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An efficient and scalable synthesis of thiazolo ring fused 2-pyridones using flow chemistry

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Dedicated to Professor Jan Bergman on the occasion of his 80th birthday

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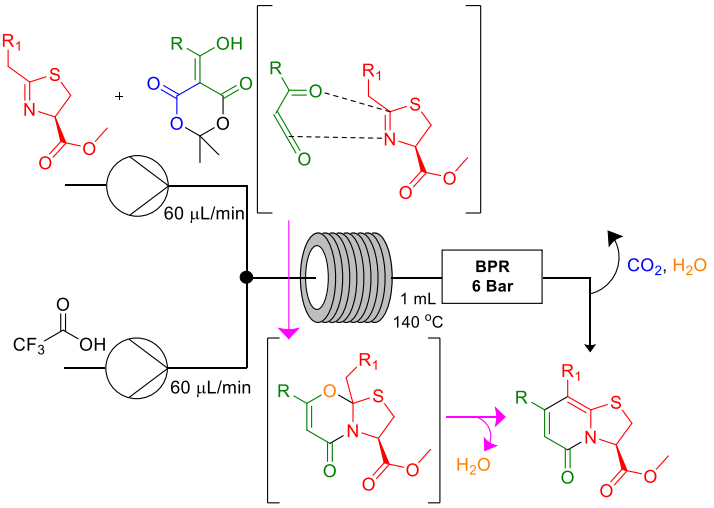
Abstract

Thiazolino ring fused 2-pyridones are a valuable scaffold with varied and substitution dependent biological activity, accessed primarily by an acyl ketene-imine cycloaddition and rearrangement. Although powerful, some aspects of this chemistry such as the requirement for excess starting material and the production of gas can make larger scale synthesis challenging. Here we describe the development, application and scaling of a continuous flow process allowing larger scale synthesis, with better handling of hazards and more reliable scaling. Optimisation and control of conditions allows for a more efficient synthesis, with a lower equivalence of the acyl ketene precursor required.



Keywords: Flow chemistry, pyridone, thiazolo, heterocycle, acyl ketene, design of experiments

Introduction

The ring fused 2-pyridone backbone has proven to be an invaluable starting point in the development of small molecules with selective biological activity. Differentially substituted variants of this privileged scaffold have previously shown activity as virulence inhibitors *in E. coli.,*[1-2] Listeria and Chlamydia infections[3-5] and modulators of protein aggregation associated with neurodegenerative diseases.[6-13] In a recent study, a cyclopropyl analogue (Fig. 1, **A**) was shown to inhibit *Mycobacterium tuberculosis* (*Mtb*) ability to convert into its tolerant state. These compounds were named Mycobacterial Tolerance Inhibitors (MTIs) and besides sensitizing *Mtb* to host defense responses (*e.g.* oxidative stress and low pH) the most striking effect was that MTIs could make isoniazid resistant *Mtb* sensitive to the front line antibiotic isoniazid again.[14] While the structure activity relationship (SAR) observed varies depending on the specific application, the combination of a rigid, peptide mimicking face and a heterocyclic framework with drug-like properties has proven widely applicable.

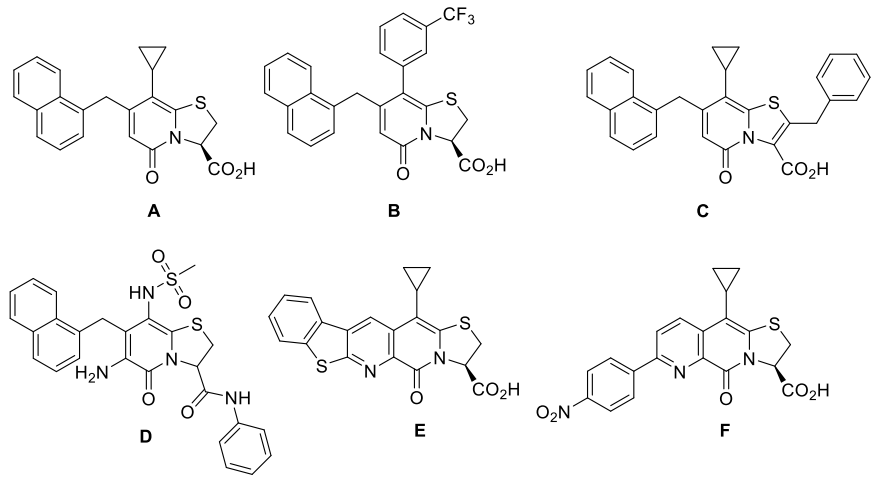


Figure 1. Biologically active thiazolino-2-pyridones

The transformation used to form these heterocycles was first discovered in an attempted β-lactam synthesis. Briefly, it comprises an acyl ketene-imine cycloaddition from an Meldrum's acid analogue and subsequent acid catalyzed rearrangement.[15] Numerous approaches have been developed for the direct functionalization of multiple positions on the bicyclic scaffold as well as its ring opening/expansion[13, 16-18] and annulation with different heterocycles. (Fig. 2).

However, all these approaches require access to the initial scaffold. The underlying methodology is powerful; multiple bonds and a new ring system is produced from simple, readily accessible starting materials. The reaction is relatively quick,[19] maintains the incorporated stereochemistry and tolerates a wide range of chemical functionality.

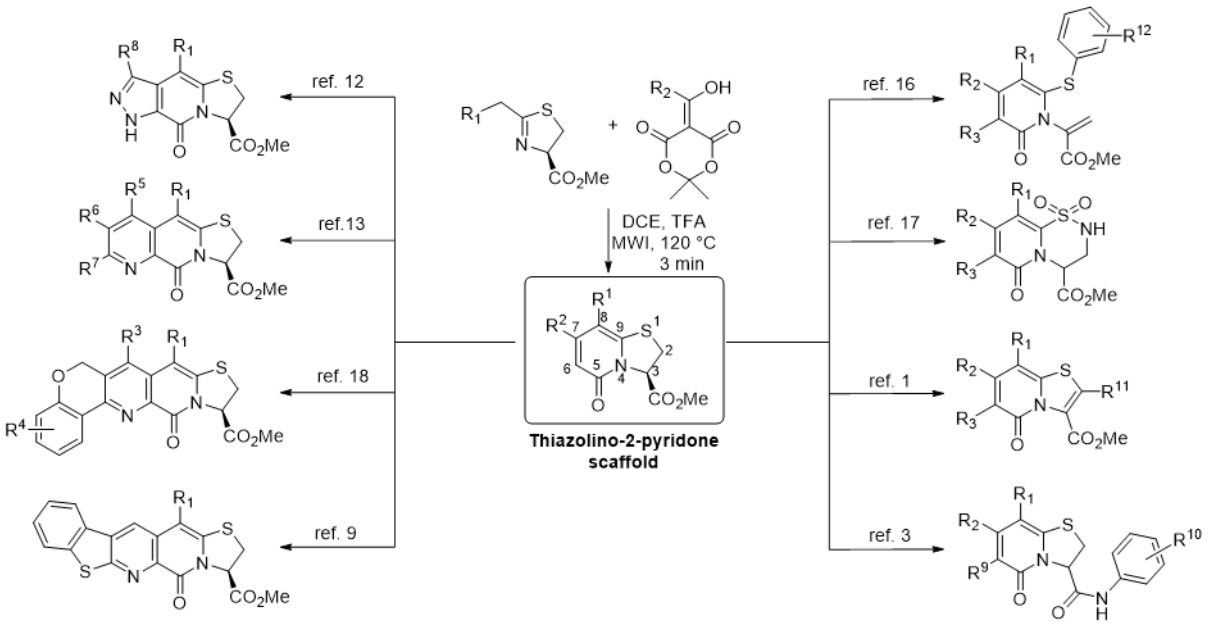


Figure 2. Synthetic applications of ring fused thiazolino-2-pyridones

There are, as with many synthetic procedures, some specific limitations of this approach. High equivalence of the acyl Meldrum's acids, which are synthesized, are usually required for good conversion, and the high temperatures and acidic conditions are not compatible with all functionalities. Side products resulting from the excess acyl Meldrum's acid cause impurities in sufficiently large quantities to require chromatographic purification. As the optimal reaction temperatures are in excess of the solvent boiling point, sealed vessels are used. Microwave heating can be used, but this typically limits the scale of reaction[20] and requires high concentrations of material to maximize throughput. At these higher concentrations, acyl Meldrum's acid starting materials differ substantially in the portion of material in solution and subsequent reactant concentration. Additionally, these conditions may decompose acyl Meldrum's acid below the optimal temperatures for subsequent cycloaddition, and slow or variable heating rates can effect yields. The $CO_{2(g)}$ generated also causes significant pressure, which represents a hazard. As analogues based on the scaffold are further developed and progress, larger quantities of material are required and these scaling issues are more problematic.

One approach to developing safe[21] and readily scalable procedures for chemistries involving unstable intermediates and intense conditions is flow chemistry.[22-26] This approach has been successfully applied to heterocycle synthesis,[27-28] cycloadditions[29-30] and even to acyl ketene formation,[31] and recent developments continue to show the potential flexibility[32] and portability[33] of this approach. Flow chemistry is often greener[34] than batch chemistry, and sometimes allows access to transformations near impossible[35] under batch conditions. At one point a mostly academic pursuit,[36] continuous flow approaches are of increasing interest for pharmaceutical API production.[36-37]

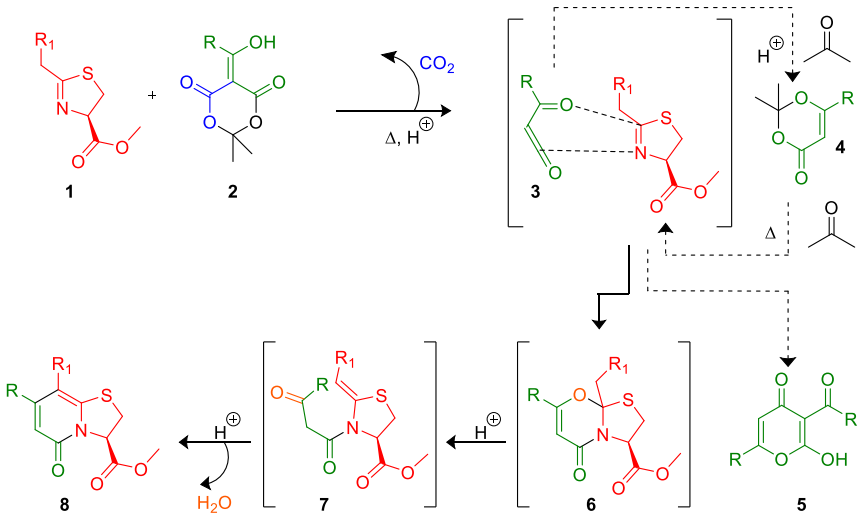


Figure 3. Synthetic applications of ring fused thiazolino-2-pyridones

To evaluate the amenability of our procedure to a flow chemistry synthesis, we first considered the mechanism. Understanding of this transformation has been refined over time; later research demonstrated that the intermediate reacting with thiazoline **1** likely took the form of an α-oxoketene (**3**),[38] with analogous reactions on related frameworks suggesting initial formation of a 1,3-oxazine-4-one **6**,[39] followed by ring opening to **7** and acid catalyzed cyclocondensation and aromatization to give the final fused 2-pyridone form **8** (Fig. 3). It is well established that dehydroacetic acid analogues (**5**) are major products of acyl-ketene self-dimerisation,[40-41] but recent results suggest that the acetone byproduct of decomposition can reversibly react with acyl-ketenes to form dioxin-4-one **4**, potentially acting as a store of this reactive intermediate.[31]

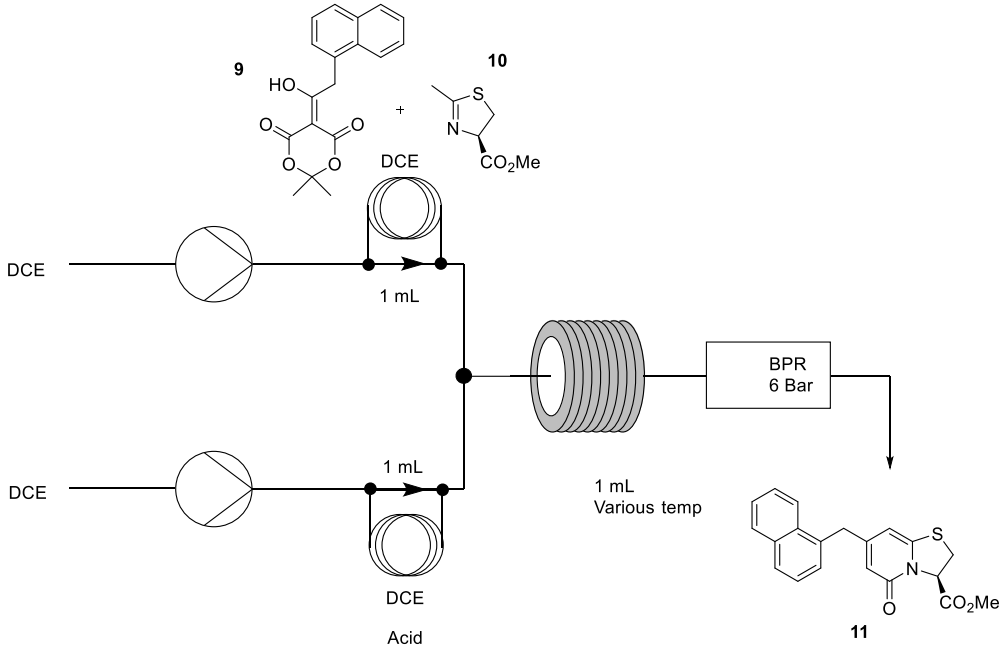
Some of the advantages to scaling our ketene-imine cycloaddition process in a flow setting are practical and obvious; heating and subsequent gas generation occur in only a small portion of our material at any time, and the $CO_{2(g)}$ is continually vented. As such, pressure build up in sealed containers is avoided. Scale can be adjusted by extending the run time, rather than running numerous batches for combination, and the containment available for the whole system is more robust than a series of microwave vials.

There are a few other advantages arguably less immediately obvious. Among these is improved and scalable mixing: in general, reagent mixing is more efficient in flow systems.[22, 26, 36, 42-44] In our system, where a reactive intermediate is generated *in-situ* and multiple steps occur within a short reaction time, efficient mixing is potentially important. Further, batch processes are frequently carried out at concentrations above the room temperature acyl Meldrum's acid solubility in order to maximize output; this increases the demand for efficient mixing. Increasing scale is much less demanding using a flow procedure and a lower concentration can be used. Variations in scale, concentration and vessel headspace can make the distribution between liquid and gas phases variable in our batch process. This is potentially important as both TFA (required for multiple steps) and our acetone byproduct boil below the reaction temperature. As previously mentioned, the acyl-ketene **3** would be expected to react reversibly with acetone to give **4**, which effectively lowers the concentration but preserves the lifespan of our acyl-ketene.[31] As such, the variation in headspace and pressure which would accompany changes in scale and vessel size may affect our process.[22] Probably most importantly, the heat transfer properties of flow reactors are usually superior, and we made use of a glass rather than polymer microfluidic reactor to maintain this advantage.[25] This improved heat transfer[20, 25] manifests as narrower temperature gradients, and these tighter temperature distributions help limit side reactions.[22] Additionally, the change in heat transfer with tube diameter scales in a linear fashion.[26]

Results and Discussion

Design of Experiments (DoE):

Another practical advantage of flow chemistry is the capacity to perform numerous trial experiments sequentially under tightly controlled conditions. In order to optimize our search for improved reaction conditions we made use of a Design of Experiments (DoE) approach in order to simultaneously optimize for multiple variables.[45] Conversion was assessed by ^{1}H NMR comparison of the residual thiazoline **10** to the newly formed pyridone. For this, the synthesis of simple napthyl pyridone **11** was used (Scheme 1). Microwave syntheses of this pyridone typically give yields of 60-70% using 2.75 eq. acyl ketene precursor. As such, there is room both for improvements in yield and reduction in excess starting reagents.



Scheme 1. The set-up for the flow system during the optimisation.

Acid screen

Trifluoroacetic acid[38] is normally used, but both stronger[15] and weaker[46] acids have been employed previously. We re-investigated this variable under flow conditions using methanesulfonic, trifluoroacetic and acetic acids for a reasonable pKa variation. We simultaneously screened a limited range of concentrations – as the relative rates of ketene formation, cycloaddition, ring opening and condensation are both unknown and likely substrate dependent, it is unclear what the optimal concentration and pKa might be from mechanistic considerations. Our results suggest TFA remains the optimal choice, with very little reaction in particular where the weaker acetic acid was employed. Further, a higher equivalence of acid proved preferable and gave greater improvement where more equivalents of acyl ketene precursor were employed.

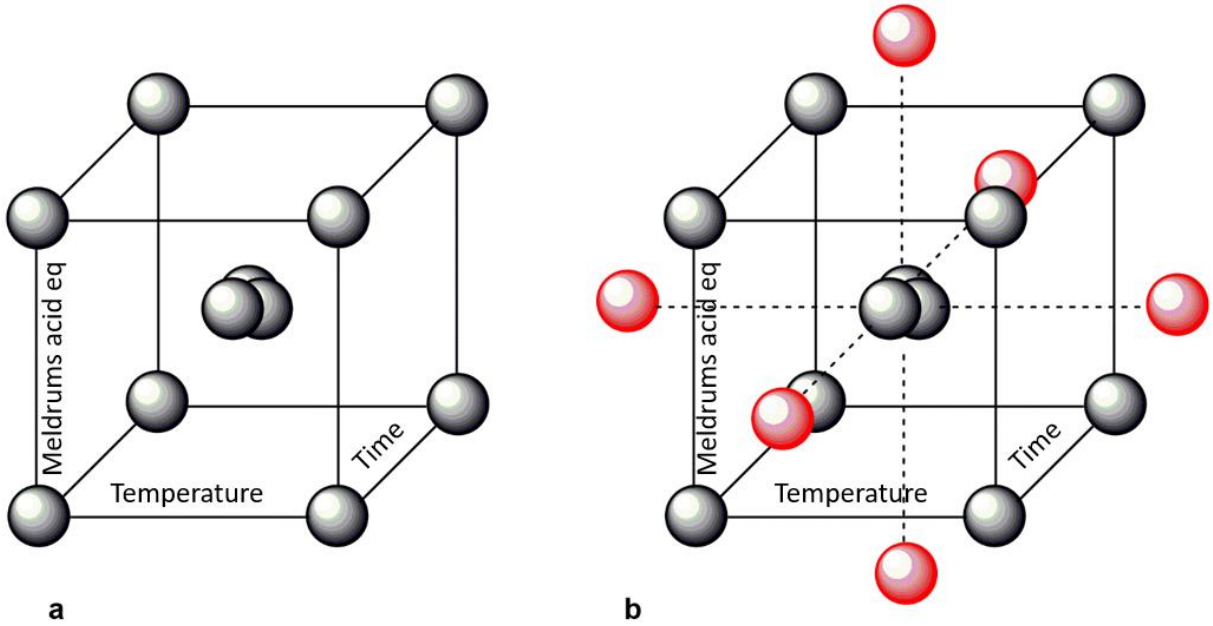


Figure 4. (a) Screening design for 2-pyridone synthesis. A full factorial design using four variables – temperature, reaction time, equivalents of Meldrums acid derivative, and concentration of thiazoline (not shown) – resulting in a total of 19 experiments including three central points. **(b)** Optimisation design for 2-pyridone synthesis. A central composite design using three variables – temperature, reaction time, and equivalents of Meldrums acid derivative – resulting in a total of 17 experiments including three central points.

Initial screen

For our initial screen, a full factorial design using four different variables were created (see Fig 5a and Table 1, Entry 1-19). The variables chosen were time (1-5 minutes), temperature (100-140 °C), concentration (0.18-0.38 M, with respect to thiazoline) and equivalence of Meldrums acid derivative (1-3). Calculated conversions from the 19 experiments were correlated with the different variables using multiple linear regression (MLR). This gave a model with ten coefficients which explained 95% of the variation in the response (R2Y = 0.95) and had good predictive properties according to cross-validation with a Q2 of 0.87. The regression coefficients (Figure 5a) revealed that, within the range studied, concentration had no significant effect on conversion. Unsurprisingly, increased ketene precursor equivalents lead to higher conversion, but more importantly so did increased reaction time. Further, a positively correlated cross-term between the reaction time and precursor equivalence indicates that an increased reaction time can compensate for lower acyl ketene precursor equivalence. This was unexpected, as residual precursor is not observed even under relatively mild conditions. While no explicit investigation was attempted with regard to the lifetime of the acyl ketene species, the combination of this time dependence and the rapid consumption of precursor, taken with previous observations on the lifetime of α-oxoketene species[38] such as **3** raises the possibility that a precursor species such as **4** persists in the reaction mixture for some time.

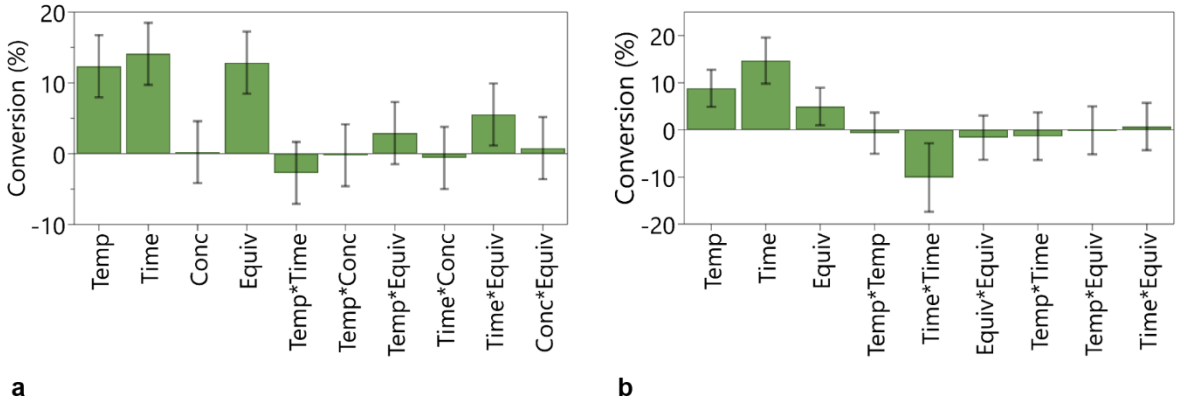


Figure 5. Multiple linear regression (MLR) models of the flow conditions used for synthesis of 2-pyridone **11 (a)** Regression coefficients of the model from the screening design (R2 = 0.95, Q2 = 0.87) showing the investigated variables and their correlation to the conversion of thiazoline to 2-pyridone. **(b)** Regression coefficients of the model from the optimization design (R2 = 0.96, Q2 = 0.64).

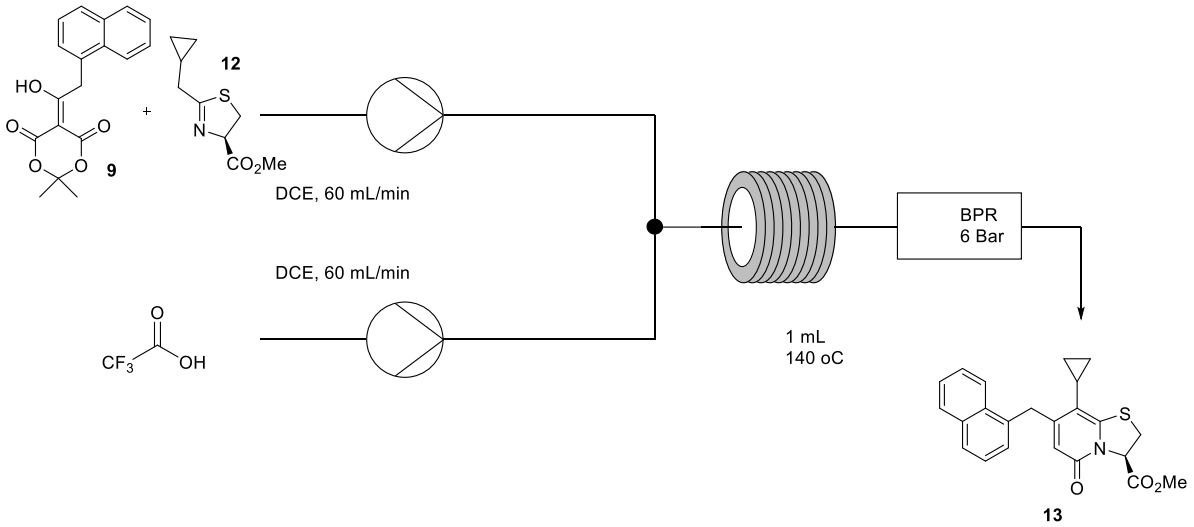
Table 1. Screening and optimisation designs

	Entry	Temp (°C)	Time (s)	Thiaz-oline conc (M)	Meld-rums acid Equiv	Conv (%)		Entry	Temp (°C)	Time (s)	Thiaz-oline conc (M)	Meld-rums acid Equiv	Conv (%)
Screening design	1	100	60	0.2	1	0,5	Optimisation design	20	130	114	0.18	1.5	40
	2	140	60	0.2	1	28		21	130	742	0.18	1.5	65
	3	100	300	0.2	1	26		22	100	114	0.18	1.5	23
	4	140	300	0.2	1	39		23	100	742	0.18	1.5	51
	5	100	60	0.38	1	3		24	130	114	0.18	2.5	48
	6	140	60	0.38	1	26		25	130	742	0.18	2.5	71
	7	100	300	0.38	1	24		26	100	114	0.18	2.5	28
	8	140	300	0.38	1	36		27	100	742	0.18	2.5	61
	9	100	60	0.2	3	11		28	140	500	0.18	2	75
	10	140	60	0.2	3	44		29	115	30	0.18	2	17
	11	100	300	0.2	3	54		30[1]	115	1486	0.18	2	74
	12	140	300	0.2	3	81		31	90	500	0.18	2	41
	13	100	60	0.38	3	12		32	115	500	0.18	1.2	44
	14	140	60	0.38	3	49		33	115	500	0.18	2.8	67
	15	100	300	0.38	3	56		34	115	500	0.18	2	67
	16	140	300	0.38	3	81		35	115	500	0.18	2	60
	17	120	187.5	0.29	2	52		36	115	500	0.18	2	64
	18	120	187.5	0.29	2	47							
	19	120	187.5	0.29	2	50							

[1]outlier, removed from the model

Optimisation screen

In light of the results from the initial screen, we chose to further interrogate the relationship between time, temperature and acyl ketene precursor equivalence to hone our preferred conditions, this time investigating some intermediate temperatures, times and equivalents in an attempt to identify any deviations from linear dependency. Thus, we set-up a central composite design with the three variables in five different levels (See Fig 4b and Table 1, Row 20-36) for a total of 17 experiments. MLR of the different variables correlated with the calculated conversions gave a model with nine coefficients having a R2Y of 0.95 and Q2 of 0.64 (Fig 5b) after removing one outlier (Entry 30, in Table 1). Also here we could obviously see the same positive correlation of time, temperature, and equivalents of Meldrum's acid derivative with the conversion. Interestingly, a negatively correlated square term of the time indicates that the optimal reaction time actually is within the investigated interval when the experiment with the longest reaction time was removed as an outlier.



Scheme 2. The set-up for the flow system during continous flow.

The results from both the designs lead us to use the higher temperature tested (140 °C) and a longer reaction time (500 s). This allowed us to reduce equivalence to 2 without loss of conversion relative to batch MWI experiments.

Table 2. Flow vs batch (microwave) synthesis of C10 methyl ester

Material	TFA eqv.	MA eqv.	T (°C)	t (s)	*ee* (%)	Yield
MWI	1[b]	2	120	180	83	59%
Flow	3	2	140	500	73	93%

[a] Based on chiral HPLC measurements. [b] Normal lab conditions; running with 3 eqv. TFA does not change the observed yield under these conditions.

Although the napthyl pyridone is useful,[47] we decided for purposes of scale up to directly synthesize C10 methyl ester **13** (Scheme 2), a molecule with very interesting anti-mycobacterial activity and frequently elaborated upon for synthesis of new analogues. This synthesis is generally somewhat higher yielding, and we carried out small scale trials which confirmed good conversion with 2 eqv. We carried out this synthesis on gram scale and then in batch microwave conditions with our prior procedure (Table 2). One important feature of this 2-pyridone synthesis is retention of stereochemistry from the thiazoline unit.[15] In practice, in more recent procedures this retention is not absolute, but a reasonable enantiomer excess is retained, and we also used chiral HPLC to check the enantiopurity of the resulting material. When using the same lower acyl-ketene precursor equivalents in all experiments, we were able to observe a 30%+ improvement in yield, which frankly exceeded our initial expectations. Unfortunately, some trade off in enantiopurity was observed, with more

epimerization in the flow material. However, definite advantages are possible in scaling: simply by switching from a 1 mL reaction chamber to a larger, off-the-shelf (16 mL) loop our results suggest up to 192 grams of material could be produced in a 24 hour period. The previous microwave approach would require 320 separate batch reactions to achieve similar quantities, and would require more starting material, be more time consuming and imply more safety issues. Scaling instead in larger, thermal batch reactions would exacerbate the issues with pressure, and be far less predictable and less efficient in scaling.

Conclusions

Application of continuous flow technology and a Design of Experiment approach to optimisation has allowed us to develop an efficient procedure for the acyl ketene-imine cycloaddition based synthesis of the thiazolo fused 2-pyridone scaffold. Accessing quantities of these materials can be challenging and we anticipate this approach could be a valuable development in our exploration of the potential applications of this scaffold. Further, the difference in yield and enantiopurity *vs* batch procedures raises questions about how these changes occur, which will be a focus of future investigation, particularly with regard to separating these properties, retaining efficient conversion while retaining higher *ee*. Our model suggests high yields might also be possible at lower reaction temperatures, given a longer reaction time, and this is a promising starting point for further investigation. Other future developments will likely include application to scaffold variants generated under other synthetic conditions,[4, 48] and addition of in-flow work-up procedures followed by further synthetic steps, such as ester hydrolysis to the procedure.

Experimental Section

General. All flow reactions were performed using a Syrris Asia flow system[49] under an inert atmosphere with dry solvents. Unless otherwise stated, purchased reactants and reagents were used as received from commercial suppliers. Microwave reactions were performed in sealed vessels using a Biotage® Initiator microwave synthesizer, temperatures were monitored by an internal IR probe, stirring was mediated magnetically. TLC was performed on purchased aluminum backed silica gel plates (median pore size 60 Å, fluorescent indicator 254 nm) and detected with UV light at 254 and 366 nm. Flash column chromatography (eluents given in brackets) employed normal phase silica gel (40-63 μm, VWR Chemicals). Automated flash column chromatography was performed using a Biotage® Isolera One system and purchased pre-packed silica gel cartridges (Biotage® SNAP Cartridge, KP-Sil). Optical rotation was measured with a Rudolph Autopol IV polarimeter 343 at 22 °C and 589 nm. [α] is reported in deg·mL·g^{-1}·dm^{-1}, concentrations (*c*) are given in g/100mL. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III Ultrashield 500 MHz or a Bruker Avance III 400 MHz spectrometer with a BBO-F/H Smartprobe^{TM} or a Bruker Avance III HD 600 MHz spectrometer with a CP BBO-H/F, 5 mm cryoprobe, at 298 K, unless other temperature is given. All spectrometers were operated by Topspin 3.5. Resonances are given in ppm relative to TMS, and calibrated to solvent residual signals [$CDCl_3$: δ_H = 7.26 ppm; δ_C = 77.16 ppm. $(CD_3)_2SO$: δ_H = 2.50 ppm; δ_C = 39.51 ppm. $(CD_3)CO$ δ_H = 2.05 ppm; δ_C = 29.84 ppm]. The following abbreviations are used to indicate splitting patterns: s = singlet; d = doublet; dd = double doublet; t = triplet; m = multiplet; bs = broad singlet. Chiral HPLC of cyclopropyl thiazoline **11** was carried out using a Diacel Chiracel OD-H (250 x 4.6 mm) column and eluting isocratically (Hexane:^{i}PrOH 90:10) at ambient temperature, then detected by UV at 254 nm. Injection was 10 μL at 1 mg/mL in $CHCl_3$. Chiral HPLC of pyridone **13** was carried out using a Lux 5 μm i-amylose-1 (250 x 4.6 mm) column and eluting with a gradient (^{i}PrOH 30:70 to 100% hexane) at ambient temperature, then detected by UV at 254 nm. Injection was 10 μL at 1 mg/mL in MeOH.

Optimisation of flow conditions for the synthesis of 2-pyridone (11). The flow equipment was set up according to Scheme 1. Naphthyl Meldrum's acid derivative **9** and methyl thiazoline **10** were taken up in DCE (1 mL) at various concentrations (see Table 1) and filled into loop 1. Acid (see Table 1) was taken up in DCE (1 mL) at various concentrations and filled into loop 2. The two loops were simultaneously injected to pass through a 1 mL chip reactor at different flow rates, pressurised by a 6 bar back pressure regulator and the outflow at a steady reaction state was collected. 50 μl of the reaction mixture was diluted with 450 μl $CDCl_3$ and analyzed with ^{1}H-NMR, comparing the α proton of the thiazoline to the C6 hydrogen in the pyridone in order to calculate the conversion rate of thiazoline **10** to 2-pyridone **11**.

Scale-up procedure for the continuous flow synthesis of 2-pyridone (13). The flow equipment was set up according to Scheme 2. Naphtyl Meldrum's acid derivative **9** (0.72 M) and cyclopropyl thiazoline **12** (0.36 M) were dissolved in DCE (15 mL). TFA (1.08 M) was dissolved in DCE (15 mL). The two solutions were simultaneously pumped (flow rate 60 μL to achieve a residence time of 500 s in the reactor) through a 1 mL chip reactor, pressurised by a 6 bar back pressure regulator, and 27.5 mL of the outflow at a steady reaction state was collected. The reaction solution was quenched with aqueous $NaHCO_3$ (sat., 30 mL) and the organic phase was collected and the aqueous phase re-extracted with CH_2Cl_2 (2 x 30 mL). The combined organic phases was washed with brine (30 mL) and dried over Na_2SO_4 before concentration then, purified by column chromatography (heptane:EtOAc, 1:2 to 1:4) to give **13** (1.8 g, 4.60 mmol, 93% yield) as a white solid. ^{1}H and ^{13}C NMR were in accordance with data previously published.[47]

MWI synthesis of 2-pyridone (13). In a microwave reaction tube equipped with a magnetic stirrer, thiazoline **12** (1.5 mmol, 1.0 eq.) and Meldrum's acid derivative **9** (0.94 g, 3.0 mmol, 2.0 eq.) were dissolved in DCE (6 ml). TFA (115 μl, 1.5 mmol, 1.0 eq.) was added, the tube was sealed and heated to 120 °C under microwave irradiation for 3 min. The reaction mixture was cooled to room temperature, diluted with DCM and washed with aqueous $NaHCO_{3(aq)}$ (sat.), followed by brine. The aqueous phases were re-extracted with DCM. The organic phases were combined, dried, filtered and evaporated and purified by column chromatography as in the above procedure.

Epimerization of thiazoline (12). In a round bottom flask equipped with magnetic stirrer, thiazoline (2.0 mmol, 1.0 eq.) was dissolved in Dichloromethane (2 ml). To this, K_2CO_3 (8.0 mmol, 4.0 eq.) was added and the mixture stirred at rt for 4 days. The reaction mixture was diluted with DCM and washed with brine twice. The organic phase was dried over Na_2SO_4, filtered and evaporated, then purified by column chromatography (heptane to EtOAc).

Naphthyl Meldrum's acid derivative (9). Meldrum's acid analogue **9** was prepared according to the earlier reported procedures.[13] Crude product was recrystallized from a dichloromethane:heptane (1:4) mixture.

Thiazolines 10 and **12** were synthesized according to literature procedures[4] and match previously published data.[50]

Acknowledgements

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Supplementary Material

^{1}H and ^{13}C NMR spectra of the material synthesis in flow and the chiral HPLC traces used for *ee* determination can be found in the supplementary material.

References

1. Chorell, E.; Pinkner, J. S.; Phan, G.; Edvinsson, S.; Buelens, F.; Remaut, H.; Waksman, G.; Hultgren, S. J.; Almqvist, F., *J Med Chem* **2010,** *53* (15), 5690-5.
https://doi.org/10.1021/jm100470t
2. Pinkner, J. S.; Remaut, H.; Buelens, F.; Miller, E.; Aberg, V.; Pemberton, N.; Hedenstrom, M.; Larsson, A.; Seed, P.; Waksman, G.; Hultgren, S. J.; Almqvist, F., *Proceedings of the National Academy of Sciences* **2006,** *103* (47), 17897-17902.
https://doi.org/10.1073/pnas.0606795103
3. Good, J. A. D.; Kulen, M.; Silver, J.; Krishnan, K. S.; Bahnan, W.; Nunez-Otero, C.; Nilsson, I.; Wede, E.; de Groot, E.; Gylfe, A.; Bergstrom, S.; Almqvist, F., *J Med Chem* **2017,** *60* (22), 9393-9399.
https://doi.org/10.1021/acs.jmedchem.7b00716
4. Kulen, M.; Lindgren, M.; Hansen, S.; Cairns, A. G.; Grundstrom, C.; Begum, A.; van der Lingen, I.; Brannstrom, K.; Hall, M.; Sauer, U. H.; Johansson, J.; Sauer-Eriksson, A. E.; Almqvist, F., *J Med Chem* **2018,** *61* (9), 4165-4175.
https://doi.org/10.1021/acs.jmedchem.8b00289
5. Good, J. A.; Andersson, C.; Hansen, S.; Wall, J.; Krishnan, K. S.; Begum, A.; Grundstrom, C.; Niemiec, M. S.; Vaitkevicius, K.; Chorell, E.; Wittung-Stafshede, P.; Sauer, U. H.; Sauer-Eriksson, A. E.; Almqvist, F.; Johansson, J., *Cell Chem Biol* **2016,** *23* (3), 404-14.
https://doi.org/10.1016/j.chembiol.2016.02.013
6. Andersson, E. K.; Bengtsson, C.; Evans, M. L.; Chorell, E.; Sellstedt, M.; Lindgren, A. E.; Hufnagel, D. A.; Bhattacharya, M.; Tessier, P. M.; Wittung-Stafshede, P.; Almqvist, F.; Chapman, M. R., *Chem Biol* **2013,** *20* (10), 1245-54.
https://doi.org/10.1016/j.chembiol.2013.07.017
7. Horvath, I.; Sellstedt, M.; Weise, C.; Nordvall, L. M.; Krishna Prasad, G.; Olofsson, A.; Larsson, G.; Almqvist, F.; Wittung-Stafshede, P., *Arch Biochem Biophys* **2013,** *532* (2), 84-90.
https://doi.org/10.1016/j.abb.2013.01.012
8. Horvath, I.; Weise, C. F.; Andersson, E. K.; Chorell, E.; Sellstedt, M.; Bengtsson, C.; Olofsson, A.; Hultgren, S. J.; Chapman, M.; Wolf-Watz, M.; Almqvist, F.; Wittung-Stafshede, P., *J Am Chem Soc* **2012,** *134* (7), 3439-44.
https://doi.org/10.1021/ja209829m
9. Singh, P.; Chorell, E.; Krishnan, K. S.; Kindahl, T.; Aden, J.; Wittung-Stafshede, P.; Almqvist, F., *Org Lett* **2015,** *17* (24), 6194-7.
https://doi.org/10.1021/acs.orglett.5b03190
10. Olsen, L. K.; Cairns, A. G.; Aden, J.; Moriarty, N.; Cabre, S.; Alamilla, V. R.; Almqvist, F.; Dowd, E.; McKernan, D. P., *Brain Behav Immun* **2019,** *80*, 525-535.
https://doi.org/10.1016/j.bbi.2019.04.036
11. Cairns, A. G.; Vazquez-Romero, A.; Mahdi Moein, M.; Aden, J.; Elmore, C. S.; Takano, A.; Arakawa, R.; Varrone, A.; Almqvist, F.; Schou, M., *ACS Chem Neurosci* **2018,** *9* (11), 2542-2547.

https://doi.org/10.1021/acschemneuro.8b00236
12. Sellstedt, M.; Almqvist, F., *Org Lett* **2008,** *10* (18), 4005-7.
https://doi.org/10.1021/ol801506y
13. Singh, P.; Adolfsson, D. E.; Aden, J.; Cairns, A. G.; Bartens, C.; Brannstrom, K.; Olofsson, A.; Almqvist, F., *J Org Chem* **2019,** *84* (7), 3887-3903.
https://doi.org/10.1021/acs.joc.8b03015
14. Flentie, K.; Harrison, G. A.; Tukenmez, H.; Livny, J.; Good, J. A. D.; Sarkar, S.; Zhu, D. X.; Kinsella, R. L.; Weiss, L. A.; Solomon, S. D.; Schene, M. E.; Hansen, M. R.; Cairns, A. G.; Kulen, M.; Wixe, T.; Lindgren, A. E. G.; Chorell, E.; Bengtsson, C.; Krishnan, K. S.; Hultgren, S. J.; Larsson, C.; Almqvist, F.; Stallings, C. L., *Proc Natl Acad Sci U S A* **2019,** *116* (21), 10510-10517.
https://doi.org/10.1073/pnas.1818009116
15. Emtenas, H.; Alderin, L.; Almqvist, F., *J Org Chem* **2001,** *66* (20), 6756-61.
https://doi.org/10.1021/jo015794u
16. Singh, P.; Cairns, A. G.; Adolfsson, D. E.; Aden, J.; Sauer, U. H.; Almqvist, F., *Org Lett* **2019,** *21* (17), 6946-6950.
https://doi.org/10.1021/acs.orglett.9b02549
17. Sellstedt, M.; Almqvist, F., *Organic Letters* **2009,** *11* (23), 5470-5472.
https://doi.org/10.1021/ol9023024
18. In press, *J Org Chem,* https://doi.org/10.1021/acs.joc.0c01699

19. Emtenas, H.; Taflin, C.; Almqvist, F., *Mol Divers* **2003,** *7* (2-4), 165-9.
https://doi.org/10.1023/B:MODI.0000006800.46154.99
20. Glasnov, T. N.; Kappe, C. O., *Chemistry* **2011,** *17* (43), 11956-68.
https://doi.org/10.1002/chem.201102065
21. Movsisyan, M.; Delbeke, E. I.; Berton, J. K.; Battilocchio, C.; Ley, S. V.; Stevens, C. V., *Chem Soc Rev* **2016,** *45* (18), 4892-928.
https://doi.org/10.1039/C5CS00902B
22. Plutschack, M. B.; Pieber, B.; Gilmore, K.; Seeberger, P. H., *Chem Rev* **2017,** *117* (18), 11796-11893.
https://doi.org/10.1021/acs.chemrev.7b00183
23. Trojanowicz, M., *Molecules* **2020,** *25* (6).
https://doi.org/10.3390/molecules25061434
24. Akwi, F. M.; Watts, P., *Chem Commun (Camb)* **2018,** *54* (99), 13894-13928.
https://doi.org/10.1039/C8CC07427E
25. Noël, T.; Su, Y.; Hessel, V., Beyond Organometallic Flow Chemistry: The Principles Behind the Use of Continuous-Flow Reactors for Synthesis. In *Organometallic Flow Chemistry*, Noël, T., Ed. Springer International Publishing: Cham, 2016; pp 1-41.
https://doi.org/10.1007/978-3-319-33243-7
26. Hartman, R. L.; McMullen, J. P.; Jensen, K. F., *Angew Chem Int Ed Engl* **2011,** *50* (33), 7502-19.
https://doi.org/10.1002/anie.201004637
27. Brandão, P.; Pineiro, M.; Pinho e Melo, T. M. V. D., *European Journal of Organic Chemistry* **2019,** *2019* (43), 7188-7217.
https://doi.org/10.1002/ejoc.201901335
28. Lehmann, J.; Alzieu, T.; Martin, R. E.; Britton, R., *Org Lett* **2013,** *15* (14), 3550-3.
https://doi.org/10.1021/ol4013525

29. Garcia-Lacuna, J.; Dominguez, G.; Perez-Castells, J., *ChemSusChem* **2020**.

30. Galaverna, R.; Fernandes, L. P.; Browne, D. L.; Pastre, J. C., *Reaction Chemistry & Engineering* **2019,** *4* (2), 362-367.
https://doi.org/10.1039/C8RE00237A
31. Galaverna, R.; McBride, T.; Pastre, J. C.; Browne, D. L., *Reaction Chemistry & Engineering* **2019,** *4* (9), 1559-1564.
https://doi.org/10.1039/C9RE00072K
32. Lai, X.; Shi, Z.; Pu, Z.; Zhang, P.; Zhang, X.; Yu, H.; Li, D., *Microsystems & Nanoengineering* **2020,** *6* (1).
https://doi.org/10.1038/s41378-020-0136-4
33. Adamo, A.; Beingessner, R. L.; Behnam, M.; Chen, J.; Jamison, T. F.; Jensen, K. F.; Monbaliu, J. C.; Myerson, A. S.; Revalor, E. M.; Snead, D. R.; Stelzer, T.; Weeranoppanant, N.; Wong, S. Y.; Zhang, P., *Science* **2016,** *352* (6281), 61-7.
https://doi.org/10.1126/science.aaf1337
34. Wiles, C.; Watts, P., *Green Chem.* **2012,** *14* (1), 38-54.
https://doi.org/10.1039/C1GC16022B
35. Yoshida, J.; Takahashi, Y.; Nagaki, A., *Chem Commun (Camb)* **2013,** *49* (85), 9896-904.
https://doi.org/10.1039/C3CC44709J
36. Baumann, M.; Moody, T. S.; Smyth, M.; Wharry, S., *Organic Process Research & Development* **2020,** *24* (10), 1802-1813.
https://doi.org/10.1021/acs.oprd.9b00524
37. Gutmann, B.; Cantillo, D.; Kappe, C. O., *Angew Chem Int Ed Engl* **2015,** *54* (23), 6688-728.
https://doi.org/10.1002/anie.201409318
38. Xu, F.; Armstrong, J. D., 3rd; Zhou, G. X.; Simmons, B.; Hughes, D.; Ge, Z.; Grabowski, E. J., *J Am Chem Soc* **2004,** *126* (40), 13002-9.
https://doi.org/10.1021/ja046488b
39. Pemberton, N.; Jakobsson, L.; Almqvist, F., *Org Lett* **2006,** *8* (5), 935-8.
https://doi.org/10.1021/ol052998e
40. Hyatt, J. A.; Feldman, P. L.; Clemens, R. J., *The Journal of Organic Chemistry* **1984,** *49* (26), 5105-5108.
https://doi.org/10.1021/jo00200a018
41. Reber, K. P.; Tilley, S. D.; Sorensen, E. J., *Chem Soc Rev* **2009,** *38* (11), 3022-34.
https://doi.org/10.1039/b912599j
42. Schwolow, S.; Hollmann, J.; Schenkel, B.; Röder, T., *Organic Process Research & Development* **2012,** *16* (9), 1513-1522.
https://doi.org/10.1021/op300107z
43. Lamberto, D. J.; Alvarez, M. M.; Muzzio, F. J., *Chemical Engineering Science* **1999,** *54* (7), 919-942.
https://doi.org/10.1016/S0009-2509(98)00275-9
44. Epps, R. W.; Volk, A. A.; Abdel-Latif, K.; Abolhasani, M., *Reaction Chemistry & Engineering* **2020,** *5* (7), 1212-1217.
https://doi.org/10.1039/D0RE00129E
45. Hone, C. A.; Boyd, A.; O'Kearney-McMullan, A.; Bourne, R. A.; Muller, F. L., *Reaction Chemistry & Engineering* **2019,** *4* (9), 1565-1570.
https://doi.org/10.1039/C9RE00180H
46. Chorell, E.; Edvinsson, S.; Almqvist, F., *Tetrahedron Letters* **2010,** *51* (18), 2461-2463.

https://doi.org/10.1016/j.tetlet.2010.02.162
47. Bengtsson, C.; Almqvist, F., *J Org Chem* **2010,** *75* (3), 972-5.
https://doi.org/10.1021/jo902458g
48. Pemberton, N.; Pinkner, J. S.; Edvinsson, S.; Hultgren, S. J.; Almqvist, F., *Tetrahedron* **2008,** *64* (40), 9368-9376.
https://doi.org/10.1016/j.tet.2008.07.015
49. Syrris webpage: http://syrris.com/flow-products/asia-flow-chemistry (accessed Oct 20).
50. Emtenas, H.; Ahlin, K.; Pinkner, J. S.; Hultgren, S. J.; Almqvist, F., *J Comb Chem* **2002,** *4* (6), 630-9.
https://doi.org/10.1021/cc020032d