

## Electrophilicity

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## Nucleophilicity of Glutathione: A Link to Michael Acceptor Reactivities

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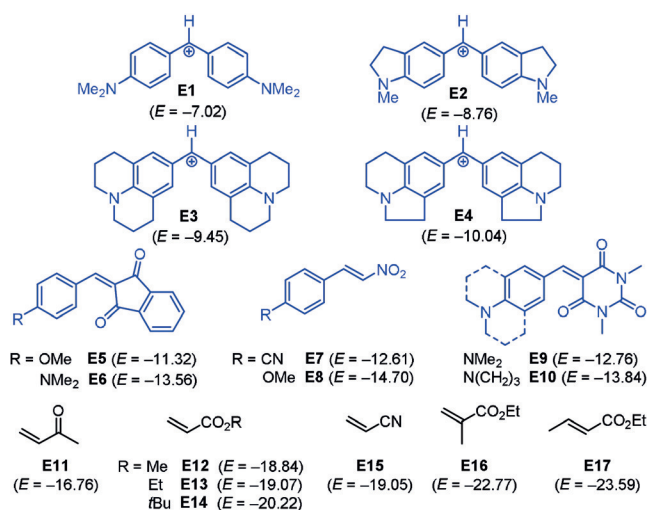
**Abstract:** Deprotonated glutathione is among the most potent biological nucleophiles and plays an important physiological role in cellular detoxification by forming covalent conjugates with Michael acceptors. The electrophilicity  $E$  of various Michael acceptors was characterized recently according to the Patz–Mayr relation  $\lg k_2 = s_N(N + E)$ . We now determined the nucleophilic reactivity ( $N$ ,  $s_N$ ) of glutathione (GSH) in aqueous solution at 20°C to connect published GSH reactivities ( $k_{\text{GSH}}$ ) with Mayr's electrophilicity scale ( $E$ ). In this way, electrophilicities  $E$  of more than 70 Michael acceptors could be estimated, which can now be used to systematically predict novel reactions with the multitude of nucleophiles whose nucleophilicity parameters  $N/s_N$  are known.

Michael acceptors are often used and versatile electrophiles in organic synthesis. Their capability to form conjugates with peptides bearing nucleophilic groups, such as the thiol of the tripeptide glutathione (GSH), supplies them with a broad spectrum of bioactivity. To assess the toxicity of xenobiotic Michael acceptors<sup>[1,2]</sup> as well as to estimate their potential as biological tools<sup>[3–5]</sup> or covalent drugs,<sup>[2,6]</sup> the kinetics of non-enzymatic GSH thiol–Michael additions have been broadly investigated under physiological conditions.<sup>[2,6,7–9]</sup> The corresponding second-order rate constants,  $k_{\text{GSH}}$ , provide the experimental basis for structure–activity relationships that comprise, for example,  $\alpha,\beta$ -unsaturated aldehydes, ketones, and esters.<sup>[2,6g]</sup>

The kinetics of Michael additions of carbon-centered reference nucleophiles were extensively studied by Mayr and co-workers, who used Equation (1) to establish an ordering system for the electrophilic reactivity of structurally diverse Michael acceptors.<sup>[10]</sup> Equation (1) is a linear free energy relationship that calculates solvent-independent electrophilicity parameters  $E$  from experimentally determined second-order rate constants  $k_2$  for the reactions of electrophiles with nucleophiles of known nucleophilic reactivities  $N$  and susceptibilities  $s_N$  (in a certain solvent).<sup>[11]</sup>

$$\lg k_2(20^\circ\text{C}) = s_N(N + E) \quad (1)$$

Kamiya, Urano, and co-workers recently demonstrated that Mayr's electrophilicities  $E$  provide a useful guide for the rational design of real-time dynamic GSH fluorescent probes.<sup>[4a]</sup> We therefore set out to interconnect electrophilicity rankings for Michael acceptors originating from rate constants of their reactions with GSH<sup>[2d,7–9]</sup> with those relying on Mayr electrophilicities  $E$ .<sup>[10,12]</sup> To achieve the contact between both reactivity scales we determined the Mayr nucleophilicity of GSH in aqueous solution from the kinetics of its reactions with the reference electrophiles **E1–E17** (Figure 1). As a consequence, the mutual interconversion of known electrophilic reactivities  $\lg k_{\text{GSH}}$  and  $E$  becomes possible.



**Figure 1.** Reference electrophiles **E1–E17** used in this study (counterion for benzhydrylium ions **E1–E4**:  $\text{BF}_4^-$ ; electrophilicities  $E$  from Refs. [10, 11b, 12]).

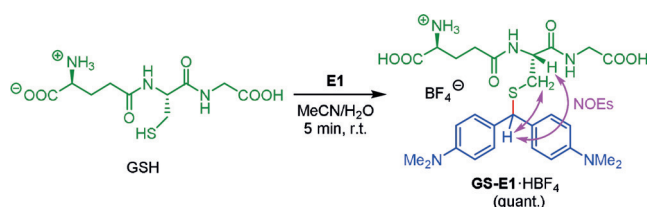
Dropwise addition of a deeply blue acetonitrile solution of the electrophile **E1**<sup>[13]</sup> to a neutral, aqueous solution of GSH led to fading of the blue color within seconds because of the rapid formation of the colorless S-benzhydrylated adduct **GS-E1**· $\text{HBF}_4$  (Scheme 1).

Thiolate attack at the cationic center of **E1** also occurred, though significantly faster, when **E1** was added to an alkaline, aqueous solution of GSH to yield **GS-E1**. Competing attack of the  $\gamma$ -glutamyl  $\text{NH}_2$  group of GSH at **E1** was not detected (see the Supporting Information), in agreement with the rate ratio of  $> 100:1$  for the  $\text{S}^-/\text{NH}_2$  attack of GSH at acrylonitrile (pH 8.1, 30°C) determined by Friedman and co-workers.<sup>[7a,14]</sup> Accordingly, the analogous reaction of **E1** with *N*-acetylcys-

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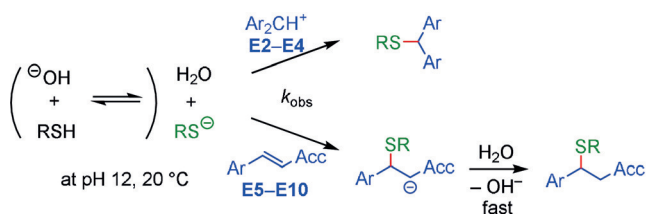


**Scheme 1.** Adduct formation between GSH and the benzhydrylium tetrafluoroborate **E1** in neutral, aqueous solution. NOESY experiments indicated a benzhydrylated Cys moiety in **GS-E1**.

teine (AcCys), which is devoid of a reactive  $\text{NH}_2$  group, gave rise to *S*-benzhydrylated AcCys (see the Supporting Information).

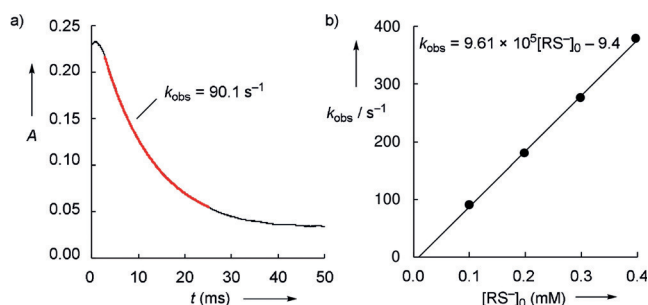
Owing to the similar acidities of the thiol and the ammonium group of  $\text{GSH}(\text{NH}_3^+/\text{SH})$ , they are deprotonated simultaneously in the pH range 7 to 12, producing two reactive thiolate species, that is,  $\text{GSH}(\text{NH}_3^+/\text{S}^-)$  and  $\text{GSH}(\text{NH}_2/\text{S}^-)$ . Depending on the pH value, variable fractions of the individual thiolates are present in the aqueous solutions, and microscopic ionization constants are needed to describe the acid–base equilibria<sup>[7a,15]</sup> (see the Supporting Information).

The rates of GSH adduct formation with reference electrophiles **E2–E10** were investigated at pH 12 in aqueous solution (20 °C). At this pH, the thiol groups of  $\text{GSH}^{[15a-c]}$  and  $\text{AcCys}^{[15d]}$  respectively, are almost quantitatively (>99%) deprotonated to the corresponding thiolates  $\text{RS}^-$  [that is,  $\text{GSH}(\text{NH}_2/\text{S}^-)$  from GSH]. The thiolates  $\text{RS}^-$  add directly to the cationic reference electrophiles **E2–E4** or undergo conjugate additions to the neutral Michael acceptors **E5–E10** (Scheme 2).



**Scheme 2.** Reactions of cationic and neutral electrophiles **E** with thiolate ions generated from GSH or AcCys in alkaline, aqueous solutions.

The kinetics of the covalent bond formation between the deprotonated GSH (or AcCys) and the electrophiles **E** were monitored by following the decay of the UV/Vis absorbance of the colored cationic or neutral electrophiles by using the stopped-flow technique (see the Supporting Information). With thiolate ions at at least tenfold higher concentrations than their electrophilic reaction partners (pseudo-first-order conditions), we observed rapid mono-exponential decays of the electrophile concentrations. First-order rate constants  $k_{\text{obs}}$  ( $\text{s}^{-1}$ ) were obtained by least-squares fitting of the single-exponential function  $A_t = A_0 e^{-k_{\text{obs}} t} + C$  to the time-dependent absorbance (Figure 2 a).



**Figure 2.** a) Decay of the absorbance  $A$  (at 383 nm) in the reaction of GSH ( $c = 1.00 \times 10^{-4}$  M) with **E5** ( $c = 1.03 \times 10^{-5}$  M) at 20 °C (aqueous solution, pH 12). The fitted mono-exponential function is depicted in red. b) The slope of the linear correlation of the first-order rate constant  $k_{\text{obs}}$  with the initial concentration of the GSH thiolate  $[\text{RS}^-]_0$  was used to derive the second-order rate constant  $k_2$  for the attack of the GSH thiolate at the Michael acceptor **E5**.

Table 1 lists the second-order rate constants  $k_2$  ( $\text{M}^{-1} \text{s}^{-1}$ ) for the attack of thiolate ions generated from GSH and AcCys, respectively, at the reference electrophiles **E2–E10**, which (according to  $k_{\text{obs}} = k_2 [\text{RS}^-]_0$ ) were obtained as the slopes of the linear correlations of  $k_{\text{obs}}$  with the thiolate concentrations

**Table 1:** Second-order rate constants  $k_2$  for the reactions of **E2–E10** with thiolates generated from glutathione (GSH), *N*-acetylcysteine (AcCys), and cysteine (Cys) by deprotonation at pH 12 in aqueous solution at 20 °C.

Electrophile	$k_2$ ( $\text{M}^{-1} \text{s}^{-1}$ )		
	GSH	AcCys	Cys <sup>[a]</sup>
<b>E2</b>	$2.70 \times 10^6$	$1.47 \times 10^6$	$1.29 \times 10^6$
<b>E3</b>	$1.37 \times 10^6$	$5.57 \times 10^5$	$6.41 \times 10^5$
<b>E4</b>	$6.60 \times 10^5$	$3.47 \times 10^5$	$3.79 \times 10^5$
<b>E5</b>	$9.61 \times 10^5$	$5.21 \times 10^5$	–
<b>E6</b>	$7.60 \times 10^4$	–	–
<b>E7</b>	$1.46 \times 10^5$	–	–
<b>E8</b>	$3.62 \times 10^4$	$3.14 \times 10^4$	–
<b>E9</b>	$2.58 \times 10^5$	$1.96 \times 10^5$	–
<b>E10</b>	$5.31 \times 10^4$	$4.12 \times 10^4$	–

[a] With  $k_2$  from Ref. [16].

(as exemplified for the reaction of GSH with **E5** in Figure 2 b and for all other combinations in the Supporting Information). Table 1 also comprises rate constants for the reactions of cysteine with benzhydrylium ions **E2–E4**, which were determined by Brotzel and Mayr.<sup>[16]</sup> For each of the electrophiles **E2–E10**, the reactivities towards GSH and the less functionalized AcCys (or Cys) agree within a factor of 2.5. We conclude from this comparison that exclusively the thiolate reactivity of  $\text{GSH}(\text{NH}_2/\text{S}^-)$  was detected in our kinetic measurements.

Kinetic assays used to investigate GSH reactivity towards electrophilic targets, such as Michael acceptors, are performed in buffered solutions at physiological pH, that is, usually at pH 7.4. In the range pH 7 to 8, only  $\text{GSH}(\text{NH}_3^+/\text{S}^-)$  is formed as a reactive thiolate. Although weakly populated (1–10% of  $[\text{GSH}]_0$ ),<sup>[15a-c]</sup> this fraction  $F$  of nucleophilic thiolate ions accounts for the observed GSH reactivity

towards electrophiles under physiological conditions. Equation (2) allows one to convert the second-order rate constants  $k_{\text{GSH}}$  into second-order rate constants  $k_2$  for the corresponding  $\text{GSH}(\text{NH}_3^+/\text{S}^-)$  thiolate reactions.

$$k_2 (\text{M}^{-1} \text{s}^{-1}) = k_{\text{GSH}}/F \quad (2)$$

Reported pH-dependent second-order rate constants  $k_{\text{GSH}}$ <sup>[2d,7]</sup> for the reactions of GSH with the Michael acceptors **E11–E17**, whose Mayr electrophilicities  $E$  are known, are compiled in Table 2 along with the second-order rate constants  $k_2$  for the corresponding thiolate reactivity [from Eq. (2)].

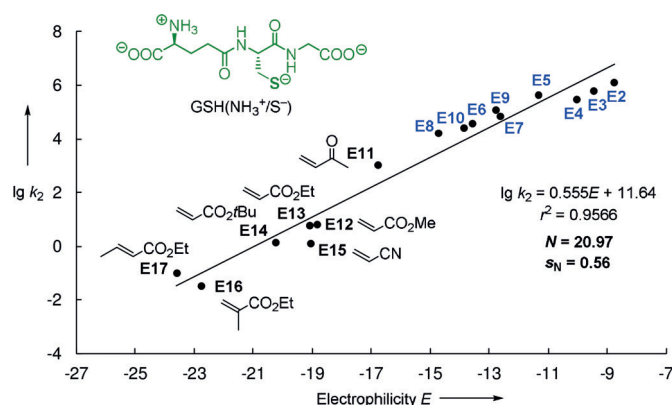
**Table 2:** Second-order rate constants  $k_{\text{GSH}}$  as reported for the reactions of GSH with electrophiles **E11–E17** at a certain pH value in aqueous, buffered solutions and second-order rate constants  $k_2$  [calculated by using Eq. (2)] for the reactions of the  $\text{GSH}(\text{NH}_3^+/\text{S}^-)$  thiolate.

Electrophile	$E^{\text{[a]}}$	$k_{\text{GSH}} (\text{M}^{-1} \text{s}^{-1})$	$k_2 (\text{M}^{-1} \text{s}^{-1})$
<b>E11</b>	-16.76	$3.19 \times 10^1$ (pH 7.4) <sup>[b]</sup>	$1.14 \times 10^3$
<b>E12</b>	-18.84	$1.90 \times 10^{-1}$ (pH 7.4) <sup>[c]</sup>	6.79
<b>E13</b>	-19.07	$1.77 \times 10^{-1}$ (pH 7.4) <sup>[c]</sup>	6.31
<b>E14</b>	-20.22	$4.17 \times 10^{-2}$ (pH 7.4) <sup>[c]</sup>	1.49
<b>E15</b>	-19.05	$1.73 \times 10^{-1}$ (pH 8.1) <sup>[d]</sup>	1.36
<b>E16</b>	-22.77	$9.67 \times 10^{-4}$ (pH 7.4) <sup>[c]</sup>	$3.45 \times 10^{-2}$
<b>E17</b>	-23.59	$3.10 \times 10^{-3}$ (pH 7.4) <sup>[c]</sup>	$1.11 \times 10^{-1}$

[a] Mayr electrophilicities  $E$  from Ref. [10]. [b] At 20 °C, from Ref. [7b];  $F = 0.0280$ . [c] At 25 °C, from Ref. [2d];  $F = 0.0280$ . [d] At 30 °C, from Ref. [7a];  $F = 0.127$ .

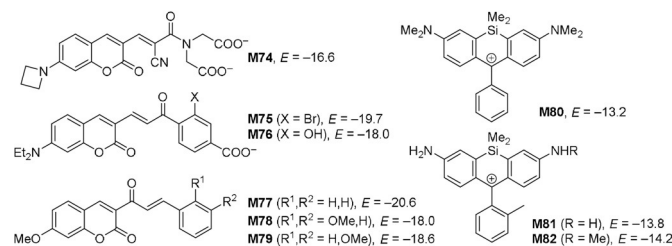
As shown in Table 1, the relative electrophilic reactivities  $E$  of **E2–E10** also hold for their reactions towards the  $\text{GSH}(\text{NH}_2/\text{S}^-)$  thiolate in aqueous solution. Friedman showed that protonation at the remote  $\gamma$ -glutamyl  $\text{NH}_2$  group in GSH reduces the thiolate reactivity by a factor of  $2^{[7a]}$  (see the Supporting Information for evidence that this factor is also appropriate in our studies). By applying Friedman's factor to convert the  $k_2$  values from Table 1, a linear correlation over 15 orders of magnitude of electrophilic reactivity results from the combined set of second-order rate constants for reactions of the thiolate  $\text{GSH}(\text{NH}_3^+/\text{S}^-)$  with **E2–E17** when correlated with their  $E$  descriptors (Figure 3).<sup>[17]</sup> The slope of the linear correlation in Figure 3 corresponds to  $s_{\text{N}}$  ( $=0.56$ ) for the thiolate  $\text{GSH}(\text{NH}_3^+/\text{S}^-)$  in water, and from the intercept with the abscissa, a nucleophilicity of  $N = 20.97$  is obtained.

Conversely, the thus determined GSH nucleophilicity ( $N$ ,  $s_{\text{N}}$ ) allows one to estimate the electrophilicity for Michael acceptors whose reactivity had thus far only been investigated in kinetic GSH assays.<sup>[2d,7–9]</sup> After converting the reported rate constants  $k_{\text{GSH}}$  into  $k_2$  [Eq. (2)], Equation (1) was used to assess  $E$  for various enones, ynones, enals, ynals, and further  $\alpha,\beta$ -unsaturated carbonyl compounds **M1–M73** (see Table S4 in the Supporting Information). For example, applying  $k_{\text{GSH}} = 8.12 \text{ M}^{-1} \text{ s}^{-1}$  (pH 7.4, 25 °C, from Ref. [2d]) for 2-octynal (**M28**) in Equation (2) yields  $k_2 = 290 \text{ M}^{-1} \text{ s}^{-1}$ . After inserting  $k_2$  into Equation (1), the electrophilic reactivity of 2-octynal is rated with  $E = -16.5$ . Analogously, electrophilicities  $E$  of another



**Figure 3.** Determination of the nucleophilicity of  $\text{GSH}(\text{NH}_3^+/\text{S}^-)$  from the linear plot of  $\lg k_2$  for its reactions with the electrophiles **E2–E17** (with  $0.5 k_2$  from Table 1 and  $k_2$  from Table 2) versus the electrophilicity parameters  $E$ .

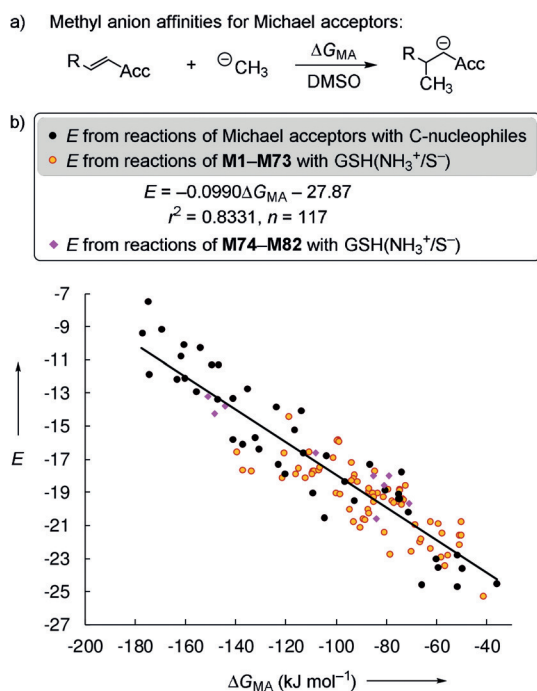
72 Michael acceptors within the range of  $-14.4 \leq E \leq -25.3$  were estimated.<sup>[18]</sup> Available rate constants furthermore create opportunities to integrate the recently developed dynamic GSH fluorescent probes **M74–M82** into Mayr's electrophilicity scale (Scheme 3).<sup>[3a,4b,19]</sup>



**Scheme 3.** Estimated electrophilicities  $E$  of the GSH fluorescent probes **M74–M82** (see Table S7 in the Supporting Information for details).

It was proposed that quantum-chemically calculated methyl anion affinities  $\Delta G_{\text{MA}}$  (Figure 4a) could be used to predict trends in the electrophilic reactivities  $E$  of Michael acceptors.<sup>[10]</sup> The  $E$  vs.  $\Delta G_{\text{MA}}$  correlation for the Michael acceptors with  $E$  derived from reactions with carbon-centered nucleophiles was reported to be linear ( $r^2 = 0.8857$ ,  $n = 44$ , black dots in Figure 4b).<sup>[10]</sup> The  $E$  parameters estimated in this work solely from a single rate constant in kinetic GSH assays cannot be expected to be as accurate as classical electrophilicities  $E$ ,<sup>[18]</sup> which are based on evaluating a set of kinetics for C–C bond-forming reactions with carbon-centered reference nucleophiles.<sup>[10]</sup> To assess the general consistency, however, we included calculated  $\Delta G_{\text{MA}}$  values for the Michael acceptors **M1–M73** with GSH-derived electrophilicities  $E$  (orange dots) in Figure 4b. Although the correlation coefficient decreases to  $r^2 = 0.8331$  ( $n = 117$ ), the scattering range of the  $E$  vs.  $\Delta G_{\text{MA}}$  correlation does not widen significantly when GSH-based  $E$  values are included. Purple rhombs in Figure 4b show additional entries for **M74–M82**. Their positions indicate that the  $E$  vs.  $\Delta G_{\text{MA}}$  correlation for prototypical Michael acceptors also holds for roughly esti-





**Figure 4.** a) Definition of the methyl anion affinities ( $\Delta G_{\text{MA}}$ ) of Michael acceptors. b) Correlation between experimentally determined electrophilicities  $E$  and methyl anion affinities  $\Delta G_{\text{MA}}$  in DMSO [calculated at the SMD(DMSO)/B3LYP/6-311 + G(3df,2pd)//B3LYP/6-31G(d,p) level of theory, see Supporting Information for details] supplemented by data for the fluorescent probes **M74–M82** (not included when calculating the correlation line).

mating the reactivity of the structurally more sophisticated fluorescent probes **M74–M82**.

Within a reactivity range that currently covers 40 orders of magnitude, using Equation (1) usually allows chemists to calculate second-order rate constants within a precision of factor  $< 100$  for reactions at  $20^\circ\text{C}$ , in which exactly one new C–X or C–C  $\sigma$ -bond is formed.<sup>[20]</sup> Table S6 collects 80 experimental second-order rate constants  $k_2^{\text{exp}}$  for the reactions of Michael acceptors with structurally diverse N-, O-, or S-centered nucleophiles in different solvents at temperatures between 19 and  $30^\circ\text{C}$ . The comparison with second-order rate constants  $k_2^{\text{eq1}}$  ( $20^\circ\text{C}$ ) calculated by using Equation (1) and the reactivity parameters  $E$ ,  $N$ , and  $s_N$  shows that  $k_2^{\text{exp}}/k_2^{\text{eq1}} < 100$  is fulfilled for 71 (of 80) Michael additions (Table S6). This analysis confirms that the GSH-derived  $E$  values for Michael acceptors can also be used to predict reaction rates for Michael additions of other classes of nucleophiles.<sup>[21]</sup> Rates of Corey–Chaykovsky cyclopropanations,<sup>[22]</sup> stepwise Huisgen reactions,<sup>[10]</sup> Weitz–Scheffer epoxidations,<sup>[23]</sup> cyanoethylyations,<sup>[24]</sup> or simple 1,4-additions of Michael acceptors with amines, alkoxide ions, and thiolates<sup>[24]</sup> can thus be assessed by using Equation (1) and the available reactivity parameters  $E$ ,  $N$ , and  $s_N$ .<sup>[12]</sup>

In conclusion, the nucleophilic reactivity for the thiolate  $\text{GSH}(\text{NH}_3^+/\text{S}^-)$  in water ( $N = 20.97$ ;  $s_N = 0.56$ ) has been established. If  $k_2 = 10^{-3} \text{M}^{-1} \text{s}^{-1}$  is considered as a minimum rate constant for practical reactions at  $20^\circ\text{C}$ ,<sup>[11c]</sup>  $\text{GSH}(\text{NH}_3^+/\text{S}^-)$  can be expected to react successfully with electrophiles of

$E > -26$ . This limiting  $E$  value may give orientation for the future development of so-called “warheads” in covalently inhibiting drugs.<sup>[2,6]</sup> The applicability of Mayr reactivity parameters for the rational design of dynamic real-time GSH-selective fluorescent probes has already been shown.<sup>[4a]</sup> The conversion of GSH reactivities ( $\lg k_{\text{GSH}}$ ) into Mayr  $E$  values (Tables S4 and S7), as proposed in this work, enables the prediction of relevant second-order rate constants for competing nucleophilic sites, which is a frequent challenge in the development of thiol-selective probe molecules.<sup>[3,4]</sup> Furthermore, the GSH-based estimated electrophilicities of  $> 70$  Michael acceptors provide new insight into general structure–reactivity relationships (Figure S4).<sup>[6g]</sup> The estimated Mayr  $E$  parameters considerably enrich the structural diversity in the chemist’s toolkit for the systematic prediction of thus far unexplored 1,4-additions of Michael acceptors to a wide range of C-, N-, P-, O-, and S-centered nucleophiles for which  $N$  and  $s_N$  are known<sup>[12]</sup> (Figure S5).

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## Conflict of interest

The authors declare no conflict of interest.

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- [1] R. M. LoPachin, T. Gavin, *Free Radical Res.* **2016**, *50*, 195–202.
- [2] a) T. W. Schultz, J. W. Yarbrough, E. L. Johnson, *SAR QSAR Environ. Res.* **2005**, *16*, 313–322; b) J. W. Yarbrough, T. W. Schultz, *Chem. Res. Toxicol.* **2007**, *20*, 558–562; c) A. Böhme, D. Thaens, A. Paschke, G. Schüürmann, *Chem. Res. Toxicol.* **2009**, *22*, 742–750; d) A. Böhme, A. Laqua, G. Schüürmann, *Chem. Res. Toxicol.* **2016**, *29*, 952–962.
- [3] a) X. Jiang, J. Chen, A. Bajić, C. Zhang, X. Song, S. L. Carroll, Z.-L. Cai, M. Tang, M. Xue, N. Cheng, C. P. Schaaf, F. Li, K. R. MacKenzie, A. C. M. Ferreón, F. Xia, M. C. Wang, M. Maletić-Savatić, J. Wang, *Nat. Commun.* **2017**, *8*, 16087; b) C. Cossetti, G. Di Giovamberardino, R. Rota, A. Pastore, *Nat. Commun.* **2018**, *9*, 1588.
- [4] a) K. Umezawa, M. Yoshida, M. Kamiya, T. Yamasoba, Y. Urano, *Nat. Chem.* **2017**, *9*, 279–286; b) G. Yin, T. Niu, T. Yu, Y. Gan, X. Sun, P. Yin, H. Chen, Y. Zhang, H. Li, S. Yao, *Angew. Chem. Int. Ed.* **2019**, *58*, 4557–4561; *Angew. Chem.* **2019**, *131*, 4605–4609.
- [5] a) X. Chen, Y. Zhou, X. Peng, J. Yoon, *Chem. Soc. Rev.* **2010**, *39*, 2120–2135; b) S. Lee, J. Li, X. Zhou, J. Yin, J. Yoon, *Coord. Chem. Rev.* **2018**, *366*, 29–68.

- [6] a) S. Amslinger, *ChemMedChem* **2010**, *5*, 351–356; b) I. M. Serafimova, M. A. Pufall, S. Krishnan, K. Duda, M. S. Cohen, R. L. Maglathlin, J. M. McFarland, R. M. Miller, M. Frödin, J. Taunton, *Nat. Chem. Biol.* **2012**, *8*, 471–476; c) N. J. Pace, E. Weerapana, *ACS Chem. Biol.* **2013**, *8*, 283–296; d) S. Krishnan, R. M. Miller, B. Tian, R. D. Mullins, M. P. Jacobson, J. Taunton, *J. Am. Chem. Soc.* **2014**, *136*, 12624–12630; e) E. H. Krenske, R. C. Petter, K. N. Houk, *J. Org. Chem.* **2016**, *81*, 11726–11733; f) R. Lonsdale, J. Burgess, N. Colclough, N. L. Davies, E. M. Lenz, A. L. Orton, R. A. Ward, *J. Chem. Inf. Model.* **2017**, *57*, 3124–3137; g) P. A. Jackson, J. C. Widen, D. A. Harki, K. M. Brummond, *J. Med. Chem.* **2017**, *60*, 839–885; h) J. M. Strelow, *SLAS Discov.* **2017**, *22*, 3–20; i) Z. Zhao, P. E. Bourne, *Drug Discovery Today* **2018**, *23*, 727–735; j) R. Lonsdale, R. A. Ward, *Chem. Soc. Rev.* **2018**, *47*, 3816–3830; k) A. Keeley, P. Ábrányi-Balogh, G. M. Keserü, *MedChemComm* **2019**, *10*, 263–267; l) for the kinetics of GSH binding to a series of acrylamides at 37°C, see: V. J. Cee, L. P. Volak, Y. Chen, M. D. Bartberger, C. Tegley, T. Arvedson, J. McCarter, A. S. Tasker, C. Fotsch, *J. Med. Chem.* **2015**, *58*, 9171–9178.
- [7] a) M. Friedman, J. F. Cavins, J. S. Wall, *J. Am. Chem. Soc.* **1965**, *87*, 3672–3682; b) H. Esterbauer, H. Zollner, N. Scholz, *Z. Naturforsch. C* **1975**, *30*, 466–473.
- [8] a) G. Eisenbrand, J. Schuhmacher, P. Gölzer, *Chem. Res. Toxicol.* **1995**, *8*, 40–46; b) K. Chan, R. Poon, P. J. O'Brien, *J. Appl. Toxicol.* **2008**, *28*, 1027–1039.
- [9] J. A. H. Schwöbel, D. Wondrousch, Y. K. Koleva, J. C. Madden, M. T. D. Cronin, G. Schüürmann, *Chem. Res. Toxicol.* **2010**, *23*, 1576–1585.
- [10] D. S. Allgäuer, H. Jangra, H. Asahara, Z. Li, Q. Chen, H. Zipse, A. R. Ofial, H. Mayr, *J. Am. Chem. Soc.* **2017**, *139*, 13318–13329.
- [11] a) H. Mayr, M. Patz, *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 938–957; *Angew. Chem.* **1994**, *106*, 990–1010; b) H. Mayr, T. Bug, M. F. Gotta, N. Hering, B. Irrgang, B. Janker, B. Kempf, R. Loos, A. R. Ofial, G. Remennikov, H. Schimmel, *J. Am. Chem. Soc.* **2001**, *123*, 9500–9512; c) H. Mayr, A. R. Ofial, *SAR QSAR Environ. Res.* **2015**, *26*, 619–646.
- [12] A free database of reactivity parameters  $E$ ,  $N$ , and  $s_N$  can be accessed at: <http://www.cup.lmu.de/oc/mayr/DBintro.html>.
- [13] R. J. Mayer, N. Hampel, P. Mayer, A. R. Ofial, H. Mayr, *Eur. J. Org. Chem.* **2019**, 412–421.
- [14] a) J. A. H. Schwöbel, Y. K. Koleva, S. J. Enoch, F. Bajot, M. Hewitt, J. C. Madden, D. W. Roberts, T. W. Schultz, M. T. D. Cronin, *Chem. Rev.* **2011**, *111*, 2562–2596; b) for the detection of the thermodynamically preferred, terminal glutamyl N-bound 1:1 adducts of GSH and hexenones by MS techniques after 24 h reaction time, see: C. Slawik, C. Rickmeyer, M. Brehm, A. Böhme, G. Schüürmann, *Environ. Sci. Technol.* **2017**, *51*, 4018–4026.
- [15] a) D. L. Rabenstein, *J. Am. Chem. Soc.* **1973**, *95*, 2797–2803; b) A. Mirzahosseini, M. Somlyay, B. Noszá, *Chem. Phys. Lett.* **2015**, *622*, 50–56; c) the pH-dependent thiolate concentrations for GSH(NH<sub>3</sub><sup>+</sup>/S<sup>−</sup>) and GSH(NH<sub>2</sub>/S<sup>−</sup>) were calculated by using the GSH ionization scheme of Rabenstein (Ref. [15a]) and the corresponding microscopic ionization constants from Ref. [15b] (see the Supporting Information for details); d) for the SH acidity of AcCys (pK<sub>a</sub> 9.62), see: A. Meißner, P. Gockel, H. Vahrenkamp, *Chem. Ber.* **1994**, *127*, 1235–1241.
- [16] F. Brotzel, H. Mayr, *Org. Biomol. Chem.* **2007**, *5*, 3814–3820.
- [17] Second-order rate constants determined at different temperatures (from 20 to 30°C) were used indiscriminately to construct Figure 3.
- [18] We estimate that  $E(\mathbf{M1-M73})$  may be assessed with a precision of  $\pm 2$  units in  $E$  if only rate constants from kinetic GSH assays are available.
- [19] a) X. Jiang, Y. Yu, J. Chen, M. Zhao, H. Chen, X. Song, A. J. Matzuk, S. L. Carroll, X. Tan, A. Sizovs, N. Cheng, M. C. Wang, J. Wang, *ACS Chem. Biol.* **2015**, *10*, 864–874; b) J. Chen, X. Jiang, S. L. Carroll, J. Huang, J. Wang, *Org. Lett.* **2015**, *17*, 5978–5981; c) O. García-Beltrán, C. González, E. G. Pérez, B. K. Cassels, J. G. Santos, D. Millán, N. Mena, P. Pavez, M. E. Aliaga, *J. Phys. Org. Chem.* **2012**, *25*, 946–952.
- [20] H. Mayr, *Angew. Chem. Int. Ed.* **2011**, *50*, 3612–3618; *Angew. Chem.* **2011**, *123*, 3692–3698.
- [21] Only for methyl crotonate (**M61**), the ratio  $k_2^{\text{exp}}/k_2^{\text{eq1}}$  slightly exceeds a factor of 100 for five of six available  $k_2^{\text{exp}}$  values at 30°C, which may in part be due to the 10 K difference in the reference temperatures for  $k_2^{\text{exp}}$  and  $k_2^{\text{eq1}}$ .
- [22] R. Appel, N. Hartmann, H. Mayr, *J. Am. Chem. Soc.* **2010**, *132*, 17894–17900.
- [23] a) R. J. Mayer, T. Tokuyasu, P. Mayer, J. Gomar, S. Sabelle, B. Mennucci, H. Mayr, A. R. Ofial, *Angew. Chem. Int. Ed.* **2017**, *56*, 13279–13282; *Angew. Chem.* **2017**, *129*, 13463–13467; b) R. J. Mayer, A. R. Ofial, *Org. Lett.* **2018**, *20*, 2816–2820; c) R. J. Mayer, A. R. Ofial, *Eur. J. Org. Chem.* **2018**, 6010–6017.
- [24] For references, see Table S6 in the Supporting Information.

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