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Identification and Suppression of a Dimer Impurity in the Development of Delafloxacin

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Abstract:

Delafloxacin is a 6-fluoroquinolone antibiotic which is under development at Rib-X Pharmaceuticals. During initial scale-up runs to prepare delafloxacin, up to 0.43% of a new impurity arose in the penultimate chlorination step. This was identified as a dimeric adduct of delafloxacin. Subsequent application of design of experiments (DoE) led to the identification of the factors responsible for this impurity. Implementation of the knowledge gained from the DoE reproducibly enabled the suppression of this impurity to acceptable levels.

Introduction

Antimicrobial resistance in the community and hospital settings has been a growing public health concern due to the continuing emergence of multidrug resistant bacterial strains.[1] Methicillin-resistant *Staphylococcus aureus* (MRSA) ranks as the most frequently isolated pathogen in hospital intensive care units in the United States where the proportion of MRSA occurrence in *S. aureus* isolates increased from 35.9% in 1992 to 64.4% in 2003.[2]

Since the introduction of nalidixic acid nearly 40 years ago,[3] quinolone antibiotics have occupied a prominent place in the array of antibiotics. 6-Fluoroquinolones such as ciprofloxacin have especially gained an expanding role in the treatment of infections, due to their broad spectrum of application.[4]

Delafloxacin is a 6-fluoroquinolone antibiotic[5] with excellent antibacterial activity against Gram-positive organisms, including both methicillin-susceptible *S. aureus* and MRSA. It is currently undergoing phase II clinical trials.

The synthesis of delafloxacin initially underwent development by Abbott Laboratories,[6] and a key step in this is a selective chlorination on the 8-position of the functionalized quinolone **1** (Scheme 1). In this process a solution of **1** in a mixture of methyl acetate and ethyl acetate is chlorinated using NCS in the presence of 3.5 mol % of H_2SO_4, affording **2**. This is followed by solvent exchange and saponification with KOH to yield **3**. Delafloxacin is obtained after salt formation with *N*-methyl-D-glucamine.

Despite initial success in implementing this process, we encountered difficulties upon scale-up of this step in that up to 0.43 area % of a new impurity was detected by HPLC at relative retention time (RRT) 1.60 after isolation of **3**. Additionally, this new impurity turned out to be difficult to purge during the final salt formation. Thus, we decided to initiate a study to identify this impurity, understand how it was being formed, and suppress its generation.

Results and Discussion

Despite numerous attempts to isolate this new impurity by preparative HPLC, we were unable to do so, and only the molecular weight was established by HPLC-MS as 880 Da. The measured molecular weight of this impurity is exactly double that of the acid **3**, which suggested a dimeric derivative of this compound. Close examination of the purity profile of the substrate of the chlorination reaction did not lead to the detection of any impurity that could similarly be assigned a dimeric structure, and its formation was therefore attributed to the chlorination−hydrolysis sequence. A number of potential dimeric adducts that could arise in this step were considered, including **4**, which would result from cleavage of the azetidine moiety in one unit of **3** and reaction with the hydroxyl group of a second. In order to investigate this possibility further, we embarked on the synthesis of **4**.

Retrosynthetically (Scheme 2), molecule **4** is readily disconnected into amino alcohol **5** and quinolone **6**; the latter is a known compound.[7] A suitably protected amino alcohol **10** was synthesized from commercially available azetidin-3-ol hydro-

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(6) (a) Haight, A. R.; Ariman, S. Z.; Barnes, D. M.; Benz, N. J.; Gueffier, F. X.; Henry, R. F.; Hsu, M. C.; Lee, E. C.; Morin, L.; Pearl, K. B.; Peterson, M. J.; Plata, D. J.; Willcox, D. R. *Org. Process Res. Dev.* **2006**, *4*, 751. (b) Barnes, D. M.; Christesen, A. C.; Engstrom, K. M.; Haight, A. R.; Hsu, M. C.; Lee, E. C.; Peterson, M. J.; Plata, D. J.; Raje, P. S.; Stoner, E. J.; Tedrow, J. S.; Wagaw, S. *Org. Process Res. Dev.* **2006**, *4*, 803.

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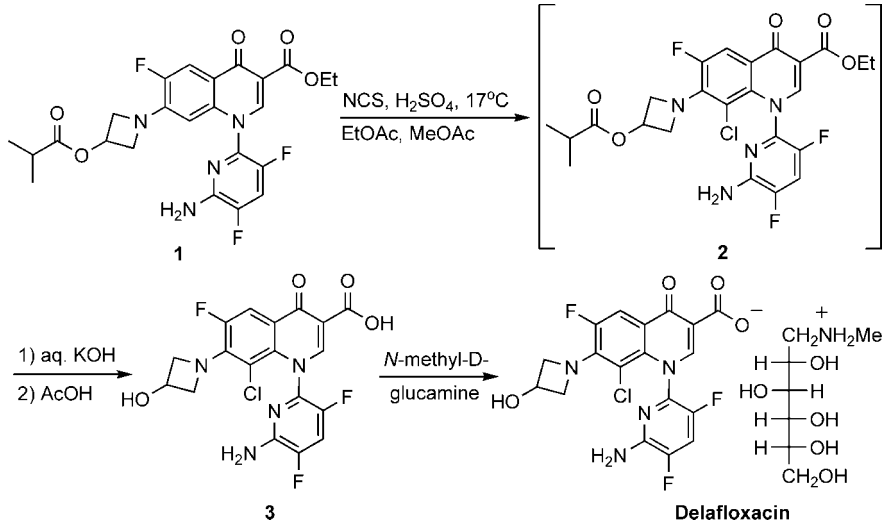
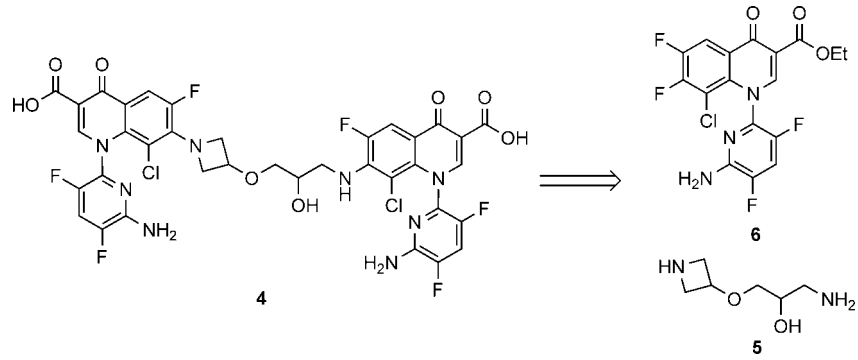
Scheme 1. **Synthesis of delafloxacin**



Scheme 2. **Retrosynthesis of proposed impurity 4**



chloride **7** (Scheme 3). Thus, amino alcohol **10** was condensed with quinolone **6** to form **11**, which after deprotection and a second condensation with **6** resulted in formation of the dimeric compound **13**. After saponification the proposed impurity **4** was obtained in 45% overall yield.

With synthetic **4** at hand, the unknown impurity in a contaminated batch of delafloxacin was compared with synthesized **4** *via* spiking experiments and comparison by HPLC-MS and HPLC-UV. To our delight, synthetic **4** matched unambiguously with the unknown impurity seen in previously manufactured batches of delafloxacin.

In order to understand the dynamics of the formation of impurity **4**, we decided to investigate the chlorination reaction further in a design of experiments (DoE) study. The following factors were chosen to be investigated in a DoE study of resolution IV, over ranges specified as follows:[8] temperature (15−25 °C), amount of NCS (1.05−1.2 equiv), amount of H_2SO_4 (2−5 mol %), water content in solvent (0−0.5%), solvent volume (2−3 vol), solvent (methyl acetate/ethyl acetate), and NCS addition rate (0.05−0.3 vol/min). A total of 19 chlorination reactions were performed in a MultiMax reactor.[9] In each case samples from the reactions were quenched after 5 h and saponified with KOH, and then the crude reaction mixtures were analyzed by HPLC.[10] The area % value for impurity **4** that resulted in each case was processed and analyzed using the DoE software.[11]

An excellent correlation of R^2 of 0.997 was obtained following processing of the data. Of the main effects, higher amounts of NCS, lower temperature, and faster addition of the NCS solution as well as the use of dry solvents[12] had the most beneficial impact on suppressing the amount of impurity **4** (Figure 1).[13] Additionally, a strong interaction was observed between the amount of NCS and solvent, in that methyl acetate is preferred when only a slight excess of NCS is used. In order to suppress any overchlorination of **2**, 1.05 equiv of NCS is preferred, and hence, methyl acetate was chosen as the solvent of choice for this step.

From a mechanistic point of view, we postulate that impurity **4** could arise from an initial acid-catalyzed activation of the azetidine ring which triggers an isobutyric ester/chloride-induced ring opening sequence to **16**. During the subsequent saponification **16** reacts with the hydrolysis intermediate **17** or **3** to **4**

(8) The experimental table and results can be found in the Supporting Information.
(9) Available from Mettler-Toledo, Inc., 1900 Polaris Parkway, Columbus, OH 43240, U.S.A.
(10) In order to determine the amount of **4**, the chlorinated samples **2** were saponified to **3**.
(11) The experimental design and analysis were conducted with JMP, Design of Experiments, Version 7, SAS Institute Inc., Cary, NC, U.S.A., 1989−2007, using a stepwise fit followed by a standard least squares method.
(12) Methyl acetate containing less than 500 ppm water was used, prior to adjustment as required by the appropriate experiment in the DoE.
(13) A more detailed analysis can be found in the Supporting Information.

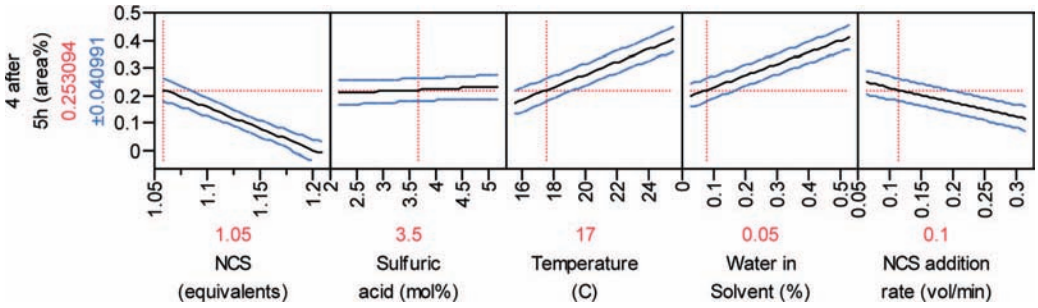
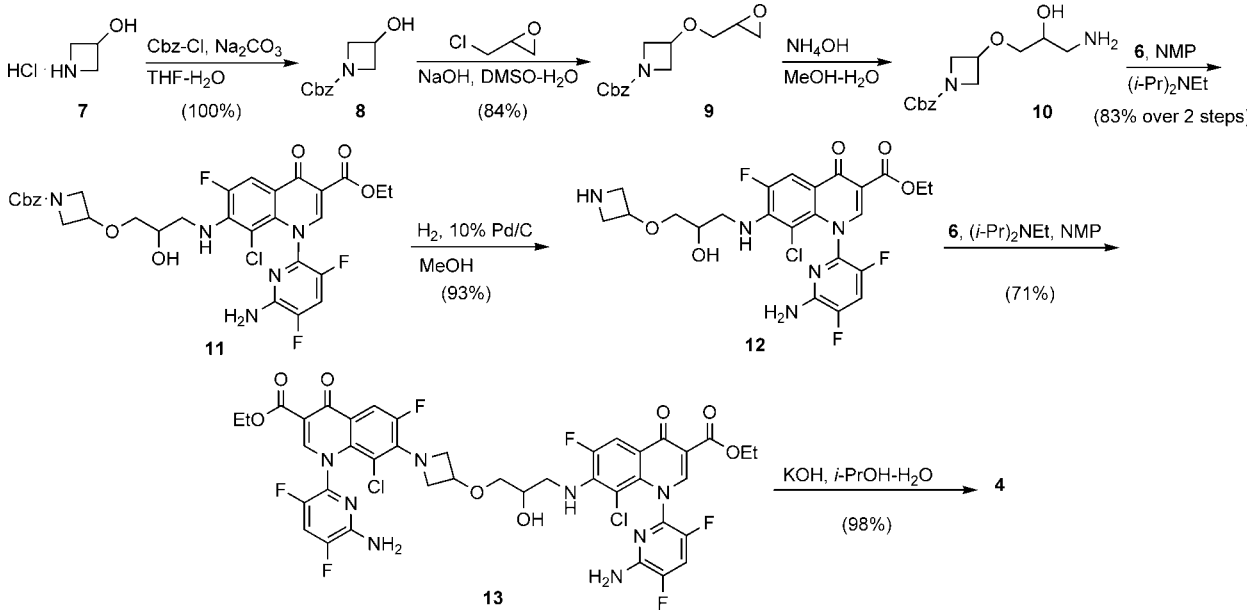


Figure 1. **Prediction profiler for ethyl acetate as the solvent.**

Scheme 3. **Synthesis of impurity 4**



(Scheme 4). [14] The validity of this sequence was further strengthened by a subsequent HPLC-MS analysis of a crude chlorination reaction before saponification. In this, an impurity with a molecular weight of 574 Da, which matches **16**, was detected in approximate equal amounts compared to **4** after saponification.

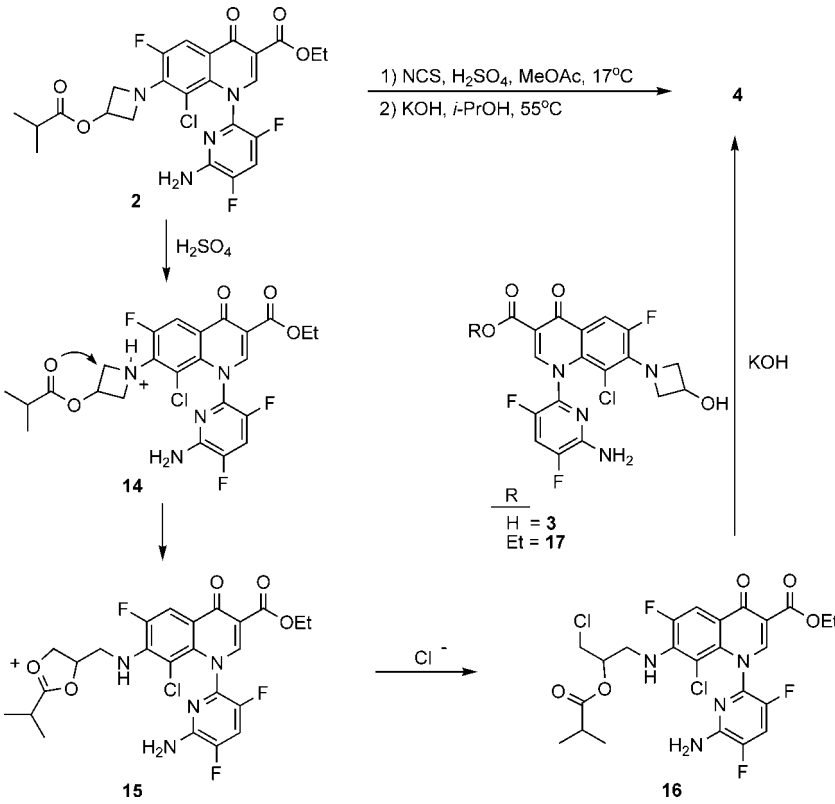
Based on this hypothetical mechanism, a time dependency for the formation of **4** during the chlorination process cannot be excluded, and since the reaction time was kept constant in the DoE study, it was decided to evaluate this parameter independently. A chlorination reaction was performed using 3.5% of H_2SO_4 and methyl acetate as solvent at 15 °C, and a sample was quenched after the reaction was deemed to be complete. Additional samples were quenched after 2 h and 6 h, saponified, and analyzed by HPLC. Not surprisingly, a steady increase of the impurity **4** over time was seen. This result has an impact on controlling the chlorination process, in that an adequate turnaround time for the HPLC monitoring of this reaction would be necessary in order to minimize the formation of **4**. However, subsequent experiments showed that decreasing the amount of H_2SO_4 to 1% diminished the amount of impurity **4** produced over time without having a significant impact on the chlorination reaction time or the quality of **3** (Figure 2). Thus, an acceptable turnaround time for the in-process control can be achieved when a level of 1% of H_2SO_4 is employed as catalyst.

After having established a good understanding of the critical parameters with respect to formation of this impurity, a second DoE study was initiated to test the robustness of the reaction in the anticipated process operating range. In this, a DoE study of resolution IV was designed with the following factors undergoing variation: temperature (13−21 °C), amount of NCS (1.04−1.07 equiv), NCS addition rate (30−75 min), and H_2SO_4 (0.8−1.2 mol %). A total of 10 chlorination reactions were performed in a MultiMax reactor. In each case samples were quenched and saponified after passing the in-process control. The resulting area % of **4** was processed and analyzed using the DoE software. As anticipated, temperature, amount of NCS, and H_2SO_4 had a statistically significant effect on the amount of impurity **4** in the studied parameter range. However, assuming the worst case scenario in the prediction profiler, impurity **4** has a value of 0.11 area % ± 0.01%, which is well within the acceptable limit that has been established from a toxicological batch of delafloxacin (Figure 3).

Two subsequent kilo laboratory runs of this reaction confirmed the effectiveness of the parameter changes, and material of high quality with impurity **4** levels of 0.07% were obtained after saponification.

(14) Saponification and subsequent epoxide formation of **16** prior to condensation with **3** or **17** cannot be ruled out.

Scheme 4. **Proposed mechanism of impurity 4**



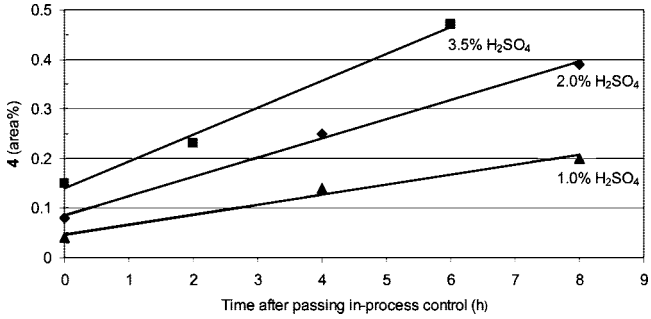


Figure 2. **Impact of H_2SO_4 on impurity 4 levels.**

In conclusion, we have successfully identified a dimer impurity which was detected during scale-up of delafloxacin. Subsequent DoE experiments enabled us to identify the means to control this impurity to acceptable levels in small scale as well as in kilo laboratory runs.

Experimental Section

1-(6-Amino-3,5-difluoropyridin-2-yl)-8-chloro-6-fluoro-7-(3-hydroxyazetidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid, 3, Improved Procedure. To a suspension of **1** (3.1 kg, 6.15 mol) in methyl acetate (8.6 kg) was added a solution of H_2SO_4 (5.9 g, 62 mmol) and NCS (0.88 kg, 6.46 mol) in methyl acetate (14.4 kg) at 10−17 °C within 45 min. The solution was stirred at 13−19 °C for 2 h and quenched with 1.6% aqueous $NaHCO_3$ (12.6 kg), and the organic layer was washed with 11% aqueous Na_2SO_3 (7 kg). The methyl acetate solution was solvent exchanged to 2-propanol at 50 °C/vacuum; then a solution of KOH (1.1 kg, 19.7 mol) in water (24.8 kg) was added, and the mixture was stirred at 55 °C for 3 h. Aqueous acetic acid (13%, 2.6 kg) was added at 40 °C, and the solution was seeded with **3** (27 g, 61 mmol). The suspension was stirred for 1 h at 40 °C, and then aqueous acetic acid (13%, 11.7 kg) was slowly added. After stirring an additional hour at 40 °C, the suspension was cooled to room temperature, filtered, washed with water (41 kg), and dried at 60 °C/vacuum to yield **3** as yellow crystals (2.5 kg, 91%). Isolated **3** had the same spectroscopic properties as reported.[6]

3-Hydroxyazetidine-1-carboxylic Acid Benzyl Ester, 8. To a solution of azetidin-3-ol hydrochloride **7** (25 g, 0.23 mol) in water (150 mL) and THF (300 mL) was added K_2CO_3 (63.1 g, 0.46 mol). The mixture was stirred for 30 min. at 20−25 °C. Then benzyl chloroformate (40.9 g, 0.24 mol) was added within 30 min. at 0−5 °C followed by stirring the mixture overnight at 20−25 °C. THF was removed on a rotavap at 30 °C/vacuum and the mixture was extracted with ethyl acetate (2 × 150 mL). The combined organic layer was washed with water (1 × 50 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography on silica gel, eluting with ethyl acetate-heptanes 1:1 and 4:1 to yield **8** as a clear oil (47.3 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.72 (1H, d, J = 6.2 Hz), 3.85 (2H, dd, J = 9.5, 4.4 Hz), 4.17 (2H, dd, J = 9.5, 6.7 Hz), 4.49−4.57 (1H, m), 5.06 (2H, s), 7.31−7.38 (5H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 59.2, 61.6, 66.9, 127.9, 128.1, 128.5, 136.5, 156.6; IR: (film) 3406, 1686, 1438 cm^{-1}; ES-HRMS m/z: (M^+ + 1H) calcd for $C_{11}H_{14}NO_3$ 208.0968, found 208.0967.

3-Oxiranylmethoxyazetidine-1-carboxylic Acid Benzyl Ester, 9. To a solution of **8** (30 g, 0.15 mol) in DMSO (250 mL) was slowly added a solution of NaOH (9.9 g, 0.25 mol) in water (195 mL) at 15−25 °C. Epichlorohydrin (93.8 g, 1.01 mol) was added, and the mixture was stirred at 20−25 °C for

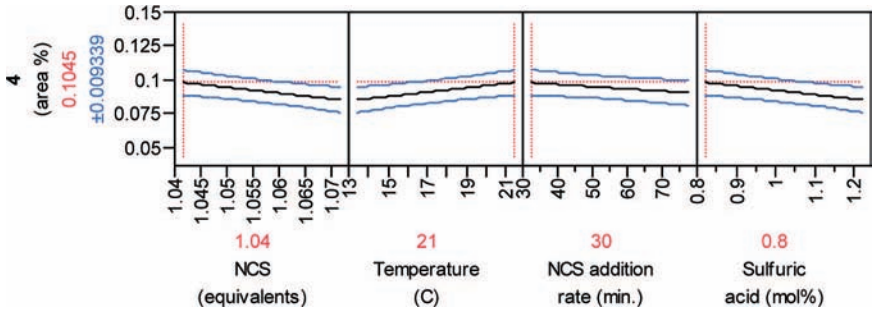


Figure 3. **Worst case scenario in prediction profiler for robustness DoE.**

24 h. The mixture was diluted with water (300 mL) and extracted with ethyl acetate (2 × 150 mL). The combined organic layer was washed with water (2 × 50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel, eluting with ethyl acetate/heptanes, 3:2, yielding **9** as a clear oil (32.1 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.60 (1H, dd, J = 4.8, 2.6 Hz), 2.81 (1H, dd, J = 4.9, 4.2 Hz), 3.09−3.16 (1H, m), 3.25 (1H, dd, J = 11.4, 6.2 Hz), 3.68 (1H, dd, J = 11.5, 2.5 Hz), 3.89−3.97 (2H, m), 4.15−4.24 (2H, m), 4.29−4.37 (1H, m), 5.09 (2H, s), 7.28−7.36 (5H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 44.2, 50.4, 56.7, 56.9, 66.7, 68.6, 70.0, 128.0, 128.1, 128.5, 136.6, 156.5; IR: (film) 2951, 1709, 1420 cm^{-1}; ES-HRMS *m/z*: (M^+ + 1H) calcd for $C_{14}H_{18}NO_4$ 264.1230, found 264.1230.

1-(6-Amino-3,5-difluoropyridin-2-yl)-7-[3-(1-benzyloxycarbonylazetidin-3-yloxy)-2-hydroxypropylamino]-8-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid Ethyl Ester, 11. A mixture of **9** (19 g, 72.2 mmol) in conc NH_4OH (380 mL) and 7 M NH_3 in MeOH (86 mL) was stirred for 5 h at room temperature. The clear solution was concentrated and azeotropically dried with toluene. The residual clear oil and **6** (20 g, 48.1 mmol) were dissolved in NMP (150 mL). *N*,*N*-Diisopropylethylamine (12.4 g, 96.2 mmol) was added, and the solution was stirred at 70 °C for 3 h. The solution was poured into 1 N citric acid/ice (300 mL) and extracted with ethyl acetate (2 × 150 mL). The combined organic layers were washed with water (2 × 100 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel, eluting with ethyl acetate/heptanes, 1:1 followed by ethyl acetate/MeOH, 95:5, yielding **11** as a yellow foam (27.1 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 (3H, t, J = 7.1 Hz), 3.35−3.52 (4H, m), 3.62−3.77 (1H, m), 3.84−3.91 (2H, m), 3.95−4.08 (1H, m), 4.15 (2H, dd, J = 9.3, 6.5 Hz), 4.23−4.30 (1H, m), 4.35 (2H, q, J = 7.1 Hz), 4.85−5.13 (3H, br s), 5.08 (2H, s), 7.18−7.25 (1H, m), 7.31−7.35 (5H, m), 7.99 (1H, dd, J = 13.7, 3.1 Hz), 8.31 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 48.5 (d, J_F = 10 Hz), 56.6, 61.1, 66.9, 68.6, 69.3, 70.8, 107.2, 111.5, 112.6 (d, J_F = 24 Hz), 113.2 (m), 120.6, 128.0, 128.1, 128.5, 134.1 (d, J_F = 5 Hz), 134.7 (m), 136.5, 139.2 (d, J_F = 13 Hz), 144.9 (d, J_F = 253 Hz), 144.4 (d, J_F = 13 Hz), 145.6 (dd, J_F = 262, 4 Hz), 149.9 (d, J_F = 246 Hz), 150.0, 156.5, 164.7, 172.9; IR: (KBr) 2949, 1700, 1615 cm^{-1}; ES-HRMS *m/z*: (M^+ + 1H) calcd for $C_{31}H_{30}ClF_3N_5O_7$ 676.1780, found 676.1762.

1-(6-Amino-3,5-difluoropyridin-2-yl)-7-[3-(azetidin-3-yloxy)-2-hydroxypropylamino]-8-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester, 12. To a slurry of 10% Pd on carbon (2.1 g) in MeOH (20 mL) was added a solution of **11** (13.7 g, 20.3 mmol) in MeOH (230 mL). The mixture was hydrogenated at 1 atm for 1 h, filtered over Hyflo, and evaporated yielding **12** as beige crystals (10.3 g, 93%). Mp 148−152 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.27 (3H, t, J = 7.1 Hz), 3.27 (1H, d, J = 5.0 Hz), 3.28−3.80 (10H, m), 4.19 (1H, br s), 4.21 (2H, q, J = 7.1 Hz), 5.86 (1H, s), 6.74 (2H, s), 7.84 (1H, d, J = 13.8 Hz), 7.94 (1H, dd, J = 9.7, 9.0 Hz), 8.43 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 48.4 (d, J_F = 10 Hz), 53.6, 60.2, 68.4, 70.4 (d, J_F = 4 Hz), 72.1, 106.4 (d, J_F = 6 Hz), 111.0, 111.3 (d, J_F = 23 Hz), 113.6 (dd, J_F = 23, 21 Hz), 118.9 (d, J_F = 6 Hz), 133.8 (d, J_F = 13 Hz), 134.2, 139.5 (d, J_F = 12 Hz), 143.3 (dd, J_F = 248, 4 Hz), 145.0 (dd, J_F = 259, 5 Hz), 145.6 (d, J_F = 14 Hz), 149.3 (d, J_F = 245 Hz), 149.5, 163.5, 171.0; IR: (KBr) 1697, 1614, 1496, 1457 cm^{-1}; ES-HRMS *m/z*: (M^+ + 1H) calcd for $C_{23}H_{24}ClF_3N_5O_5$ 542.1413, found 542.1391.

1-Amino-3-(azetidin-3-yloxy)-propan-2-ol-bis(*N*,*N*′-quinolone Diester), 13. A solution of **12** (9.6 g, 17.7 mmol), **6** (7.8 g, 18.6 mmol), and *N*,*N*-diisopropylethylamine (4.6 g, 35.4 mmol) in NMP (150 mL) was stirred at 55 °C for 3 h. The solution was poured into 1 N citric acid/ice (300 mL) and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with water (2 × 100 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel, eluting with ethyl acetate/MeOH, 95:5. The obtained yellow foam was crystallized with CH_2Cl_2/MeOH, 9:1 (160 mL), yielding **13** as beige crystals (11.8 g, 71%). Mp 184−187 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.26 (6H, t, J = 7.1 Hz), 3.29−3.48 (3H, m), 3.49−3.62 (1H, m), 3.73−3.82 (1H, m), 4.12−4.30 (3H, m), 4.21 (4H, q, J = 7.1 Hz), 4.52−4.65 (2H, m), 5.13−5.22 (1H, m), 5.83−5.92 (1H, m), 6.72 (4H, s), 7.73 (1H, d, J = 13.9 Hz), 7.82 (1H, d, J = 13.9 Hz), 7.92 (1H, t, J = 9.6 Hz), 7.93 (1H, t, J = 8.7 Hz), 8.41 (2H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.3 (2×), 46.4 (d, J_F = 11 Hz), 58.4 (2×), 61.9 (2×), 66.7, 67.3 (d, J_F = 4 Hz), 69.1, 103.4 (d, J_F = 6 Hz), 104.6 (d, J_F = 6 Hz), 108.7 (d, J_F = 23 Hz), 109.2, 109.4 (d, J_F = 23 Hz), 109.5, 111.7 (dd, J_F = 25, 24 Hz), 111.8 (dd, J_F = 25, 24 Hz), 117.1 (d, J_F = 7 Hz), 117.8 (d, J_F = 6 Hz), 132.1 (dd, J_F = 17, 4 Hz), 132.2, 132.5, 133.5, 137.7 (d, J_F = 12 Hz), 139.4 (d, J_F = 12 Hz), 141.0 (dd, J_F = 247, 5 Hz), 141.5 (dd, J_F = 248, 5 Hz), 143.0 (dd, J_F = 259, 5 Hz), 143.3 (dd, J_F = 259, 5 Hz), 143.8 (2×), d, J_F = 15 Hz), 147.5 (d, J_F = 245 Hz), 147.7, 147.8, 148.1 (d, J_F = 247 Hz), 161.7 (2×), 169.1, 169.2; IR:

(KBr) 1728, 1615, 1491, 1448 cm^{-1}; ES-HRMS *m*/*z*: (M^+ + 1H) calcd for $C_{40}H_{33}Cl_2F_6N_8O_8$ 937.1697, found 937.1696.

1-Amino-3-(azetidin-3-yloxy)-propan-2-ol-bis(*N*,*N*′-quinolone Carboxylic Acid), 4. To a suspension of **13** (17.0 g, 18.1 mmol) in 2-propanol (75 mL) was added a 1 N KOH solution (127 mL, 126.7 mmol). After stirring the mixture at 55 °C for 3.5 h, the solution was cooled to 30 °C, and a solution of AcOH (12.4 g, 206.5 mmol) dissolved in water (94 mL) was added within 1 h. The suspension was stirred at room temperature for 2 h, filtered, washed with water (3 × 40 mL), and dried at 50 °C/vacuum, yielding **4** as yellow crystals (15.7 g, 98%). Mp 198−205 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.28−3.45 (2H, m), 3.45−3.78 (2H, m), 3.79−3.88 (1H, m), 4.16−4.33 (3H, m), 4.61−4.75 (2H, m), 5.25 (1H, br s), 6.23−6.35 (1H, m), 6.76 (4H, s), 7.79 (1H, d, J = 13.7 Hz), 7.90 (1H, d, J = 13.8 Hz), 7.93 (2H, dd, J = 9.7, 2.4 Hz), 8.70 (1H, s), 8.71 (1H, s), 14.59 (2H, br s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 48.1 (d, J_F = 11 Hz), 63.8, 68.4, 69.0 (d, J_F = 5 Hz), 70.6 (d, J_F = 6 Hz), 104.5 (d, J_F = 6 Hz), 105.9 (d, J_F = 7 Hz), 107.8, 108.2, 109.8 (d, J_F = 23 Hz), 110.8 (d, J_F = 23 Hz), 113.4 (d, J_F = 23 Hz), 113.7 (d, J_F = 23 Hz), 115.8 (d, J_F = 8 Hz), 116.6 (d, J_F = 8 Hz), 133.3 (dd, J_F = 14, 3 Hz), 133.5 (dd, J_F = 14, 4 Hz), 134.8, 135.9, 141.0 (d, J_F = 12 Hz), 142.1 (d, J_F = 12 Hz), 142.8 (dd, J_F = 249, 5 Hz), 143.3 (dd, J_F = 249, 5 Hz), 145.1 (dd, J_F = 259, 5 Hz), 145.4 (dd, J_F = 260, 5 Hz), 145.6 (2×, d, J_F = 15 Hz), 149.5 (d, J_F = 248 Hz), 150.1 (2×), 150.2 (d, J_F = 249 Hz), 164.7, 164.8, 175.8 (d, J_F = 3 Hz), 175.9 (d, J_F = 3 Hz); IR: (KBr) 1727, 1622, 1489, 1439 cm^{-1}; ES-HRMS *m*/*z*: (M^+ + 1H) calcd for $C_{36}H_{25}Cl_2F_6N_8O_8$ 881.1071, found 881.1090.

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Supporting Information Available

Experimental tables and analyses of the DoE studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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