

Determination of kinetic parameters within a single nonisothermal on-flow experiment by Nanolitre-NMR Spectroscopy

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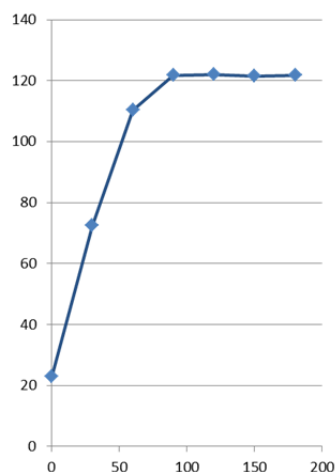
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ABSTRACT

Conventional methods to determine the kinetic parameters for a certain reaction require multiple, separate isothermal experiments resulting in time and material-consuming processes. Here, an approach to determine the kinetic information within a single nonisothermal on-flow experiment is presented, consuming less than ten micromoles of reagents and having a total measuring time of typically 10 minutes. This approach makes use of a microfluidic NMR chip hyphenated to a continuous-flow microreactor and is based on the capabilities of the NMR chip to analyse sub-nanomole quantities of material in the 25 nL detection volume. Importantly, useful data are acquired from the microreactor platform in specific isothermal and non-isothermal frames. A model fitting the experimental data enables rapid determination of kinetic parameters, as demonstrated for a library of isoxazole and pyrazole derivatives



Time (seconds)	Temperature (°C)
0	22.9
30	72.4
60	110.4
90	121.8
120	122.1
150	121.5
180	121.9

Figure S1. Graphical of temperature data versus time measured with a Pt-100 sensor when the Labtrix® Start works at 14 V. Table S1. Temperature data versus time measured with a Pt-100 sensor when the Labtrix® Start works at 14 V.

Control experiments using the Labtrix® Start-NMR hyphenated setup

Temperature (K)	1/ T (K) ($\times 10^{-3}$)	Kinetic constant ($M s^{-1}$)
368	2.7	0.008
382	2.6	0.0171
404	2.5	0.07
420	2.4	0.23

Table S2. Kinetic constant values obtained for a certain temperature range (368-420K) when the Labtrix® Start worked in steady-state conditions by means of plotting $1/[A]$ vs time (s) for the synthesis of 5-methyl-3-phenylisoxazole (**3**). [A] represents the reagent concentration (M).

The Arrhenius plot ($\ln k$ vs $1/T$) allows the determination of the activation energy as reported conventionally¹, rendering an $E_a = 82.80$ kJ/mol (19.81 kcal/mol)

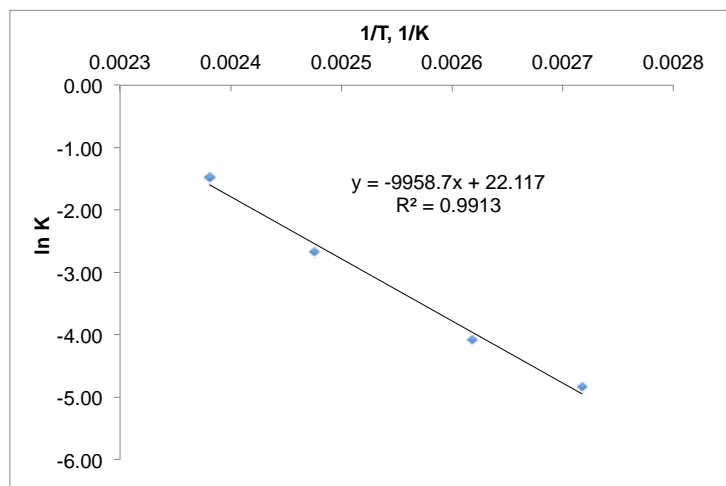


Figure S2. Arrhenius plot for the determination of activation energy following the conventional approach, employing the Labtrix® Start-NMR hyphenated setup, for the synthesis of 5-methyl-3-phenylisoxazole (**3**).

An experiment time of 30 min is required for every temperature value when working under steady state conditions (conventional way) by means of the Labtrix® Start-NMR hyphenated setup. Considering triplicates for an estimation of the error data, it sums up 90 min per experiment. Therefore, 6 h of experiment time were required to calculate the activation energy in our control experiment, where four different temperatures were considered, in contrast to the 30 min (10 x 3 (triplicates) min) per experiment of the developed methodology herein reported. If conventional laboratory equipment (batch vessels) were used for the determination of this information, it would require not only much more time than when using the Labtrix® Start-NMR hyphenated setup, as explained above, but also much more material (solvent and reagents) due to its higher scale.

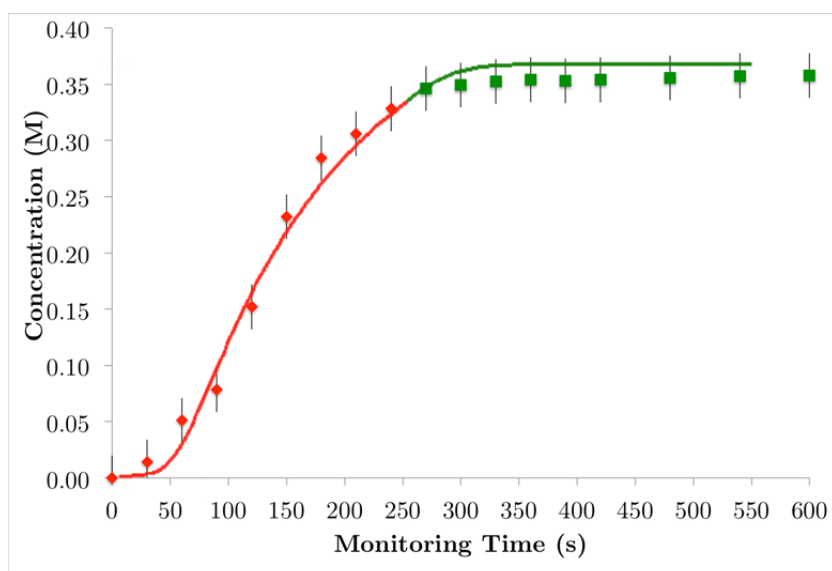


Figure S3. Reaction product concentration (M) versus monitoring time (seconds) for the synthesis of 1-benzyl-3,5-dimethylpyrazole (**9**) for an overall flow rate 5 $\mu\text{l}/\text{min}$. The points correspond to experimental data. The curve along the data points has been obtained from fitting to a model using Maple software. The use of a flow rate of 5 $\mu\text{l}/\text{min}$ enables the extraction of the kinetic parameters after a single on-flow experiment of just 600 seconds

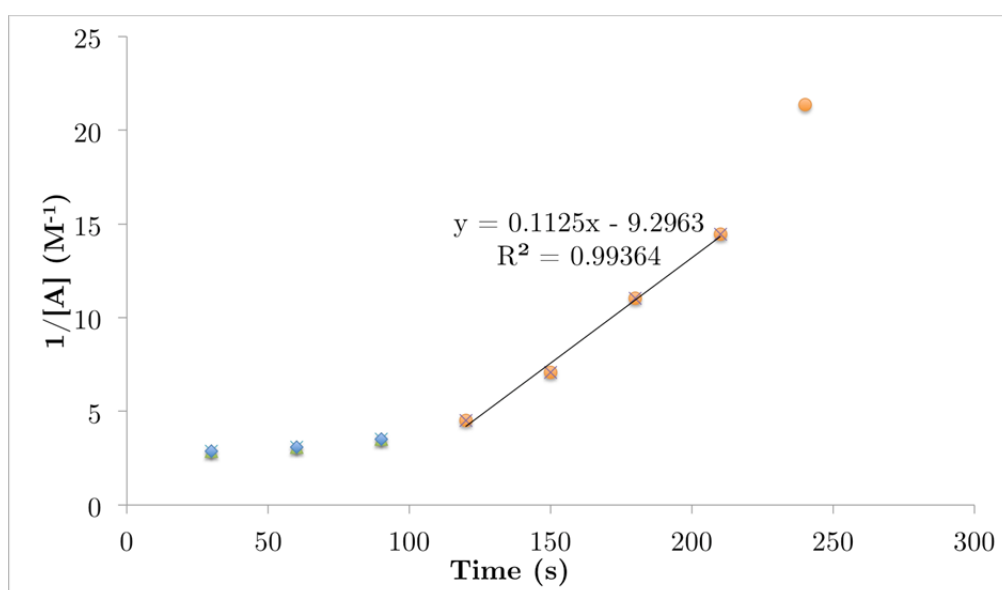


Figure S4. $1/[A]$ (M^{-1}) versus residence time (seconds) for the synthesis of 1-benzyl-3,5-dimethylpyrazole (**9**) for a flow rate of 5 $\mu\text{l}/\text{min}$. $[A]$ represents the reagent concentration. The straight line emphasizes the zone b' data points, that represents a 'conventional' data set with incremental residence time and a constant temperature. Note: because of the accumulated time interval of one datapoint is 1 minute, in some plots the straight line goes from datapoints 4-7 rather than from 5-8.

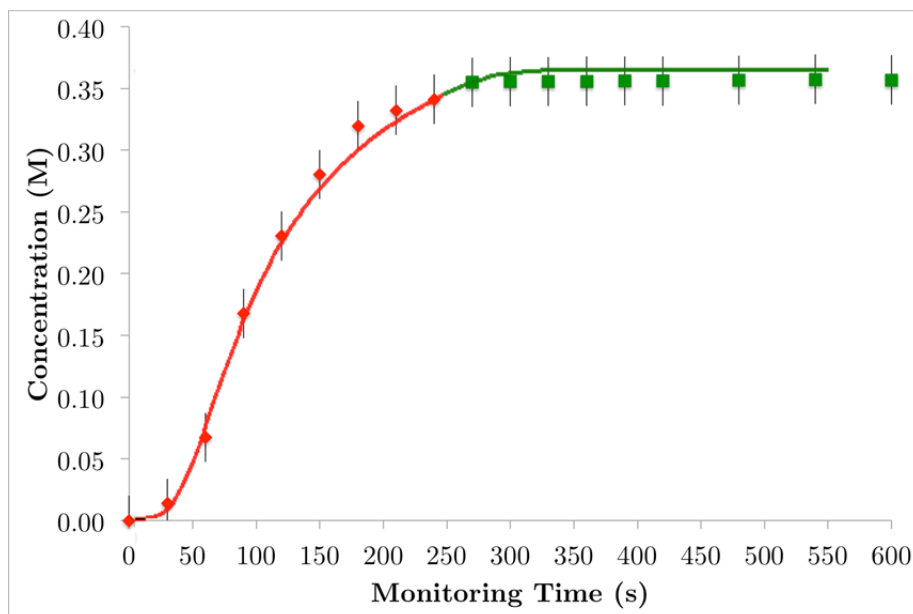


Figure S5. Reaction product (M) concentration versus monitoring time (seconds) for the synthesis of 1-benzyl-3,4,5-dimethyl-pyrazole (**10**) for an overall flow rate of 5 $\mu\text{l}/\text{min}$. The points correspond to experimental data. The curve along the data points has been obtained from fitting to a model using Maple software. The use of a flow rate of 5 $\mu\text{l}/\text{min}$ enables the extraction of the kinetic parameters after a single on-flow experiment of just 600 seconds.

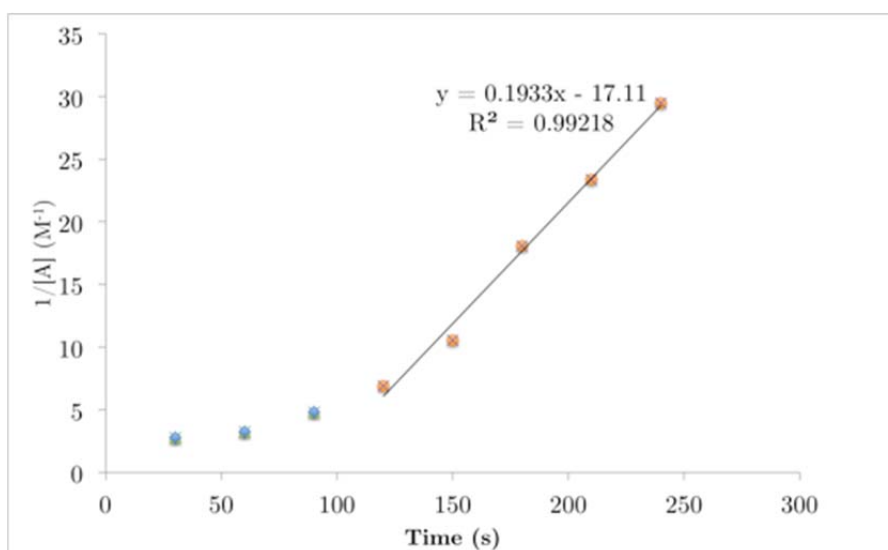


Figure S6. $1/[A]$ (M^{-1}) versus residence time (seconds) for the synthesis of 1-benzyl-3,4,5-dimethyl-pyrazole (**10**) for a flow rate of 5 $\mu\text{l}/\text{min}$. $[A]$ represents the reagent concentration. The straight line emphasizes the zone b' data points, that represents a 'conventional' data set with incremental residence time and a constant temperature.

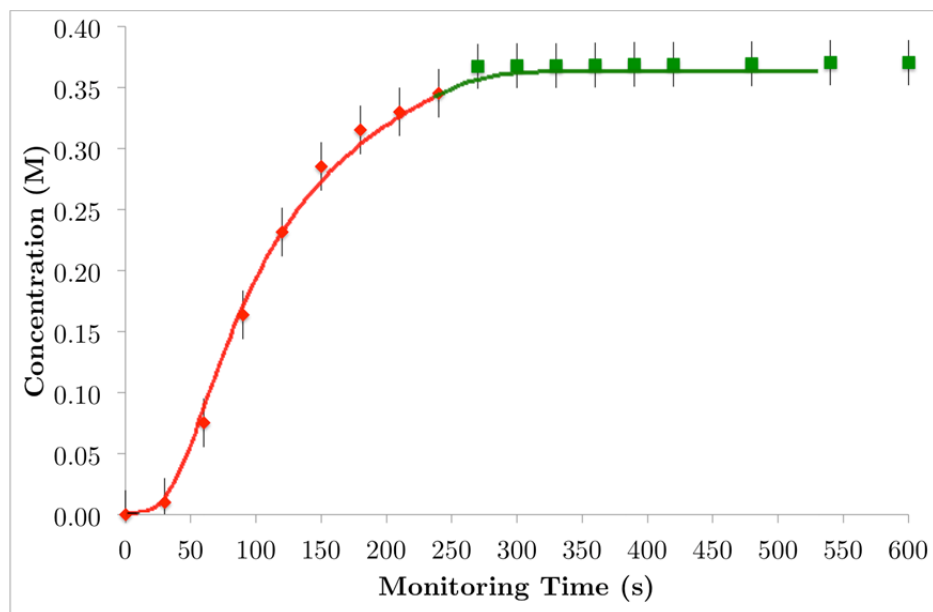


Figure S7. Reaction product (M) versus monitoring time (seconds) for the synthesis of 1-benzyl-3-methyl-5-phenyl-pyrazole (**11**) for an overall flow rate of 5 $\mu\text{l}/\text{min}$. The points correspond to experimental data. The curve along the data points has been obtained from fitting to a model using Maple software. The use of a flow rate of 5 $\mu\text{l}/\text{min}$ enables the extraction of the kinetic parameters after a single on-flow experiment of just 600 seconds.

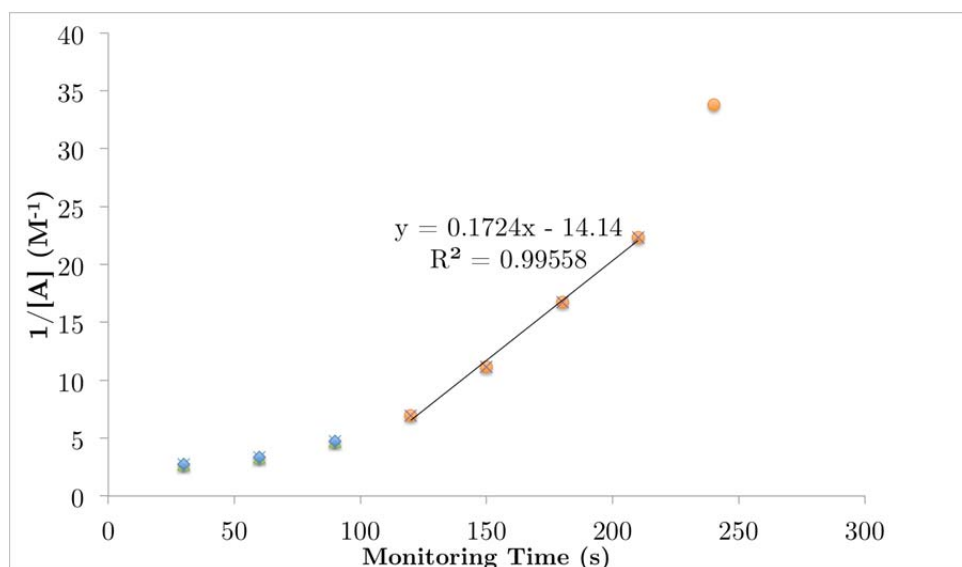


Figure S8. $1/[A]$ (M^{-1}) versus residence time (seconds) for the synthesis of 1-benzyl-3-methyl-5-phenyl-pyrazole (**11**) for a flow rate of 5 $\mu\text{l}/\text{min}$. $[A]$ represents the reagent concentration. The straight line emphasizes the zone b' data points, that represents a 'conventional' data set with incremental residence time and a constant temperature.

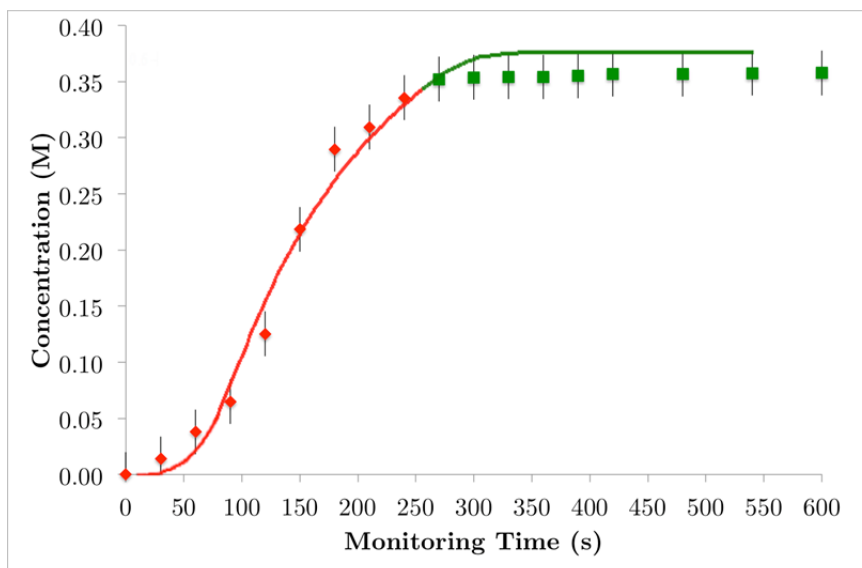


Figure S9. Reaction product concentration (M) versus monitoring time (seconds) for the synthesis of 1-(2-fluorophenyl)-3,5-dimethylpyrazole (**12**) for an overall flow rate of 5 $\mu\text{l}/\text{min}$. The points correspond to experimental data. The curve along the data points has been obtained from fitting to a model using Maple software. The use of a flow rate of 5 $\mu\text{l}/\text{min}$ enables the extraction of the kinetic parameters after a single on-flow experiment of just 600 seconds.

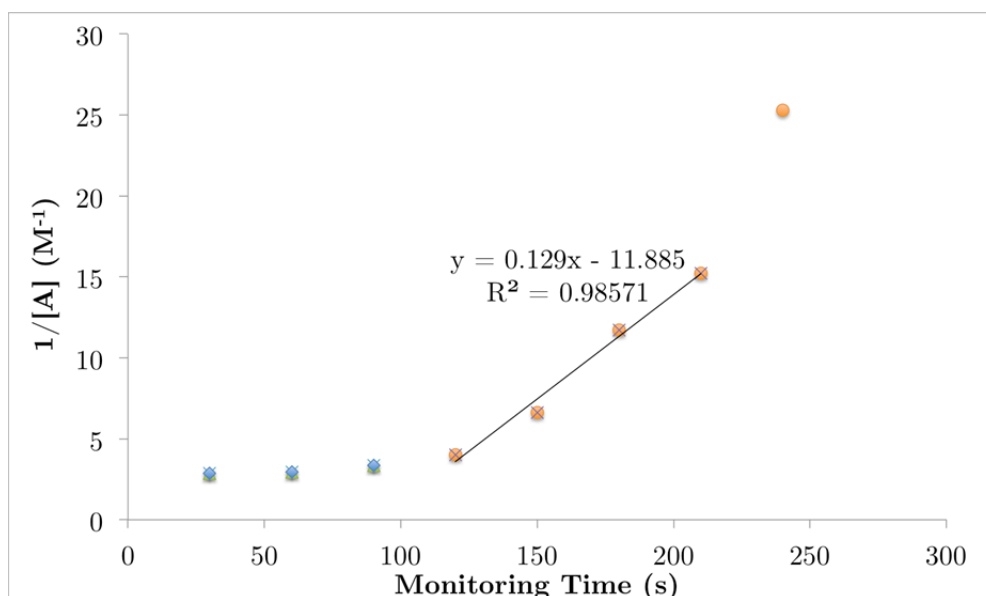


Figure S10. $1/[A]$ (M^{-1}) versus residence time (seconds) for the synthesis of 1-(2-fluorophenyl)-3,5-dimethylpyrazole (**12**) for a flow rate of 5 $\mu\text{l}/\text{min}$. $[A]$ represents the reagent concentration. The straight line emphasizes the zone b' data points, that represents a 'conventional' data set with incremental residence time and a constant temperature.

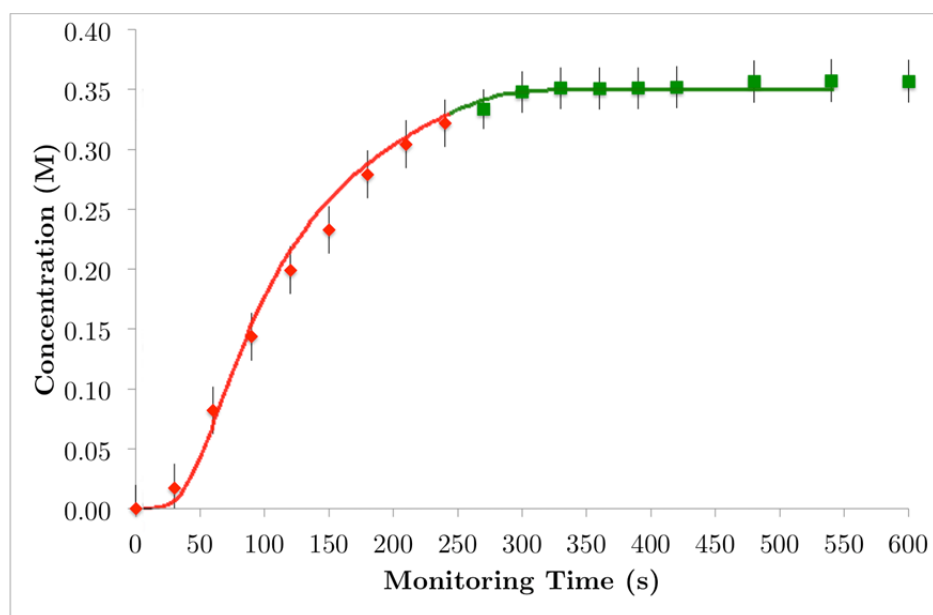


Figure S11. Reaction product (M) concentration *versus* monitoring time (seconds) for the synthesis of 1-(2-fluorophenyl)-3,4,5-trimethylpyrazole (**13**) for an overall flow rate of 5 $\mu\text{l}/\text{min}$. The points correspond to experimental data. The curve along the data points has been obtained from fitting to a model using Maple software. The use of a flow rate of 5 $\mu\text{l}/\text{min}$ enables the extraction of the kinetic parameters after a single on-flow experiment of just 600 seconds.

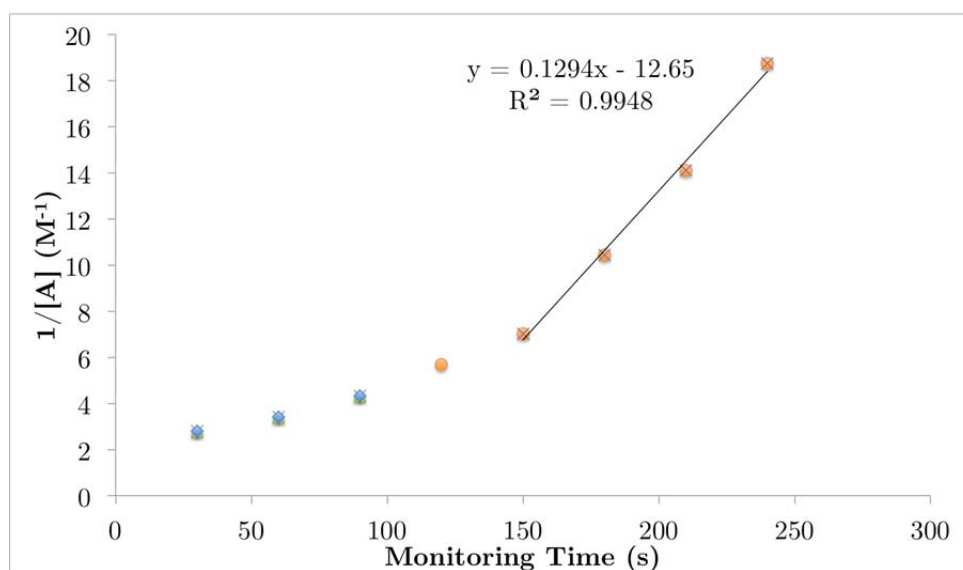


Figure S12. $1/[A]$ (M^{-1}) *versus* residence time (seconds) for the synthesis of 1-(2-fluorophenyl)-3,4,5-trimethylpyrazole (**13**) for a flow rate of 5 $\mu\text{l}/\text{min}$. $[A]$ represents the reagent concentration. The straight line emphasizes the zone b' data points, that represents a 'conventional' data set with incremental residence time and a constant temperature.

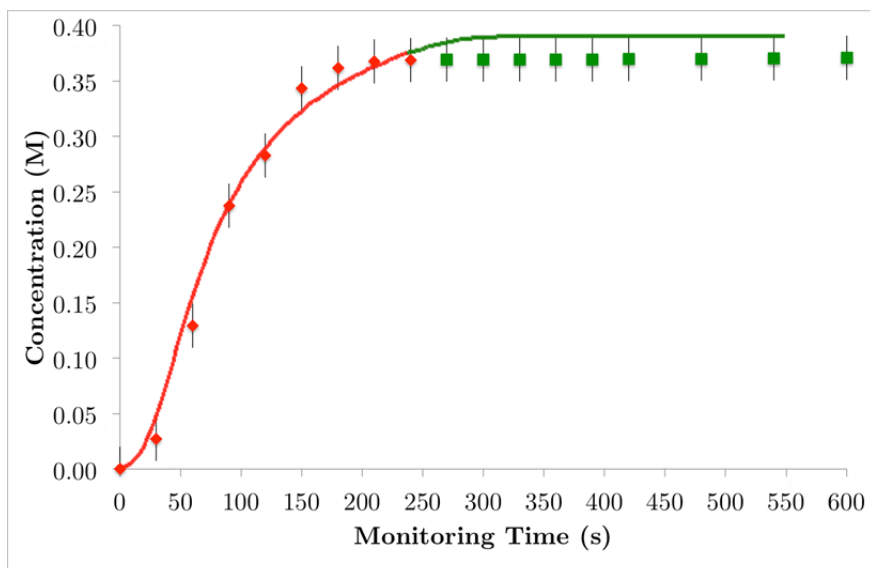


Figure S13. Reaction product (M) concentration *versus* monitoring time (seconds) for the synthesis of 1-(2-fluorophenyl)-5-methyl-3-phenyl-pyrazole (**14**) for an overall flow rate of 5 $\mu\text{l}/\text{min}$. The points correspond to experimental data. The curve along the data points has been obtained from fitting to a model using Maple software. The use of a flow rate of 5 $\mu\text{l}/\text{min}$ enables the extraction of the kinetic parameters after a single on-flow experiment of just 600 seconds.

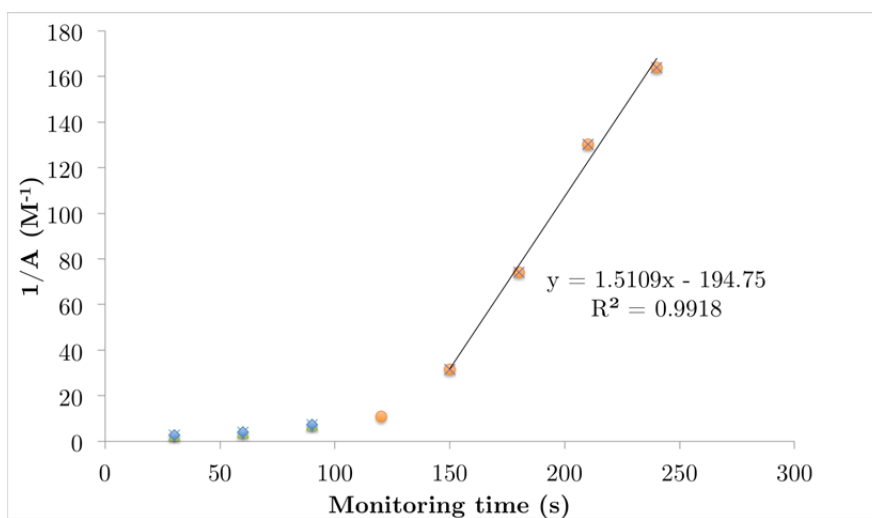


Figure S14. $1/[A]$ (M^{-1}) *versus* residence time (seconds) for the synthesis of 1-(2-fluorophenyl)-5-methyl-3-phenyl-pyrazole (**14**) for a flow rate of 5 $\mu\text{l}/\text{min}$. $[A]$ represents the reagent concentration. The straight line emphasizes the zone b' data points, that represents a 'conventional' data set with incremental residence time and a constant temperature.

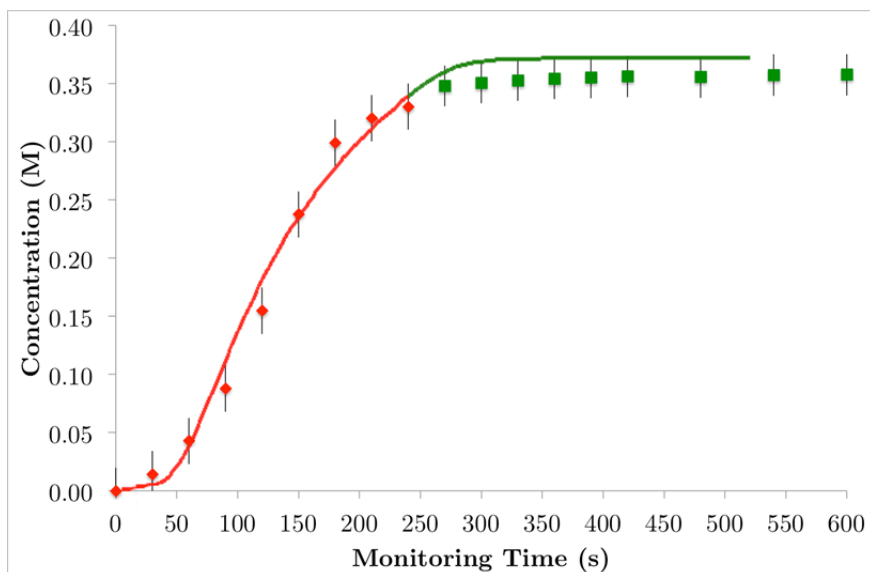


Figure S15. Reaction product (M) *versus* monitoring time (seconds) for the synthesis of 1-pentafluorophenyl-3,5-dimethylpyrazole (**15**) for an overall flow rate of 5 $\mu\text{l}/\text{min}$. The curve along the data points has been obtained from fitting to a model using Maple software. The use of a flow rate of 5 $\mu\text{l}/\text{min}$ enables the extraction of the kinetic parameters after a single on-flow experiment of just 600 seconds.

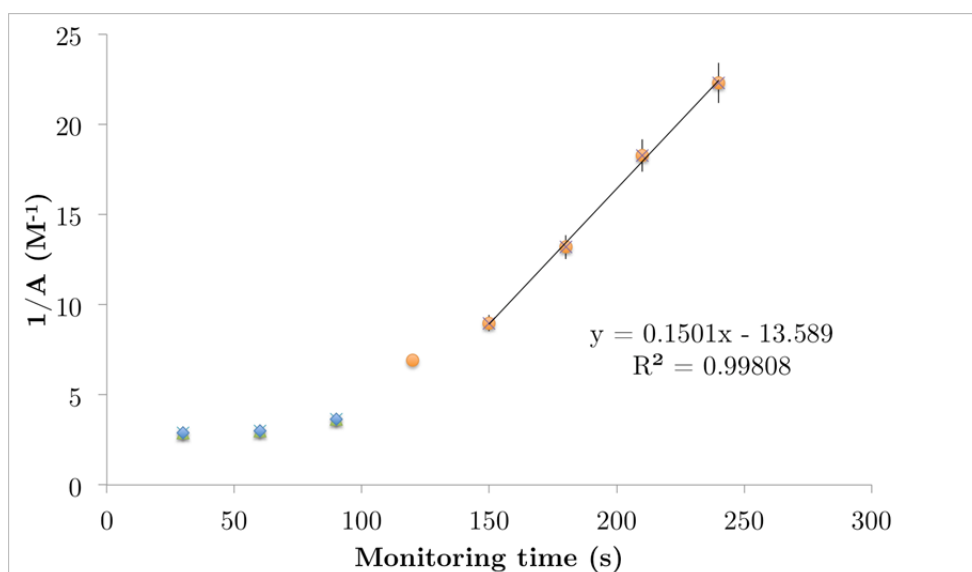


Figure S16. $1/[A]$ (M^{-1}) *versus* residence time (seconds) for the synthesis of 1-pentafluorophenyl-3,5-dimethylpyrazole (**15**) for a flow rate of 5 $\mu\text{l}/\text{min}$. $[A]$ represents the reagent concentration. The straight line emphasizes the zone b' data points, that represents a 'conventional' data set with incremental residence time and a constant temperature.

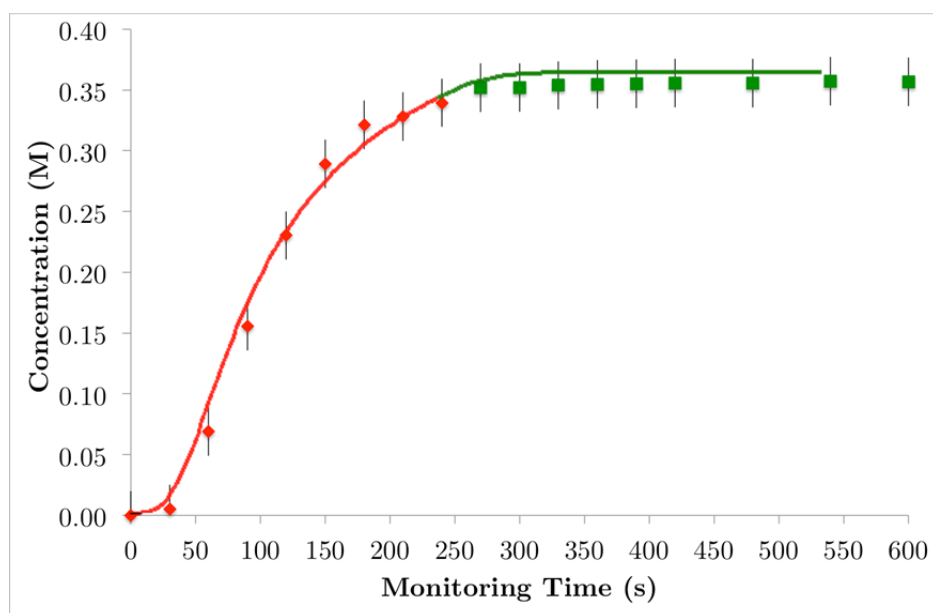


Figure S17. Reaction product (M) concentration *versus* monitoring time (seconds) for the synthesis of 1-pentafluorophenyl-3,4,5-trimethylpyrazole (**16**) for an overall flow rate of 5 $\mu\text{l}/\text{min}$. The points correspond to experimental data. The curve along the data points has been obtained from fitting to a model using Maple software. The use of a flow rate of 5 $\mu\text{l}/\text{min}$ enables the extraction of the kinetic parameters after a single on-flow experiment of just 600 seconds.

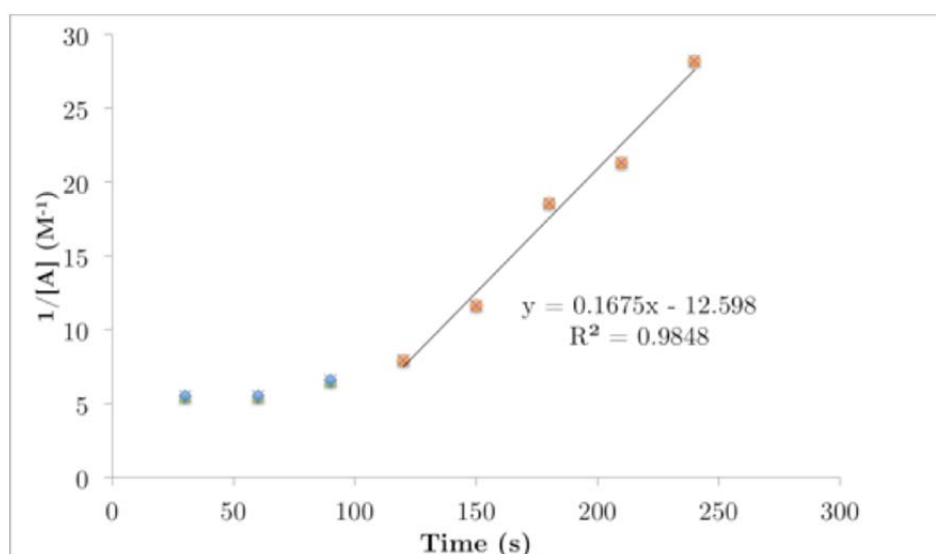


Figure S18. $1/[A]$ (M^{-1}) *versus* residence time (seconds) for the synthesis of 1-pentafluorophenyl-3,4,5-trimethylpyrazole (**16**) for a flow rate of 5 $\mu\text{l}/\text{min}$. $[A]$ represents the reagent concentration. The straight line emphasizes the zone b' data points, that represents a 'conventional' data set with incremental residence time and a constant temperature.

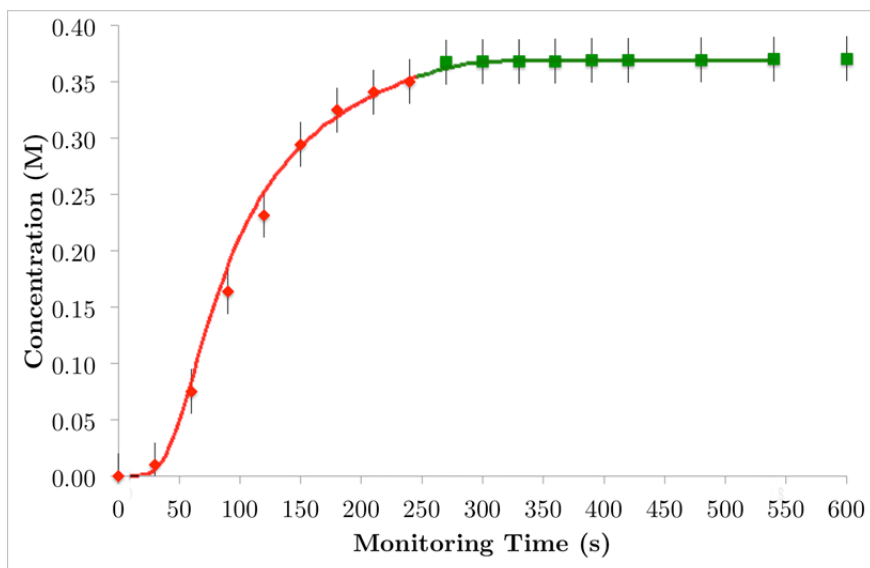


Figure S19. Reaction product (M) concentration *versus* monitoring time (seconds) for the synthesis of 1-pentafluorophenyl-5-methyl-3-phenyl-pyrazole (**17**) for an overall flow rate of 5 $\mu\text{l}/\text{min}$. The points correspond to experimental data. The curve along the data points has been obtained from fitting to a model using Maple software. The use of a flow rate of 5 $\mu\text{l}/\text{min}$ enables the extraction of the kinetic parameters after a single on-flow experiment of just 600 seconds.

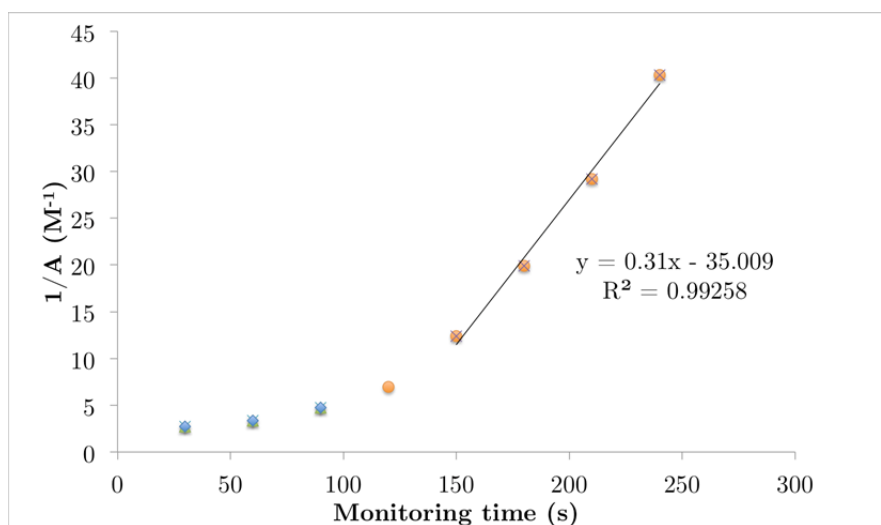


Figure S20. $1/[A]$ (M^{-1}) *versus* residence time (seconds) for the synthesis of 1-pentafluorophenyl-5-methyl-3-phenyl-pyrazole (**17**) for a flow rate of 5 $\mu\text{l}/\text{min}$. $[A]$ represents the reagent concentration. The straight line emphasizes the zone b' data points, that represents a 'conventional' data set with incremental residence time and a constant temperature.