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ORIGINAL PAPER

Fast Claisen condensation reaction optimization in a continuous flow reactor

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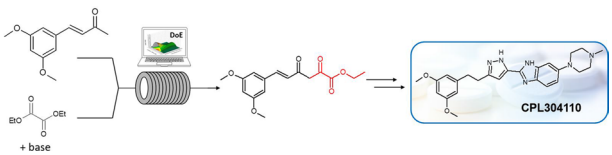
Abstract

In our previous study, we described the batch synthesis of CPL304110, an innovative pan-FGFR inhibitor. Herein, we transferred the Claisen condensation reaction, one of the synthesis steps to a continuous flow reactor. A simple solvent switch from ethanol to tetrahydrofuran shortened the original reaction time from 20 h to 10 min. With the use of the design of experiment method and program Statistica®, we optimized reaction parameters and increased the reaction yields from 73 to 84% with greatly shortened reaction times (20 h vs. 2 min), improved productivity (74.4 g h^{-1}), and increased space–time yield (3720 kg h^{-1} m^{-3}).

Graphical abstract



Keywords Antitumor agents · CPL304110 · Design of experiment · Enols · FGFR · Nucleophilic substitution

Introduction

FGFRs (fibroblast growth factor receptors) have become therapeutic targets in cancer treatment over the last decade. FGFR1 to FGFR4 are receptor tyrosine kinases with morphologically congruent cell surfaces. Fibroblast growth factor (FGF) ligands binding interactions with FGFR play an essential role in cell functions such as proliferation, growth, migration, apoptosis, and differentiation [1–6]. Miscellaneous aberrations in the FGF–FGFR axis, such as gene translocations, amplifications, and mutations, are considered to be oncogenic drivers [7–10]. Despite the progress in diagnostics and numerous innovative therapies, the prognoses of advanced cancer are very poor [11, 12]. Several small molecule FGF/FGFR inhibitors have been approved for clinical use and many are in clinical trials [13, 14]. In our previous study, we reported a new clinical candidate CPL304110 with FGFR1, FGFR2, and FGFR3 inhibition activity IC50s of 0.75 nM, 0.50 nM, and 3.05 nM, respectively [15]. Currently, clinical trials are still ongoing to achieve the maximal safety and clinical benefit of CPL304110 (01FGFR2018; NCT04149691) [16]. The synthetic pathway has already been presented, but as the compound reaches the next clinical phases, more and more material will be needed. In this study, we focused on one of the synthesis steps: the Claisen condensation of (3*E*)-4-(3,5-dimethoxyphenyl)but-3-en- 2-one (**2**) with diethyl oxalate (Scheme 1).

Claisen condensation is a widely known reaction, with numerous examples in the literature either in batch or flow reactors [17–23]. Our goal was to transfer the batch reaction to a continuous flow reactor with the perspective of transferring the whole synthesis to the flow process. This would

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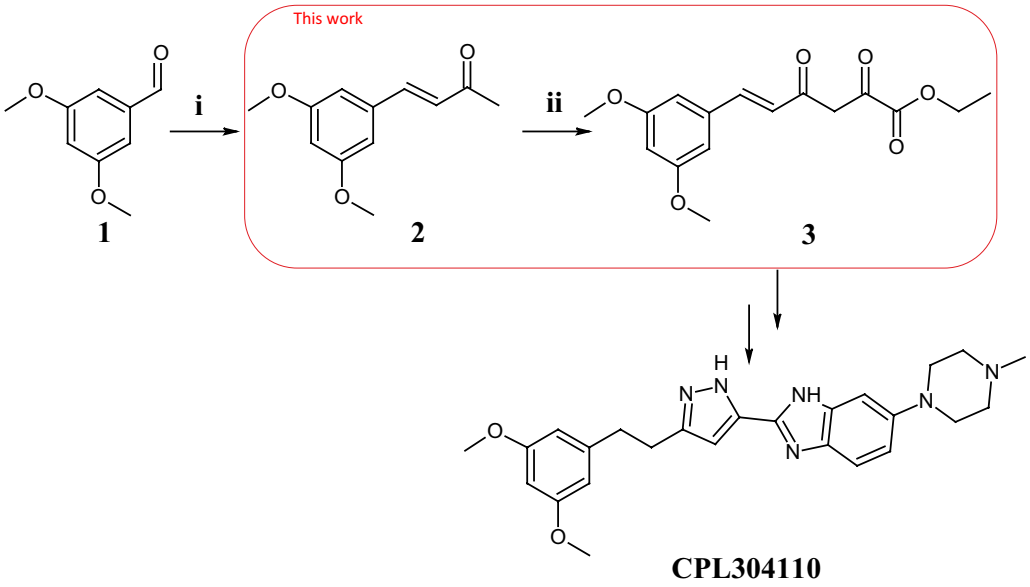
Scheme 1



i) acetone, 3.3 M NaOH, RT, 3 h; **ii**) ethanol, 1.2 eq EtONa, 1.2 eq diethyl oxalate RT, 20 h

Table 1 DoE response surface methodology plan and results

Entry	Time /min	Temperature/°C	Diethyl oxalate and sodium ethoxide equivalents	Conversion/%	Product yield[a]/%
1	2	20	1.0	88.1	83.6
2	2	20	1.4	93.9	90.1
3	2	40	1.0	85.9	81.2
4	2	40	1.4	91.9	88.5
5	8	20	1.0	91.5	86.0
6	8	20	1.4	93.7	88.9
7	8	40	1.0	86.7	82.2
8	8	40	1.4	92.8	88.6
9	2	30	1.2	99.4	96.0
10	8	30	1.2	99.6	96.2
11	5	20	1.2	99.7	96.5
12	5	40	1.2	99.5	95.9
13	5	30	1.0	84.1	79.0
14	5	30	1.4	93.4	88.6
15 (C)	5	30	1.2	99.8	97.0
16 (C)	5	30	1.2	99.7	97.0
17 (C)	5	30	1.2	99.7	97.3

[a]Yield determined by UHPLC

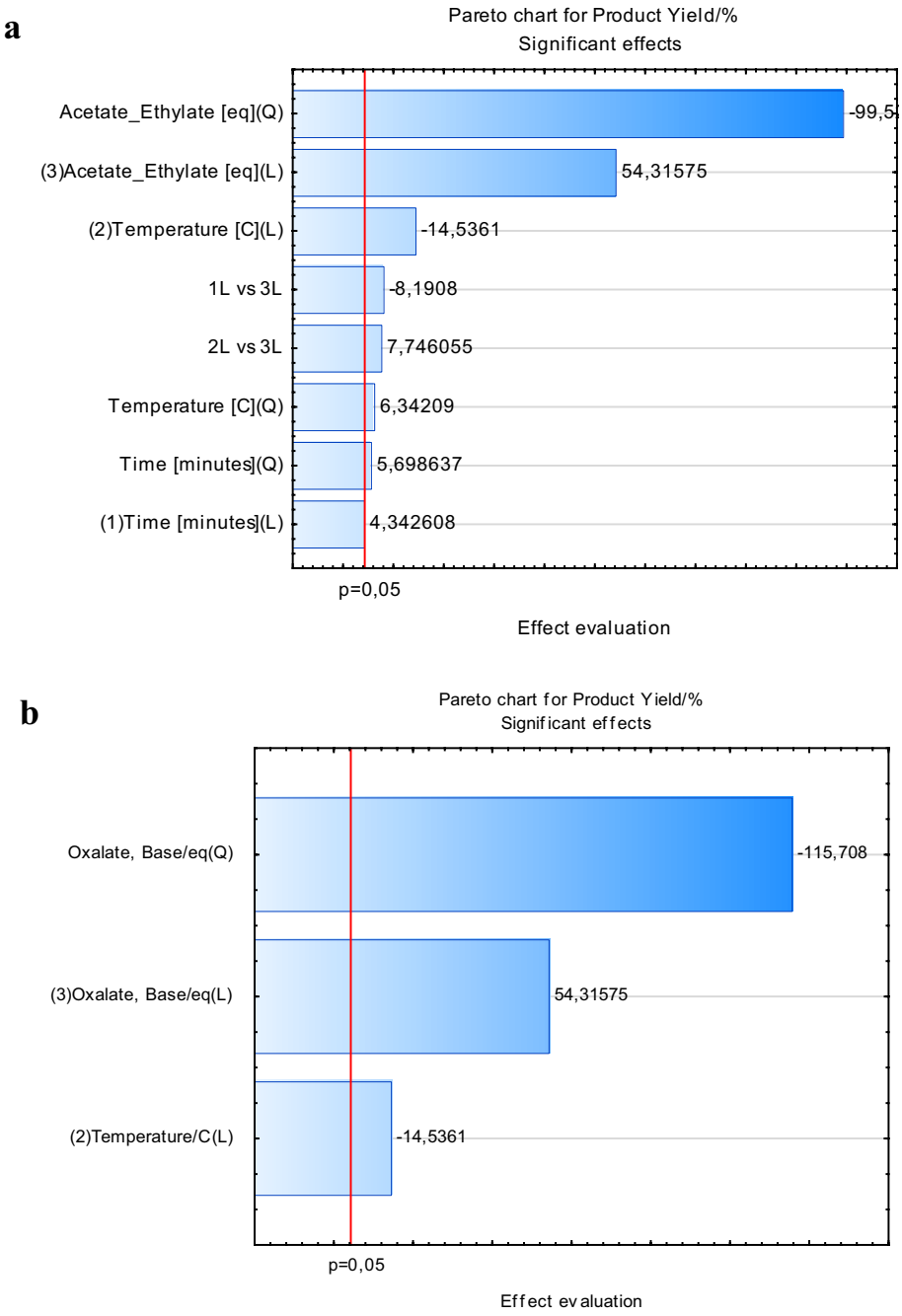


Fig. 1 **a** Pareto chart for significant effects. **b** Pareto chart for the most significant effects

allow us to eliminate the need for isolating byproducts and reduce the amount of waste.

Results and discussion

In batch synthesis, intermediate **3** was obtained by the reaction of intermediate **2** with ethyl oxalate and sodium ethoxide (EtONa) in ethanol as solvent at room temperature for 20 h. The reaction yield was 73% yield after purification.

Due to the poor solubility of **2** in ethanol (1 g/40 cm^3), we decided to change ethanol to another solvent. Other alcohols were protic, and the solubility of **2** was also poor. Esters and acetonitrile were unsuitable for basic conditions. Hydrocarbons, DCM and chloroform, DMF, and DMSO are seen as problematic or hazardous [24]. Although THF is also viewed as problematic, it was chosen because it is aprotic and has a relatively low boiling point, and the solubility of **2** in it is up to 1 g/2 cm^3. Unfortunately, sodium ethoxide is insoluble in THF. To tackle this issue, we decided to use ethanol

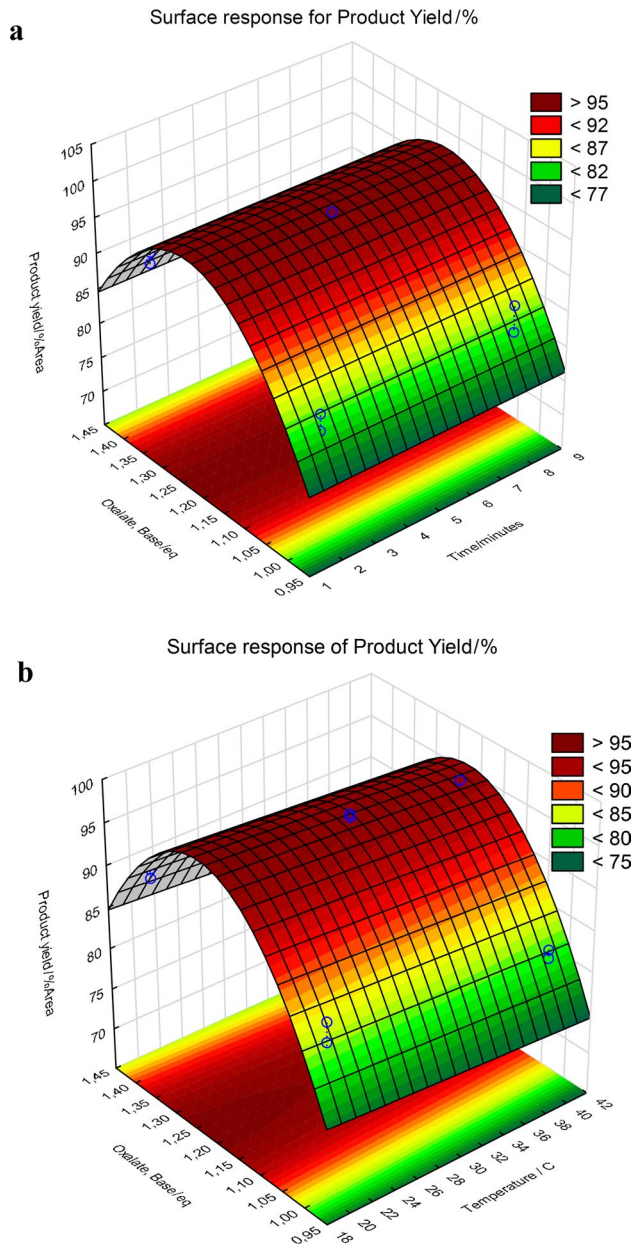


Fig. 2 **a** Surface response for product yield/% at reaction temperature = 20 °C. **b** Surface response for product yield/% at reaction time = 2 min

as a co-solvent and 2 M EtONa/EtOH to assure solubility and use as little ethanol as possible. The use of this setup resulted in the formation of **3** in 10 min with an 87% yield after purification.

The Design of Experiment (DoE) study and statistical analysis were performed by using the design of experiment tools of STATISTICA software (v.13.3) [25, 26]. The DoE study was performed using central composite design (CCD) and response surface methodology (RSM). We explored three variable parameters: reaction temperature, residence time, and the equivalent of diethyl oxalate and sodium ethoxide (Table 1). To reduce the number of experiments, we used the same number of equivalents of diethyl oxalate and sodium ethoxide and regarded them as one parameter.

The CCD model has a good fit ($R^2 = 0.96$). The equivalents of diethyl oxalate and sodium ethoxide have major statistically significant effects (Fig. 1a) on the reaction yields. They have a negative quadratic effect but a positive linear effect. The positive effect may be caused by the increased concentration of oxalate, while the negative effect is probably caused by the increased presence of a protic solvent—ethanol used to keep sodium ethoxide soluble. The temperature has a negative linear effect and a smaller positive quadratic effect. The residence time has a positive effect, both quadratic and linear. However, the combined linear effect of time and the equivalents of oxalate and ethoxide is negative (Fig. 1a). Finally, we considered only the most statistically significant effects to build the model: linear and squared equivalents of diethyl oxalate and sodium ethoxide and a linear term of temperature (Fig. 1b). Noteworthy, a higher amount of ethanol added with ethoxide slows reaction speed which may lower total yield (Fig. 2a, b).

Additionally, the short optimization based on a two-level design has been performed in a full factorial design with three repetitions at the center to verify the influence of diethyl oxalate and EtONa on the product yield separately (Table 2). Temperature and reaction time were constant at 30 °C and 5 min, respectively.

Because of the small data set, the obtained model has not had an ideal fit ($R^2 = 0.63$), but it provides sufficient information about the general dependency between tested variables.

The interaction effect of equivalents of diethyl oxalate and sodium ethoxide has major statistically significant negative effects (Fig. 3) on the reaction yields. Separately, diethyl oxalate equivalent and sodium ethoxide have a positive effect. The optimal, maximum yield is achieved when one or both of the equivalents are above 1.2, but when both equivalents are above 1.3, the yield slightly decreases (Fig. 4).

We calculated optimal reaction parameters using Statistica® software: temperature = 20 °C, diethyl oxalate, and sodium ethoxide equivalents = 1.23. These parameters were used to check how an increased starting concentration of **2** would affect the product yield (Table 3). The starting reagent concentrations had almost no impact on the reaction yield.

The reaction with parameters from Table 2, entry 4, was repeated at a 1 g scale. The reaction yield after purification was almost the same as in the batch (84 vs. 87%, respectively). We speculate that an improved work-up protocol may increase isolated reaction yield and thus should be studied further.

Table 2 DoE full factorial 2^2 plan and results

Entry	Time/min	Temperature/°C	Diethyl oxalate eq	Sodium ethoxide eq	Conversion/%	Product yield[a]/%
1	5	30	1.0	1.4	98.6	93.5
2	5	30	1.4	1.0	98.9	96.3
3	5	30	1.0	1.0	84.1	79.0
4	5	30	1.4	1.4	93.4	88.6
5 (C)	5	30	1.2	1.2	99.7	97.0
6 (C)	5	30	1.2	1.2	99.7	97.0
7 (C)	5	30	1.2	1.2	99.9	97.3

[a]Yield determined by UHPLC

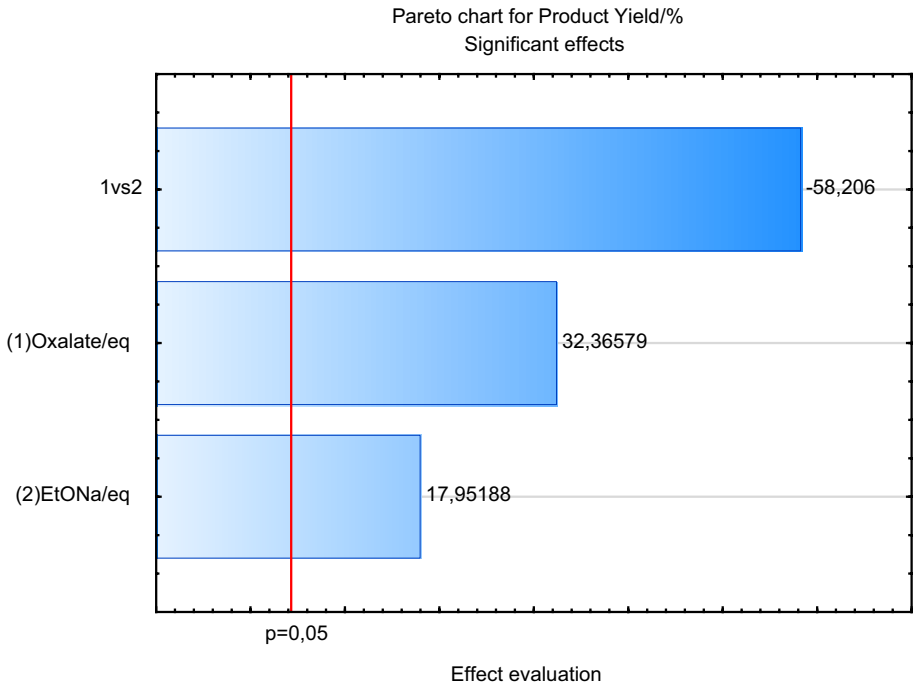


Fig. 3 Pareto chart for significant effects

Conclusion

Only with the change of the reaction solvent, we managed to shorten the batch reaction time from 20 h to 10 min and increased yield from 73 to 87%. Successfully, we transferred the reaction from batch to continuous flow reactor without a change in product yield. With the use of DoE, we were able to optimize flow synthesis parameters in a couple of experiments which resulted in reduced reaction time (2 min), almost the same isolated yield (84%), a high space–time yield (3720 kg h^{-1} m^{-3}), and a total product throughput = 74.4 g h^{-1}. Our study opens the possibility of transferring further synthesis steps to continuous flow and combining them into a telescopic process.

Experimental

Solvents and chemicals were obtained from Sigma-Aldrich and VWR and were used without any further purification unless otherwise noted. The (3*E*)-4-(3,5-dimethoxyphenyl)but-3-en-2-one (**2**) was synthesized according to the procedure published earlier [15], with a purity of 99%. THF was purchased from VWR and dried by adding 3 Å molecular sieves at least 48 h before the

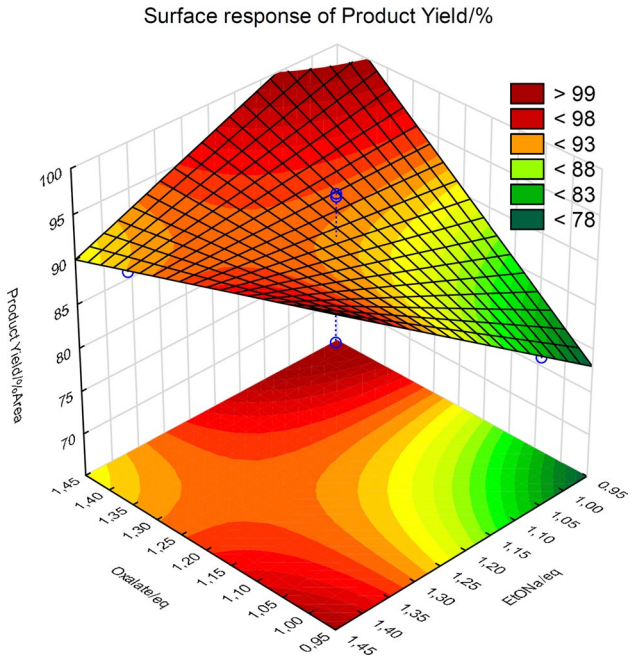


Fig. 4 Surface response for product yield/%

Table 3 Yield of **3** depending on starting substrate concentration

Entry	Substrate dilution/g cm^{-3}	Substrate concentration/mol dm^{-3}	Yield[a]/%
1	1/40	0.121	96.8
2	1/20	0.242	97.9
3	1/10	0.485	97.8
4	1/5	0.970	96.9

Reaction parameters: reaction time 2 min, temperature 20 °C, diethyl oxalate and sodium ethoxide equivalents = 1.23

[a]Yield determined by UHPLC

reaction. 2 M EtONa/EtOH was prepared by dissolving sodium rod in absolute ethanol and kept under argon for a maximum time of 1 week. ^{1}H NMR and ^{13}C NMR data were recorded on a JOEL JNMR-ECZS 400 MHz spectrometer with the residual solvent peak as an internal reference (DMSO-d_6 = 2.49 ppm). UHPLC analysis was performed on a Thermo Scientific Kinetex® C18 column (100 mm; 2.6 µm). Phase: A) H_2O + 0.1% formic acid; B) ACN + 0.1% formic acid. A 5-point calibration plot was prepared using previously synthesized **2** and **3** in a batch reaction as an internal standard for the calculation of the reaction yields. The MS analysis was acquired with an Agilent QTOF 6545 equipped with electrospray ionization (AJS ESI).

Flow experiments were performed using a Vapourtec R2C + with two tubular reactors (10 cm^3, id = 1 mm, each). All tubes (id = 1 mm) and mixers were bought from Vapourtec. Pressure in the system was maintained using a back pressure regulator of 8 bar (BPR) (Fig. 5).

Batch synthesis of ethyl 6-(3,5-dimethoxyphenyl)-2,4-dioxohex-5-enoate (3)

Reaction in ethanol

To the solution of 787 mm^3 diethyl oxalate (5.7 mmol, 1.2 eq) in 20 cm^3 dry ethanol under argon, 2.85 cm^3 of 2 M EtONa/EtOH was added (5.7 mmol, 1.2 eq). Therefore, 1.0 g (4.85 mmol) of **2** was dissolved in 20 cm^3 of hot, dry ethanol, cooled to RT, and added to the reaction mixture. The reaction was stirred for 20 h at room temperature and quenched with 1 M HCl. 20 cm^3 of brine was added, and the reaction mixture was extracted with ethyl acetate (3 × 25 cm^3). Organic phases were combined, washed with brine, and additionally dried with sodium sulfate before being evaporated, resulting in 1.45 g of yellow oil. The crude product was purified by flash chromatography (100% DCM) to give 1.08 g of yellow solid (yield = 73%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.69–7.65 (d, 1H), 7.16–7.12 (d, 1H), 6.92 (s, 2H), 6.61 (s, 1H), 6,57 (s, 1H), 4.30–4.25 (q, 2H), 3.79–3.76 (s, 6H), 1.30–1.27 (t, 3H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 184.4, 174.0, 161.5, 160.7, 142.7, 136.3, 124.1, 106.5, 103.2, 101.0, 62.1, 55.4, 13.9 ppm; HRMS: *m/z* calculated for $[M+H]^+$ 307.1176, found 307.1172.

Reaction in THF

To the solution of 787 mm^3 diethyl oxalate (5.7 mmol, 1.2 eq) in 20 cm^3 dry THF under argon, 2.85 cm^3 of 2 M EtONa/EtOH (5.7 mmol, 1.2 eq) was added. Therefore, 1.0 g (4.85 mmol, 1 eq) of **2** in 20 cm^3 of dry THF was added. The reaction was stirred for 10 min and quenched with 1 M HCl. 20 cm^3 of brine was added, and the reaction mixture was extracted with ethyl acetate (3 × 25 cm^3). Organic phases were combined, washed with brine, and additionally dried with sodium sulfate before being evaporated, resulting in 1.55 g of yellow oil. The crude product was purified by flash chromatography (100% DCM) to give 1.27 g of yellow solid (yield = 87%). ^{1}H NMR (DMSO-d_6): δ = 7.70–7.66 (d, 1H), 7.17–7.13 (d, 1H), 6.96 (s, 2H), 6.61 (s, 1H), 6,57 (s, 1H), 4.31–4.25 (q, 2H), 3.80–3.76 (s, 6H), 1.31–1.27 (t, 3H) ppm.

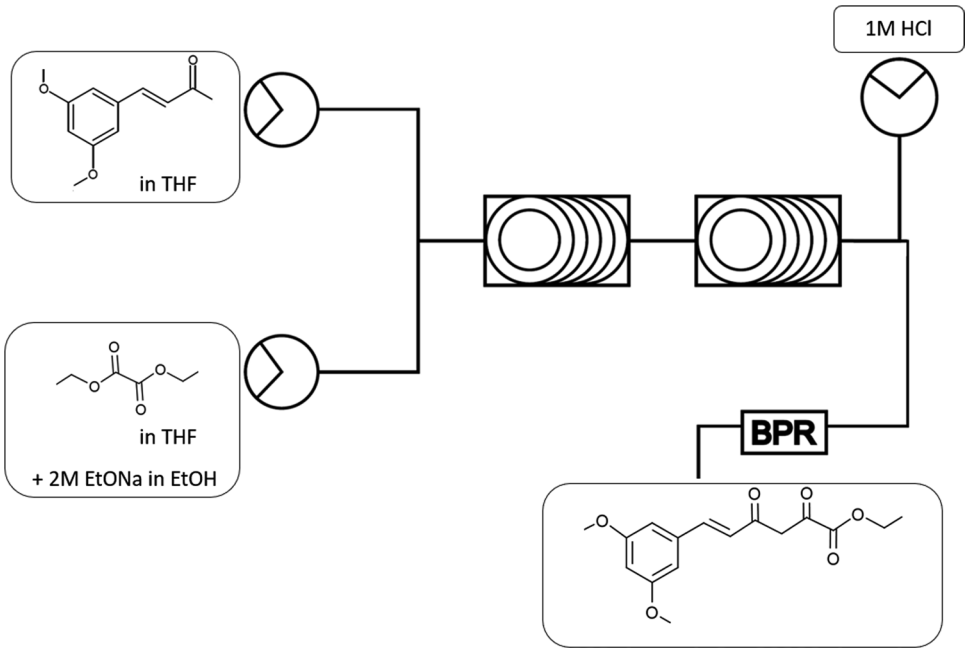


Fig. 5 Flow schematic for synthesis of **3**

Flow synthesis

General procedure for DoE experiments

Reagent A: 0.5 g of **2** was dissolved in 20 cm^3 dry THF; reagent B: appropriate amounts of diethyl oxalate and 2 M EtONa/EtOH were dissolved in dry THF to gain 20 cm^3 of each solution with the required amounts of equivalents. The solvent bottle was filled with dry THF (for both reagents).

Reagent A and reagent B feeds were mixed in a standard Y-shaped mixer at the same flow speeds, then allowed to react in two 10 cm^3 PFA reactors (id = 1 mm, each) at a set temperature. Just after the last reactor outlet, the reaction mixture was quenched in a Y-shaped mixer with 1 M HCl feed at the same flow speed as each reagent. Samples were collected into vials and analyzed offline by UHPLC.

Flow synthesis in 1 g scale

Reagent A: 8.4 g of **2** was dissolved in 42 cm^3 of dry THF; reagent B: 6.6 cm^3 of diethyl oxalate (1.23 eq) and 24 cm^3 of 2 M EtONa/EtOH (1.23 eq) were dissolved in 11.4 cm^3 of dry THF. The solvent bottle was filled with dry THF (for both reagents).

For the reaction, 40 cm^3 of each reagent was used. Both reagents were pumped at a 5 cm^3 min^{-1} flow rate. Reagent A and reagent B feeds were mixed in a standard Y-shaped mixer at the same flow speeds, then reacted in two 10 cm^3 PFA reactors (id = 1 mm) at the set temperature. Just after the reactor outlet, the reaction mixture was quenched with 1 M HCl (flow rate: 5 cm^3 min^{-1}) in a Y-shaped mixer. The reaction mixture was collected at a steady state (after 4 min of residence time) for 2 min (which equals 2.0 g, 9.7 mmol of the substrate) into a 100 cm^3 glass bottle and extracted with ethyl acetate (3×35 cm^3). Organic phases were combined, washed with brine, and additionally dried with sodium sulfate before being evaporated, resulting in 1.78 g of dark yellow solid. The crude product was purified by flash chromatography (100% DCM) to give 1.26 g of yellow solid (yield = 84%).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00706-023-03121-z.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

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