Electronic Supplementary Material (ESI) for Chemical Science.

Development of microfluidic platforms for the synthesis of metal complexes and evaluation of their DNA affinity using online FRET melting assays

Viktoria Rakers, Paolo Cadinu, Joshua B. Edel* and Ramon Vilar*

Supplementary Information

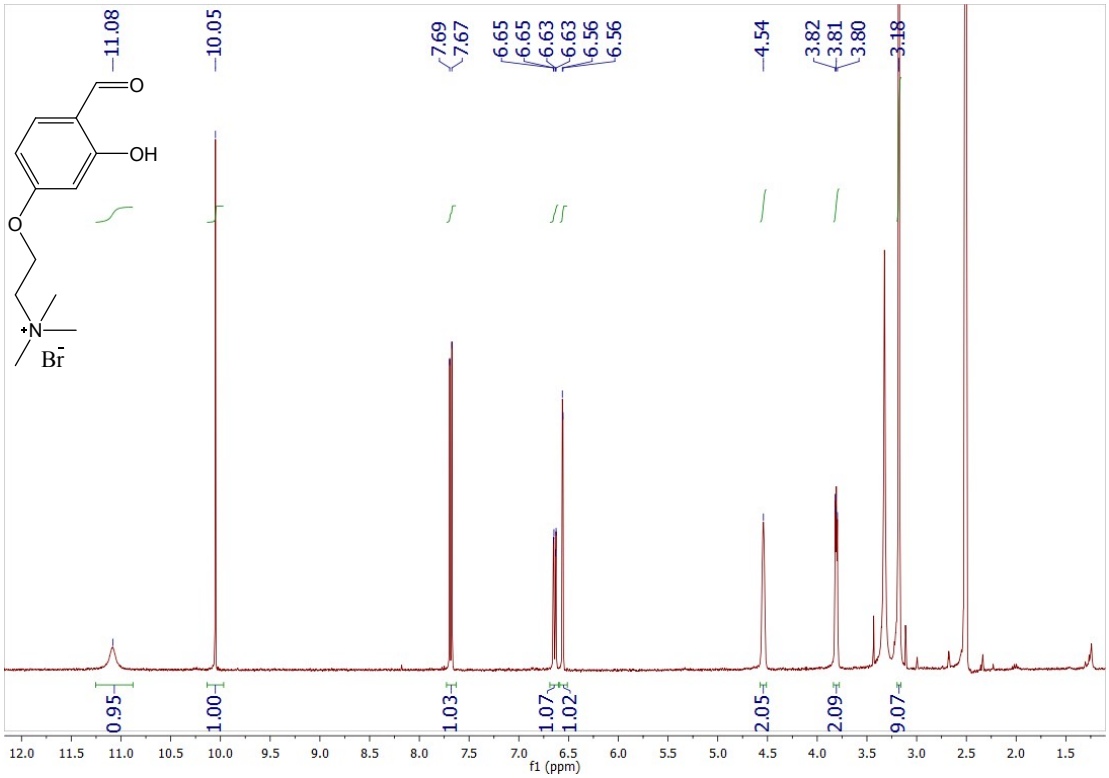


Figure S1. ^{1}H NMR spectrum of **1** (d^{6}-DMSO, 400 MHz, 298 K) prepared by conventional methods.

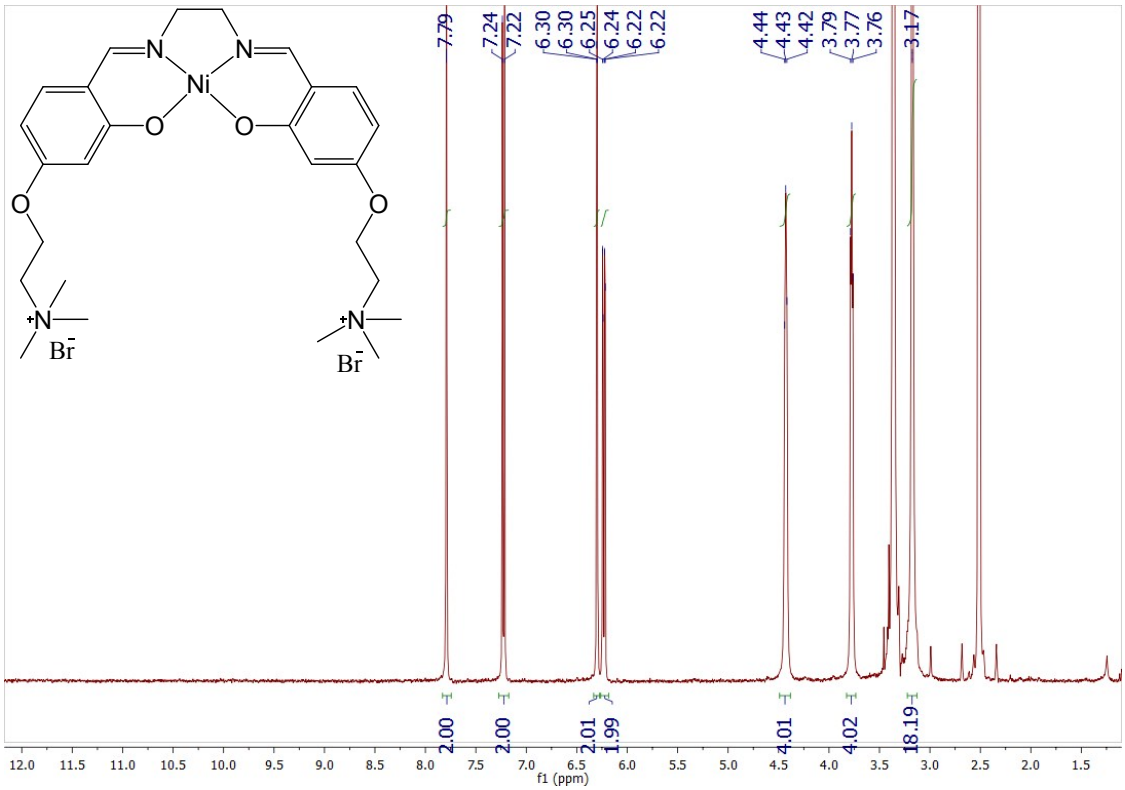


Figure S2. ^{1}H NMR spectrum of **2** (d^{6}-DMSO, 400 MHz, 298 K) prepared by conventional methods.

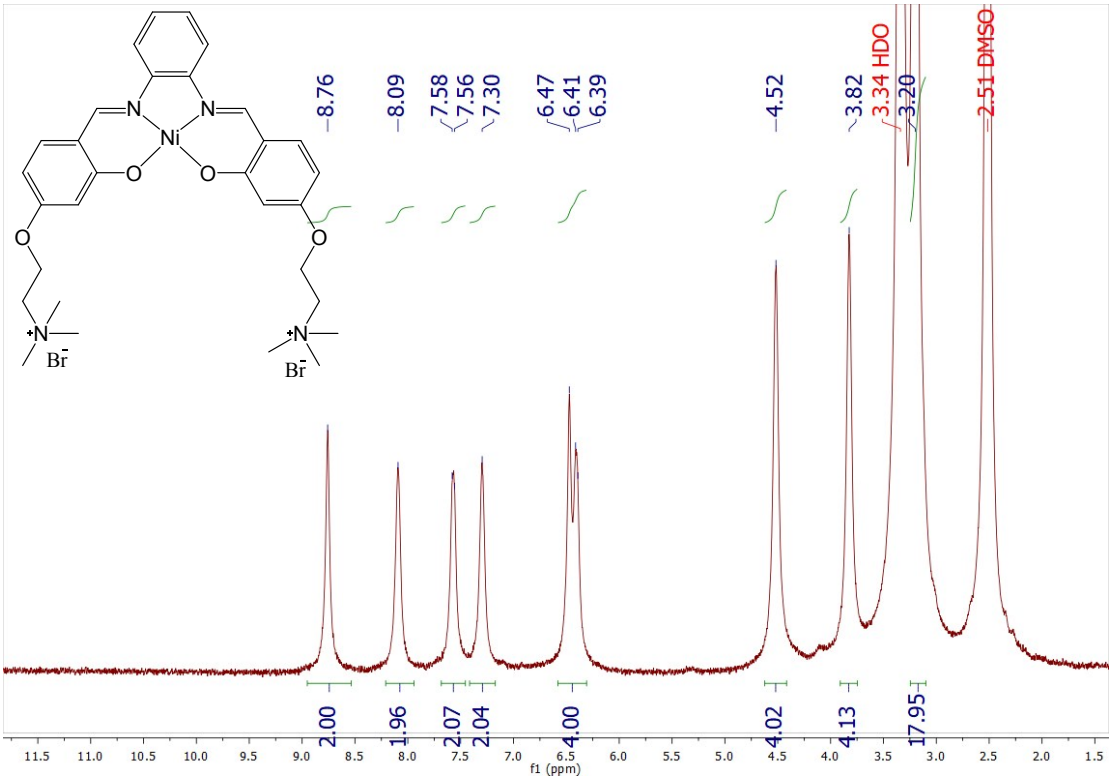


Figure S3. ^{1}H NMR spectrum of **3** (d^{6}-DMSO, 400 MHz, 298 K) prepared by conventional methods.

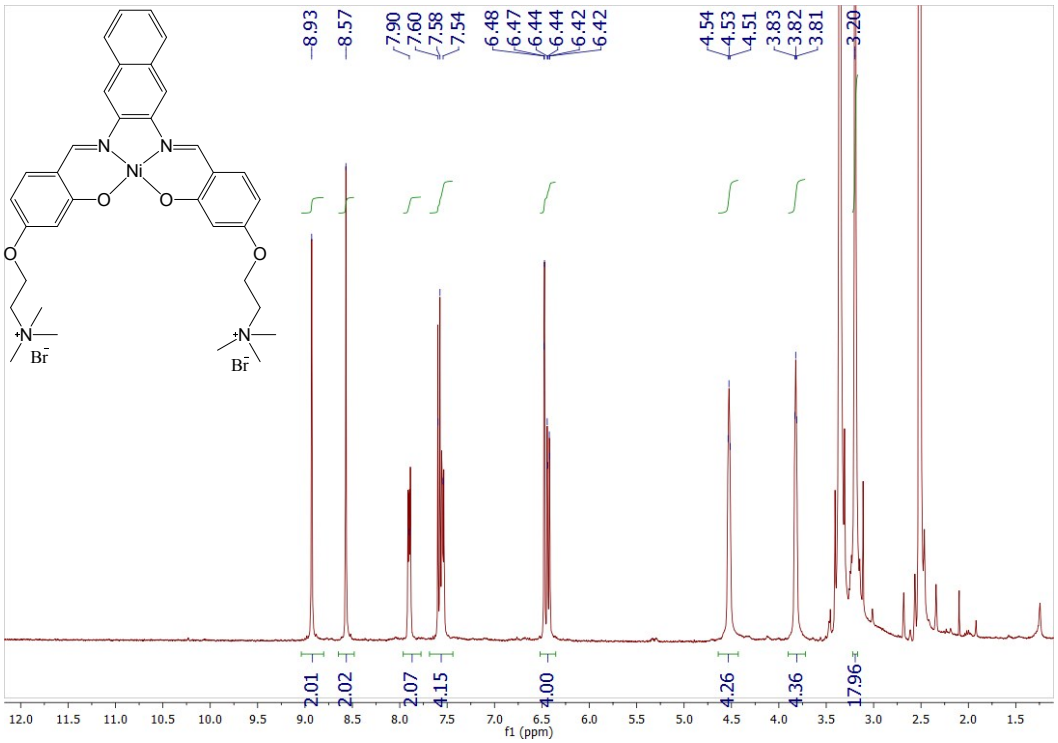


Figure S4. ^{1}H NMR spectrum of **4** (d^{6}-DMSO, 400 MHz, 298 K) prepared by conventional methods.

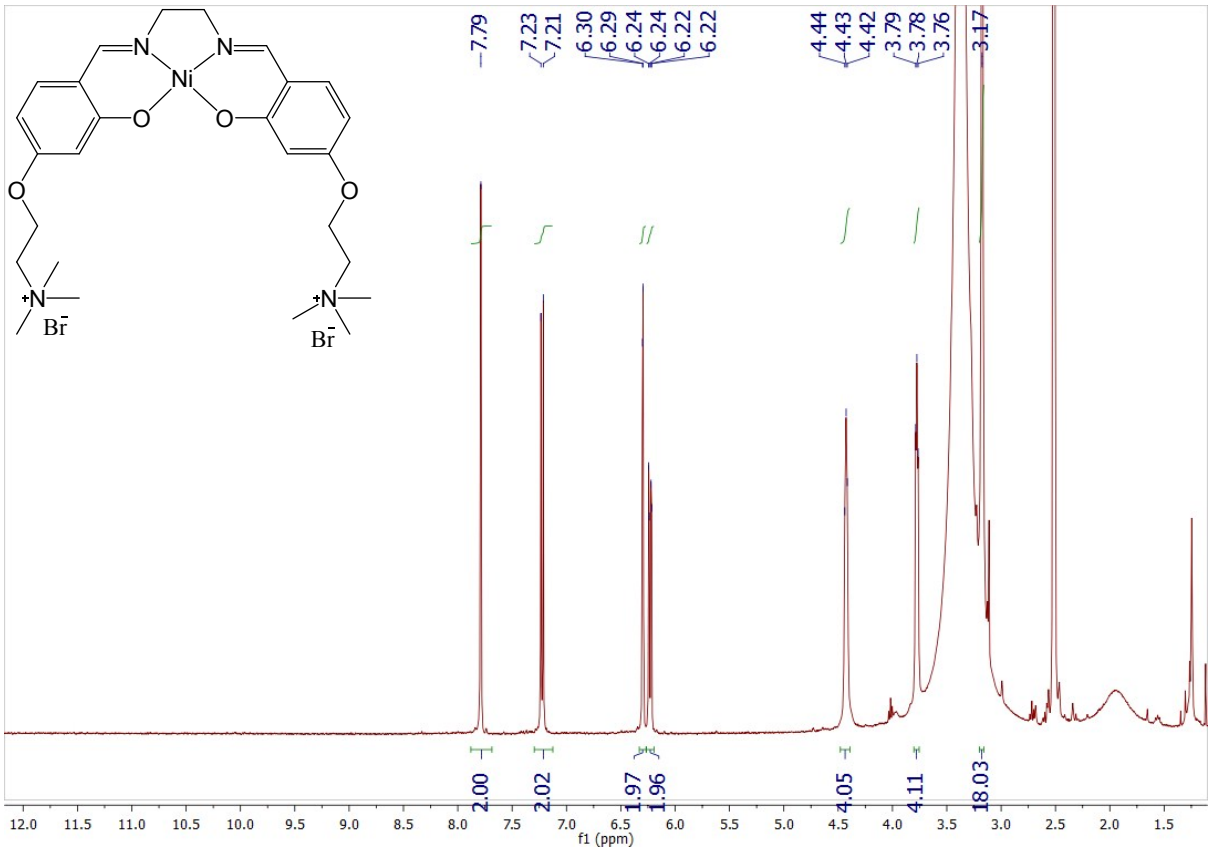


Figure S5. ^{1}H NMR spectrum of **2** (d^{6}-DMSO, 400 MHz, 298 K) prepared "*one-pot*".

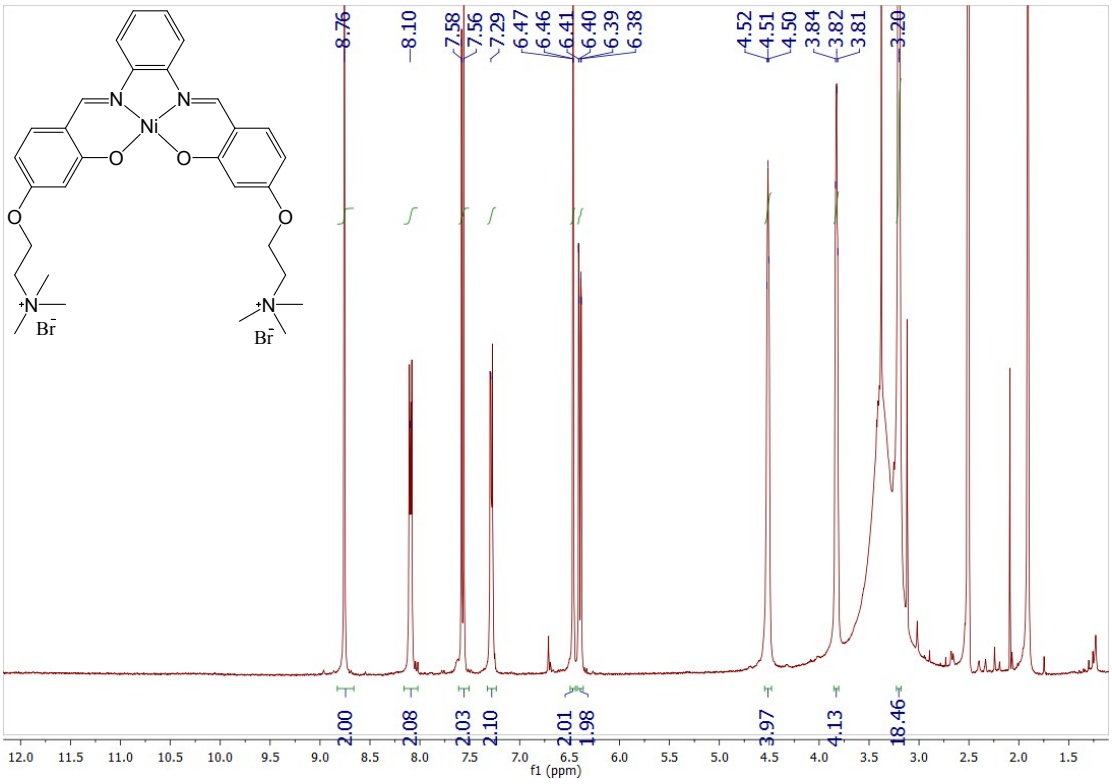


Figure S6. ^{1}H NMR spectrum of **3** (d^{6}-DMSO, 400 MHz, 298 K) prepared "*one-pot*".

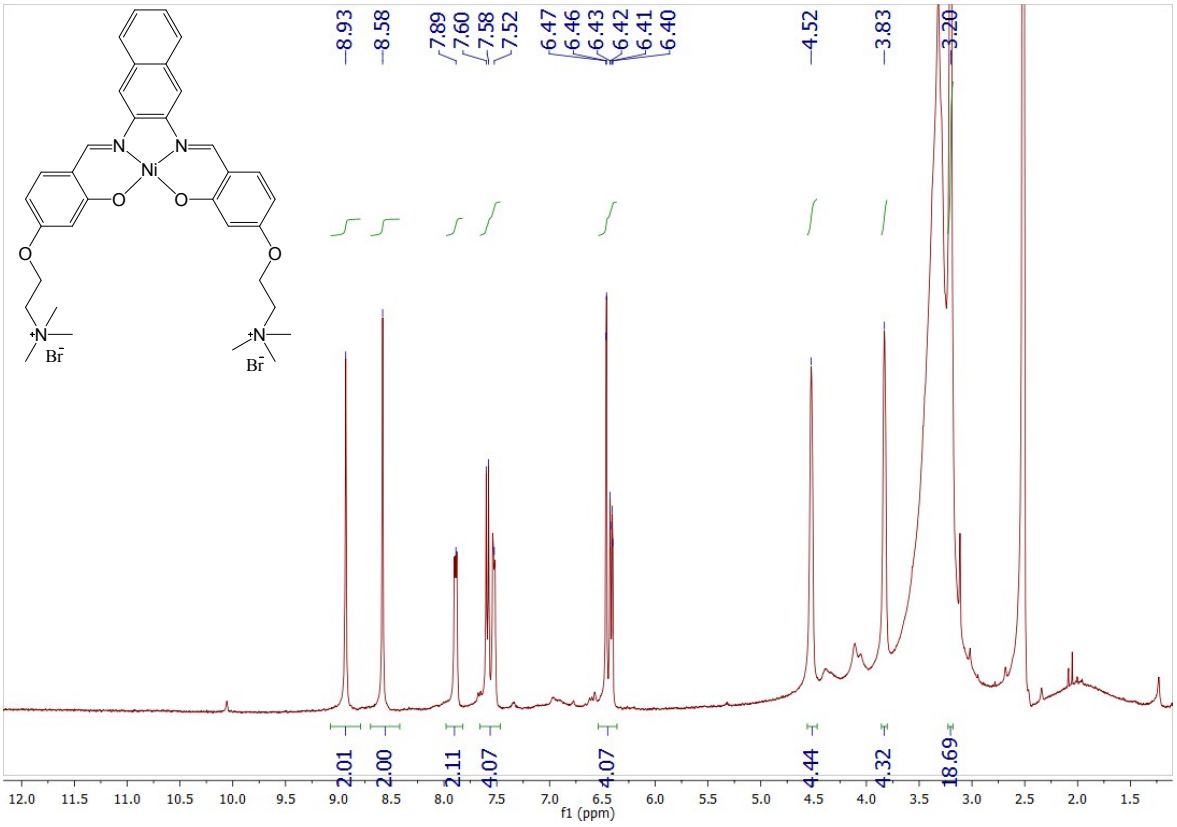


Figure S7. ^{1}H NMR spectrum of **4** (d^{6}-DMSO, 400 MHz, 298 K) prepared "*one-pot*".

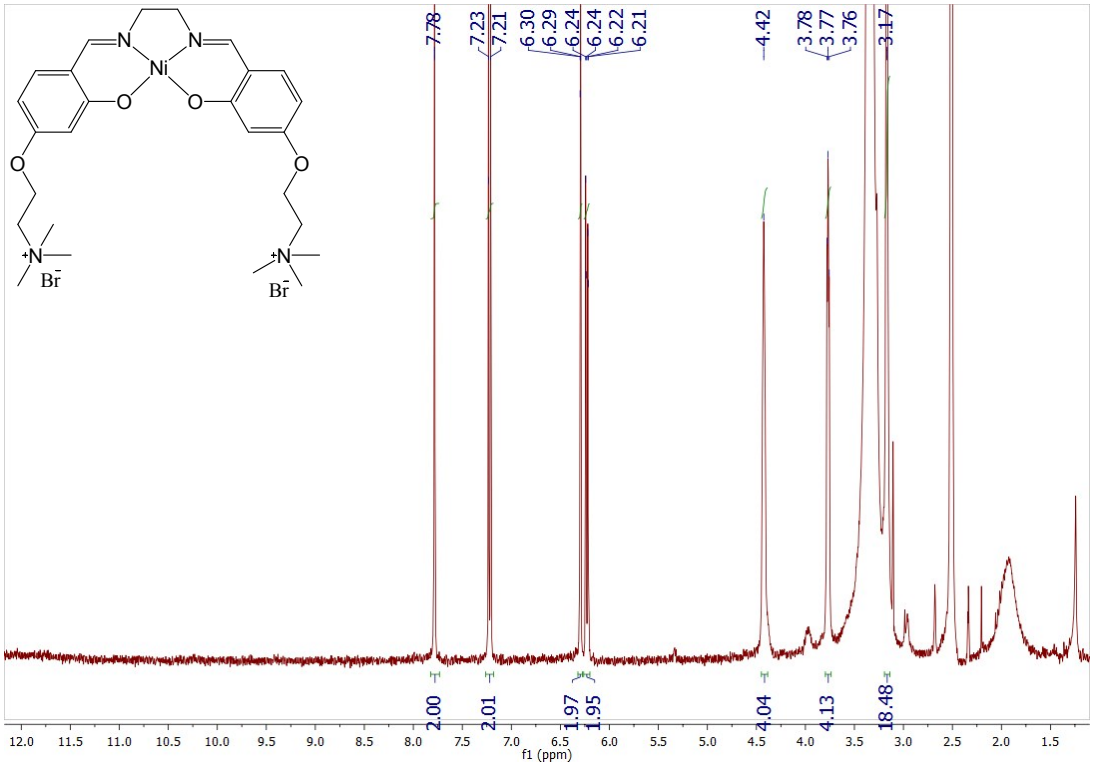


Figure S8. ^{1}H NMR spectrum of **2** (d^{6}-DMSO, 400 MHz, 298 K) prepared *in flow*.

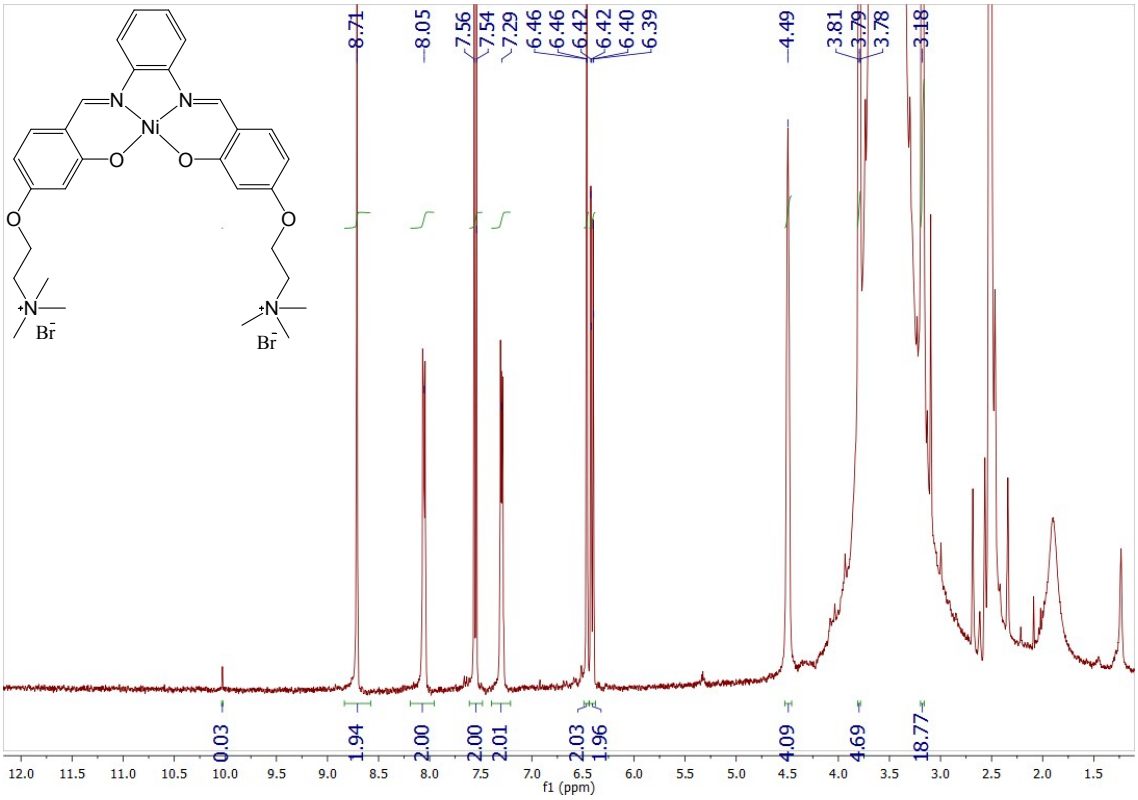


Figure S9. ^{1}H NMR spectrum of **3** (d^{6}-DMSO, 400 MHz, 298 K) prepared *in flow*.

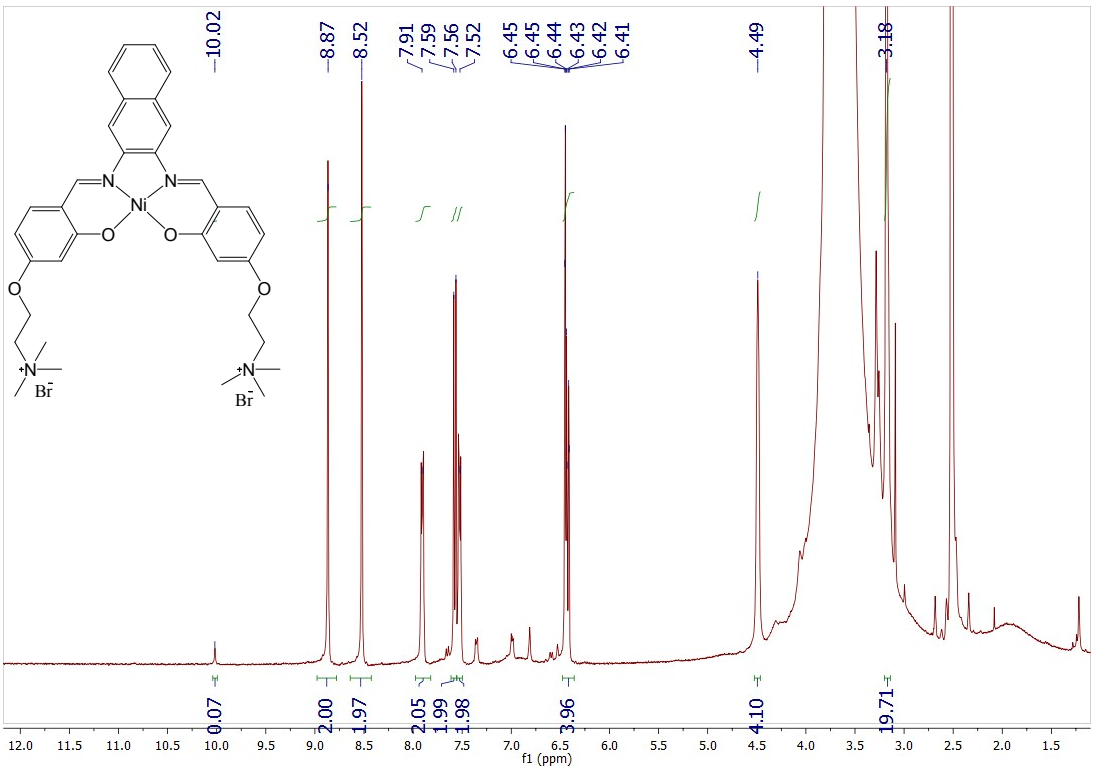


Figure S10. ^{1}H NMR spectrum of **4** (d^{6}-DMSO, 400 MHz, 298 K) prepared *in flow*.

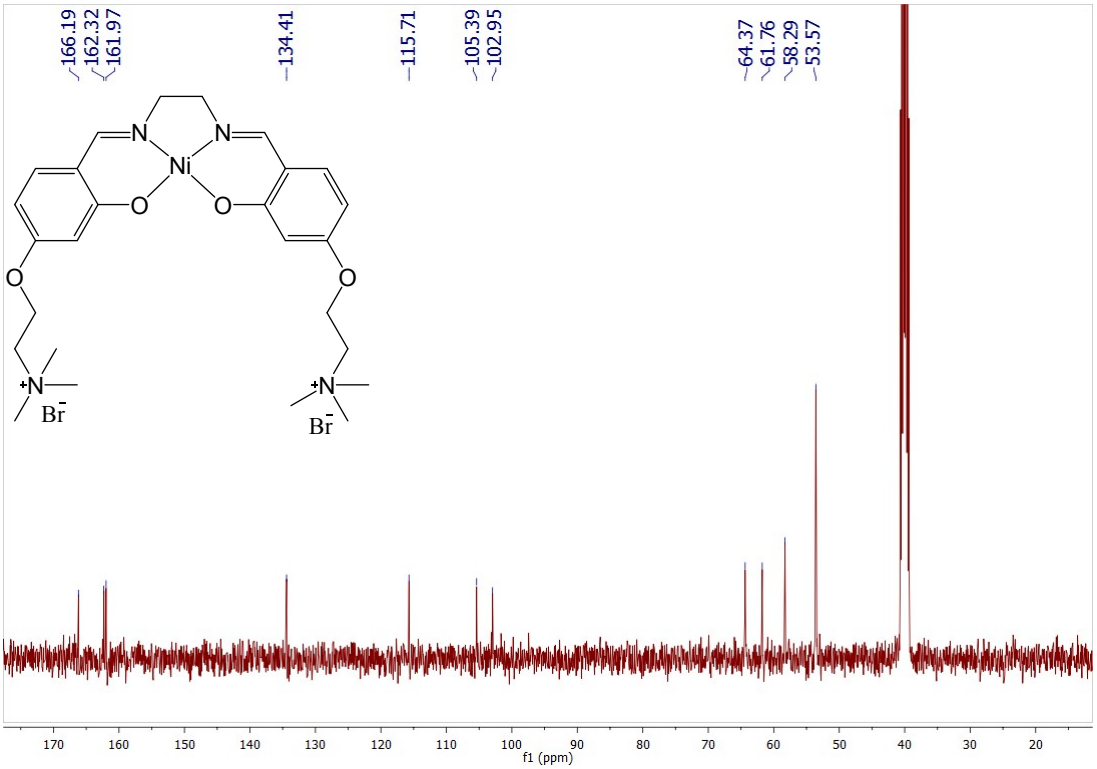


Figure S11. ^{13}C NMR spectrum of **2** (d^6-DMSO, 400 MHz, 298 K) prepared by conventional methods.

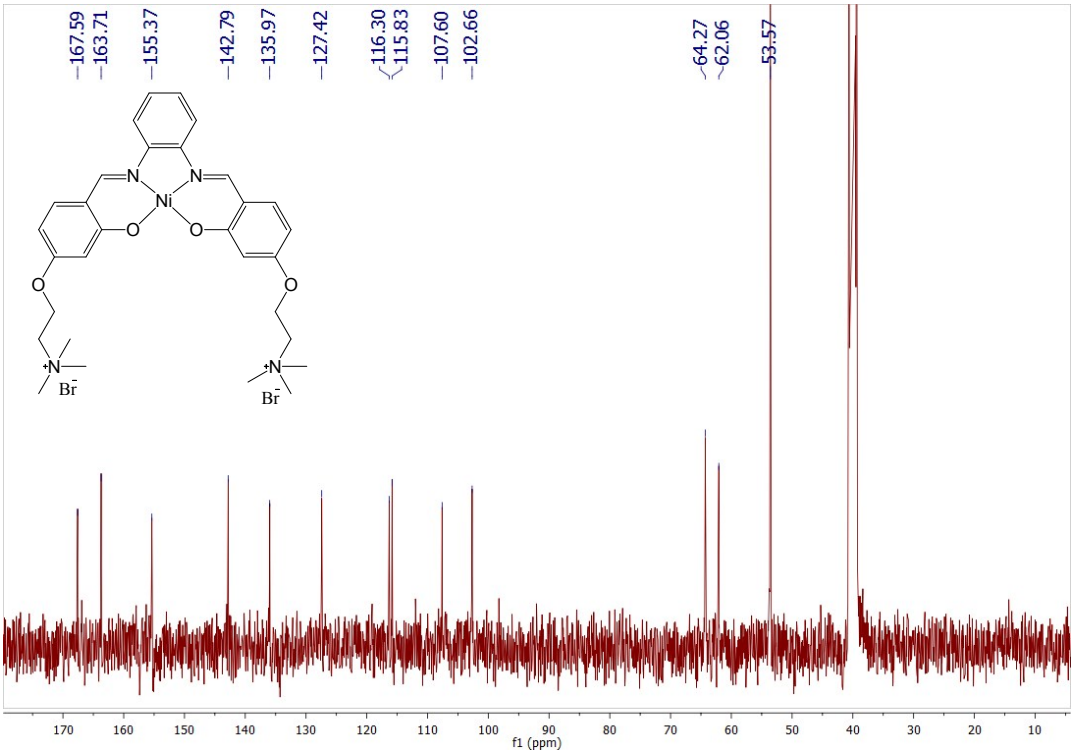


Figure S12. ^{13}C NMR spectrum of **3** (d^6-DMSO, 400 MHz, 298 K) prepared by conventional methods.

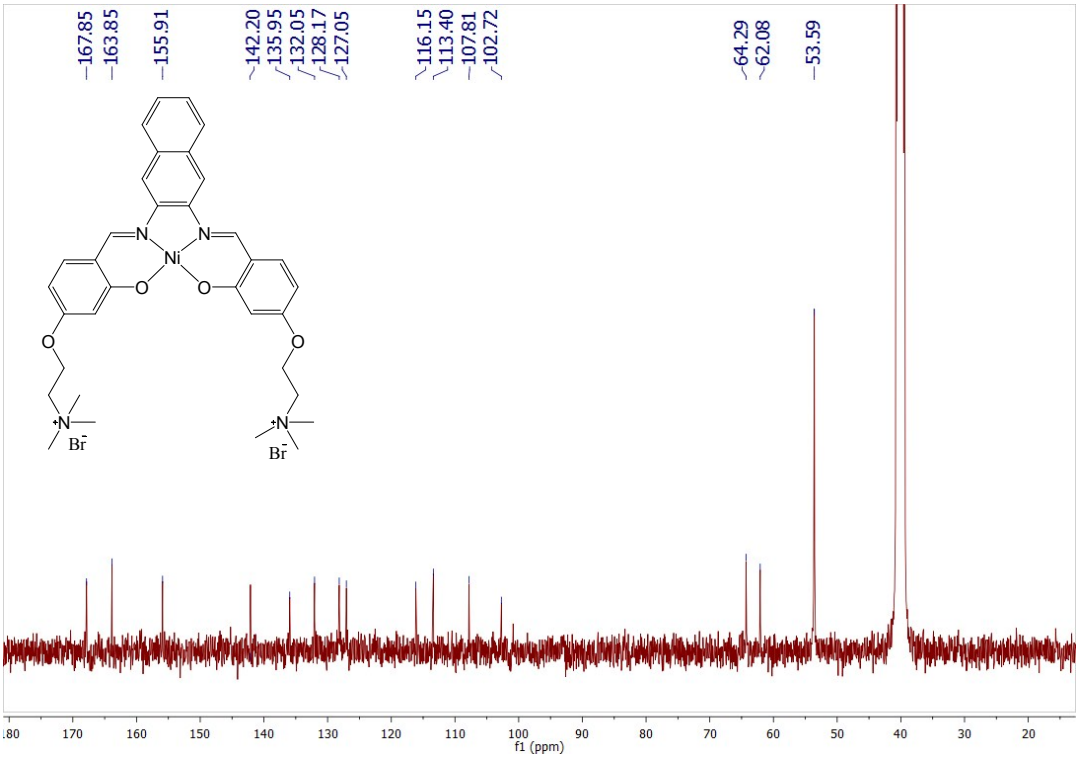


Figure S13. ^{13}C NMR spectrum of **4** (d^6-DMSO, 400 MHz, 298 K) prepared by conventional methods.

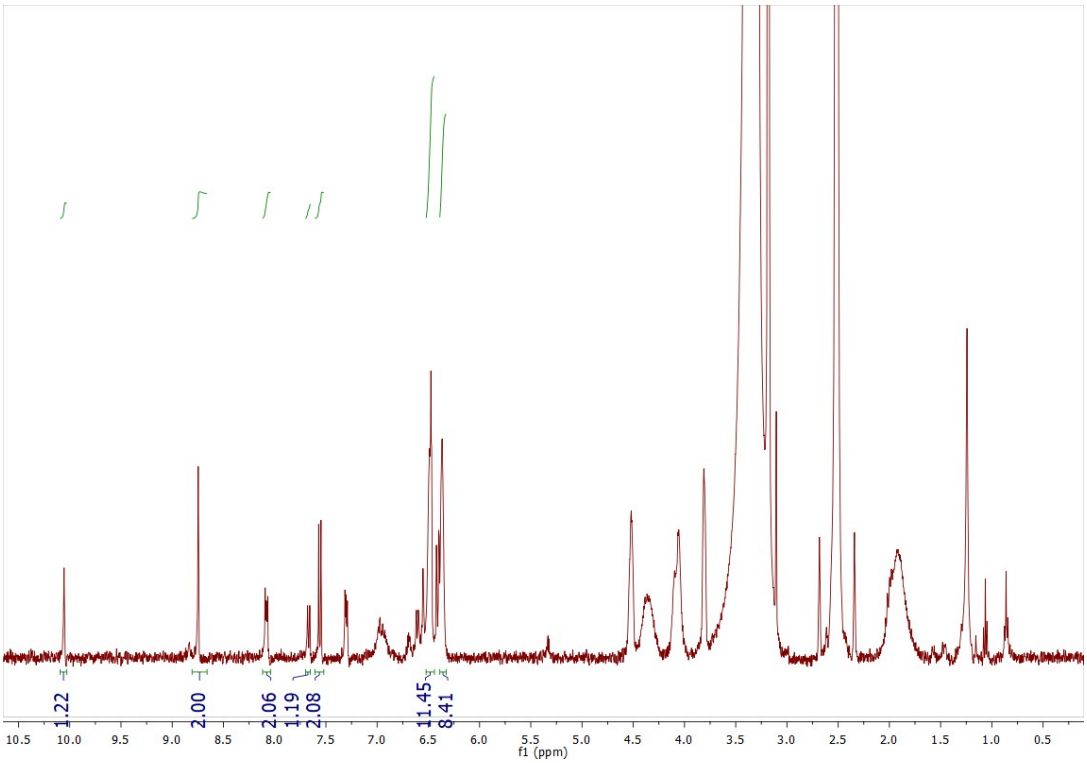


Figure S14. ^{1}H NMR spectrum of **3** after 10 min heating in continuous flow (d^6-DMSO, 400 MHz, 298 K).

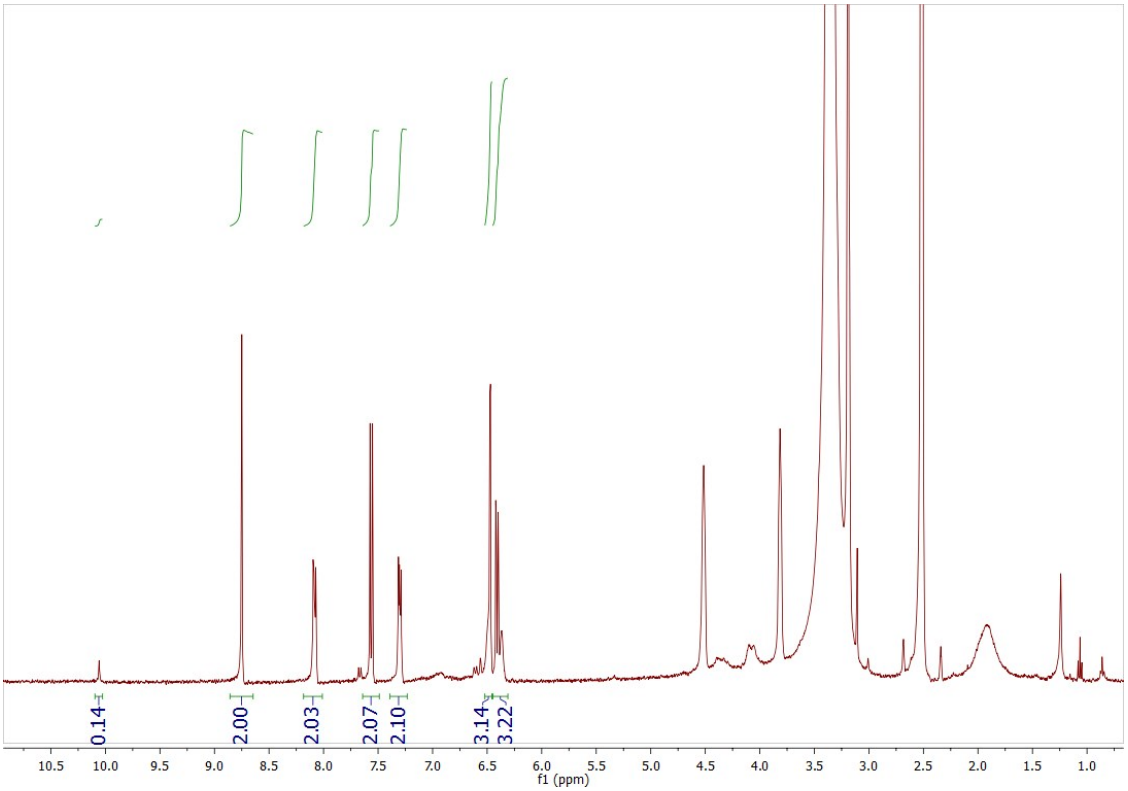


Figure S15. ^{1}H NMR spectrum of **3** after 30 min heating in continuous flow (d^{6}-DMSO, 400 MHz, 298 K).

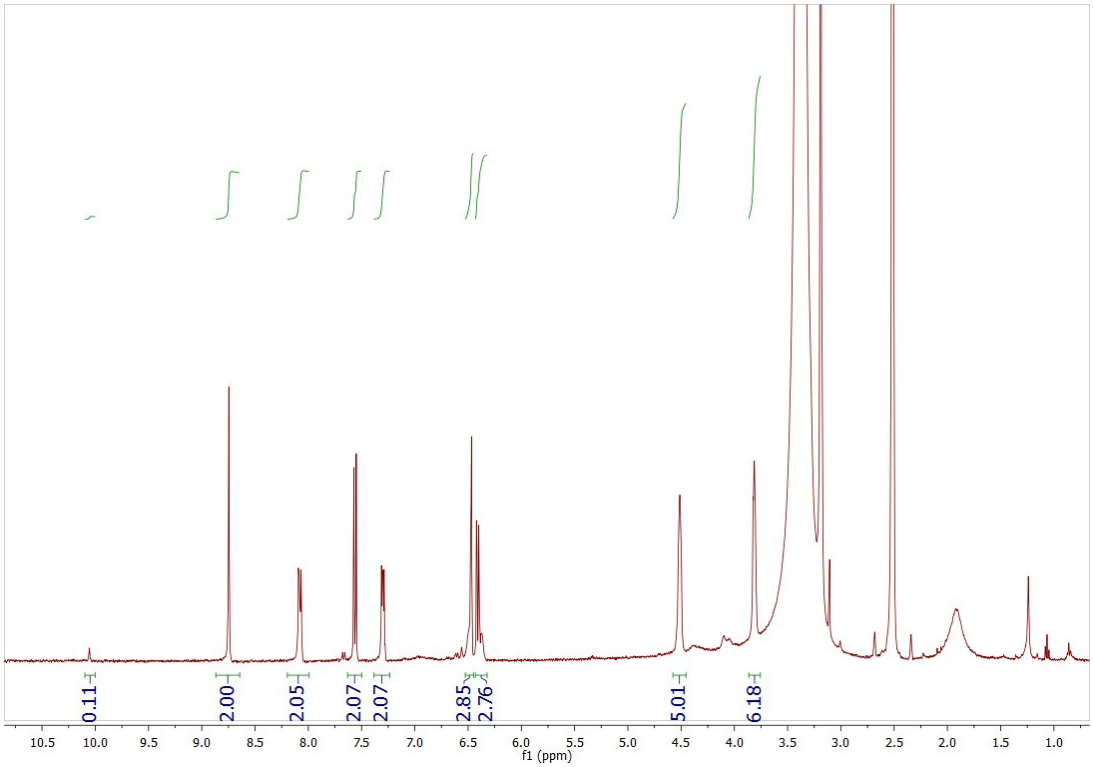


Figure S16. ^{1}H NMR spectrum of **3** after 150 min heating in continuous flow (d^{6}-DMSO, 400 MHz, 298 K).

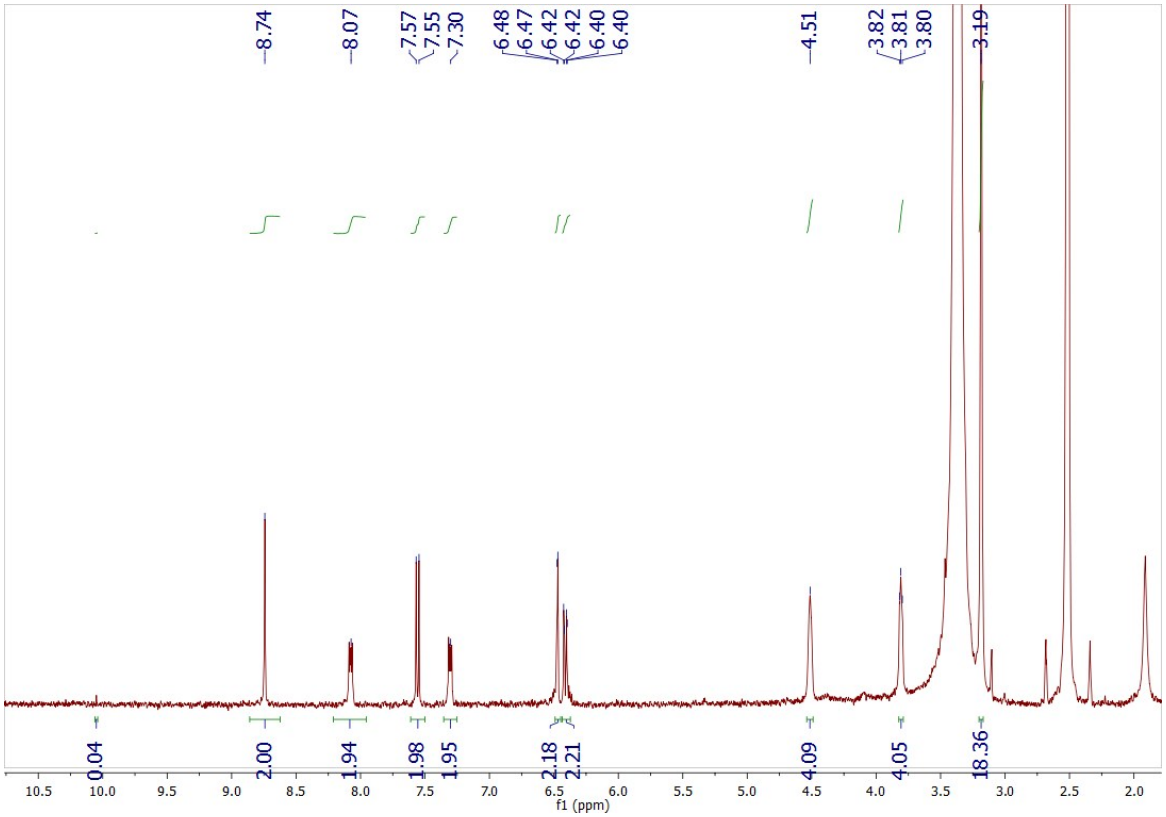


Figure S17. ^{1}H NMR spectrum of **3** after 9h heating in continuous flow (d^{6}-DMSO, 400 MHz, 298 K).

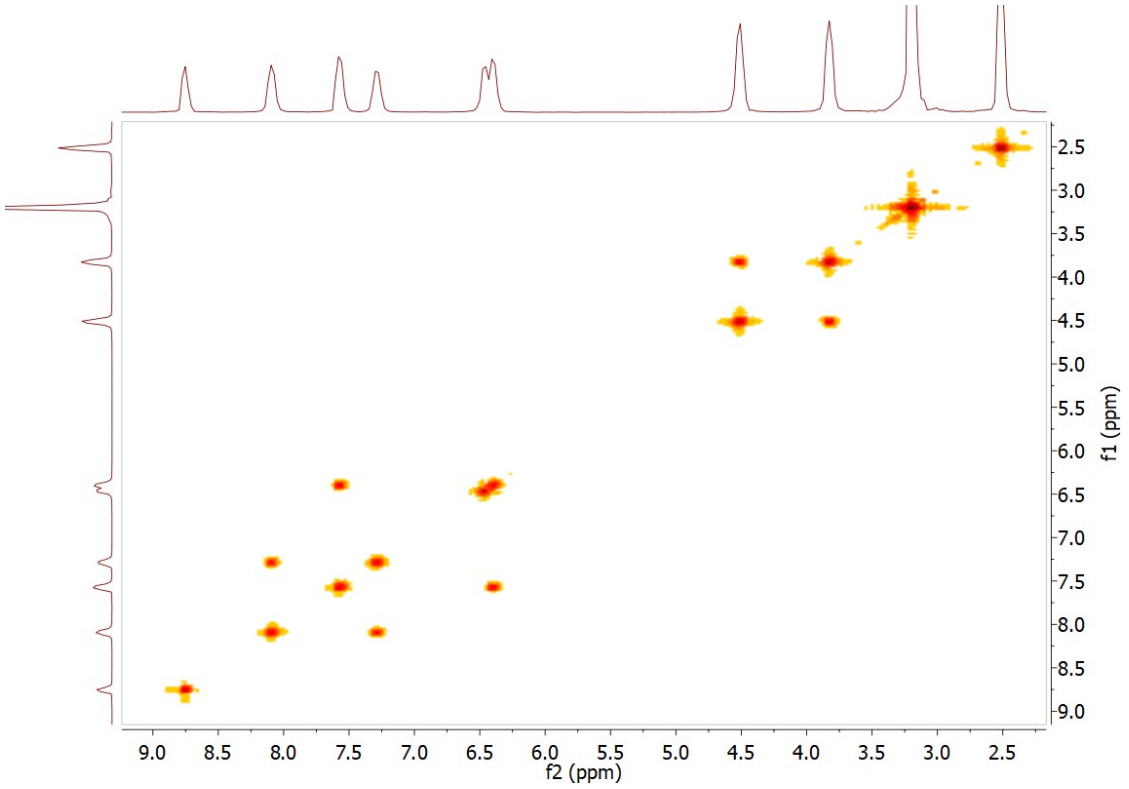


Figure S18. ^{1}H – ^{1}H-2D COSY-NMR spectrum of 23 mM "One-pot" **3** (d^{6}-DMSO, 400 MHz, 298 K).

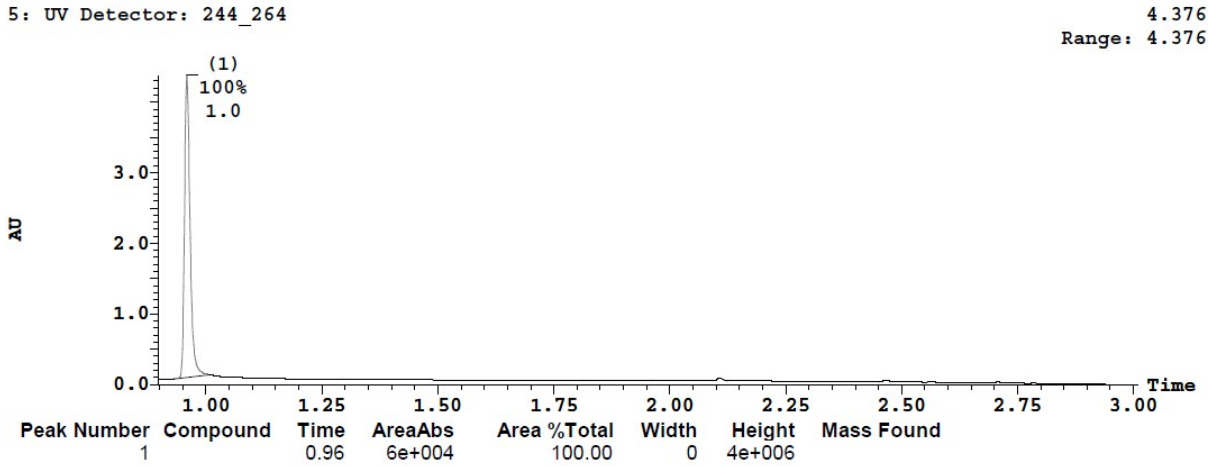


Figure S19. LC-MS chromatogram of convent. **2**.

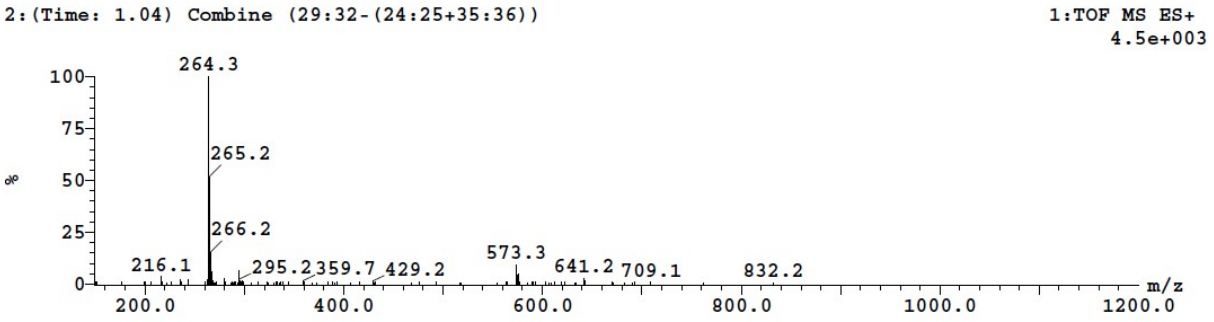
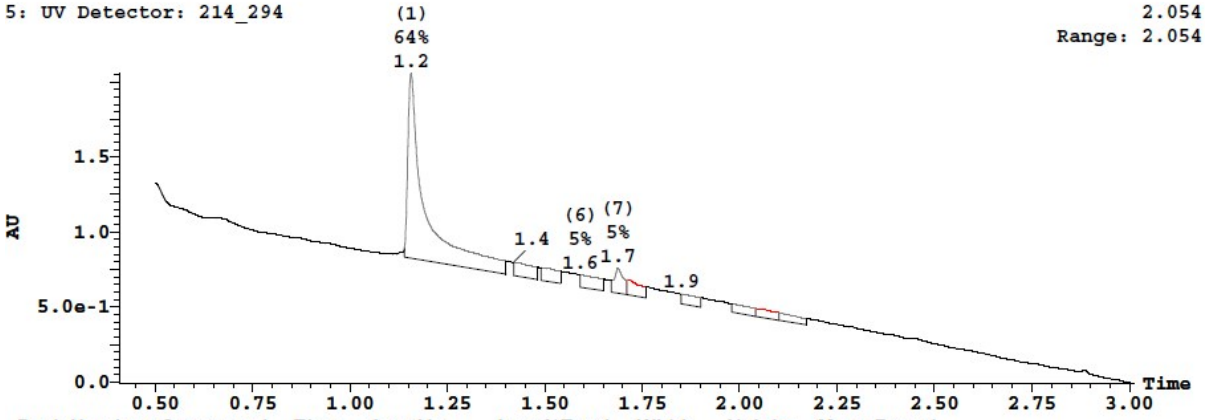


Figure S20. ESI(+) mass spectrum of convent. **2**.



Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1		1.16	6e+004	63.81	0	1e+006	
3		1.42	5e+003	5.20	0	9e+004	
4		1.49	4e+003	3.85	0	8e+004	
6		1.59	5e+003	4.56	0	8e+004	
7		1.69	5e+003	4.78	0	2e+005	
8		1.72	4e+003	4.52	0	1e+005	
9		1.85	3e+003	3.30	0	7e+004	
10		1.98	4e+003	3.59	0	6e+004	
11		2.04	3e+003	3.28	0	5e+004	
12		2.10	3e+003	3.12	0	5e+004	

Figure S21. LC-MS chromatogram of convent. **3**.

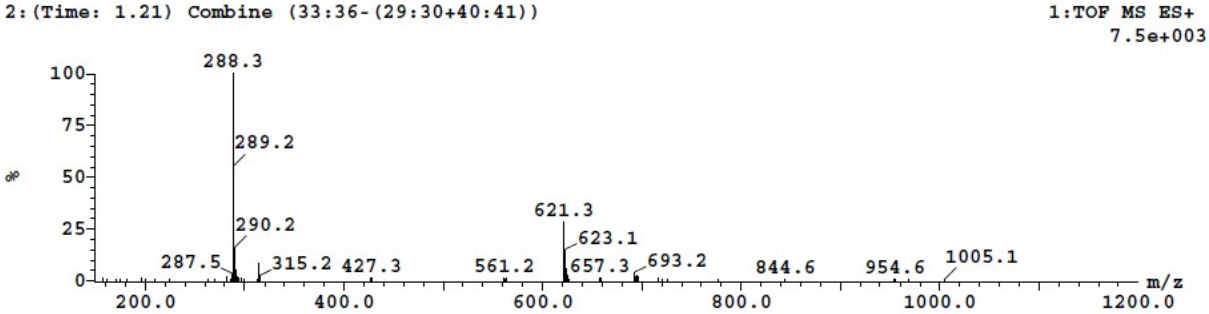
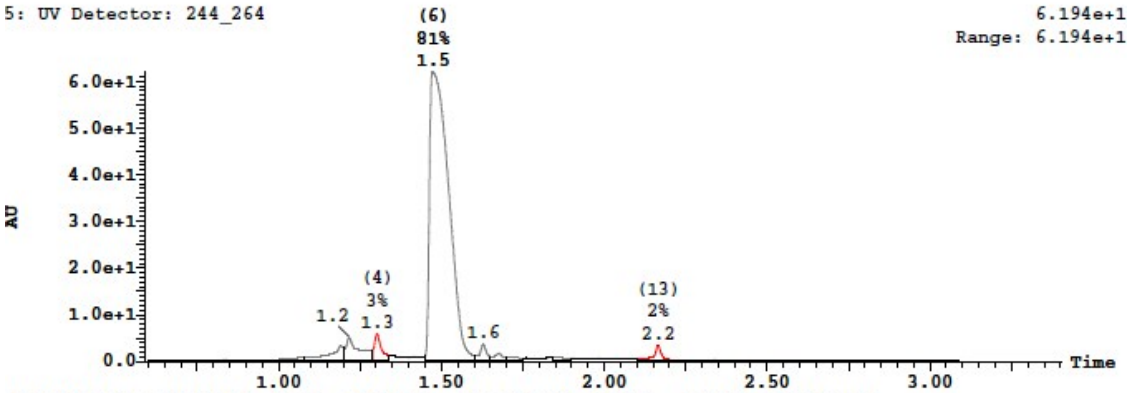


Figure S22. ESI(+) mass spectrum of convent. **3**.



Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1		1.07	7e+004	1.31	0	7e+005	
2		1.19	2e+005	3.37	0	3e+006	
3		1.22	2e+005	4.63	0	5e+006	
4		1.30	2e+005	3.18	0	6e+006	
6		1.47	4e+006	81.08	0	6e+007	
8		1.63	9e+004	1.77	0	4e+006	
9		1.68	5e+004	1.02	0	2e+006	
10		1.71	4e+004	0.72	0	9e+005	
11		1.85	4e+004	0.82	0	8e+005	
13		2.17	1e+005	2.09	0	3e+006	

Figure S23. LC-MS chromatogram of convent. **4**.

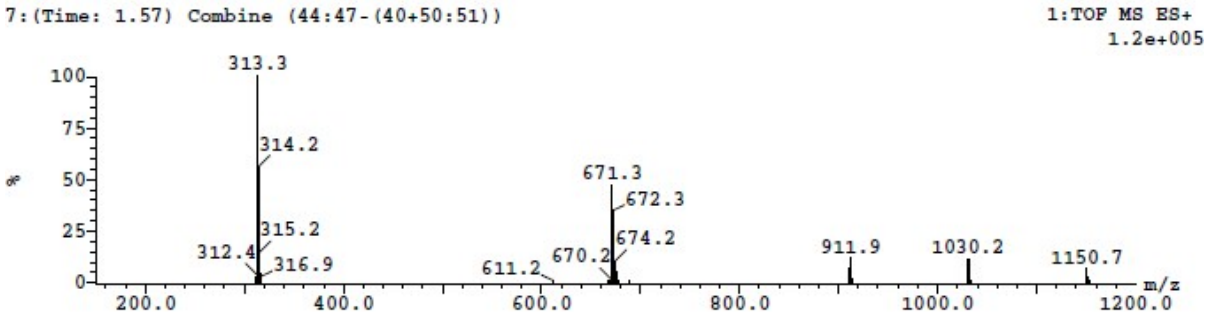


Figure S24. ESI(+) mass spectrum of convent. **4**.

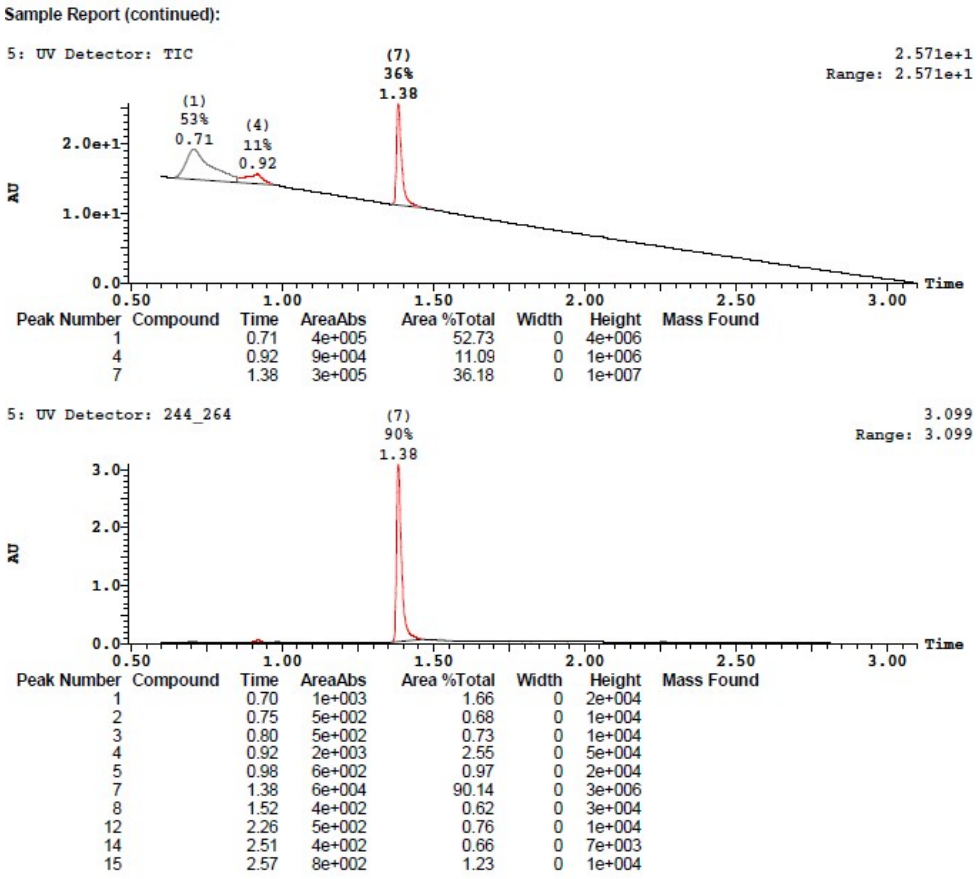


Figure S25. Chromatogram showing "*One pot*" product **3** after freeze drying it from d^6-DMSO mixture and redissolving it in H_2O. It elutes at 1.38 min.

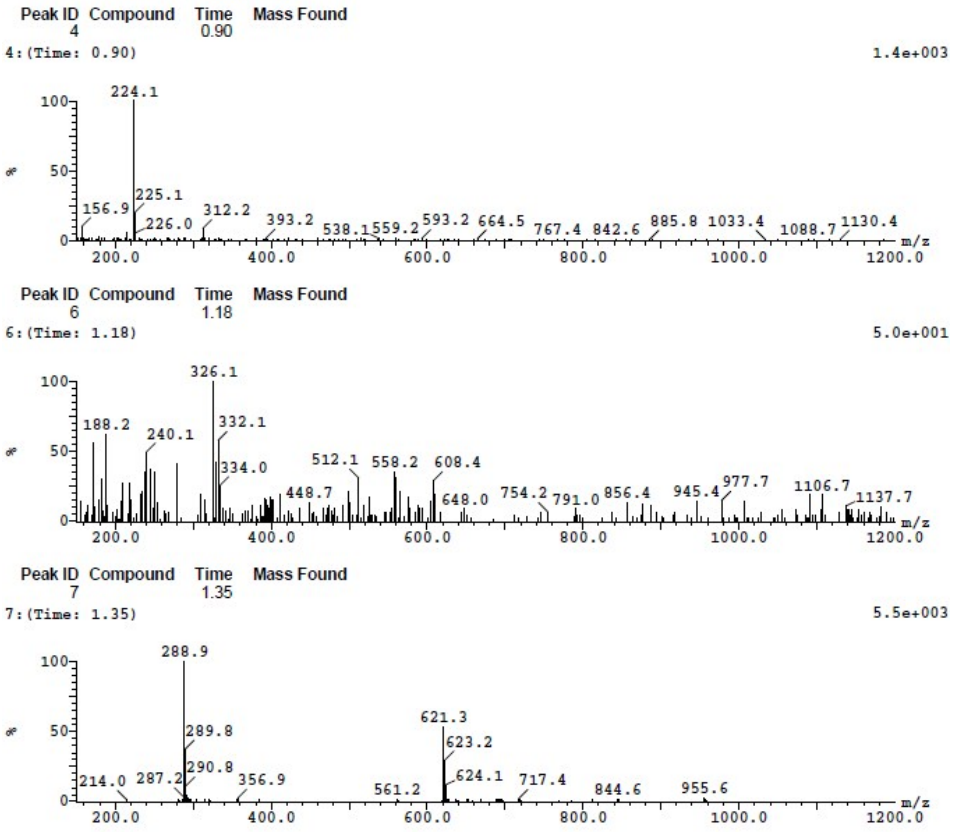


Figure S26. The corresponding ESI(+) MS spectrum for **Figure S35**. The $[M\text{-}2Br^-]^{2+}$ ion of "*One-pot*" product **3** shows m/z = 288.9. Residual aldehyde **1** elutes at 0.9 min (peak 4) and $[M\text{-}Br^-]^+$ corresponds to 224.1.

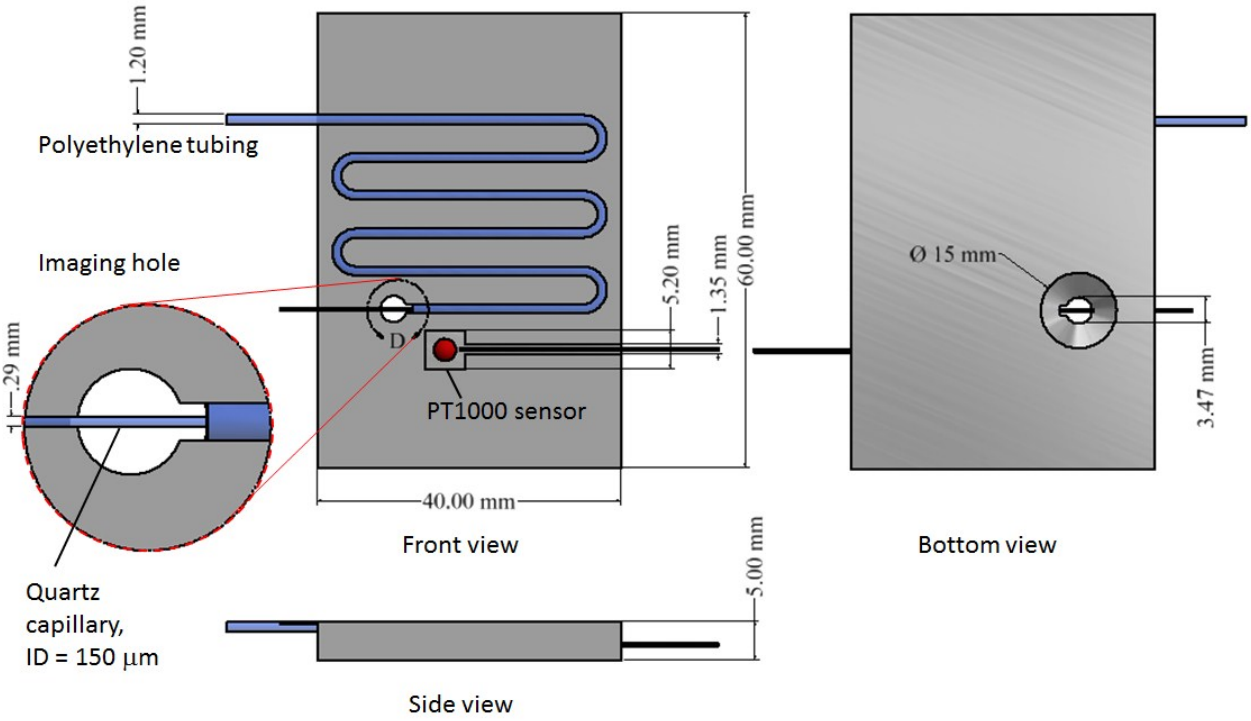


Figure S27. Orthographic drawing showing bottom and side view of the Al platform (created with Autodesk ®).

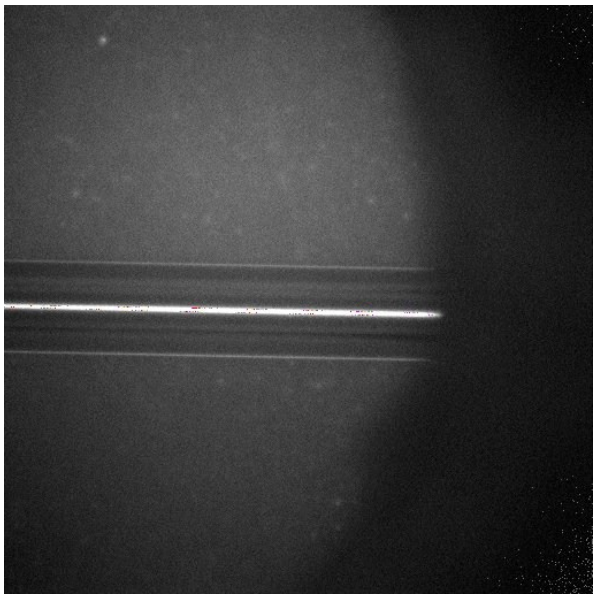


Figure S28. 150 µm thick quartz capillary which is being imaged by a laser. Part of the hole through which the objective approaches the capillary is shown on the right side of the image (5x magnification).

Figure S29. Photograph of the set-up used for the *in flow* synthesis showing syringe pump, solid state heater, around which the PTFE taped is wrapped, and PID controller.

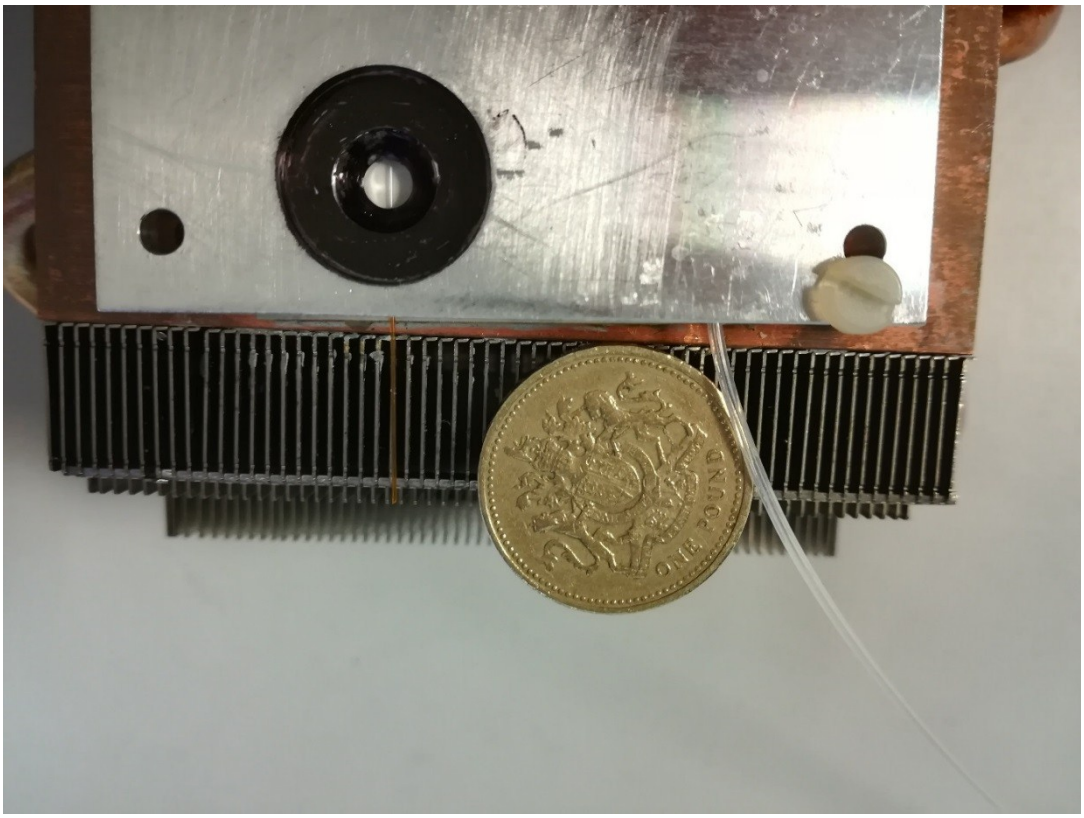


Figure S30. Photograph showing the Al platform with imaging hole, heat sink and capillary. A one-pound coin is shown for size comparisons.

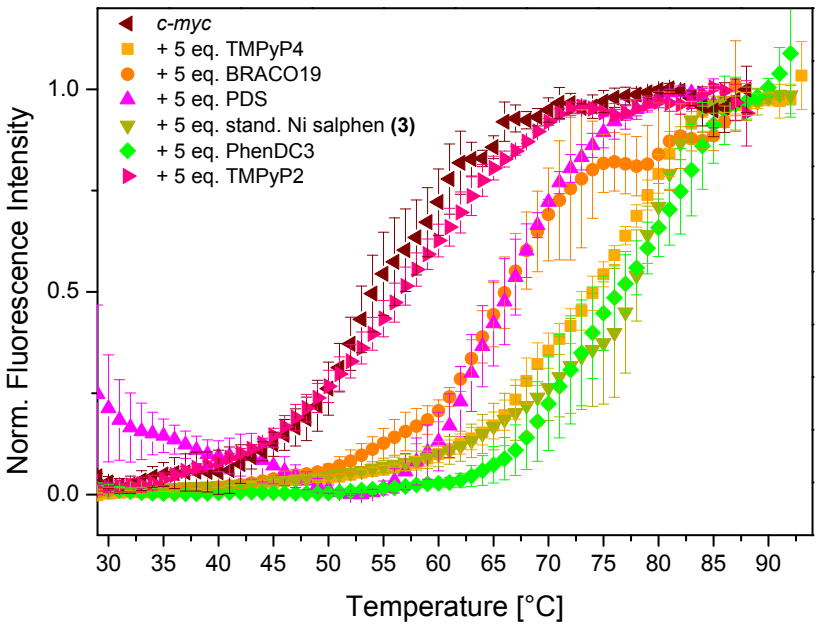


Figure S31. FRET melting data with FAM-TAMRA labelled *c-Myc* and various ligands (obtained with the FRET melting platform).

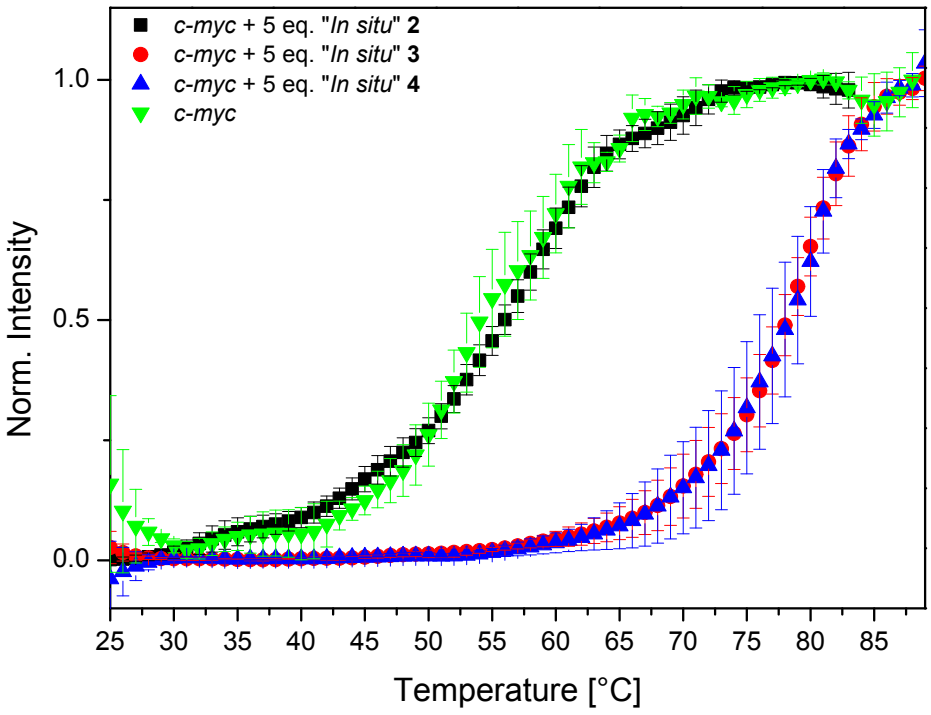


Figure S32. FRET melting data with FAM-TAMRA labelled *c-Myc* and "*One-pot*" prepared compounds **2** – **4** (obtained with the FRET melting platform).

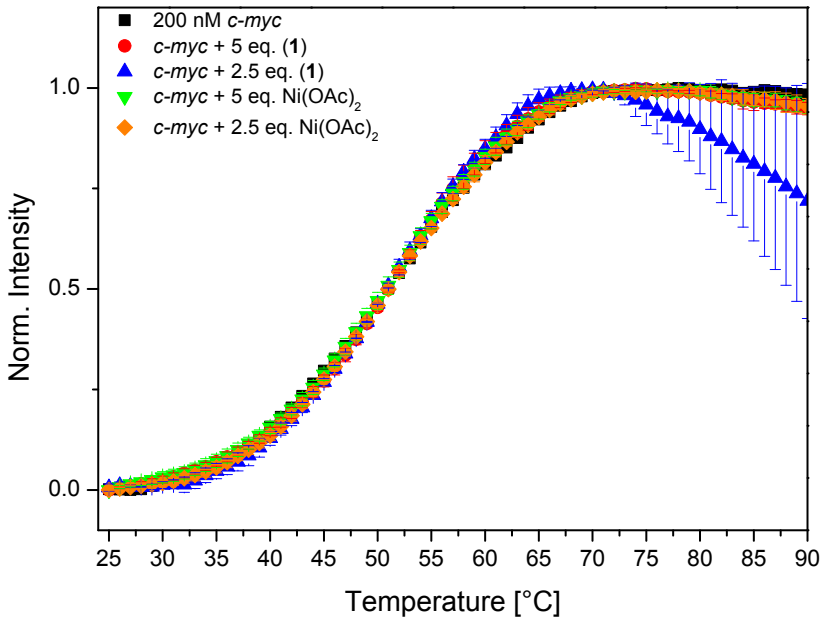


Figure S33. FRET melting control (carried out on Stratagene Mx3005P qPCR machine (Agilent Technologies)). No stabilisation of *c-Myc* is seen in the presence of $Ni(OAc)_2$ or Aldehyde **1** under the used concentrations.

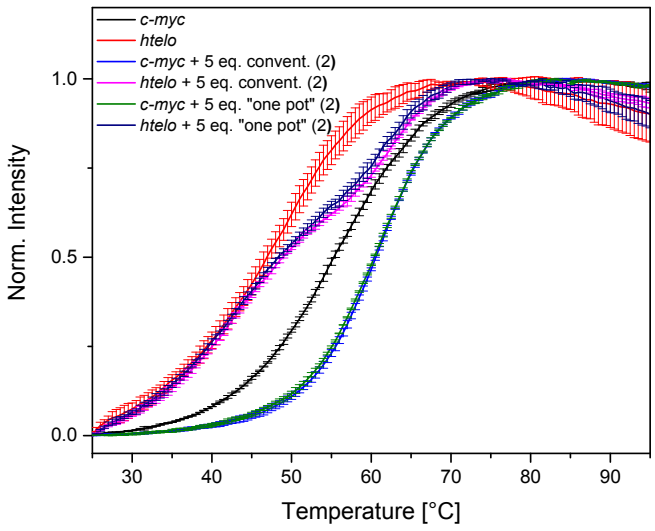


Figure S34. FRET melting curves (obtained from Stratagene Mx3005P qPCR machine (Agilent Technologies)) of compound **2** with 0.2 µM *h-Telo* G4 (10 mM KCl, 90 mM LiCl, 10 mM LiCac, pH = 7.4) and 0.2 µM *c-Myc* G4 (1 mM, 99 mM KCl, 10 mM LiCac, pH = 7.4). The error bars are shown for 2 – 3 replicates.

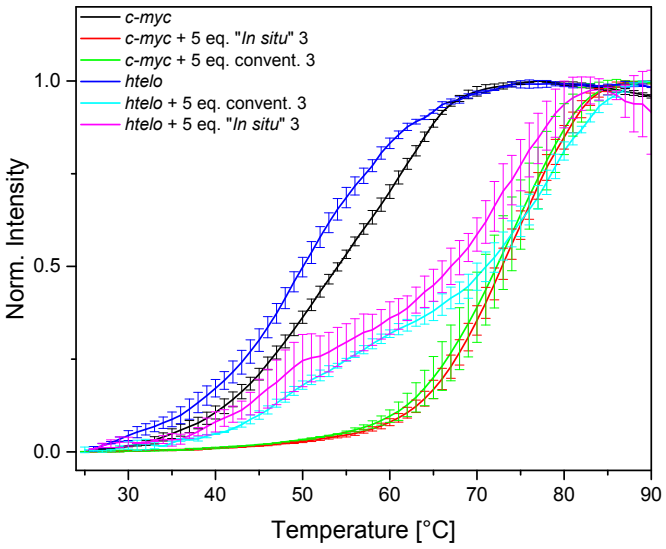


Figure S35. FRET melting curves (obtained from Stratagene Mx3005P qPCR machine (Agilent Technologies)) of compound **3** with 0.2 µM *h-Telo* G4 (10 mM KCl, 90 mM LiCl, 10 mM LiCac, pH = 7.4) and 0.2 µM *c-myc* G4 (1 mM KCl, 99 mM LiCl, 10 mM LiCac, pH = 7.4). The error bars are shown for 2 – 3 replicates.

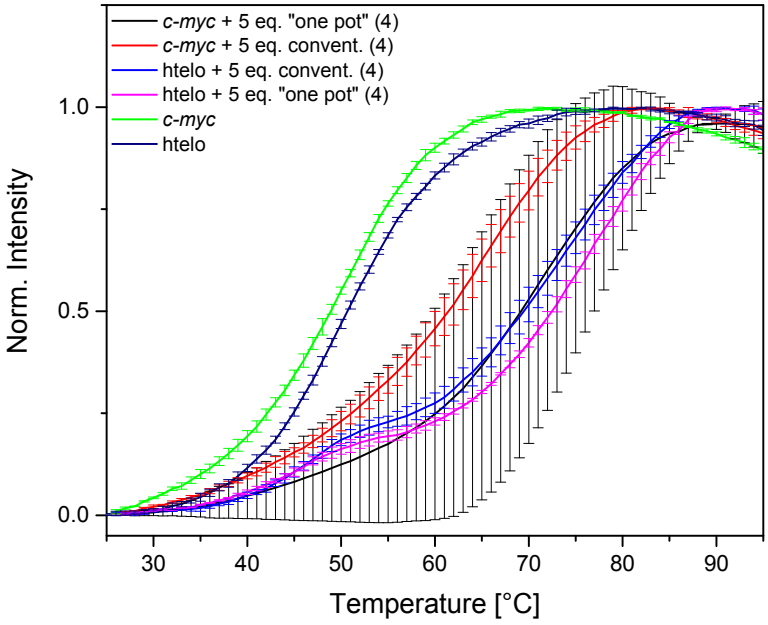


Figure S36. FRET melting curves (obtained from Stratagene Mx3005P qPCR machine (Agilent Technologies)) of compound **4** with 0.2 µM *h-Telo* G4 (10 mM KCl, 90 mM LiCl, 10 mM LiCac, pH = 7.4) and 0.2 µM *c-myc* G4 (1 mM, 99 mM KCl, 10 mM LiCac, pH = 7.4). The error bars are shown for 2 – 3 replicates.

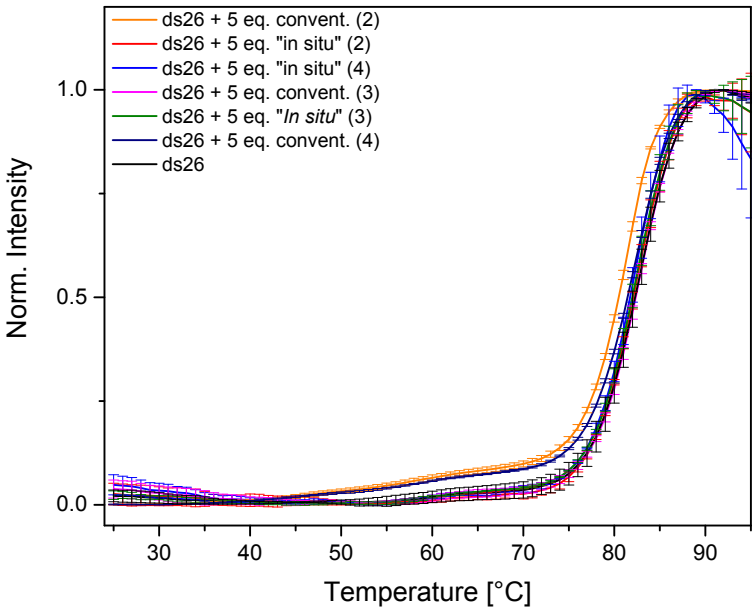


Figure S37. FRET melting curves (obtained from Stratagene Mx3005P qPCR machine (Agilent Technologies)) of compounds **2** – **4** with 0.2 µM *ds26* (10 mM KCl, 90 mM LiCl, 10 mM LiCac, pH = 7.4). No significant stabilization is seen. Error bars are shown for 2 – 3 replicates.

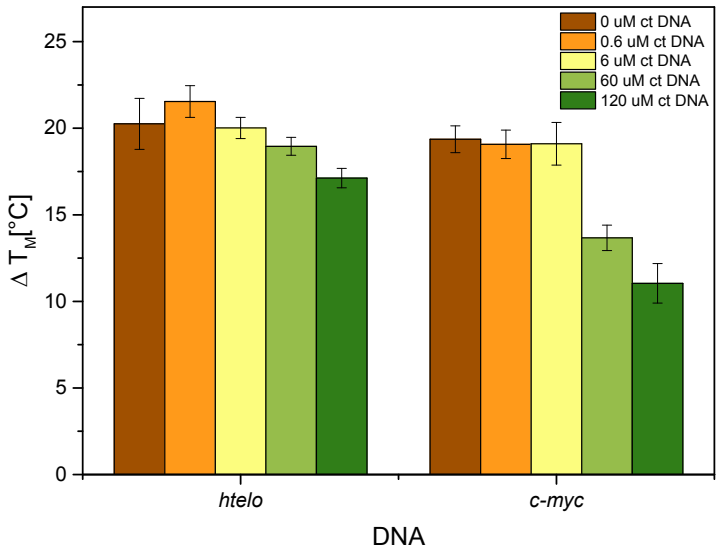


Figure S38. FRET competition assay with CT DNA: Increasing concentrations of CT DNA (0 – 120 µM) were added to a mixture of 0.2 µM G4 with 1 µM nickel(II)-salphen **3**. Regarding its binding to *h-Telo* the compound seems selective up to a 60-fold excess of CT DNA, with *c-Myc* it seems selective up to a 6 fold excess of CT DNA.

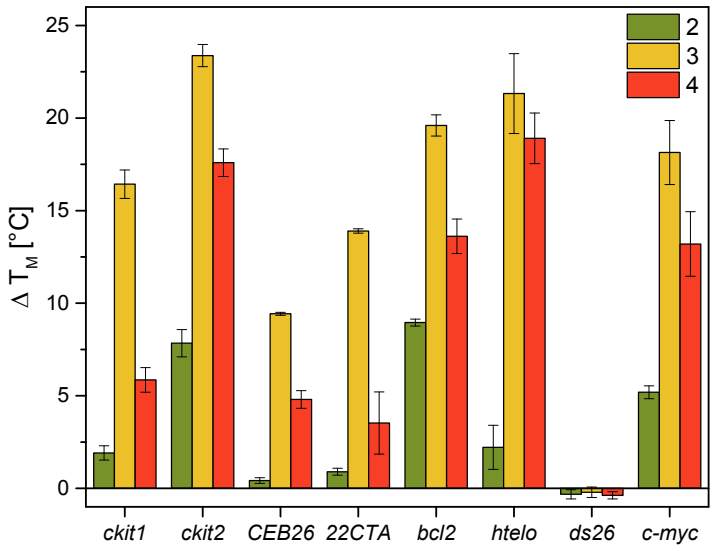


Figure S39. ΔT_m (°C) values for eight different DNA topologies (including G4 and duplex DNA) in the presence of the new metal complexes **2-4** synthesised using the conventional method. The ΔT_m values were determined (in triplicate) by conventional FRET melting assays using 0.2 μM of oligonucleotide and 1 μM of the compound being tested.

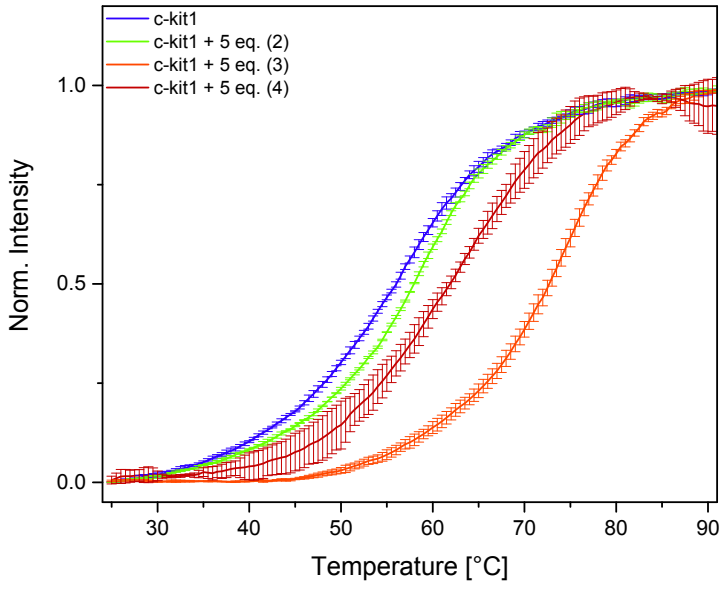


Figure S40. FRET melting curves (obtained from Stratagene Mx3005P qPCR machine (Agilent Technologies)) of compounds **2 – 4** with 0.2 μM *c-kit1* G4 (10 mM KCl, 90 mM LiCl, 10 mM LiCac, pH = 7.4). The error bars are shown for 3 replicates.

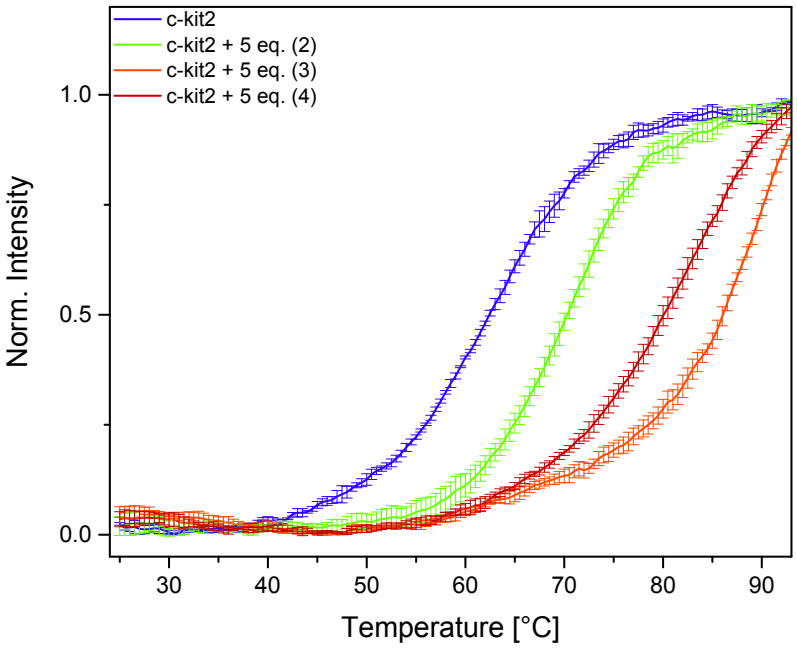


Figure S41. FRET melting curves (obtained from Stratagene Mx3005P qPCR machine (Agilent Technologies)) of compounds **2 – 4** with 0.2 µM *c-kit2* G4 (10 mM KCl, 90 mM LiCl, 10 mM LiCac, pH = 7.4). The error bars are shown for 3 replicates.

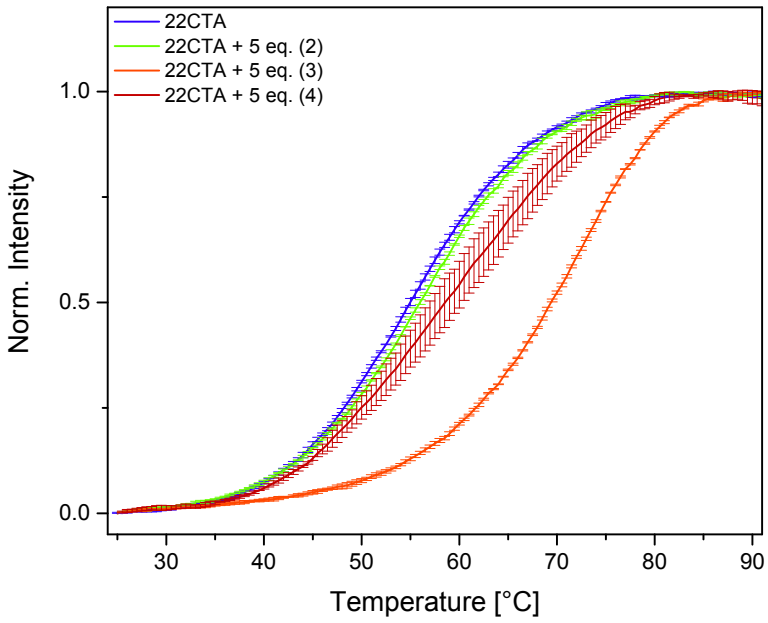


Figure S42. FRET melting curves (obtained from Stratagene Mx3005P qPCR machine (Agilent Technologies)) of compounds **2 – 4** with 0.2 µM *22CTA* G4 (10 mM KCl, 90 mM LiCl, 10 mM LiCac, pH = 7.4). The error bars are shown for 3 replicates.

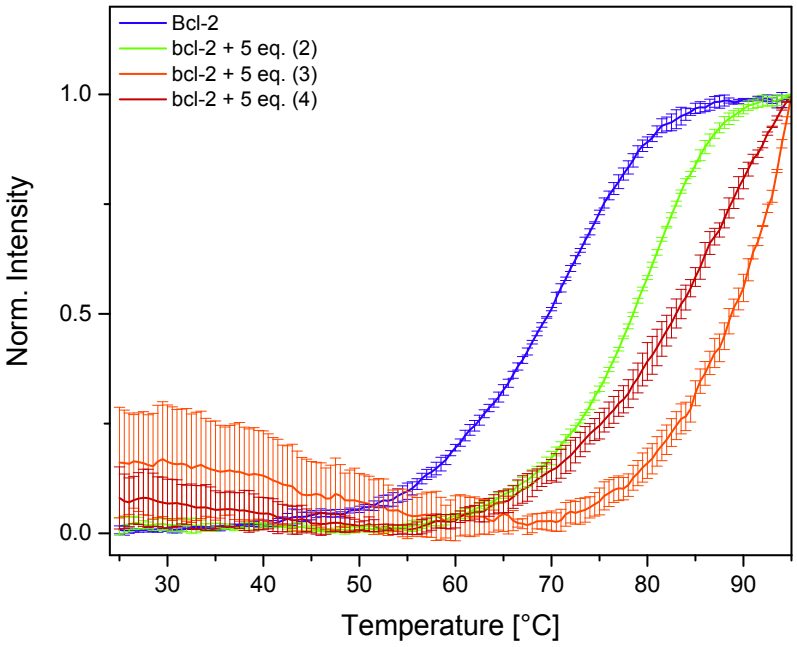


Figure S43. FRET melting curves (obtained from Stratagene Mx3005P qPCR machine (Agilent Technologies)) of compounds **2** – **4** with 0.2 µM *bcl-2* G4 (100 mM KCl, 10 mM LiCac, pH = 7.4). The error bars are shown for 3 replicates.

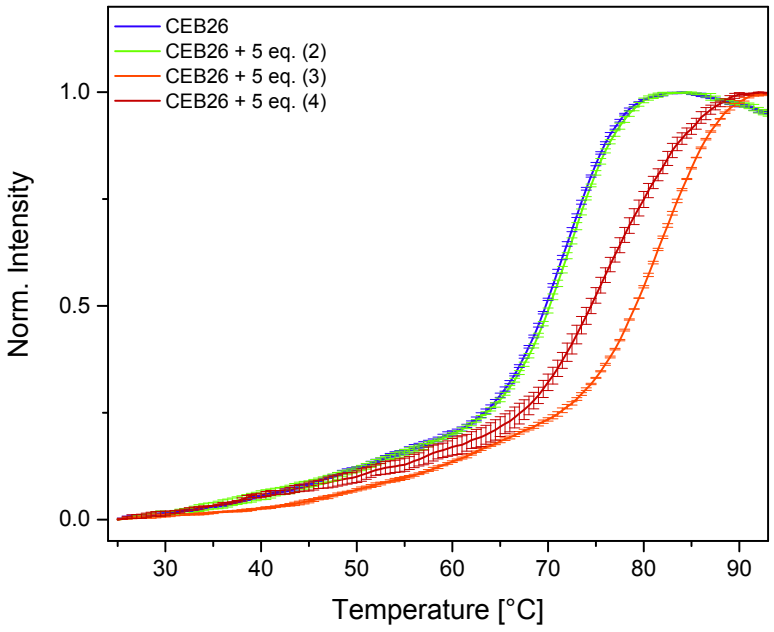


Figure S44. FRET melting curves (obtained from Stratagene Mx3005P qPCR machine (Agilent Technologies)) of compounds **2** – **4** with 0.2 µM *CEB26* G4 (10 mM KCl, 90 mM LiCl, 10 mM LiCac, pH = 7.4). The error bars are shown for 3 replicates.