

## A Microfluidic Platform to Synthesise a G-Quadruplex Binding Ligand

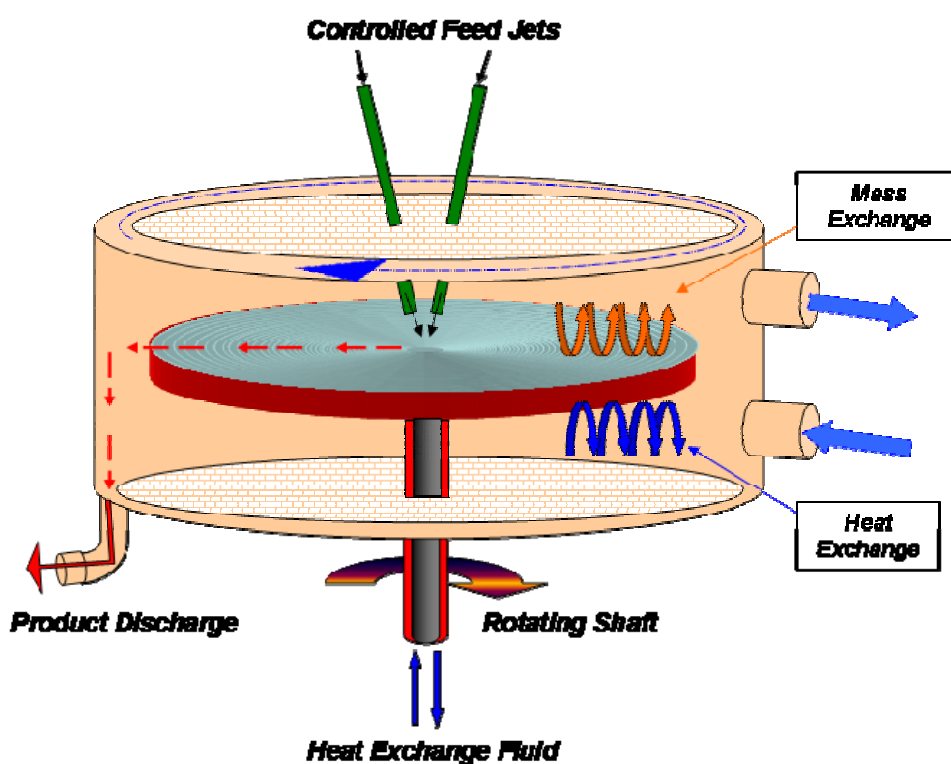
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### SUPPORTING INFORMATION



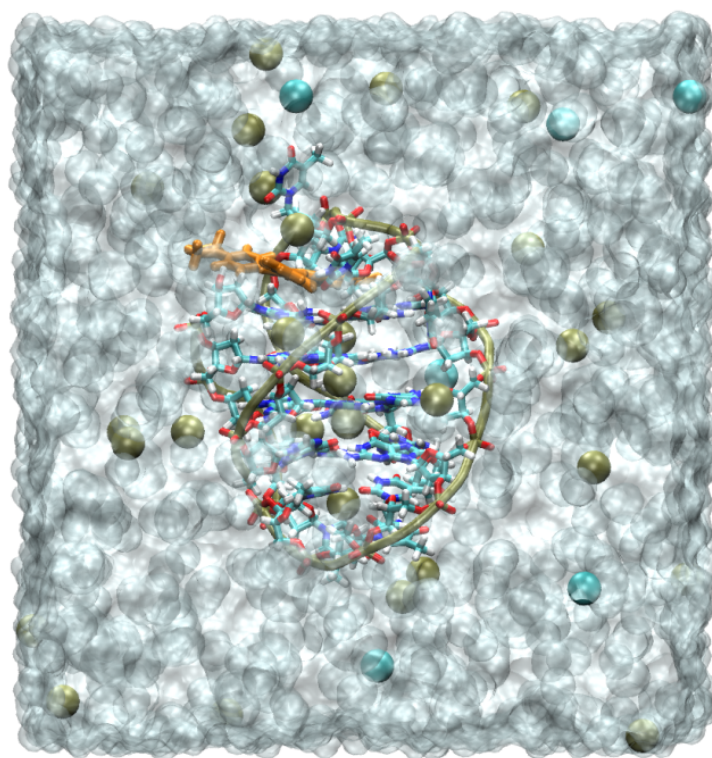
**Supplementary Figure 1:** Schematic of a Spinning Disc Processor (SDP).

## **SI.2. Experimental**

### **2.1. Molecular Dynamics Simulation**

To conduct molecular dynamics simulations, dimethylamino functionalized 4'-aryl-2,6-bis(4-aminophenyl)pyridine was first parameterised using ab initio calculations. Partial charges were calculated using an electrostatic fitting method in the program GAUSSIAN03 after optimizing the structure with Hartree-Fock theory and a 6-31+G\* basis set.<sup>33-34</sup> Force constants for bond stretching, angle bending, and dihedral torsions as well as Lennard-Jones parameters were taken from similar atoms in the CHARMM27 force field. The initial position of the compound within the DNA quadruplex was determined by aligning the pyridine core with that of 3,6-bis-[3-pyrrolidino-propionamide] acridine (BSU6039) in a crystal structure with it bound to the telomeric sequence of *Oxytrichia nova* d(GGGGTTTTGGGG).<sup>35</sup> The compound-DNA complex was solvated in a 55x55x55Å TIP3P water box neutralized with 300mM KCl (Supplementary Figure 2). To equilibrate the structure, the DNA and dimethylamino functionalized 4'-aryl-2,6-bis(4-aminophenyl)pyridine were initially held fixed and the system minimized for 1000 steps and equilibrated for 25ps. Then harmonic constraints of 2kcal/mol/Å<sup>2</sup> were placed on the compound and DNA which were slowly reduced over 75ps of simulation. Simulations were conducted at a pressure of 1 atm using a 1fs timestep using the program NAMD with the CHARMM27 force field for nucleic acids.<sup>36-38</sup> Initial simulations were conducted at a temperature of 298K, however additional simulations were also run in which this was slowly increased at a rate of 5 K per ns. Given the well known stability of the *Oxytrichia nova* quadruplex arising from the presence of 4 guanine tetrads it is unlikely that we could witness the melting of this structure in the timescale of our simulations (indeed the quadruplex did not melt when heated to 373K over 15 ns). To

overcome this, in our simulations of quadruplex melting, we cropped the quadruplex structure such that it contained only 2 of the guanine tetrads and one of the connecting thymine loops. Due to the short length of our simulations, caution should be applied in directly relating the melting temperatures seen here with experimental measurements. To calculate the free energy of binding of dimethylamino functionalized 4'-aryl-2,6-bis(4-aminophenyl)pyridine with the DNA quadruplex we conducted alchemical free energy perturbation simulations in which the compound bound to the DNA was slowly removed and an additional compound was slowly added to the aqueous medium over 2.6 ns.<sup>39</sup>



**Supplementary Figure 2:** The dimethylamino functionalised 4'-aryl-2,6-bis(4-aminophenyl)pyridine -DNA complex solvated in a 55x55x55Å TIP3P water box neutralized with 300mM KCl.

**2.2.Batch processing in propan-1-ol:** *p*-Aminoacetophenone (1.1 mmol, 149 mg) and sodium hydroxide (NaOH) (2.2 mmol, 88 mg) were added to propan-1-ol (10 cm<sup>3</sup>) with stirring and the mixture heated to dissolve the base and ketone. *p*-

Dimethylaminobenzaldehyde (0.55 mmol, 82.1 mg) was then added and the reaction mixture refluxed for 13 hours. An orange precipitate was evident in the reaction mixture and the reaction was cooled to room temperature. TLC of the reaction mixture was carried out in hexane:ethylacetate (1:2). The orange precipitate was collected by suction filtration and characterised by <sup>1</sup>HNMR in CDCl<sub>3</sub> (see **SI.3 section 3.1, Supplementary figure 3**). The filtrate was quenched with 1 M HCl then basified with ammonia solution (28% w/w). The solvent was removed *in vacuo* affording a dark brown powder (561.2 mg) which was characterised by <sup>1</sup>HNMR in DMSO.

**2.3. Batch processing in polyethyleneglycol (PEG300):** *p*-Aminoacetophenone (1.1 mmol, 149 mg) and NaOH (2.2 mmol, 88 mg) were added to PEG 300 (10 cm<sup>3</sup>) with stirring and the mixture was heated to dissolve the base and ketone. *p*-Dimethylaminobenzaldehyde (0.55 mmol, 82.1 mg) was then added and the reaction mixture was heated at 140 °C for 14 hours. On cooling to room temperature, water (100 cm<sup>3</sup>) was added affording an orange colour precipitate which was too fine to collect by filtration. TLC of the reaction mixture was carried out in hexane:ethylacetate (1:2). The solution was acidified with 1 M HCl and the product extracted into ethyl acetate (3 x 100 cm<sup>3</sup>). The solvent was removed *in vacuo* and the product dried under high vacuum affording a red/brown oil (736.6 mg) which was characterised by <sup>1</sup>HNMR in DMSO.

**2.4. Batch processing in a microwave reactor:**

Attempts to synthesize compound 5 in microwave was not successful. The following two experiments were undertaken: A) Ketone (27 mg), Aldehyde (14.1 mg) with Mg/Al Hydrotalcite-Rh (50%wt, 13.5 mg, Mg/Al ratio of 2.5), 110degC, 300W, 8 bar, 5-30 mins – to get schiff base plus starting materials. B) Ketone (27 mg),

Aldehyde (14.1 mg), PEG300 (5ml) with with Mg/Al Hydrotalcite-Rh (100%wt, 27 mg, Mg/Al ratio of 2.5), 110degC, 300W, 8 bar, 5- 30 mins – to get schiff base plus starting materials.

### **2.5. SDP experiments:**

**(a) General** For each experiment a ketone solution containing *p*-aminoacetophenone (6.684 g, 0.0495 mol) and NaOH (3.96 g, 0.099 mol) in propan-1-ol (300 cm<sup>3</sup>) and an aldehyde solution containing *p*-dimethylaminobenzaldehyde (7.38 g, 0.0495 mol) in propan-1-ol (300 cm<sup>3</sup>) were prepared unless otherwise stated. Percent conversion was obtained from the <sup>1</sup>HNMR of fractions collected.

**(b) Variable temperature studies** Five different experiments were conducted with different disc temperatures of 90, 110, 120, 130 and 140 °C. The disc rotational speed was fixed at 2500 rpm. For the first pass of each experiment one feed jet for the ketone solution was calibrated to 1 ml/s and the other jet feed for the aldehyde solution was calibrated to 0.5 ml/s. For all consecutive passes the solution was fed through one feed jet at 0.5 ml/s. Ten passes were conducted for all except the 140 °C experiment (only 7 passes) as precipitation in the feed jets clogged up the pipes during the 8<sup>th</sup> pass. Fractions were collected from each pass for analysis. The fractions were allowed to cool to room temperature, and TLC was carried out in hexane:ethylacetate (1:2). The orange precipitate from the 10<sup>th</sup> pass of the 110 °C experiment was collected by suction filtration and characterized by <sup>1</sup>HNMR in CDCl<sub>3</sub>. All fractions were quenched with HCl (1 M) and basified with ammonia solution (28% w/w). The solvent was removed *in vacuo* and the crude material was analysed by <sup>1</sup>HNMR in DMSO.

**(c) Varying the feed rate** The disc speed and temperature were fixed at 2500 rpm and 140 °C respectively. For the first pass one feed jet for the ketone solution was

calibrated to 1 ml/s and the other for the aldehyde solution was calibrated to 0.5 ml/s. The resulting mixture was then passed through one feed jet at 1.5 ml/s for the 2<sup>nd</sup> to 10<sup>th</sup> pass. Fractions were collected from each pass for analysis. The fractions were allowed to cool to room temperature. TLC of each fraction was carried out in hexane:ethylacetate (1:2). The fractions were quenched with HCl (1 M) and basified with ammonia solution (28% w/w). The solvent was removed *in vacuo* and the crude material was analysed by <sup>1</sup>H NMR in DMSO.

**(d) Varying the disc speed** The disc temperature and speed were fixed at 140 °C and 500 rpm respectively. For the first pass one jet feed the ketone solution was calibrated to 1 ml/s and the other for the aldehyde solution was calibrated to 0.5 ml/s. The resulting mixture was then passed through one feed jet at 0.5 ml/s for the 2<sup>nd</sup> to 10<sup>th</sup> pass. Fractions were collected from each pass for analysis. The fractions were allowed to cool to room temperature. TLC of each fraction was carried out in hexane:ethylacetate (1:2). The fractions were quenched with HCl (1 M) and basified with ammonia solution (28% w/w). The solvent was removed *en vacuo* and the crude was analysed by <sup>1</sup>HNMR in DMSO.

**(e) Isolated yield experiment** The disc speed and temperature were maintained at 2500 rpm and 130°C respectively. A 220ml solution containing *p*-Aminoacetophenone (3.268g, 0.0242 mol), NaOH (1.94 g, 0.0484 mol) and *p*-dimethylaminobenzaldehyde (1.804 g, 0.0121 mol) was fed into the SDP at 0.5 ml/s. The 7<sup>th</sup> pass was collected for analysis. The mixture was cooled to room temperature. TLC was carried out in hexane:ethylacetate (1:2). Water was added to aid precipitation. The crude precipitate was collected by filtration, dissolved in chloroform, filtered and the solvent was evaporated to give a brown solid (2.07 g). The brown solid was characterised by <sup>1</sup>HNMR in DMSO (see **SI.3** section **3.2 (d)**,

**Supplementary figure 4).** The solid was then purified by column chromatography over silica gel using hexane:ethyl acetate (1:2) as eluent to obtain pure 1,5-bis(4-aminophenyl)-3-(4-(dimethylamino)phenyl)pentane-1,5-dione, **5**, in 15% yield (see **SI.3 section 3.2 (d), Supplementary figure 5**) and the chalcone, **3**, in 35% yield (see **SI.3 section 3.2 (d), Supplementary figure 6**).

#### **2.6. Cyclisation of 5 in PEG300:**

A mixture of 1,5-bis(4-aminophenyl)-3-(4-(dimethylamino)phenyl)pentane-1,5-dione, **5** (600 mg, 0.00149 mol), PEG300 (10 cm<sup>3</sup>) and ammonium acetate ( 2.30 g, *excess*) were heated at 100 °C for 3 h with stirring. The reaction was monitored by TLC in hexane: ethylacetate (1:2). After 3 h the mixture was cooled to RT and water (100 cm<sup>3</sup>) was added to afford a brown precipitate of the triarylpyridine, **6**. This was collected by suction filtration and resuspended in a stirred solution of ethanol/water (10 cm<sup>3</sup> : 100 cm<sup>3</sup>) to remove any PEG300 still present. After stirring for 20 mins compound **6** was collected as a brown powder (535 mg, 94 % yield). The powder was characterised by <sup>1</sup>HNMR in DMSO.

#### **2.7. Acylation of 6:**

4-Chlorobutylchloride (4 cm<sup>3</sup>, 0.0355 mol) was added to Compound **6** (1.634 g, 0.00429 mol) with stirring and the mixture was heated at 60 °C overnight (~16 h). The reaction mixture was then cooled to room temperature. Diethyl ether (40 cm<sup>3</sup>) was added to aid precipitation. The resulting precipitate was collected by suction filtration as a red/brown solid. The solid was dissolved in methanol, filtered to remove any salt precipitate and the filtrate was then evaporated to give red/brown oil. Acetone was added to solidify the oil and the resulting brown precipitate was collected by suction filtration. The solid was re-dissolved in acetone/water (50 cm<sup>3</sup>: 1 cm<sup>3</sup>) and left to cool to room temperature. The solution was then filtered by suction filtration and

the filtrate was evaporated to give red/brown oil. Acetone was added to aid solidification of the oil and the resulting red/brown powder was collected by suction filtration (1.44 g, 57 % yield), compound **7**. The powder was dried on a high vacuum line and characterised by <sup>1</sup>HNMR in DMSO.

### **2.8. Aminolysis of 7:**

Pyrrolidine (1 cm<sup>3</sup>, 0.0122 mol) was added to Compound **7** (55.4 mg, 0.094 mmol) with stirring and the mixture was left stirring at room temperature overnight (~ 17 h). Ice-cold saturated bicarbonate solution was added to get an orange/brown precipitate. The mixture was centrifuged and the solution was decanted, the orange/brown precipitate was washed with ice-cold saturated bicarbonate solution (x 3) to give a red/brown oily precipitate. The oil was dissolved in methanol and filtered to remove excess salt. The filtrate was evaporated to give compound **8** as orange/yellow oil (58 mg, 94 % yield). HPLC (gradient: starting from Acetonitrile (0.2% TFA): Water (0.2% TFA) 30:70 to 70% Acetonitrile (0.2% TFA) over 20 min, hold for 5 min, R<sub>t</sub>=23.44 min).

### **2.9. Fluorescence Resonance Energy Transfer (FRET)**

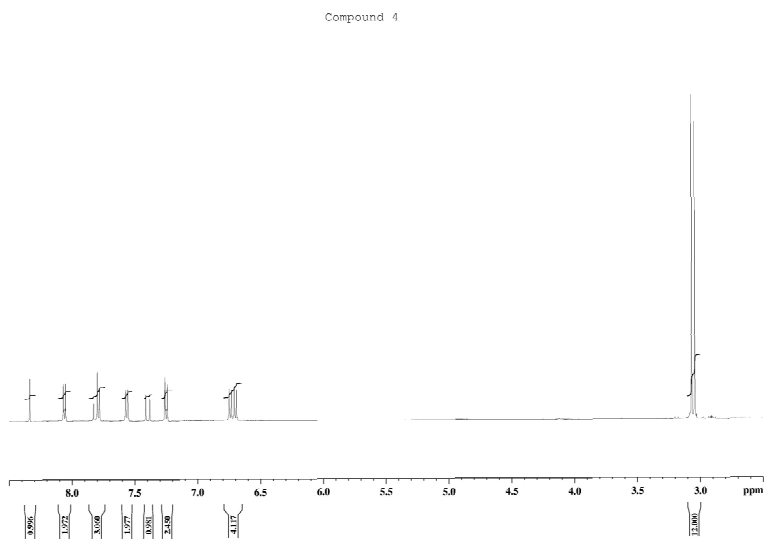
The labelled oligonucleotide c-kit: 5'-FAM-d(GGG CGG GCG CGA GGG AGG GG)-TAMRA-3' [donor fluorophore FAM is 6-carboxyfluorescein and acceptor fluorophore TAMRA is 6-carboxytetramethyl-rhodamine] was initially dissolved as a 100 μM stock solution in purified water and stored in a freezer until required. A 1 mM stock solution of compound **8** was made up in purified water and stored in a freezer until required. A 400 nM solution of c-kit was prepared by diluting the stock solution in a 60 mM sodium cacodylate buffer (pH 7.4). The solutions was then



heated at 90 °C for 2 min and allowed to cool to room temperature overnight. A 1  $\mu$ M solution of compound **8** was prepared by diluting the stock solution in a 60 mM sodium cacodylate buffer (pH 7.4). A 96-well plate was prepared by aliquoting: 100  $\mu$ l of 60 mM sodium cacodylate buffer into well A01, 50  $\mu$ l of 60 mM sodium cacodylate & 50  $\mu$ l of 400 nM c-kit solution into well C01 and 50  $\mu$ l of 400 nM c-kit solution & 50  $\mu$ l of 1  $\mu$ M solution of compound **8** into well H01. Fluorescence melting curve was determined in a EnVision 2102 Multilabel Plate Reader. Measurements were made in duplicate at each wavelength with excitation at 480 nm and detection at 590 nm and 515 nm. Final analysis of the data was carried out using Origin 7.5.

### **SI.3. Additional Results**

**3.1. Batch processing in propan-1-ol and PEG300:** No 1,5-diketone was evident by <sup>1</sup>HNMR and TLC analysis. The major product, which precipitated out of solution as an orange precipitate in propan-1-ol was the Schiff-base adduct of the Claisen-Schmidt condensation product, compound **4** (**Supplementary Figure 3**). As the orange precipitate in the PEG300 reaction was too fine to collect by filtration <sup>1</sup>HNMR of the crude product after acidification and basifying with HCl (1 M) and Ammonia solution (28% w/w) respectively was identical to the <sup>1</sup>HNMR of pure compound **4** after acidification and basifying with HCl (1 M) and Ammonia solution (28% w/w) respectively. For both methods, 90% of the crude product was Compound **4**, with the other 10% consisting of a mixture of ketone, aldehyde and chalcone.

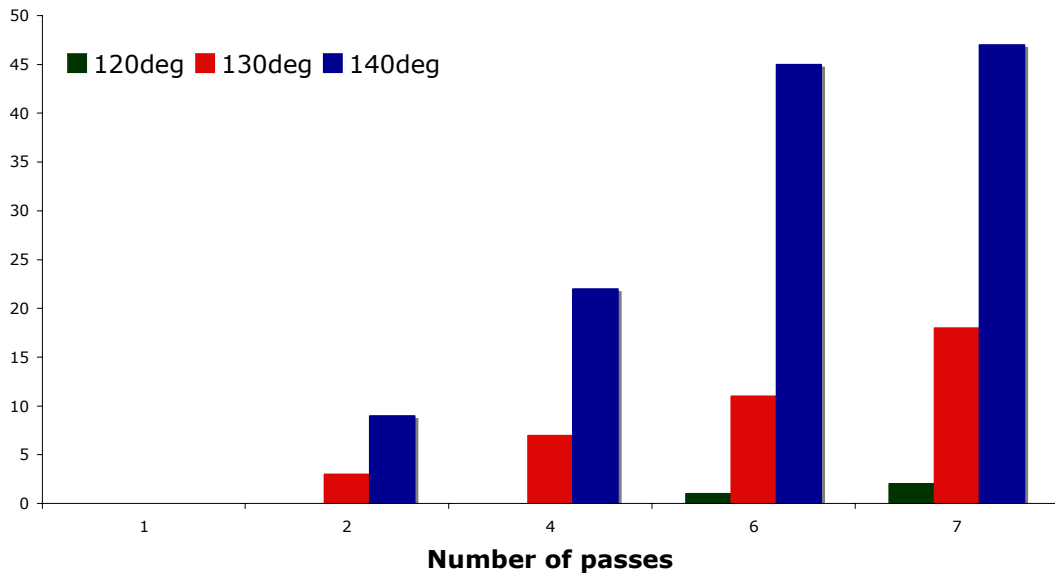


**Supplementary Figure 3:** <sup>1</sup>HNMR in CDCl<sub>3</sub> of orange precipitate from Batch processing in propan-1-ol.

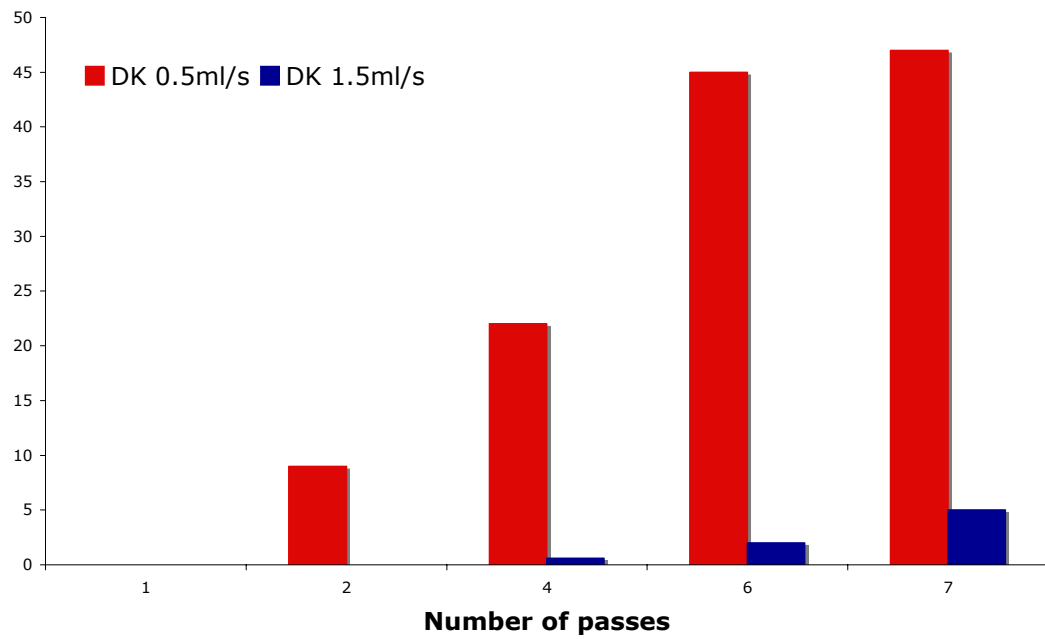
### **3.2. SDP experiments**

#### ***(a) Variable temperature studies***

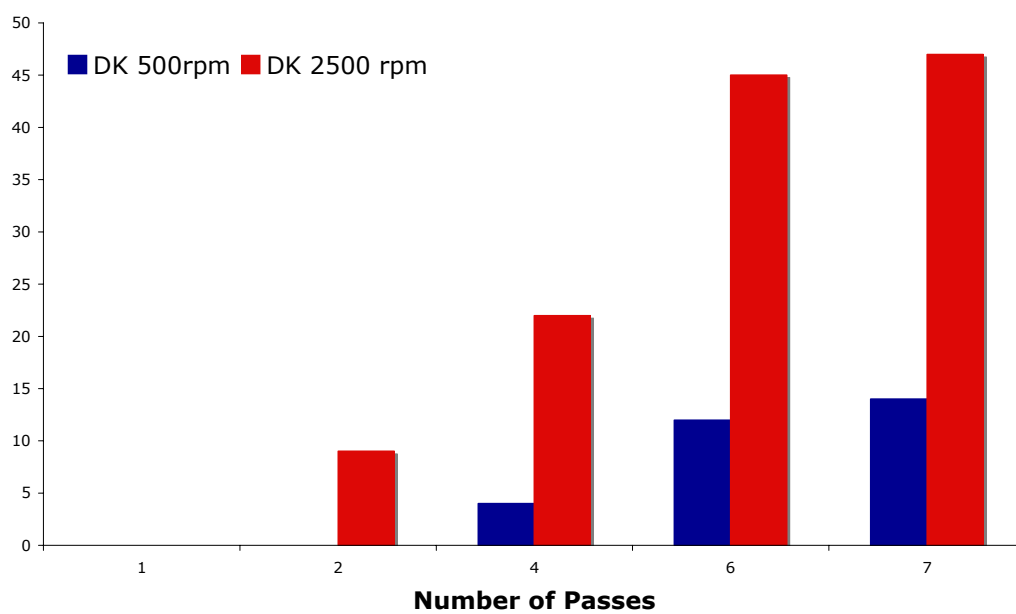
No 1,5-diketone formation was observed at 90 and 110 °C. The major product obtained at these temperatures was compound **4**. The orange precipitate obtained from the 10<sup>th</sup> pass of the 110 °C experiment was identified as compound **4** by <sup>1</sup>HNMR analysis. From the above graph it is evident that 1,5-diketone conversion increased with an increase in temperature, with maximum conversion (>45%) achieved at 140 °C. Compound **4** was not evident by TLC and <sup>1</sup>HNMR analysis at 130 °C and above, suggesting that Schiff-base formation has been shut down.



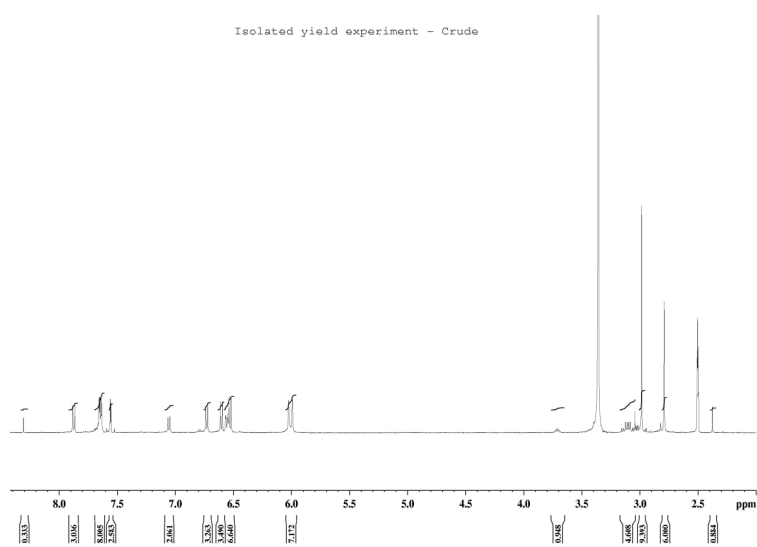
*(b) Varying the feed rate*



***(c) Varying the disc speed***

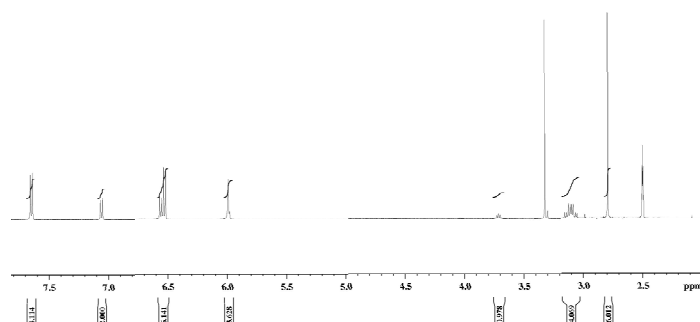


***(d) Isolated yield experiment***



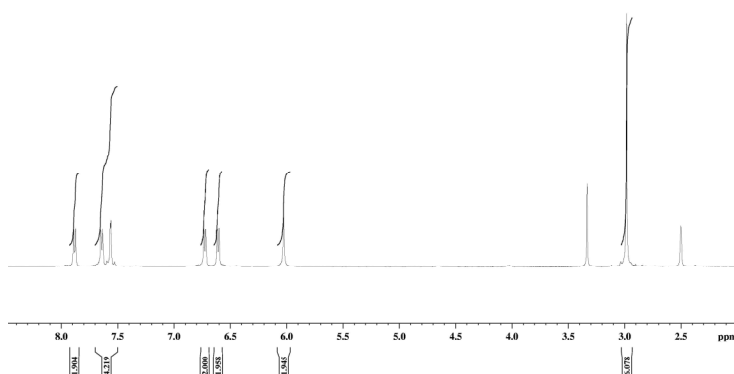
**Supplementary Figure 4:** <sup>1</sup>H NMR in DMSO of crude solid from isolated yield experiment.

Compound 5



**Supplementary Figure 5:**  $^1\text{H}$ NMR of Compound 5 from isolated yield experiment.

Compound 3



**Supplementary Figure 6:**  $^1\text{H}$ NMR in DMSO of Compound 3 from isolated yield experiment.

### **3.3 NMR Characterization of Products**

**3:**  $\delta_{\text{H}}$  (500 MHz, DMSO) 7.89 (d,  $J = 8.82$  Hz, 2H), 7.64 (d,  $J = 8.82$  Hz, 2H), 7.57 (d,  $J = 2.63$  Hz, 2H), 6.72 (d,  $J = 8.94$  Hz, 2H), 6.62 (d,  $J = 8.82$  Hz, 2H), 6.03 (s, 2H), 2.98 (s, 6H).  $\delta_{\text{C}}$  (125 MHz, DMSO) 185.85, 153.36, 151.53, 142.47, 130.68, 130.14,

125.97, 122.55, 116.62, 112.68, 111.79, 39.74. MS ( $\text{EI}^+$ , 70 eV, 200 °C) for  $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}$  ( $[\text{M}]^+$ ): calcd: 266.1419; found: 266.1418 (100 %).

**4:**  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 8.33 (s, 1H), 8.06 (d,  $J = 8.48$  Hz, 2H), 7.81 (d,  $J = 15.24$  Hz, 1H), 7.78 (s, 2H), 7.56 (d,  $J = 8.94$ , 2H), 7.39 (d,  $J = 15.46$ , 1H), 7.25 (d,  $J = 8.48$  Hz, 2H), 6.74 (d,  $J = 8.82$  Hz, 2H), 6.70 (d,  $J = 8.94$  Hz, 2H), 3.06 (s, 6H), 3.04 (s, 6H).  $\delta_{\text{C}}$  (125 MHz,  $\text{CDCl}_3$ ) 189.52, 161.22, 156.74, 152.87, 152.04, 145.28, 135.62, 130.93, 130.44, 129.80, 124.11, 122.92, 121.07, 116.92, 113.98, 111.93, 111.62, 40.22. MS ( $\text{EI}^+$ , 70 eV, 200 °C) for  $\text{C}_{26}\text{H}_{27}\text{N}_3\text{O}$  ( $[\text{M}]^+$ ): calcd: 397.2154; found: 397.2155 (100 %).

**5:**  $\delta_{\text{H}}$  (500 MHz, DMSO) 7.65 (d,  $J = 8.71$  Hz, 4H), 7.06 (d,  $J = 8.71$  Hz, 2H), 6.56 (d, 8.82 Hz, 2H), 6.53 (d,  $J = 8.71$  Hz, 4H), 5.99 (s, 4H), 3.71 (m, 1H), 3.10 (m, 4H), 2.79 (s, 6H).  $\delta_{\text{C}}$  (125 MHz, DMSO) 195.91, 153.49, 148.82, 132.46, 130.35, 127.96, 124.79, 112.47, 112.37, 43.89, 40.30, 36.67. MS ( $\text{EI}^+$ , 70 eV, 200 °C) for  $\text{C}_{25}\text{H}_{27}\text{N}_3\text{O}_2$  ( $[\text{M}]^+$ ): calcd: 401.2103; found: 401.2102 (100 %).

**6:**  $\delta_{\text{H}}$  (500 MHz, DMSO) 7.97 (d,  $J = 8.71$  Hz, 4H), 7.81 (d,  $J = 8.82$  Hz, 2H), 7.71 (s, 2H), 6.83 (d,  $J = 8.82$  Hz, 2H), 6.67 (d,  $J = 8.48$  Hz, 4H), 5.36 (s, 4H), 2.98 (s, 6H).  $\delta_{\text{C}}$  (125 MHz, DMSO) 156.42, 150.87, 149.72, 148.52, 127.71, 127.61, 127.04, 125.36, 113.75, 112.34, 111.49, 39.94. MS ( $\text{EI}^+$ , 70 eV, 200 °C) for  $\text{C}_{25}\text{H}_{24}\text{N}_4$  ( $[\text{M}]^+$ ): calcd: 380.2001; found: 381.2001 (100%).

**7:**  $\delta_{\text{H}}$  (500 MHz, DMSO) 10.47 (s,  $\text{NH}(\text{C}=\text{O})$ ), 8.23 (d,  $J = 8.25$  Hz, 4H), 8.16 (s, 2H), 8.08 (d,  $J = 8.82$  Hz, 2H), 7.35 (d,  $J = 8.25$  Hz, 4H), 7.11 (d,  $J = 8.82$  Hz, 2H), 4.25 (t,  $J = 6.87$  Hz, 4H), 3.06 (s,  $\text{N}(\text{CH}_3)_2$ ), 2.41 (t,  $J = 7.22$  Hz, 4H), 2.13 (tt,  $J = 6.87$  Hz, 4H).  $\delta_{\text{C}}$  (125 MHz, DMSO) 170.66, 153.63, 141.41, 134.61, 130.59, 130.04, 129.51,

129.22, 128.99, 128.78, 118.87, 115.98, 45.02, 39.94, 33.49, 27.86. MS (EI<sup>+</sup>, 70 eV, 200 °C) for : C<sub>33</sub>H<sub>34</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>2</sub> ([M]<sup>+</sup>): calcd: 588.2059: found: 588.2053 (100%).

**8:** δ<sub>H</sub> (500 MHz, MeOD) 8.11 (d, J = 8.59 Hz, 4H), 7.85 (s, 2H), 7.72 (d, J = 8.82 Hz, 2H), 7.70 (d, J = 8.59 Hz, 4H), 6.85 (d, J = 8.82 Hz, 2H), 3.00 (s, N(CH<sub>3</sub>)<sub>2</sub>), 2.57 (m, 8H), 2.54 (m, 4H), 2.43 (t, J = 7.22 Hz, 4H), 1.93 (tt, J = 7.45 Hz, 4H), 1.81 (m, 8H).  
δ<sub>C</sub> (125 MHz, MeOD) 173.97, 158.11, 152.83, 151.55, 140.77, 136.69, 128.79, 128.67, 126.78, 120.97, 116.06, 113.73, 56.88, 54.97, 40.52, 35.99, 25.74, 24.19. MS (EI<sup>+</sup>, 70 eV, 200 °C) for C<sub>41</sub>H<sub>50</sub>N<sub>6</sub>O<sub>2</sub> ([M]<sup>+</sup>): calcd: 658.3995: found: 659.3995 (100%).

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#### **SI. 4. Supplementary Notes**

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