

Determination of binding constants by NMR titration

An overview on a current project by Kira Hof and Katharina
Lippert

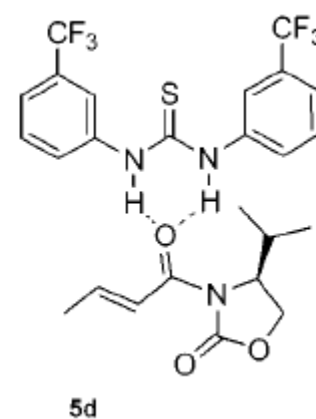
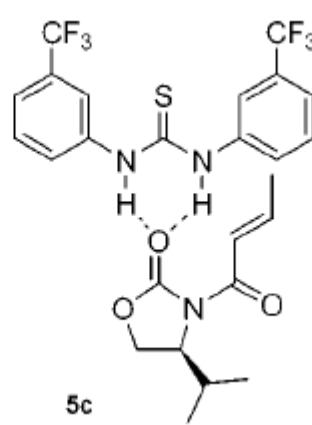
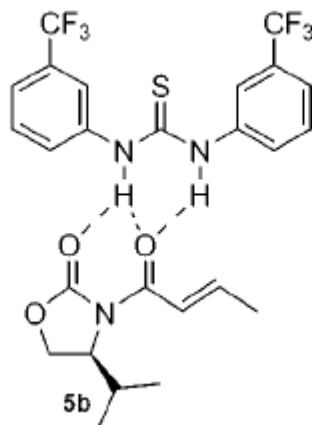
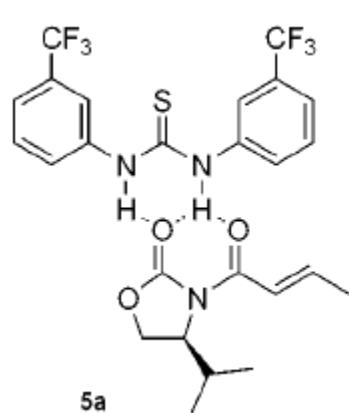
Table of content

- ▶ Goals
- ▶ Introduction
- ▶ Determination of stoichiometry
- ▶ Determination of association constant
- ▶ Advantages
- ▶ Our results
- ▶ Summary
- ▶ Outlook



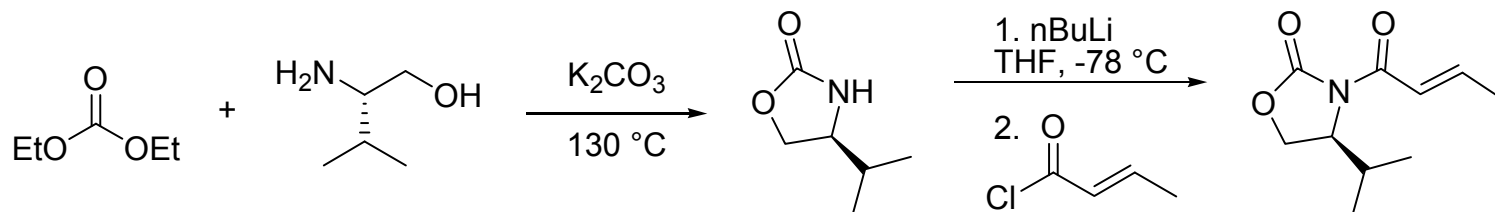
H-bonding complex

- ▶ Dr. Bredenbeck is interested in an organocatalytic complex
- ▶ Investigation with 2D IR spectroscopy

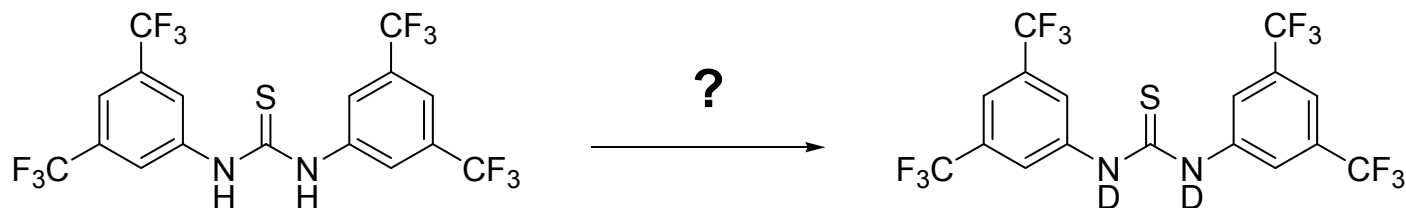


Goals

► Synthesis of acyloxazolidinone



► Deuteration of thiourea TI



► Determination of the association constant of TI and acyloxazolidinone via NMR titration

H-bonding complex with thioureas

- ▶ Thiourea $pK_s = 21$, Urea $pK_s = 27$

Bordwell, F. G.; Algrim, D. J.; Harrelson, J. A., *J. Am. Chem. Soc.* **1998**, 110, 5903-5904

- ▶ Sulfur lower electronegativity, decreasing self-association of thioureas Wittkopp,

A.; Schreiner, P. R., *Chem. Eur. J.* **2003**, 9, 407-414

- ▶ Cocrystallization of ureas with hydrogen bond acceptors

Etter, M. C., *Acc. Chem. Res.* **1992**, 23, 120-126, Etter et al., *J. Am. Chem. Soc.* **1992**, 112, 8415-8426

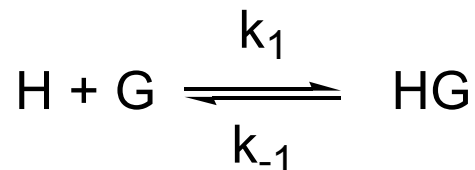
- ▶ Thioureas more soluble in organic solvents

Curran, D. P.; Lung, H. K., *Tetrahedron Lett.* **1995**, 36, 6647-6650



Introduction

- ▶ Complexation between a host [H] and a guest [G]



- ▶ Strength of complex: K

$$K = \frac{[\text{H} \cdot \text{G}]}{[\text{G}][\text{H}]} = \frac{[\text{H} \cdot \text{G}]}{([\text{H}]_0 - [\text{H} \cdot \text{G}]) * ([\text{G}]_0 - [\text{H} \cdot \text{G}])}$$

- ▶ Measured shift δ_{obs} :

$$\delta_{\text{obs}} = X_G \cdot \delta_G + X_H \cdot \delta_H$$

- ▶ Relationship between δ_{obs} and K is non-linear!

Preparing a NMR titration

- ▶ Solvent: No hydrogen bonding solvent
- ▶ Dry solvents
- ▶ Working under argon
- ▶ Concentration area (0.1 mM)
stock solution
- ▶ Right dilution
- ▶ Cooling of stock solutions
- ▶ Filling height of NMR tubes



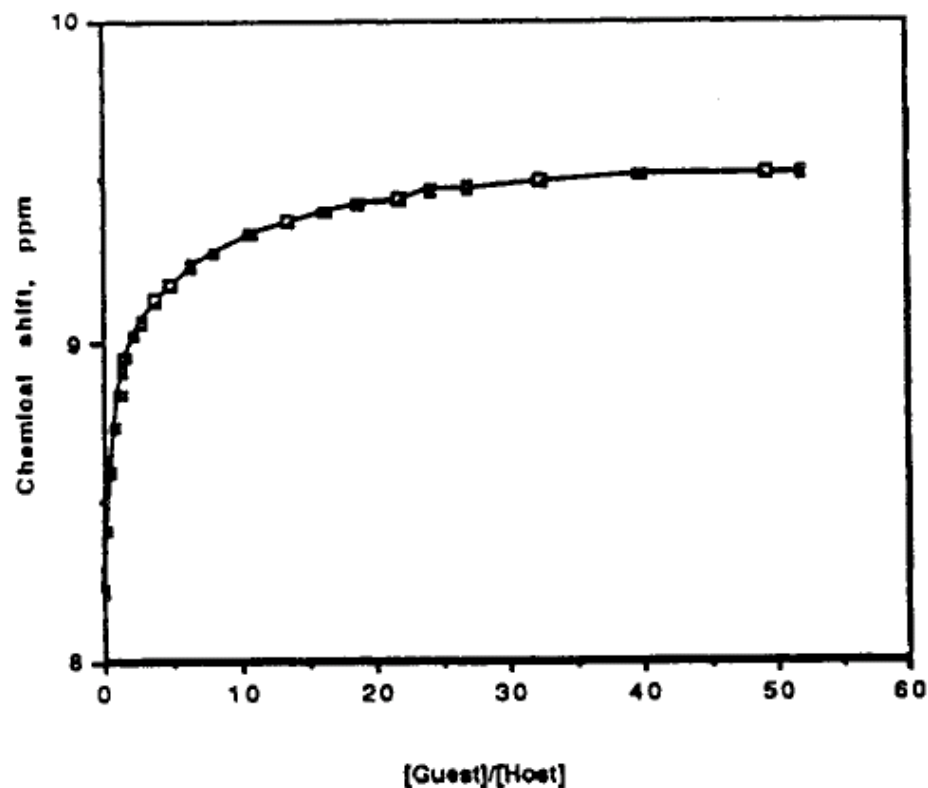
Titration Method

- ▶ Measuring δ_0 of $[H]_0$
- ▶ Adding small amounts of Guest; measuring δ_{obs} of $[H^*G]$
- ▶ Measurement of δ_{max}

$$K = \frac{\alpha}{(1 - \alpha) \cdot ([G] - \alpha[H])}$$

$$\alpha = \frac{(\delta_{\text{obs}} - \delta_0)}{(\delta_{\text{max}} - \delta_0)}$$

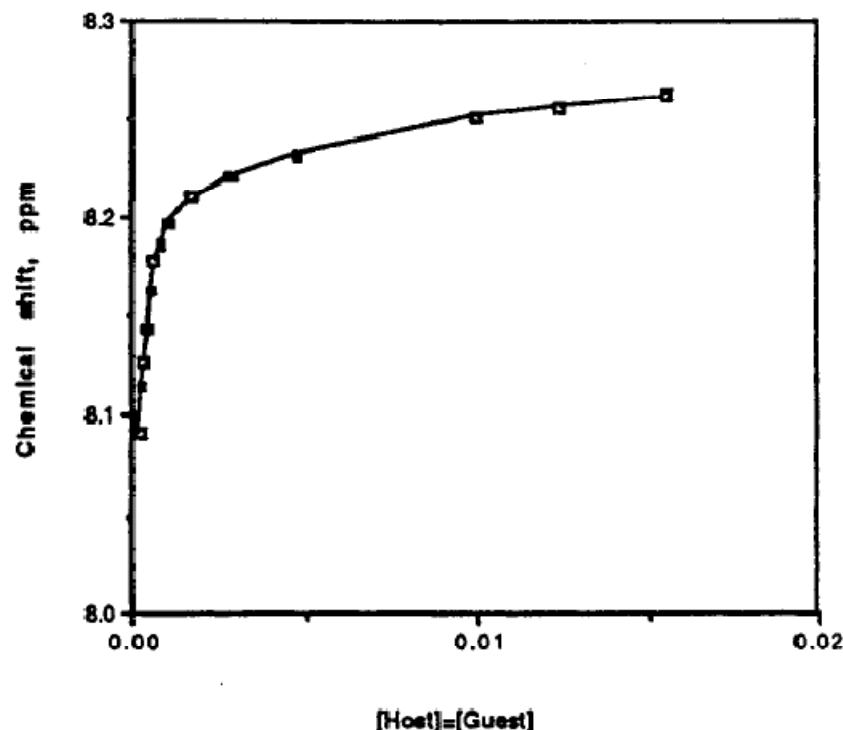
- ▶ Calculation of averaged K



Dilution Method

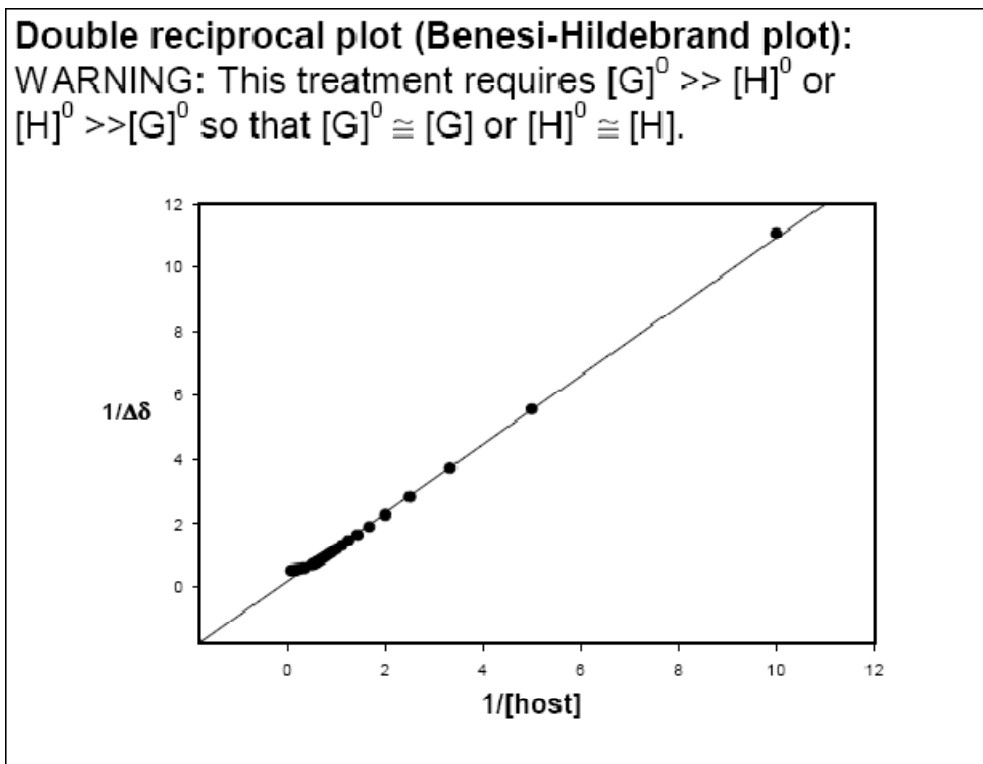
- ▶ Solution of 1:1 complex
- ▶ Measuring of δ_{obs} of $[H^*G]$ and δ_{max}
- ▶ Calculation of averaged K

$$K = \frac{\alpha}{(1-\alpha)^2 \cdot ([HG])}$$
$$\alpha = \frac{(\delta_{\text{obs}} - \delta_0)}{(\delta_{\text{max}} - \delta_0)}$$

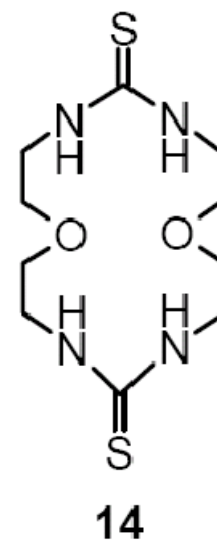
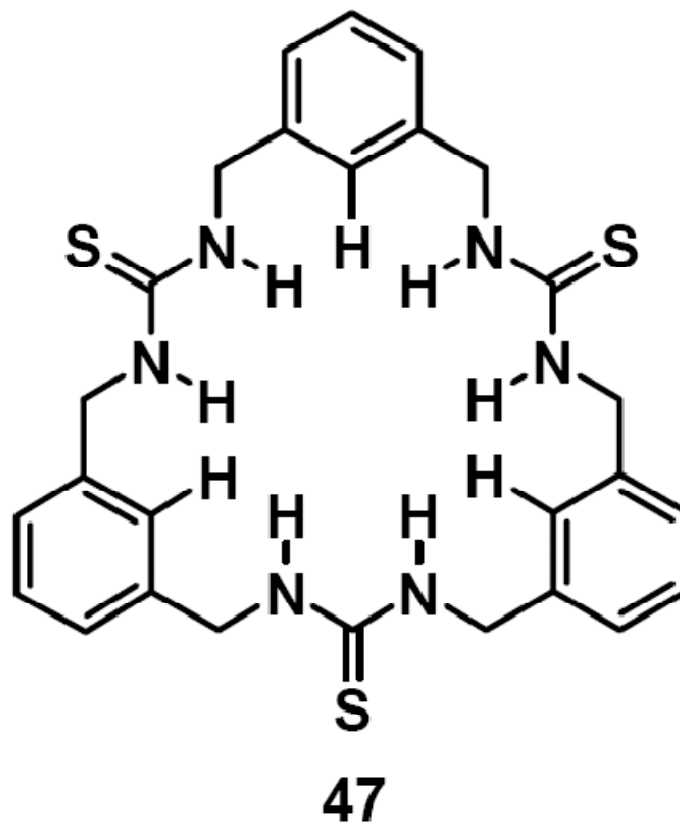
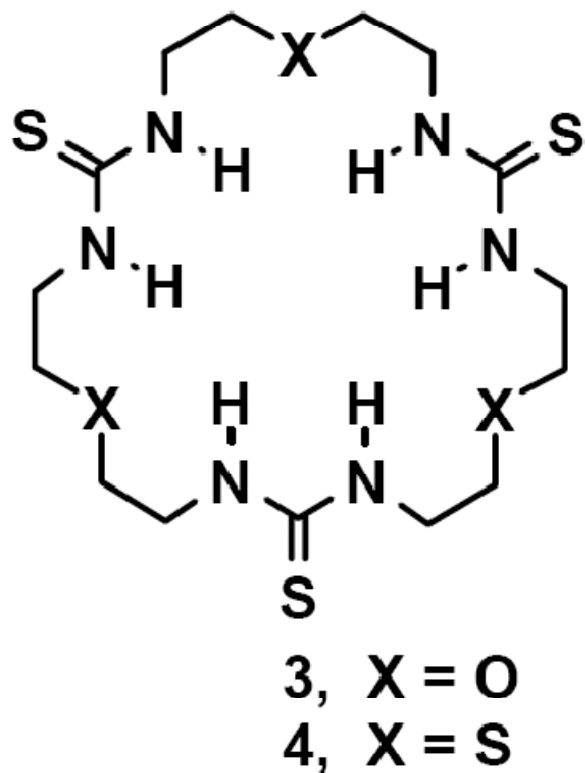


Benesi Hildebrand

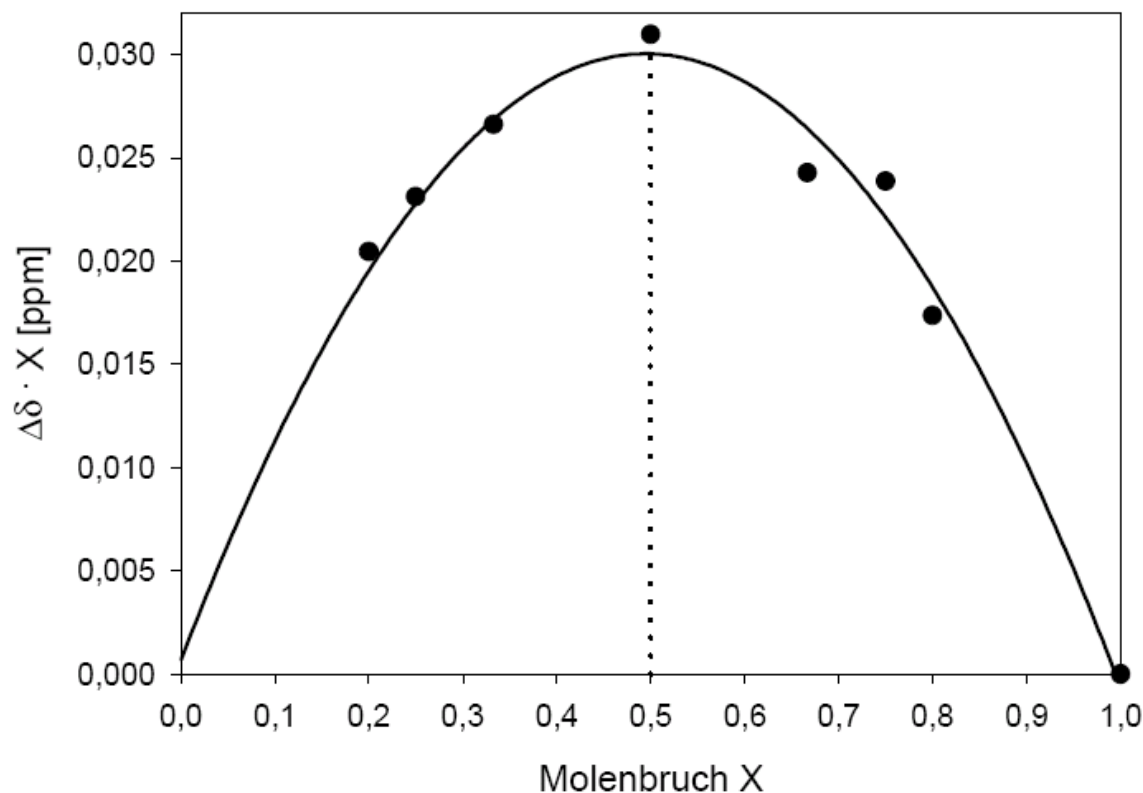
- Unuseful for small association constants



Example: Macrocyclic thioureas

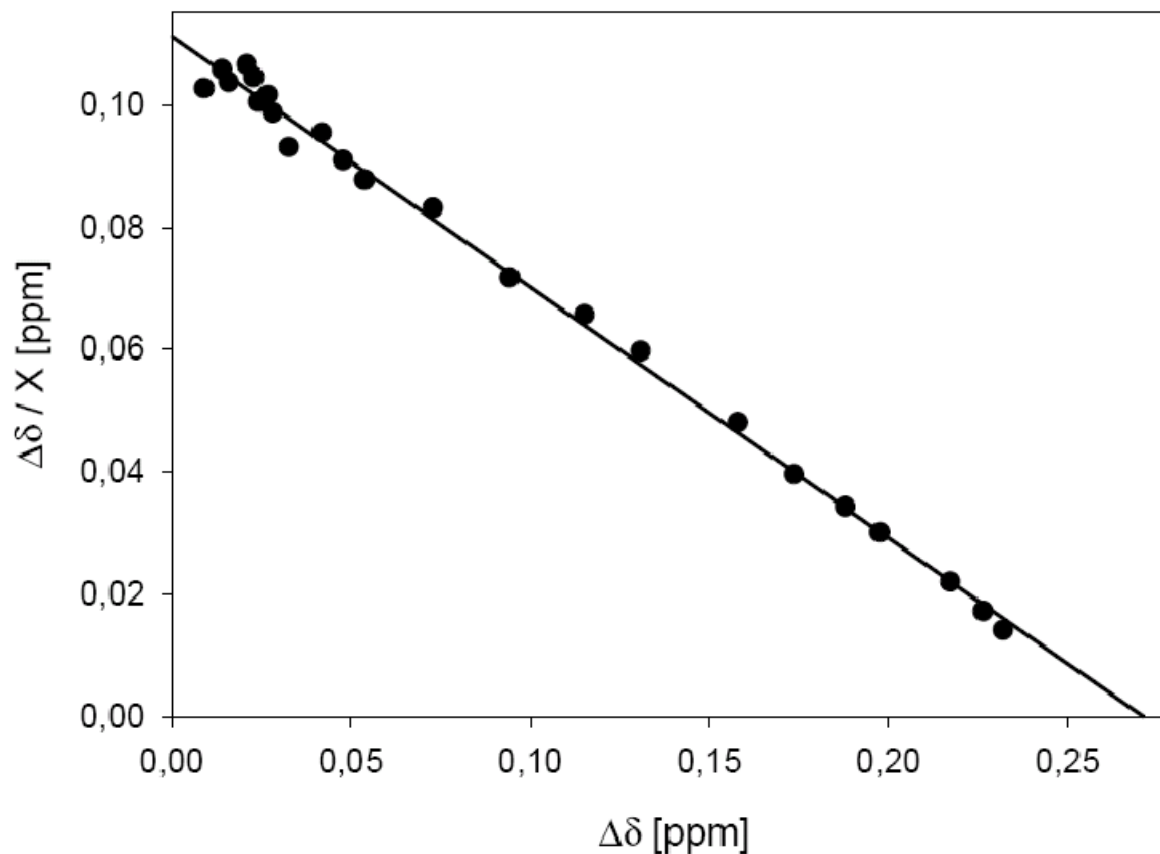


Determination of stoichiometry: Job's Plot



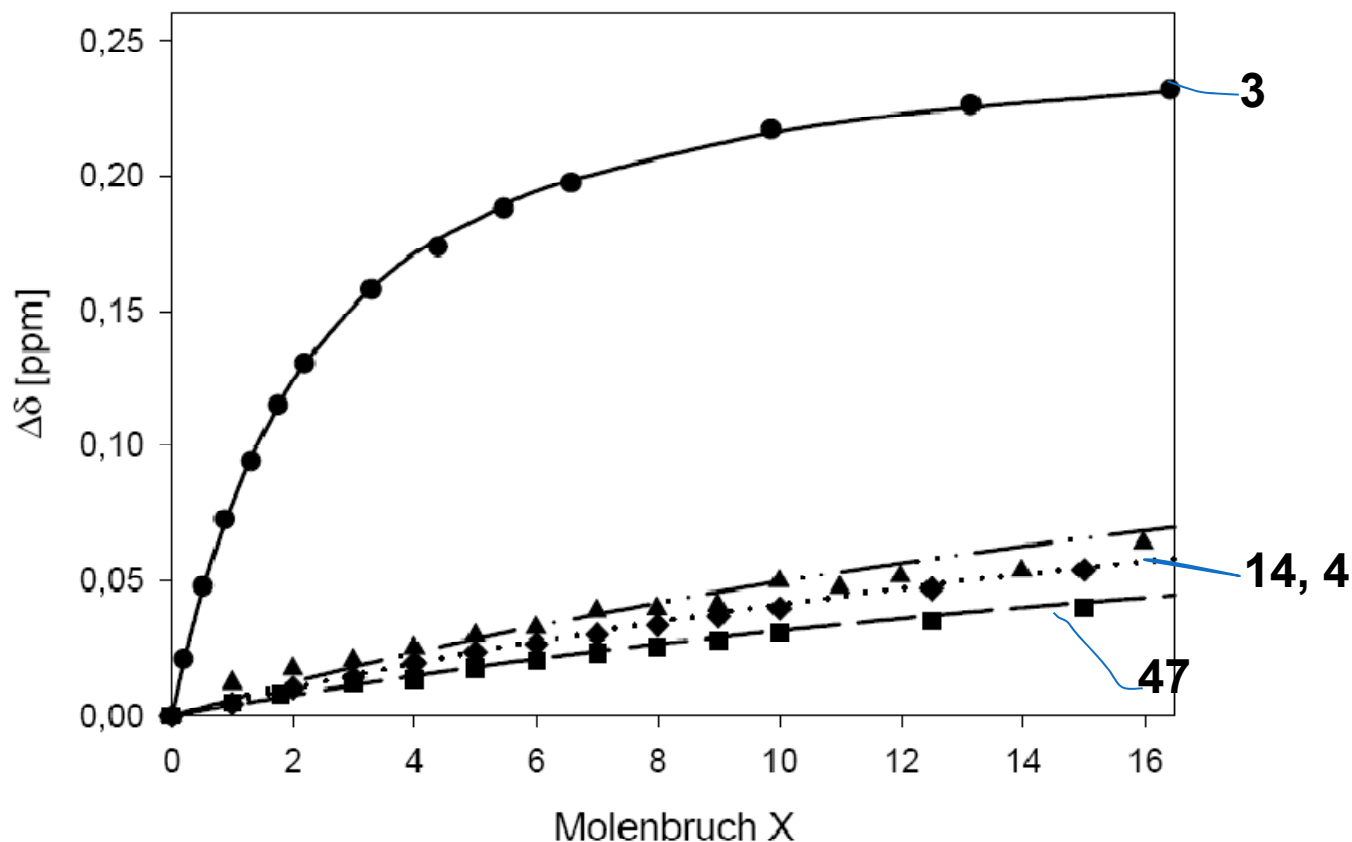
17: *Job*-Auftragung für das **3** : NBu_4NO_3 Assoziat in D_6 -DMSO

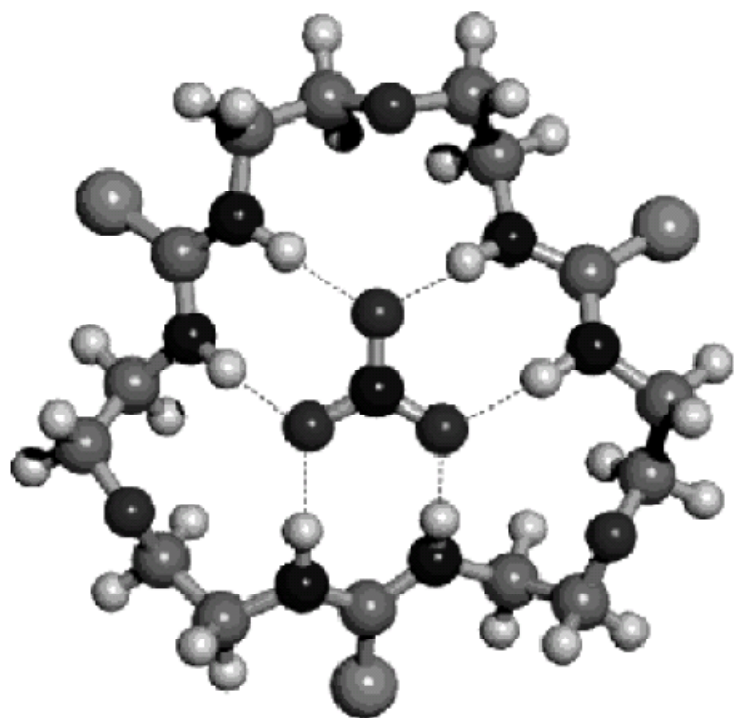
Scatchard method



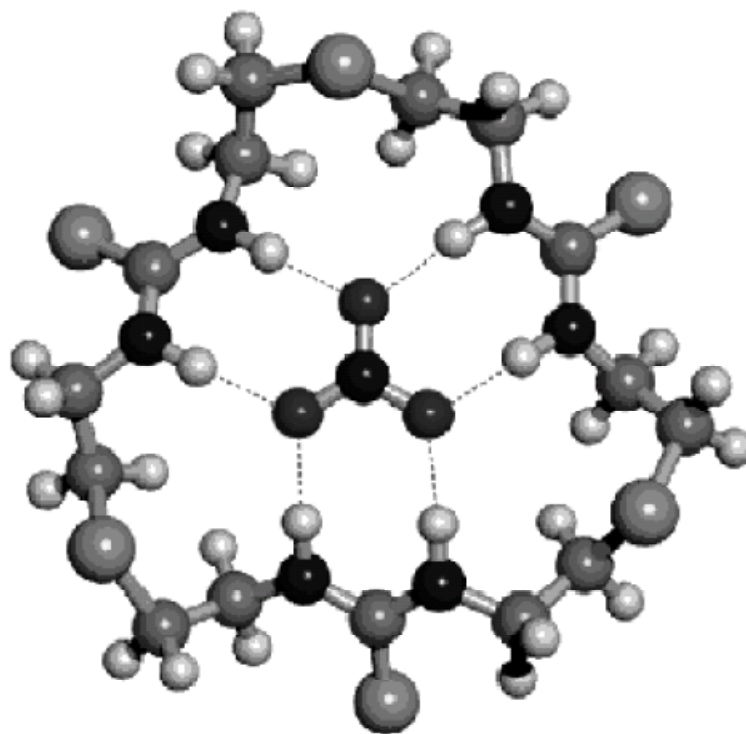
ng 18: *Scatchard*-Auftragung für das **3** : NBu₄NO₃ Assoziat in D₆-DMSO

Determination association constant





3 (X = O)

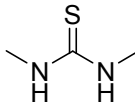
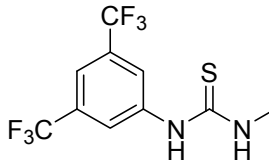
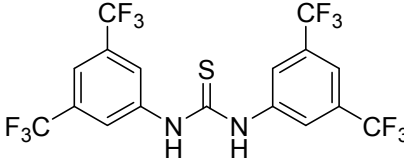


4 (X = S)

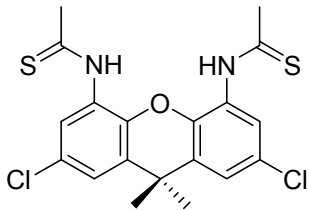
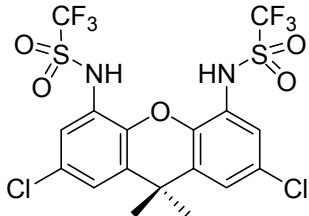
Abbildung 8: B3LYP/6-31G* optimierte Struktur der Ionophore **3** und **4** mit assoziiertem Nitration

Muñiz et al.

Host = Triphenylarsene oxide, measured in CDCl_3 and at rt

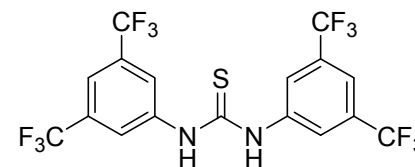
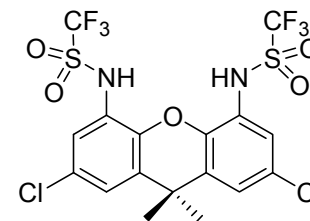
Entry	Receptor	K [M^{-1}]
1		16
2		$5.3 * 10^2$
3		$1.0 * 10^5$

Muñiz et al.

Entry	Receptor	K [M ⁻¹]
1	 <chem>CC(=S)Nc1cc(Cl)cc2c1O[C@H](C2)c3cc(Cl)cc(NC(=S)C)c3</chem>	$6.0 * 10^3$
2	 <chem>OS(=O)(=O)Nc1cc(Cl)cc2c1O[C@H](C2)c3cc(Cl)cc(NS(=O)(=O)C(F)(F)F)c3</chem>	$4.5 * 10^5$

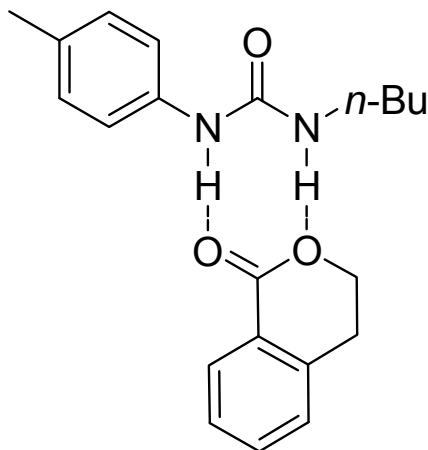
Muñiz et al.

Entry	Guest	$K_{\text{rel}} = [K_{\text{xanthene}}/K_{\text{T1}}]$
1	$\text{Ph}_3\text{As}=\text{O}$	4.3
2	$\text{Ph}_3\text{P}=\text{O}$	0.43
3	$\text{Oc}_3\text{P}=\text{O}$	6
4	tetramethylsulfoxide	7.1
5	cyclohexanone	1.9



Ureas in different solvents

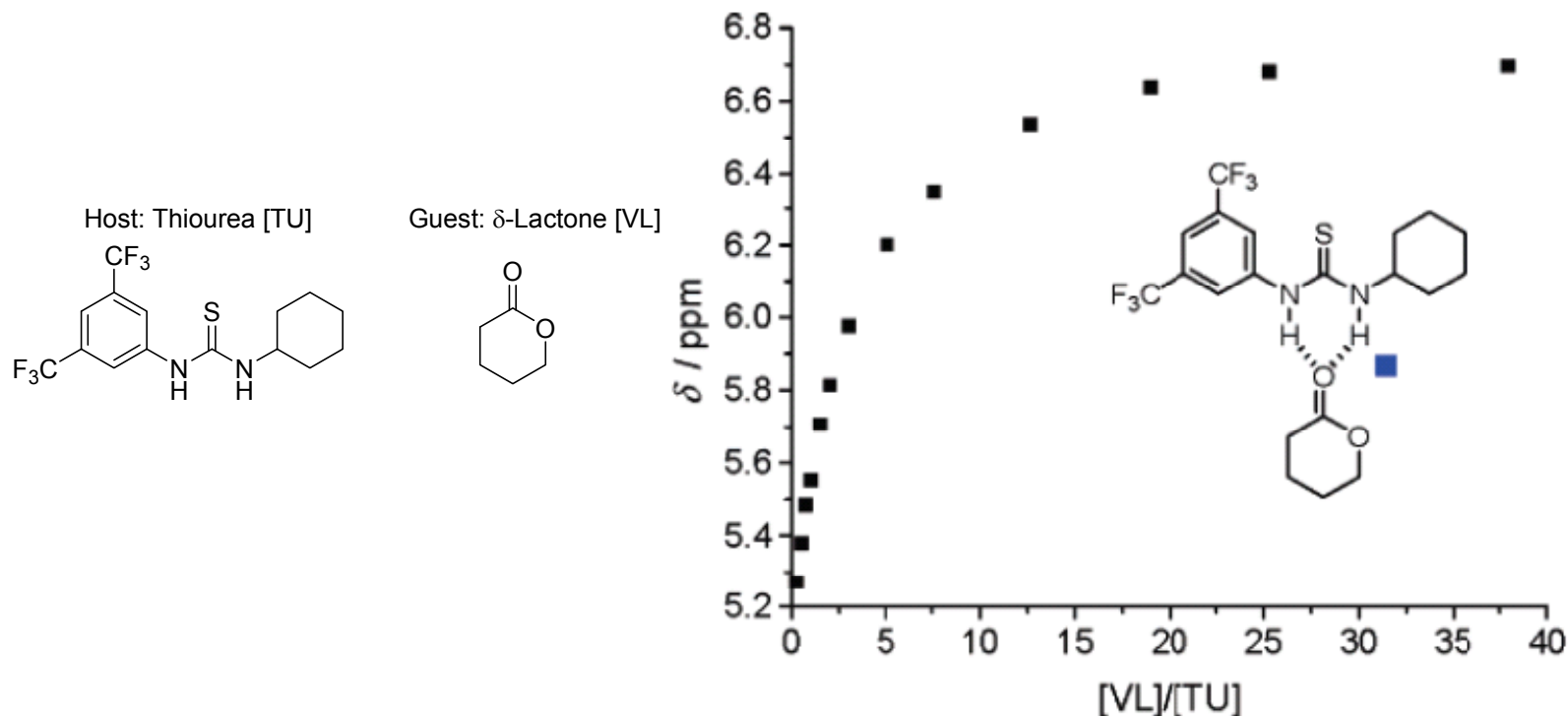
- ▶ Further example for uncharged substrates



- ▶ CDCl_3 : $K_{\text{ass}} = 13 (\pm 1) \text{ M}^{-1}$
- ▶ DMSO : $K_{\text{ass}} < 1 \text{ M}^{-1}$
- ▶ CCl_4 : $K_{\text{ass}} = 330 (\pm 120) \text{ M}^{-1}$

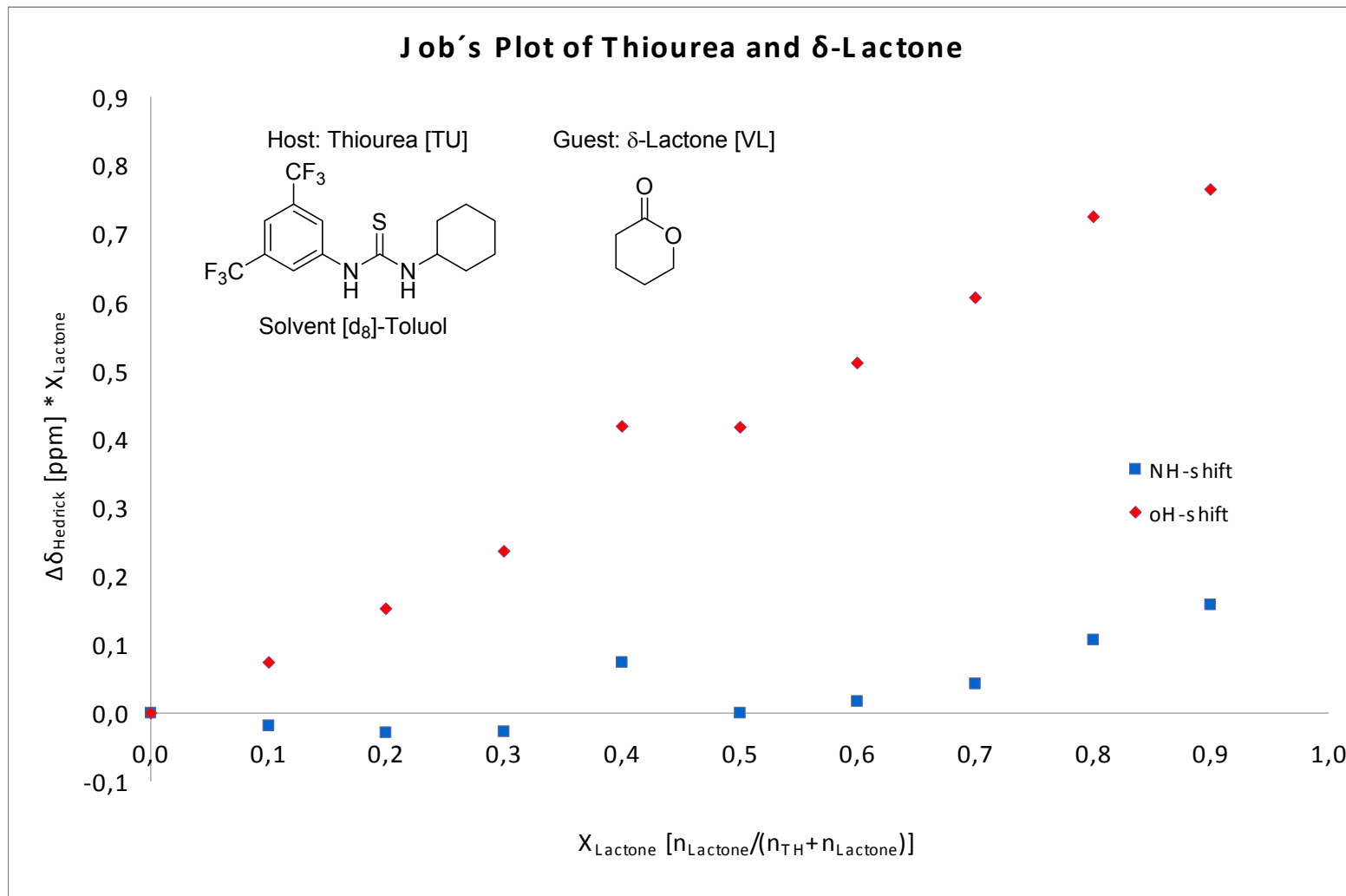
Reproducing literature

- ▶ Hedrick et al. found $K = 35 \pm 5$ L/mol
- ▶ Measured in $[d_6]$ -benzene, we utilized $[d_8]$ -toluene



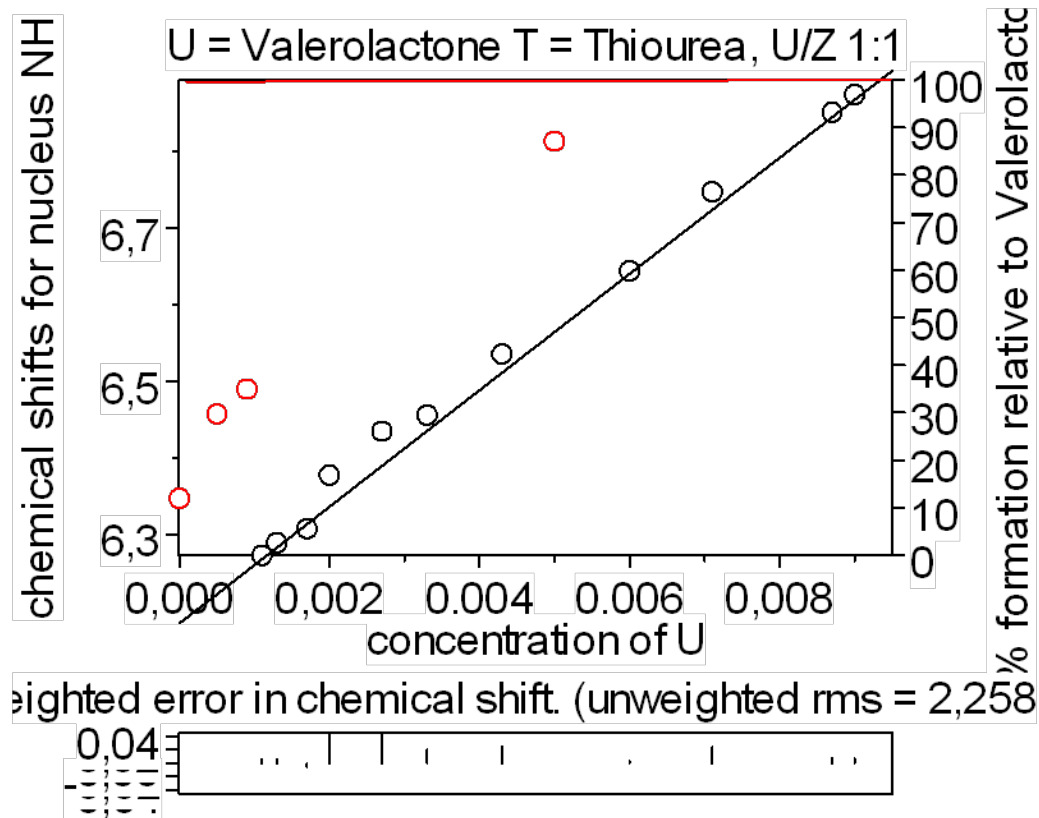
Job's Plot

$n_{\text{ges}} = 0,003 \text{ mmol}$



Our result with curve fitting method

- ▶ U/Z 1:1, 4 iterations, $K = 35.26 \pm 0.78$ L/mol



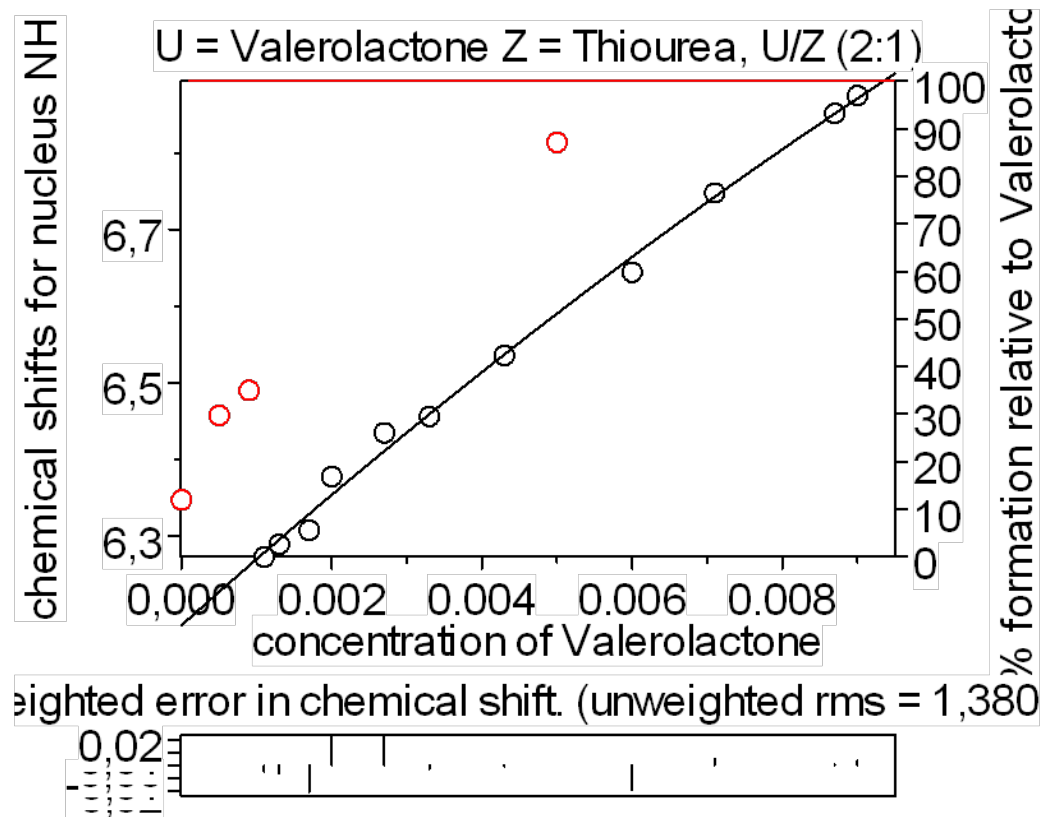
- ▶ U/Z 1:1, when 2:1 is constant, 4 iterations, $K = 23.35 \pm 21.83$

- ▶ $c_{TH} = 0.005$ mmol/mL, $c_{Lactone} = 0.01$ mmol/mL

Our result with curve fitting method

► U/Z 2:1, 5 iterations $K_{1:1} = 1.15 \cdot 10^{-2} \pm 1.94 \text{ L/mol}$
 $K_{2:1} = 746.45 \pm 37.18 \text{ L/mol}$

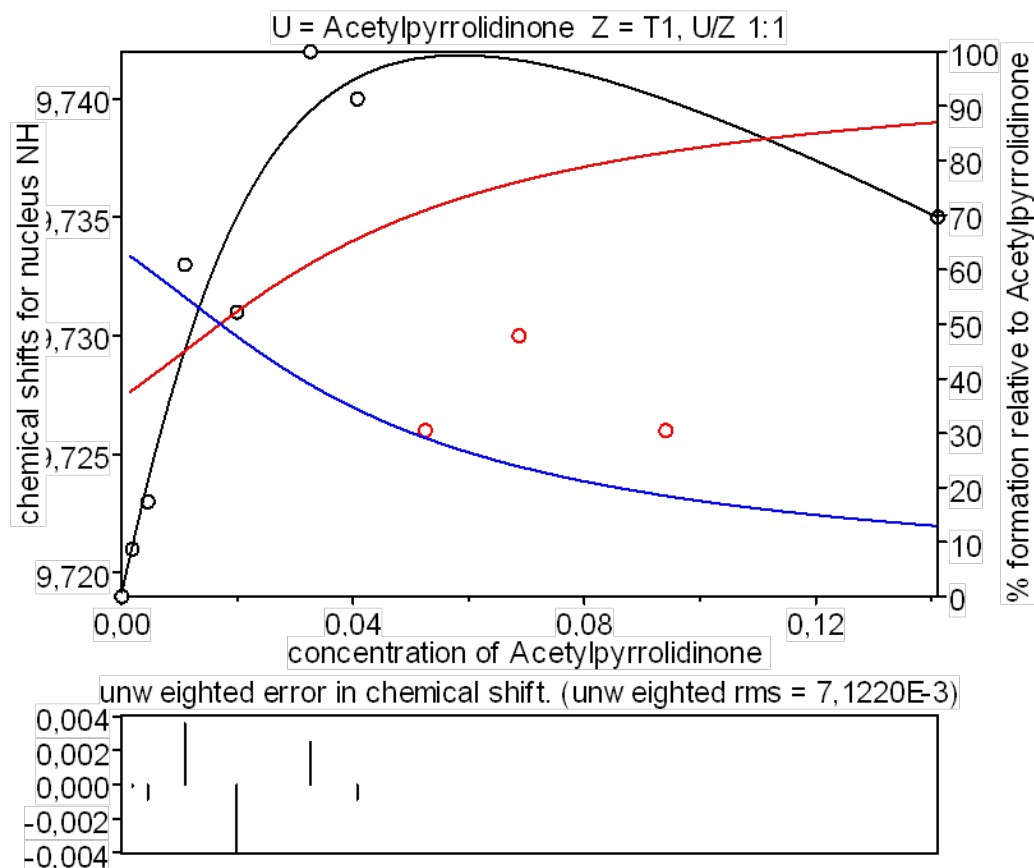
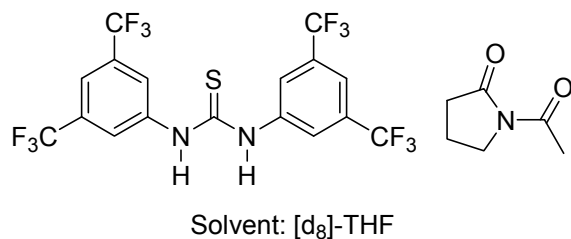
► $\text{Log}K = 4.81$



► $c_{\text{TH}} = 0.005 \text{ mmol/mL}$, $c_{\text{Lactone}} = 0.01 \text{ mmol/mL}$

T1 and acetylpyrrolidinone

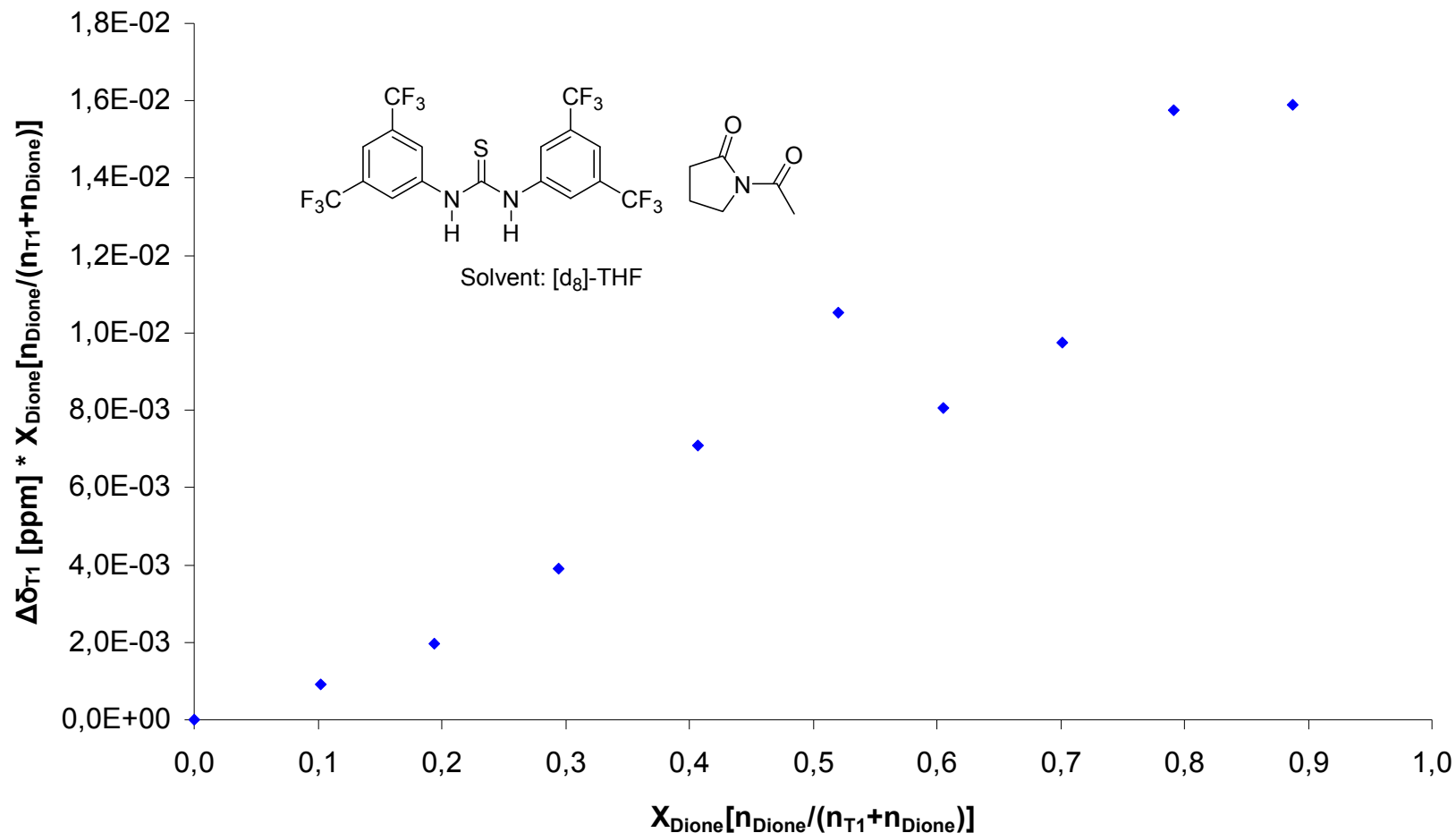
- ▶ Strong spreading of data points
- ▶ No exact calculation of association constant



▶ $c_{\text{TH}} = 0.005 \text{ mmol/mL}$, $c_{\text{Dione}} = 580 \text{ mmol/mL} - 1.8 \text{ mmol/mL}$

Checking stoichiometry

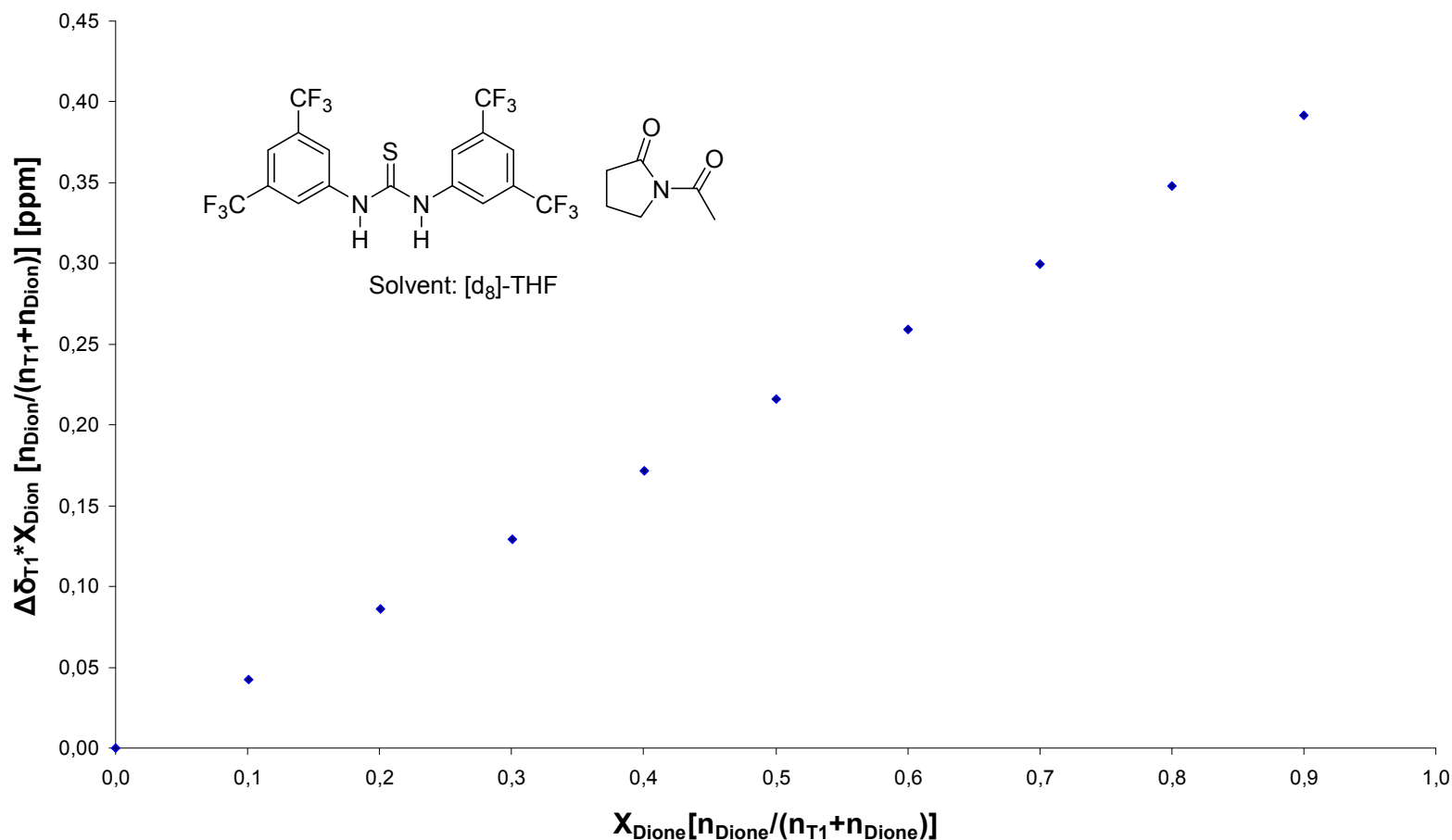
Job's Plot of T1 and acetylpyrrolidinone



► $n_{\text{ges}} = \sim 0.22 \text{ mmol}$

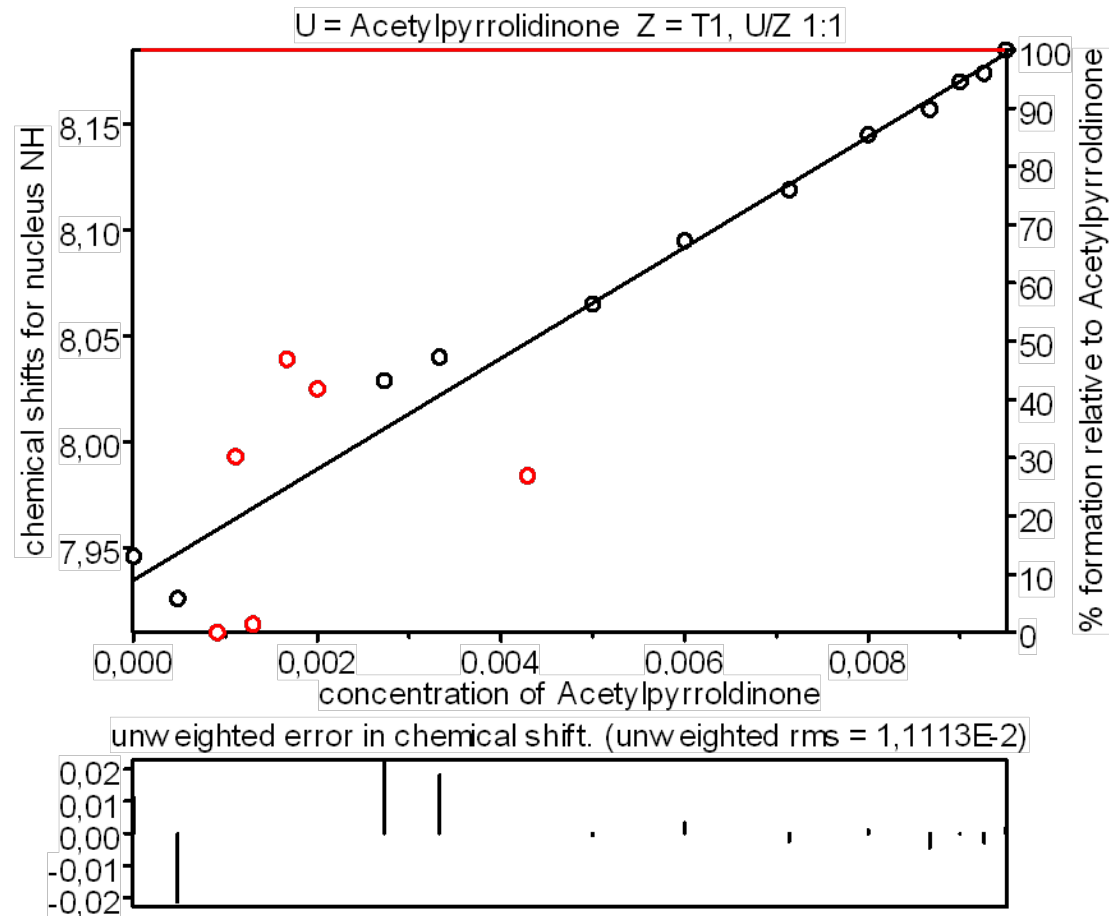
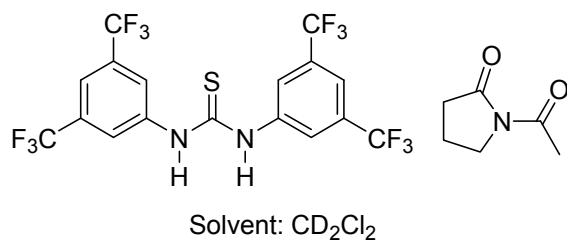
Repeat of checking stoichiometry

Job's Plot of T1 and acetylpyrrolidinone



► $n_{ges} = 0.2 \text{ mmol}$

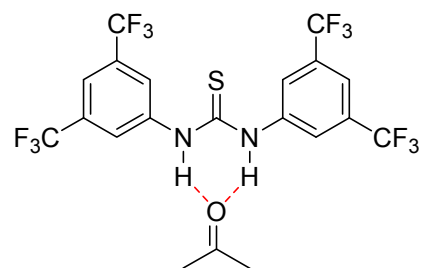
- U/Z 1:1, 3 iterations, $K = 39.09 \pm 1.02 \text{ L/mol}$



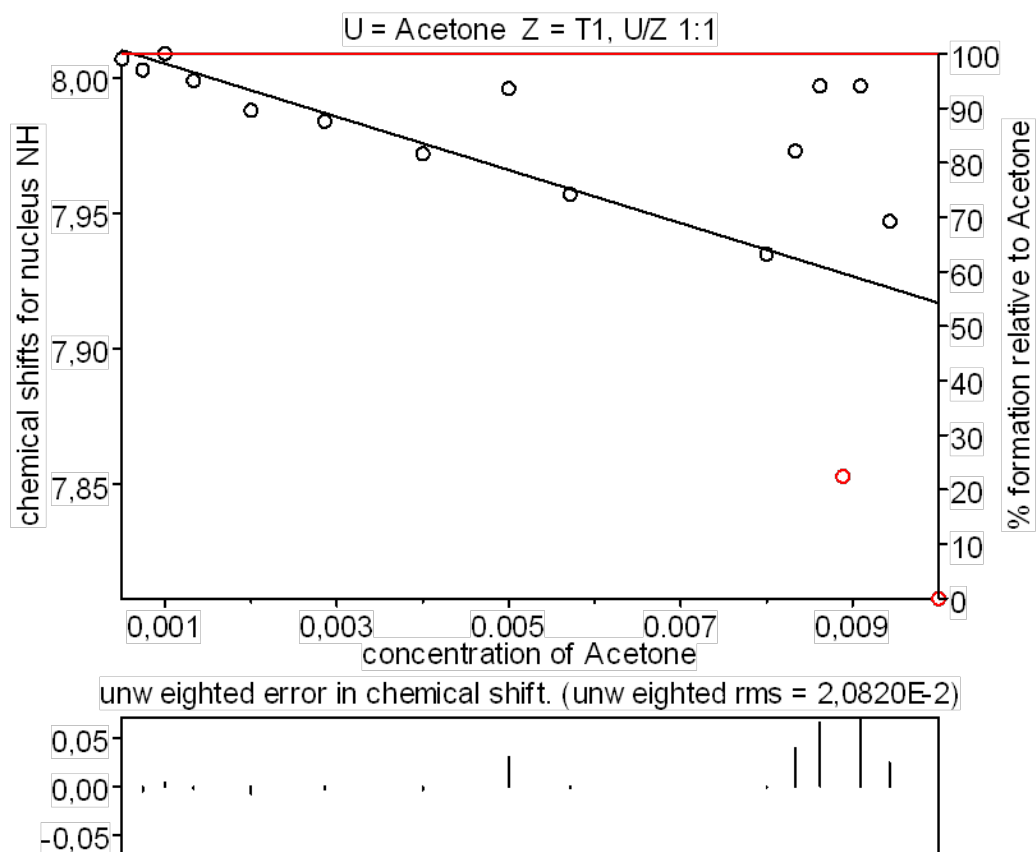
- $c_{\text{T1}} = 0.01 \text{ mmol/mL}$, $c_{\text{Dione}} = \text{until } 0.01 \text{ mmol/mL}$

T1 and acetone

- U/Z 1:1, 5 iterations, $K = 613.49 \pm 2.178 \text{ L/mol}$



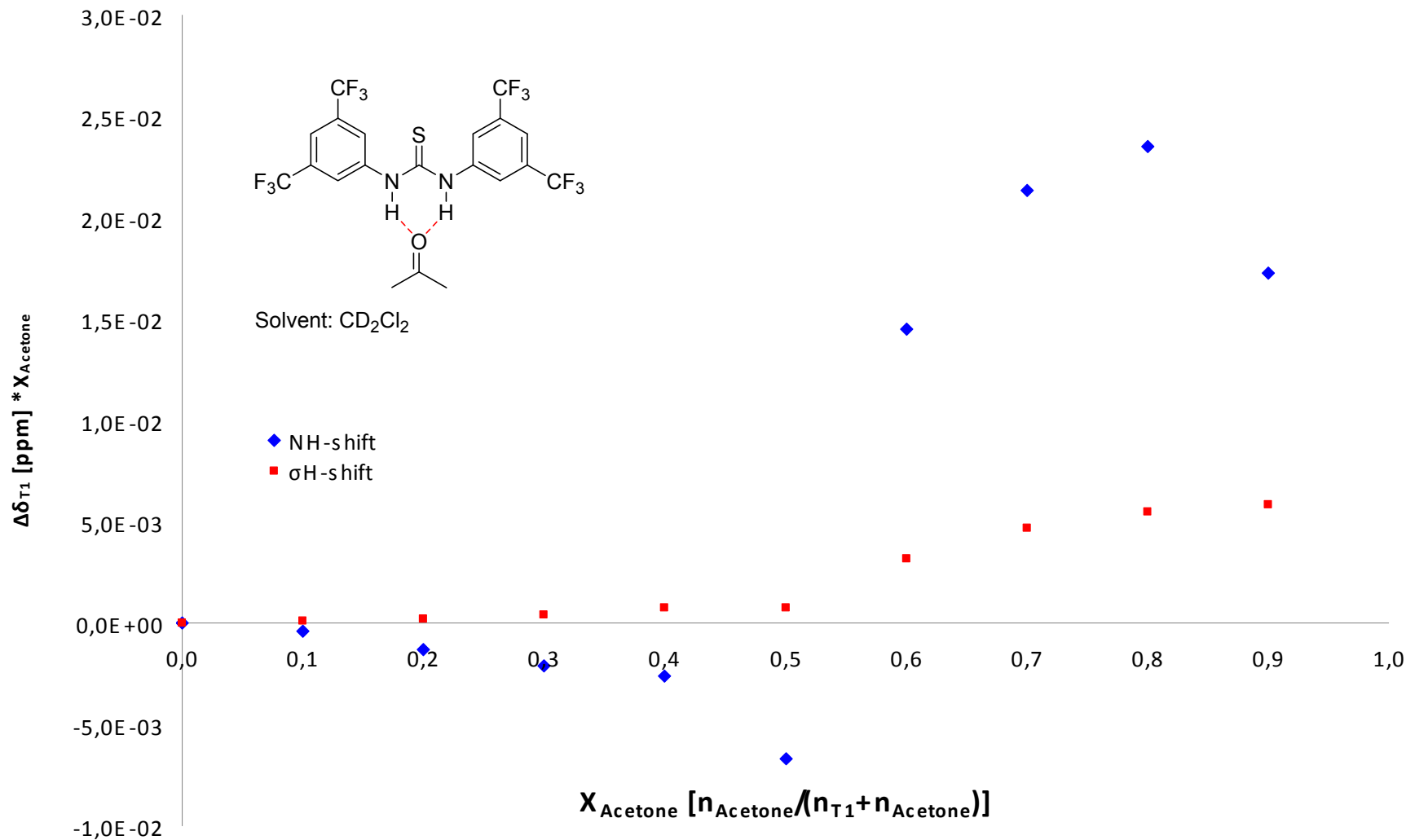
Solvent: CD_2Cl_2



- $c_{\text{T1}} = 0.005 \text{ mmol/mL}$, $c_{\text{Dione}} = 0.01 \text{ mmol/mL}$

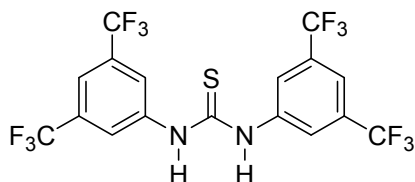
Job's Plot of T1 and acetone

$n_{\text{ges}} = 0.003 \text{ mmol}$

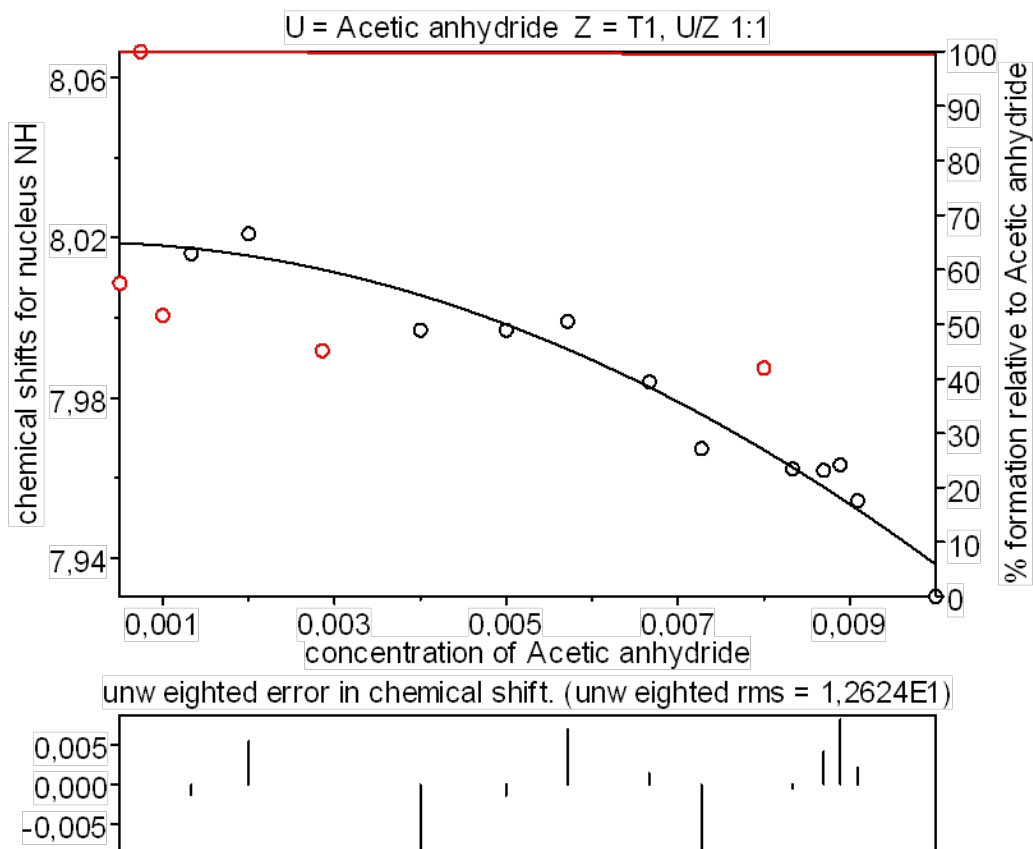
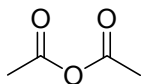


T1 and acetic anhydride

- ▶ U/Z 1:1, 5 iterations, $K = -26.9 \pm 1.36 \text{ L/mol}$
- ▶ U/Z 2:1, 4 iterations, $K = 2.88 \cdot 10^{17} \pm 2.81 \text{ L/mol}$



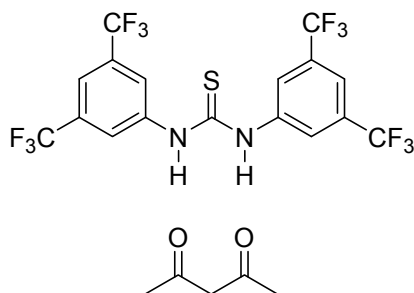
Solvent: CD_2Cl_2



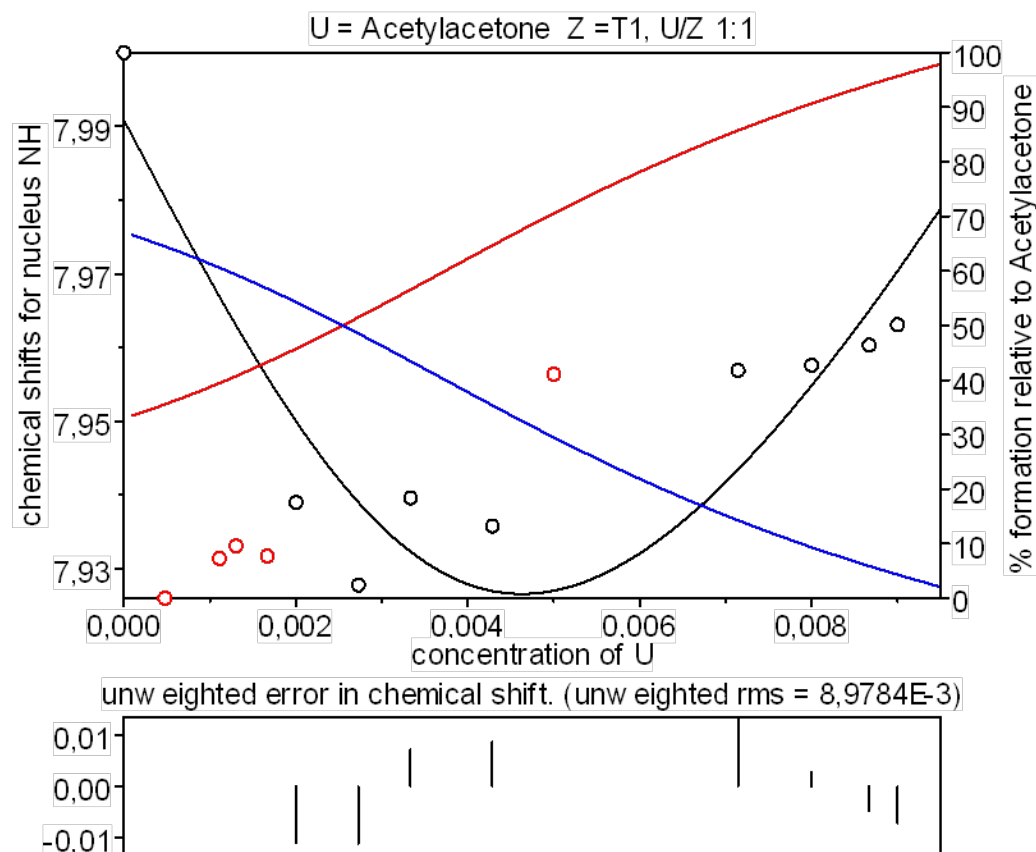
- ▶ $c_{\text{T1}} = 0.005 \text{ mmol/mL}$, $c_{\text{Acetic anhydride}} = 0.01 \text{ mmol/mL}$

T1 and acetylacetone

- ▶ U/Z 1:1, 3 iterations, $K = 207.1 \pm 3.63 \text{ L/mol}$
- ▶ U/Z 2:1, 7 iterations, $K = 1.39 \cdot 10^7 \pm 6.70 \text{ L/mol}$
- ▶ $\text{Log}K = 4.53$



Solvent: CD_2Cl_2

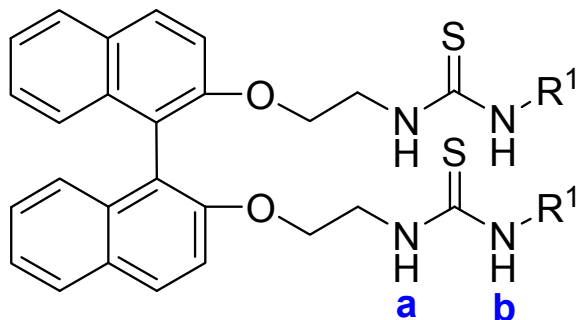


- ▶ $c_{\text{T1}} = 0.005 \text{ mmol/mL}$, $c_{\text{acetylacetone}} = 0.01 \text{ mmol/mL}$

Self-association tendency of T1?

1a: R¹ = pNO₂Ph

1b: R¹ = Ph



2a: R¹ = pNO₂Ph

2b: R¹ = Ph

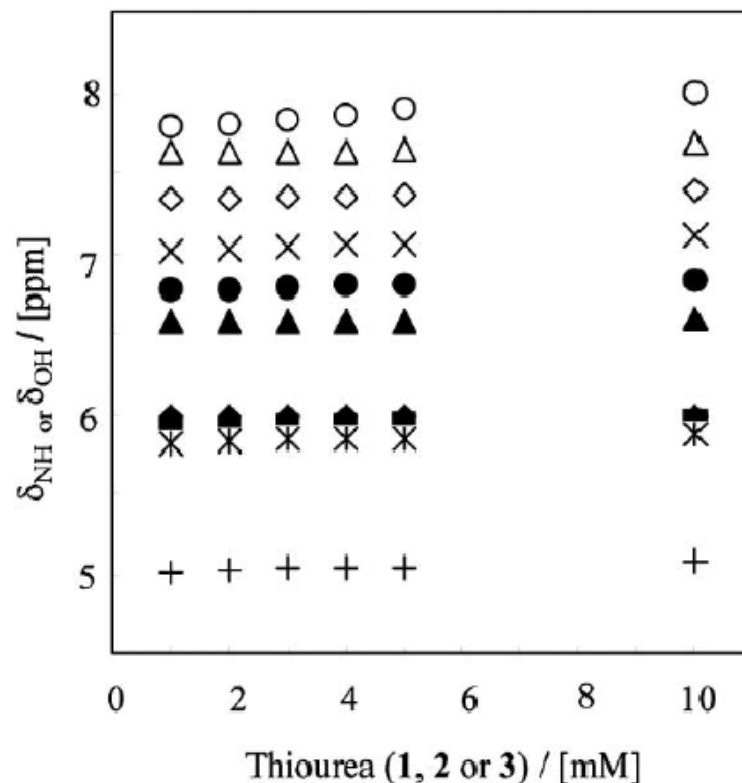
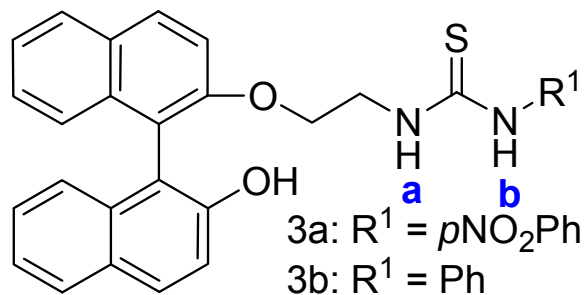
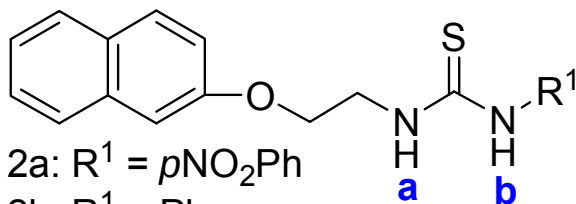
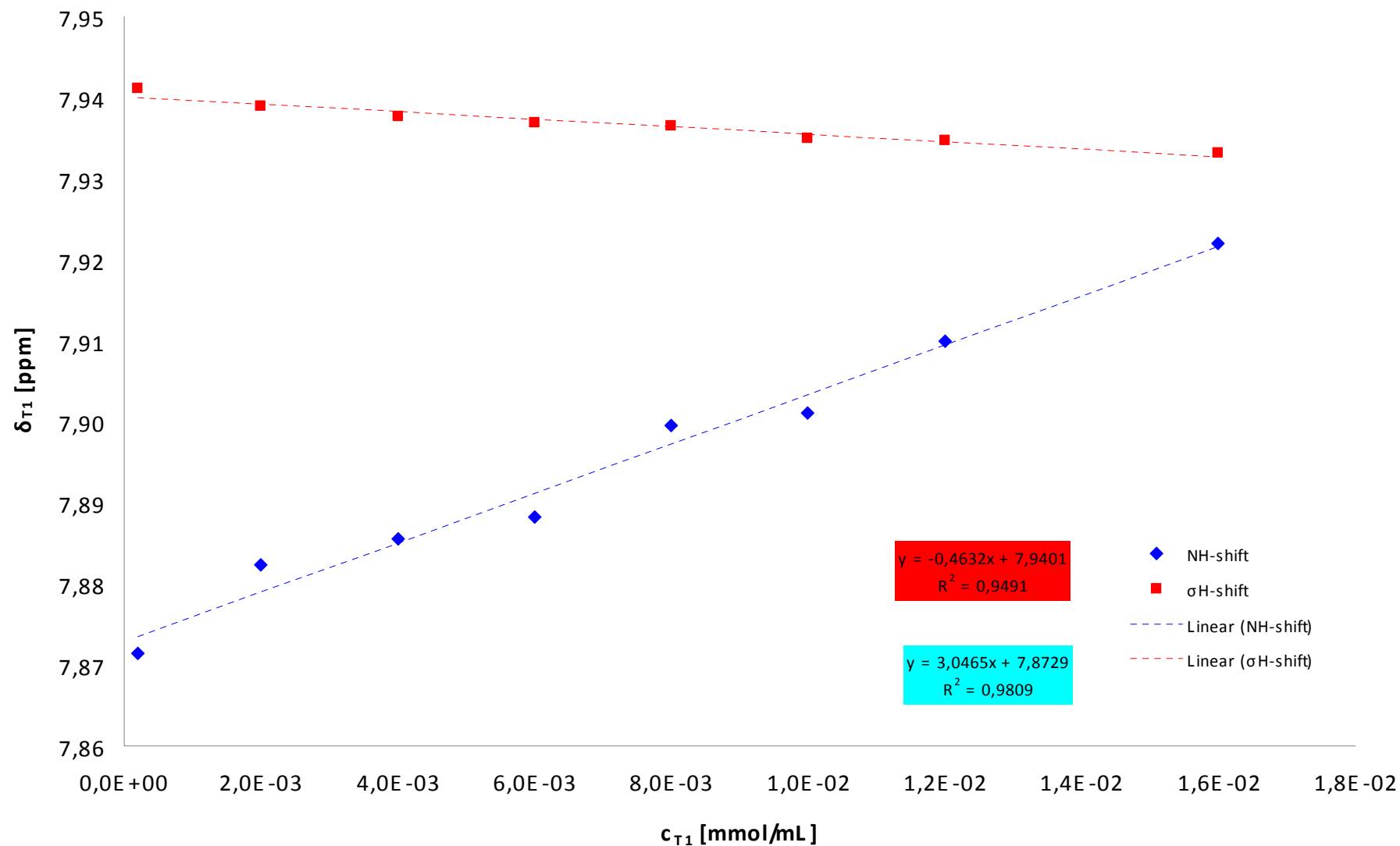


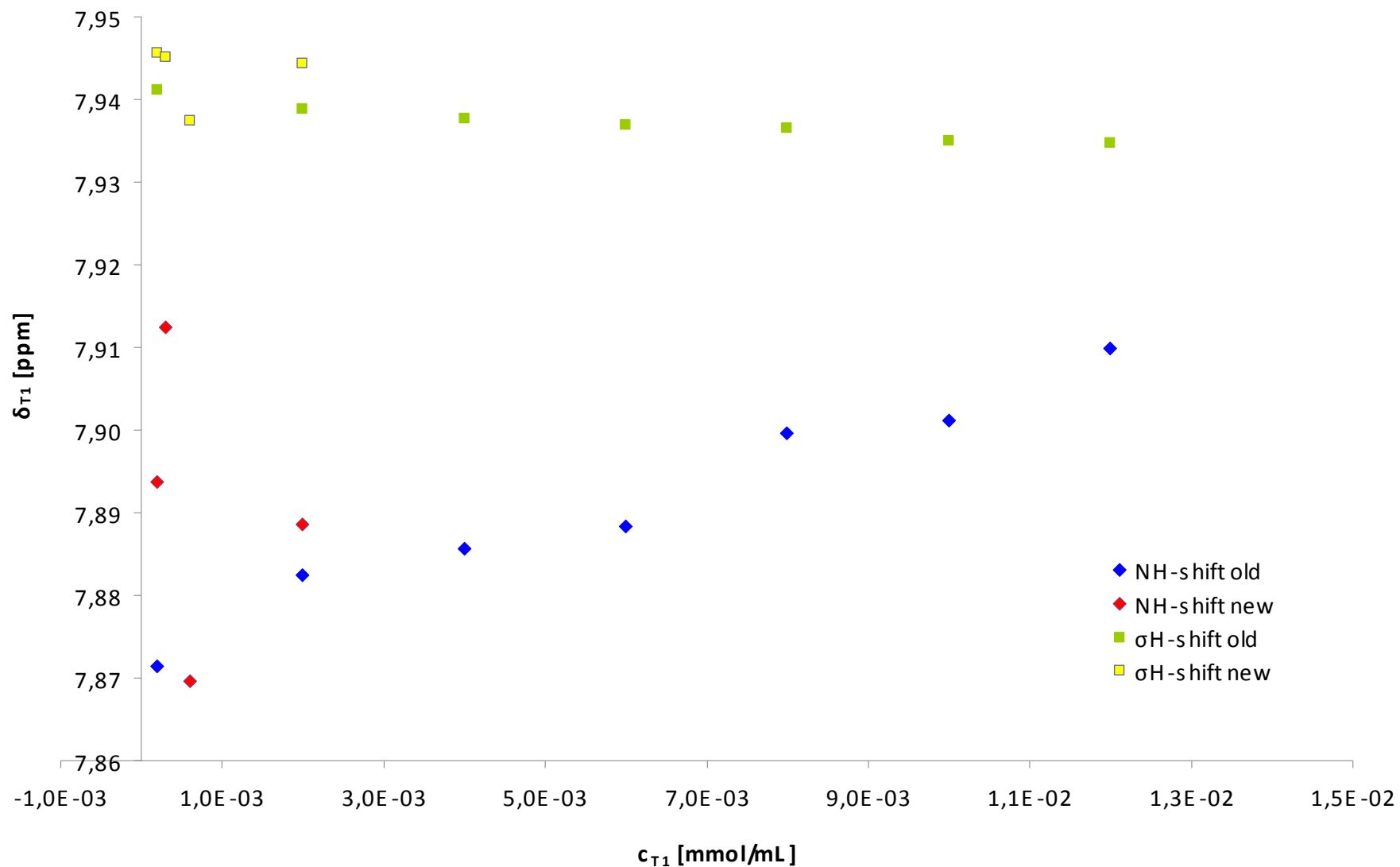
Fig. (2). ¹H NMR Chemical Shifts (δ values) of NH Protons of **1** as a Function of Concentration (1 mM to 10 mM) in CDCl₃. (1a: NH_a(■), NH_b(□), 1b: NH_a(◆), NH_b(◇), 2a: NH_a(●), NH_b(○), 2b: NH_a(▲), NH_b(△), 3a: NH_a(×), NH_b(*), OH(+))

Self-association of T1



► $c_{T1} = 18 \text{ mmol/mL} - 0.2 \text{ mmol/mL}$

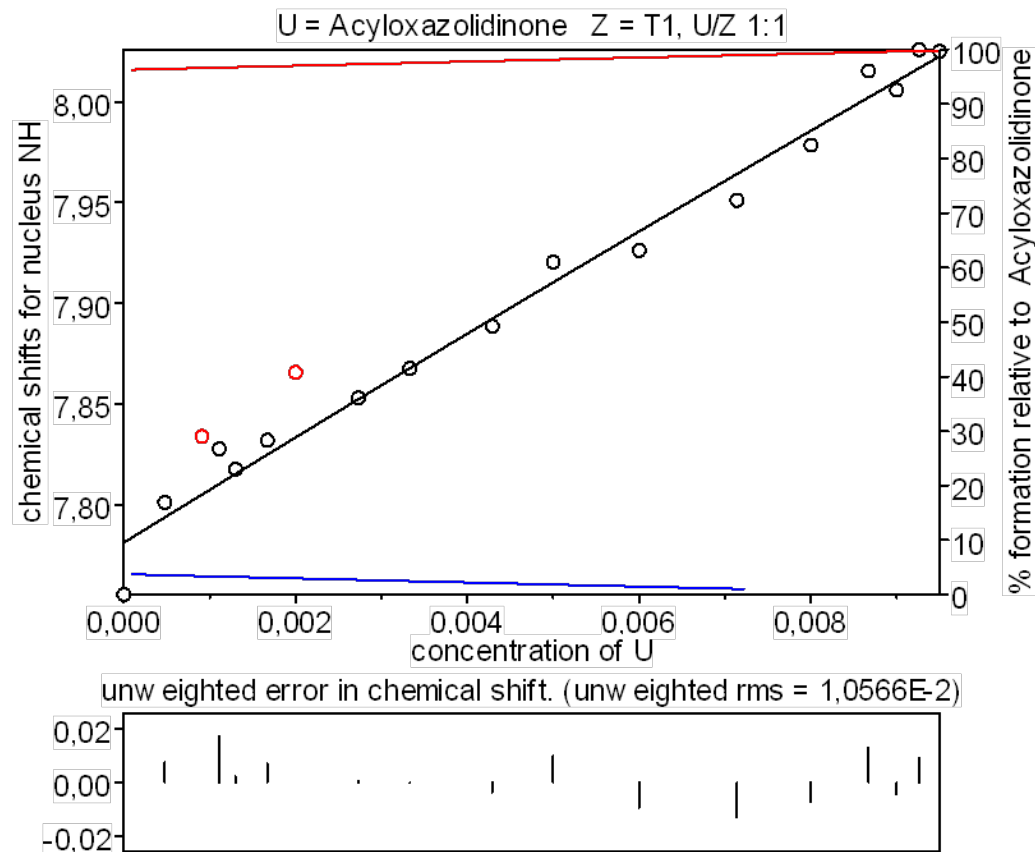
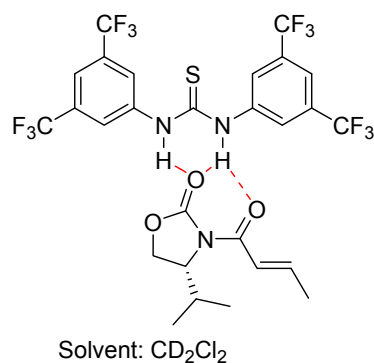
Self-association of T1



► $c_{T1} = 18 \text{ mmol/mL} - 1.8 \text{ }\mu\text{mol/mL}$

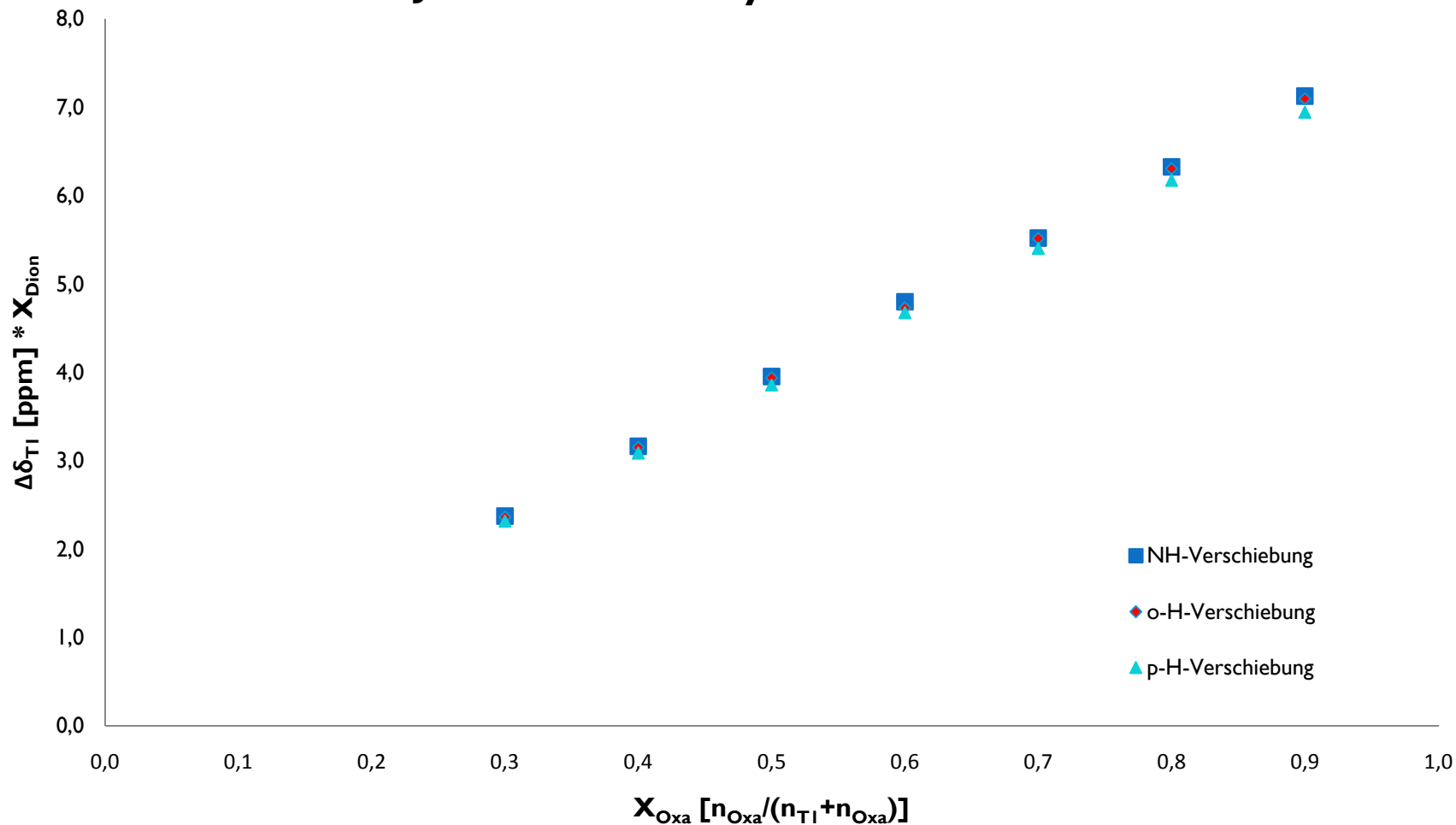
T1 and acyloxazolidinone

- U/Z 1:1, 2 iterations, $K = 5.98 \pm 5.17$ L/mol



- $c_{\text{T1}} = 0.005$ mmol/mL, $c_{\text{acyloxazolidinone}} = 0.01$ mmol/mL

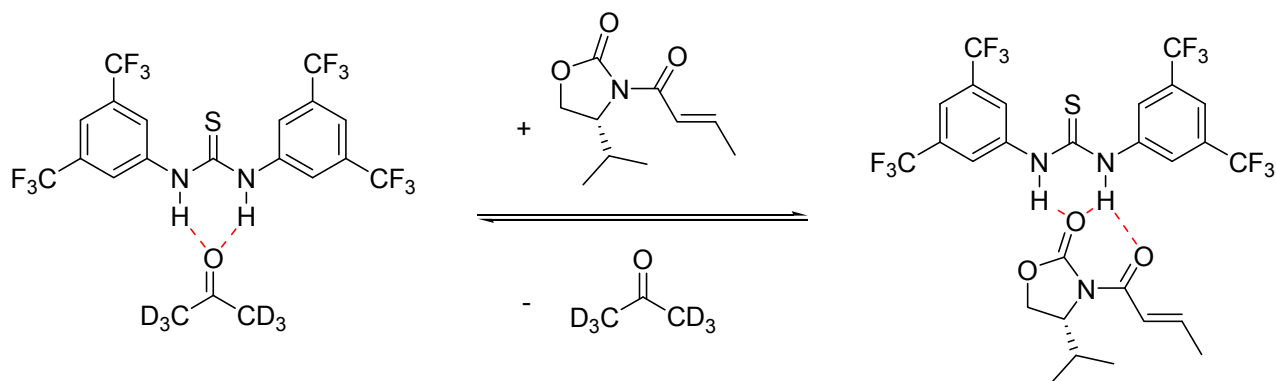
Job's Plot - TI/Acyloxazolidinone



► $n_{\text{ges}} = 0.003 \text{ mmol}$

Displacement experiment

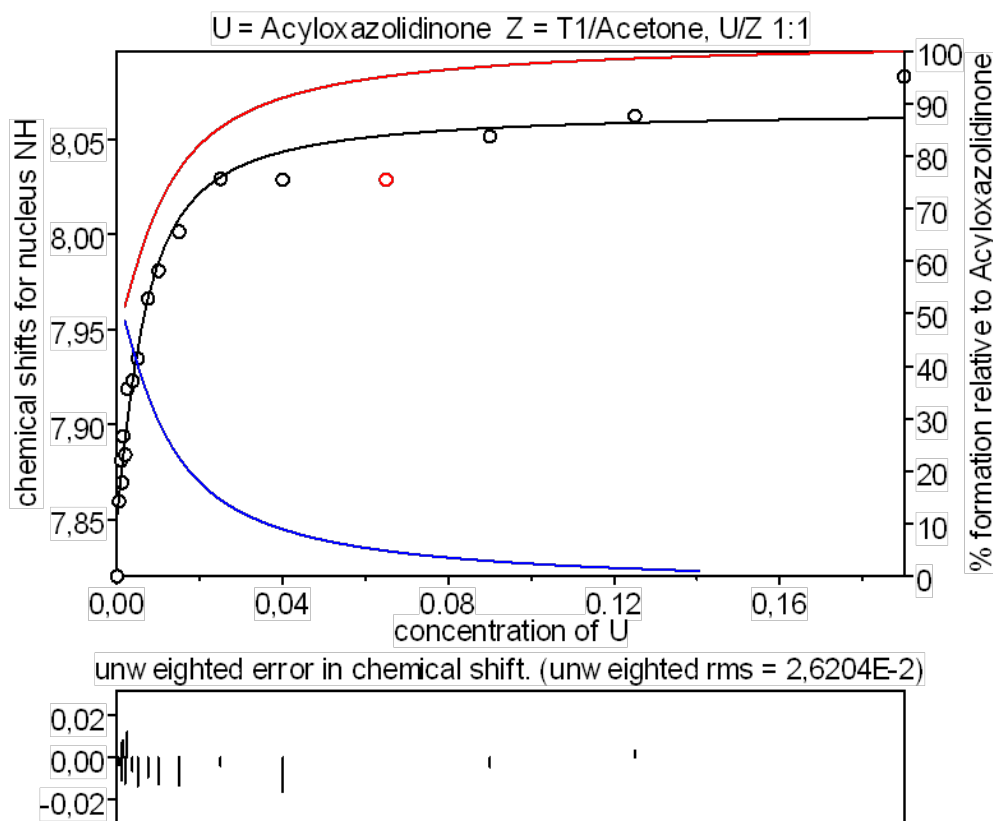
► Solvent: CD_2Cl_2



► $c_{\text{T1/acetone}} = 0.005 \text{ mmol/mL}$, $c_{\text{acyloxazolidinone}} = 0.01 \text{ mmol/mL}$

Displacement experiment

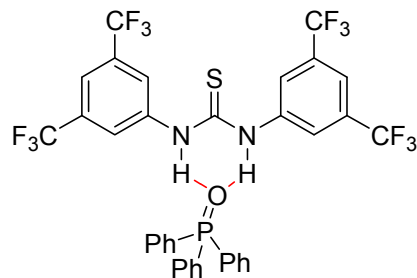
- ▶ U/Z 1:1, 3 iterations, $K = 196.72 \pm 0.42$ L/mol
- ▶ U/Z 2:1, 26 iterations, $K = -837.44 \pm 1.81$ L/mol



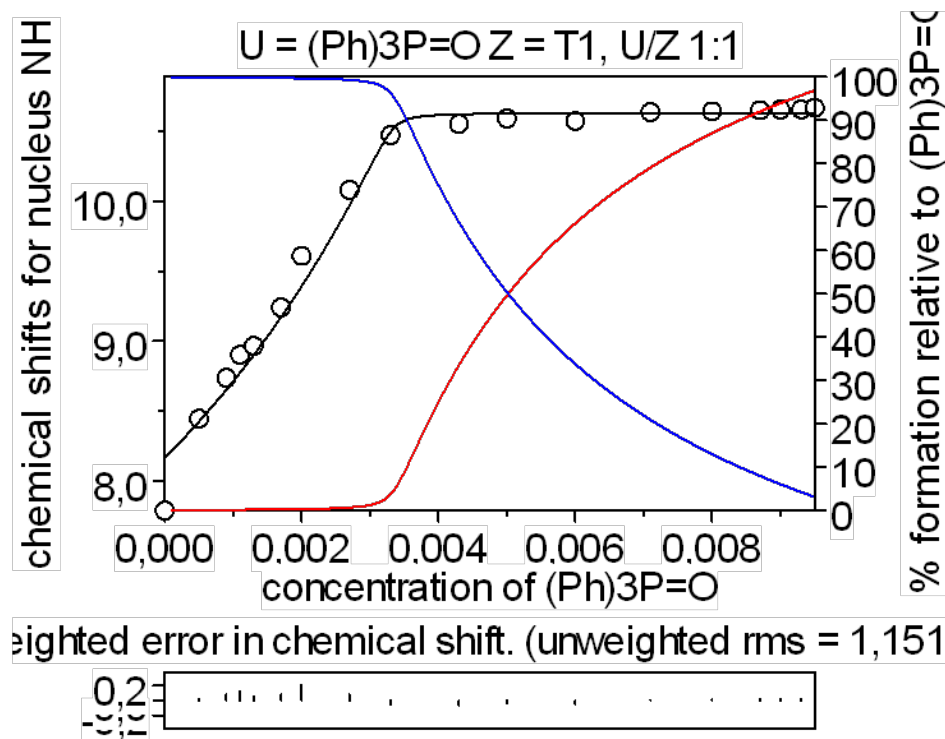
- ▶ One day later: U/Z 1:1, 5 iterations, $K = 72.93 \pm 0.31$ L/mol
- ▶ $c_{T1/acetone} = 0.005$ mmol/mL, $c_{acyloxazolidinone} = 0.01$ mmol/mL

Reproducing Muñiz' work

- ▶ U/Z 1:1, 9 iterations,
- ▶ $K = 18288 \pm 1.56$ L/mol



Solvent: CDCl_3



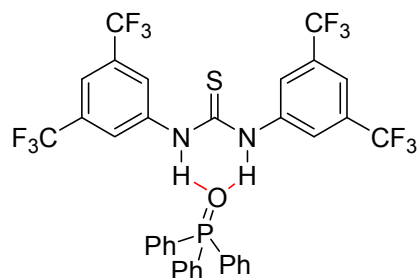
- ▶ One day later:
U/Z 1:1, 13 iterations,
 $K = 6882.4 \pm 1.24$ L/mol

$$c_{T1} = 0.005 \text{ mmol/mL},$$

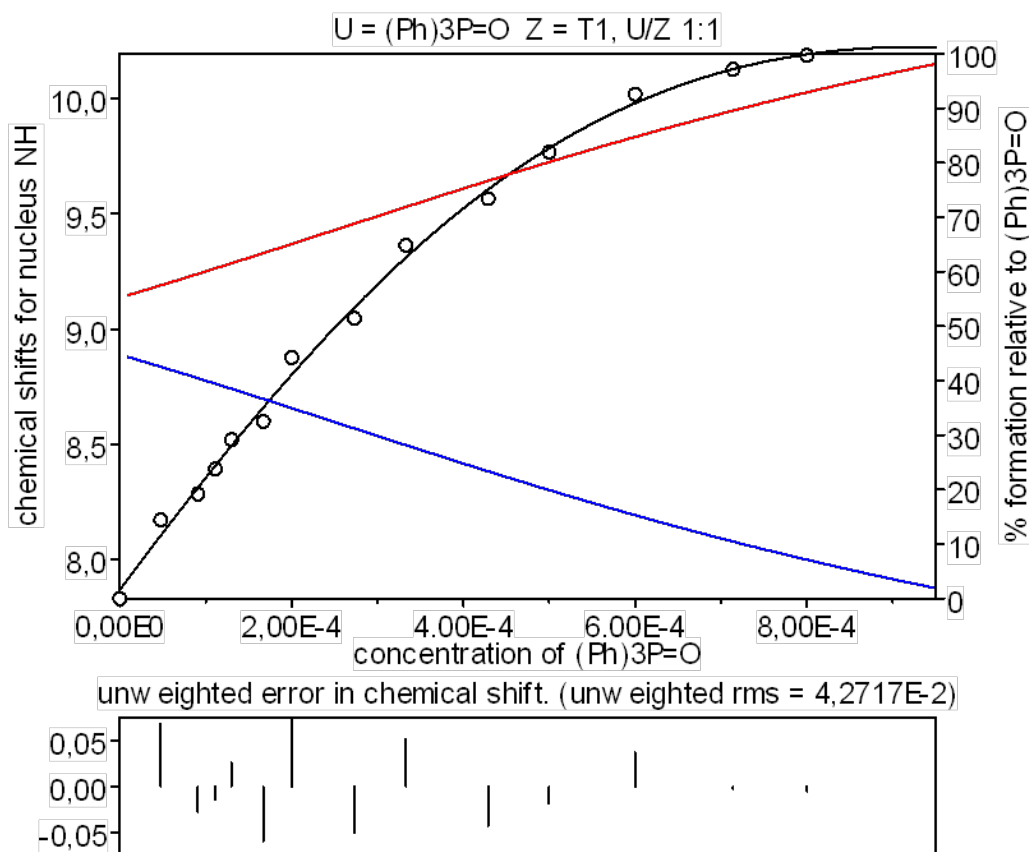
$$c_{\text{Phosphine oxide}} = 0.01 \text{ mmol/mL}$$

Reproducing Muñiz' work

- ▶ U/Z 1:1, 9 iterations, $K = 519.53 \pm 5.52$ L/mol



Solvent: CDCl_3



$c_{\text{T1}} = 0.0005$ mmol/mL,
 $c_{\text{Phosphine oxide}} = 0.001$ mmol/mL

Summary

- ▶ Difficult to reproduce literature
- ▶ Measurement of K via NMR is difficult to realize because of finding the right concentration area, solvent and substrate
- ▶ In most cases substrates build complexes with higher stoichiometry as 1:1



Summary

- ▶ Curve fitting method of HYPNMR could bear failure
 - ▶ Because the program needs models
 - ▶ Calculation for one complex stoichiometry differs, if different models are used during one calculation
- ▶ We can't give a degree of the quality of the calculated models



Outlook

- ▶ Inhibition of self-association:
 - ▶ Using lower concentrations?
 - ▶ Using dilution method
 - ▶ Using another solvent
(CDCl₃, [d₈]-Toluene)
- ▶ Using bis(thiourea)
- ▶ Using sterically hindered substrate



Acknowledgement

- ▶ We thank Prof. Schreiner for the opportunity to work in cooperation with the group of Dr. Bredenbeck
- ▶ We thank Dr. Hausmann for NMR measurements and data analysis
- ▶ We thank Dr. Reisenauer for IR measurements
- ▶ We also thank Volker Lutz and Jörg Glatthaar for discussions
- ▶ And we thank the colleagues of our working group

