Supporting Information

A Light-induced Reversible Phase Separation and its Coupling to a Dynamic Library of Imines

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Experimental part:

Irradiations were carried out with a Müller Elektronik Optik Light Source Model LAX 1000 / SVX 1000 with a xenon short arc lamp XBO 1000 W / HS OFR from Osram. The experiments were performed in a glass thermostated bath at 25°C.

Synthesis of deuterated (α-d,6-d)1-E:

6-deuterio-2-pyridinecarboxaldehyde-α-2-pyridylhydrazone ((α-d,6-d)1-E) - A solution of 2-pyridine-6-d-methan-α,α-d2-ol-d [1] (1.26 g, 11.1 mmol) in 50 mL of CHCl3 was treated with MnO2 (4.00 g, 46.0 mmol) and heated to reflux for 14 hours under nitrogen. The suspension was cooled to room temperature and filtered through a pad of celite. The filter cake was washed with CHCl3, and the combined filtrates were evaporated, giving the crude 6-deuterio-2-pyridinecarboxaldehyde-alpha-d, which was immediately carried over to the next step.

The crude aldehyde was dissolved in 50 mL of absolute ethanol and treated with 2-hydrazinopyridine (1.21 g, 11.1 mmol). The mixture was heated to reflux under nitrogen for 12 hours and evaporated to dryness, giving a tan solid. Recrystallization from CHCl3/Et2O gave 1-E as colorless needles (1.38 g, 6.89 mmol, 62%, 95% D).

1H NMR (CDCl3): 8.20 (d, J = 4.49, 1H), 7.99 (d, J = 8.03, 1H), 7.69 (t, J = 7.63, 1H), 7.63 (t, J = 7.84, 1H), 7.42 (d, J = 8.49, 1H), 7.19 (d, J = 7.20, 1H), 6.82 (dd, J = 7.40, J = 5.51, 1H); 13C NMR (CDCl3): 156.7, 154.3, 149.1 (t, J = 26.9), 147.4, 139.3 (t, J = 24.3), 138.4, 136.4, 122.9, 119.9, 116.4, 107.9. MS calculated for [C11H8D2N4+H]+ 201.1104, found 201.1109. Traces of [C11H9D1N4+H]+ 200.1041, found 200.1039 and [C11H10N4+H]+ 199.0978, found 199.0981.
Figures:

*Calcium binding by hydrazone 1.*

Fig. 1 Solid state molecular structure of the \([1\text{-E}2,\text{Ca}]\text{Cl}_2\) complex showing that each chloride anion is hydrogen bonded to one of the N-H sites of the pyridyl-hydrazone of another complex. One ligand is omitted for clarity and only its N binding sites are shown. Crystals were obtained by vapor diffusion of diisopropylether into a solution of 1-E and CaCl\(_2\) in methanol. CCDC 971153 contains the supplementary crystallographic data for this structure. (gray = carbon; green = chloride; blue = nitrogen, purple = calcium)

Fig. 2 UV/Vis spectra of 1-E resulting from titration with incremental additions of CaCl\(_2\) in 3:2 AN/W. These data were analyzed by the software Chem-Equili.\(^{1}\) Stock solutions of ligand 1-E were prepared in 3:2 AN/W (10 \(\mu\)M) and were titrating with CaCl\(_2\). UV/Vis spectra were recorded after each addition.
The observed changes of the ligand absorption as a function of concentration of CaCl$_2$ were analyzed to give the association constant by the software ChemEquili.$^{[2]}$

**Fig. 3** Fitting for the experimental data of NMR shifts for three different protons (blue: H3, red: H10 and black: H1) of 1-E upon addition of CaCl$_2$ in 3:2 AN/W at 25°C.

**Fig. 4** Job plot of 1-E with CaCl$_2$ showing the formation of a 1:1 complex in 10 mM 3:2 AN/W solution in agreement with the NMR observations. The lines just connect the experimental points for easier viewing.
Fig. 5  A portion of the 400 MHz $^1$H NMR spectra showing the evolution of the chemical shifts (ppm) of 1-E upon addition of Ca(OTf)$_2$ in 10 mM AN-$d_3$ solution at -40°C (from bottom trace to top trace, 0, 0.15, 0.25, 0.43, 0.5, 0.6, 0.7, 0.8, 1, 1.5 eq. Ca(OTf)$_2$). The three complexes [(1-E)$_n$Ca](OTf)$_2$ ($n = 1, 2$ and 3) are visible in slow exchange (the assignment of NH protons are indicated in the figure). The complex [(1-E)$_3$Ca](OTf)$_2$ presents a doublet at 10.3 ppm due to the lack of symmetry of the complex (see X-ray structure in Figure 4 in the publication).

Fig. 6  DOSY NMR spectrum of 1-E with 0.15 eq. Ca(OTf)$_2$ in 10 mM solution AN-$d_3$ at -40°C showing the two species 1-E and [(1-E)$_3$Ca](OTf)$_2$ and their different sizes.
Fig. 7 Job plot of 1-E with Ca(OTf)$_2$ showing the formation of 1:1 (□ trace) and 3:1 (■ trace) complexes on progressive addition of Ca(OTf)$_2$ in 10 mM AN solution at 25°C. The lines just connect the experimental points for easier viewing.

Fig. 8 Fitting for the experimental data of NMR shifts for two different protons (blue: H9, red: H5 and black: H1) of 1-Z upon addition of Ca(OTf)$_2$ in AN at 25°C.
Fig. 9 Job plot of 1-Z with Ca(OTf)₂ showing the formation of a 1:1 complex in 10 mM AN solution. The lines just connect the experimental points for easier viewing.

Fig. 10 A portion of the 400 MHz ¹H NMR competition spectrum (bottom trace) between Ca(OTf)₂, 1-E and 1-Z at -40°C (10 mM each) in AN-d₃ solution showing a change in the chemical signals ratio of NH (10.3-10.7 ppm) for the complexes of 1-E compared to the spectrum (top trace) with Ca(OTf)₂ and 1-E (10 mM each) in AN-d₃ solution.
Calcium binding by hydrazone 2-E.

The stability constant of the complex (2-E,Ca)Cl₂ in 3:2 AN/W was found to be 5.4 ±1.1 M⁻¹ at 25°C as determined by analysis of the UV-vis spectroscopy titration data obtained on progressive addition of CaCl₂ to 2-E (see Fig. 11, SI). Nevertheless, in pure AN, no difference of bindings between 1-E and 2-E were noticed. A ¹H NMR competition experiment in pure AN did not show preferential binding of calcium cations with 1-E or 2-E. The experiment showed the formation of the complexes [(1-E)₂,Ca]Cl₂, [(2-E)₂,Ca]Cl₂ and [(1-E),(2-E),Ca]Cl₂ in almost the same quantity, proving that the complexes have the same stability in pure AN (see Fig. 12, SI). This experiment had been studied at -40°C to distinguish the different species in slow exchange.

Fig. 11 UV/Vis spectra of 2-E resulting from titration with incremental additions of CaCl₂ in 3:2 AN/W. These data were analyzed by the software Chem-Equili.[1]
Fig. 12 A portion of the 400 MHz $^1$H NMR competition spectrum (bottom trace) between Ca(OTf)$_2$, 1-E and 2-E (10 mM each) in AN-$d_3$ solution at -40°C showing two singulets at 10.5 ppm coming from a mixture of 2:1 complexes [(1-E)$_2$,Ca]Cl$_2$ and (1-E,2-E,Ca)Cl$_2$, compared to a portion of the 400 MHz $^1$H NMR spectrum (top trace) of 1-E with Ca(OTf)$_2$ (10 mM each) in AN-$d_3$ solution at -40°C showing the formation of the complex [(1-E)$_2$,Ca]Cl$_2$.

**Strontium binding by hydrazone 1-E.**

Fig. 13 UV/Vis spectra of 1-E resulting from titration with incremental additions of SrCl$_2$ in 3:2 AN/W. These data were analyzed by the software Chem-Equili.$^{[1]}$
**Calcium binding by acyl-hydrazone 3.**

**Fig. 14** Solid state molecular structure of the dimeric association of two neutral 2:1 3-E:Ca\(^{2+}\) complexes with the ligands in their ionized form at the N-H site. Crystals obtained by slow vapor diffusion of diisopropylether into a solution of 3-E and CaCl\(_2\) in AN. CCDC 971407 contains the supplementary crystallographic data for this structure. (gray = carbon; red = oxygen; blue= nitrogen, purple = calcium).

**Fig. 15** UV/Vis spectra of 3-E resulting from titration with incremental additions of CaCl\(_2\) in 3:2 AN/W. These data were analyzed by the software Chem-Equili.\(^1\)
Fig. 16 Job plot of 3-E with CaCl$_2$ showing the formation of a 1:1 complex in 10 mM 3:2 AN/W solution. The lines just connect the experimental points for easier viewing.

Fig. 17 Fitting for the experimental data of NMR shifts for two different protons (red: H1 and black: H3) of 3-E upon addition of Ca(OTf)$_2$ in AN at 25°C.
Fig. 18 Fitting for the experimental data of NMR shifts for two different protons (red: H3 and black: H5) of 3-Z upon addition of Ca(OTf)2 in AN at 25°C.

To confirm the preferential binding of Ca²⁺ with 3-E compared to 3-Z, a ¹H NMR competition experiment had been performed in pure AN 10 mM solution at 25°C in presence of Ca(OTf)₂, 3-E and 3-Z (10 mM each) and was compared to the ¹H NMR titration on progressive addition of Ca(OTf)₂ to 3-Z (see Fig. 19, SI). The results showed that the signals of 3-Z were found in the position where 0.3 eq. Ca²⁺ was bound to 3-Z, so that 0.7 eq. Ca²⁺ was bound to 3-E which confirmed the stronger binding of Ca²⁺ with 3-E.

Fig. 19 A portion of the 400 MHz ¹H NMR competition spectra (a) between Ca(OTf)₂, 3-E and 3-Z (10 mM each) in AN-d₃ solution at 25°C compared to portions of the 400 MHz ¹H NMR spectra (b-j) of 3-Z (10 mM) with progressive addition of Ca(OTf)₂ in AN-d₃ solution, showing a preferential binding of Ca²⁺ to 3-E because the chemical shifts of 3-Z in the competition experiment (a) superpose with the chemical shifts of the spectrum (e) with 0.3 eq. Ca(OTf)₂.
Fig. 20 Job plot of 3-E with Ca(OTf)$_2$ showing the formation of a 2:1 complex in 10 mM AN solution. The lines just connect the experimental points for easier viewing.

Fig. 21 Job plot of 3-Z with Ca(OTf)$_2$ showing the formation of a 2:1 complex in 10 mM AN solution. The lines just connect the experimental points for easier viewing.
Fig. 22 Distribution of the dynamic covalent library of imines 8-11 (40 mM each) in a single phase of 3:2 AN/W in the presence of 306 mM CaCl₂. a) 400 MHz ¹H NMR spectrum of the mixture: -CH=N- proton signals of imines 8-11 in the 8.1-8.4 ppm region; -CHO proton signals of unreacted 4 and 5 between 9.3 and 9.5 ppm. The arrow indicates the remaining proton signal H₆ of partially deuterated 1-E. b) Amounts and fractions of the four different imine constituents 8-11, determined by integration of the corresponding –CH=N- proton signals against an external standard.

Fig. 23 Distribution of the dynamic covalent library of imines 8-11 (40 mM each), in the presence of 305 mM CaCl₂, upon phase separation of a 3:2 AN/W solution into a biphasic system by light irradiation for 9h. (Top) 400 MHz ¹H NMR spectrum of the aqueous (a) and organic phase (b); -CH=N- and -CHO proton signals in the 8.0-8.5 and 9.1-9.8 ppm region respectively. (c) Distributions of the four different imine constituents in the respective phases, aqueous (blue, left) and organic (yellow, right), determined by integration of the corresponding –CH=N proton signals against an external standard. (d) Integrated amounts and fractions of the four different imine constituents 8-11 summed over both phases.
References:


(2) CHEM-EQUILI is a computer program for the calculation of equilibrium constants from many types of equilibrium data (IR, NMR, UV/Vis and fluorescence spectrophotometry, potentiometry, calorimetry, conductimetry…). It is possible to use any combination of such kinds of methods simultaneously for reliable calculations of equilibrium constants. For a detailed description see: Solov’ev, V. P.; Vnuk, E.; Strakhova, N. N.; Kazachenko, V. P.; Belsky, V. K.; Varnek, A. A.; Volkova, T. A.; Wipff, G. *J. Chem. Soc. Perkin Trans. 2* 1998, 1489.