Bioconjugation

International Edition: DOI: 10.1002/anie.201510321
German Edition: DOI: 10.1002/ange.201510321

Fast and Tight Boronate Formation for Click Bioorthogonal Conjugation

*Burcin Akgun and Dennis G. Hall**

Abstract: *A new click bioorthogonal reaction system was devised to enable the fast ligation ($k_{ON} \approx 340\ M^{-1}s^{-1}$) of conjugatable derivatives of a rigid cyclic diol (nopoldiol) and a carefully optimized boronic acid partner, 2-methyl-5-carboxymethylphenylboronic acid. Using NMR and fluorescence spectroscopy studies, the corresponding boronates were found to form reversibly within minutes at low micromolar concentration in water, providing submicromolar equilibrium constant ($K_{eq} \approx 10^5$–$10^6\ M^{-1}$). Efficient protein conjugation under physiological conditions was demonstrated with model proteins thioredoxin and albumin, and characterized by mass spectrometry and gel electrophoresis.*

The development of rapid and bioorthogonal chemical reactions has greatly expanded the utility of bioconjugation chemistry in the service of chemical biology, even allowing molecular imaging in live cells or animals.[1] To this end, click reactions, spontaneous bond-forming processes that can occur rapidly in aqueous media, are particularly desirable.[2] Unfortunately, the repertoire of click reactions between abiotic and biocompatible functional groups is relatively small, and some of the most popular and efficient reactions present inconveniences, such as slow rates and side-reactions that may limit some of their applications in living systems.[3,4] Most importantly, many common bioorthogonal reactions are not mutually orthogonal due to possible cross-reactions,[5] and thus new reactions would provide more options for interrogating cellular processes in tandem. In this context, boronic ester formation, a process better known in the field of carbohydrate sensing,[6] has remained an underexplored option in bioconjugation despite recent applications of boronic acids in surface immobilization,[7] detection of reactive oxygen/nitrogen species,[8] and protein modification.[9] Simplicity (no catalyst required), bioorthogonality (boronic acids complex biological diols with low affinities), fast on-rates, and potential reversibility are many possible attributes of boronate bioconjugation. Although a recent phenylboronic acid–salicylhydroxamic acid conjugation system shows promise, it displays moderate binding affinity ($K_{eq} \approx 17\,800\ M^{-1}$).[10] The design of a tight boronate conjugation system is hampered by mechanistic ambiguities[11] and a dearth of comprehensive kinetic data (k_{ON}, k_{OFF}, K_{eq}) on boronate formation in water.[12] This information would enable a systematic optimization of both diol and boronic acid partners. As a condensation reaction, boronic ester formation is intrinsically unfavored in water owing to Le Châtelier's principle. Thermodynamically, the process is driven solely by the enthalpy of ring formation because the balance of bond enthalpy is neutral (two O–H and two B–O bonds are broken to form four similar bonds). It is also well-established that hindered, pre-organized vicinal diols mitigate the loss of entropy in the diol substrate, and are thus preferred.[13] It is therefore unsurprising that pinanediol affords some of the most hydrolytically robust boronic esters.[14] There is no knowledge, however, about the rates of pinanediol boronate formation in aqueous solvents, the question of its reversibility, and the structural requirements (sterics and electronics) for an optimal boronic acid partner. Herein, using the conjugatable pinanediol derivative nopoldiol, we report the design and proof-of-concept of a fast ($k_{ON} \approx 340\ M^{-1}s^{-1}$) and tight-binding ($K_{eq} \approx 10^5$–$10^6\ M^{-1}$) click boronate bioconjugation system (Figure 1).[15]

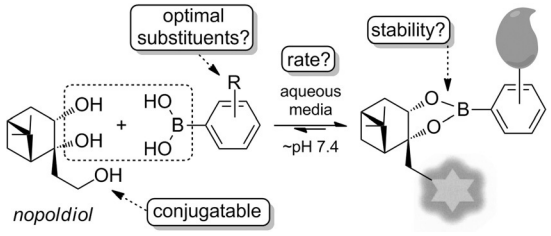


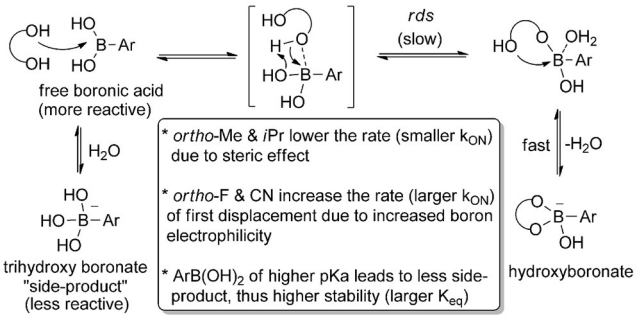
Figure 1. Design of a bioorthogonal click boronate conjugation using conjugatable nopoldiol derivatives and optimal arylboronic acids in neutral aqueous media.

To identify fast-forming boronates in aqueous medium, a model water-soluble nopol-PEG-diol (**1a**, Table 1) was synthesized (Supporting Information, Scheme S1).[16] We chose ^{1}H NMR spectroscopy as the initial method to screen various arylboronic acids owing to its convenience, even though its capability to monitor fast reactions is limited.[17] The low intrinsic sensitivity of NMR makes it difficult to detect very low concentrations ($< 100\ \mu M$), therefore it requires higher reaction concentration at which fast processes ($> 50\ M^{-1}s^{-1}$) are not readily monitored. Moreover, early rates (< 40 s) cannot be captured owing to the essential gradient shimming. Thus, because boronate formation is likely reversible, forward rate data obtained from NMR kinetics provide an underestimation of rates, which is however sufficient for a preliminary comparison and identifica-

[*] B. Akgun, Prof. Dr. D. G. Hall
Department of Chemistry, 4-010 CCIS, University of Alberta
Edmonton, Alberta, T6G 2G2 (Canada)
E-mail: dennis.hall@ualberta.ca

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under http://dx.doi.org/10.1002/anie.201510321.

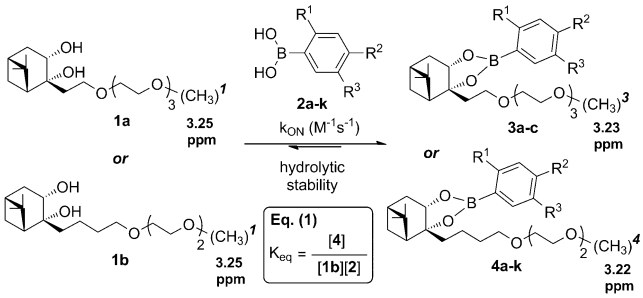
tion of promising reagents. In this way, NMR analysis allowed us to rapidly obtain relative hydrolytic stability data and forward rate constants (k_{ON}, $M^{-1}s^{-1}$) for a large set of arylboronic acids.[16] It was found that *ortho*-methyl or isopropyl groups improved the hydrolytic stability likely because of their bulkiness, while halogens and other electron-withdrawing groups decreased boronate stability, which is probably due to the increased acidity of these arylboronic acids. Hydrolytic stability showed a near-linear correlation with the acidity of arylboronics.[11d,16] This is indeed consistent with a recent report suggesting that more acidic arylboronic acids tend to form the sp^3 trihydroxyborate, a "side-product" shown to be less reactive compared to a neutral (sp^2) boronic acid, thereby shifting the equilibrium to the left and lowering boronate stability (Scheme 1).[11g] Moreover, the *ortho*-methyl substituent slowed down boronate formation (lower k_{ON}) while *ortho*-halogens and especially a cyano group accelerated it to a significant measure. The proposed reaction mechanism starts with the exchange of one of the two hydroxy groups of the boronic acid, which is likely the rate-determining step, followed by a fast cyclization to form the Lewis acidic boronic ester resting as a tetrahedral hydroxyboronate ion (Scheme 1).



Scheme 1. Proposed boronate formation mechanism.

Our initial NMR-based screening with diol **1a** led to a selection of three promising boronic acids (Table 1, entries 1–3). These superior reagents are *ortho*-tolylboronic acid (**2a**), which provided better boronate stability than phenylboronic acid,[16] *ortho*-fluorophenylboronic acid (**2b**), and *ortho*-cyanophenylboronic acid (**2c**), which both showed excellent k_{ON} values. We suspected that the proximal oxygen of **1a** might be detrimental by assisting hydrolysis. Thus, another nopol-PEG-diol (**1b**) was synthesized to examine this potential issue on the stability and k_{ON}.[16] Boronic acids **2a–c** were then tested with diol **1b**. To our satisfaction, their hydrolytic stability and k_{ON} both improved (entries 4–6). Other arylboronic acids were also evaluated with diol **1b**, but **2a–c** remained superior. We then proceeded to design, synthesize, and evaluate conjugatable derivatives of **2a–c** (entries 7–14). Their equilibrium constants (K_{eq}, M^{-1}) were also determined via ^{1}H NMR beside their forward rate constants (k_{ON}). Consistent with our initial screening results, the presence of an electron-withdrawing group (CO_2Me or $CONMe_2$) at either *meta or para* positions enhanced the

Table 1: Results of the hydrolytic stability study, forward rate constant k_{ON}, and association constant measurement K_{eq}[a]



Entry (**1a**/**b**)	**2a–k:** R^1, R^2, R^3	Hydrolytic stability[b] (**3**/**1a** or **4**/**1b**)	K_{eq} $\times 10^3$ [M^{-1}][c]	k_{ON} [$M^{-1}s^{-1}$][d]
1 (**1a**)	**2a**: Me, H, H	**3a**/**1a**: 87:13	–	1.6 ± 0.1
2 (**1a**)	**2b**: F, H, H	**3b**/**1a**: 78:22	–	18 ± 4
3 (**1a**)	**2c**: CN, H, H	**3c**/**1a**: 70:30	–	> 50[e]
4 (**1b**)	**2a:** Me, H, H	**4a**/**1b**: 93:7	180	2.3 ± 0.2
5 (**1b**)	**2b:** F, H, H	**4b**/**1b:** 84:16	27	33 ± 2
6 (**1b**)	**2c:** CN, H, H	**4c**/**1b:** 83:17	25	> 50[f]
7 (**1b**)	**2d:** Me, H, CO_2Me	**4d**/**1b:** 91:9	120	6.9 ± 0.6
8 (**1b**)	**2e:** Me, CO_2Me, H	**4e**/**1b:** 90:10	91	7.8 ± 0.7
9 (**1b**)	**2f:** Me, H, OMe	**4f**/**1b:** 92:8	130	3.3 ± 0.6
10 (**1b**)	**2g:** Me, OMe, H	**4g**/**1b:** 94:6	330	1.0 ± 0.2
11 (**1b**)	**2h:** F, H, $CONMe_2$	**4h**/**1b:** 75:25	15	> 50[f]
12 (**1b**)	**2i:** F, H, OMe	**4i**/**1b:** 82:18	25	> 50[f]
13 (**1b**)	**2j:** F, OMe, H	**4j**/**1b:** 85:15	40	18 ± 1
14 (**1b**)	**2k:** CN, H, CO_2Me	**4k**/**1b:** 78:22	12	> 50[f]

[a] See the Supporting Information, Table S1 for more detailed screening conditions. [b] Hydrolytic stability of boronates **3**/**4** was studied at 1 mM concentration by ^{1}H NMR in 0.1 M D_2O phosphate buffer (pD 7.4)/CD_3CN (65:35). The integral ratio of $(CH_3)^{3/4}$/$(CH_3)^1$ was monitored after 24 h and recorded as **3**/**1a** or **4**/**1b**. [c] Association constant measurement (K_{eq}, M^{-1}) was performed via ^{1}H NMR as described in the Supporting Information. [d] Second-order rate constant (k_{ON}, $M^{-1}s^{-1}$) was monitored by ^{1}H NMR. Compound **2** (1 equiv, 1 mM) and diol **1a** or **1b** (1 equiv, 1 mM) were mixed in 0.05 M D_2O phosphate buffer (pD 7.4)/CD_3CN (70:30) (The disappearance of $(CH_3)^1$ was monitored and converted to a forward rate constant k_{ON}). Average of at least three measurements ± standard deviation. [e] This reaction showed a circa 50% conversion in 30 seconds at room temperature. It slowed down at 0 °C and provided $k_{ON} = 49 \pm 2$ $M^{-1}s^{-1}$. [f] This reaction could not be monitored by NMR owing to its very fast conversion.

reactivity compared to boronic acids **2a–c**, and a decrease in K_{eq} was noted (compare entry 4 vs. 7,8; 5 vs. 11).

On the other hand, whereas an electron-donating group (OMe) impeded the reactivity (lower k_{ON}), it improved K_{eq} specifically when placed in the *para* position (compare entry 4 vs. 9,10; 5 vs. 12,13). Even though *ortho*-fluoro derivatives **2h–2j** enabled high reactivity, their lower hydrolytic stability caused concerns towards their use under the conditions of bioorthogonal conjugation at micromolar concentrations. Boronic acid **2k** was eliminated because of its lower K_{eq} and its susceptibility to deboronation. In the end, the *ortho*-tolylboronic acid derivative **2d** was selected because it best combines a high reactivity with excellent hydrolytic stability for the resulting boronates. Moreover, it is easily synthesized and the carboxyl handle is ideal for conjugation. Yet because the reaction of *ortho*-fluorophenylboronic acid derivative **2h**

was too fast to be monitored by NMR, a more sensitive fluorescence quenching assay was designed to compare the forward rate constants of **2d** and **2h**.

A dansyl-tagged nopoldiol (**5**) and dabsyl derivatives of the corresponding boronic acids (**6a–b**) were synthesized,[16] and initial forward rate constants (k_{ON}) were determined at 10 μM concentration (Figure 2). The results (**6a**; 340 ± 40 $M^{-1}s^{-1}$ and **6b**: 1200 ± 300 $M^{-1}s^{-1}$) are significantly higher than that obtained via ^{1}H NMR spectroscopy. As indicated above, it is likely that the NMR method underestimated the actual rates of these fast reactions. Their rough K_{eq} were also determined by allowing the corresponding boronate products (**7a**,**7b**) to reach equlibrium in the fluorescence quenching assay, giving K_{eq} values of about 15×10^6 and $1.3 \times 10^6\,M^{-1}$ for *ortho*-methyl and *ortho*-fluoro derivatives, respectively. High K_{eq} values for substrates **5** and **6** relative to ^{1}H NMR values (for **2d/2h** and **1b**) may be due to hydrophobic and π interactions between fluorescent moieties, dansyl and dabsyl. Nonetheless, equilibration times obtained from both methods are consistent, with **6a**/**7a** taking about 20 min to reach a steady state made of 85 % **7a** (from both directions, at 10 μM conc.), whereas **6b**/**7b** needed only 2 min to reach 60 % of **7b**.[16]

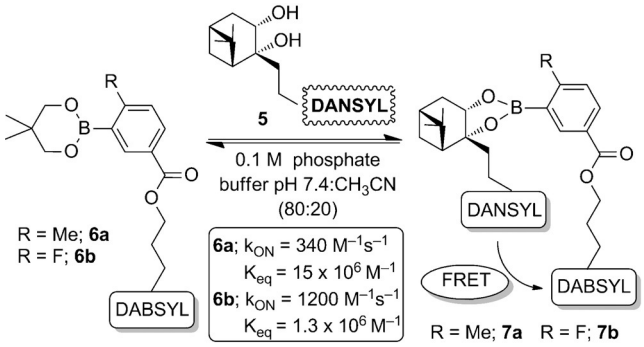


Figure 2. Kinetic analysis using a fluorescence quenching experiment to measure the initial rate of boronate formation. Reported k_{ON} and K_{eq} are the average of at least three measurements.[16]

A biological competition assay was designed to determine whether the optimal nopoldiol boronates compete favorably with biological polyols such as glucose (4–7 mM), fructose (8 μM), or catecholamines (ca. 0.0014 μM) found in the blood stream.[6a,18] Thus, diol **5** was allowed to form boronates with **2d** and **2h** in the presence of a mixture of these biocompetitors used at concentrations higher than that found in the blood (Figure 3).[16] This experiment was analyzed by HPLC. Fortunately, **2d** preserved its high affinity towards diol **5** in the presence of the biocompetitors. In contrast, **2h** showed none or little conversion, even in the absence of the biocompetitors. Although **2h** showed promise at higher concentrations, its conversion to boronate product is too low at micromolecular concentrations to be useful in bioconjugation.

The optimal boronic acid derivative **2d**, which fulfills the criteria of bioorthogonality, reactivity and stability, was used to demonstrate boronate ligation on proteins. Towards this end, bovine serum albumin (BSA), which contains one free

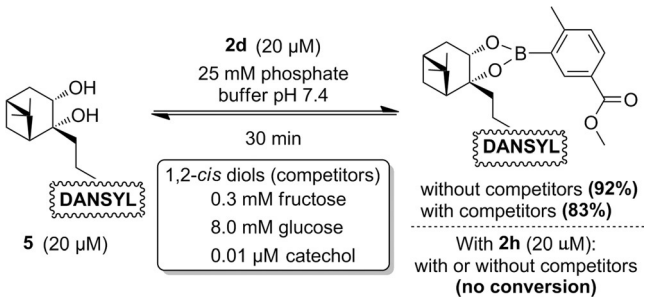


Figure 3. Competitive effect of biological polyols on boronate formation. Boronate conversions were immediately monitored and determined by HPLC under both UV and fluorescence after diol **5** and **2d**/**2h** were allowed to react together for 30 minutes.[16]

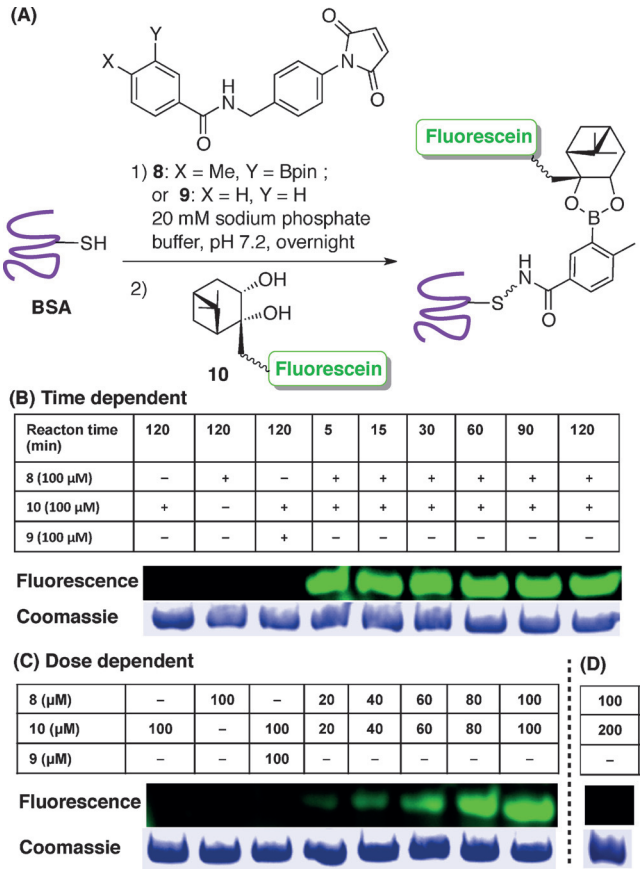


Figure 4. Labeling of boronic acid modified BSA with nopoldiol-fluorescein-diol (**10**) and gel results. A) Modification of free cysteine with **8** or **9** and labeling with **10**. B) Time-dependent and C) dose-dependent fluorescent labeling of BSA on gel containing phosphate buffer. Protein loading was determined by Coomassie blue staining. Compound **9** is a negative control probe devoid of a boronic acid. D) Fluorescent labeling of BSA on gel containing tris buffer, a competing triol that causes breakdown of conjugates.

cysteine, was allowed to react with the boronyl-containing maleimide **8** to form a boronic acid–BSA adduct (Figure 4 A). Then, click conjugation with fluorescein-derivatized nopoldiol **10** was conducted in both time and dose dependent fashion and monitored by gel electrophoresis and gel fluorescence imaging (Figure 4 B, C). As depicted in Fig-

ure 4B, the reaction between **10** and the boronic acid–BSA conjugate was complete within 5 minutes when 100 μM of **10** was employed. Even at lower concentrations of **8** and **10** (20 and 40 μM), a significant level of conjugation was detected (Figure 4C). As expected, when a maleimide adduct **9** devoid of a boronic acid head was used, no labeling was detected.[16]

With this promising result in hand, another selective protein labeling experiment was performed. This time, thioredoxin (Trx, 11.7 kDa; 50 μM), a protein with a single disulfide, was reduced with TCEP (1 mM) overnight followed by functionalization with boronyl-containing maleimide **8** (100 μM) in ammonium acetate buffer for 30 minutes (Figure 5). Following the addition of **1b** (200 μM) to the

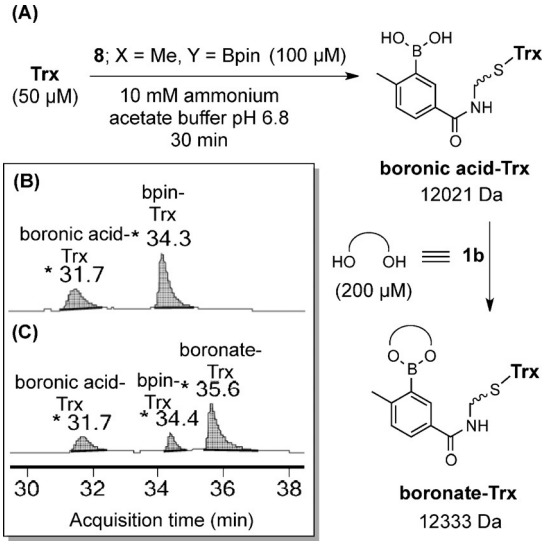


Figure 5. Boronate formation on thioredoxin (Trx) analyzed by HPLC-MS. A) Modification of Trx with **8** and subsequent labeling with **1b**. B) HPLC result without **1b**. C) HPLC result in the presence of **1b**.

mixture, HPLC and LC-MS were used to monitor and detect the boronate–Trx conjugate after a 30-minute reaction time. As shown in Figure 5B,C, the desired bioconjugate was formed with >50% conversion. Additionally, boronic acid–Trx was reacted with **1b** in the presence of fetal bovine serum. Even though the exact conversion could not be determined due to spectral overlap with serum compounds, a large amount of boronate–Trx was detected.[16] Moreover, to address the orthogonality of the reaction in complex protein mixtures, this experiment was repeated by mixing the boronic acid–Trx adduct with an equivalent volume of complete cell media prior to addition of dansyl-conjugated diol **5**. Boronate–Trx (83% conversion) was detected by HPLC-fluorescence and LC-MS, while no other new peaks from unselective labeling were observed (see the Supporting Information for full LC-MS chromatograms).[16]

In conclusion, we have developed a click bioorthogonal reaction system enabling the fast ligation (ca. 340 $M^{-1}s^{-1}$) of easily synthesized, conjugatable derivatives of nopoldiol and 2-methyl-5-carboxyphenylboronic acid (**2d**) to form tightly bound boronates with submicromolar equilibrium dissociation constant. For the first time, boronate formation with a rigid diol was studied thoroughly in water with an optimization of both reaction partners, thus providing useful knowledge of this important condensation process. Measured k_{ON} rates are faster than many existing bioconjugation systems, and a high K_{eq} enables high conversion with proteins under physiological conditions. Coupled with the ability to site-specifically encode boronic acid containing aminoacids in proteins,[9b,c] reversibility can be an additional asset when target turnover or time-based profiling are required. This preliminary account also suggests applications in affinity purification, surface immobilization, and materials chemistry.

Acknowledgements

This work was funded by the Natural Sciences and Engineering Research Council (NSERC) of Canada (Discovery Grant to DGH) and the University of Alberta. BA thanks Alberta Innovates—Technology Futures for a Graduate Scholarship. We thank Dr. Sylvain Bernard (preliminary experiments), Ed Fu (HPLC), Bela Reiz (Mass Spectrometry Laboratory), Gareth Lambkin (Biological Services), Nupur Dabral and Mark Miskolzie (NMR Laboratory), and Prof. Ratmir Derda for their help and suggestions.

Keywords: bioorthogonality · boronates · boronic acids · click chemistry · protein labeling

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 3909–3913
Angew. Chem. **2016**, *128*, 3977–3981

[1] See recent reviews: a) M. Zheng, L. Zheng, P. Zhang, J. Li, Y. Zhang, *Molecules* **2015**, *20*, 3190–3205; b) K. Lang, J. W. Chin, *ACS Chem. Biol.* **2014**, *9*, 16–20; c) C. P. Ramil, Q. Lin, *Chem. Commun.* **2013**, *49*, 11007–11022; d) E. M. Sletten, C. R. Bertozzi, *Acc. Chem. Res.* **2011**, *44*, 666–676; e) Y.-X. Chen, G. Triola, H. Waldmann, *Acc. Chem. Res.* **2011**, *44*, 762–773.

[2] Reviews: a) C. S. McKay, M. G. Finn, *Chem. Biol.* **2014**, *21*, 1075–1101; b) H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021; *Angew. Chem.* **2001**, *113*, 2056–2075.

[3] N. J. Agard, J. A. Prescher, C. R. Bertozzi, *J. Am. Chem. Soc.* **2004**, *126*, 15046–15047.

[4] a) J. Šečkutė, N. K. Devaraj, *Curr. Opin. Chem. Biol.* **2013**, *17*, 761–767; b) M. L. Blackman, M. Royzen, J. M. Fox, *J. Am. Chem. Soc.* **2008**, *130*, 13518–13519; c) D. S. Liu, A. Tangpeerachaikul, R. Selvaraj, M. T. Taylor, J. M. Fox, A. Y. Ting, *J. Am. Chem. Soc.* **2012**, *134*, 792–795.

[5] H.-W. Shih, D. N. Kamber, J. A. Prescher, *Curr. Opin. Chem. Biol.* **2014**, *21*, 103–111.

[6] Reviews: a) X. Wu, Z. Li, X.-X. Chen, J. S. Fossey, T. D. James, Y.-B. Jiang, *Chem. Soc. Rev.* **2013**, *42*, 8032–8048; b) S. Jin, Y. Cheng, S. Reid, M. Li, B. Wang, *Med. Res. Rev.* **2010**, *30*, 171–257.

[7] Recent examples: a) H. Liu, Y. Li, K. Sun, J. Fan, P. Zhang, J. Meng, S. Wang, L. Jiang, *J. Am. Chem. Soc.* **2013**, *135*, 7603–7609; b) G. Pan, B. Guo, Y. Ma, W. Cui, F. He, B. Li, H. Yang, K. J. Shea, *J. Am. Chem. Soc.* **2014**, *136*, 6203–6206; Reviews: c) S. D. Bull, M. G. Davidson, J. M. H. Van Den Elsen, J. S. Fossey, A. T. A. Jenkins, Y.-B. Jiang, Y. Kubo, F. Marken, K. Sakurai, J. Zhao, T. D. James, *Acc. Chem. Res.* **2013**, *46*, 312–326.

[8] a) A. R. Lippert, G. C. Van De Bittner, C. J. Chang, *Acc. Chem. Res.* **2011**, *44*, 793–804; b) F. Wang, W. Niu, J. Guo, P. G. Schultz,

Angew. Chem. Int. Ed. **2012**, *51*, 10132 – 10135; *Angew. Chem.* **2012**, *124*, 10279 – 10282; c) V. S. Lin, B. C. Dickinson, C. J. Chang, *Methods Enzymol.* **2013**, *526*, 19 – 43; d) Z.-J. Chen, W. Ren, Q. E. Wright, H.-W. Ai, *J. Am. Chem. Soc.* **2013**, *135*, 14940 – 14943; e) X. Sun, Q. Xu, G. Kim, S. E. Flower, J. P. Lowe, J. Yoon, J. S. Fossey, X. Qian, S. D. Bull, T. D. James, *Chem. Sci.* **2014**, *5*, 3368 – 3373.

[9] a) T. Hoeg-Jensen, S. Havelund, P. K. Nielsen, J. Markussen, *J. Am. Chem. Soc.* **2005**, *127*, 6158 – 6159; b) E. Brustad, M. L. Bushey, J. W. Lee, D. Groff, W. Liu, P. G. Schultz, *Angew. Chem. Int. Ed.* **2008**, *47*, 8220 – 8223; *Angew. Chem.* **2008**, *120*, 8344 – 8347; c) C. C. Liu, A. V. Mack, E. M. Brustad, J. H. Mills, D. Groff, V. V. Smider, P. G. Schultz, *J. Am. Chem. Soc.* **2009**, *131*, 9616 – 9617; d) G. A. Ellis, M. J. Palte, R. T. Raines, *J. Am. Chem. Soc.* **2012**, *134*, 3631 – 3634; For examples of protein conjugation using boronic acid-enhanced imine/hydrazone formation, see: e) P. M. S. D. Cal, J. B. Vicente, E. Pires, A. V. Coelho, L. F. Veiros, C. Cordeiro, P. M. P. Gois, *J. Am. Chem. Soc.* **2012**, *134*, 10299 – 10305; f) O. Dilek, Z. Lei, K. Mukherjee, S. Bane, *Chem. Commun.* **2015**, *51*, 16992 – 16995.

[10] S. B. Y. Shin, R. D. Almeida, G. Gerona-Navarro, C. Bracken, S. R. Jaffrey, *Chem. Biol.* **2010**, *17*, 1171 – 1176.

[11] a) J. P. Lorand, J. O. Edwards, *J. Org. Chem.* **1959**, *24*, 769 – 774; b) L. Babcock, R. Pizer, *Inorg. Chem.* **1980**, *19*, 56 – 61; c) R. Pizer, C. Tihal, *Inorg. Chem.* **1992**, *31*, 3243 – 3247; d) J. Yan, G. Springsteen, S. Deeter, B. Wang, *Tetrahedron* **2004**, *60*, 11205 – 11209; e) E. Watanabe, C. Miyamoto, A. Tanaka, K. Iizuka, S. Iwatsuki, M. Inamo, H. D. Takagi, K. Ishihara, *Dalton Trans.* **2013**, *42*, 8446 – 8453; f) T. Okamoto, A. Tanaka, E. Watanabe, T. Miyazaki, T. Sugaya, S. Iwatsuki, M. Inamo, H. D. Takagi, A. Odani, K. Ishihara, *Eur. J. Inorg. Chem.* **2014**, 2389 – 2395; g) Y. Furikado, T. Nagahata, T. Okamoto, T. Sugaya, S. Iwatsuki, M. Inamo, H. D. Takagi, A. Odani, K. Ishihara, *Chem. Eur. J.* **2014**, *20*, 13194 – 13202; Review: h) J. A. Peters, *Coord. Chem. Rev.* **2014**, *268*, 1 – 22.

[12] a) N. Ni, S. Laughlin, Y. Wang, Y. Feng, Y. Zheng, B. Wang, *Bioorg. Med. Chem.* **2012**, *20*, 2957 – 2961; b) J. W. Tomsho, S. J. Benkovic, *J. Org. Chem.* **2012**, *77*, 2098 – 2106; c) H. Monajemi, M. H. Cheah, V. S. Lee, S. Mohd. Zain, W. A. T. Wan Abdullah, *RSC Adv.* **2014**, *4*, 10505.

[13] D. S. Matteson, H.-W. Man, *J. Org. Chem.* **1996**, *61*, 6047 – 6051.

[14] a) C. D. Roy, H. C. Brown, *Monatsh. Chem.* **2007**, *138*, 747 – 753; b) C. D. Roy, H. C. Brown, *Monatsh. Chem.* **2007**, *138*, 879 – 887.

[15] R. F. Baggio, A. L. Sparks, R.-R. Juo, J. E. Arenas, US Patent 0277143 A1 December 15, **2005**. The use of nopoldiol and phenylboronic acid for conjugation purposes was described in this patent; however, it lacks an optimization of the boronic acid and detailed kinetics characterization.

[16] See the Supporting Information for full details.

[17] P. Thordarson, *Chem. Soc. Rev.* **2011**, *40*, 1305 – 1323.

[18] a) J. de Champlain, L. Farley, D. Cousineau, M. R. van Ameringen, *Circ. Res.* **1976**, *38*, 109 – 114; b) T. Kawasaki, H. Akanuma, T. Yamanouchi, *Diabetes Care* **2002**, *25*, 353 – 357.

Received: November 6, 2015
Published online: February 23, 2016

