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"Kinetic NMR Titration": Including Chemical Shift Information in the Kinetic Analysis of Supramolecular Reaction Systems such as Organic Replicators

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Minimal self-replicating systems[1−5] typically reveal characteristic changes of NMR signal shifts in the course of the reactions. Usually, the kinetic analysis of such systems is based on the monitoring of NMR integrals, while the changes of chemical shifts are not taken into account here. Thermodynamic information on the supramolecular complexes involved is usually derived from independent NMR titrations, typically following Wilcox's procedure.[6] We here introduce a kinetic method for a combined analysis of integral and shift changes, which is applicable to supramolecular reaction systems employing species rapidly equilibrating on the NMR time scale. Our example is based on a Diels−Alder ligation reaction which is a variant of a recently described replicator (Scheme 1).[5] As in the latter system, there is no NMR evidence either for the synthesis of exo isomers or of other diastereomers within the precision of NMR quantification.

Figure 1 shows the time-resolved changes of the pyridine signals 3-H and 4-H in precursor **1** and products **3b** and **4b**. The latter regioisomers are formed with very similar rates, and the overlap of their 3-H signals results in an apparent triplet structure, while they are not distinguishable with respect to their 4-H signals. For the sake of simplicity, we thus treat both isomers and their enantiomers as nondistinguishable, always dealing with the sum of their concentrations in the kinetic analysis. The reaction model is shown in Figure 2. We assumed a single rate parameter for all background reactions, which was fixed to the value found for the model reactions between **2a**/**b** and **5**. We furthermore assumed identical equilibrium constants for all single amidopyridine−carboxylate recognition events, resulting in a set of three rate parameters to be optimized during SimFitting of integral versus time data.[7,8]

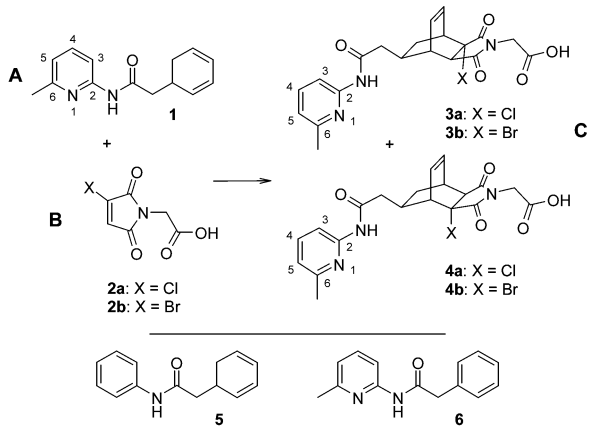
Analysis of shift versus time data[9] is based on the equation

$$\delta_{\mathrm{obs}}(t) = \sum_{i=1}^{n} \gamma_i(t)\delta_i$$

in which $\delta_{\mathrm{obs}}(t)$ is the observable chemical shift of a given proton at time t, $\gamma_i(t)$ is the mole fraction of a proton bearing species i at time t, and δ_i is the chemical shift of the respective proton in the pure species. The mole fraction is computable from the actual concentration of species i and the total concentration of all species contributing to the signal of the respective proton. The chemical shift for the pure species is either obtainable from individual NMR measurements of isolated compounds or needs to be approximated as a fit parameter in the case of supramolecular complexes. We expanded our SimFit program to deal with shift observables (Supporting Information) in kinetic and conventional NMR titrations. Figure 3 shows as an example the fits for concentrations (from integrals) as well as chemical shifts of a product (3-H, Figure 3b) and precursor proton (3-H, Figure 3c).

The respective curves for the decrease of the maleimide could not be created due to overlaying NMR signals. Kinetic titration

Scheme 1. Precursors and Template Isomers of the Analyzed Self-Replicating Diels−Alder System: **5** and **6** are Model Compounds (see text)



was applied for a whole range of temperatures, leading to the results shown in Table 1.

In addition conventional NMR titrations were performed for the same range of temperatures for the interaction of the nonreactive

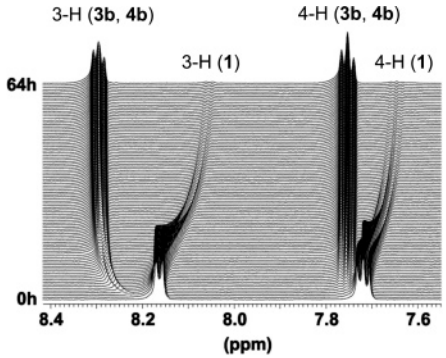


Figure 1. ^{1}H NMR stack plot for the reaction of **1** and **2b** to **3b** (600 MHz, $C_2D_2Cl_4$, 323 K, c = 15 mM).

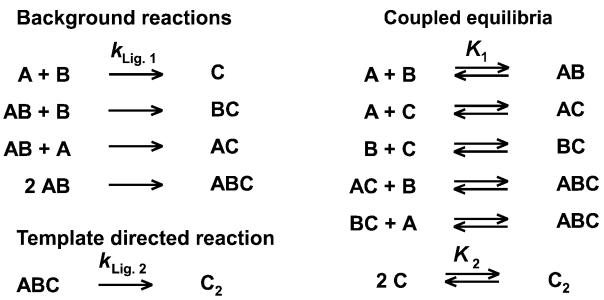


Figure 2. Reaction model for the analysis of the kinetic experiments. For definition of A, B, and C, see Scheme 1. Species describing supramolecular complexes are noted as letter combinations. C_2 refers to the self-complementary template duplex.

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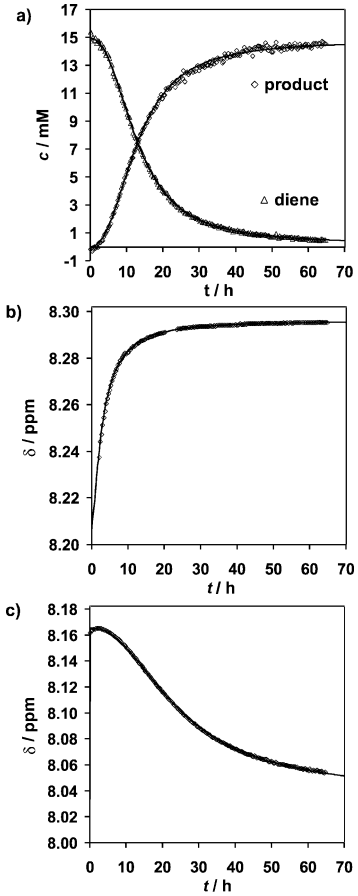

Figure 3. Experimental and theoretical profiles for (a) concentrations as a function of time, (b) chemical shift of the product signal for 3-H (**3b**, **4b**) as a function of time, and (c) chemical shift of the diene proton 3-H (**1**) as a function of time. Experimental conditions: $T = 323$ K, $c = 15$ mM for both precursors, $C_2D_2Cl_4$. The NMR data were processed using WinNMR (Bruker) and transformed into SimFit data files with an Excel macro (Supporting Information).

Table 1. Results of the Kinetic NMR Titration Analysis According to the Reaction Model Shown in Figure 2 and the Structures in Scheme 1

X	T (K)	k_{Lig1}[a] (10^{-5} M^{-1} s^{-1})	k_{Lig2} (10^{-5} s^{-1})	K_1 (10^3 M^{-1})	K_2 (10^5 M^{-1})	rms (%)
Cl	323	2.88	68 ± 2	0.243	1.82	0.97
	343	7.32	209 ± 3	0.0779	0.132	1.16
Br	303	0.59 ± 0.25	8.0 ± 0.3	1.90	20.421 ± 0.008	0.87
	313	1.70	17.1 ± 0.2	1.08	9.419 ± 0.001	0.82
	318	1.9 ± 0.5	29.6 ± 1.5	0.538	1.43	2.63
	323	4.15	99 ± 3	0.256	0.738 ± 0.001	1.24
	330	6.0 ± 2.0	162.6 ± 1.5	0.202	0.308	1.49
	337	7.1 ± 1.3	124 ± 1	0.140	0.104	1.37
	343	14.32	327 ± 4	0.0617	0.0224	1.16

[a] k_{Lig1} (background reaction) was determined independently using model diene compound **5** (X = Br, $T = 313$, 323, 343 K) or iterated during the analysis (X = Br, $T = 303$, 318, 330, 337 K).

model compound **6** with **2a**/**b** (Table 2). The data for K_1 can be directly compared to each other. While at low temperature they are in good agreement, they differ to a higher extent at elevated temperatures. Whether this is due to a methodological or structural difference remains to be reinvestigated for systems of lower complexity.

Table 2. Results of NMR Titration Experiments between Model Compound **6** as Host and Maleimide **2a/b** as the Guest[a]

	T (K)	293	303	313	323	333	343
Cl	K_1/ 10^3 M^{-1}	$1.7 \le K \le 6.5$	1.6 ± 0.2	$0.71 \le K \le 1.25$	$0.28 \le K \le 1.33$	0.28 ± 0.02	0.16 ± 0.02
Br	K_1/ 10^3 M^{-1}	$2.5 \le K \le 4.0$	1.9 ± 0.3	1.2 ± 0.2	$0.63 \le K \le 0.90$	$0.38 \le K \le 0.58$	$0.22 \le K \le 0.35$

[a] c(host) between 0.5 and 1 mM; guest/host ratio between 0 and 10.

We have shown that kinetic NMR titration, that is, the extraction and simultaneous kinetic modeling of temporal shift and integral changes, allows one to harvest kinetic and thermodynamic information on supramolecular reaction systems much more efficiently, reliably, and accurately than in conventional kinetic data fitting based on integrals alone. For the latter, the variation of initial template concentration and the utilization of thermodynamic information from independent sources, such as conventional NMR titrations, were a must. We believe that our method which combines colligative and structural information is generally applicable for a whole range of supramolecular reaction systems in which temporal chemical shift changes are observable. In our hands, it has been proven to be a valuable new tool in the systems chemistry toolbox.

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Supporting Information Available: SimFit files including calculation mode for chemical shift data of NMR signals; time dependent concentration and chemical shift profiles for the analyzed experiments; Excel macro for transformation of NMR data into SimFit data files. This material is available free of charge via the Internet at http://pubs.acs.org.

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