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Scalable Flow Chemistry

Application Note 21

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Chemtrix BV

Continuous Flow Synthesis of Biologically Interesting 2-Aminothiazole Derivatives – mg-Scale Optimisation (Labtrix®) and kg-Scale Production (KiloFlow®) of *N*-(Phenylthiazol-2-yl)acetamide

Introduction: With 2-aminothiazoles representing a pharmaceutically important scaffold that possess with wide ranging biological activity such as anti-microbial,[1] anti-bacterial,[2] anti-inflammatory,[3] anti-fungal, anti-HIV[4] and anti-prion [5], significant research has been undertaken into the construction of combinatorial libraries. Methods for their preparation include the Hantzsch reaction[6] between α-haloketones and thioureas, α-tosyloxyketones[7] or *in-situ* bromination of acetophenone derivatives.[8] However, despite the large number of biologically active molecules containing the thiazole ring (Figure 1), many reported methods suffer from drawbacks such as harsh reaction conditions, low yields and difficulties in isolating the materials from the solvent system employed.[9]

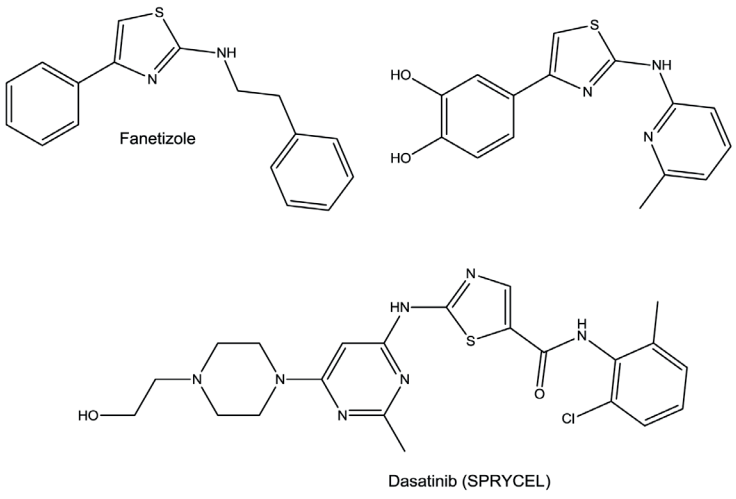


Figure 1. A selection of biologically interesting thiazole derivatives.

Literature Precedent: In an early example of micro reactor synthesis, Garcia-Egido *et al*.[10] assessed the Hantzsch reaction for the preparation of Fanetizole utilising NMP (63 % conversion, at 7.0 x 10^{-3} M) as the reaction solvent – owing to the use of EOF as a fluid manipulation technique, production rates were limited to the ng level – proving useful as a screening tool but not suitable for material production. Utilising segmented flow, Thompson and co-workers [11] again evaluated the preparation of a small molecule library with isolated yields ranging from 9 to 29 % at the 10's mg level. More recently, Cosford *et al*.[12] utilised the Hantzsch thiazole reaction as the first step in the formation of 5-(thiazol-2-yl)-3,4-dihydropyrimidin-2-(1*H*)-ones – where yields of 49 to 91 % (0.375 M) were obtained after purification by silica gel chromatography – necessary to remove un-reacted starting materials.

With a view to improving the methodology available for the preparation of this important class of biologically interesting molecules, the Hantzsch synthesis of *N*-(4-phenylthiazol-2-yl)acetamide **1** underwent detailed assessment and optimisation in Labtrix®-S1 ahead of scaling to KiloFlow®, a pilot-scale production unit, for the preparation of a kg of material (Figure 2).[13,14]

Figure 2. Photographs illustrating the turnkey scalable flow chemistry platform from Chemtrix BV; (left) Labtrix®-S1 and (right) KiloFlow®.

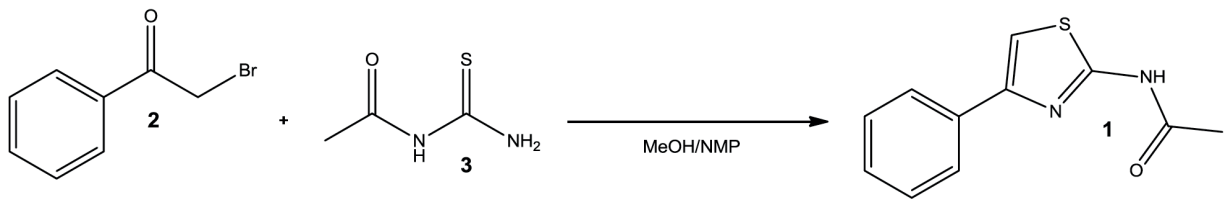
Materials Used: Unless otherwise stated, all reaction and wash solvents were of HPLC grade and used as received (Fisher Scientific, UK). 2-Bromoacetophenone **2** (98 %) and *N*-methyl-2-pyrrolidinone (NMP) (99 % extra pure) were obtained from Acros Organics (Belgium) and *N*-acetyl thiourea (+99 %) was sourced from Aldrich (UK).

Instrumentation: Analytical assessment of the micro flow reactions was performed using a Varian Prostar HPLC fitted with a Luna C_{18} (Phenomenex, UK) column. A mobile phase of 80 % MeOH and 20 % H_2O was employed at a flow rate of 1.5 ml min^{-1}, with analyte detection performed at 254 nm.

Product purity was assessed by NMR spectroscopy with ^{1}H and ^{13}C NMR spectra obtained at room temperature as solutions in deuteriochloroform ($CDCl_3$) with TMS as an internal standard. The spectra were recorded using a Jeol GX400 spectrometer and all spectral data compared with the literature. Samples were analysed by Varian GC-FID in NMP fitted with a CP-Sil (30 m (long) x 0.25 mm (o.d.) x 0.25 µm (film thickness); Phenomenex, UK) column, employing a Helium flow rate of 1.6 ml min^{-1} (99.9999 %; Energas, UK) and a thermal program utilising an initial oven temperature of 50 ºC (0 min hold) ramping to 240 ºC at 40 ºC min^{-1} (0 min hold) to 300 ºC at 100 ºC min^{-1} (3.0 min hold). Mass spectra were recorded as solutions in NMP using an Agilent 6890 Series GC-MS fitted with a HB-1 column (0.20 mm (o.d.) x 0.33 µm (film thickness) x 12 m (length)) employing a helium flow rate of 1.0 ml min^{-1} and a thermal program of 50 ºC (1 min hold) ramping to 320 ºC at 30 ºC min^{-1} (10 min hold); mass spectra were compared with the NIST 02 database.

At the lab-scale, flow reactions were performed in a standard Labtrix®-S1 system fitted with a 3223 device (Reactor Volume = 10 µl) and at the pilot-scale, reactions were performed in a KiloFlow® Basic system (Reactor Volume = 13 ml) at a back pressure of 7.0 bar – both systems contained PEEK wetted parts. Reagents were dosed using the standard KiloFlow® pump rack fitted with ETFE check valves and bottle pressurisation (N_2) at 0.4 bar. Thermal regulation (10 to 150 ºC) was achieved using a re-circulating thermostat (Lauda, XT 150, Germany) utilising Kryo 55 thermal fluid.

Results and Discussion: Prior to performing reactions in KiloFlow® Basic, initial investigations were performed at the lab-scale to assess the optimal conditions for the reaction illustrated in Scheme 1, with a view to maximising productivity by employing high concentration reagent solutions.



Scheme 1. Illustration of the synthetic route selected for the continuous flow synthesis of *N*-(4-phenylthiazol-2-yl)acetamide **1**.

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Labtrix® Reaction Screening: An initial challenge for the development of a high-throughput continuous flow synthesis of *N*-(phenylthiazol-2-yl)acetamide **1** was the limited solubility of *N*-acetyl thiourea **3** in conventional solvents utilised for this transformation. After an initial screening of alcohols (MeOH, EtOH), acetonitrile, *N,N*-dimethylformamide (DMF), *N*-methylpyrrolidinone (NMP) and dimethylsulfoxide (DMSO), NMP was identified as highly solubilising solvent allowing the facile preparation of 1.0 M solutions. With product isolation in mind, a combined solvent system was assessed as a means of reducing the proportion of NMP employed – leading to the assessment of MeOH as solvent for the **2**-bromoacetophenone 2 feed and 50:50 NMP:MeOH for the thiourea **3** feed.

Employing a solution of 2-bromoacetophenone **2** (1.0 M in MeOH) and *N*-acetyl thiourea **3** (1.0 M in 50 % NMP/MeOH) as the reagents and aqueous dimethylamine in NMP (10 wt. %) as the quench solution,[15] the formation of *N*-(4-phenylthiazol-2-yl)acetamide **1** was assessed using the automated micro reactor system Labtrix®-S1 (Chemtrix BV). Evaluating the effect of reaction time (30 to 120 s) and reaction temperature (25 to 175 ºC) within a 10 µl micro reactor (Labtrix® 3223) (Figure 3), the most efficient condition was sought prior to translation of the flow method to a 13 ml KiloFlow® reactor.

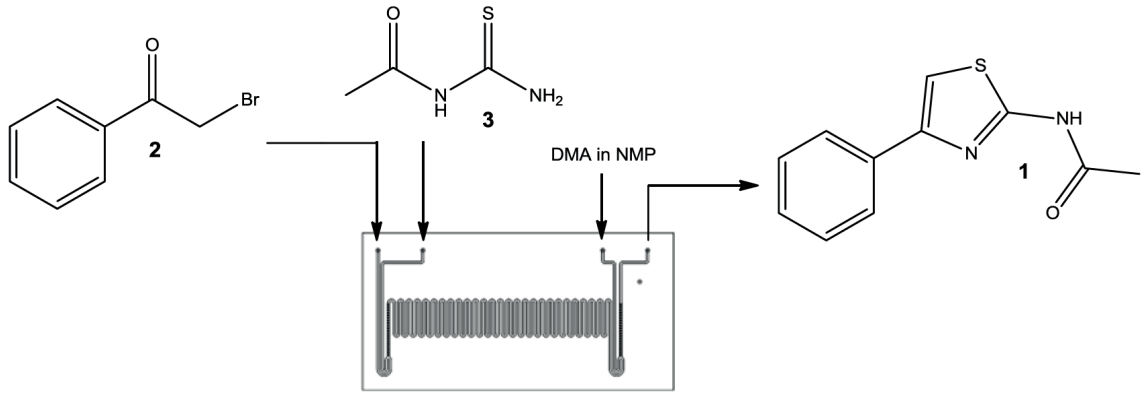

Figure 3. Schematic illustrating the reaction manifold assessed for the synthesis of *N*-(4-phenylthiazol-2-yl)acetamide **1**.

At a reaction time of 120 s, quantitative conversion of **2**-bromoacetophenone 2 to the target 2-aminothiazole was observed at reactor temperatures > 50 ºC. Looking towards accessing reaction conditions that would afford the target product **1** in both high yield and purity, suitable for large-scale material preparation, the effect of increased reaction temperature was assessed. Utilising the same reactant solutions as previously described the use of higher reaction temperatures was evaluated for a shorter reaction time of 30 s, whereby incomplete conversion was obtained at temperatures < 75 ºC. At temperatures exceeding 75 ºC, precipitation of the reaction product was observed in the collection tube/vessel (100 to 150 ºC), along with partial decomposition of the reaction product (between 175 to 195 ºC) to the de-acetylated 2-aminothiazole.

In order to obtain the target product **1** in high purity, the effect of reaction time was assessed at a T_{max} of 75 ºC – revealing 40 s as the optimal condition for *N*-(4-phenylthiazol-2-yl)acetamide **1** synthesis (quantitative conversion).

Long-term Reactor Performance: With these observations in hand, a reaction time of 40 s and a reactor temperature of 75 ºC were assessed for long-term operation within Labtrix®-S1 in the absence of a quench solution. Under the aforementioned conditions, the system was run for 2 h, collecting ~ 1 ml of reaction product (in 2 x 0.5 ml aliquots) with stable system pressure and product collection obtained. Over the assessment period, no precipitation was observed in the reactor, collection tube, BPR or collection vessel confirming suitability for translation of the methodology to KiloFlow®. In order to confirm the product formed was the target aminothiazole **1**, the reaction products were combined and the aminothiazole **1** precipitated by addition to 0.5 ml DI H_2O. Filtration under suction, followed by washing with DI H_2O (20 ml) and drying in a vacuum dessicator (24 h) afforded *N*-(4-phenylthiazol-2-yl)acetamide **1** as a free-flowing, white solid (0.108 g, 99.1 % yield). Analysis by HPLC, ^{1}H & ^{13}C NMR and GC-MS confirmed the product formed to be analytically pure *N*-(4-phenylthiazol-2-yl)acetamide **1**.

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Scaling to KiloFlow®: Having optimised the reaction in a glass micro reactor, the following conditions were selected for application in KiloFlow® Basic, with a view to preparing a kg batch of a biologically active compound within the system;

- 1.0 M stock solutions (1:1 2-bromoacetophenone **2**:*N*-acetyl thiourea **3**)
- NMP/MeOH as the solvent system
- 40 s reaction time at 75 ºC reaction temperature
- No quench – collection into aq. $N(CH_3)_2$

Initially the reactor was filled with 50 % NMP in MeOH, then the feed lines were filled with stock solution A (1.0 M 2-bromoacetophenone **2** in MeOH) and stock solution B (1.0 M *N*-acetyl thiourea **3** in 50 % NMP/MeOH). The reactor was warmed to 75 ºC using a Lauda XT150 thermostat and reagent dosing at a set flow rate of 9.75 ml min^{-1} (affording a 40 s reaction time). Upon reaching steady-state (three system volumes), the reaction products were collected over 8.5 h into a plastic vessel, maintained at atmospheric pressure, containing 2 litres aq. dimethylamine solution (10 wt. %) – affording a white precipitate. The reaction mixture was cooled overnight and the product isolated using vacuum filtration and washing with DI H_2O – a second crop was isolated from the filtrate upon additional cooling. The resulting white product was dried in a vacuum dessicator for 48 h prior to determination of the isolated yield (1.08 kg; 99.0 % yield) (Figure 4). Analysis of the reaction product **1** by NMR and MS confirmed that the target material was obtained in analytical purity (Figure 5); spectra consistent with the literature.[1]

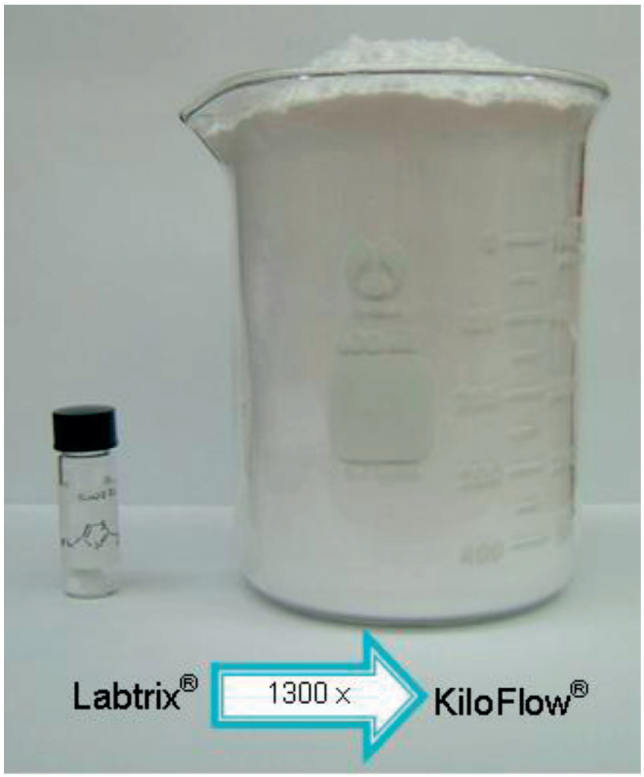

Figure 4. Photograph illustrating the aminothiazole product **1** synthesised in Labtrix® (~100 mg) and KiloFlow® Basic (~ 1 kg).

N-(Phenylthiazol-2-yl)acetamide **1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.78 (3H, s, CH_3), 7.15 (1H, s, CH), 7.34-7.38 (1H, m, ArH), 7.41-7.45 (3H, m, 3 x ArH), 7.80-7.83 (2H, m, 2 x ArH) and 11.67 (1H, br s, NH); ^{13}H NMR (100 MHz, $CDCl_3$) δ 22.6 (CH^3), 107.8 (CH), 126.3 (2 x CH), 128.4 (CH), 128.9 (2 x H), 133.9 (C_0), 149.1 (C_0), 159.8 (C_0N) and 168.5 (CO); *m/z* (EI) 219 (M^++1, 4 %), 218 (32), 176 (100), 134 (41), 121 (4), 104 (7), 89 (7) and 77 (7). All data is consistent with literature standards.

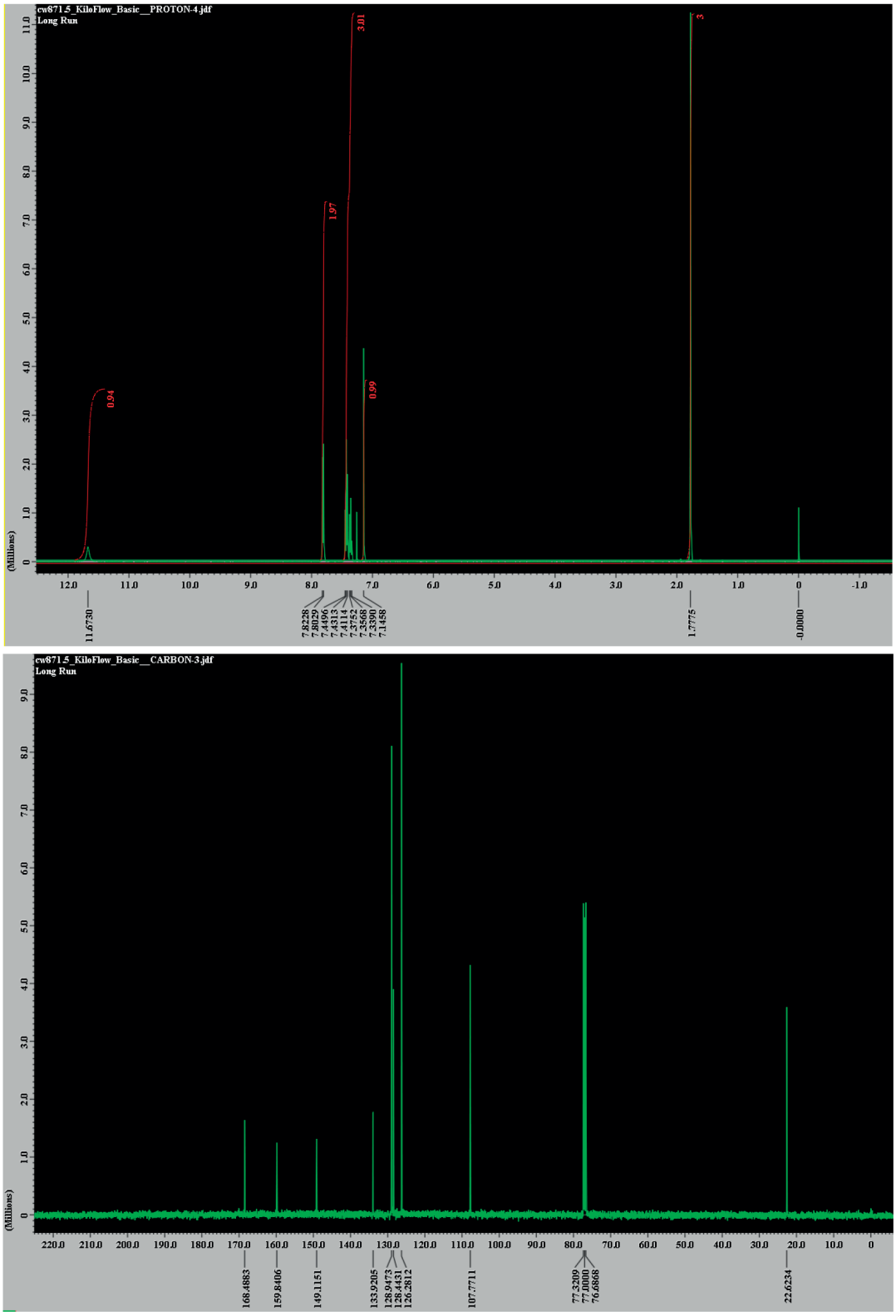

Figure 5. Illustration of the (top) ^{1}H and (bottom) ^{13}C NMR spectra of the aminothiazole **1** product prepared using KiloFlow® Basic ($CDCl_3$).

NOTE: When preparing stock solutions on the 1 to 10 litre scale reagents can require stirring to aid dissolution. Herein, once prepared the solutions were homogeneous and could be stored for in excess of 2 weeks without precipitation. All equipment described herein must be used within a closed, functioning fume cupboard.

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Conclusion: Using the synthesis of *N*-(4-phenylthiazol-2-yl)acetamide **1**, we were able to demonstrate the direct 1300x scaling of a continuous flow reaction optimised in Labtrix®-S1 (Reaction Volume = 10 µl) to KiloFlow® Basic (Reaction Volume = 13 ml), producing under identical reaction conditions (75 ºC, 40 s) in excess of 1 kg of a biologically interesting material.

References:

[1]. P. Morales-Bonilla, A. Pérez-Cardeña, E. Quintero-Mármol, J. L. Arias-Téllez and G. J. Mena-Rejón, *Heteratom Chem.*, 2006, **17**,(4), 254.
[2]. K. Tsuji and H. Ishikawa, *Bioorg. Med. Chem*. Lett., 1994, **4**, 1601.
[3]. B. S. Jayashree, D. Anuradha and N. K. Venugopala, *Asian J. Chem.*, 2005, **17**, 2093.
[4]. F. W. Bell, A. S. Cantrell, M. Högberg, S. R. Jaskunas, N. G. Johansson, C. L. Jordan, M, D, Kinnick, P. Lind, J. M. Morin, R. Noréen, B. Öberg, J. A. Palkowitz, C. A. Parrish, P. Prane, C. Sahlberg, R. J. Ternansky, R. T. Vasileff, L. Vrang, S. J. West, H. Zhang and X.-X. Zhou, *J. Med. Chem.*, 1995, **38**, 4929.
[5]. A. Gallardo-Godoy, J. Gever, K. L. Fife, B. M. Silber, S. B. Prusiner and A. R. Renslo, J. *Med. Chem.*, 2011, **54**, 1010.
[6]. A. R. Hantzsch and J. H. *Weber, Ber.*, 1887, **20**, 3118.
[7]. P.-Y. Lin, R.S. Hou, H.-M. Wang, I.-J. Kang and L.-C. Chen, *J. Chin Chem*. Soc., 2009, **56**, 455.
[8]. S. I. Zav'yalov, O. V. Dorofeeva, E. E. Rumyantseva, L. B. Kulikova, G. I. Ezhova, N. E. Kravchenko and A. G. Zavozin, *Pharma. Chem. J.*, 2001, **35**, 96.
[9]. G. Kabalka and A. R. Mereddy, *Tet. Lett.*, 2006, **747**, 5171.
[10]. E. Garcia-Egido, S. Y. F. Wong and B. H. Warrington, *Lab Chip*, 2002, **2**, 31.
[11]. C. M. Thompson, J. L. Poole, J. L. Cross, I. Akritopoulou-Zanze and S. W. Djuric, *Molecules*, 2011, **16**, 9161.
[12]. N. Pagano, A. Herath and N. D. P. Cosford, *J. Flow Chem.*, 2011, **1**, 28.
[13]. See Application Note 15: The Scale-up of (*Z*)-Ethyl-2-cyano-3-phenylacrylate Synthesis; www.chemtrix.com
[14]. See Application Note 17: 13,000 x Scale-up of 3-Acetylcoumarin – Demonstration of Scalable Flow Synthesis; from R&D to Production without Parameter Re-optimisation; www.chemtrix.com
[15]. N. Bailey, A. W. Dean, D. B. Judd, D. Middlemiss, R. Storer and S. P. Watson, Bioorg. *Med. Chem. Lett.*, 1996, **6**, 1409.