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Communication

Quinolone Natural Products

The Synthesis of Quinolone Natural Products from *Pseudonocardia* sp.

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Abstract: The synthesis of four quinolone natural products from the actinomycete *Pseudonocardia* sp. is reported. The key step involved a sp^2–sp^3 Suzuki–Miyaura reaction between a common boronic ester lateral chain and various functionalised quinolone cores. The quinolones slowed growth of *E. coli* and *S. aureus* by inducing extended lag phases.

Introduction

Quinolone natural products have been noted for their biological activities since the 1950s, and in the 1980s the introduction of synthetic fluoroquinolones provided potent new antibiotics.[1] More recently, quinolones have been found to play a role in bacterial cell-cell signalling, known as quorum sensing (QS), in the pathogen *Pseudomonas aeruginosa*[2] which produces over 50 quinolones.[3] Since this discovery it has become apparent that other bacterial species including *Burkholderia* and *Altermonas* spp. also produce quinolones.[4] Such findings have led to the hypothesis that in the bacterial world quinolones may play an underlying role in gene regulation and inter-species modulation in complex multi-bacterial communities.[5,6] Intriguingly, both *Burkholderia* sp. and *P. aeruginosa* which can be found in the natural environment, including soil and water sources, are major pathogens in cystic fibrosis patients and form mixed biofilms.[7] Quinolones produced by *P. aeruginosa* have also been found to influence a number of phenotypes in other bacterial species.[6]

Considering this role, and our research interests involving quinolone signalling and bacterial signaling,[8] we became interested in the natural quinolones produced by the actinomycete *Pseudonocardia* sp. CL38489, isolated from a soil sample in India by Dekker et al.[9] (Figure 1). A number of these quinolone natural products were originally noted for their potent antibacterial activity against the Gram-negative bacteria *Helicobacter pylori*, which is implicated in pathogenesis of chronic gastritis, peptic ulcers and gastric cancers.[9] Quinolones **1**–**4** also bear structural resemblance to a number of natural alkyl-substituted quinolones produced by *P. aeruginosa* and *Burkholderia* spp.[3,4]

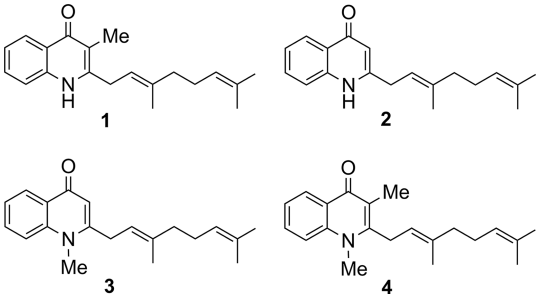
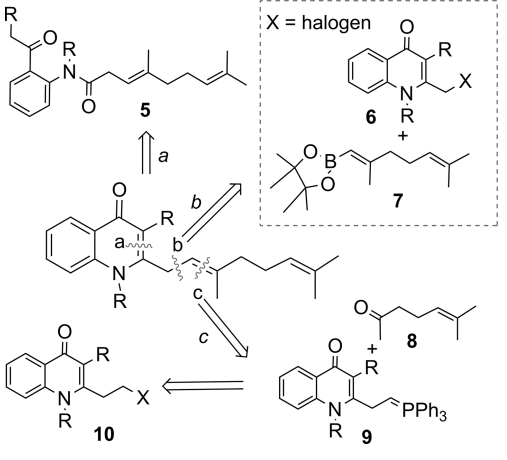


Figure 1. Four quinolone natural products isolated from *Pseudonocardia* sp.[9]

We envisaged a divergent total synthesis[10] that will be efficient for the production of quinolone natural products **1**–**4** for further biological evaluation of their effects on QS, inter-species signaling, and antibacterial activities. The first total synthesis of **1** was recently reported by Abe et al.[11] when synthetic efforts within our group was also aiming at these natural products.



Scheme 1. Retrosynthetic analysis of quinolones **1**–**4**. Disconnection b proved successful.

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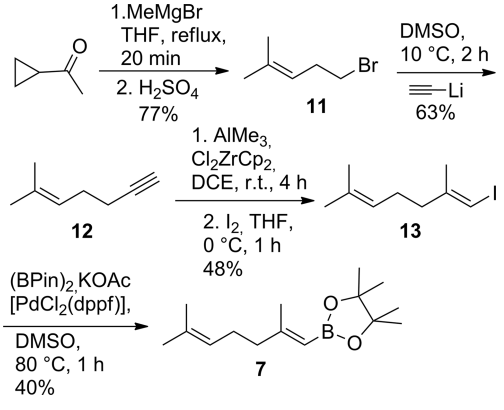
Our retrosynthetic analysis of quinolones **1**–**4**, similarly to Abe et al., incorporated three main disconnection strategies (a, b and c, Scheme 1). Disconnection (a) involved a final intramolecular cyclisation step with **5** to set up the quinolone core. Disconnection (b) would require the preparation of halogen functionalised quinolone building blocks **6** and a common boronic ester lateral chain **7** that would be combined via palladium sp^2–sp^3 coupling chemistry. Cross-couplings of this sort are notoriously challenging; however, this would enable a very expedient and modular route towards the quinolone natural products that should prove highly amenable to analogue preparation. Disconnection (c) required quinolone building blocks of the type **9** and the commercially available ketone **8**, which could be converted into the final quinolone natural products utilizing Wittig chemistry.

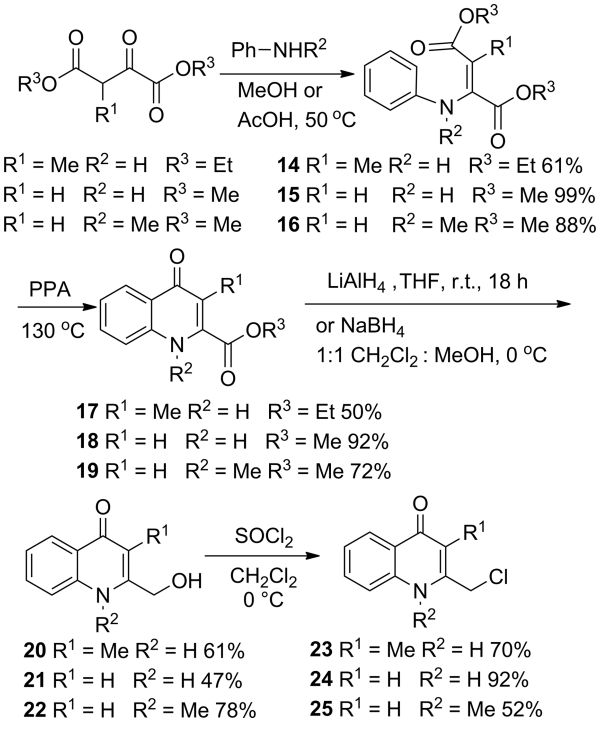
Results and Discussion

Initial studies focused upon the exploration of disconnection strategies (a) and (c) (Scheme 1). Unfortunately, these both proved to be unsatisfactory. Under various cyclisation conditions with intermediate **5** only the undesired pyridone regiosiomer could be detected and isolated. Preparation of the quinolone intermediate **10** was very low yielding as upon formation of **10** elimination of the halogen readily occurred to give the undesired alkene substituted quinolone.

Attempts to perform Wittig chemistry between **8** and **9** resulted in complex mixtures, with no detected desired product. Attention thus shifted towards disconnection strategy (b), which was initially regarded as being the more challenging of the three proposed options. 5-Bromo-2-methyl-pent-2-ene **11** which could be purchased, or readily prepared by literature procedure,[12] served as the starting material for the synthesis of the lateral chain **7** (Scheme 2). Compound **11** was subjected to an S_N2 reaction with lithium acetylide to afford the alkyne **12**.[13] Zr-catalysed carboalumination followed by reaction with iodine generated compound **13** as a single stereoisomer in modest yield.[14] The last step was the formation of the pinacolboronate **7** via the Miyaura borylation reaction, a single stereoisomer was isolated and the desired (*E*) geometry was confirmed by NOESY analysis. For the synthesis of the quinolone building blocks for natural products **1**–**3**, esters **17**–**19** were synthesized in a two-step sequence, utilizing the Conrad–Limpach method in good to moderate yields (Scheme 3).[15] The subsequent reduction of esters **17**–**19** in the presence of lithium aluminium hydride or sodium borohydride afforded the alcohols **20**–**22** in good yields. Lithium aluminum hydride proved to be a non-chemo selective reducing agent in the case of *N*-methyl-substituted quinolone **19**, with reduction of both the quinolone core and the ester group observed. Substitution of the hydroxy group of **20**–**22** by chlorination with thionyl chloride led to the formation of the halide substituted quinolone cores **23**–**25** in good yields. Unfortunately, the dimethyl-substituted quinolone core **29**, required for quinolone natural product **4**, could only be prepared in poor yields using the Conrad–Limpach reaction that was utilized for the formation of esters **17**–**19**.



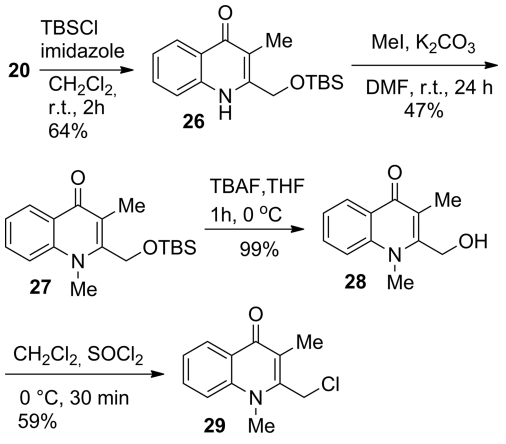
Scheme 2. Preparation of common boronic ester lateral chain.



Scheme 3. Preparation of quinolone cores for natural products **1**–**3**. For full experimental details see the Supporting Information.

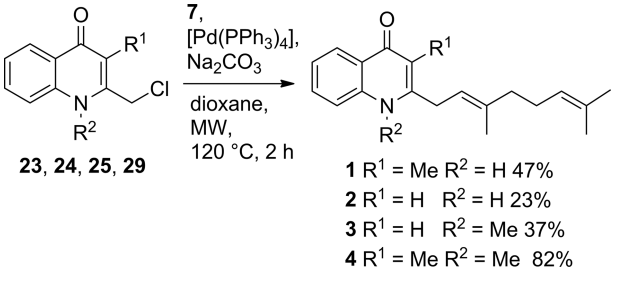
Thus, an alternative strategy was employed for the synthesis of **4**, based upon the use of compound **20**. The hydroxy group of **20** was protected with TBDMS-Cl to afford the desired silyl ether in good yield (Scheme 4). Methylation of the nitrogen resulted in the formation of **27** in moderate yield; the *O*-methylated regioisomer of **27** was also detected as a side product. Deprotection of **27** using TBAF and chlorination using thionyl chloride afforded **29** in good yield.

With the halide substituted quinolones **23**, **24**, **25**, **29** and pinacolborate **7** in hand, we were ready to attempt the key Suzuki–Miyaura coupling reaction (Scheme 5). Pleasingly, microwave irradiation of a suspension of **7**, quinolone **23**, **24**, **25** or **29**, sodium carbonate and tetrakis(triphenylphosphine)palladium(0) in 1,4-dioxane led to the formation of desired products **1**–**4** respectively in good to moderate yields, aside from **2** (final



Scheme 4. Preparation of dimethyl-substituted quinolone core.

purification of **2** for biological testing proved particularly challenging), however significant quantities were obtained for biological studies. The four natural products **1**–**4** were obtained with high levels of purity and stereoselectivity (≥ 99 % *E*-selectivity; the *E*-geometry of the double bond was confirmed by NOESY analysis). The bromine analogues of quinolones **23**, **24**, **25** and **29** were also prepared however there was no significant improvement in yield of the final coupling step compared to the chlorine substituted quinolones.



Scheme 5. Preparation of quinolone natural products **1**–**4**.

With the natural products in hand we next sought to investigate their effects on quinolone signaling on other bacterial species. Our initial assay involved focusing on PqsR (also known as MvfR), the receptor protein utilized by *P. aeruginosa* for quinolone mediated signalling, in a heterologous *E. coli* system.[16,8b] The natural products **1**–**4** failed to modulate PqsR dependent transcriptional activity in any agonistic or antagonistic fashion (data not shown). However, it cannot be ruled out that other phenotypes independent of PqsR could be modulated by analogues **1**–**4**, as *Burklhoderia* and *Altermonas* spp produce quinolones but no PqsR homologue has been identified to date. Inspired by the antibacterial activities of compounds **1**–**4** against *Helicobacter pylori*, we decided to screen the natural products to see if they had any noticeable bioactivity in a range of bacterial species. We initially screened four bacterial species; *Pseudomonas aeruginosa* wild-type strain PA01, *Staphylococcus*

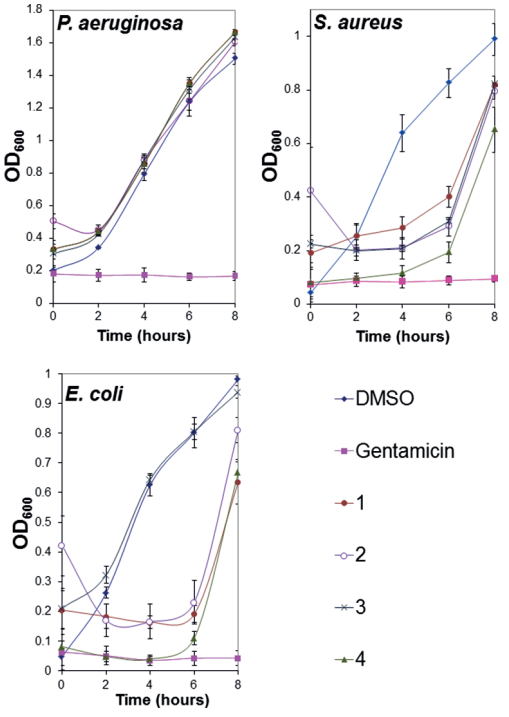


Figure 2. Growth profile of bacterial species in presence of natural products 1–4. Error bars represent standard deviation of triplicate cultures. As a control growth was also measured in the presence of solvent DMSO alone and in the presence of a known antibiotic gentamicin. The compounds were tested at 200 μM.

aureus 25923, *Escherichia coli* ESS (Figure 2) and *Serratia marcescens* 274 (data not shown). The growth of *P. aeruginosa* and *S. marcescens* were not affected by the presence of the natural products. Intriguingly, there was a large effect on the growth of the *E. coli* and *S. aureus* strains;[17] however this effect was notably different to the gentamicin control (which prevented growth of all the species tested).

In *S. aureus* cultures, all four natural products caused a marked delay in the onset of growth. A similar effect was observed in *E. coli*, although natural product **3** was inactive. This observed delay in the onset of growth resembles an extended lag phase in the growth profile of the bacteria. Thus, quinolones **1**–**4** are slowing growth (decreased OD_{600} values for the first 6 hours of growth compared to the DMSO control) rather than directly inhibiting growth (bacteriostatic antibiotic) or killing the bacteria (bacteriolytic antibiotic). In the case of a bacteriolytic or bacteriostatic antibiotic, similar to the gentamicin control, growth would be completely inhibited over the 8 hour period. Toyofuku and co-workers have reported that the structurally related signalling molecule utilized by *P. aeruginosa* [2-heptyl-3-hydroxy-4(1*H*)-quinolone] also known as the *Pseudomonas* Quinolone Signal (PQS), also effects the growth of several Gram-negative and Gram-positive bacterial species by slowing growth and inducing an extended lag phase.[18] Although the underlying biochemical basis for this induced extended lag phase is not known, it is tempting to speculate that the quinolones may be interfering with the electron transport chain and affecting cellular respiration, as has previously been reported for other quinolone natural products.[19] Previous

studies have also shown that the uptake of hydrophobic quinolone antibiotics by *S. aureus* and *E. coli* occurs by passive diffusion, whereas uptake by *P. aeruginosa* was found to be more complex.[20] An inadequate uptake may explain why *P. aeruginosa* showed to be insensitive to the tested natural products. One hypothesis for the observed growth defect by the natural product quinolones such as **1**–**4**, PQS and possibly other natural quinolones produced by bacteria, is that such quinolones could be functioning in multi-bacterial communities in the natural environment to perturb the growth of competing bacterial species.

Conclusions

In conclusion we have developed an efficient and direct synthetic route to four quinolone natural products **1**–**4** produced by the actinomycete *Pseudonocardia* sp. The route involved a traditionally challenging sp^2–sp^3 Suzuki step between various functionalised quinolone cores and a common lateral chain, which proceeded in high stereoselectivity. The quinolone core building blocks and lateral chain could be readily prepared from easily accessible starting materials (longest linear sequence of four steps). It was established that the natural products **1**–**4** do not interfere with signalling by the quinolone receptor protein PqsR of *P. aeruginosa*. However, similar to PQS the natural products were found to slow the growth of other bacterial species, including *E. coli* and *S. aureus*. Further studies of such quinolones and their biological activities in bacterial species should lead to a greater understanding of inter-species signaling in the natural environment and potential novel antibacterial agents.

Experimental Section

Supporting Information (see footnote on the first page of this article): Full experimental protocols, characterisation data, and ^1H and ^{13}C NMR spectra.

Acknowledgments

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Keywords: Synthetic methods · Cross-coupling · C–C coupling · Nitrogen heterocycles · Natural products · Antibiotics

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