

Synthesis of Novel Antibacterial Agents Targeting Bacterial Topoisomerases: 8-Methyl & 8-Desmethyl Fluoroquinolones

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Introduction

Bacterial resistance to currently available antimicrobial agents continues to be a growing threat to public health. The CDC recently reported that each year in the United States at least 2 million people acquire serious infections caused by bacteria resistant to one or more antibacterial agents, with 23,000 of them dying as a direct result.¹ Many more die from underlying medical conditions that are exacerbated by these difficult-to-treat infections. Multidrug-resistant (MDR) strains of the so-called 'ESKAPE' pathogens are of particular concern because of their association with considerable morbidity and mortality in the hospital setting.² The Gram-negative 'KAPE' organisms – *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp. – are especially problematic because of the dearth of new and effective agents found in the existing clinical development pipeline. The recent emergence of infections caused by Gram-negative pathogens such as carbapenem-resistant Enterobacteriaceae (CRE) has further increased the magnitude of the problem.¹

The fluoroquinolones (FQs), exemplified by moxifloxacin (*vide infra*), are generally broad-spectrum antibacterial agents that have been on the market for many years and have been useful, at least in part, in treating Gram-negative infections.³ The FQs target bacterial DNA gyrase (A subunit)⁴ and topoisomerase IV (C subunit), tetrameric enzymes that can now be considered as clinically validated.

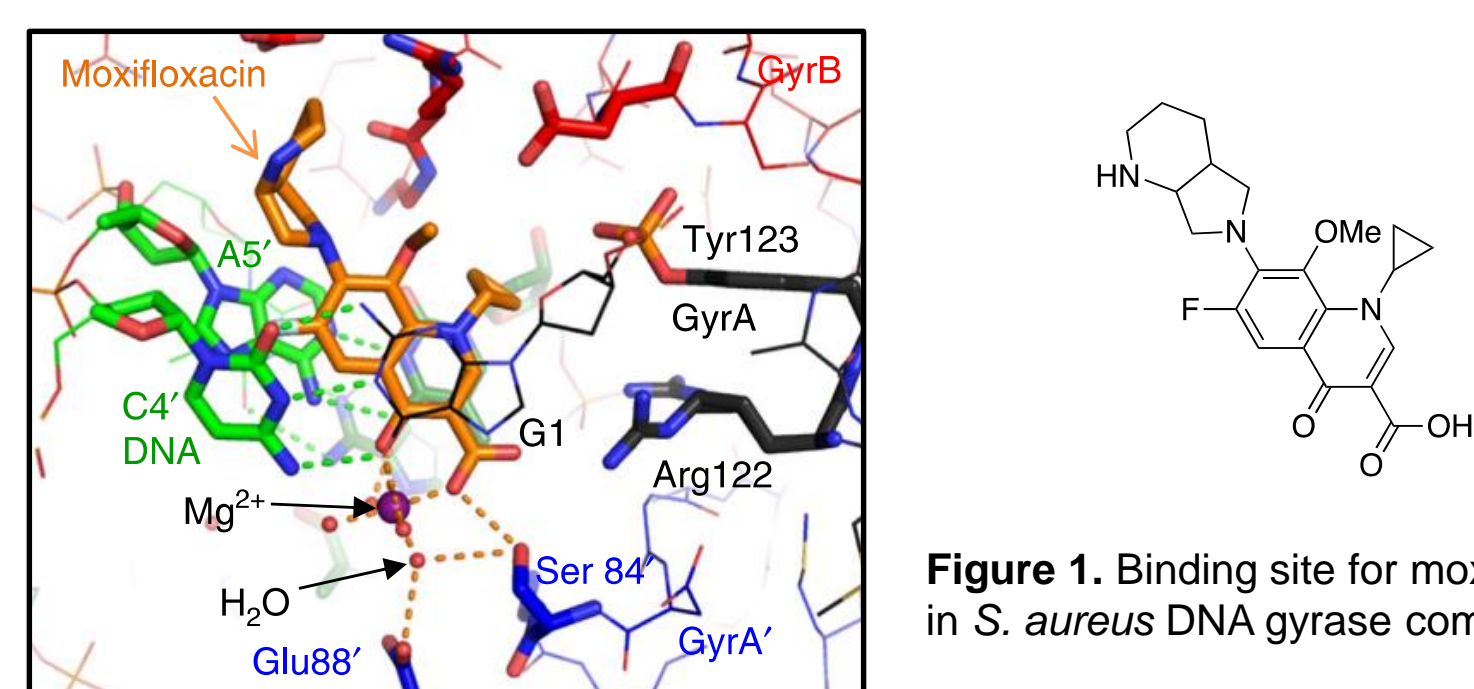
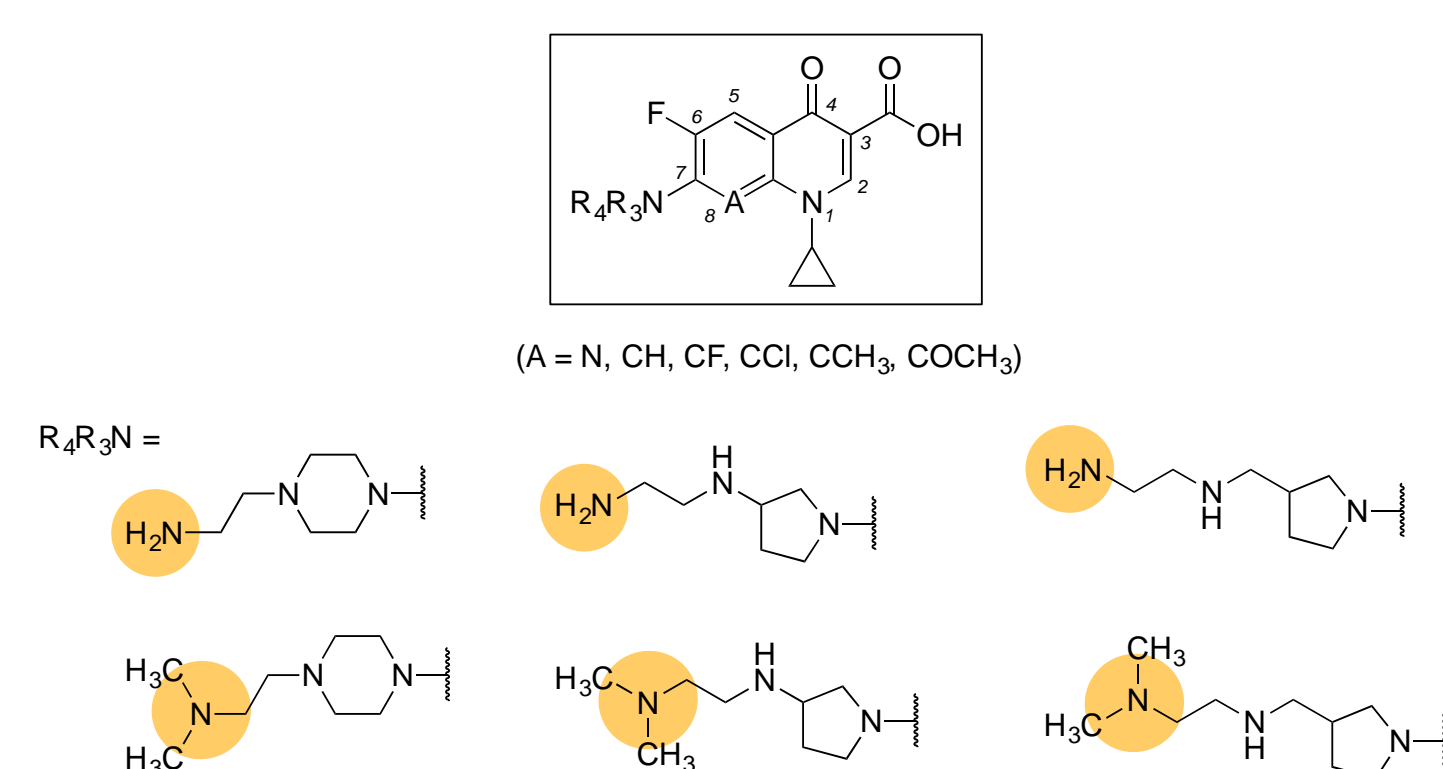


Figure 1. Binding site for moxifloxacin in *S. aureus* DNA gyrase complex.⁴

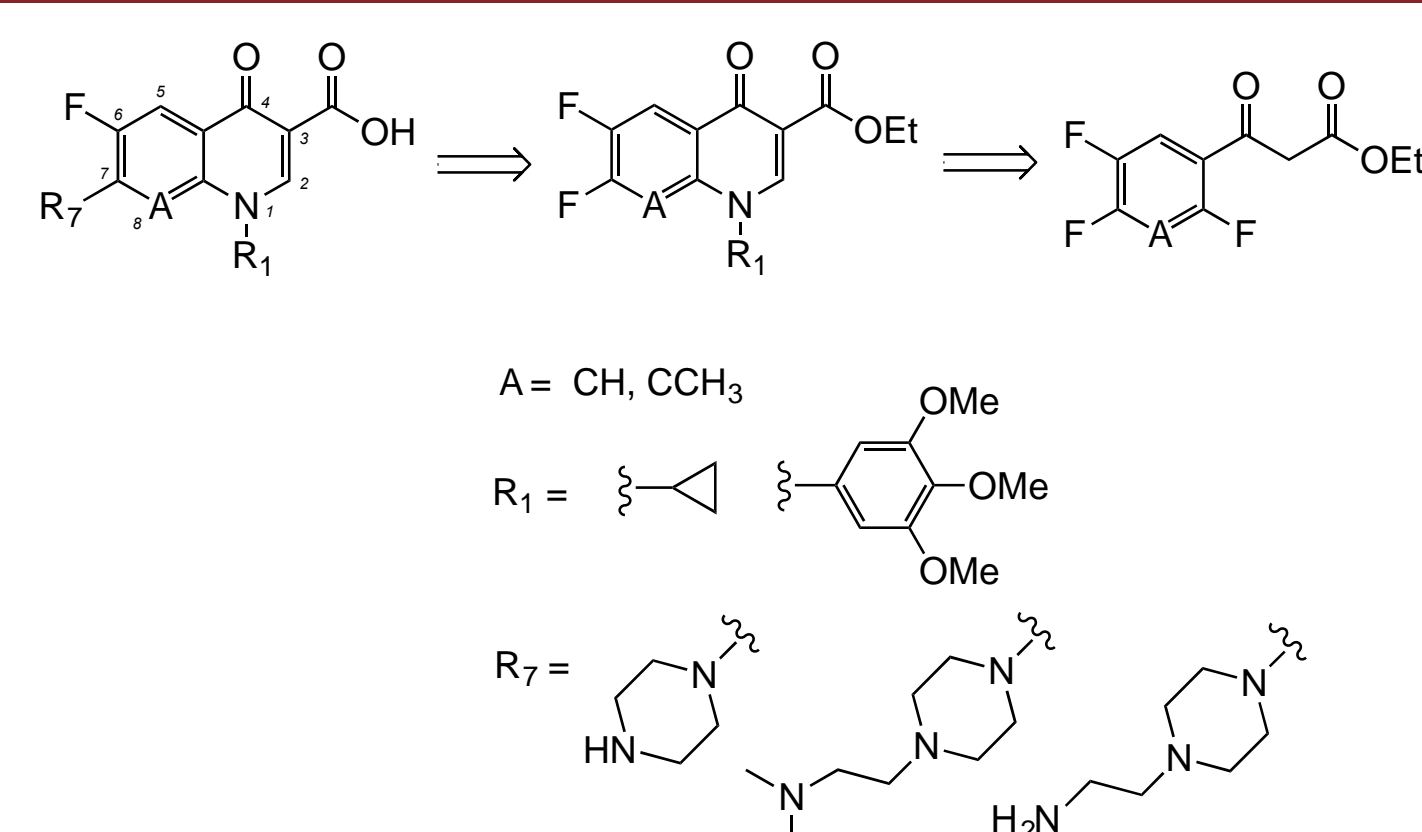
One way to potentiate the activity of antibiotics against Gram-negative bacteria is to incorporate additional basic amino groups. At physiologic pH, such groups are generally protonated. The resulting quaternary ammonium salts enhance penetration of the Gram-negative outer membrane. Polymyxin B is an exemplar in this area. We hypothesized that the Gram-negative activity of fluoroquinolones would be further enhanced by appending an additional basic amino group, highlighted below, to the usual C-7 diamine substituent.



Principal Objectives

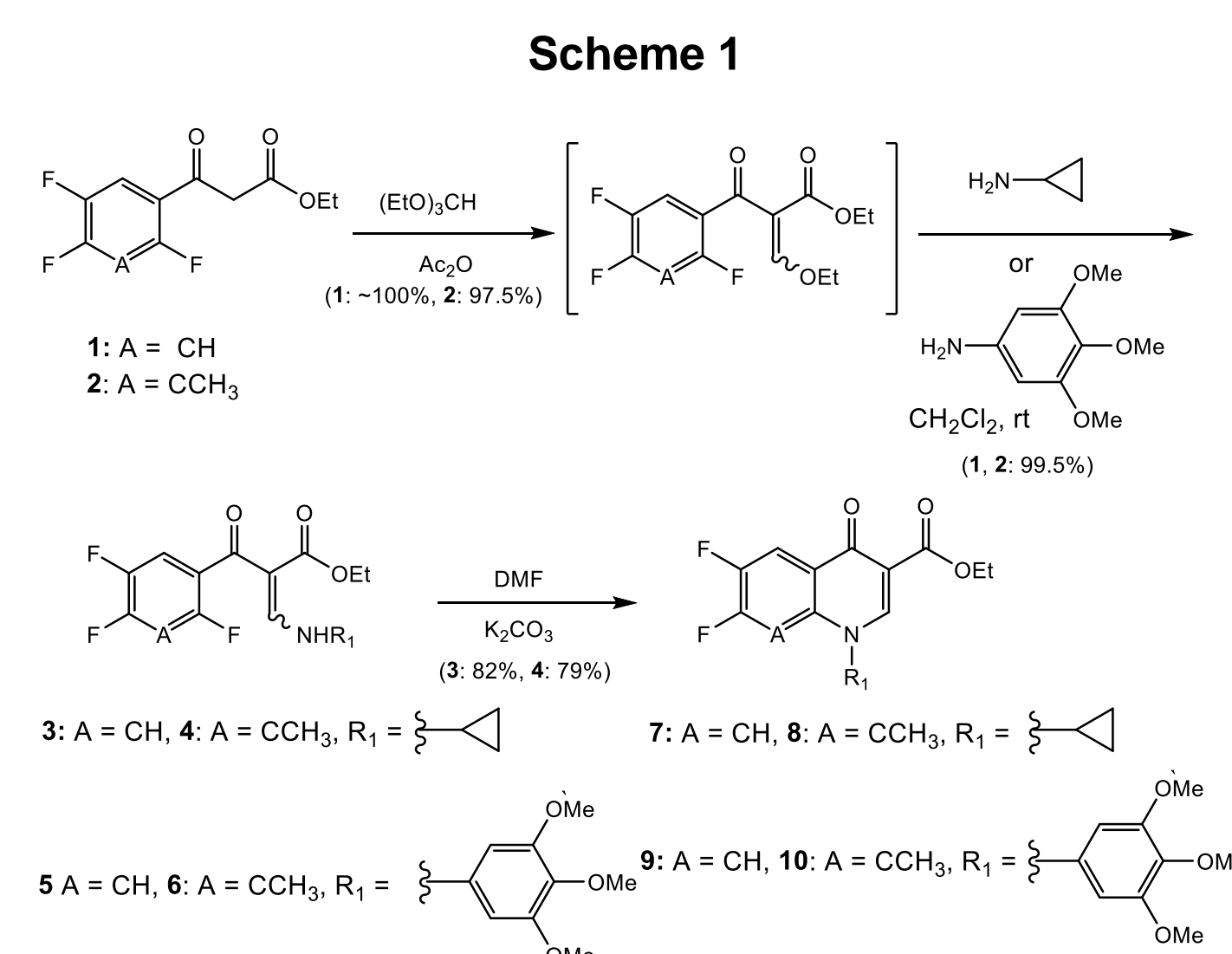
- Generate adequate supplies of key N-1 substituted 6,7-difluoroquinolone ester intermediates to explore the subsequent nucleophilic aromatic substitution reaction
- Identify the best method for introducing the desired triamino substituents to the C-7 position
 - Direct amination with a triamine fragment
 - Aminoalkylation of a diamine-substituted quinolone
- Begin exploring compounds bearing a 3,4,5-trimethoxyphenyl moiety at N-1
- Purify and characterize compounds for testing at Walter Reed Army Institute of Research

Retrosynthetic Analysis



Results: Synthesis of Quinolone Ester Intermediates

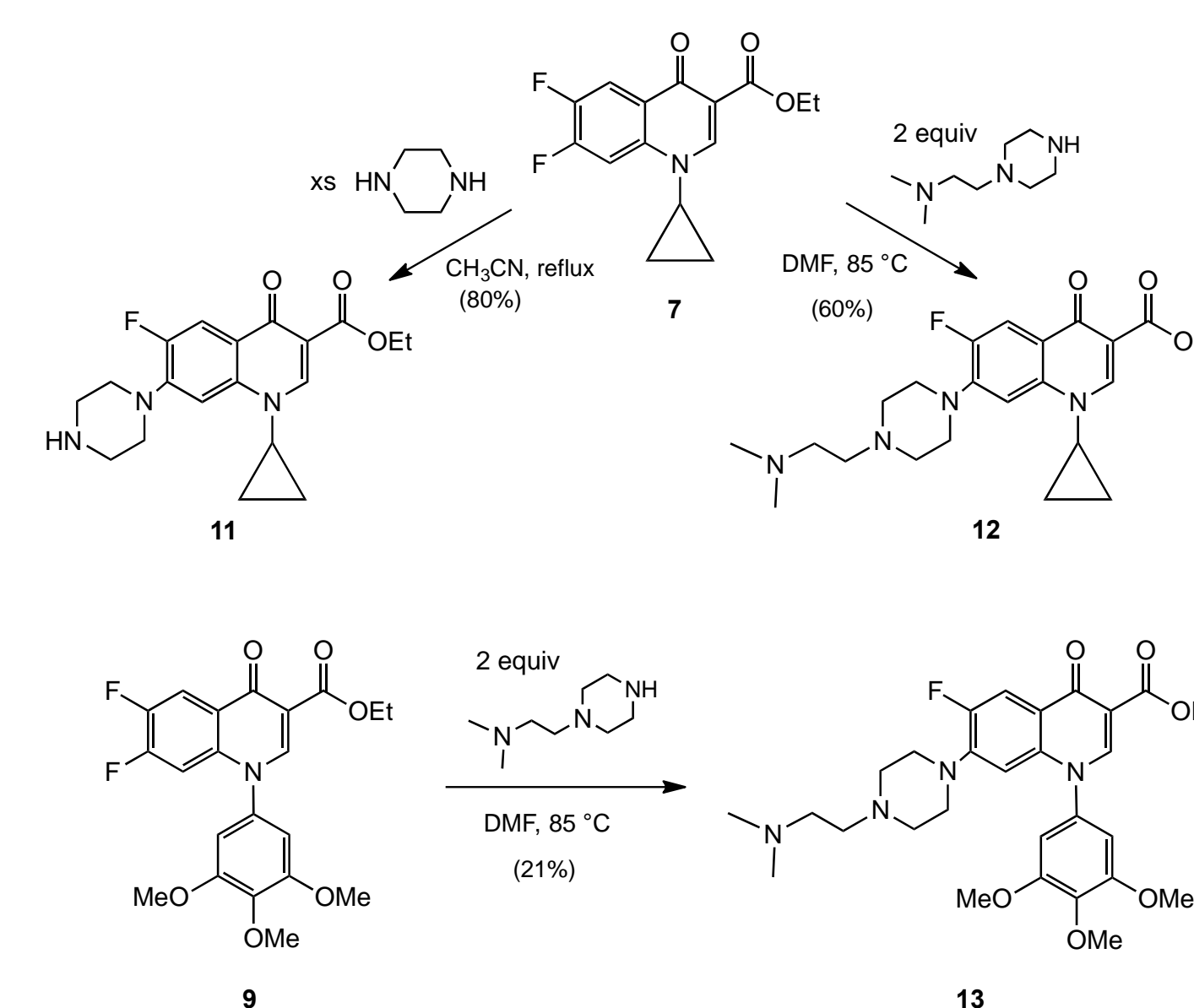
Ethyl (2,4,5-trifluorobenzoyl)acetate (1) or ethyl (2,4,5-trifluoro-3-methylbenzoyl)acetate (2) was used as the starting material. Conversion to the ethoxymethylene derivative with triethylorthoformate and acetic anhydride, followed by the addition of cyclopropyl amine or 3,4,5-trimethoxyaniline, afforded the corresponding enamine products 3 or 4 and 5 or 6, respectively, in good yields (Scheme 1). Base-induced cyclization then provided the key 6,7-difluoroquinolone ester intermediates 7-10.



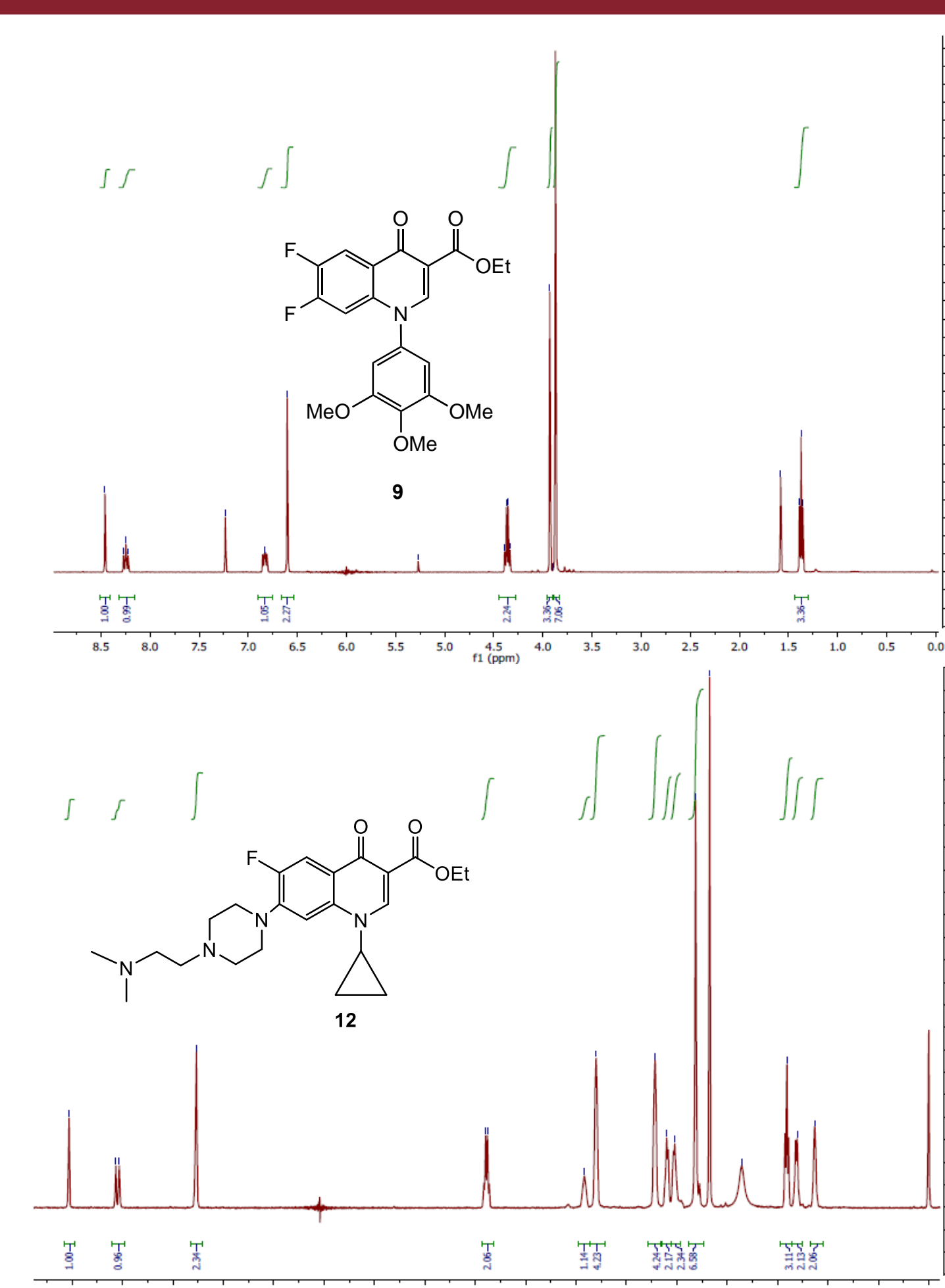
Results: C-7 Nucleophilic Aromatic Substitution

With quinolone esters 7, 8, 9 and 10 in hand, the stage was set for exploring the nucleophilic aromatic substitution reaction at C-7, with a wide range of amine nucleophiles within scope. We initially examined the reaction of quinolone ester 7 with nucleophilic partners piperazine, 1-[2-(dimethylamino)ethyl]piperazine and Boc-pyrrolidine (not shown). The indicated C-7 substituted quinolone ester products 11 and 12 were obtained in good isolated yields (Scheme 2). Similarly, intermediate ester 9 was reacted with 1-[2-(dimethylamino)ethyl]piperazine to afford the adduct 13 in modest yield (unoptimized).

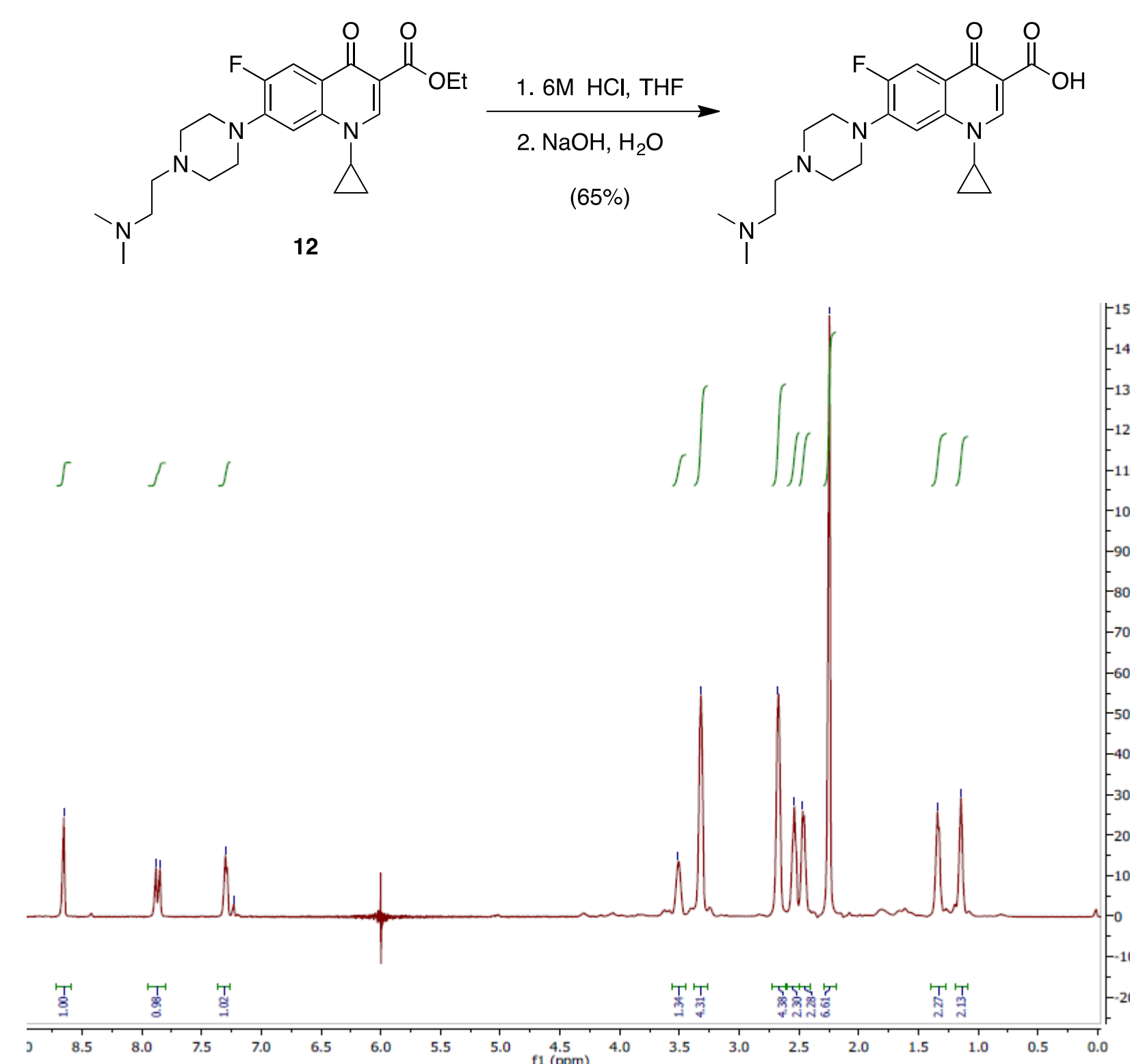
Scheme 2



Representative NMR Spectra of Quinolone Esters



Results: Ester Hydrolysis and NMR Spectrum of Fluoroquinolone Final Product



Conclusions

- N-1 cyclopropyl and 3,4,5-trimethoxyphenyl analogs were synthesized and characterized.
- Initial microbiological assessment of the final products will be done at the Walter Reed Army Institute of Research (WRAIR).
- Structure-activity relationships (SAR) in this series will be evaluated once minimum inhibitory concentration (MIC) data is available.

References

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2. Pendleton, J.N.; Gorman, S.P.; Gilmore, B.F. *Expert Rev. Anti Infect. Ther.* **2013**, *11*, 297-308.
3. Van Bambeke, F.; Michot, J.-M.; Van Eldere, J.; Tulkens, P.M. *Clin Microbiol Infect.* **2005**, *11*, 256-80.
4. Chan, P. F.; et al. *Nat. Commun.* **2015**, *6*, 10048.

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