Application Note 4: Evaluation of the Effect of Reaction Conditions on the Base-catalysed Knoevenagel Condensation using Labtrix® S1

The Knoevenagel Condensation \[1\] is a synthetically useful route to the formation of α,β-unsaturated compounds and comprises of a reaction between an aldehyde or ketone with an activated methylene compound in the presence of a weak base, usually a 1°, 2° or 3° amine or their salts. Demonstrating wide substrate scope, examples of the Knoevenagel Condensation reaction have been found as key steps in the synthesis of natural products such as the antiinsectan (±)-leporine A \[2\] and an indole alkaloid (±)-hirsutine \[3\], lumefantrine, an active ingredient in the antimalarial Coartem \[4\], along with the preparation of charge transfer materials such as 1,3-diethyl-5-(2-methoxybenzylidene)-2-thioxohydroprymidine-4,6(1H,5H)-dione \[5\]. With this in mind, the Knoevenagel Condensation between benzaldehyde 1 and ethyl cyanoacetate 2 was investigated in the presence of piperidine 3 under continuous flow to afford (E)-ethyl-2-cyano-3-phenylacrylate 4 as the sole reaction product.

Reaction Conditions: Reactions were performed using a Labtrix® S1 system, as illustrated in Figure 1a, fitted with a 10 μl glass micro reactor (3123) containing static micro mixers (Figure 1b). Reactants were delivered to the reactor using 3 x 1000 μl gas-tight syringes (SGE) and the system maintained under a positive pressure of 25 bar using an ULDV-BPR (Upchurch Scientific). Two reactant stock solutions (5 ml) were prepared, the first comprised of benzaldehyde 1 (1.0 M) and piperidine 3 (0 to 10 mol %) in EtOH and the second ethyl cyanoacetate 2 (1.0 M) in EtOH. In order to terminate the reactions prior to analysis, a quench solution of ethanolic HCl (10 %) was employed.

Figure 1. Illustration of (a). Labtrix® S1, the micro reactor development equipment used for the automated evaluation of synthetic reactions under continuous flow conditions and (b). the static micro mixers available within Labtrix® S1 micro reactors.
Prior to performing a micro reaction, the micro reactor was filled with EtOH at a total flow rate of 25 μl min⁻¹ for 5 min, after which time the syringes were replaced with those containing the aforementioned stock solutions and the reactor primed, again at a total flow rate of 25 μl min⁻¹, for 20 min (25 °C) (Figure 2). Reaction products were collected in 50 μl aliquots, using the automated sample collection function, and analysed offline using a Varian GC-FID (Zebron ZB-5 capillary column (Phenomenex, UK) = 30 m (long) x 0.25 mm (i.d.) x 0.25 μm film thickness, oven program = 50 °C (initial), ramped to 300 °C at 60 °C min⁻¹ and held for 10 min), with compound retention times compared to commercially available or fully characterised synthetic standards. Reaction progress was quantified by determining the percentage conversion of benzaldehyde 1 to (E)-ethyl-2-cyano-3-phenylacrylate 4, with 100 % conversion denoting complete consumption of the aldehyde 1. EtOH was selected as the reaction solvent due to its high solubilising properties, enabling use of high reactant concentrations (1.0 M) and affording increased system throughputs. When using high reaction temperatures, transesterified products can result and it is therefore important to bear this in mind if considering adapting the methodology to the reaction of other activated methylenes.

![Figure 2](image)

Figure 2. Illustration of the micro reactor (3123) manifold used for the Knoevenagel Condensation reaction described herein.

Results and Discussion: Using the aforementioned conditions, a series of micro reactions were performed under automated control, enabling rapid exploration of the effect of reaction time, temperature and reactant stoichiometry. Initial investigations centred on establishing the effect of base 3 concentration on the reaction conducted at room temperature. With this in mind, a series of reactions (n = 5) were performed using 0, 1, 2.5, 5.0, 7.5, 10.0 and 15.0 mol % piperidine 3 with the resulting reaction products diluted using EtOH (0.5 ml) prior to offline analysis. As expected, Figure 3 illustrates an increasing proportion of product 4 formation with increasing mol % of piperidine 3. The proportions employed however are limited based on the relative insolubility of piperidine hydrochloride that forms upon quenching the reaction mixture. In addition, when developing an efficient synthetic methodology, it is desirable to employ small quantities of catalytic material in order to reduce processing costs and the complexity of subsequent purification steps. With this in mind, the effect of reaction temperature on the system was investigated with 5.0 mol % of base 3.
Having identified that the reaction could be dramatically improved by employing elevated reactor temperatures, the effect of reaction time was investigated with a view to quantitatively converting benzaldehyde 1 and ethyl cyanoacetate 2 into (E)-ethyl-2-cyano-3-phenylacrylate 4. To achieve this, reactant concentrations of 1.0 M and and 5.0 mol % of piperidine 3 were maintained and a fixed reactor temperature of 150 °C was employed, varying reaction time from 30 to 300 s. Using this approach it can be seen in Figure 5 that a reaction time of 91 s is optimal for the quantitative conversion of the starting materials to the target product 4. Interestingly, no decomposition of the reaction product 4 was observed with extended reaction times ranging from 91 to 300 s.
Operating under the aforementioned conditions, the device afforded (E)-ethyl-2-cyano-3-phenylacrylate 4 at a theoretical throughput of 39.8 mg h⁻¹. With this in mind, the reaction products were collected in a single vessel over a period of 1.25 h as a means of synthesising sufficient material for isolation and full characterisation. In order to remove the resulting piperidine HCl, the reaction products were concentrated in vacuo and subjected to an aqueous extraction (Organic = DCM, Aqueous = sat. NH₄Cl). The organic extract was dried over MgSO₄, filtered and concentrated in vacuo to afford of (E)-ethyl-2-cyano-3-phenylacrylate 4 as a white crystalline solid in 97.9 % yield (48.7 mg). As Figure 6 illustrates, dissolution of the resulting reaction product 4 in CDCl₃ followed by analysis by 1H NMR spectroscopy confirmed the synthesis of (E)-ethyl-2-cyano-3-phenylacrylate 4 in analytical purity.
Summary: The present application illustrates the steps taken to optimise a synthetic reaction under continuous flow conditions. Using static micro mixers, it was found that the Knoevenagel Condensation reaction could be efficiently performed with reaction times of 91 s and only 5.0 mol % of catalyst 3. Subsequent aldehydes were also investigated and the reaction conditions identified found to be general; in the case of less soluble aldehydes, reactant concentrations of 0.1 to 0.5 M can be employed. As discussed, a limitation of this flow reaction was found to be the low solubility of the quaternary ammonium salt formed during the condensation reaction; as such reaction rate could not be increased by the use of more catalyst leaving reaction time and temperature as the only variables. An alternative therefore would be to use a solid-supported base, which would remove the issue of intermediate insolubility and the need for an aqueous extraction of the reaction products, affording a increased productivity per micro reactor. Solid-supported reagents, catalysts and scavengers can be employed under continuous flow conditions using our new packed-bed reactor (Device 3026, Figure 7), details of which can be found at www.chemtrix.com and will be the subject of subsequent application notes.

Figure 7. Schematic of Chemtrix BV packed-bed reactor (3026), suitable for using commercially available solid-supported reagents, catalysts and scavengers.

References:

Note: Care must be taken when selecting the reaction solvent and activated methylene under investigation, in order to avoid transesterification of the reaction product upon quenching with ethanolic HCl.

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