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6th April 2004

Evaluation of Cooled Carousel™ 6 Place Reaction Station
Radleys Discovery Technologies

A range of experiments have been performed to establish the utility of the Cooled Carousel 6 Place Reaction Station. These include experiments designed to specifically test the Carousel 6 Place with examples of our research activities. Intended key points for observation:

- Cooling performance
- Ease of handling
- Safety of handling
- Affect of temperature on stirring efficiency.

1. Evaluation of Cooling Performance

Test runs have been undertaken to ascertain the success of the Cooled Carousel Cooling Reservoir (RR99501) at maintaining the chilled environment for prolonged periods of time. Two set-ups were trialled:

i) With the Carousel cover. Runs performed with standard flasks (RR99041) and screw on reflux necks (RR99042). Bath temperature only was monitored for these runs.
ii) Without the Carousel cover. This set-up is necessary when using flasks with side arms in conjunction with side arm accessories e.g. pressure equalising dropping funnel for reagent addition (RR99047/RR99048). For these runs, in addition to bath temperature, we were also able to monitor reaction temperature using thermometers in side arm ports.

In both cases the flasks were set-up as described in the Radleys supporting literature. Acetone was added to a depth of approximately 2cm. Dry ice (approximately 5kg total) was then added, slowly at first, until the cooling reservoir was nearly full. The bath temperature was allowed to reach its minimum point, approximately -80ºC, and the timer started. Each of the six flasks contained 100ml solvent (THF or DCM as noted). A variety of stirrer bars were also evaluated concurrently.

Data obtained for these experiments is shown graphically in figure 1. For both set-ups low temperatures were maintained for considerable times. With the Carousel cover in place (i) the temperature stayed below -40°C for over 13 hours and still hadn't reached room temperature after 20 hours. Without the Carousel cover (ii) temperatures stayed below -40ºC for over 7 hours.

The stirring bars evaluated were the same as those in our previous assessment of the heated Carousel 6 Place;

RR99064	elliptical, rare earth, 25mm (as currently supplied with Carousel 6 Place Reaction Station)
001.2638.RE	elliptical, rare earth, 38mm
001.325.10	pivot ring, 25mm x 10mm
001.125.RE	tapered, 25mm x 8mm
1.1925	cylindrical, rare earth, 25mm x 6mm.

The maximum stirring speeds achievable for each type were independent of temperature and in line with those for the standard heated Carousel.

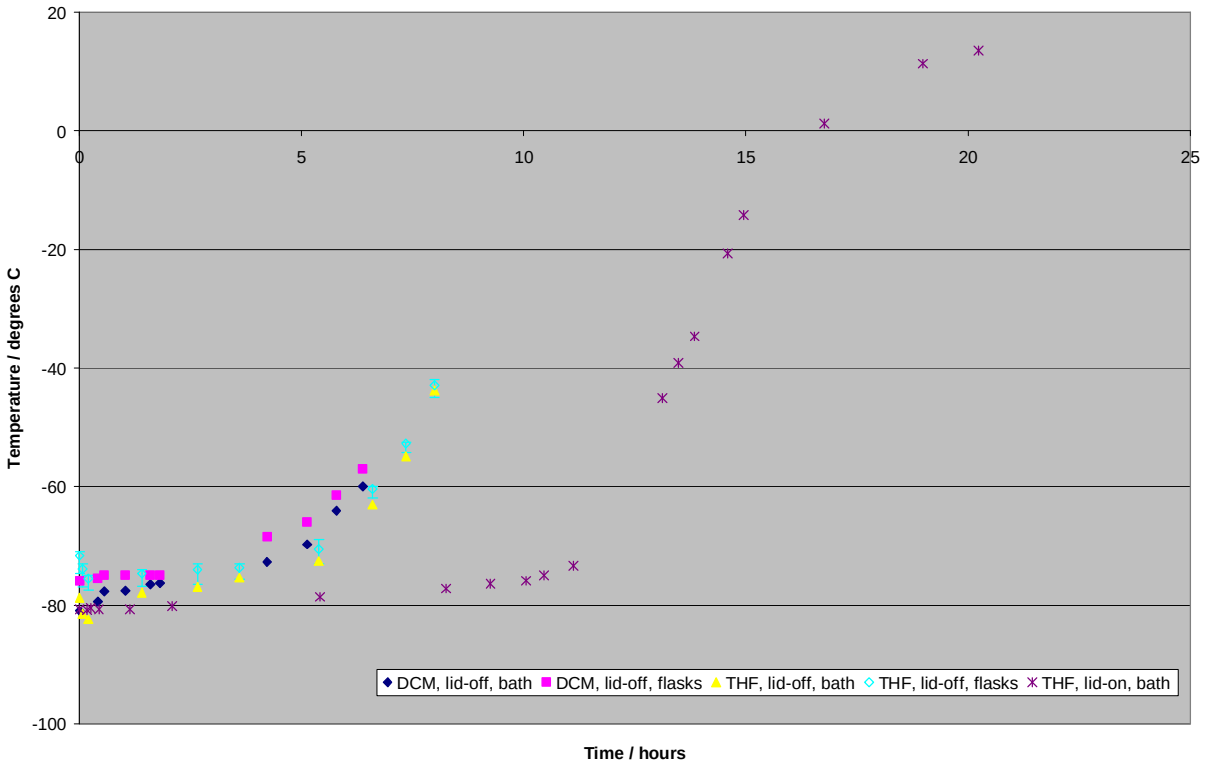


Figure 1. Cooled Carousel™ warming profiles of dry ice / acetone bath mixture.

2. Comments on Glassware

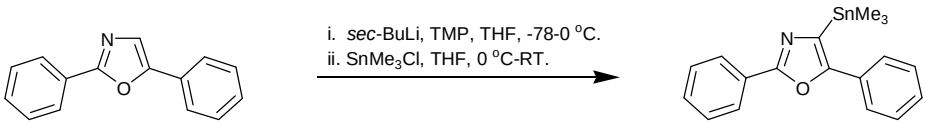
The glassware was quick and easy to assemble, as with all other modular RDT Carousel systems. The flasks fitted easily into the aluminium holder and dropping funnels, solid addition funnels, etc., could be added as per normal laboratory glassware. The additional Rodaviss sealing system provided a secondary gas tight seal and prevented cone/socket jams. The rotational ability of the stainless steel reactor head also proved to be useful, allowing easy access to all six flasks to both add reagents and monitor temperature.

3. Other Comments

Overall the Cooled Carousel 6 Place system met its requirements well. Filling the cooling reservoir with dry ice and acetone and subsequently using the cooling bath was as easy as a conventional one flask set-up. Certainly it was felt that the bath size, shape and accessibility offered no additional safety concerns over those normally required for dry ice/acetone use. The outer of the plastic bath remained at a hand touchable temperature throughout all experiments. When the optional bath cover was not used some icing of the flasks was visible above the dry ice/acetone level but this is to be expected with a conventional glassware set-up.

4. Examples of Synthesis

A series of chemically functionalised polymer supports that incorporate scintillant covalently were required for application in solid-phase synthesis and subsequent scintillation proximity assays. The construction of these scintillant-containing polymer supports requires the incorporation of a scintillant monomer in the comonomer mixture. 2,5-Diphenyloxazole was chosen as the scintillant moiety of choice since it is well known to scintillate in the presence of ionising radiation and has a chemically robust oxazole skeleton. The scintillant monomer (4'-vinyl)-4-benzyl-2,5-diphenyloxazole has been synthesised previously by a Stille coupling reaction between chloromethylstyrene and 2,5-diphenyl-4-trimethylstannanyloxazole. The 2,5-diphenyl-4-trimethylstannanyloxazole was synthesised by treating a cooled solution of 2,5-diphenyloxazole and tetramethylpiperidine (TMP) in tetrahydrofuran (THF) with *sec*-butyl lithium to generate the 4-lithio-2,5-diphenyloxazole intermediate. This reaction mixture was then added to a solution of trimethylstannyl chloride in THF at 0 °C to provide 2,5-diphenyl-4-trimethylstannanyloxazole (Scheme 1).



Scheme 1: Synthesis of 2,5-diphenyl-4-trimethylstannanyloxazole.

We wished to determine the optimal stoichiometric amount of *sec*-butyllithium to be used in this reaction to give the highest yield of 2,5-diphenyl-4-trimethylstannanyloxazole. Herein is reported the parallel synthesis of 2,5-diphenyl-4-trimethylstannanyloxazole using the Cooled Carousel 6 Place. In this evaluation, three batches of 2,5-diphenyl-4-trimethylstannanyloxazole were synthesised. The three reactions used identical conditions and molar quantities of reagents with the exception of the stoichiometric amount of *sec*-butyl lithium used to generate the 4-lithio-2,5-diphenyloxazole intermediate.

The Cooled Carousel 6 Place was assembled as follows. The Reactor Head (RR99502) was fitted into the high density polyethylene (HDPE) Cooling Reservoir (RR99501), which was then mounted upon a Carousel Stirring Hotplate (RR98072). In triplicate, a Rare Earth – 25mm Elliptical PTFE Stirring Bar (RR99064) was placed in a 250ml 3-neck round bottomed flask (custom design) that was fitted with a Reflux Tube & Connecting Set (RR99042), thermometer and Liquid Additions Dropping Funnel (50ml) (R99048), which had all been dried in an oven (140°C). These three glassware assemblies were loaded in positions 1, 3 and 5 of the Reactor Head (RR99502) and held in place by the built-in 'spring clips' of the Reactor Head (RR99502). The Reflux Tube (RR99042) threaded top was connected to the Gas Tight Threaded PTFE Cap (RR98059), which was fitted with a silicone Suba Seal (RR98076) and tubing (RR99066). The Gas Tight Threaded PTFE Cap (RR98059) and attached tubing (RR99066) was then connected to the stainless steel barb of the radial gas distribution system of the Reactor Head (RR99502), which was connected via a 3-way tap to a vacuum pump and nitrogen gas supply. Simultaneously, the three glassware assemblies were evacuated, then purged with nitrogen gas and cooled to room temperature.

2,5-Diphenyloxazole (3.3650 g, 15.056 mmol) was added to flasks 1, 3 and 5 using a B14/23 Solid Additions Funnel (RR99049). This procedure was facilitated by the free rotation of the cooled Carousel 6 place Reaction Station. Rotation was made possible by the Reactor Head (RR99502) central stainless steel barb being connected to the vacuum pump/nitrogen gas supply by tubing that incorporated a Quick Release Barbed Coupling (RR99062), which rotates freely.

The three glassware assemblies were evacuated and then charged with nitrogen gas. A solution of 2,2,6,6-tetramethylpiperidine (254µl, 1.506 mmol) dissolved in dry THF (50ml) was added to each flask (custom design) via the dropping funnel (R99048), followed by THF (50ml).

A Temperature Probe (with Digital Thermometer, RR99905) was placed into the HDPE Cooling Reservoir (RR99501) by sliding the probe through the drilled hole in the Reactor Head. The Cooling Reservoir was filled with acetone (1.5 litres) and dry-ice added using a Dry Ice Scoop (RR99908) until the desired temperature (-70°C) was attained. The Carousel Stirring Hotplate (RR98072) was used to set the stirring speed (speed 6) of the reaction mixtures. The three reaction mixtures were stirred equally and powerfully to provide a deep vortex. Each of the three reaction mixtures was cooled to -70°C (11 min). The acetone/dry-ice temperature was maintained during the course of the reaction. In addition, the Carousel Stirring Hotplate (RR98072) did not freeze and condensation/ice did not form on outer surfaces of the HDPE Cooling Reservoir (RR99501). However, ice did form on the 3-neck flasks because the HDPE Cooled Carousel 6 Place Cover (RR99515) could not be fitted upon the HDPE Cooling Reservoir (RR99501) due to the Liquid Additions Dropping Funnel (50ml) (R99048) and temperature probes being connected to each 3-neck flask (custom design).

sec-Butyllithium (**Flask 1**: 11ml 15.056 mmol, **Flask 3**: 13ml 18.821 mmol, **Flask 5**: 16ml 22.585 mmol) was added to the appropriate reaction mixture via the Liquid Additions Dropping Funnel (50ml) (R99048) (1 drop/sec). This resulted in an increase in temperature (-63°C) and formation of brick-red coloration of the reaction mixture. Upon complete addition of *sec*-butyllithium, the three Gas Tight Threaded PTFE Cap (RR98059) valves were closed and the Reactor Head (RR99502) radial gas distribution system central Quick Release Barbed Coupling (RR99062) was disconnected. The Reactor Head (RR99502) was the removed form the Cooling Reservoir (RR99501), with flasks *in-situ*, and placed temporarily on the HDPE Stand (RR99503).

The acetone/dry-ice in the HDPE Cooling Reservoir (RR99501) was replaced with ice/water. The Reactor Head (RR99502), with flasks in-situ, was then removed from the Stand (RR99503), returned to the Cooling Reservoir (RR99501), and the reaction mixture stirred (speed 6) whilst warming to 10°C (15 min).

Trimethylstannyl chloride (3.0000 g, 15.056 mmol) was added to each of three 250ml reaction Flasks with B14/23 Sidearm & Septa Ports (RR99047) using a B14/23 Solid Additions Funnel (RR99049). These three flasks were fitted with a Reflux Tube & Rodaviss Connecting Set (RR99042) and B14/23 Rodaviss Connecting Caps (RR99068) and B14/23 Septa (RR99080) then loaded in positions 2, 4 and 6 of the Cooled Carousel 6 Place Reactor Head (RR99502). These three glassware assemblies were evacuated and then purged with nitrogen gas. The trimethylstanyl chloride was dissolved in tetrahydrofuran (40 ml) and cooled to 0°C. The glassware assemblies in positions 1, 3 and 5 of the Reactor Head (RR99502) had their Gas Tight Threaded PTFE Cap (RR98059) valves closed to isolate each apparatus.

The 4-lithio-2,5-diphenyloxazole intermediate in positions 1, 3 and 5 of the Reactor Head (RR99502) were then transferred to trimethylstanyl chloride solutions in positions 2, 4 and 6 of the Reactor Head (RR99502) respectively. This transfer procedure was achieved by use of a cannula and nitrogen gas line. The resultant reaction mixtures were stirred (16 hr) whilst warming to room temperature.

Saturated ammonium chloride aqueous solution (40ml) was added to each reaction mixture in positions 2, 4 and 6 of the Reactor Head (RR99502) via the Liquid Additions Dropping Funnel (R99048) and stirring continued (10 min). The Rare Earth – 25mm Elliptical PTFE Stirring Bar (RR99064) was removed from each reaction mixture using a PTFE Magnetic Stirring Bar Retriever (RR98094). The organic phase was separated from the aqueous phase and then dried over anhydrous magnesium sulfate. The filtrate was separated from the magnesium sulfate by filtration into a single neck 250ml Reaction Flask (RR99041). The 250ml Reaction Flask (RR99041) was fitted with a Rotary Evaporator Adapter (RR99046) and connected to a rotary evaporator. The filtrate was concentrated under reduced pressure to give oil that was brick red coloured. The oil was dried *in-vacuo* (40°C, 16 hr) and the crude, now solid, product recrystallised from methanol. The resultant crystals were separated from the filtrate by filtration, washed *in-situ* with cold methanol (3x1 ml) and then dried *in-vacuo* (33°C) to constant mass to give 4-trimethylstannanyloxazole as white needle like crystals (**Flask 2**: 1.1921g 20%, **Flask 4**: 0.7585g 14%, **Flask 6**: 0.6934g 12%).

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May 2004