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Measurement of Atropisomer Racemization Kinetics Using Segmented Flow Technology

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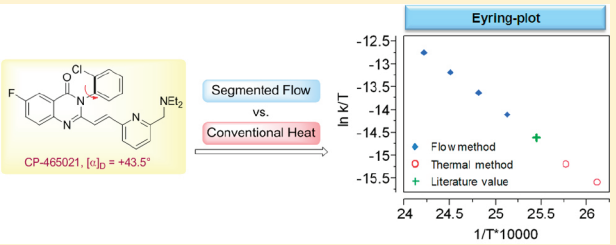
Supporting Information

ABSTRACT: When stable atropisomers are encountered by drug discovery teams, they can have important implications due to potential differences in their biological activity, pharmacokinetics, and toxicity. Knowledge of an atropisomer's activation parameters for interconversion is required to facilitate informed decisions on how to proceed. Herein, we communicate the development of a new method for the rapid measurement of atropisomer racemization kinetics utilizing segmented flow technology. This method leverages the speed, accuracy, low sample requirement, safety, and semiautomated nature of flow instrumentation to facilitate the acquisition of kinetics data required for experimentally probing atropisomer activation parameters. Measured kinetics data obtained for the atropo isomerization of AMPA antagonist CP-465021 using segmented flow and traditional thermal methods were compared to validate the method.



KEYWORDS: *Atropisomer, Reaction kinetics, Segmented flow chemistry*

The incidence of axial chirality known as atropisomerism[1] in chemical research is steadily increasing due to the advent of new chemical methodologies, which have made sterically encumbered bonds more facile to form.[2] Their existence in a drug discovery setting can have important implications due to possible differences in their biological activity, pharmacokinetics, and toxicity.[3] Knowledge of atropisomer activation parameters facilitates informed decisions about appropriate handling, storage, and reaction conditions. These data can also influence preclinical development strategy.[4] Herein, we report the rapid measurement of atropisomer racemization kinetics utilizing segmented flow technology. This method leverages the speed, accuracy, low sample requirement, safety, and semiautomated nature of flow instrumentation[5] to facilitate convenient acquisition of the kinetics data required for experimentally probing activation parameters.

We demonstrate the utility of this method by employing it to generate kinetics data for CP-465021 (Figure 1), a potent and selective noncompetitive α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist whose synthesis and resolution are reported elsewhere.[6−9] This molecule has an axial element of chirality due to hindered rotation of the *N*-aryl bond. Previously, serial measurements of the chiral stability of this molecule were conducted in a thermally controlled oil bath in 3-methylbutan-1-ol at 120 °C. These experiments suggest that interconversion of CP-465021 occurs via a planar, nonionic transition state with a rate constant k_{rac} = 1.07×10^{-2} min^{-1}.[10]

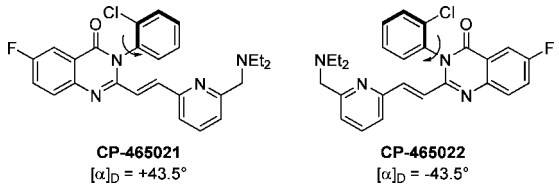


Figure 1. AMPA antagonist CP-465021 and its atropoenantiomer CP-465022.

Segmented flow technology[11] is uniquely suited for measuring thermal interconversion kinetics and has several advantages over traditional methods. First, the reaction temperature can be maintained safely above the boiling point of solvent due to the instrument's ability to contain pressure, thus accelerating reaction times and data acquisition. Second, the thermal mass of the system is much greater than the reaction fluid permitting near instantaneous heating and cooling, which translates into more accurate data (Figure 2). Last, many flow systems feature programmable methods and automated sample collection for convenient hands-off "overnight" acquisition.

Operationally, programmed segments of blank system solvent and solubilized substrate are alternatingly delivered to a small diameter tube reactor as shown schematically in Figure 3. The segments are separated by an immiscible perfluorodecalin spacer that acts as a physical barrier to minimize

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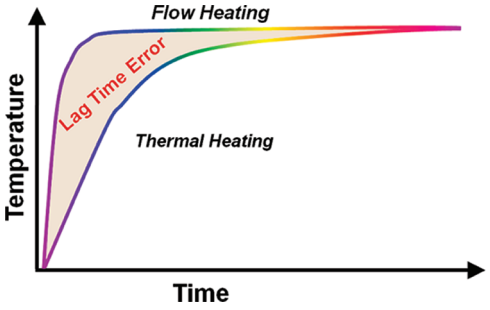

Figure 2. Representation of flow heating vs thermal heating. With flow heating, the relative thermal mass of the reactant compared to the heating block permits near instantaneous heating of a sample compared to a traditional system.

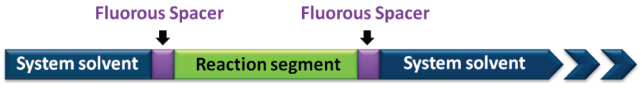

Figure 3. Schematic of a reaction segmented.

diffusion. As the sample segment exits the reactor, it passes a UV detector, which registers its residence time and triggers collection. The degree of racemization is then measured by an appropriate analytical method.[12]

The interconversion of CP-465021[13] was measured in 3-methyl-butane-1-ol with 3% toluene added to assist UV detection.[10] Because flow technology is automated and programmable, samples for six time points were collected in duplicate at four temperatures (125, 130, 135, and 140 °C) in a single overnight run (Table 1, entries 1−5). The chiral purity

Table 1. Measured First-Order Rate Constants for the Racemization of CP-465021 in 3-Methyl-butane-1-ol

entry	T (°C)	k_{rac} (min^{-1})	R^2	$t_{1/2}$ (min)
1	140	7.20×10^{-2}	0.997	9.60
2	135	4.64×10^{-2}	0.969	14.9
3[a]	135	4.81×10^{-2}	0.970	14.4
4	130	2.93×10^{-2}	0.962	23.7
5	125	1.80×10^{-2}	0.993	38.7
6	120[b]	1.07×10^{-2}	not reported	64.5
7	115[c]	5.89×10^{-3}	0.994	118
8	110[c]	3.91×10^{-3}	0.989	177

[a]Performed on different day and reaction block to assess reproducibility. [b]Literature value.[10] [c]Data obtained via conventional thermal flask method.

was measured by chiral supercritical fluid chromatography (SFC), and first-order rate constants, k_{rac}, for the rate of racemization were obtained from the plots of ln(% ee) vs time.[14] As a control, rate constants were similarly derived from four thermal runs performed at two temperatures, 110 and 115 °C, in duplicate (Table 1, entries 7 and 8). In every case, the reactions were monitored for between one and three interconversion half-lives.[15,16] The rate constants and R^2 values from these experiments are shown in Table 1.

Figure 4 is an overlay of the Eyring plots for the flow and thermal methods, showing excellent agreement. Using the four flow points, the Eyring expression gives $\Delta H^{\ddagger}$ = 29.5 kcal/mol and $\Delta S^{\ddagger} = -1.07 \times 10^{-3}$ kcal/mol K. From these parameters, we calculate Gibbs energy of activation $\Delta G^{\ddagger}$ to be 29.9 kcal/mol at physiological temperature (37 °C). This corresponds to

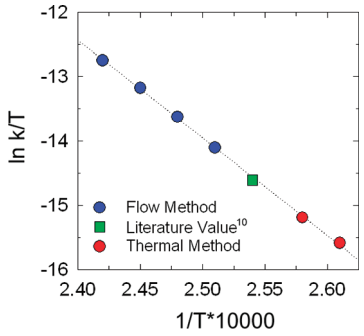

Figure 4. Eyring plot for the atropo isomerization of CP-465021 in 3-methyl-butane-1-ol.

a $t_{1/2}$ of approximately 4 years.[17] An identical $\Delta G^{\ddagger}$ was obtained from the thermally derived data points out to three significant figures (Table 2).

Table 2. Extrapolated Activation Parameters for CP-465021 and BINOL

		kcal/mol		
solute	T (°C)	flow $\Delta G^{\ddagger}$	thermal $\Delta G^{\ddagger}$	extrapolated $t_{1/2}$ (years)
CP-465021	37	29.9	29.9	4
S-BINOL	37	37.1	ND	460 000

Encouraged with the strong correlation of the thermal and flow kinetics data obtained from CP-465021, we applied the method to two compounds whose measured $t_{1/2}$ values at 37 °C were between 4 and 7 h. For these compounds, collectively, the R^2 values were lower than those obtained from CP-465021. Background epimerization of the stock solution over the course of the experiment and during the analytical phase would account for some of the observed drift. There was still excellent correlation between the data obtained from segmented flow and that obtained thermally with R^2 in the generated Eyring plots typically >0.97. Therefore, while we believe there are still some automation and accuracy benefits to using the flow system for measuring rapidly interconverting atropisomers, they are less pronounced.

To complete our study, *S*-1,1′-bi-2-naphthol (S-BINOL), a compound with a much higher barrier to interconversion, was also tested.[18] For this substrate, we measured 6−7 time points in duplicate at four temperatures (280, 285, 290, and 295 °C) using diglyme as solvent. From the Eyring equation, we obtained an extrapolated $\Delta G^{\ddagger}$ of 37.1 kcal/mol at 37 °C (Table 2), which corresponds to a $t_{1/2}$ of nearly 500 000 years. The R^2 value for the Eyring plot generated from S-BINOL data was 0.98, lending further support that segmented flow technology is a robust method for measuring reaction kinetics.

Ignoring atropisomerism can have deleterious consequences in drug development settings. Measured activation parameters combined with powerful computational models[19] can guide the use of compounds with this unique form of chirality. We have adopted segmented flow as a reproducible and convenient method for generating the samples necessary for measuring high temperature atropo isomerization kinetics. Because of its programmable semiautomated nature, once appropriate temperatures are identified, a run can potentially be completed overnight without intervention. It is compatible with a wide range of solvents over a broad range of pH values (1−12), temperatures (−20 to 300 °C), and pressure (up to 2500 psi),

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making it useful not only for pharmaceutical examples but also for chiral ligands, which must stand up to high temperatures for prolonged periods of time to convey their chirality.[20−22] A report detailing the use of experimentally and computationally derived activation parameters to guide decisions around atropisomers in drug discovery is currently in preparation.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures for kinetics experiments, SFC chromatograms, raw kinetics data, and Eyring analyses for CP-465021 and S-BINOL. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(15) At equlibrium, a mixture of atropoenantiomers will approximate 0% ee; therefore, $t_{1/2}$ is defined as the time that it takes a sample of a single enantiomer to reach 50% ee.

(16) The latter requirement is to confirm that the interconversion approximates a first-order reaction.

(17) Alternatively, the Arrhenius equation can be used to calculate the relationship between the rate constant (k) to the activation energy (E_a) and the preexponential factor (A).

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