Microwave-assisted preparation of the quorum-sensing molecule 2-heptyl-3-hydroxy-4(1*H*)-quinolone and structurally related analogs

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An optimized procedure for the efficient preparation of 2-heptyl-3-hydroxy-4(1*H*)-quinolone (*Pseudomonas* quinolone signal or PQS) and a diverse range of structurally related 2-alkyl-4-quinolones with biological activity is presented. The two-step synthesis begins with the formation of α-chloro ketones by the coupling of a Weinreb amide (2-chloro-*N*-methoxy-*N*-methylacetamide) and an appropriate Grignard reagent. The resulting α-chloro ketones can be reacted with commercially available anthranilic acids under microwave irradiation conditions to furnish the desired 2-alkyl-4-quinolone products. As a typical example, the synthesis of PQS, a molecule involved in quorum sensing in the pathogenic bacterium *Pseudomonas aeruginosa*, is described in detail. The first step of this process (α-chloro ketone formation) takes ~10 h in total to complete from commercially available bromoheptane and 2-chloro-*N*-methoxy-*N*-methylacetamide. The second step (microwave-assisted reaction with anthranilic acid) takes ~14 h in total to complete (the reaction typically proceeds in ~30 min, with work-up and purification requiring ~13 h).

INTRODUCTION

Background

Quorum sensing is a mechanism of intercellular communication used by many species of bacteria[1]. This signaling process is mediated by small molecules termed autoinducers, which are produced and detected by the bacterial cells themselves. The bacterium *P. aeruginosa* uses (at least) three different types of quorum sensing systems with each regulated by a different autoinducer. One such molecule is PQS (**Fig. 1**)[1]. *P. aeruginosa* is a clinically important pathogen, which is associated with a range of life-threatening hospital-acquired infections and is also a common cause of mortality in cystic fibrosis sufferers[2–4]. The virulence of this bacterium is known to be dependent (at least in part) on PQS-based quorum sensing[2,3,5–7]. Thus, the selective disruption of PQS-based signaling represents a strategy for attenuating the virulence of the bacterium; in the case of human infections, this would allow the host immune system a better chance of clearing the infection before the bacteria cause too much tissue damage[2,3]. This would be of huge clinical importance given the fact that *P. aeruginosa* is known to have resistance to many commonly prescribed antibiotics[8].

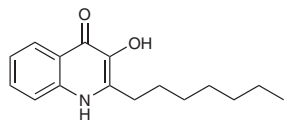
In recent years, the identification of quinolone-based analogs of PQS that can modulate PQS quorum sensing has attracted considerable attention; such molecules could be used as probes for chemical biology studies and may potentially find application in a therapeutic context[1,3].

Development of the protocol

Within the Spring group we were interested in carrying out a structure-activity relationship study of PQS with the intention of determining what aspects of its structure are important for its activity or how the molecule might be altered so as to change its biological effects[2,3]. This required access to PQS itself as well as to a diverse collection of structurally related 2-alkyl-4-quinolones. Literature routes to PQS typically follow a route pioneered by Pesci *et al.*[9], which involves 2-heptyl-4-quinolone (HHQ), the biosynthetic precursor to PQS, as the key intermediate (**Fig. 2**). HHQ can be synthesized by a three-step process from octanoic acid (**1**) by the method of Pritchard *et al.*[10]. HHQ can then be converted to PQS by two steps: (i) a Duff reaction to furnish **2** followed by (ii) a Dakin oxidation.

There are several drawbacks associated with this overall route to PQS[3]. First, the whole sequence is relatively lengthy and inefficient. Furthermore, in our hands both the Duff reaction and Dakin oxidation steps were somewhat capricious and unreliable. We therefore recently developed a more efficient, rapid and reliable synthetic route to PQS and a range of 2-alkyl-4-quinolone analogs (**Fig. 3**)[2,3] by adaption of methodology previously reported by Hradil *et al.*[11] Our two-step synthesis begins with the coupling of a Weinreb amide **3** (2-chloro-*N*-methoxy-*N*-methylacetamide) and an appropriate readily synthesized Grignard reagent **4**. The resulting α-chloro ketones **5** can be reacted with commercially available anthranilic acids **6** under microwave irradiation conditions to furnish the desired 2-alkyl-4-quinolone products **7** (ref. 3).

In addition to the synthesis of compounds designed to modulate PQS-based quorum sensing, this protocol should prove valuable in a wider synthetic context as it offers a new, efficient method for the synthesis of quinolone derivatives in general; as such, it may find application in other target-oriented and diversity-oriented synthetic campaigns. For a recent discussion of diversity-oriented synthesis, see ref. 12.



PQS

Figure 1 | Structure of PQS.

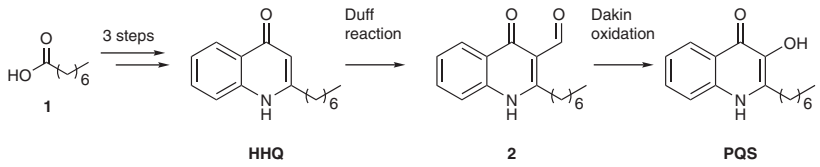


Figure 2 | Outline of the synthetic route to PQS developed by Pesci *et al.*[9]. A three-step synthesis of HHQ from octanoic acid (**1**) has been described by Pritchard *et al.*[10].

Example procedures

This protocol describes, in detail, the generation of PQS by using optimized variants of the synthetic procedures recently reported by Hodgkinson *et al.*[3] (**Fig. 4**). Specifically, (i) the synthesis of heptyl Grignard reagent **8** as an ~1 N solution in anhydrous tetrahydrofuran (THF) from bromoheptane (**9**), (ii) the formation of α-chloro ketone **10** by the reaction of the Weinreb amide 2-chloro-*N*-methoxy-*N*-methylacetamide (**3**) and **8**, and (iii) the reaction of **10** with commercially available anthranilic acid (**11**) under microwave irradiation conditions, which leads to the desired PQS product in a good overall yield (**Fig. 4**). This represents the most efficient chemical synthesis of this molecule reported to date.

This synthetic sequence is readily amenable to the generation of non-natural 2-alkyl-4-quinolone analogs of PQS through the variation in the anthranilic acid and α-chloro ketone building blocks used[2,3]. When adapting this route for the synthesis of analogs, the reaction times for the three synthetic steps (synthesis of the Grignard reagent, α-chloro ketone formation and reaction with the appropriate anthranilic acid) need to be optimized on a case-by-case basis. Whenever possible, the progression of each of these reaction processes should be monitored by the methods described below. No substantial deviation from the reaction times listed for the synthesis of PQS is expected. The PQS analogs that have previously been successfully prepared by this method are shown in **Figure 5** (refs. 2,3). Notably, both electron donating moieties (e.g., a methoxy group in **12g**) and electron withdrawing moieties (e.g., a fluorine atom in **12e**) can be installed on the aryl ring. In general, yields are comparable to those for PQS itself. However, analog **12d**, with a Cl atom positioned *ortho* to the amino group, was isolated in poor yield. In addition, an anthranilic acid analog with a methoxy group *ortho* to the amino group failed to react (data not shown). These results indicate that substituents *ortho* to the amino group interfere with the reaction; this could possibly be due to unfavorable steric interactions during the cyclization step. Modifications at the 2 position of the quinolone ring can also be accessed (compounds **14**–**16**).

This microwave-based protocol represents a highly efficient and robust method for the synthesis of PQS and a range of PQS analogs on a 10 mg to 1 g scale. For the synthesis of larger amounts (multiple grams) of material, continuous-flow conditions have been found to be more suitable; compound purity is comparable to that obtained using the microwave-assisted protocol, but the overall yield of the reaction sequence is slightly reduced[3].

MATERIALS

REAGENTS

! CAUTION Exercise care when handling all organic materials. A laboratory coat, gloves and safety goggles should be worn. Synthetic operations should be performed in a chemical fume hood whenever possible.

- Acetonitrile (Fisher Scientific, cat. no. A/0626/17, certified HPLC)
- Ammonium acetate
- Anhydrous 1-methyl-2-pyrrolidinone (NMP; Sigma-Aldrich, cat. no. 328634)
- Anhydrous *N*, *N*-diisoproylethylamine (DIPEA, Sigma-Aldrich, cat. no. 387849) **▲ CRITICAL** Anhydrous DIPEA must be used.
- Anthranilic acid (Sigma-Aldrich, cat. no. 10680)
- Brine
- 1-Bromoheptane (Sigma-Aldrich, cat. no. B67570)
- 2-Chloro-*N*-methoxy-*N*-methylacetamide (Sigma-Aldrich, cat. no. 362425).
- Deuterated chloroform ($CDCl_3$; Euriso-top, cat. no. D007HC)
- Distilled water
- Dry ice
- Ethyl acetate (Fisher Scientific, cat. no. E/0850/25, distilled from calcium hydride prior to use)
- Formic acid
- Hydrochloric acid (HCl, 10% (vol/vol) aqueous solution)
- Ice
- Magnesium metal turnings (Fisher Scientific, product code M/0200/50)
- Magnesium sulfate ($MgSO_4$)
- Tetrahydrofuran (THF; Fisher Scientific) **▲ CRITICAL** Anhydrous THF must be used. In our laboratory, THF is dried over sodium wire and distilled under argon from a mixture of lithium aluminum hydride and calcium hydride.
- Toluene (Fisher Scientific, cat. no. T/2200/17) **▲ CRITICAL** Anhydrous toluene must be used. In our laboratory, toluene is distilled under argon from calcium hydride.

EQUIPMENT

- Büchner flask
- Büchner funnel
- Cotton wool

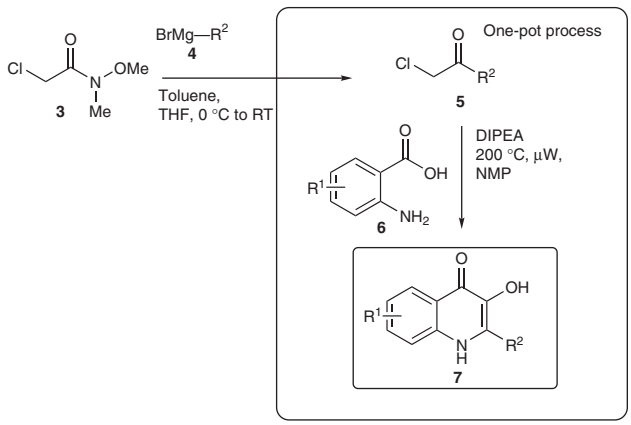


Figure 3 | Synthesis of PQS (R^1 = H, R^2 = $(CH_2)_6CH_3$) and related 2-alkyl-4-quinolones **7** using a microwave-assisted process.

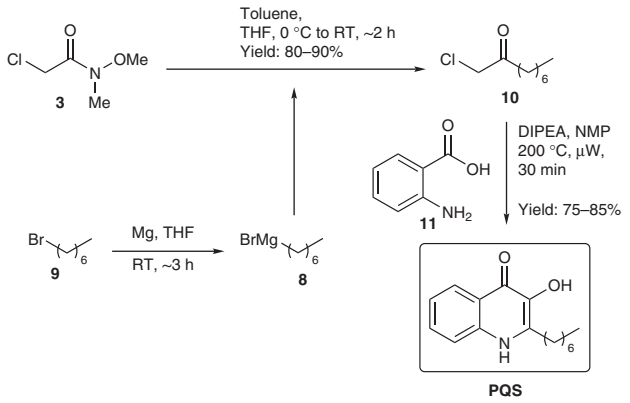


Figure 4 | Synthesis of PQS. The microwave reactor was programmed with a ramp time of 10 min and a hold time of 30 min.

- Discover microwave apparatus (CEM Corporation)
- Disposable 21-gauge needles
- Dual nitrogen-vacuum manifold with high vacuum line
- Erlenmeyer flasks (various sizes)
- Filter paper
- Glass columns for flash column chromatography (Fisher Scientific, various sizes, sintered)
- Glass funnels (various sizes)
- Heat gun (Bosch, PHG 500-2)
- LC-MS (HP/Agilent MSD LC-MS APCI 120–1,000 full gradient machine)
- LC-MS vials
- Magnetic stirrer with temperature probe (Heidolph MR 3001 K (Heidolph) and IKA-Werke RCT basic (IKA-Werke))
- Magnetic stir bar retriever
- Microwave reaction vial (10 ml; CEM Corporation)
- NMR tubes
- Plastic bowl (for ice-water baths)
- Plastic syringes, slip tip (various sizes)
- Reflux condenser
- Round-bottom flasks (various sizes, one and three necks)
- Rubber septa (various sizes)
- Separatory funnels (various sizes)
- Snap-cap lids for LC-MS vials
- Supelcosil ABZ + PLUS columns (3.3 cm × 4.6 mm, particle size = 3 μm; Sigma-Aldrich, cat. no. 59191)
- Teflon-coated magnetic stir bars for round-bottom flasks
- Teflon-coated magnetic stir bars for microwave vial (Biotage, cat. no. 355543)
- Teflon septum clip caps (CEM Corporation)
- Ultrasonic bath (Branson, 2210)
- UV lamp

EQUIPMENT SETUP

Synthesis apparatus Round-bottom flasks, stirrer bars and microwave reaction vials used in the reaction stages should be either oven dried (~80 °C) for at least 12 h before use or flame dried while attached to a high-vacuum line using a blowtorch. Glassware should be allowed to cool to room temperature (~20 °C) before use either under a flow of nitrogen gas or under high vacuum by attachment to a high-vacuum line using a suitable adaptor; microwave reaction vessels should be cooled under a flow of nitrogen gas.

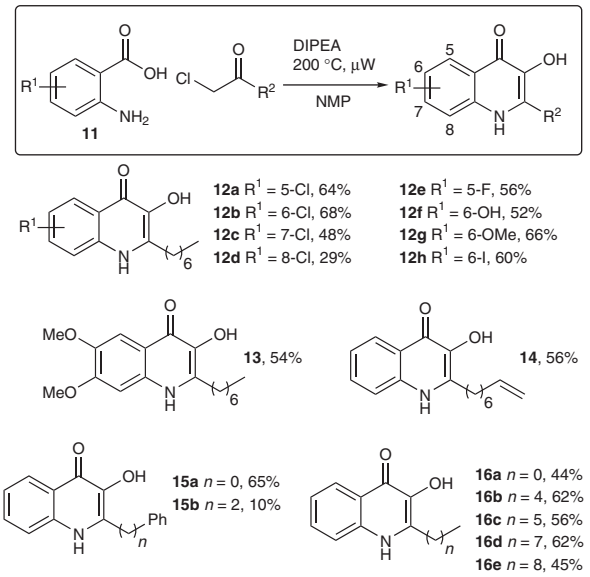


Figure 5 | PQS analogs successfully generated using this protocol[2,3]. The quoted yields are calculated over two steps (i.e., from Weinreb amide **3**)[3].

LC-MS The purities of isolated products can be measured using LC-MS analysis (UV trace). LC-MS can also be used to monitor reaction progression as indicated in the table below. Samples were prepared using the following procedure: the compounds were dissolved at a concentration of ~1 mg ml^{-1} in acetonitrile/water (1:1) and filtered into a LC-MS vial through a small pad of cotton wool contained in a glass pipette to remove any insoluble material. In cases where a reaction was monitored, a small aliquot of the reaction solution was removed (~0.02 ml) and added to a glass vial containing 1 ml of acetonitrile/water (1:1). The contents of the vial were filtered into an LC-MS vial through a small pad of cotton wool contained in a glass pipette to remove any insoluble material.

Equipment	Agilent 1200 Series HPLC with a Waters Micromass ZQ ESCi detector
Column	Supelcosil ABZ + PLUS, 3.3 cm × 4.6 mm, particle size = 3 μm
Solvent A	H_2O, 10 mM NH_4OAc, 0.1% HCOOH
Solvent B	95% MeCN, 5% H_2O, 0.05% HCOOH
Gradient	0.70 min (0% solvent B); 4.2 min (100% solvent B); 7.70 min (100% solvent B); 8.50 min (0% solvent B)
UV absorption	Range: 190 nm–600 nm; interval: 2.0 nm
Flow rate	1 ml min^{-1}

PROCEDURE

Preparation of heptyl Grignard reagent 8 as an ~1 N solution in anhydrous THF ● TIMING ~4 h

1| Place a Teflon-coated magnetic stir bar in a 50-ml three-necked round-bottom flask.

2| Weigh out 176 mg (7.27 mmol) of magnesium turnings and add it to the flask.

3| Cap two necks of the flask with rubber septa. Place a reflux condenser in the third neck, cap with a rubber septum and attach water in/out tubing. Attach the system to a dual nitrogen-vacuum manifold with vacuum line using a 21-gauge needle piercing through the septum attached to the top of the reflux condenser (**Figs. 6** and **7**). Turn the water flow on.

4| Evacuate the flask under vacuum and backfill it with nitrogen (**Fig. 7**). Repeat this process three times and then maintain the vessel under a positive pressure of nitrogen.
▲ **CRITICAL STEP** The reaction vessel must be kept under a nitrogen atmosphere to exclude air and water, which could destroy the Grignard reagent.

5| By using a disposable 10-ml syringe and 21-gauge needle, transfer 7.5 ml of anhydrous THF into the reaction vessel through a side arm of the flask so that the magnesium turnings are submerged (**Fig. 8**).
▲ **CRITICAL STEP** Anhydrous THF must be used. If the solvent is not dry, residual water could destroy the Grignard reagent **8**.
? TROUBLESHOOTING

6| By using a disposable 10-ml syringe and a 21-gauge needle, transfer 1.20 ml (7.30 mmol) of bromoheptane dropwise into the reaction vessel through a side arm of the flask with vigorous stirring.
! CAUTION The reaction of bromoheptane with the magnesium turnings (Grignard formation) is an exothermic process and the solution will self-reflux. Slow addition of the bromoheptane is necessary in order to maintain a gentle reflux, which prevents the reaction from proceeding too rapidly and in an uncontrollable fashion.
▲ **CRITICAL STEP** Vigorous stirring is necessary in order to prevent precipitation of the Grignard reagent after formation.
? TROUBLESHOOTING

Figure 6 | Equipment setup for Grignard formation (Step 3).

7| Stir the self-refluxing reaction mixture until the majority of the magnesium turnings have dissolved and no more heat is evolved, as determined by touching the flask (~3 h), to generate the heptyl Grignard reagent **8** as an ~1 N solution in anhydrous THF.
▲ **CRITICAL STEP** The Grignard solution must be stored under nitrogen and should be used as soon as possible in the subsequent reaction (on the same day).
? TROUBLESHOOTING

Preparation of α-chloro ketone 10 ● **TIMING ~6 h**

8| Place a Teflon-coated magnetic stir bar in a 50-ml round-bottom flask.

9| Weigh out 0.5 g (3.63 mmol) of 2-chloro-*N*-methoxy-*N*-methylacetamide (**3**) and add it to the flask.

10| Cap the flask with a rubber septum. Attach the flask to a dual nitrogen-vacuum manifold with vacuum line using a 21-gauge needle piercing through the septum. Evacuate the flask under vacuum and backfill it with nitrogen. Repeat this process three times and then maintain the vessel under a positive pressure of nitrogen.
▲ **CRITICAL STEP** The reaction vessel must be kept under a nitrogen atmosphere to exclude air and water, which could destroy the Grignard reagent added in Step 14.

11| By using a disposable 10-ml syringe and a 21-gauge needle, transfer 10 ml of anhydrous THF into the reaction vessel.
▲ **CRITICAL STEP** Anhydrous THF must be used. If the solvent is not dry, residual water could destroy the Grignard reagent added in Step 14.

12| Cool the reaction vessel to 0 °C using an ice-water bath.
▲ **CRITICAL STEP** Cooling the reaction vessel during addition of the Grignard reagent (Step 13) helps to control the exothermic reaction process.

13| By using a disposable 10-ml syringe and a 21-gauge needle, transfer the Grignard reagent **8** (5.45 ml, ~1 N solution in anhydrous THF, 5.45 mmol) into the reaction vessel dropwise over the course of 5 min.
▲ **CRITICAL STEP** Slow addition of the Grignard reagent is necessary in order to prevent the exothermic reaction from proceeding too rapidly and in an uncontrollable fashion.

14| Remove the vessel from the cooling bath and stir the reaction mixture at room temperature for 2 h. The solution will appear cloudy.

15| Use a disposable 20-ml syringe and a 21-gauge needle to transfer 15 ml of anhydrous toluene into the reaction vessel.

16| Cool a 250-ml Erlenmeyer flask containing HCl (50 ml, 10% (vol/vol) aqueous solution) to 0 °C using an ice-water bath.

Figure 7 | Close-up of attachment of reaction system to dual nitrogen-vacuum manifold (Step 3).

17| Pour the reaction mixture and the stirrer bar into the Erlenmeyer flask. Stir the mixture in the ice-water bath for 10 min.

18| Transfer the mixture into a 250-ml separatory funnel.

19| Separate the aqueous and organic layers.

20| Wash the organic layer with brine (15 ml) and transfer the organic layer to a 250-ml Erlenmeyer flask.

21| Add $MgSO_4$ to the organic layer in portions with swirling until a free-flowing powder is seen. Remove the $MgSO_4$ by filtration under suction using a Büchner funnel, fitted with filter paper, attached to a Büchner flask connected to a low-vacuum pump or water aspirator.

22| Transfer the filtrate to a 100-ml round-bottom flask and remove the solvent using a rotary evaporator (water bath temperature 80 °C).

23| Remove residual solvent under high vacuum by fitting the flask with a rubber septum and connecting the flask to a dual nitrogen-vacuum manifold with vacuum line using a 21-gauge needle piercing through the septum.

24| Check purity of crude product material (colorless oil) by LC-MS and ^{1}H NMR (see the method described in the LC-MS setup). The crude product material is usually of sufficient purity to use directly in the next step of synthesis.
? TROUBLESHOOTING
■ **PAUSE POINT** Product **10** can be stored under nitrogen at −20 °C for several months.

Preparation of PQS ● **TIMING ~1 h**
25| Place a Teflon-coated magnetic stir bar for a microwave vial in a 10-ml microwave reaction vial.

26| Add anthranilic acid (0.15 g, 1.1 mmol), anhydrous NMP (2.25 ml), α-chloro ketone **10** (0.19 g, 1.1 mmol) and anhydrous *N*, *N*-diisoproylethylamine (0.23 ml, 1.3 mmol), in sequence, to the microwave vial.
▲ **CRITICAL STEP** Even though the microwave vial can be left open to air during the addition of the reagents, the yield of the reaction is considerably reduced if anhydrous solvents are not used.

27| Fit the microwave vial with a Teflon septum clip cap.
▲ **CRITICAL STEP** The cap on the microwave vial must be secure; otherwise leakage of the tube contents can occur under microwave irradiation.

28| Stir the contents of the microwave tube at room temperature until the anthranilic acid dissolves to produce a homogeneous solution.

29| Program the microwave reactor using the following parameters (these settings correspond to the use of the 'Standard' method on the Discover microwave apparatus with a temperature of 200 °C and a time of 30 min):

Temperature type	Infrared
Release limits	60 °C, 2.8 bar
Hold time	30 min
Ramp time	10 min
Temperature	200 °C
Microwave power	150 Watt
Stirring	On
Premix time	Off
Pressure	17.2 bar
Cooling time	20 min
PowerMax	Off

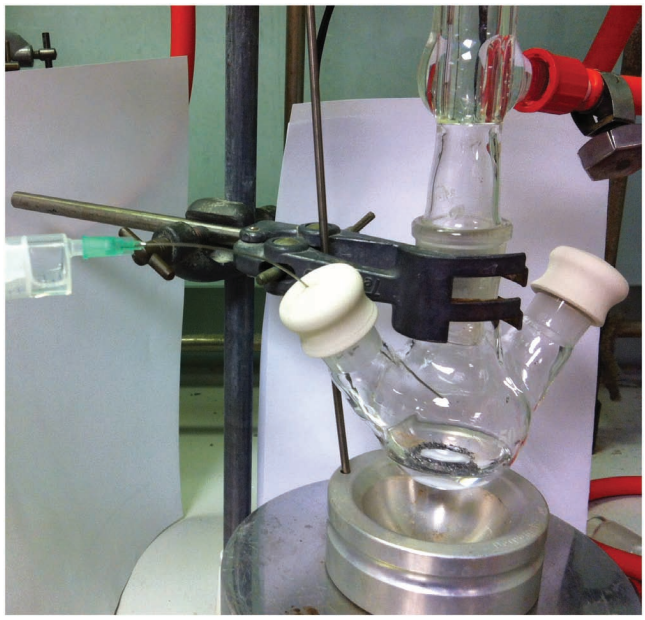


Figure 8 | Transfer of anhydrous THF into the reaction vessel (Step 5).

30| After the program is complete, remove the microwave vial from the reactor and allow the reaction mixture to cool to room temperature.

31| Check the progress of the reaction by LC-MS (compare with anthranilic acid; see the method described in EQUIPMENT SETUP). If LC-MS analysis indicates that anthranilic acid is still present, repeat Steps 29 and 30.

Purification of PQS ● TIMING Option A ~13 h, option B ~7 h (both including drying time)

32| This step can be performed using purification option A or option B, which involves precipitation of the desired solid material from water and acetonitrile, respectively. Purification by option A takes longer but typically furnishes the desired product in a greater overall yield. Purification by option B is more rapid but the isolated yield of the desired product is typically lower as a greater proportion of the product material remains in solution. The product material isolated using purification option A is not always as pure as that obtained using purification option B; typically ~90% from option A (by ^{1}H NMR and LC-MS analysis) compared with >95% from option B.

(A) Precipitation from water

(i) Half-fill a 50-ml Erlenmeyer flask with crushed ice and add 25 ml of distilled water.

(ii) Remove the stirrer bar from the microwave vial using a magnetic stir bar retriever.

(iii) Pour the contents of the microwave tube into the Erlenmeyer flask. Wash out the tube with a small volume (~2 ml) of anhydrous NMP (two times) and pour it into the Erlenmeyer flask.

(iv) Allow the contents of the flask to settle for 20 min. A precipitate will be seen.

(v) Collect the precipitate by filtration under suction using a Büchner funnel, fitted with filter paper, attached to a Büchner flask (250 ml) connected to a low-vacuum pump or water aspirator. Wash the precipitate on the filter with ice-cold water (~100 ml).

▲ **CRITICAL STEP** It is important that ice-cold water is used; the use of water at a higher temperature will lead to a lot of PQS being lost in the washings.

(vi) Transfer the white solid to a 50-ml round-bottom flask.
(vii) Fit the flask with a rubber septum and connect the flask to a dual nitrogen-vacuum manifold with vacuum line using a 21-gauge needle piercing through the septum. Place the flask under vacuum for ~12 h to remove residual solvent.
■ **PAUSE POINT** The flask can be left under vacuum for long periods of time (~24-h maximum period examined).

(B) Precipitation from acetonitrile

(i) Remove the stirrer bar from the microwave vial using a magnetic stir bar retriever.
(ii) Cool the microwave vial containing the reaction mixture to 0 °C using an ice-water bath.
(iii) Add 2 ml of acetonitrile to the vial and leave it at this temperature for 20 min. A white precipitate will be seen.
(iv) Collect the white precipitate by filtration under suction using a Büchner funnel, fitted with filter paper, attached to a Büchner flask connected to a low-vacuum pump or water aspirator. Wash the precipitate on the filter with ice-cold acetonitrile (~100 ml).
▲ **CRITICAL STEP** It is important that ice-cold acetonitrile is used; the use of acetonitrile at a higher temperature will lead to a lot of PQS being lost in the washings.
(v) Transfer the white solid to a 50-ml round-bottom flask.
(vi) Fit the flask with a rubber septum and connect the flask to a low-vacuum system using a 21-gauge needle piercing through the septum. Place the flask under vacuum for ~6 h to remove residual solvent.
■ **PAUSE POINT** The flask can be left under vacuum for long periods of time (~24-h maximum period examined).

Analysis of purity of desired product ● TIMING 30 min

33| Analyze the purity of the desired product using LC-MS and ^{1}H NMR. Further purify the desired product if necessary (Steps 34–40).
? TROUBLESHOOTING

Further purification of PQS by recrystallization ● TIMING ~3 h

34| Transfer the product material to a 100-ml Erlenmeyer flask.

35| Add hot (~50 °C) ethyl acetate to the flask slowly with swirling. Gently warm the flask during the addition process periodically with a heat gun.
! CAUTION The flask should not be heated continuously or too vigorously, or hot solvent may be ejected from the flask in an uncontrollable fashion. The use of heat-resistant gloves is advised.

36| When all product material has dissolved, stop heating and allow the flask to cool undisturbed to room temperature slowly. Leave the flask for 2 h.

37| Isolate the white solid precipitate by filtration under suction using a Büchner funnel, fitted with filter paper, attached to a Büchner flask connected to a low-vacuum pump or water aspirator.

38| Transfer the white solid to a 50-ml round-bottom flask.

39| Fit the flask with a rubber septum and connect the flask to a low-vacuum system using a 21-gauge needle piercing through the septum. Place the flask under vacuum for ~6 h to remove residual solvent.
■ **PAUSE POINT** The flask can be left under vacuum for long periods (~24-h maximum period examined).

40| Analyze the purity of the desired product using LC-MS and ^{1}H NMR. Further purify the desired product if necessary (repeat Steps 34–39).
? TROUBLESHOOTING

? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 1**.

TABLE 1 | Troubleshooting table.

Step	Problem	Possible reason	Solution
5	Some of the magnesium turnings are distributed around the inner surface of the side arm(s) of the flask rather than submerged in the center of the flask	Magnesium turnings may have been displaced from the center of the flask prior to the addition of THF when handling the flask or when the flask was evacuated and backfilled with nitrogen	A small quantity (maximum 2 ml) of anhydrous THF can be added to the reaction vessel through the side arm(s) in such a way that it runs along the inner glass surface of the side arm(s), which should wash the magnesium turnings into the central area of the flask
6	Grignard formation does not appear to occur (no dissolution of the magnesium turnings and/or evolution of heat)	The reaction is sluggish (or has failed) to initiate, possibly because the magnesium turnings are coated with a protective layer of magnesium oxide	A small quantity ~0.1 ml of 1,2-dibromoethane can be added to the reaction vessel to stimulate initiation. Alternatively, the flask can be heated gently (with a heat gun or to ~50 °C using a magnetic stirrer with temperature probe) to try to stimulate initiation
7	Observation of solid material in the flask containing the Grignard reagent	The solid material is most likely to be the Grignard reagent, which can precipitate out of solution on standing	Gently heat the reaction flask (with a heat gun or to ~50 °C using a magnetic stirrer with temperature probe) until the material dissolves
24	Poor conversion of **3** to α-chloro ketone **10**	Grignard reagent may have been destroyed by reaction with adventitious moisture	Repeat the reaction (Steps 1–24) using freshly prepared Grignard reagent; use extra vigilance to maintain water-free conditions
	Isolated α-chloro ketone **10** is not pure (according to LC-MS and/or ^{1}H NMR)	Undesired side-reactions may have occurred	The α-chloro ketone **10** can be purified by flash column chromatography on silica gel (elutant: hexanes/diethyl ether, 9:1)
33, 40	Isolated PQS product is contaminated with solvent (e.g., NMP, acetonitrile or water) but is otherwise analytically pure	The product was not attached to the low-vacuum line for long enough, or extra washings with water required	Leave the product attached to a low-vacuum line for a longer length of time until all the solvent(s) is removed or dry the product on a high-vacuum line. Alternatively, the product material can be treated in the following fashion: transfer to a round-bottom flask. Add water to form a suspension. Stir in an ultrasonic bath for 20 min. Reisolate the solid material by filtration and dry under vacuum (see Step 32A(v–vii))

● **TIMING**

Steps 1–7, preparation of heptyl Grignard reagent **8** as an ~1 N solution in anhydrous THF: 4 h
Steps 8–24, preparation of α-chloro ketone **10**: 6 h
Steps 25–31, preparation of PQS: 1 h
Step 32A, purification of PQS using option A: 13 h
Step 32B, purification of PQS using option B: 7 h
Step 33, analysis of purity of desired product: 30 min
Steps 34–40, further purification of PQS by recrystallization: 3 h

ANTICIPATED RESULTS

Typical isolated yield of α-chloro ketone **10** is 80–90% from **3**. Typical isolated yield of PQS (purified by option A) is 75–85% (from α-chloro ketone **10**) with a purity of ~90%. Typical isolated yield of PQS (purified by option A) is 75–85% (from α-chloro ketone **10**) with a purity >95%.

Analytical data

α-chloro ketone 10

Colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.09 (2H, s), 2.60 (2H, t, J = 7.5 Hz), 1.64 (2H, quintet, J = 7.5 Hz), 1.25–1.35 (8H, m), 0.90 (3H, t, J = 7.5 Hz).

^{13}C NMR (125 MHz, $CDCl_3$) δ 202.8 (C), 48.2 (CCl) 39.7 (CH_2), 31.6 (CH_2), 29.0 (CH_2), 28.4 (CH_2), 23.6 (CH_2), 22.6 (CH_2), 14.1 (CH_3).

HRMS (ESI): *m/z*: calcd for $C_9H_{17}ONaCl$ $[M + Na]^+$: 199.0868090 found 199.0860140.
IR (neat, cm^{-1}) 2,926; 2,856; 1,710.

PQS

White crystalline solid.
m.p. 190–192 °C (EtOAc).
^1H NMR (400 MHz, d_6-DMSO) δ 11.42 (1H, br s), 8.10 (1H, d, J = 8.0 Hz), 8.00 (1H, br s), 7.52–7.55 (2H, m), 7.19–7.24 (1H, m), 2.74 (2H, t, J = 7.5 Hz), 1.68 (2H, quintet, J = 7.5 Hz), 1.20–1.40 (8H, m), 0.86 (3H, t, J = 7.0 Hz).
^{13}C NMR (125 MHz; d_6-DMSO) 168.9 (C), 137.9 (C), 137.5 (C), 135.6 (C) 130.0 (CH), 124.6 (CH), 122.3 (C), 121.6 (CH), 117.9 (CH), 31.3 (CH_2), 28.9 (CH_2), 28.6 (CH_2), 28.2 (CH_2), 27.9 (CH_2), 22.1 (CH_2), 14.0 (CH_3).
LC-MS: UV detector retention time 4.57 min, ESI^+ found 260.19, corresponds to $[M + H]^+$.
IR (neat, cm^{-1}) 3,249; 2,949; 2,924; 2,849; 1,639.

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