

Microwave and flow syntheses of *Pseudomonas* quinolone signal (PQS) and analogues†James T. Hodgkinson,^a Warren R. J. D. Galloway,^a Shreya Saraf,^a Ian R. Baxendale,^a Steven V. Ley,^a Mark Ladlow,^b Martin Welch^c and David R. Spring^{*a}

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Expedient syntheses of *Pseudomonas* quinolone signal (PQS) and related structural analogues using microwave and flow methods are reported.

Quorum sensing is the intercellular signalling mechanism, mediated by small molecules, that numerous species of bacteria use to co-ordinate their gene expression in a cell-density dependent manner.¹ Several clinically relevant pathogens use quorum sensing systems to regulate processes associated with virulence. However, quorum sensing is not directly involved in biological processes essential for bacterial survival.² Thus selective disruption of quorum sensing represents a strategy to attenuate bacterial pathogenicity without imposing an intense selective pressure for the development of resistant mutants.³ Recent years have witnessed significant efforts towards identifying small molecules that can modulate quorum sensing pathways, with either agonist or antagonist activity, and thereby enhance understanding of this form of intercellular communication.⁴

It has emerged that some 2-alkyl-4-quinolone derivatives, most notably 2-heptyl-3-hydroxy-4(1*H*)-quinolone (*Pseudomonas* quinolone signal, PQS), serve as signalling molecules in the quorum sensing of *Pseudomonas aeruginosa* (Fig. 1).^{5,6} This bacterium is associated with a range of life-threatening nosocomial infections and is the primary cause of mortality in cystic fibrosis (CF) patients.⁷

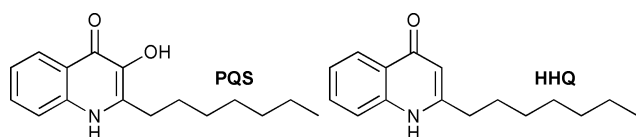
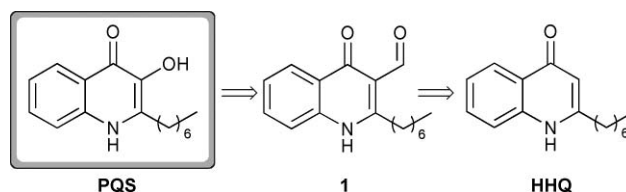


Fig. 1 The structure of PQS together with its biosynthetic precursor 2-heptyl-4-quinolone (HHQ); both have been shown to be involved in the quorum sensing of *P. aeruginosa*.⁶

PQS has been implicated as an important factor in the virulence of *P. aeruginosa*. For example, PQS is known to control the production of multiple virulence determinants⁸ and the molecule has also been reported in the sputa of CF patients, indicating that it may play a central role in the establishment of chronic infections.^{9,10} Despite the biological importance of PQS, synthetic approaches towards PQS and related analogues are much less numerous than those for other quorum sensing molecules, such as the widely studied *N*-acyl-L-homoserine lactones (AHLs).¹¹ Consequently there is a relative paucity of information regarding structure–activity relationships (SAR) exhibited by these quinolone-based small molecules in bacterial quorum sensing systems.¹² Such data should facilitate our understanding of quorum sensing in general and consequently enhance our ability to manipulate such systems in a useful fashion. PQS analogues may prove to be valuable tools for chemical biology studies and could potentially be used in a therapeutic context for the treatment of human bacterial infections.

Recently we reported the first systematic SAR analysis of PQS.¹⁰ Herein we describe how the analogues examined in that study were generated. The synthetic route centres around a novel one-pot microwave-assisted procedure that allows expedient access to PQS and a range of other 2-alkyl-4-quinolone derivatives from readily available starting materials. In addition, we report the successful adaptation and optimisation of these microwave-based conditions for use in a continuous flow reaction, which has enabled the synthesis of PQS on a multi-gram scale.

The majority of PQS syntheses described in the literature are based around a method developed by Pesci *et al.* which involves 2-heptylquinoline (HHQ) as a key intermediate (Scheme 1).⁵ A Duff reaction is used to convert HHQ to **1** and subsequent Dakin oxidation furnishes PQS. However, there are several drawbacks associated with this procedure. HHQ itself is not commercially available and methods for its synthesis are somewhat lengthy.¹³ In addition, we have found both the Duff formylation and Dakin



Scheme 1 Synthesis of PQS by Pesci *et al.*⁵ proceeds via HHQ.

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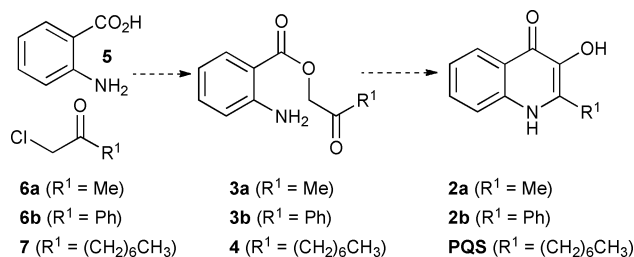
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† Electronic supplementary information (ESI) available: Full experimental details and analytical data for all novel compounds. See DOI: 10.1039/c0ob00652a

oxidation steps to be capricious, which significantly detracts from the efficiency of the synthesis.

Hradil *et al.* have previously reported a two-step procedure for the synthesis of quinolinones **2** ($R^1 = \text{Me}$ or Ph) by the cyclisation of phenacyl anthranilates **3** derived from the reaction of anthranilic acids **5** and α -chloro ketones **6** (Scheme 2).¹⁴ It was envisaged that this method could be adapted for the synthesis of PQS ($R^1 = (\text{CH}_2)_6\text{CH}_3$), which would require the use of anthranilic acid (**5**) and α -chloro ketone **7**.¹⁵



Scheme 2 Outline of proposed synthetic route towards PQS based on methodology developed by Hradil *et al.*¹¹ for the synthesis of quinolinones **2**.¹²

Towards this end we developed a high-yielding, one-step method for the synthesis of α -chloro ketone **7** starting from readily available 2-chloro-*N*-methoxy-*N*-methylacetamide **8** and heptyl Grignard reagent **9** (Scheme 3).¹⁶ In addition, we theorised that acylation of anthranilic acid and subsequent cyclisation could be carried out without isolation of the intermediate ester derivative **4**.

Extensive optimisation studies culminated in the development of a highly efficient one-pot reaction protocol for the synthesis of PQS from commercially available anthranilic acid and readily synthesised α -chloro ketone **7** under microwave irradiation conditions (Scheme 3). In line with previous studies on the formation of related 3-amino systems, we propose that this one-pot process proceeds *via* a tandem $\text{S}_\text{N}2$, enamine formation, nucleophilic rearrangement sequence (Scheme 3).¹⁷ Upon completion of the

reaction, the desired product could be obtained in high yield simply by precipitation induced by the addition of water.¹⁸

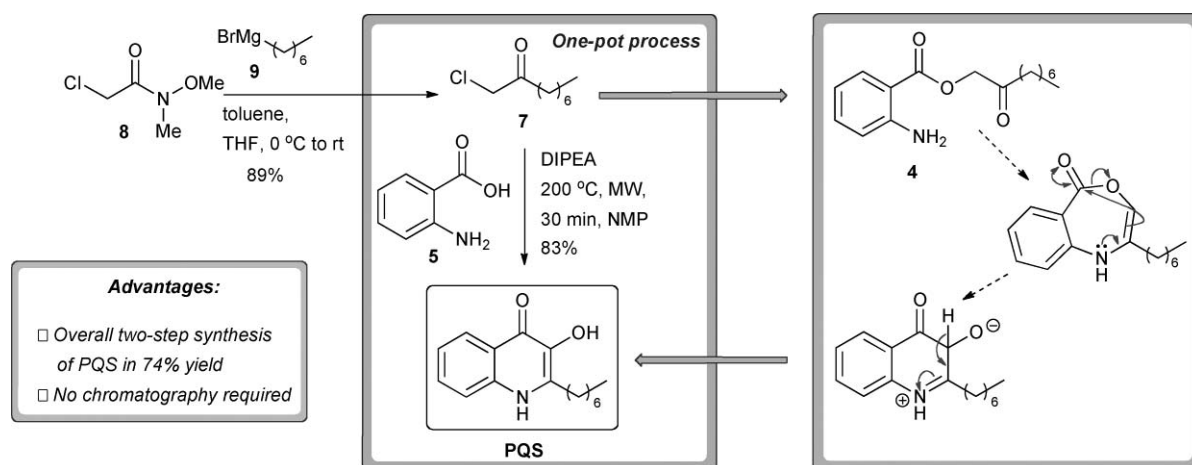
There are several notable features associated with this protocol:

1. Both reaction set-up and purification are operationally simple.
2. The intermediate adduct **4** does not need to be isolated.
3. The starting materials for the reaction are readily available (either commercially available or easily synthesised).
4. The reaction time is relatively short (30 min).
5. The reaction is reproducible and isolated yields of pure product are usually good.

A further point worthy of note is that no purification of the intermediate α -chloro ketone **7** is required; the crude material obtained after work-up can be used directly in the next reaction step. Consequently column chromatography is avoided at all stages of PQS synthesis.

Use of this tandem reaction process allows a two-step synthesis of PQS from readily obtainable or commercially available materials with an overall yield of 74%. To the best of our knowledge this is the shortest and most efficient reported synthesis of PQS from readily available starting materials to date.¹⁹

In the context of PQS analogue synthesis, this route is also advantageous due to its inherent flexibility. For example, substitution at the 2-position and the introduction of heteroatoms around the aromatic ring could, in principle, simply be achieved through variation in the anthranilic acid and α -chloro ketone building-blocks used; as there are a wide range of such compounds available commercially (or readily synthesised), the method offers the potential for rapid modification. A variety of different α -chloro ketones were easily obtained simply by the treatment of **8** with different Grignard reagents. Reaction with **5** under the optimised one-pot conditions led to a variety of PQS analogues with different groups present at the 2-position of the quinolone ring (**10**, **11** and **12**, Fig. 2). Similarly a range of analogues with different heteroatoms around the aryl ring (**13** and **14**) were obtained through the use of a variety of different anthranilic acid derivatives (Fig. 2). In all cases pure product was obtained without recourse to column chromatography. Isolated yields of the desired products were typically moderate to good.



Scheme 3 One-pot synthesis of PQS from readily synthesised α -chloro-ketone **7** and commercially available anthranilic acid (**5**). A tentative mechanistic proposal is shown. DIPEA = di-*isopropyl*-ethylamine. MW = microwave irradiation. NMP = *N*-methylpyrrolidone.

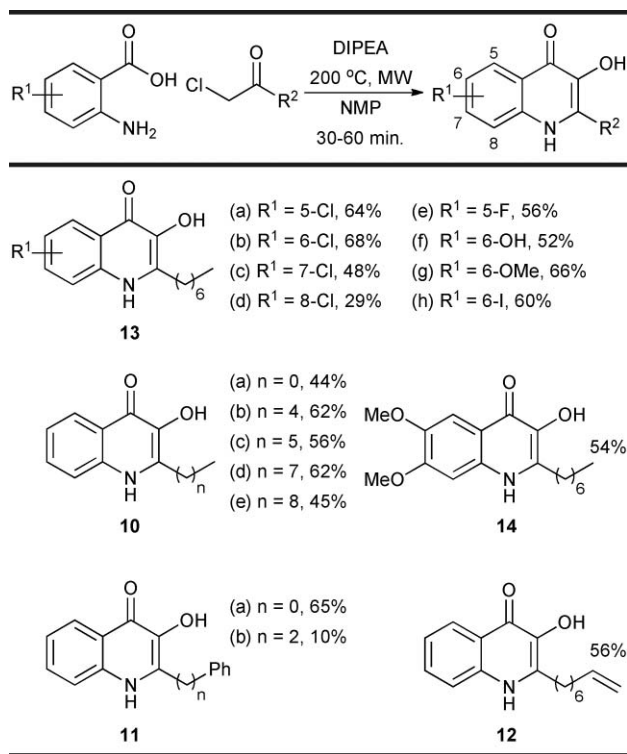


Fig. 2 PQS analogues synthesised in this study. The quoted yields are calculated over two steps (*i.e.* from Weinreb amide **8** to isolated pure product).

The PQS analogues described in this report were tested in a range of biological assays.¹⁰ One such assay focused upon the interaction of these quinolone molecules with the protein PqsR. PqsR (otherwise known as the multiple virulence factor regulator or MvfR) is a transcriptional regulator that plays a critical role in *P. aeruginosa* pathogenicity.^{20a} PQS is known to bind to PqsR and is thought to act as a co-inducer; for example, PQS in concert with PqsR regulates the production of a variety of extracellular virulence factors such as pyocyanin and hydrogen cyanide.^{20a-c} We were thus interested in examining how structural modifications of the PQS molecule affected its ability to bind to, and thus activate, PqsR. Towards this end the PQS analogues described in this report were tested for their ability to stimulate PqsR-dependent *pqsA* transcription from the *pqsA* promoter in *Escherichia coli* strain DH5 α containing the reporter plasmid pEAL08-2.¹⁰ This plasmid expresses PqsR (MvfR) and also carries a *lacZ* open reading frame cloned behind the *pqsA* promoter region.^{10,20b} The *lacZ* gene encodes β -galactosidase. In the presence of co-inducer, PqsR will bind to the *pqsA* promoter and stimulate transcription of the downstream *lacZ* ORF, thereby leading to β -galactosidase production. Therefore a comparison of the β -galactosidase activity of the *E. coli* cells grown in the presence of PQS with that of cells grown in the presence of a PQS analogue provides an indication of the relative ability of the different quinolone molecules to bind to the PqsR protein.¹⁰ Using this assay system a structure–activity profile of the PQS analogues for this phenotype was established.¹⁰ Some selected results are shown in Fig. 3.²¹ It was found that agonism was strongly dependent upon the alkyl chain length. Small modifications in chain length **10b**, **10c**, **10d** and **10e** resulted in a slight loss whereas a major

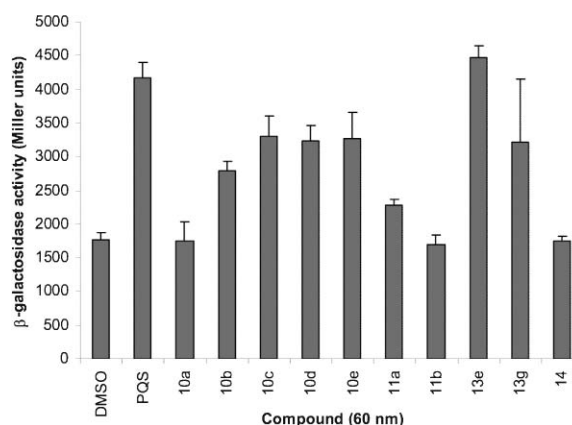
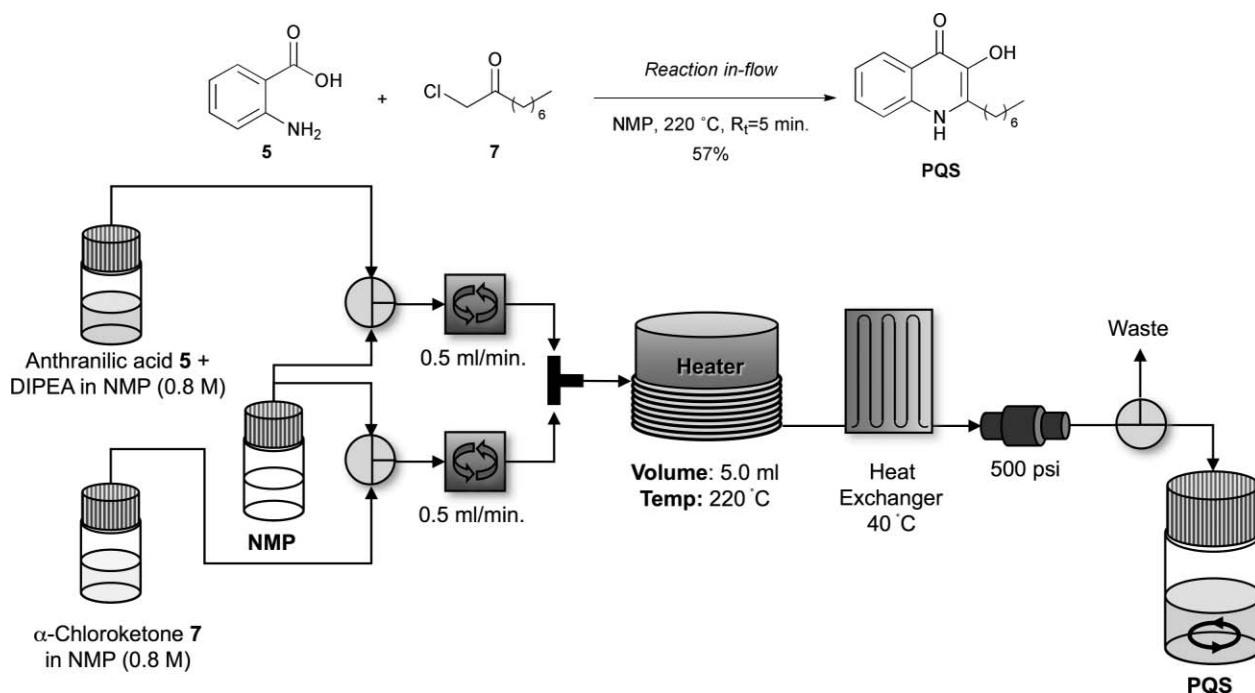


Fig. 3 Stimulation of MvfR-dependent promoter activity (as measured by levels of β -galactosidase activity) by a selection of the PQS analogues. DMSO was added as a control. The data represent the averages and standard deviations from the results of three independent repeats.¹⁰

reduction in chain length **10a** or the substitution of the heptyl chain for a phenyl ring **11a** and **11b** greatly reduced activity. In addition, this analysis suggested that the electron density around the quinolone aromatic ring has an effect upon the level of PqsR binding. The introduction of electron donating groups at position 6 led to a large reduction in activity **13e**, **13g** and **14**. For a full account of the biological assessment of the PQS analogues the reader is directed to the original report.¹⁰

The microwave-based protocol outlined in Scheme 3 proved to be a highly efficient and robust method for the synthesis of a range of PQS derivatives. However, one limitation associated with this route is its amenability to scale-up. For the synthesis of large amounts (grams) of material the need to empty and refill conventional batch microwave vessels coupled with repetitive heating up and cooling down cycles is both inconvenient and decreases throughput. We were thus interested in exploring the synthesis of PQS and analogues under continuous flow conditions as this would provide a more efficient means of preparing the larger quantities of these materials required for any further, more detailed biological and pharmacological studies. Towards this end we investigated the adaptation and optimisation of our previously developed microwave-based PQS synthesis conditions for use in a Uniqsis FlowSynTM continuous flow reactor (Scheme 4).²² Pleasingly, under slightly modified reaction conditions (reaction temperature of 220 °C, residence time of 5 min) it proved possible to synthesise PQS on a multi-gram scale and with comparable purity to the PQS generated using the microwave-based protocol (albeit in a slightly reduced yield). In principle, through the use of different anthranilic acid and α -chloro ketone building-blocks in the reaction channels, (Scheme 4) it should prove to be possible to achieve the large scale synthesis of a range of PQS analogues in-flow.²³

In conclusion, we have developed an efficient microwave-based reaction process that allows expedient access to PQS and a range of novel non-natural analogues from readily available starting materials. Use of this chemistry facilitated the first systematic SAR analysis of PQS to be carried out.¹⁰ Furthermore we have described the successful adaptation and optimisation of these microwave-based conditions for use in a continuous flow reaction, which has enabled the synthesis of PQS on a multi-gram scale. Additional



Scheme 4 Schematic of the synthesis of PQS using a Uniqsis FlowSyn™ continuous flow reactor.²²

analogue syntheses and biological studies should further enhance our understanding of PQS-mediated quorum sensing systems; this may facilitate the development of methods that can modulate these signalling networks in a useful fashion, with possible therapeutic applications in the treatment of human bacterial infections.

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- 23 The synthesis of PQS analogue **10d** was also achieved in-flow (see ESI†).