Aniline Catalysed Hydrazone Formation Reactions Show a Large Variation in Reaction Rates and Catalytic Effects

Trausel, Fanny; Fan, Bowen; van Rossum, Susan A.P.; van Esch, Jan H.; Eelkema, Rienk

DOI
10.1002/adsc.201800342

Publication date
2018

Document Version
Final published version

Published in
Advanced Synthesis and Catalysis

DOI: 10.1002/adsc.201800342

Advanced Synthesis & Catalysis

Aniline Catalysed Hydrazone Formation Reactions Show a Large Variation in Reaction Rates and Catalytic Effects

Fanny Trausel,[a] Bowen Fan,[a] Susan A. P. van Rossum,[a] Jan H. van Esch,[a] and Rienk Eelkema[a,*]

[a] Department of Chemical Engineering, Delft University of Technology, van der Maasweg 9, 2629 HZ Delft, the Netherlands
Phone: (+31)-15-27-81035
E-mail: r.eelkema@tudelft.nl

Received: March 16, 2018; Revised: April 26, 2018; Published online: May 23, 2018

Supporting information for this article is available on the WWW under https://doi.org/10.1002/adsc.201800342

Abstract: Hydrazone formation reactions from aldehydes and hydrazides have the remarkable qualities that they proceed in water and the kinetics can be controlled by organocatalysis. For these reasons, this class of reactions finds widespread use in biological as well as material settings. We recently reported a protected aniline catalyst for hydrazone formation that can be activated using a chemical signal. In our search to find a suitable hydrazone formation reaction to investigate the activation of this pro-catalyst, we found a wide variety in reaction rates and response to catalysis. Here we report an overview of hydrazone formation reactions, their reaction rates and response to aniline catalysis, their compatibility for kinetic analysis by UV/Vis spectroscopy, and their compatibility with the reaction environment and with the pro-catalyst pro-aniline.

Keywords: Hydrazones; Bioorthogonal chemistry; Click chemistry; Organocatalysis; UV/vis spectroscopy

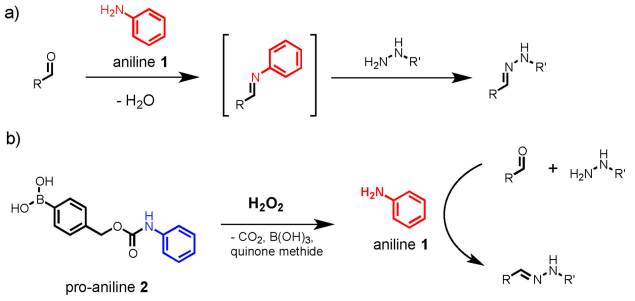
Introduction

Non-biological reactions that proceed in water and can be accelerated by catalysis are uncommon, but of high interest for the design of responsive (bio-) materials and for functionalization of biomolecules.[1] Hydrazone formation reactions, between aldehydes and hydrazides, are a convenient class of bioorthogonal copper-free click reactions as they proceed in water.[2] Furthermore, because hydrazone formation reactions proceed at ambient conditions and are susceptible to catalysis, hydrazone formation reactions are widely applied in dynamic combinatorial chemistry.[3] Hydrazone reactions proceed rapidly at pH 5 or lower, but are unpractically slow at physiological pH.[3a,4] Jencks found that hydrazone formation reactions can be accelerated by nucleophilic catalysis using the organocatalyst aniline at physiological pH (Scheme 1, a).[5] Kool reported alternative organocatalysts for hydrazone formation that are more efficient and less toxic than aniline.[2c,6] However, the rate of hydrazone formation and response to catalysis depends heavily on the type of hydrazide and aldehyde coupling partners. Overviews reporting reaction rate constants can thus be useful in allowing scientists to choose a suitable hydrazone formation system for their purposes.[2d,7]

Recently we reported a protected aniline catalyst for hydrazone formation (pro-aniline **2**, Scheme 1, b).[8] Addition of the chemical signal H_2O_2 leads to deprotection of pro-aniline **2** and release of aniline **1** which can then catalyse hydrazone formation. We used **2** to control the formation of hydrogels featuring hydrazone bonds, introducing signal response in soft materials.[9] To find a suitable hydrazone formation reaction to investigate the activation of **2**, we compared a selection of hydrazone formation reactions. Our goal was to find a hydrazone formation reaction that shows a detectable conversion within 15 hours when catalysed by **1** at room temperature in aqueous buffer. To enable a clearly observable signal response, the reaction should show at least a three-fold increase in reaction rate when catalysed by **1**.

Furthermore, the reaction mixture should not show any side reactions with **2** or any other component, which would complicate analysis.

Finally, as we ideally wanted to follow the progress of the reaction by UV/Vis spectroscopy, the starting materials, intermediates and products should be soluble in the reaction medium (aqueous buffer with



Scheme 1. Catalysis of hydrazone formation. (**a**) Hydrazone formation: the reaction between an aldehyde and a hydrazide catalysed by aniline **1**. (**b**) The pro-catalyst pro-aniline **2** and the chemical signal H_2O_2 react to release the organocatalyst aniline **1** which catalyses hydrazone formation between an aldehyde and a hydrazide.[8]

20% dimethylformamide as co-solvent) and product formation should give a detectable change in the UV/Vis spectrum above a wavelength of 250 nm.

Here, we disclose our findings on this topic. We find that the reaction rate constants between different hydrazone formation reactions may vary by orders of magnitude, that aniline **1** only catalyses some of the hydrazone formation reactions that we tested and that some hydrazides degrade in the solvent system or react with pro-aniline **2**. These findings may help the reader to choose a suitable hydrazone formation reaction for his or her experimental purposes.

Results and Discussion

Table 1 shows the selection of hydrazone reactions for which we investigated the response to aniline catalysis and unwanted reactivity towards **2**. The reaction rates were determined by following the change in absorbance in UV/vis spectroscopy. Pseudo-first -order rate constants were determined by using the Guggenheim time lag method.[10] The graphs were fitted using linear regression. All reactions were carried out using the same conditions: 0.020 mM hydrazide, 0.5 mM aldehyde, 0.5 mM aniline **1** or 0.5 mM pro-aniline **2**, 20% (v/v) DMF (dimethylformamide) in 100 mM sodium phosphate buffer pH 7.4, 25 °C. The concentrations of the reagents were chosen such that all reactions are in the right absorbance window to follow the reaction using UV/vis spectroscopy. We used 20% DMF as a co-solvent to ensure solubility of all reagents, catalysts and products. To measure the response on hydrazone formation rate to catalysis by **1** we determined the ratio between the reaction rate constant for the reaction catalysed by **1** and the reaction rate constant of the uncatalysed reaction. We report the wavelength at which we followed each reaction (rate analysis wavelength, Table 1). Full range UV/vis spectra of the hydrazone reactions at t0 and t = 15 h are shown in Supplementary Figure 1, graphs of the absorbance at the rate analysis wavelength over time are shown in Supplementary Figure 2 and the Guggenheim fits are shown in Supplementary Figure 3.

Reaction 1 is catalysed by **1** but only shows a 1.7-fold increase in reaction rate in the presence of **1**, the difference in rate between the catalysed reaction and the uncatalysed reaction is modest. Reactions 2 and 3 show a more promising response to aniline catalysis: the reaction rate constant of reaction 2 shows a 3.3-fold increase and reaction 3 a 3.7-fold increase in the presence of **1**: the kinetics of these reactions can be controlled by aniline catalysis. Kool found an 11-fold increase of reaction rate for reaction 3 upon addition of **1**, using 1 mM of aldehyde and 1 mM of **1**.[6b] The difference for this response to aniline catalysis can be due to the amount of DMF as a co-solvent (Kool used 10% DMF, whereas we used 20% DMF). When testing cross-reactivity with pro-catalyst **2**, the absorbance of a mixture of hydrazide **3** and pro-aniline **2** changes over time, indicating that the two compounds react or form a non-covalent interaction with each other. Therefore, we were unable to use reactions 1–3.

Reaction 4 only shows a 1.1-fold increase in reaction rate with **1**, a value that is barely significant. Reactions 5, 6 and 7 are not catalysed by **1**. The 2nd order rate constants for reactions 4, 5, 6 and 7 were also reported by Kool.[6b] He found a very similar response to aniline catalysis for reaction 5, but more convincing responses to aniline catalysis for reactions 4, 6 and 7: the reaction rates increased between 1.8–2.1 fold upon aniline catalysis. Again, this discrepancy with the results of Kool can be due to the difference in amount of DMF that was used as a co-solvent. An explanation for the lack of response to aniline catalysis can be that hydrazide **7** reacts already efficiently with aldehydes without catalysis, and the activation of the aldehydes by aniline in the form of the imine intermediate does not increase the reaction rate.

The reaction rate of reaction 8 increases 2.6-fold when catalysed by **1**. For reactions 10, 14 and 16, there is no detectable change in UV/Vis absorption over the course of 15 hours during both the catalysed and uncatalysed reactions. Reaction 11 only shows a 1.2-fold increase in reaction rate upon addition of **1**. Reactions 12, 15 and 17 are promising reactions: they do not show any conversion within 15 hours without catalyst and with **1** the reactions have considerable rate constants of $(2.1 \pm 0.12) \times 10^{-4}\ s^{-1}$, $5.0 \times 10^{-4}\ s^{-1}$ and $2.5 \times 10^{-4}\ s^{-1}$, respectively. Because the uncatalysed reactions were found to be immeasurably slow, we could not reliably calculate the ratio between the catalysed reaction and uncatalysed reaction rates. A slight disadvantage of these reactions is that the change in absorbance during the reactions is very small, which makes the reactions less reliable to

Table 1. Overview of hydrazone formation reactions tested with aniline **1**.[a]

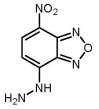
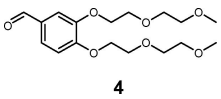
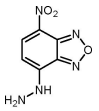
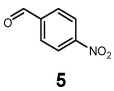
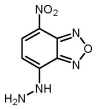
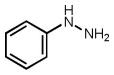
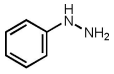
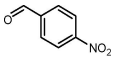
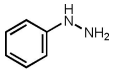
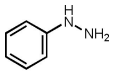
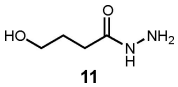
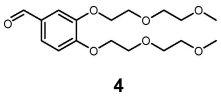
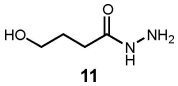
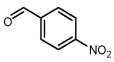
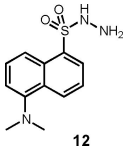
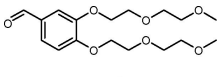
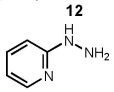
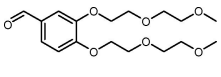
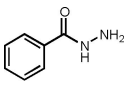
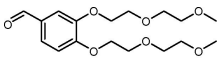
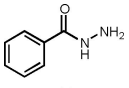
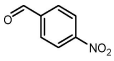
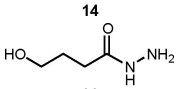
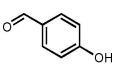
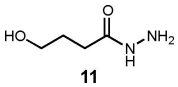
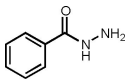
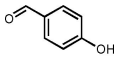
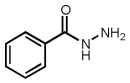
	Hydrazide	Aldehyde	$k_{1,\,cat}$[b] (s^{-1})	$k_{2\,(app)}$[c] ($M^{-1}\,s^{-1}$)	k_{rel}[d]	Rate analysis[e] wavelength (nm)
1		 **4**	$(1.8\pm0.015)\times10^{-6}$	$(3.7\pm0.033)\times10^{-3}$	1.7	500
2	**3** 	 **5**	$(5.0\pm2.0)\times10^{-5}$	$(1.0\pm0.39)\times10^{-1}$	3.3	500
3	**3** 	 **6**	$(6.7\pm1.1)\times10^{-6}$	$(1.3\pm0.21)\times10^{-2}$	3.7	500
4	**3**  **7**	 **8**	$(2.3\pm0.016)\times10^{-4}$	$(4.6\pm0.033)\times10^{-1}$	1.1	350
5	 **7**	 **5**	$(3.2\pm0.078)\times10^{-4}$	$(6.4\pm0.16)\times10^{-1}$	1.0	450
6	 **7**	 **9**	$(4.3\pm1.6)\times10^{-4}$	$(8.6\pm3.1)\times10^{-1}$	1.0	350
7	 **7**	 **10**	$(5.4\pm0.57)\times10^{-4}$	1.1 ± 0.11	1.0	350
8	 **11**	 **4**	$(3.7\pm0.14)\times10^{-4}$	$(7.3\pm0.28)\times10^{-1}$	2.6	340
9	 **11**	 **5**	$(3.1\pm0.20)\times10^{-4}$	$(6.2\pm0.40)\times10^{-1}$	47	330
10	 **12**	 **4**	N.A.[f]	N.A.	N.A.	N.A.
11	 **13**	 **4**	$(5.2\pm0.065)\times10^{-5}$	$(1.0\pm0.013)\times10^{-1}$	1.2	340
12	 **14**	 **4**	$(2.1\pm0.12)\times10^{-4}$	$(4.2\pm0.24)\times10^{-1}$	N.A.	340
13	 **14**	 **5**	$(1.8\pm0.013)\times10^{-4}$	$(4.6\pm0.033)\times10^{-1}$	24	340
14	 **11**	 **16**	N.A.	N.A.	N.A.	N.A.
15	 **11**	 **8**	5.0×10^{-4}	1.0	N.A.	330

Table 1. continued

	Hydrazide	Aldehyde	$k_{1,\,cat}$[b] (s^{-1})	$k_{2\,(app)}$[c] ($M^{-1}\,s^{-1}$)	k_{rel}[d]	Rate analysis[e] wavelength (nm)
16	 14	 16	N.A.	N.A.	N.A.	N.A.
17	 14	 8	2.5×10^{-4}	5.0×10^{-1}	N.A.	330

[a] Reaction conditions: 0.020 mM hydrazide, 0.5 mM aldehyde and 0.5 mM aniline **1** in 20% DMF in 100 mM sodium phosphate buffer pH 7.4.
[b] $k_{1,\,cat}$ is the pseudo-first-order rate constant of the aniline catalysed reaction, reported with the standard error of the mean (SEM, $n \geq 2$).
[c] $k_{2\,(app)}$ is de calculated second order rate constant of the aniline catalysed reaction, calculated with $k_{2(app)} = k_{1,cat}/[\text{aldehyde}]$.
[d] k_{rel} is the ratio of the rate constant of the reaction catalysed by **1** and the rate constant of the uncatalysed reaction ($k_{rel} = k_{cat}/k_{uncat}$).
[e] We report the wavelengths at which we followed the hydrazone formation reaction using UV/vis spectroscopy.
[f] N.A.: not applicable.

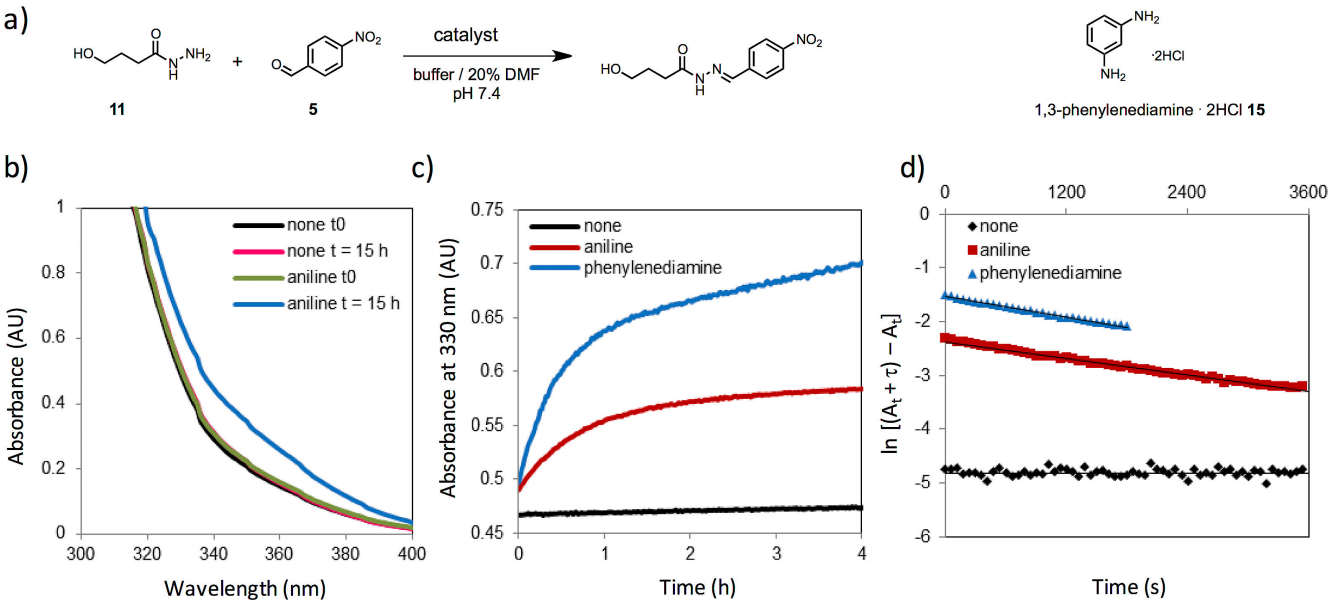


Figure 1. The hydrazone formation reaction 9 and response to aniline **1** and 1,3-phenylenediamine ·2HCl **15** catalysis. Reaction conditions: 0.020 mM hydrazide **11**, 0.5 mM aldehyde **5**, 0.5 mM **1** or 0.5 mM **15** in 20% DMF in 100 mM sodium phosphate buffer pH 7.4 (**a**) Reaction 9 between hydrazide **11** and aldehyde **5**. (**b**) Absorbance spectra of the reaction mixtures for the uncatalysed reaction at t0 (black line) and at t = 15 h (magenta line), for the reaction catalysed by **1** at t0 (green line), at t = 15 h (blue line). (**c**) Hydrazone formation followed over time for the uncatalysed reaction (black), for the reaction catalysed by **1** (red) and for the reaction catalysed by **15** (blue). (**d**) The first-order-rate constants were determined by Guggenheim fits, uncatalysed reaction (black), reaction catalysed by **1** (red), reaction catalysed by **15** (blue).

follow. Besides the coupling partners of hydrazides with aromatic aldehydes, we also measured the reaction between hydrazide **14** with the aliphatic aldehyde propanal. Again, there is no detectable change in UV/Vis absorption over the course of 15 hours with or without catalyst **1**. It may be that aliphatic aldehydes are less suited to our analysis method because of their lack of a chromophore.

Reaction 13 responds well to aniline catalysis: the reaction rate is increased 24-fold in the presence of **1**. There is a clear change in absorbance during the

reaction, making reaction 13 a promising benchmark reaction for aniline catalysis.

With a 47-fold increase in reaction rate in the presence of **1**, reaction 9 shows, apart from reactions 12, 15 and 17, by far the largest increase in reaction rate among the reactions investigated in the current work. Because there is also a clear change in absorbance during the reaction and as we did not find any side reactions with **2** (Supplementary Table 2), we chose this reaction as a benchmark reaction for the activation of pro-aniline **2** in the 2017 publication.[8]

We also investigated the activity of the catalyst 1,3-phenylenediamine·2HCl **15** (Figure 1a, c, d) in reaction 9.

Whereas **1** gives a 47-fold increase in reaction rate for reaction 9, catalysis by **15** results in a 74-fold increase in reaction rate (Supplementary Table 1), making catalyst **15** 1.5-times more active than aniline. This result is in agreement with studies from the literature: catalyst **15** was reported by Kool as 2.1-times more active than aniline.[11]

We tested each aldehyde and hydrazide combination for possible side reactions with pro-aniline **2**. None of the aldehydes showed any change in absorbance in the presence of **2**, indicating that the aldehydes do not react with **2**. The absorbance spectrum of hydrazide **3** changes in the presence of **2**, which indicates that the two compounds react or non-covalently bind to each other.

We found that the absorbance spectrum of another nitro-bearing hydrazide, 2,4-dinitrophenylhydrazine (DNPH), also changes in the presence of **2** (Supplementary Figure 4). This might indicate that the nitro-group causes this apparent side reaction. A possible explanation might be that nitro-bearing hydrazides can coordinate or react with the boronic acid group on **2**.[12]

The side reaction of hydrazide **3** with **2** reduced the usefulness of hydrazide **3** under our reaction conditions. However, as long as no compounds similar to pro-aniline **2** are applied, hydrazide **3** may still be a good probe to analyse hydrazone formation.

Furthermore, we studied the stability of the aldehydes and hydrazides in the reaction solvent. The absorbance spectra of the aldehydes and hydrazides **3**, **13** and **14** did not change over a course of 15 h, indicating that the compounds remain stable. In contrast, the absorbance spectra of hydrazide **7** and hydrazide **12** alone changed in the presence of DMF or DMSO (dimethyl sulfoxide), which suggests that these compounds react with the solvents or degrade (Supplementary Figure 5).

Overall it appears that relatively unreactive hydrazides, such as hydrazides **3**, **11**, and **14**, benefit from aniline catalysis. Relative reactive hydrazides, such as hydrazides **7** and **13** react efficiently with the aldehydes without activation by aniline and do not seem to benefit from aniline catalysis.

Conclusions

In summary, the hydrazone formation reactions we discussed show large variation in reactivity, stability and response to aniline catalysis. Reactions 2, 3, 13 and especially reactions 12, 15, 17 and 9 show a large increase in reaction rate in the presence of aniline **1**. Only a moderate increase in reaction rate in the presence of **1** was found for reactions 1, 4, 8 and 11. Aniline **1** does not show any significant catalytic activity in reactions 5, 6 and 7. Reactions 10, 14, and 16 show no detectable change in absorption, with or without **1**. The organocatalyst **15** is 1.5 times more active in reaction 9, when compared to **1**. Overall, hydrazone formation of NBD-hydrazide **3** and sulfonated benzaldehyde **6** (reaction 3), or acylhydrazide **11** or **14** and *p*-nitrobenzaldehyde **5** or benzaldehyde **8** (reactions 12, 15, 17, 13 and 9) are significantly accelerated by aniline catalysis without observed side reactions, making them useful benchmark reactions to test aniline catalysis or in designing responsive materials where aniline catalysis plays a role.

Experimental Section

General Procedure to Follow a Hydrazone Reaction in UV/Vis Spectroscopy

The hydrazone reactions were performed in 20% (v/v) DMF (dimethylformamide) in a 100 mM sodium phosphate buffer pH 7.4. The quartz cuvettes contained a total reaction volume of 2 mL. All reactions were carried out using the same conditions: 0.020 mM hydrazide, 0.5 mM aldehyde, 0.5 mM aniline **1** or pro-aniline **2**, 20% DMF in 100 mM phosphate buffer pH 7.4, 25 °C. The stock solutions of the reagents were added as follows: aldehyde solution (100 μL, 10 mM in DMF), phosphate buffer, DMF, catalyst solution (100 μL, 10 mM in DMF), the hydrazide solution (100 μL, 0.4 mM in DMF). Stock solutions were made fresh for every reaction and used within 1 h. The cuvettes were closed using Teflon caps and thoroughly mixed by turning the cuvette upside down 4 times. The spectra of the reaction mixtures at $t = 0$ were measured (reference measurement using a cuvette with only solvent as the reference cuvette, 10 nm s^{-1}). The change in absorbance was followed at the rate analysis wavelength using a 6-sample holder (standard absorption measurement, scan every 30 s). At $t = 15$ h single scans were measured again using the same settings as for the starting reaction mixtures. The pseudo-first-order rate constants were determined using the Guggenheim time lag fit.[10] The graph was fitted using linear regression to yield the pseudo-first-order reaction rate constant.

Acknowledgements

This work was supported by the Netherlands Organization for Scientific Research through a VIDI grant and by the European Research Council (*ERC consolidator grant 726381*).

References

[1] a) D. D. McKinnon, D. W. Domaille, J. N. Cha, K. S. Anseth, *Chem. Mater.* **2014**, *26*, 2382–2387; b) D. Yang, E. Gustafsson, T. C. Stimpson, A. Esser, R. H. Pelton, *ACS Appl. Mater. Interfaces* **2017**, *9*, 21000–21009; c) D. E. Apostolides, T. Sakai, C. S. Patrickios, *Macromolecules* **2017**, *50*, 2155–2164; d) N. Boehnke, C. Cam, E. Bat, T. Segura, H. D. Maynard, *Biomacromolecules* **2015**, *16*, 2101–2108; e) M. A. Azagarsamy, I. A. Marozas, S. Spaans, K. S. Anseth, *ACS Macro Lett.* **2016**, *5*, 19–23; f) J. Boekhoven, J. M. Poolman, C. Maity, F. Li, L. van der Mee, C. B. Minkenberg, E. Mendes, J. H. van Esch, R. Eelkema, *Nat. Chem.* **2013**, *5*, 433–437; g) J. M. Poolman, J. Boekhoven, A. Besselink, A. G. L. Olive, J. H. van Esch, R. Eelkema, *Nat. Protoc.* **2014**, *9*, 977–988; h) R. P. Brinkhuis, F. de Graaf, M. B. Hansen, T. R. Visser, F. P. J. T. Rutjes, J. C. M. van Hest, *Polym. Chem.* **2013**, *4*, 1345–1350.

[2] a) T. Ganguly, B. B. Kasten, D.-K. Bucar, L. R. MacGillivray, C. E. Berkman, P. D. Benny, *Chem. Commun.* **2011**, *47*, 12846–12848; b) Craig S. McKay, M. G. Finn, *Chem. Biol.* **2014**, *21*, 1075–1101; c) P. Crisalli, E. T. Kool, *Org. Lett.* **2013**, *15*, 1646–1649; d) E. T. Kool, D.-H. Park, P. Crisalli, *J. Am. Chem. Soc.* **2013**, *135*, 17663–17666.

[3] a) A. Dirksen, S. Dirksen, T. M. Hackeng, P. E. Dawson, *J. Am. Chem. Soc.* **2006**, *128*, 15602–15603; b) Nguyen, R. Ivan Huc, *Chem. Commun.* **2003**, 942–943; c) M. G. Simpson, M. Pittelkow, S. P. Watson, J. K. M. Sanders, *Org. Biomol. Chem.* **2010**, *8*, 1181–1187; d) G. R. L. Cousins, S.-A. Poulsen, J. K. M. Sanders, *Chem. Commun.* **1999**, 1575–1576.

[4] A. Dirksen, P. E. Dawson, *Bioconjugate Chem.* **2008**, *19*, 2543–2548.

[5] E. H. Cordes, W. P. Jencks, *J. Am. Chem. Soc.* **1962**, *84*, 826–831.

[6] a) P. Crisalli, E. T. Kool, *J. Org. Chem.* **2013**, *78*, 1184–1189; b) D. Larsen, M. Pittelkow, S. Karmakar, E. T. Kool, *Org. Lett.* **2015**, *17*, 274–277.

[7] a) E. T. Kool, P. Crisalli, K. M. Chan, *Org. Lett.* **2014**, *16*, 1454–1457; b) D. K. Kölmel, E. T. Kool, *Chem. Rev.* **2017**, *117*, 10358–10376.

[8] F. Trausel, C. Maity, J. M. Poolman, D. S. J. Kouwenberg, F. Versluis, J. H. van Esch, R. Eelkema, *Nat. Commun.* **2017**, *8*, 879.

[9] F. Trausel, F. Versluis, C. Maity, J. M. Poolman, M. Lovrak, J. H. van Esch, R. Eelkema, *Acc. Chem. Res.* **2016**, *49*, 1440–1447.

[10] a) E. A. Guggenheim, *Philos. Mag.* **1926**, *2*, 538–543; b) M. R. K. Hemalatha, I. Noorbatcha, *J. Chem. Educ.* **1997**, *74*, 972.

[11] a) L. H. Yuen, N. S. Saxena, H. S. Park, K. Weinberg, E. T. Kool, *ACS Chem. Biol.* **2016**, *11*, 2312–2319. b) M. Rashidian, M. M. Mahmoodi, R. Shah, J. K. Dozier, C. R. Wagner, M. D. Distefano, *Bioconjugate Chem.* **2013**, *24*, 333–342.

[12] a) R. Nishiyabu, Y. Kubo, T. D. James, J. S. Fossey, *Chem. Commun.* **2011**, *47*, 1124–1150. b) C. J. Stress, P. J. Schmidt, D. G. Gillingham, *Org. Biomol. Chem.* **2016**, *14*, 5529–5533.

