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Reversible Covalent Reactions of Aldehydes and Salicylaldehydes Using a Lysine-Model Substrate

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Supplementary information

Contents

1. NMR reactivity assay

All amines, aldehydes, and components of buffer solutions were obtained from commercial suppliers and used without further purification. Nα-Acetyl-L-lysine methyl amide was obtained from Enamine. All reactions were performed at room temperature. Imine formation reactions were carried out in deuterated phosphate buffer at pH 7.4. To determine the correct concentration of each solution, quantitative NMR (qNMR) was performed using an external reference in the same buffer. Maleic acid, with a known concentration, was used as the reference, and the integration of this signal was used to determine the unknown concentration of the solution using the ERETIC module of Bruker Topspin (qNMR with TOPSPIN). The concentration of each stock solution (aldehyde and amine) was determined by qNMR before mixing them together.

All reactions were performed on a Bruker Avance III HD 600 MHz (14.1 T) and a Bruker Avance NEO 600 MHz (14.1 T) NMR spectrometer. Each tube was prepared with 1 equivalent of aldehyde and 2 equivalents of lysine in phosphate buffer (pH 7.4) in D_2O, using a 3 mm NMR tube. The tubes were

immediately placed into the magnet. Initially, a 1D qNMR spectrum was measured, followed by automatic and continuous measurement of 80 proton spectra over several hours.

In the following figure, an overlay of the aromatic region from the first time point of the reaction and original aldehyde is shown. The formation of the imine is indicated by a peak between 8 and 9 ppm. For the following example at approximately 8.7 ppm.

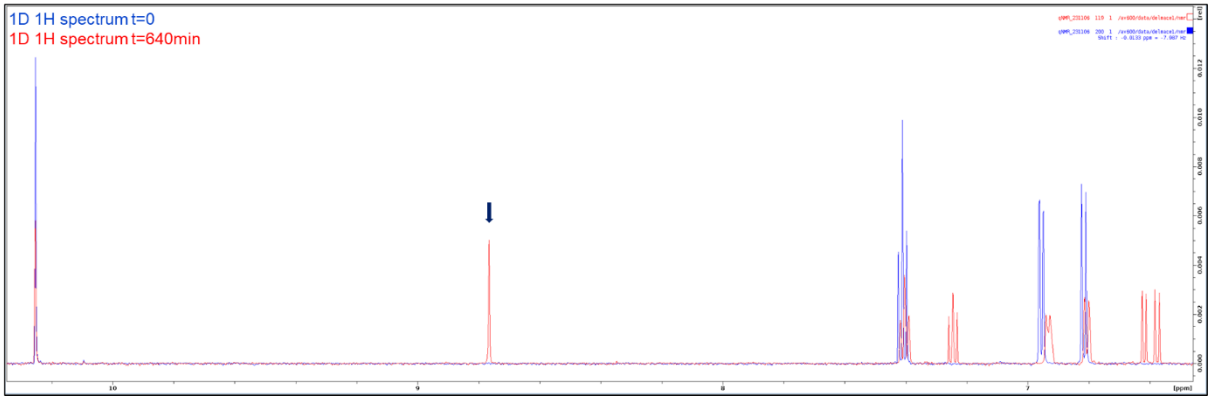


Figure 1: In the aliphatic region, the formation of the imine is also evident, as shown in the following figure, with the peaks marked by an arrow.

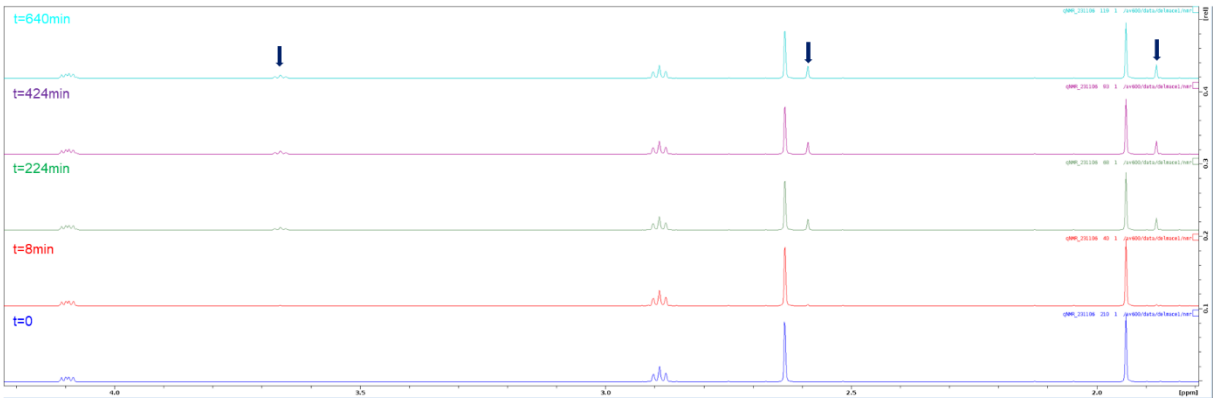
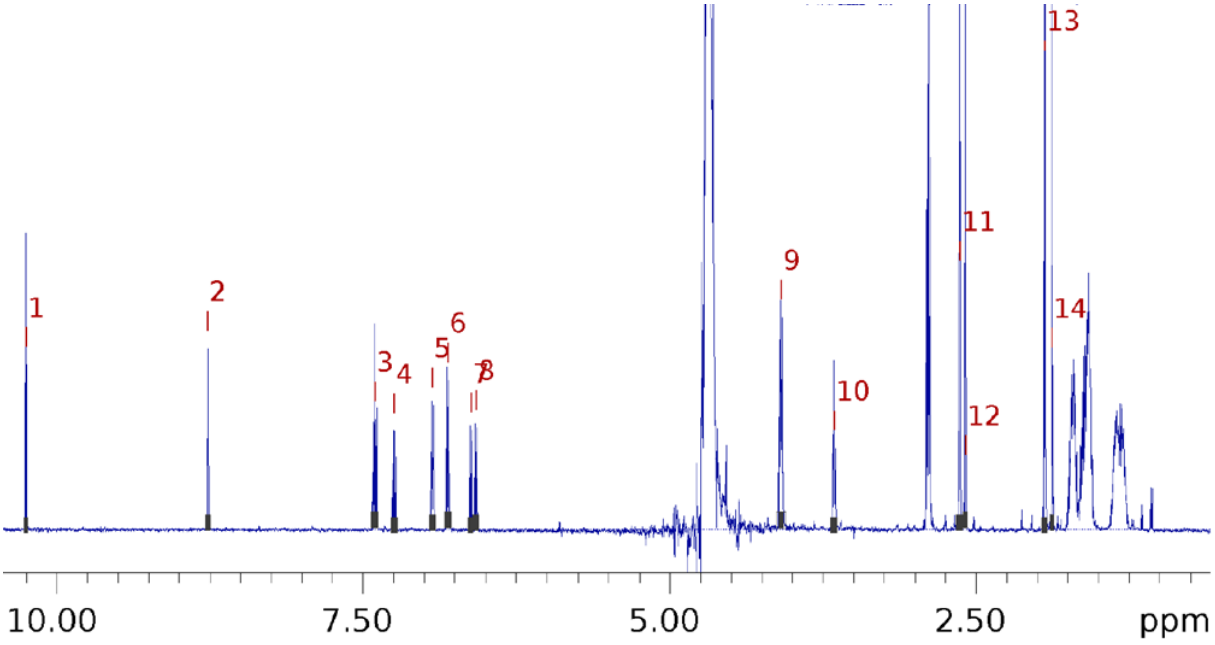


Figure 2: The 80 measured spectra were analyzed using the Dynamics Center module of TOPSPIN. The integration of various peaks was considered as follow for this example: for the aldehyde (peaks 1, 3, 5, and 6), for lysine (peaks 9, 11, and 13), and for the imine adduct (peaks 2, 4, 7, 8, 10, and 14), as illustrated in the following figure.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
10.00
7.50
5.00
2.50
ppm

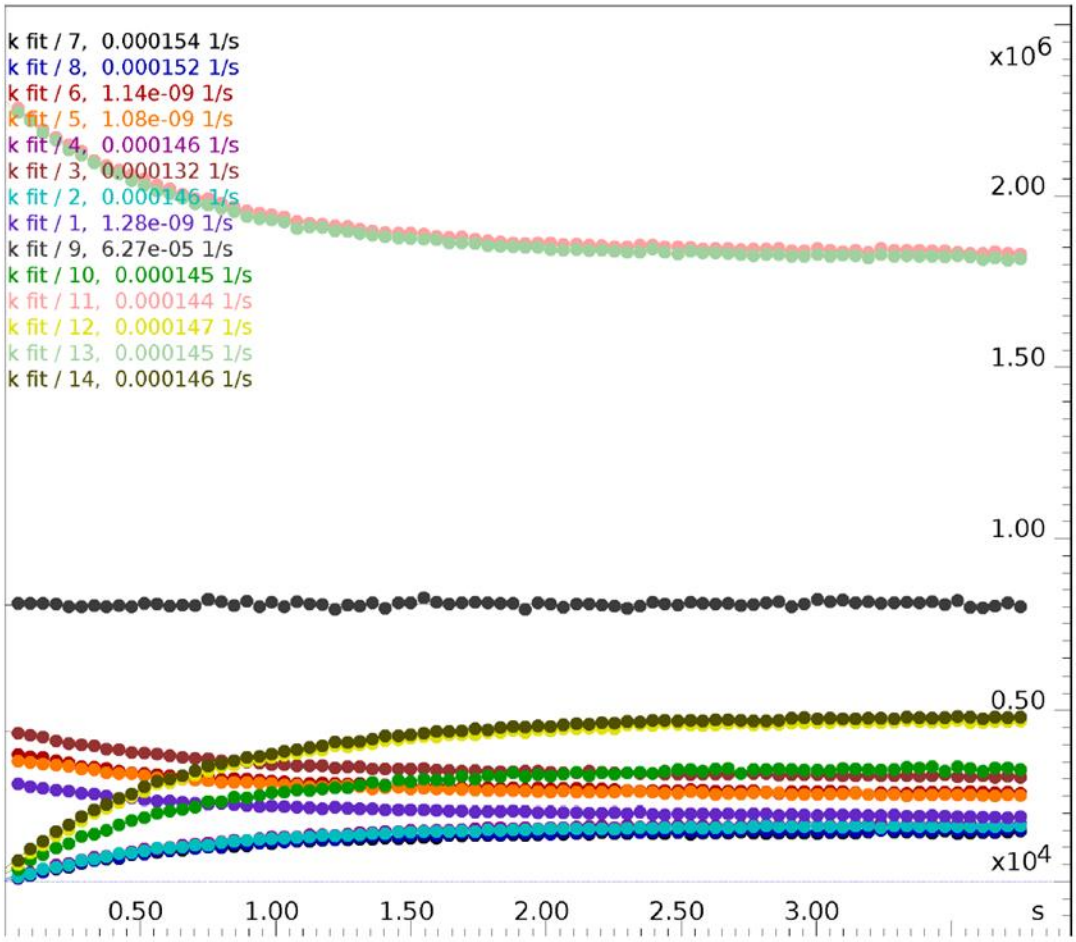
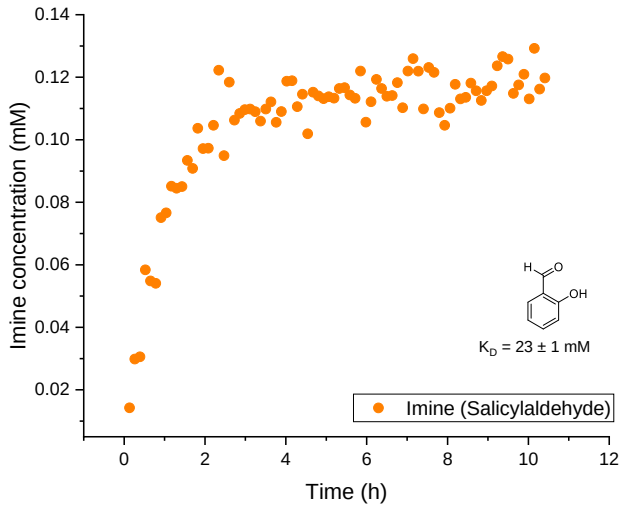


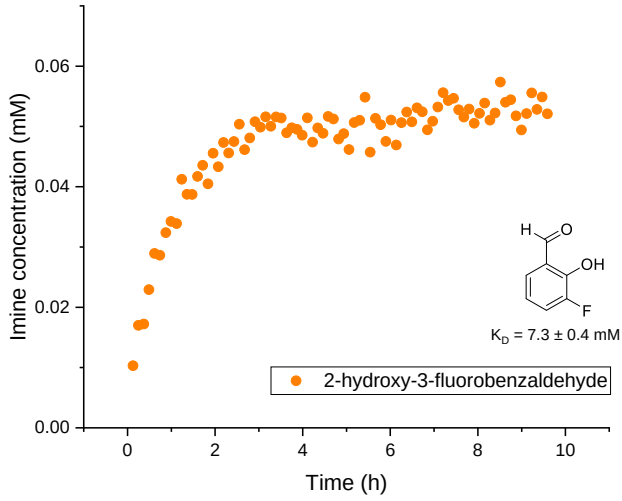
Figure 3: All one-dimensional proton NMR spectra were phased and baseline corrected and using the Dynamics center module of TOPSPIN (Bruker). Data were exported as an Excel table.

1. Kinetic profiles of salicylaldehydes

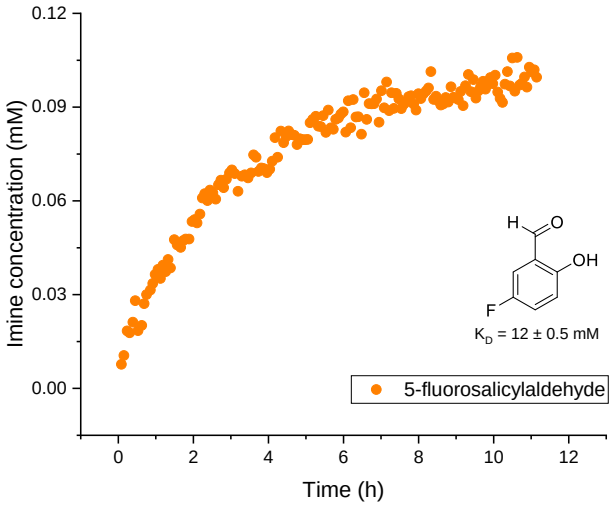
i. Salicylaldehyde (SA1)



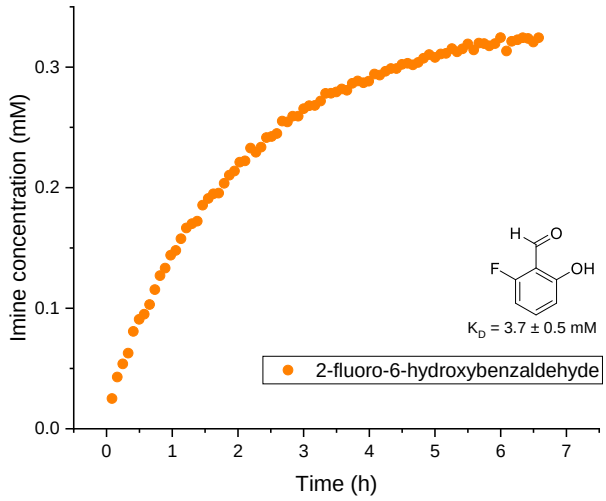
ii. 2-hydroxy-3-fluorobenzaldehyde (SA2)



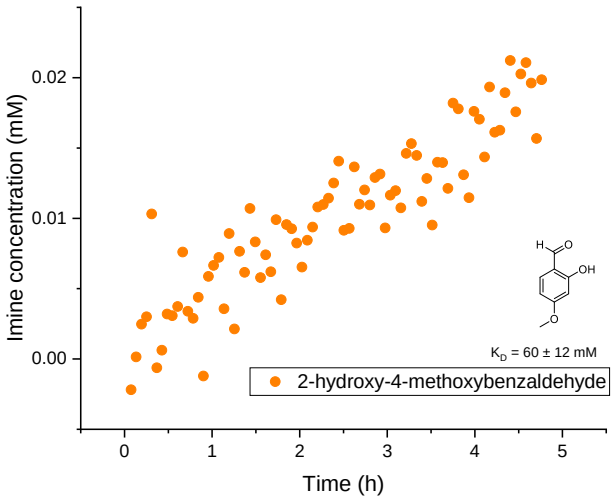
iii. 5-fluorosalicylaldehyde (SA3)



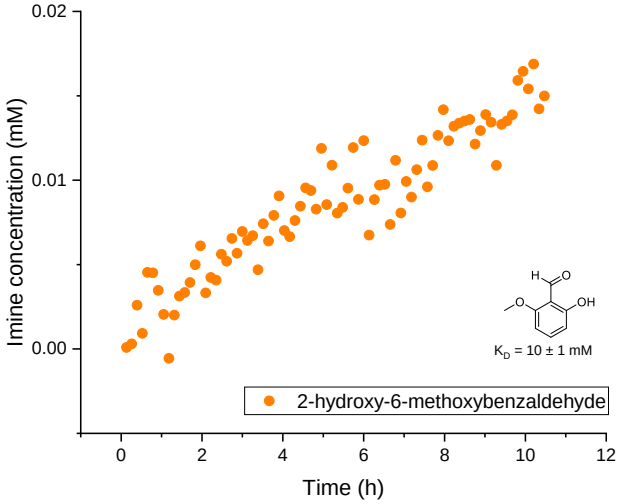
iv. 2-fluoro-6-hydroxybenzaldehyde (SA4)



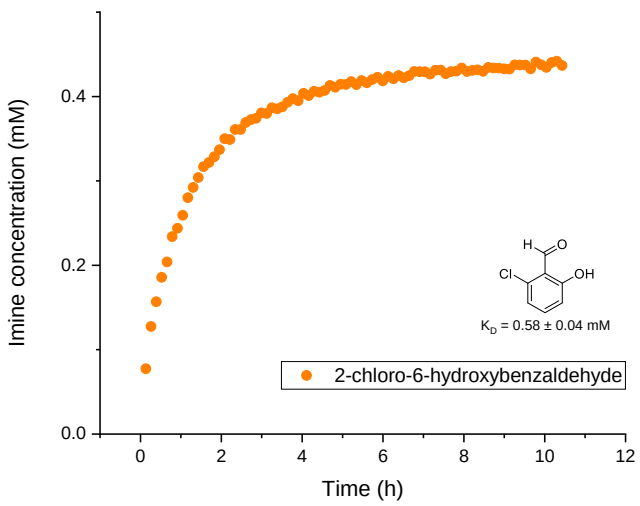
v. 2-hydroxy-4-methoxybenzaldehyde (SA5)



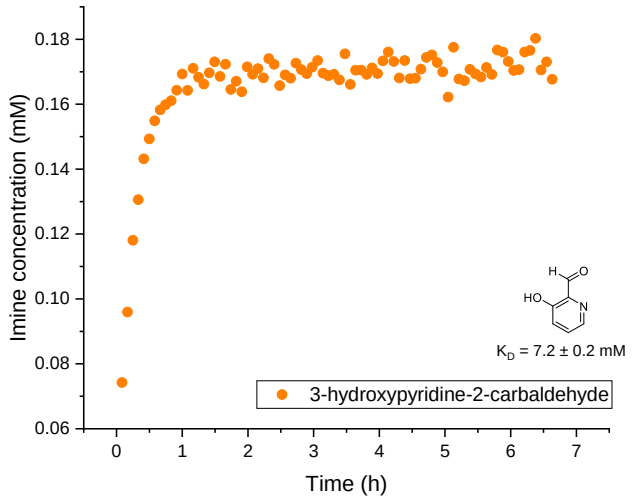
vi. 2-hydroxy-6-methoxybenzaldehyde (SA6)



vii. 2-chloro-6-hydroxybenzaldehyde (SA7)

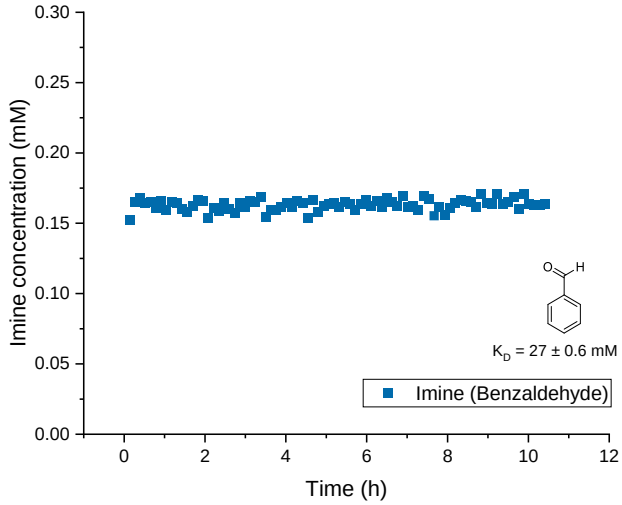


viii. 3-hydroxypyridine-2-carbaldehyde (SA8)

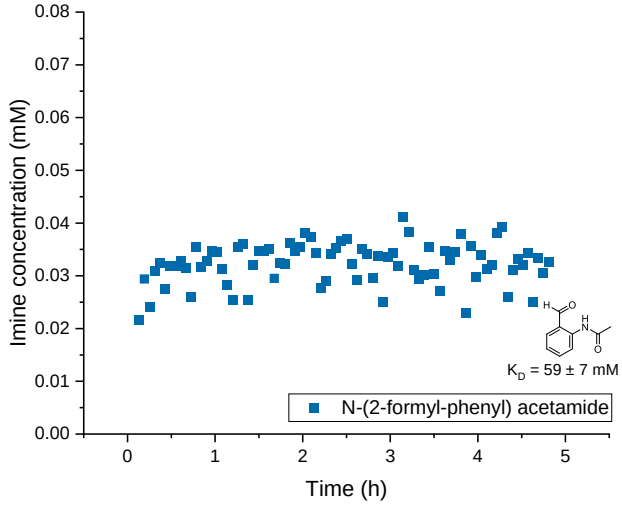


2. Kinetic profiles of aldehydes

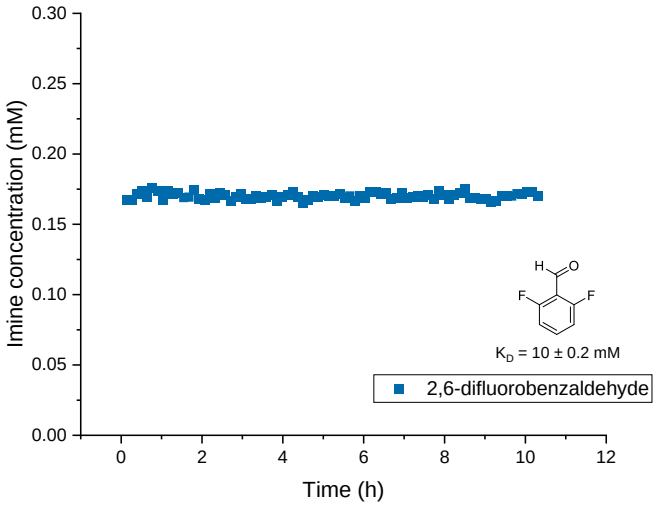
i. Benzaldehyde (A1)



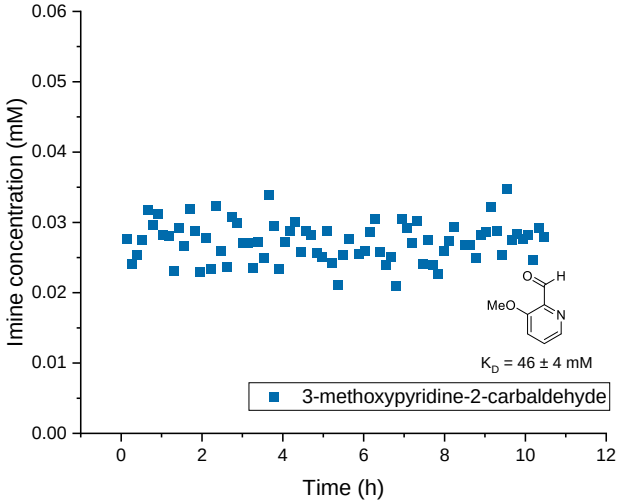
ii. N-(2-formyl-phenyl) acetamide (A2)



iii. 2,6-difluorobenzaldehyde

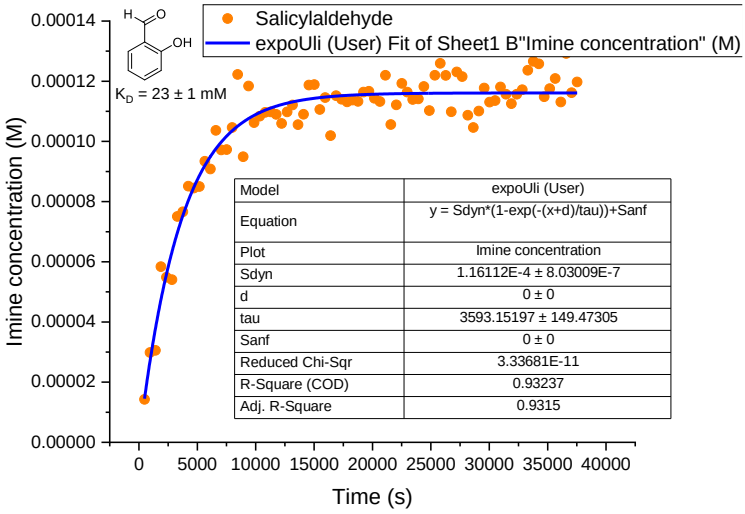


iv. 3-methoxypyridine-2-carbaldehyde



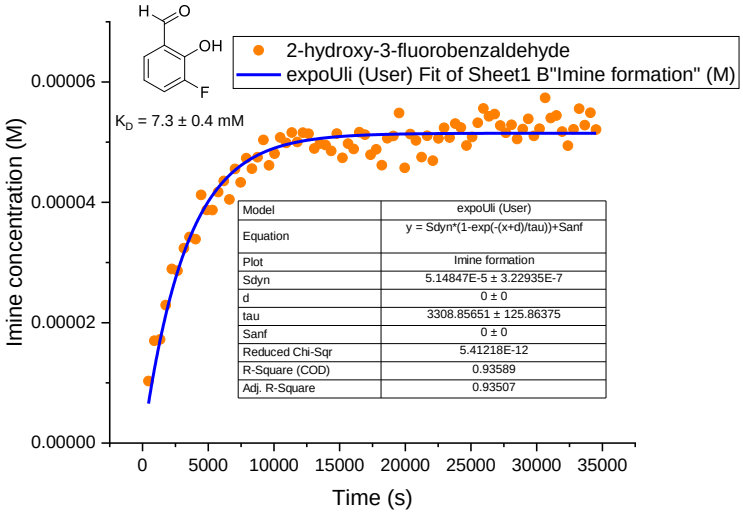
3. Kinetic fits of salicylaldehydes

i. Salicylaldehyde (SA1)



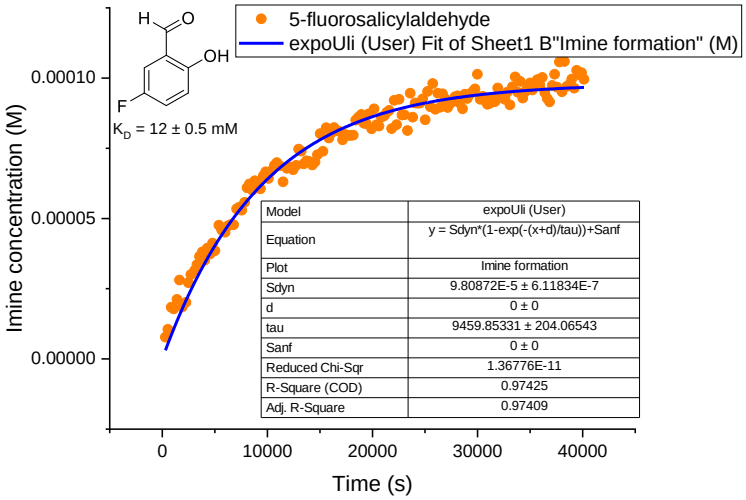
Model	expoUli (User)
Equation	y = Sdyn*(1-exp(-(x+d)/tau))+Sanf
Plot	Imine concentration
Sdyn	1.16112E-4 ± 8.03009E-7
d	0 ± 0
tau	3593.15197 ± 149.47305
Sanf	0 ± 0
Reduced Chi-Sqr	3.33681E-11
R-Square (COD)	0.93237
Adj. R-Square	0.9315

ii. 2-hydroxy-3-fluorobenzaldehyde (SA2)



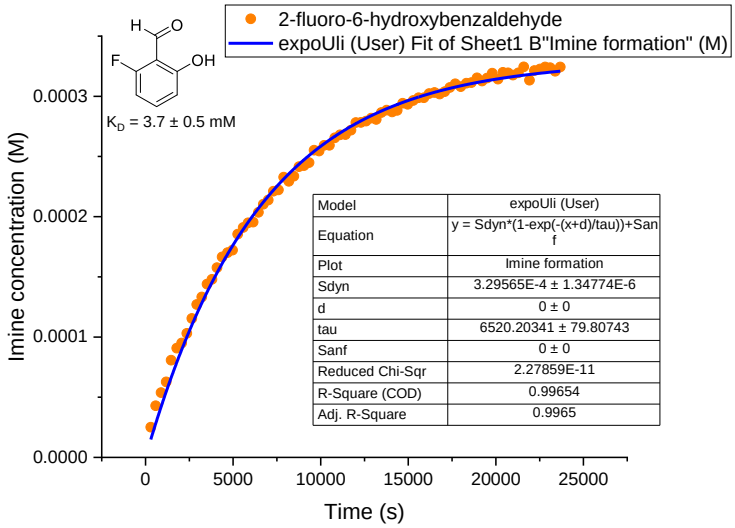
Model	expoUli (User)
Equation	y = Sdyn*(1-exp(-(x+d)/tau))+Sanf
Plot	Imine formation
Sdyn	5.14847E-5 ± 3.22935E-7
d	0 ± 0
tau	3308.85651 ± 125.86375
Sanf	0 ± 0
Reduced Chi-Sqr	5.41218E-12
R-Square (COD)	0.93589
Adj. R-Square	0.93507

iii. 5-fluorosalicylaldehyde (SA3)



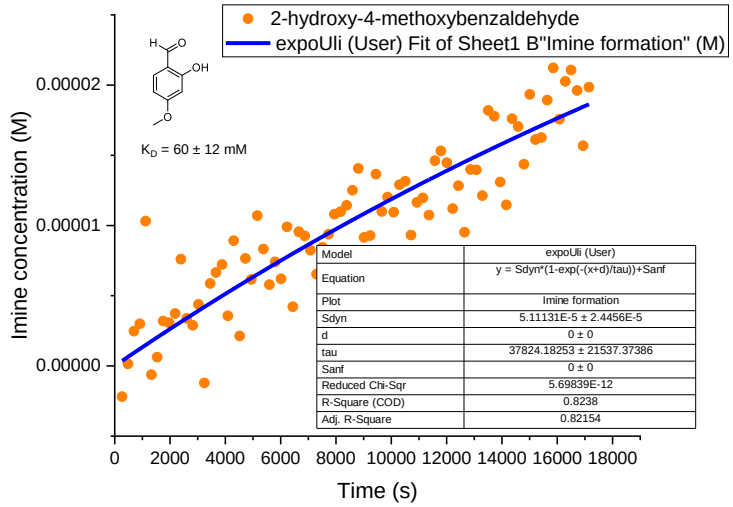
Model	expoUli (User)
Equation	y = Sdyn*(1-exp(-(x+d)/tau))+Sanf
Plot	Imine formation
Sdyn	9.80872E-5 ± 6.11834E-7
d	0 ± 0
tau	9459.85331 ± 204.06543
Sanf	0 ± 0
Reduced Chi-Sqr	1.36776E-11
R-Square (COD)	0.97425
Adj. R-Square	0.97409

iv. 2-fluoro-6-hydroxybenzaldehyde (SA4)



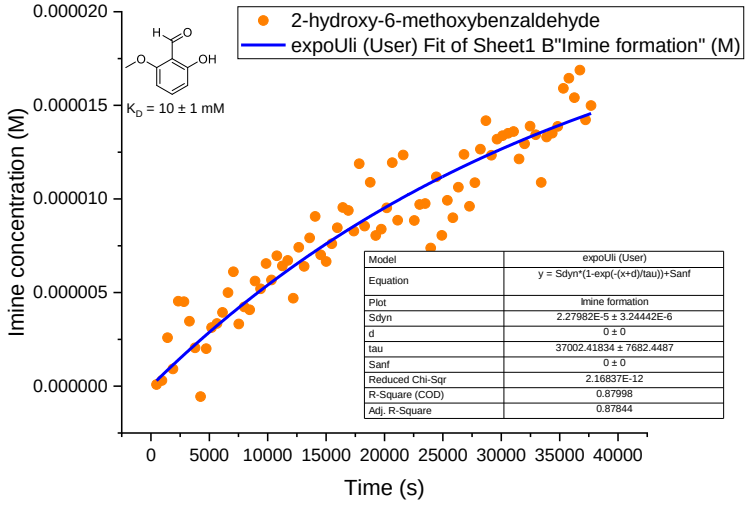
Model	expoUli (User)
Equation	y = Sdyn*(1-exp(-(x+d)/tau))+Sanf
Plot	Imine formation
Sdyn	3.29565E-4 ± 1.34774E-6
d	0 ± 0
tau	6520.20341 ± 79.80743
Sanf	0 ± 0
Reduced Chi-Sqr	2.27859E-11
R-Square (COD)	0.99654
Adj. R-Square	0.9965

v. 2-hydroxy-4-methoxybenzaldehyde (SA5)



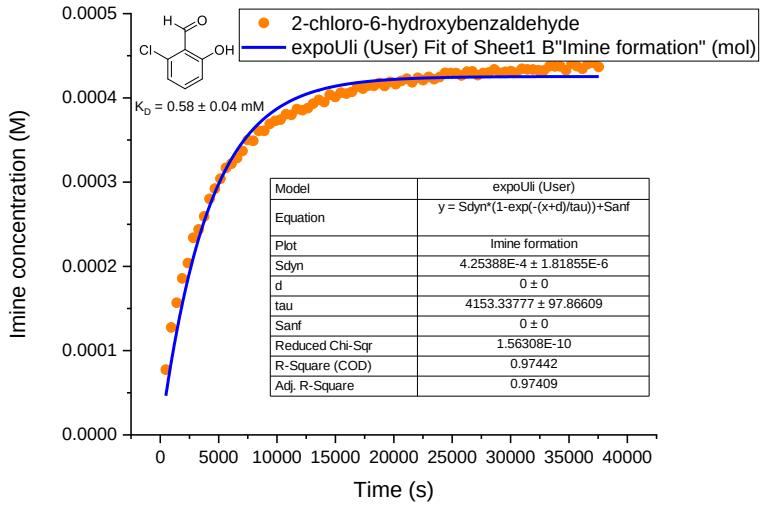
Model	expoUli (User)
Equation	y = Sdyn*(1-exp(-(x+d)/tau))+Sanf
Plot	Imine formation
Sdyn	5.11131E-5 ± 2.4456E-5
d	0 ± 0
tau	37824.18253 ± 21537.37386
Sanf	0 ± 0
Reduced Chi-Sqr	5.69839E-12
R-Square (COD)	0.8238
Adj. R-Square	0.82154

vi. 2-hydroxy-6-methoxybenzaldehyde (SA6)



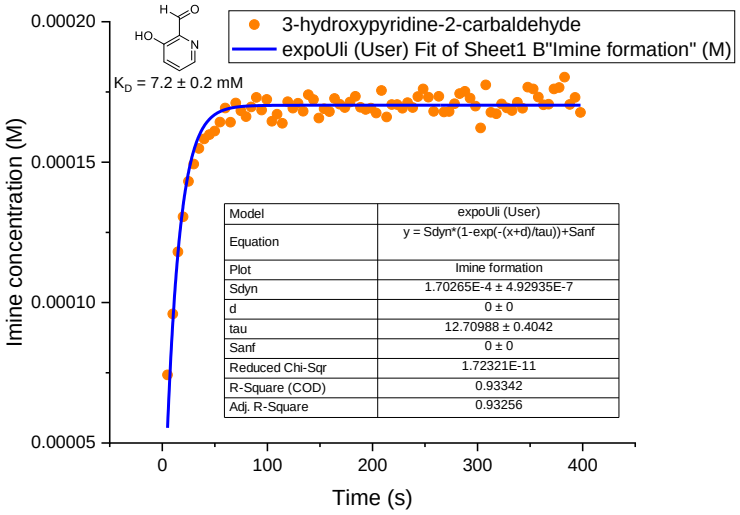
Model	expoUli (User)
Equation	y = Sdyn*(1-exp(-(x+d)/tau))+Sanf
Plot	Imine formation
Sdyn	2.27982E-5 ± 3.24442E-6
d	0 ± 0
tau	37002.41834 ± 7682.4487
Sanf	0 ± 0
Reduced Chi-Sqr	2.16837E-12
R-Square (COD)	0.87998
Adj. R-Square	0.87844

vii. 2-chloro-6-hydroxybenzaldehyde (SA7)



Model	expoUli (User)
Equation	y = Sdyn*(1-exp(-(x+d)/tau))+Sanf
Plot	Imine formation
Sdyn	4.25388E-4 ± 1.81855E-6
d	0 ± 0
tau	4153.33777 ± 97.86609
Sanf	0 ± 0
Reduced Chi-Sqr	1.56308E-10
R-Square (COD)	0.97442
Adj. R-Square	0.97409

viii. 3-hydroxypyridine-2-carbaldehyde (SA8)



Model	expoUli (User)
Equation	y = Sdyn*(1-exp(-(x+d)/tau))+Sanf
Plot	Imine formation
Sdyn	1.70265E-4 ± 4.92935E-7
d	0 ± 0
tau	12.70988 ± 0.4042
Sanf	0 ± 0
Reduced Chi-Sqr	1.72321E-11
R-Square (COD)	0.93342
Adj. R-Square	0.93256