



University of Oxford
Department of Chemistry
South Parks Road
Oxford OX1 3QY

Professor S. G. Davies Laboratory
The Dyson Perrins Laboratory
University of Oxford
South Parks Road
Oxford
OX1 3QY

Tel: +44 (0)1865 275662
Fax: +44 (0)1865 275674
<http://chem.ox.ac.uk/dp/sgdavies>

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Evaluation of RDT GreenHouse Parallel Synthesiser™

The RDT GreenHouse Parallel Synthesiser (RR99600) was evaluated for the following performance criteria:

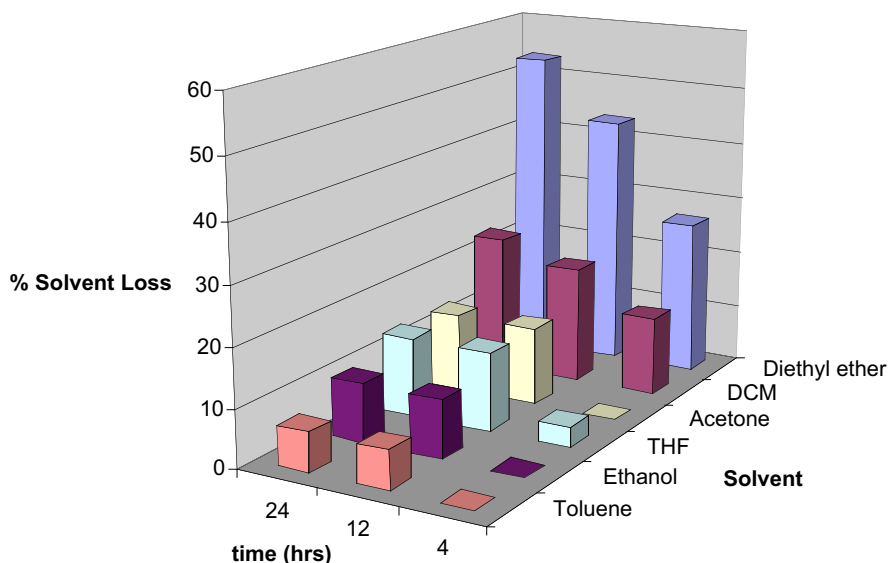
- The efficiency of the Reflux Head in preventing solvent loss.
- The application to the parallel array synthesis of pharmacologically relevant heterocycles.
- The cooling efficiency of the GreenHouse Cooling Reservoir

Efficiency of the Reflux Head

To determine the performance of the GreenHouse when refluxing organic solvents over extended time periods, a series of evaluations were performed using six widely used solvents, representing a range of boiling points from 37°C (Et₂O) to 111°C (toluene). The GreenHouse was set up in a fume hood with 24 GreenHouse reaction tubes (RR99603) containing GreenHouse PTFE magnetic stirrer bars (RR99607) in the reaction block. The reflux head was tightly secured using the GreenHouse Knobs (RR99628) and connected to the laboratory water supply (water temperature 10 to 12°C). The radial gas inlet was connected to an argon supply and the reaction tubes were charged with the appropriate solvent (3mL).

A Carousel Digital temperature controller (RR98073) was inserted directly into one of the reaction tubes through the cap and the stirred solvents brought to a gentle reflux for the designated time, allowed to cool and the volume of the solvent remaining in the tube measured. The results of this study using six different solvents are listed in **Chart 1**.

Chart 1: Solvent loss at reflux over time in the GreenHouse ^{a, b}



Notes:

^a The evaluation was performed a total of three times to determine the reproducibility of each run.

^b Starting volume 3.0mL of each solvent.

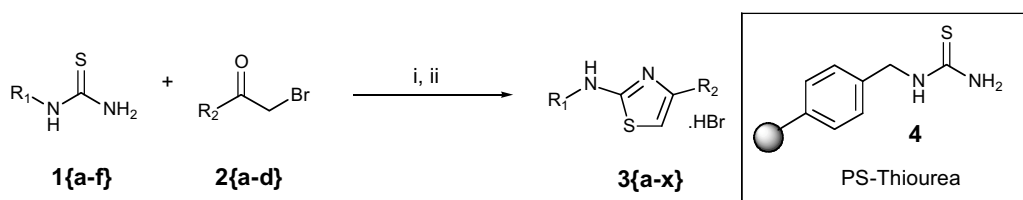
These data illustrate that for all but the most volatile of solvents, the loss of solvent from the reaction tubes at reflux is minimal with over 85 % (2.6mL) of the solvent remaining after 24h of reflux. With the volatile solvents such as diethyl ether and dichloromethane, solvent loss is more pronounced, with 53% (1.4mL) and 23% (0.7mL) respectively of solvent evaporation over a 24h period. It is of note that with the higher boiling solvents, there was an initial loss of solvent in each case (approximately 10-15%) after which time little or no extra solvent loss was encountered. This suggests that the upper limit for reaction volume for the higher boiling solvents is 2.6-2.7mL. Although the effect on solvent evaporation when using a water chiller was not determined, experiments with the Carousel Reaction Station¹ would suggest that solvent loss with the more volatile solvents such as diethyl ether would be reduced significantly.

Evaluation of the GreenHouse for parallel array synthesis:

The Hantzsch synthesis of 4-substituted 2-aminothiazoles









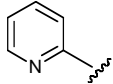
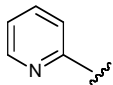
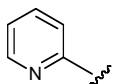
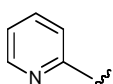
To evaluate the suitability of the GreenHouse for parallel synthesis, the ease by which the Hantzsch Thiazole synthesis² could be adapted to the GreenHouse apparatus was investigated. Hence six thioureas **1{a-f}** were combined with four α -bromoketones **2{a-d}** to furnish an array of twenty four 4-substituted 2-aminothiazoles **3{a-x}** (**Scheme 1**), this protocol being representative of a typical 2-component cyclodehydration reaction employed for the synthesis of 2-aminothiazoles, an important class of biologically active heterocycles.³ GreenHouse reaction tubes (RR99603) equipped with PTFE magnetic stirrer bars (RR99607) were charged with DMF stock solutions of the α -bromoketone (750 μ L, 0.24M, 0.18mMol, 1.2eq.). DMF stock solutions of the thioureas (750 μ L, 0.20M, 0.15 mMol) were added *via* pipette (Eppendorf Multipette[®] Pro) and the mixture heated with vigorous stirring⁴ at 80°C for 8 hours. The excess of α -bromoketone was sequestered by the addition of PS-thiourea (3.0 eq.) and heating at 80°C for a further 3h. The reaction mixtures were cooled then filtered through a fritted tube to remove the spent resin and the filtrates collected into weighed tubes and concentrated using vacuum centrifugal evaporation (GeneVac Atlas HT-8). The crude yields were recorded and each individual array member characterised by HPLC,⁵ MS and ¹H NMR (400MHz). The yields and purity data for individual array members are presented in **Table 1**.

Scheme 1:



Reagents and conditions: (i) 1.2 eq. α -bromoketone, DMF 80°C, 8h; (ii) 3 eq PS-thiourea, 80°C, 3h.

Table 1: Parallel synthesis of a 4-substituted 2-aminothiazole array using the GreenHouse.

Product	R ₁	R ₂	Yield (%)	Purity ^a
3a	Me	Ph	82	95
3b	Me	CF ₃	84	98
3c	Me	<i>t</i> -Bu	50	94
3d	Me	2-Naphthyl	96	80
3e		Ph	100	85
3f		CF ₃	77	98
3g		<i>t</i> -Bu	99	87
3h		2-Naphthyl	99	70
3i		Ph	54	90
3j		CF ₃	99	92
3k		<i>t</i> -Bu	60	90
3l		2-Naphthyl	100	91
3m	Bn	Ph	58	97
3n	Bn	CF ₃	99	94
3o	Bn	<i>t</i> -Bu	99	86
3p	Bn	2-Naphthyl	99	95
3q	Ph	Ph	99	82
3r	Ph	CF ₃	99	>99
3s	Ph	<i>t</i> -Bu	98	>99
3t	Ph	2-Naphthyl	99	95
3u		Ph	96	98
3v		CF ₃	99	>99
3w		<i>t</i> -Bu	99	>99
3x		2-Naphthyl	99	95

^a Yields refer to crude product following centrifugal evaporation of the solvent;

^b As determined by LC-determination at 3 wavelengths concurrently (λ_{\max} 220, 254 and 290nm)

This protocol furnished between 50-70mg of the respective 4-substituted 2-aminothiazoles **3{a-x}** corresponding to an average yield for the array of 85 per cent. HPLC and ¹H NMR data indicated that the average purity of the array was

92 per cent, comfortably exceeding the 80 per cent criteria stipulated by the ACS for publication in the *Journal of Combinatorial Chemistry*.⁶

The Cooling Efficiency of the GreenHouse Cooling Reservoir

Following our evaluation of the Radleys Cooled Carousel (Oxford University report: www.radleys.co.uk/rdt/html/pdf/bulletins/B49REP.DOC.pdf)⁷ an identical protocol was followed for evaluation of the cooling efficiency of the RR99600 GreenHouse Parallel Synthesiser (referred to henceforth as the GreenHouse).

The GreenHouse was assembled in a standard fume-hood. Twenty-four GreenHouse Reaction Tubes (RR99603) containing GreenHouse PTFE Stirring Bars (RR99607) were placed in the GreenHouse Reaction Block (RR99601) and charged with THF (3mL) and the GreenHouse sealed with the Additions Head. The cooling mixtures listed in **Table 2** were evaluated with temperature measurements taken from the cooling reservoir, inside the reaction block and the THF held within the reaction tubes using a Digital Thermometer (RR99905). In the case of the acetonitrile/dry ice cooling mixture the reaction block and solvent attained the requisite temperature within 30 minutes. However in the case of the freezing mixture (ice/sodium chloride) and the acetone/dry ice cooling mixtures, the maximum theoretical possible temperatures were never attained. With the freezing mixture the lowest temperature that the THF reached was -13°C (against the block and cooling bath temperature of -15°C and -18°C respectively) while with acetone/dry ice the lowest attainable temperature of the THF was -72°C (**Table 2**).

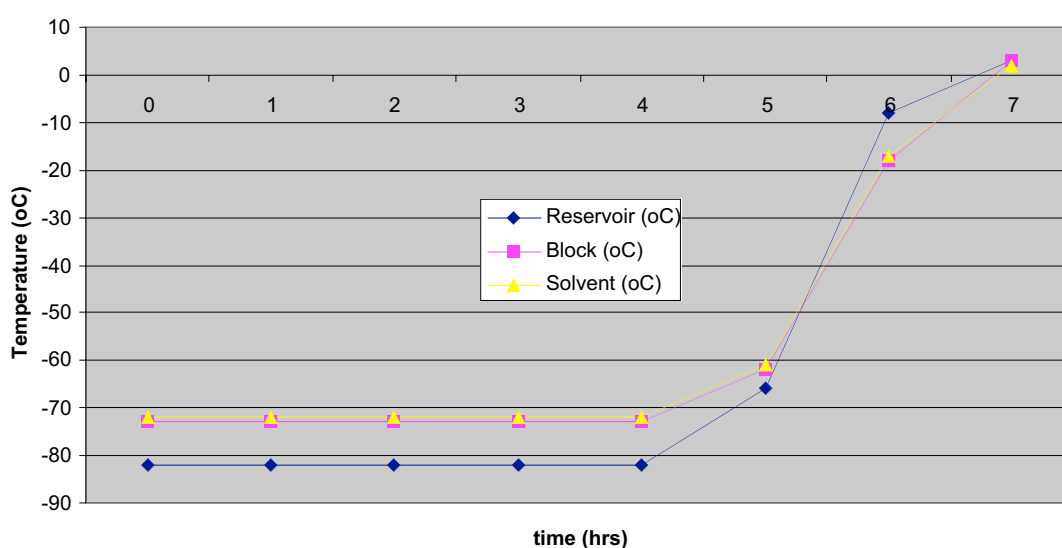
Table 2. Evaluation of cooling performance with different cooling mixtures

	Cooling Bath Temp ($^{\circ}\text{C}$)	Block Temp ($^{\circ}\text{C}$)	Solvent Temp ($^{\circ}\text{C}$)
Ice/sodium chloride	-18	-15	-13
acetonitrile/dry ice	-52	-44	-42
Acetone/dry ice	-82	-73	-72

The time course over which these temperatures could be sustained when the user is not present to top up the cooling mixture with dry ice and/or solvent (e.g. in the case of an overnight reaction) was then evaluated. This study was conducted

using dry ice/acetone as the cooling mixture; the cooling reservoir was charged with dry ice such that the level of the dry ice was level with the rim of the reservoir and acetone was added *via* a wash bottle to give a slurry of the coolant. It was noted that approximately sixty minutes elapsed before the reaction block and THF reached the lowest attainable temperatures. The temperature of the coolant in the reservoir and the THF was then monitored at sixty-minute intervals over a seven-hour period. The results are displayed in **Chart 2**.

Chart 2: Evaluation of Temperature vs. Time using GreenHouse Parallel Synthesiser^{a,b}



Notes

^a The evaluation was performed a total of three times to determine the reproducibility of each run. There was little observed deviation in the data between each run.

^b Time=0 was taken as 60 minutes after the cooling mixture had been added to the cooling reservoir after which time the reaction block and THF had attained the lowest possible temperature. The cooling mixture was fully replenished at this point.

These results indicate that when the cooling reservoir is fully charged with dry ice/acetone as the coolant, the reaction temperature can be expected to remain at a temperature of -72°C for a period of four to five hours. It was noted that when using the ultra-low temperature cooling mixtures such as dry ice/ethanol and dry ice/acetone, frosting of the GreenHouse glass resulted in the reaction block being completely obscured (although this is readily removed by rinsing with solvent from a wash bottle). Additionally, a large amount of condensation of moisture onto the aluminium reactor head was found to occur. Previous experience with the Cooled Carousel has shown that addition of a lid or cover to the cooling bath

not only serves to reduce the condensation problem but also leads to a dramatic extension (~40%) in the time period over which low-temperature cooling mixtures can be contained. It is the author's opinion that a similar lid designed for the GreenHouse would be a useful addition for this apparatus.

Comment

The GreenHouse Parallel Synthesiser, developed by RDT in conjunction with Medicinal Chemists at GlaxoSmithKline, makes a very welcome addition to the armoury of instruments that RDT offer to the organic chemist for parallel synthesis. Like forerunners of RDT equipment in routine use within this laboratory, we have found the GreenHouse to be extremely robust, requiring little or no maintenance and with the minimal of training required for its operation. Furthermore, because the GreenHouse was designed to be used in conjunction with the Stacker™, Lollipop Phase Separator™ and Genevac evaporation system for post-synthetic manipulations, the GreenHouse represents a low cost and reliable platform for parallel synthesis.

References

- ¹ Dr. I. Baxendale. Cambridge University Report: <http://www.radleys.co.uk/rdt/html/pdf/bulletins/B17REP.DOC.pdf>
- ² Hantzsch, A. R.; Weber, J.H. *Ber.* **1887**, 20, 3118.
- ³ For recent reports on the synthesis of 2-aminothiazoles see: (a) Bailey, N.; Dean, A.W.; Judd, D.B.; Middlesmiss, D.; Storer, R.; Watson, S.P. *Biorg. Med. Chem. Lett.* **1996**, 6, 1409; (b) Kearney, P.C.; Fernandez, M.; Flygare, J.A. *J. Org.Chem.*, **1998**, 63, 196.
- ⁴ The PTFE stirrer bars were found to provide extremely efficient stirring performance at high stirrer speeds.
- ⁵ Reverse-phase high-pressure liquid chromatography (RP-HPLC) was performed on a Gilson instrument comprising of Gilson 306 pumps, Gilson 811C dynamic mixer, Gilson 806 manometric module with automated sample injection on a Gilson 215 Liquid handler, configured with a Gilson 819 valve actuator. Separations were performed on a Varian Omnisphere 5 C18 column (5µM particle size, 150.0mm x 4.6mm) for analytical separations. Experiments were performed under gradient elution: solvent A (H₂O containing 0.1% TFA) and solvent B (CH₃CN), starting from 95%A, 5%B to 5%A, 95%B over 8 mins then isocratic for 4 mins. Flow rates were 1.0mL/min. Detection was at λ220, 254 and 290nm using a Gilson 170 Diode Array Detector.
- ⁶ *Journal of Combinatorial Chemistry*, American Chemical Society. <http://pubs.acs.org>
- ⁷ Dr. A. W. Mulvaney, Miss. A.J. Wright. Oxford University Report

Evaluation and report performed and compiled by Dr. Richard Vickers and Dr. Andy Mulvaney.