- Supplementary Information-

Real-time monitoring of a dynamic molecular system using ^{1}H-^{13}C HSQC NMR spectroscopy with optimized ^{13}C window

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Contents:

1. Characterization data for compounds 1C and 1D.

Hydrazones **1C** and **1D** were synthesized using a literature procedure (G. Gasparini, M. Martin, L.J. Prins, P. Scrimin, *Chem.Commun., 2007*, 1340-1342).

HYDRAZONE 1C

^{1}H-NMR (300 MHz, CD_3OD) δ (ppm): 1.01 (t, *J* = 7.31, 7.31 Hz, 12H), 1.20 (dt, *J* = 18.96, 7.62 Hz, 3H), 1.40 (m, 8H), 1.58-1.82 (m, 10H), 3.18-3.23 (m, 8H), 6.94-7.03 (m, 1H), 7.09-7.14 (m, 1H), 7.22-7.44 (m, 5H), 8.17 (d, *J* = 7.75 Hz, 1H), 8.79 (s, 1H)

^{13}C-NMR (63 MHz, CD_3OD) δ (ppm): 8.13 (d, J_{P-C} = 6.44), 14.00, 20.74 (t, J_{P-N} = 1.51 Hz), 21.79 (d, J_{P-C} = 139.64 Hz), 24.80, 59.48 (t, J_{P-N} = 2.86 Hz), 115.67, 119.58, 120.28, 122.37 (d, J_{P-C} = 2.83 Hz), 124.45, 127.09 (d, J_{P-C} = 4.47 Hz), 127.63, 130.86, 132.56, 135.61, 147.09, 153.79 (d, J_{P-C} = 7.90 Hz), 159.09, 167.133

ESI-MS(-) MeOH: $[M\text{-}TBA]^-$ 347m/z

HYDRAZONE 1D

^{1}H-NMR first isomer (84%) (300 MHz, CD_3OD) δ (ppm): 1.03 (t, *J* = 7.26 Hz, 12H), 1.20 (dt, *J* = 18.98, 7.65Hz, 3H), 1.42 (m, 8H), 1.61-1.84 (m, 10H), 3.22-3.28 (m, 8H), 6.07 (s, 2H), 7.12 (t, *J* = 7.42 Hz, 1H), 7.33-7.46 (m, 2H), 8.01 (d, *J* = 7.69 Hz, 1H), 8.18 (t, *J* = 6.71 Hz, 2H), 8.46 (s, 1H), 8.61-8.75 (m, 1H), 9.00 (d, *J* = 5.65 Hz, 2H)

^{1}H-NMR second isomer (16%) (300 MHz, CD_3OD) δ (ppm): 1.03 (t, *J* = 7.26 Hz, 12H), 1.20 (dt, *J* = 18.98, 7.65Hz, 3H), 1.42 (m, 8H), 1.61-1.84 (m, 10H), 3.22-3.28 (m, 8H), 5.92 (s, 2H), 7.12 (t, *J* = 7.42 Hz, 1H), 7.33-7.46 (m, 2H), 8.01 (d, *J* = 7.69 Hz, 1H), 8.18 (t, *J* = 6.71 Hz, 2H), 8.46 (s, 1H), 8.61-8.75 (m, 1H), 9.00 (d, *J* = 5.65 Hz, 2H)

^{13}C-NMR first isomer (84%) (63 MHz, CD_3OD) δ (ppm): 8.12 (d, J_{P-C} = 6.45 Hz), 14.01, 20.76 (t, J_{P-N} = 1.51 Hz), 21.75 (d, J_{P-C} = 139.60 Hz), 24.84, 59.54 (t, J_{P-N} =2.882 Hz), 63.01, 122.44 (d, J_{P-C} = 2.79 Hz), 124.49, 126.72 (d, J_{P-C} = 4.60 Hz), 127.13, 128.95, 132.57, 144.19, 147.56, 147.85, 153.57 (d, J_{P-C} = 7.77 Hz), 167.60.

^{13}C-NMR second isomer (16%) (63 MHz, CD_3OD) δ (ppm): 8.12 (d, J_{P-C} = 6.45 Hz), 14.01, 20.76 (t, J_{P-N} = 1.51 Hz), 21.75 (d, J_{P-C} = 139.60 Hz), 24.84, 59.54 (t, J_{P-N} =2.882 Hz), 63.01, 122.44 (d, J_{P-C} = 2.79 Hz), 124.56, 126.55 (d, J_{P-C} = 4.35 Hz), 127.58, 129.08, 132.96, 147.48, 147.71, 147.77, 153.57 (d, J_{P-C} = 7.77 Hz), 163.09

ESI-MS(-) MeOH: $[M\text{-}TBA]^-$ 347m/z

2. Procedure for measuring the ^{1}H-^{13}C HSQC NMR spectra.

Hydrazide stocksolutions were prepared as concentrated as possible (0.7-0.8 M in CD_3OD) also using gentle heating in order to minimize dilution of the initial solution of hydrazone **1A** (50 mM). After hydrazide addition a time lap of around 20 minutes was required before acquisition of the first spectrum could be initiated. HSQC spectra were recorded on a Bruker 600 MHz spectrometer equipped with a TXI-cryoprobe using procedures published before (ref. 10 manuscript). Specific details for these experiments are given below.

The spectral width proposed by the optimization program was doubled and set to 742.2 Hz (4.94 ppm at 150 MHz ^{13}C Larmor frequency) in order to minimize the discrepancy between expected and observed chemical shifts and to account for changes in chemical shifts of the hydrazones. Multiplying the spectral width with an integer is possible because a property of spectral aliasing insures that signals do not overlap for any spectral width *n**SWa, where *n* is in integer, provided 1) signals do not overlap for SWa, and 2) the number of time increments is multiplied by *n* (or more).

Because of the risk of signal overlap, the resolution should be pushed to the maximum allowed by the relaxation rates. The number of increments should therefore be set to 2 * $t_{1,max}$ [s] * SW [Hz] where $t_{1,max}$ is the time beyond which the signal has decreased to the level of the noise. In the case of small molecules 0.5 s is a good value to use but when the complexity of the mixture requires top performances one can make a better choice after measuring the relevant relaxation rates. We set the number of time increments to 400 which is a good choice given the imine relaxation rates (see Table S1, next section) and because this allows to decrease the experimental time of each spectrum to 10 min. The T_2 during the t_1 evolution time were measured by fitting the signal decay within a series of HSQC in which a $\tau/2-\pi_{carbon}-\tau/2$ as inserted before the first evolution time to the exponential relaxation equation $exp(-\tau/T_2)$.

3. Quantification of signals in the HSQC spectra.

In order to quantify signals of any given CH in the different molecules of the mixture, one has to take into account the deviation of the $^1J_{CH}$ coupling constants to the value used for the INEPT delay and the differences in relaxation rates during t_1. The differences in $^1J_{CH}$ turned out to be negligible as Table S1 shows but relaxation being quite significant during the long ^{13}C evolutions of high-resolution experiments could not be neglected. Nevertheless, extrapolation of the kinetic profile in Figure 2 (manuscript) using these corrected values revealed that this had only a small effect on the final ratio of components.

Table S1. Corrections applied to the signal amplitudes of HSQC spectra

Imine	$^1J_{CH}$[a]	Corr. fact.[b]	T_2[c]	Corr. fact[d]
$1A_M$	165.9	1.003	385	1.384
$1A_m$	165.6	1.003	314	1.483
$1B_M$	163.9	1.001	279	1.551
$1B_m$	164.1	1.002	296	1.515
1C	166.9	1.005	161	2.043
$1D_M$	165.1	1.003	347	1.431
$1D_m$	162.6	1.001	293	1.521

a) Measured in the proton dimension of an HSQC where the ^{13}C decoupler was turned off during acquisition

b) Due to the deviation relative to 160 Hz, the value used for the INEPT

c) Relaxation time in ms

d) Rel. to negligible relaxation and considering a maximal t_1 evolution time of 270 ms reached with a 747.2 Hz spectral width in ^{13}C and 400 time increments.

4. Changes in thermodynamic composition upon dilution.

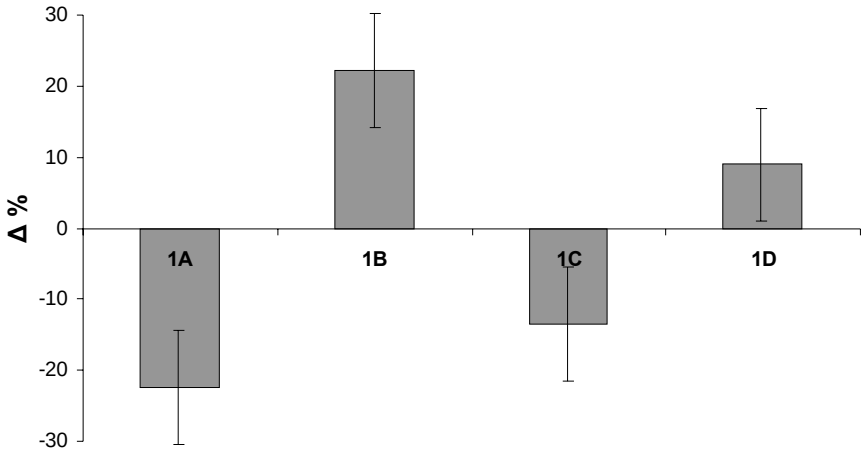

Fig. SI-1 Relative changes in the signal intensities of hydrazones **1A-1D** upon an eight-fold dilution of the mixture. The final mixture was diluted from to a final concentration **1** of 5 mM and compositional changes were followed for 24 hours.

5. Micromath Scientist (version 2.01) model files used for fitting

Knowing the total hydrazone concentration during the experiment (taken into account the dilutions at the beginning of each section) the concentrations of each separate hydrazone are directly calculated from the peak intensities. The respective mass balances give the concentration of free hydrazides. The three sections in Figure 1 are fitted separately, fixing the rate constants obtained from the previous sections.

```
// MicroMath Scientist Model File
// section 1
// 1A+B = 1B +A
// kAB; kBA

IndVars: T
DepVars: HA, HB, A, B
Params: kAB, kBA, HA0, HB0, A0, B0

HA'=-kAB*HA*B+kBA*HB*A
HB'=kAB*HA*B-kBA*HB*A

A'=-kBA*HB*A+kAB*HA*B
B'=kBA*HB*A-kAB*HA*B
//initial conditions
t=0
HA=HA0
HB=HB0
A=A0
B=B0
***
```

```
// MicroMath Scientist Model File
// section 2 (parameter kAB, kBA fixed)
// 1A + B = 1B + A
// kAB, kBA

// 1A + C = 1C+ A
// kAC, kCA

// 1B + C = 1C +B
// kBC, kCB

IndVars: T
DepVars: HA, HB, HC, A, B, C, ATOT, BTOT, CTOT
Params: kAB, kBA, kAC, kCA, kBC, kCB, HA0, HB0, HC0, A0, B0, C0

HA'=-kAB*HA*B+kBA*HB*A-kAC*HA*C+kCA*HC*A
HB'=-kBA*HB*A+kAB*HA*B-kBC*HB*C+kCB*HC*B
HC'=-kCA*HC*A+kAC*HA*C-kCB*HC*B+kBC*HB*C

A'=kAB*HA*B-kBA*HB*A-kCA*HC*A+kAC*HA*C
B'=kBA*HB*A-kAB*HA*B-kCB*HC*B+kBC*HB*C
C'=kCA*HC*A-kAC*HA*C-kBC*HB*C+kCB*HC*B
ATOT=HA+A
BTOT=HB+B
CTOT=HC+C
//initial conditions
t=0
```

```
HA=HA0
HB=HB0
HC=HC0
A=A0
B=B0
C=C0
***
```

// MicroMath Scientist Model File
// section 3 (parameter kAB, kBA, kAC, kCA, kBC, kCB fixed)

```
// 1A + B = 1B + A
// kAB, kBA

// 1A + C = 1C+ A
// kAC, kCA

//1A + D = 1D + A
// kAD, kDA

// 1B + C = 1C +B
// kBC, kCB

// 1B + D = 1D + B
// kBD, kDB

// 1C+ D = 1D + C
// kCD, kDC

IndVars: T
DepVars: HA, HB, HC, HD, A, B, C, D, ATOT, BTOT, CTOT, DTOT
Params: kAB, kBA, kAC, kCA, kAD, kDA, kBC, kCB, kBD, kDB, kCD, kDC, HA0, HB0, HC0, HD0, A0, B0,
C0, D0

HA'=-kAB*HA*B+kBA*HB*A-kAC*HA*C+kCA*HC*A-kAD*HA*D+kDA*HD*A
HB'=-kBA*HB*A+kAB*HA*B-kBC*HB*C+kCB*HC*B-kBD*HB*D+kDB*HD*B
HC'=-kCA*HC*A+kAC*HA*C-kCB*HC*B+kBC*HB*C-kCD*HC*D+kDC*HD*C
HD'=-kDA*HD*A+kAD*HA*D-kDB*HD*B+kBD*HB*D-kDC*HD*C+kCD*HC*D

A'=kAB*HA*B-kBA*HB*A-kCA*HC*A+kAC*HA*C+kAD*HA*D-kDA*HD*A
B'=kBA*HB*A-kAB*HA*B-kCB*HC*B+kBC*HB*C+kBD*HB*D-kDB*HD*B
C'=kCA*HC*A-kAC*HA*C-kBC*HB*C+kCB*HC*B+kCD*HC*D-kDC*HD*C
D'=kDA*HD*A-kAD*HA*D+kDB*HD*B-kBD*HB*D+kDC*HD*C-kCD*HC*D

ATOT=HA+A
BTOT=HB+B
CTOT=HC+C
DTOT=HD+D
//initial conditions
t=0
HA=HA0
HB=HB0
HC=HC0
HD=HD0
A=A0
B=B0
C=C0
D=D0
***
```