A Chemoselective Strategy for Late-Stage Functionalization of Complex Small Molecules with Polypeptides and Proteins

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The conjugation of small molecules to biopolymers has gained tremendous interest over the last several decades. These hybrid molecules can potentially harness the strengths of the small molecules and proteins to deliver new classes of therapeutics that were previously inaccessible with each component independently. Antibody drug conjugates (ADCs) for targeted cancer treatment highlight the promise of conjugate therapies in modern medicine.[1] Methods to construct these classes of molecules typically rely on the reaction of the nucleophilic residue within the biopolymer (i.e. cysteine or lysine) in combination with a preinstalled electrophilic handle (i.e. maleimide or activated ester) on the small molecule of interest. Professor Bradley Pentelute, from the Massachusetts Institute of Technology (MIT, USA), explained that due to the abundant nature of nucleophilic residues in these biopolymers, the corresponding conjugates are typically heterogeneous and the site of modification is often challenging to predict. "Additionally, multistep syntheses are often required to introduce the electrophilic handles on the small molecules to enable the conjugation step to proceed," he said.

Selenocysteine (Sec, U), the 21st proteinogenic amino acid, is a structural analogue of cysteine, but with a selenol in place of the thiol. "Due to its inherent redox profile, Sec favors the oxidized form rather than the corresponding selenol," said co-author Dr. Daniel Cohen of biopharmaceutical company AbbVie North Chicago, USA), who continued: "Amidst our investigation into the utilization of Sec as a bioconjugation handle for the preparation of small-molecule–biopolymer conjugates, we determined that the Se–S bond could behave as a latent electrophile such that a new Se–C bond can be formed in the presence of a copper reagent and a nucleophilic (hetero)aryl boronic acid (Figure 1, A, eq. 1).[2]" Further exploration by the authors into this innate electrophilicity revealed that (hetero)aryl with electron-rich functional groups can react directly even in the absence of copper reagent to form new Se–C bonds. "The significance of this work is that this strategy uses inborn native nucleophilicity of small molecules with different biological function in combination with oxidized Sec to provide conjugation to biopolymers (Figure 1, A, eq. 2)," explained Dr. Cohen, continuing: "The approach is selective in regards to both the biopolymer and the small molecule, but more importantly no pre-functionalization of the small molecule is necessary."

Professor Pentelute said: "We have been able to demonstrate this approach with numerous classes of small molecules (Figure 1, B), in particular, vancomycin." Utilizing the native nucleophilicity of vancomycin, the authors were able to prepare new peptide–vancomycin conjugates with excellent site-selectivity, with regard to both the biopolymer and the small molecule. Professor Pentelute also noted that, importantly, the aforementioned conjugates showed improved *in vitro* potency against Gram-positive and Gram-negative pathogens than either the peptide or vancomycin alone. "Expansion of this work into the protein area was demonstrated by conjugating vancomycin to a 6 kDa affibody protein, and genistein to a 150 kDa immunoglobulin-G antibody, in a two-step sequence (Figure 1, C)," said Professor Pentelute, who concluded: "Notably, labeling these proteins using our method did not result in diminished binding of the proteins to their corresponding targets."

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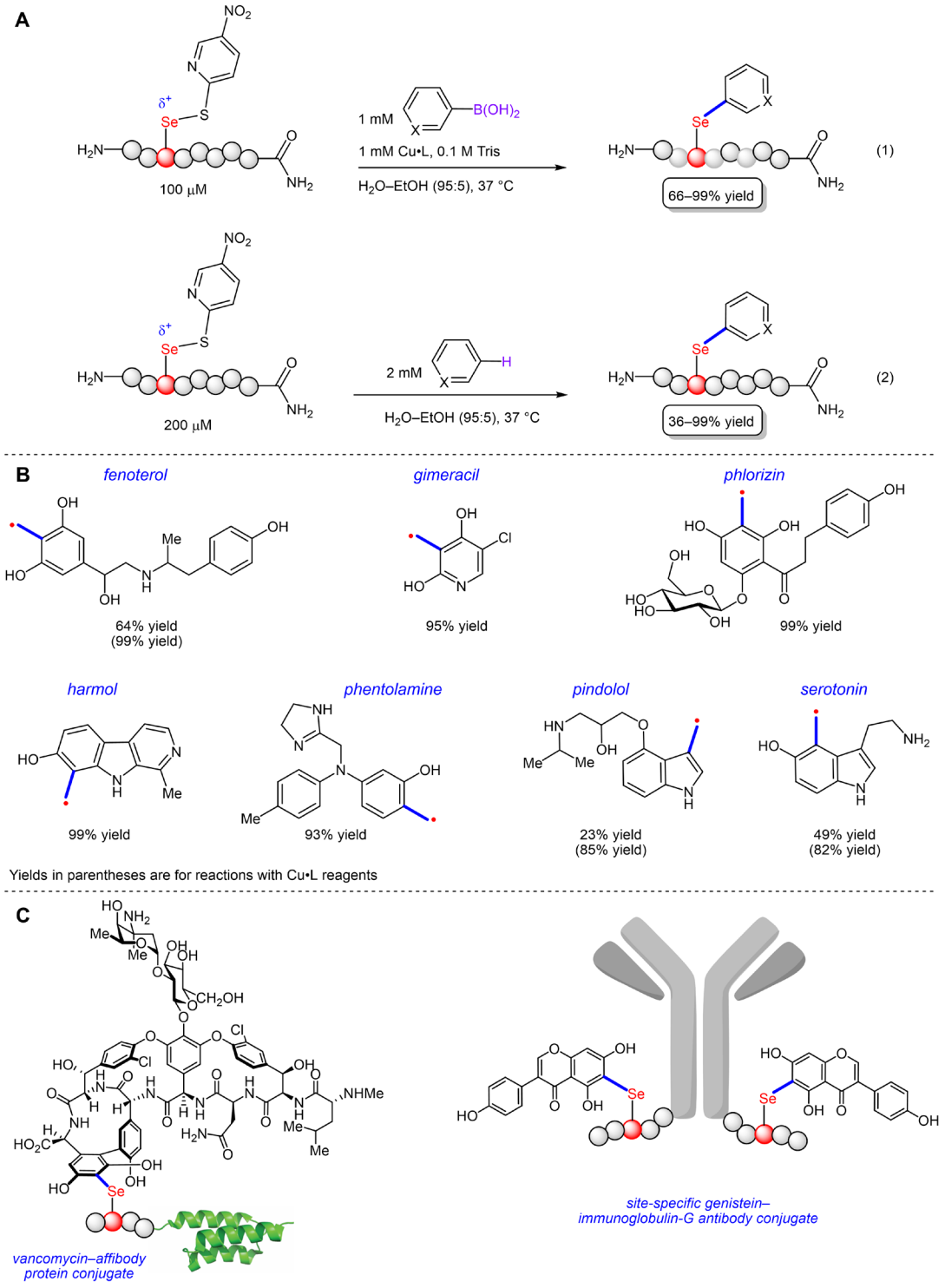

Scheme 1 (A) The Se–S bond behaves as a latent electrophile. (B) Small molecules conjugated to a selenocysteine peptide (indicated with a red dot). (C) Vancomycin and genistein conjugates.

About the authors

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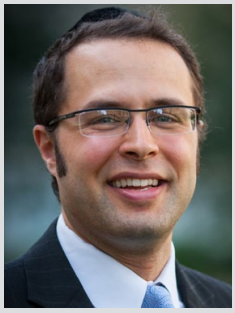
Stephen L. Buchwald has been a faculty member at Massachussetts Institute of Technology (MIT; USA) since 1984 and is currently the Camille Dreyfus Professor and an Associate Head of the Department of Chemistry. During his time at MIT he has been the coauthor of over 495 published or accepted papers and 52 issued patents. He has received a number of honors and awards, most recently the 2018 Tetrahedron Prize for Creativity in Organic Chemistry, the 2018 Dr. Karl Wamser Innovation Award (from the Technische Universität München) and the 2019 Roger Adams Award from the American Chemical Society.



Dr. D. Cohen

Daniel Cohen graduated salutatorian from State University of New York New Paltz (USA) with a B.A. in chemistry in 2007. As an undergraduate in the Dhar lab, he studied the antimicrobial and antifungal activity of α-pinene derivatives. Daniel received his Ph.D. in 2013 from Northwestern University (USA). Under the tutelage of his graduate advisor Karl A. Scheidt, Daniel developed new annulation strategies in N-heterocyclic carbene catalysis. From there Daniel moved on to complete his postdoctoral studies at MIT (USA) with Stephen L. Buchwald and Bradley L. Pentelute. His research involved metal catalysis to construct new bonds in small molecules and biomolecules. Currently, Daniel is a Senior Scientist at AbbVie where he works as a Medicinal Chemist in the Oncology Discovery Department.

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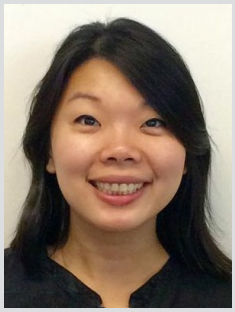
Colin Fadzen obtained his B.A. in physics and biochemistry and M.S. in chemistry from the University of Pennsylvania (USA) in 2013, where he worked in the laboratory of Prof. E. James Peterson on minimalist chromophores to monitor conformational changes in proteins using unnatural amino acid mutagenesis and native chemical ligation. He then moved to Boston (USA) to join the Harvard/MIT M.D.-Ph.D. program. He completed his graduate work in the laboratory of Prof. Bradley Pentelute in the MIT Department of Chemistry, graduating in 2018. His dissertation focused on the design of perfluoroaryl-based macrocyclic peptides for the improved delivery of chemotherapeutics across the blood–brain barrier and for the improved delivery of phosphorodiamidate morpholino oligonucleotides into muscle cells for the treatment of Duchenne muscular dystrophy. He is currently completing his M.D. at Harvard Medical School (USA) and will graduate in 2020. Notable awards include the Roy and Diana Vagelos Science Challenge Award (2012) and Dean's Scholar, Penn College of Arts & Sciences (2013).



Dr. L. Hie

Liana Hie earned her BSc at University of California Davis (USA), working with Prof. Xi Chen on chemoenzymatic synthesis of oligosaccharides. She then moved to University of California Los Angeles (USA) to study nickel catalysis under the guidance of Prof. Neil K. Garg. Following her doctoral work, she joined the laboratory of Prof. Scott Miller at Yale University (USA) as an NIH Postdoctoral Fellow studying site-selective modification of natural products via glycosylation reactions. Liana is currently employed by the FMC Corporation as a Discovery Research Investigator.

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Scott J. Miller was born in Buffalo, NY (USA). He received his B.A. (1989), M.A. (1989) and Ph.D. (1994) degrees from Harvard University (USA), where he worked with David Evans as a National Science Foundation Predoctoral Fellow. Subsequently, he traveled to the California Institute of Technology (USA) where he was a National Science Foundation Postdoctoral Fellow with Robert Grubbs until 1996. For the following decade, he was a member of the faculty at Boston College (USA), until joining the faculty at Yale University (USA) in 2006. In 2008, he was appointed as the Irénée duPont Professor of Chemistry.

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Dr. C. Zhang

Chi Zhang received his Ph.D. from MIT (USA) in 2017 under the supervision of Prof. Brad Pentelute. He is currently a postdoctoral associate with Prof. Ed Boyden at MIT Media Lab. His research focuses on developing tools for highly multiplexed protein analysis in complex biological systems. Chi is a recipient of several awards: 2015 Bristol–Myers Squibb Fellowship in Synthetic Organic Chemistry, 2016 Regeneron Prize for Creative Innovation, 2016 Genentech Chemical Research Award, 2016 Alfred R. Bader Award for Student Innovation, and 2018 IUPAC-Solvay International Award for Young Chemists.

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