was suspended in H_2O (800 mL), and the pH was immediately adjusted to pH 8 by the addition of 1 M KOH, with the concomitant slow dissolution of the product. The solution was concentrated in vacuo at 30 °C and purified by reverse-phase chromatography (gradient elution, 100% water to 30% methanol/water). The pure product **36** was obtained as a white fluffy solid (1.26 g, 33% recovery based on tetrapotassium salt of product). ^{1}H NMR (400 MHz, D_2O) δ 0.88−1.02 (m, 2H), 1.08−1.22 (m, 2H), 1.17 (d, J = 6.2, 3H), 1.77−2.14 (m, 5H), 2.72−3.30 (m, 10H), 3.79 (s, 3H), 4.06−4.14 (m, 1H), 4.21 (t, J = 6.4, 2H), 7.14 (d, J = 8.8, 2H), 7.73 (d, J = 12.8,1H), 8.05 (d, J = 8.8, 2H), 8.52 (s, 1H). ^{19}F (376 MHz, D_2O) δ −122.44 (d, J = 12.0, 1F). ^{31}P (162 MHz, D_2O) δ 20.88 (s, 2P). MS: (MH^+) 740.2.

Preparation of Acyloxymethyl Carbamate Prodrugs. Tetraethyl 3-Carboxypropylene-1,1-bisphosphonate (37). To a solution of alcohol **32** (12.7 g, 36.7 mmol) in 200 mL of MeCN and 200 mL of a phosphate buffer solution made by mixing equal volumes of 0.67 M Na_2HPO_4 and 0.67 M NaH_2PO_4 solutions at 35 °C was added a catalytic amount of TEMPO (430 mg, 2.75 mmol). The reaction flask, maintained at 35 °C, was fitted with two addition funnels. One was filled with a solution of $NaClO_2$ (8.3 g, 91.7 mmol) in 75 mL of H_2O. The other one was filled with a solution of household bleach (5.25%, 25 mL) in 250 mL of H_2O. About $^1/_5$ of the $NaClO_2$ solution was added, followed by about $^1/_5$ of the bleach solution to initiate the reaction. The remainder of both solutions was added dropwise, simultaneously, with a rate adjusted so that both additions finished concurrently. The reaction mixture was stirred at 35 °C for 4 h, then at room temperature for 18 h. It was diluted with 300 mL of H_2O, and the pH of the solution was adjusted to 8.0 by adding 1 M NaOH. The resulting solution was cooled to 0 °C, and a cold solution of Na_2SO_3 (6.1 wt %, 185 mL) was added slowly. The mixture was stirred at 0 °C for 30 min, after which a portion of Et_2O was added. After being stirred vigorously, the mixture was poured into an extraction funnel and the Et_2O layer was separated and discarded. The aqueous layer was acidified to pH 3.4 with concentrated HCl and extracted 3× with $CHCl_3$/*i*-PrOH mixture (4:1). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to dryness, yielding **37** as a pale-yellow oil (12.9 g, 98%), which could be used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (t, J = 7.0 Hz, 12H), 2.18−2.28 (m, 2H), 2.60 (tt, J = 23.9, 6.5 Hz, 1H), 2.69 (t, J = 7.3 Hz, 2H), 4.14−4.23 (m, 8H).

Tetraisopropyl 5-(2-Tetrahydro-2*H*-pyranyloxy)pentylene-1,1-bisphosphonate (39). To a suspension of sodium hydride (60%, 342.5 mg, 8.563 mmol) in 15 mL of THF was carefully added tetraisopropyl methylenebisphosphonate (2.80 mL, 8.61 mmol), and the resultant pale-yellow clear solution was stirred at room temperature for 30 min. Then neat compound **38** (2.0194 g, 8.516 mmol) was introduced by pipet plus 5 mL of THF rinse. The mixture was refluxed for 8 h and allowed to cool to room temperature before quenching with saturated NH_4Cl. The mixture was extracted with ethyl acetate (3×) and dried over sodium sulfate and concentrated in vacuo. Flash chromatography with 10:1 EtOAc/MeOH as eluent provided an inseparable mixture of the desired product **39** and unreacted tetraisopropyl methylenebisphosphonate, and the mixture was used directly in the next step. Selected ^{1}H NMR signals (400 MHz, $CDCl_3$) δ 1.48−2.02 (m, 12H), 2.14 (tt, J = 24.2, 5.9, 1H), 3.36−3.42 (m, 1H), 3.46−3.52 (m, 1H), 3.71−3.77 (m, 1H), 3.83−3.89 (m, 1H), 4.57−4.58 (m, 1H).

Tetraisopropyl 5-Hydroxypentylene-1,1-bisphosphonate (40). The mixture of **39** from the flash chromatography in the previous step was dissolved in 4 mL of MeOH, and 24.5 mg (0.127 mmol) of *p*-toluenesulfonic acid monohydrate was added. After being stirred overnight at room temperature, the mixture was concentrated and subjected to flash chromatography with 12:1 EtOAc/MeOH as eluent to afford **40** as a colorless oil (1.2 g, 50% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33−1.36 (m, 24H), 1.54−1.61 (m, 2H), 1.65−1.72 (m, 2H), 1.84−1.98 (m, 2H), 2.15 (tt, J = 24.1, 6.1, 1H), 2.28 (t, J = 5.7, 1H), 3.66 (q, J = 6.1, 2H), 4.72−4.82 (m, 4H).

Tetraisopropyl 5-Carboxypentylene-1,1-bisphosphonate (41). Compound **40**(365.5 mg, 0.9083 mmol) and pyridinium dichromate (1.22 g, 3.18 mmol) were dissolved in 3 mL of *N*,*N*-dimethylformamide and stirred at room temperature overnight. After the reaction was complete as monitored by TLC, the mixture was diluted with water and extracted with EtOAc (3×), dried over sodium sulfate, and concentrated in vacuo. Flash chromatography on silica gel with 19:1 EtOAc/acetic acid afforded **41** as a colorless oil (246.8 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29−1.35 (m, 24H), 1.90−1.99 (m, 4H), 2.18 (tt, J = 24.4, 5.5, 1H), 2.34 (t, J = 6.8, 2H), 4.73−4.82 (m, 4H).

General Procedure for the Acylation of Fluoroquinolones with Chloroethylchloroformate. 1-Chloroethyl chloroformate (0.27 mL, 2.478 mmol) was added to a solution of fluoroquinolone (2.477 mmol) and 547.9 mg (2.557 mmol) of Proton Sponge in 25 mL of chloroform. The clear yellow solution was stirred at room temperature for 5 h before being washed with water (3×) and dried over anhydrous sodium sulfate. Removal of the solvent yielded the desired product. In the cases where Proton Sponge was still present after the water wash, the crude product was passed through a short silica gel column with the elution of 19:1 dichloromethane/methanol.

7-((4a*S*,7a*S*)-1-((1-Chloroethoxy)carbonyl)octahydropyrrolo[3,4-*b*]pyridin-6-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (43a). Yield: 98%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78−0.86 (m, 1H), 1.03−1.17 (m, 2H), 1.25−1.35 (m, 1H), 1.47−1.64 (m, 1H), 1.78−1.90 (m, 5H), 2.32 (bs, 1H), 2.95−3.05 (m, 1H), 3.24−3.64 (m, 2H), 3.58 (s, 3H), 3.86−4.03 (m, 2H), 4.04−4.22 (m, 2H), 4.70−4.98 (m, 1H), 6.63 (q, J = 5.9, 1H), 7.82 (dd, J = 1.6, 13.7, 1H), 8.79 (s, 1H).

7-(4-((1-Chloroethoxy)carbonyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (43b). Yield: 98%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.94−1.04 (m, 2H), 1.21−1.26 (m, 2H), 1.40 (d, J=7.1, 3H), 1.85 (d, J = 5.9, 3H), 3.31−3.34 (m, 2H), 3.41−3.53 (m, 4H), 3.74 (s, 3H), 3.98−4.07 (m, 2H), 4.45 (bs, 1H), 6.65 (dq, J=1.8, 5.7, 1H), 7.92 (d, J = 12.0, 1H), 8.84 (s, 1H).

7-(4-(Chloromethoxycarbonyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic Acid (49). A suspension of gatifloxacin **1b** (8.65 g, 22.9 mmol) and Proton Sponge (4.90 g, 22.9 mmol) in anhydrous CH_2Cl_2 (100 mL) was cooled in an ice bath followed by the dropwise addition of chloroformic acid chloromethyl ester (2.03 mL, 22.9 mmol). The resulting mixture was stirred at the same temperature for 4 h. The reaction mixture was diluted by the addition of CH_2Cl_2 (300 mL), washed with cold aqueous HCl (5%) and saturated NaCl, and dried over anhydrous sodium sulfate. The organics were removed under reduced pressure to give **47** as a yellow solid that was used without purification (10.2 g, 95%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94−1.04 (m, 2H), 1.17−1.27 (m, 2H), 1.40 (d, J=6.7, 3H), 3.28−3.53 (m, 5H), 3.73 (s, 3H), 3.98−4.10 (m, 2H), 4.45 (bs, 1H), 5.85 (m, 2H), 7.92 (d, J = 12.3, 1H), 8.83 (s, 1H).

General Procedure for the Reaction of Fluoroquinolone Chloroethyl Carbamates with Bisphosphonated Acids. The carboxylic acid (1.45 mmol) was dissolved in 4 mL of THF, and 1.45 mL (1.45 mmol) of 1 N sodium hydroxide aqueous solution was added. The mixture was stirred at room temperature for 4 h, and the organic solvent was removed. The residual water was removed either by applying high vacuum overnight or by freeze-drying. A mixture of this sodium salt and a fluoroquinolone chloroethyl carbamate (1.460 mmol) in 7 mL of anhydrous acetonitrile was heated in a 60 °C oil bath for 2 days. After cooling to room temperature, the reaction mixture was filtered through a pad of Celite. The filtrate was concentrated and purified by chromatography through a Waters C18 Sep-Pak cartridge (35 cm^3) with gradient elution from neat water to 2:1 water/methanol to 1:2 water/methanol to methanol.

Mixed Acetal 44a. From acid **42** and carbamate **43a**. Yield: 43%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78−0.86 (m, 1H), 1.04−1.20 (m, 2H), 1.28−1.36 (m, 12H), 1.46−1.64 (m, 4H), 1.74−2.03 (m, 5H), 2.26−2.44 (m, 4H), 2.90−3.02 (m, 1H), 3.22−3.50 (m, 3H), 3.58 (s, 3H), 3.84−4.03 (m, 3H), 4.03−4.28 (m, 8H), 4.64−4.94 (bs, 1H), 5.02 (dt, J = 10.0, 21.9, 1H), 6.15 (d, J = 10.2, 1H), 6.84 (q, J = 4.7, 1H), 7.82 (d, J = 13.9, 1H), 8.79 (s, 1H).

Mixed Acetal 44b. From acid **42** and carbamate **43b**. Yield: 43%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96−1.04 (m, 2H), 1.16−1.29 (m, 2H), 1.34 (t, J = 7.1, 12H), 1.52 (d, J = 5.5, 3H), 1.69 (bs, 3H), 1.99 (quint, J = 7.0, 2H), 2.35 (t, J = 7.2, 2H), 2.42 (t, J = 7.2, 2H), 3.22−3.53 (m, 5H), 3.74 (s, 3H), 3.98−4.04 (m, 2H), 4.14−4.24 (m, 8H), 4.36−4.44 (m, 1H), 5.03 (dt, J = 10.2, 21.7, 1H), 6.18−6.21 (m, 1H), 6.86 (q, J = 5.5, 1H), 7.91 (d, J = 12.1, 1H), 8.83 (s, 1H).

Mixed Acetal 46. From acid **41** and carbamate **43b**. Yield: 74%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88−1.04 (m, 2H), 1.14−1.25 (m, 2H), 1.28−1.42 (m, 24H), 1.51 (d, J=5.5, 3H), 1.61 (bs, 3H), 1.86−2.00 (m, 4H), 2.13 (tt, J = 4.7, 24.3, 1H), 2.35 (t, J = 6.0, 2H), 3.22−3.53 (m, 5H), 3.73 (s, 3H), 3.96−4.04 (m, 2H), 4.34−4.44 (m, 1H), 4.78 (septet, J = 6.1, 1H), 6.86 (q, J = 5.3, 1H), 7.90 (d, J = 12.2, 1H), 8.83 (s, 1H).

Mixed Acetal 50. Acid **37** (3.70 g, 10.3 mmol) was dissolved in CH_3CN (20 mL), followed by the addition of KOH (0.634 g, 11.3 mmol) in H_2O. The resulting solution was stirred for 5 min and then concentrated under reduced pressure. This sodium salt was dissolved in DMF (25 mL) followed by the addition of carbamate **49** (2.09 g, 4.47 mmol). The resulting solution was stirred at room temperature for 3 h and then quenched by the addition of ice-cold H_2O (150 mL). The product was extracted with EtOAc (3 × 100 mL), and the combined organics were washed with water and brine and then dried over Na_2SO_4. After the drying agent was filtered off, the organics were removed under reduced pressure, resulting in **50** as yellow solid that was used without purification (3.3 g, 93%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92−1.04 (m, 2H), 1.17−1.27 (m, 2H), 1.34 (t, J = 6.9, 12H), 1.39 (d, J=10.5, 3H), 1.95 (bs, 2H), 2.19−2.33 (m, 2H), 2.47 (tt, J = 7.5, 31.1, 1H), 2.77 (t, J = 7.6, 2H), 3.28−3.52 (m, 5H), 3.73 (s, 3H), 3.98−4.23 (m, 8H), 4.42 (bs, 1H), 5.87 (m, 2H), 7.90 (d, J = 11.9, 1H), 8.83 (s, 1H).

General Procedure for Bisphosphonate Deprotection. To a solution of bisphosphonated tetraester (0.63 mmol) in 5 mL of CH_2Cl_2 was added 0.83 mL (6.29 mmol) of bromotrimethylsilane. After being stirred at room temperature for 6 h, the mixture was concentrated and the residue was kept at high vacuum for at least 30 min. The resulting material was dissolved in dilute sodium hydroxide solution (≤2 equiv of NaOH; this process was fairly time-consuming, and ultrasound sonication was needed from time to time to maintain the solution) prior to the careful adjustment of pH to 7.20 with 1 N sodium hydroxide solution. The aqueous solution obtained was subjected to a Waters C18 Sep-Pak cartridge (20 cm^3) with gradient elution from neat water to 10:1 water/methanol. All fractions containing the desired product were immediately combined and frozen in an acetone/dry ice cold bath. The solvents were removed by freeze-drying, and the material obtained was washed with CH_2Cl_2.

Mixed Acetal 45a. Yield: 18%. ^{1}H NMR (400 MHz, D_2O) δ 0.75−0.83 (m, 1H), 0.96−1.04 (m, 1H), 1.04−1.13 (m, 1H), 1.18−1.27 (m, 1H), 1.46−1.60 (m, 2H), 1.52 (d, J = 5.5, 3H), 1.73−1.86 (m, 2H), 1.93 (quint, J = 7.6, 2H), 2.28−2.40 (m, 1H), 2.38 (t, J = 7.1, 2H), 2.49 (t, J = 7.2, 2H), 2.98−3.12 (m, 1H), 3.30 (d, J = 9.4, 1H), 3.43−3.53 (m, 1H), 3.59 (s, 3H), 3.90−4.10 (m, 4H), 4.24 (t, J = 19.4, 1H), 6.77 (q, J = 5.5, 1H), 7.64 (d, J = 14.5, 1H), 8.46 (s, 1H) ppm. ^{31}P NMR (162 MHz, D_2O) δ 14.23 ppm. ^{19}F NMR (376 MHz, D_2O) δ −123.52 (bs) ppm. MS (*m*/*e*): 777 (M + H).

Mixed Acetal 45b. Yield: 30%. ^{1}H NMR (400 MHz, D_2O) δ 0.89−1.00 (m, 2H), 1.08−1.17 (m, 2H), 1.37 (t, J = 7.2, 3H), 1.53 (d, J = 5.5, 3H), 1.93 (quint, J = 7.2, 2H), 2.38 (t, J = 7.6, 2H), 2.50 (t, J = 7.0, 2H), 3.24−3.34 (m, 2H), 3.42−3.50 (m, 3H), 3.75 (s, 3H), 3.93 (bs, 1H), 4.06−4.12 (m, 1H), 4.22 (t, J = 19.0, 1H), 4.34 (bs, 1H), 6.79 (q, J = 5.5, 1H), 7.73 (d, J = 12.7, 1H), 8.51 (s, 1H). ^{31}P NMR (162 MHz, D_2O) δ 14.27. ^{19}F NMR (376 MHz, D_2O) δ −122.32 (bs). MS (*m*/*e*): 751 (M + H).

Mixed Acetal 47. Yield: 30%. ^{1}H NMR (400 MHz, D_2O) δ 0.88−1.02 (m, 2H), 1.07−1.20 (m, 2H), 1.37 (t, J = 7.3, 3H), 1.55 (d, J=5.5, 3H), 1.72−1.85 (m, 5H), 2.48 (t, J = 6.4, 2H), 3.25−3.34 (m, 2H), 3.43−3.52 (m, 4H), 3.75 (s, 3H), 3.93 (bs, 1H), 4.10 (septet,, J = 3.5, 1H), 4.36 (bs, 1H), 6.80 (dq, J = 0.8, 5.5, 1H), 7.73 (d, J = 12.7, 1H), 8.52 (s, 1H). ^{31}P NMR (162 MHz, D_2O) δ −20.87. ^{19}F NMR (376 MHz, D_2O) δ −122.32 (bs). MS (*m*/*e*): 708 (M + H).

Mixed Acetal 51. A solution of crude **53** (3.25 g, 4.11 mmol) and 2,6-lutidine (9.53 mL, 82.1 mmol) in CH_2Cl_2 (30 mL) was cooled in an ice-bath followed by the dropwise addition of TMSBr (8.13 mL, 61.6 mmol). The resulting yellow solution was stirred while warming to room temperature over 24 h. The solvent and excess lutidine were then removed under reduced pressure. The residue was resuspended in H_2O and purified by C18 reverse phase chromatography (0−60% CH_3CN in water) on a Biotage flash chromatography system. The CH_3CN was removed under reduced pressure and the water was removed by lyophization to give the pale-yellow mono 2,6-lutidine salt of **54** (1.58 g, 49%): ^{1}H NMR (400 MHz, D_2O) δ 0.88−1.11 (m, 2H), 1.20−1.31 (m, 2H), 1.36 (d, J = 6.9, 3H), 2.04−2.22 (m, 3H), 1.79 (t, J = 7.7, 2H), 2.71 (s, 6H), 3.28−3.55 (m, 5H), 3.75 (s, 3H), 3.97 (bd, J = 12.0, 1H), 4.20−4.26 (m, 1H), 4.38 (bs, 1H), 5.82 (s, 2H), 7.41 (d, J = 12.8, 1H), 7.62 (d, J = 8.2, 1H), 8.26 (t, J = 7.7, 2H), 8.84 (s, 1H). ^{31}P NMR (162 MHz, D_2O) δ −20.94 (s, 1P). ^{19}F NMR (376 MHz, D_2O) δ −119.05 (d, J = 10.5, 1F). LCMS: −98.8% (254 nm), 98.8% (220 nm), 98.9% (320 nm). MS: (MH^+) 680.2.

Biology. Determination of Minimum Inhibitory Concentrations against *S. aureus* ATCC13709. The antimicrobial activity of the compounds were tested against *S. aureus* strain ATCC 13709. Minimum inhibitory concentration (MIC) testing was performed by the microdilution method according to the guidelines set by the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards).[46]

Determination of Levels of Bone Binding in Vitro. An individual compound was dissolved in PBS and added, at a final concentration of 1 mg/mL, to a slurry of bone meal powder (Now Foods, Bloomingdale, IL) 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. 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 control binding experiments, >99% of input parent (nonbisphosphonated) drug was recovered in the supernatant.

Regeneration of Parent Drug from Bone-Bound Prodrug. Washed bone powder-bound prodrugs from the previous section were resuspended in 400 μL of PBS or in 400 μL of 50% (v/v in PBS) human or rat serum. The suspension was incubated for 24 h at 37 °C and 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, section M7 A7), which always matched those of parent drugs but not those of prodrugs.

Prophylactic Use of Prodrugs in a Rat Model of Bone Infection. *S. 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 phosphate-buffered saline (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 used to check the CFU count by serial dilution plating. 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[47,48] to generate the bone infection. Female CD rats (age, 57−70 days; $n = 5$/group; Charles River, St-Constant, Canada) were anesthetized 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 condyle) 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 intratibially with 0.05 mL of 5% sodium morrhuate (sclerosing agent) and then with 0.05 mL of *S. aureus* suspension (about 5 × 105 CFU/rat). The hole was sealed by applying a small amount of dry dental cement that immediately absorbs fluids and adheres to the site. The wound was closed using three metal skin clips. Moxifloxacin **3** (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 of 0.9% NaCl, serially diluted, and processed for quantitative cultures. Treatment efficacy was measured in terms of the logarithm of the amount of 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 was 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. Differences were considered significant when the P value was <0.05 when comparing treated infected animals to the untreated infected ones.

Supporting Information Available: Chromatographic methods, HPLC purities, and LC/MS traces for compounds **4a**, **13a**,**b**, **20**, **29a**−**c**, **36**, **45a**,**b**, **47**, and **51**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Lew, D. P.; Waldvogel, F. A. Osteomyelitis. *Lancet* **2004**, *364*, 369–379.

(2) Mandal, S.; Berendt, A. R.; Peacock, S. J. *Staphylococcus aureus* Bone and Joint Infection. *J. Infect.* **2002**, *44*, 143–151.

(3) Peter, K.; Henke, P. K.; Blackburn, S. A.; Wainess, R. W.; Cowan, J.; Alicia Terando, A.; Proctor, M.; Wakefield, T. W.; Upchurch, G. R., Jr.; Stanley, J. C.; Greenfield, L. J. Osteomyelitis of the Foot and Toe in Adults Is a Surgical Disease: Conservative Management Worsens Lower Extremity Salvage. *Ann. Surg.* **2005**, *241*, 885–894.

(4) Tice, A. D.; Hoaglund, P. A.; Shoultz, D. A. Outcomes of Osteomyelitis among Patients Treated with Outpatient Parenteral Antimicrobial Therapy. *Am. J. Med.* **2003**, *114*, 723–728.

(5) Tice, A. D.; Hoaglund, P. A.; Shoultz, D. A. Risk Factors and Treatment Outcomes in Osteomyelitis. *J. Antimicrob. Chemother.* **2003**, *51*, 1261–1268.

(6) Nelson, C. L.; McLaren, S. G.; Skinner, R. A.; Smeltzer, M. S.; Thomas, J. R.; Olsen, K. M. The Treatment of Experimental Osteomyelitis by Surgical Debridement and the Implantation of Calcium Sulfate Tobramycin Pellets. *J. Orthop. Res.* **2002**, *20*, 643–647.

(7) Baro, M.; Sánchez, E.; Delgado, A.; Perera, A.; Évora, C. In Vitro−in Vivo Characterization of Gentamicin Bone Implants. *J. Controlled Release* **2002**, *83*, 353–364.

(8) Castro, C.; Sánchez, E.; Delgado, A.; Soriano, I.; Núñez, P.; Baro, M.; Perera, A.; Évora, C. Ciprofloxacin Implants for Bone Infection. In Vitro−in Vivo Characterization. *J. Controlled Release* **2003**, *93*, 341–354.

(9) Mäkinen, T. J.; Veiranto, M.; Lankinen, P.; Moritz, N.; Jalava, J.; Törmälä, P.; Aro, H. T. In Vitro and in Vivo Release of Ciprofloxacin from Osteoconductive Bone Defect Filler. *J. Antimicrob. Chemother.* **2005**, *56*, 1063–1068. Joostena, U.; Joist, A.; Goshegerc, G.; Liljenqvist, U.; Brandt, B.; von Eiff, C. Effectiveness of Hydroxyapatite−Vancomycin Bone Cement in the Treatment of Staphylococcus Aureus Induced Chronic Osteomyelitis. *Biomaterials* **2005**, *26*, 5251–5258.

(10) Hirabayashi, H.; Fujisaki, J. Bone-Specific Drug Delivery Systems: Approaches via Chemical Modification of Bone-Seeking Agents. *Clin. Pharmacokinet.* **2003**, *42*, 1319–1330.

(11) Uludag, H. Bisphosphonates as a Foundation of Drug Delivery to Bone. *Curr. Pharm. Des.* **2002**, *8*, 1929–1944.

(12) Vepsäläinen, J. J. Bisphosphonate Prodrugs. *Curr. Med. Chem.* **2002**, *9*, 1201–1208.

(13) Russell, R. G. G.; Rogers, M. J. Bisphosphonates: From the Laboratory to the Clinic and Back Again. *Bone* **1999**, *25*, 97–106.

(14) Licata, A. A. Discovery, Clinical Development, and Therapeutic Uses of Bisphosphonates. *Ann. Pharmacother.* **2005**, *39*, 668–677.

(15) Hirabayashi, H.; Takahashi, T.; Fujisaki, J.; Masunaga, T.; Sato, S.; Hiroi, J.; Tokunaga, Y.; Kimura, S.; Hata, T. Bone-Specific Delivery and Sustained Release of Diclofenac, a Non-Steroidal Anti-Inflammatory Drug, via Bisphosphonic Prodrug Based on the Osteotropic Drug Delivery System (ODDS). *J. Controlled Release* **2001**, *70*, 183–191.

(16) Hirabayashi, H.; Sawamoto, T.; Fujisaki, J.; Tokunaga, Y.; Kimura, S.; Hata, T. Dose-Dependent Pharmacokinetics and Disposition of Bisphosphonic Prodrug of Diclofenac Based on Osteotropic Drug Delivery System (ODDS). *Biopharm. Drug Dispos.* **2002**, *23*, 307–315.

(17) Gil, L.; Han, Y.; Opas, E. E.; Rodan, G. A.; Ruel, R.; Seedor, J. G.; Tyler, P. C.; Young, R. N. Prostaglandin E_2-Bisphosphonate Conjugates: Potential Agents for Treatment of Osteoporosis. *Bioorg. Med. Chem.* **1999**, *7*, 901–919.

(18) Page, P. C. B.; McKenzie, M. J.; Gallagher, J. A. Novel Synthesis of Bis(phosphonic acid)−Steroid Conjugates. *J. Org. Chem.* **2001**, *66*, 3704–3708.

(19) Fujisaki, J.; Tokunaga, Y.; Takahashi, T.; Hirose, T.; Shimojo, F.; Kagayama, A.; Hata, T. Osteotropic Drug Delivery System (ODDS) Based on Bisphosphonic Prodrug. I: Synthesis and in Vivo Characterization of Osteotropic Carboxyfluorescein. *J. Drug Target.* **1995**, *3*, 273–282.

(20) Fujisaki, J.; Tokunaga, Y.; Sawamoto, T.; Takahashi, T.; Kimura, S.; Shimojo, F.; Hata, T. Osteotropic Drug Delivery System (ODDS) Based on Bisphosphonic Prodrug. III: Pharmacokinetics and Targeting Characteristics of Osteotropic Carboxyfluorescein. *J. Drug Targeting* **1996**, *4*, 117–123.

(21) Adzamli, I. K.; Gries, H.; Johnson, D.; Blau, M. Development of Phosphonate Derivatives of Gadolinium Chelates for NMR Imaging of Calcified Soft Tissues. *J. Med. Chem.* **1989**, *32*, 139–144.

(22) Ogawa, K.; Mukai, T.; Arano, Y.; Ono, M.; Hanaoka, H.; Ishino, S.; Hashimoto, K.; Nishimura, H.; Saji, H. Development of a Rhenium-186-Labeled MAG3-Conjugated Bisphosphonate for the Palliation of Metastatic Bone Pain Based on the Concept of Bifunctional Radiopharmaceuticals. *Bioconjugate Chem.* **2005**, *16*, 751–757.

(23) Kubíek, V.; Rudovsk, J.; Kotek, J.; Hermann, P.; Vander Elst, L.; Muller, R. N.; Kolar, Z. I.; Wolterbeek, H. Th.; Peters, J. A.; Luke, I. A Bisphosphonate Monoamide Analogue of DOTA: A Potential Agent for Bone Targeting. *J. Am. Chem. Soc.* **2005**, *127*, 16477–16485.

(24) Bansal, G.; Wright, J. E. I.; Kucharski, C.; Uludağ, H. A Dendritic Tetra(bisphosphonic acid) for Improved Targeting of Proteins to Bone. *Angew. Chem., Int. Ed.* **2005**, *44*, 3710–3714.

(25) Wanga, D.; Miller, S. C.; Kopečková, P.; Kopeček, J. Bone-Targeting Macromolecular Therapeutics. *Adv. Drug Delivery Rev.* **2005**, *57*, 1049–1076.

(26) Wright, J. E. I.; Gittens, S. A.; Bansal, G.; Kitov, P. I.; Sindrey, D.; Kucharski, C.; Uludağ, H. A Comparison of Mineral Affinity of Bisphosphonate−Protein Conjugates Constructed with Disulfide and Thioether Linkages. *Biomaterials* **2006**, *27*, 769–784.

(27) Herczegh, P.; Buxton, T. B.; McPherson, J. C.; Kovács-Kulyassa, Á.; Brewer, P. D.; Sztaricskai, F.; Stroebel, G. G.; Plowman, K. M.; Farcasiu, D.; Hartmann, J. F. Osteoadsorptive Bisphosphonate Deriva-

tives of Fluoroquinolone Antibacterials. *J. Med. Chem.* **2002**, *45*, 2338–2341.
(28) Rogers, M. J.; Gordon, S.; Benford, H. L.; Coxon, F. P.; Luckman, S. P.; Monkkonen, J.; Frith, J. C. Cellular and Molecular Mechanisms of Action of Bisphosphonates. *Cancer* **2000**, *88*, 2961–2978.
(29) Henderson, B.; Nair, S. P. Hard Labour: Bacterial Infection of the Skeleton. *Trends Microbiol.* **2003**, *11*, 570–577.
(30) Mitscher, L. A. Bacterial Topoisomerase Inhibitors: Quinolone and Pyridone Antibacterial Agents. *Chem. Rev.* **2005**, *105*, 559–592.
(31) Van Bambeke, F.; Michot, J.-M.; Van Eldere, J.; Tulkens, P. M. Quinolones in 2005: An Update. *Clin. Microbiol. Infect.* **2005**, *11*, 256–280.
(32) Greenberg, R. N.; Newman, M. T.; Shariaty, S.; Pectol, R. W. Ciprofloxacin, Lomefloxacin, or Levofloxacin as Treatment for Chronic Osteomyelitis. *Antimicrob. Agents Chemother.* **2000**, *44*, 164–166.
(33) Shirtliff, M. E.; Calhoun, J. H.; Mader, J. T. Comparative Evaluation of Oral Levofloxacin and Parenteral Nafcillin in the Treatment of Experimental Methicillin-Susceptible *Staphylococcus aureus* Osteomyelitis in Rabbits. *J. Antimicrob. Chemother.* **2001**, *48*, 253–258.
(34) Shirtliff, M. E.; Calhoun, J. H.; Mader, J. T. Gatifloxacin Efficacy in Treatment of Experimental Methicillin-Sensitive *Staphylococcus aureus*-Induced Osteomyelitis in Rabbits. *Antimicrob. Agents Chemother.* **2002**, *46*, 231–233.
(35) Degenhardt, C. R.; Burdsall, D. C. Synthesis of Ethenylidenebis(phosphonic acid) and Its Tetraalkyl Esters. *J. Org. Chem.* **1986**, *51*, 3488–3490.
(36) Alexander, J.; Bindra, D. S.; Glass, J. D.; Holahan, M. A.; Renyer, M. L.; Rork, G. S.; Sitko, G. R.; Stranieri, M. T.; Stupienski, R. F.; Veerapanane, H.; Cook, J. J. Investigation of (Oxodioxolenyl)methyl Carbamates as Nonchiral Bioreversible Prodrug Moieties for Chiral Amines. *J. Med. Chem.* **1996**, *39*, 480–486.
(37) Sakamoto, F.; Ikeda, S.; Kondo, H.; Tsukamoto, G. Studies on Prodrugs. IV. Preparation and Characterization of *N*-(5-Substituted 2-oxo-1, 3-dioxol-4-yl)methyl Norfloxacin. *Chem. Pharm. Bull.* **1985**, *33*, 4870–4877.
(38) Luke, G. P.; Shakespeare, W. C. A Simple and Efficient Preparation of (Arylphosphinyl)-methylphosphonates. *Synth. Commun.* **2002**, *32*, 2951–2957.
(39) Amsberry, K. L.; Borchardt, R. T. The Lactonization of 2′-Hydroxyhydrocinnamic Acid Amides: A Potential Prodrug for Amines. *J. Org. Chem.* **1990**, *55*, 5867–5877.
(40) Amsberry, K. L.; Gerstenberger, A. L.; Borchardt, R. T. Amine Prodrugs Which Utilize Hydroxy Amide Lactonization. II. A Potential Esterase-Sensitive Amide Prodrug. *Pharm. Res.* **1991**, *8*, 455–461.
(41) Agarwal, S. P.; Bala, H.; Ali, M. M. Ciprofloxacin Prodrug via Mannich Reaction. *Indian J. Pharm. Sci.* **1999**, *61*, 223–226.
(42) See, for example, the following: Svahn, C. M.; Merenyi, F.; Karlson, L.; Widlund, L.; Grälls, M. Tranexamic Acid Derivatives with Enhanced Absorption. *J. Med. Chem.* **1986**, *29*, 448–453.
(43) Alexander, J.; Cargill, R.; Michelson, S. R.; Schwam, H. (Acyloxy) alkyl Carbamates as Novel Bioreversible Prodrugs for Amines: Increased Permeation through Biological Membranes. *J. Med. Chem.* **1988**, *31*, 318–322.
(44) Alexander, J.; Fromtling, R. A.; Bland, J. A.; Pelak, B. A.; Gilfillan, E. C. (Acyloxy)alkyl Carbamate Prodrugs of Norfloxacin. *J. Med. Chem.* **1991**, *34*, 78–81.
(45) Fujisaki, J.; Tokunaga, Y.; Takahashi, T.; Shimojo, F.; Kimura, S.; Hata, T. Osteotropic Drug Delivery System (ODDS) Based on Bisphosphonic Prodrug. IV. Effects of Osteotropic Estradiol on Bone Mineral Density and Uterine Weight in Ovariectomized Rats. *J. Drug Targeting* **1998**, *5*, 129–138.
(46) *National Committee for Clinical Laboratory Standards, Performance Standards for Antimicrobial Susceptibility Testing*; Fifth Information Supplement, Approved Standard M100-S5; NCCLS: Villanova, PA, 1994.
(47) Mader, J. T. Animal Models of Osteomyelitis. *Am. J. Med.* **1985**, *78*, 213–217.
(48) O'Reilly, T.; Kunz, S.; Sande, E.; Zak, O.; Sande, M. A.; Täuber, M. G. Relationship between Antibiotic Concentration in Bone and Efficacy of Treatment of Staphylococcal Osteomyelitis in Rats: Azithromycin Compared with Clindamycin and Rifampin. *Antimicrob. Agents Chemother.* **1992**, *36*, 2693–2697.

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