

A Concise Synthesis of Tetrodotoxin

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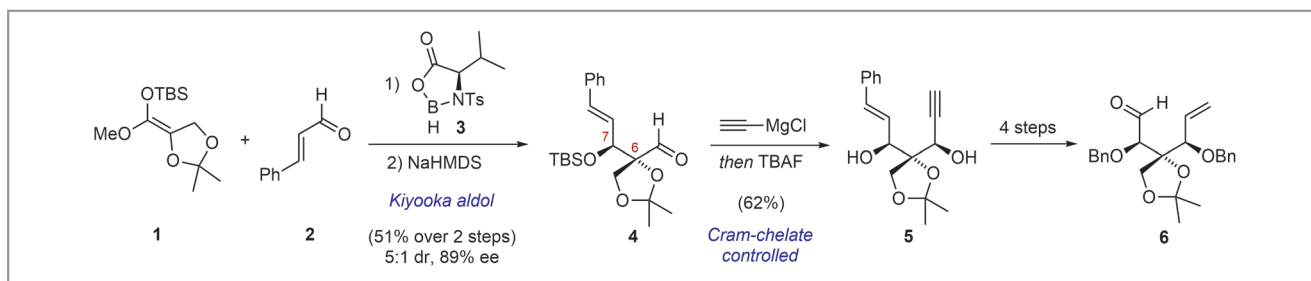
Tetrodotoxin (TTX) is an extremely potent poison found in different organs of some fish, such as puffer fish, as well as in some amphibian, octopus, and shellfish species, acting as a selective sodium channel blocker. Chemically, TTX is a complex quinazoline alkaloid, whose structure was elucidated by Hirata, Tsuda, Amakasu, and Woodward and later confirmed by X-ray crystallography. Its first synthesis – in racemic form – dates back to the work of Yoshito Kishi (1972), whereas the synthesis of the stereopure compound was first accomplished over three decades later. Recently, the group of Professor Dirk Trauner (New York University, USA) reported in *Science* a conceptually new and extremely concise stereoselective synthesis of TTX, which could also be amenable to readily accessing biologically active TTX derivatives of pharmaceutical interest.

Professor Trauner said: “Since my undergraduate days, I have been fascinated by the chemistry and biology of TTX, and I have always admired how Kishi solved this synthetic challenge more than five decades ago. For many years, I had been teaching his synthesis in comparison with more recent work in my graduate courses on “Synthetic Design” and “Chemical Neuroscience”. Browsing through an issue of *Org. Lett.* after one of my classes, I realized that the product of a Kiyooka aldol reaction reported by Marcus Kalesse, compound **4**, mapped onto C5, C6, C7 and C11 of TTX (Scheme 1) (*Org. Lett.* **2011**, *13*, 6038–6041). This prompted us to work on TTX in hopes that we could adopt this powerful opening step, “somehow” forge the central cyclohexane ring, and then install the missing carbons, the all-important α -tertiary nitrogen, the remaining hydroxy groups, and the heterocycles of TTX.” The group found that a Cram-chelate controlled acetylide addition to **4** indeed set the C5 stereocenter with the correct configuration and they were able to access a key aldehyde intermediate **6** within

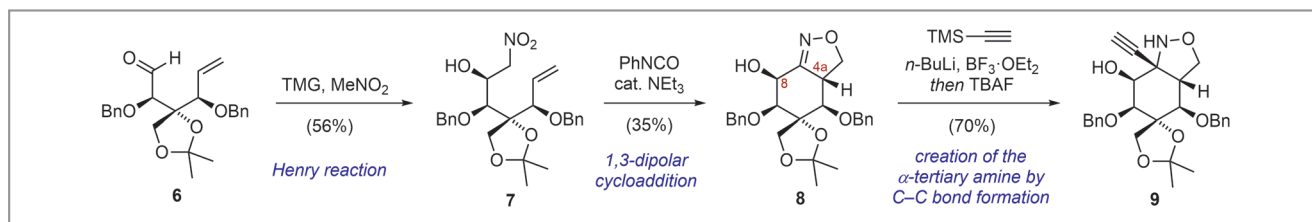
seven steps. David Konrad, the graduate student with whom Professor Trauner started this project, also used an intermediate similar to **5** in the first total synthesis of kweichowenol A (*Synlett* **2019**, *30*, 383–386).

Mr. Konrad then went ahead and investigated a subsequent Henry reaction, dehydration and cycloaddition sequence (Scheme 2). “Despite extensive experimentation, the diastereoselectivity of the Henry reaction remained low,” said Professor Trauner. He continued: “Dehydration of the unprotected nitro-aldol product **7** to form a nitrile oxide with subsequent (3+2) cycloaddition afforded an isoxazoline product **8** in moderate yields. We confirmed the wrong configuration at C4a by X-ray crystallography; however, we were optimistic that we could epimerize this stereocenter at a later stage. Another distinct strategic difference of our approach was the establishment of the α -tertiary amine.” Indeed, all previous syntheses of TTX established this stereocenter by C–N bond formation using intramolecular rearrangements [Beckmann (Kishi), Overman (Isobe), Fukuyama or Curtius (Fukuyama)], an intramolecular nitrene insertion (Du Bois) or nucleophilic substitution with an azide (Sato). Professor Trauner’s group, however, instead aimed to create this stereocenter by formation of a C–C bond, specifically by addition of a C2 nucleophile into the isoxazoline. They found that steric hindrance prevented the addition of many nucleophiles, but TMS-acetylide formidably performed the addition into the isoxazoline after activation with a Lewis acid. Importantly, the C8-alcohol needed to be unprotected to promote an efficient nucleophilic addition.

Professor Trauner explained: “Although our route was scalable, the material throughput was limited by the necessity to use high volumes of carcinogenic HMPA as a cosolvent



Scheme 1 Initial attempts to access aldehyde **6** using a Kalesse–Kiyooka aldol approach



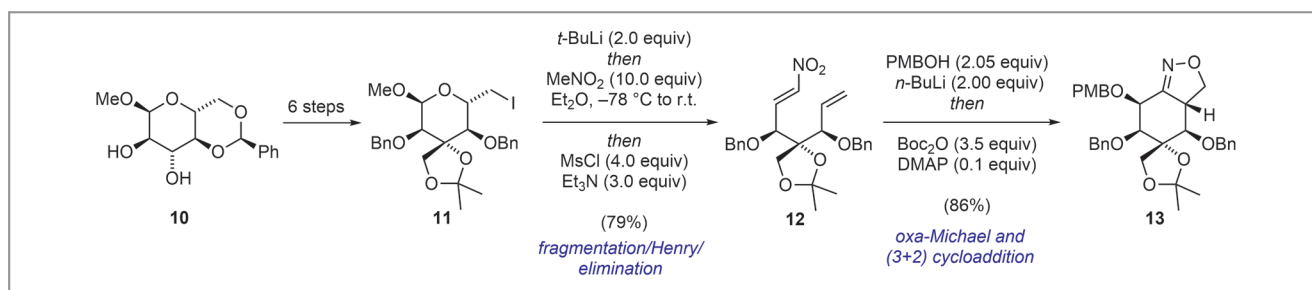
Scheme 2 Henry reaction, 1,3-dipolar cycloaddition and ATA formation

for the formation of silyl ketene acetal **1** and stoichiometric amounts of the oxazaborolidinone **3** for the Kiyooka aldol reaction.” At this stage, Bryan Matsuura joined the project as a postdoc and investigated alternative, more scalable strategies towards the key aldehyde **6**. Together with Hiroyasu Ando, he developed a highly scalable (100 g scale) and reliable route towards an iodoglycoside (**11**), starting from commercially available methyl 4,6-*O*-benzylidene- α -D-glucopyranoside (**10**). “With this new strategy, we no longer had any issues of material supply,” commented Professor Trauner. He added: “Initially, we used original Bernet–Vasella fragmentation conditions (Zn, I_2) to obtain crude aldehyde **6** and used the same Henry and cycloaddition conditions as before. Trying to optimize this low-yielding two-step sequence, Peter Rühmann followed conditions reported by Soengas and Silva and found that a tandem fragmentation/Henry reaction sequence successfully yielded nitroaldol products **7**, albeit still with low diastereoselectivities after extensive experimentation” (*Eur. J. Org. Chem.* **2013**, 5022–5027). The group’s solution to this problem was to eliminate the secondary alcohol in the same pot, which exclusively yielded (*E*)-nitroalkene **12** (Scheme 3). They were delighted to find that an oxa-Michael addition into this nitroalkene system solely yielded a single diastereomer. Professor Trauner said: “We could extend this finding by trapping the intermediate nitronate with Boc anhydride, which triggered the dehydration and subsequent 1,3-dipolar cycloaddition cascade in a single reaction step. Using phenyl iso-

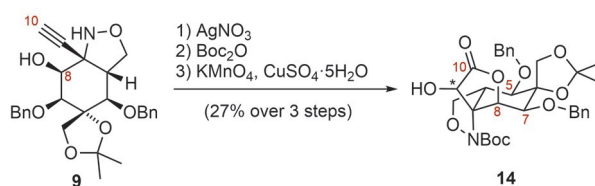
cyanate, we observed similar results, however obtained lower yields. Intrigued by the selectivity of this oxa-Michael attack and curious about which would be the actual 1,3-dipole that undergoes the cycloaddition (nitronate vs. nitrile oxide), we approached Prof. Ken Houk and Nina Strassner (UCLA) for computational assistance. Nina calculated diastereomeric transition states for the oxa-Michael attack which validated the lowest-energy transition state to yield an addition from the desired face. She further confirmed the pathway of the (3+2) cycloaddition to proceed via a nitrile oxide 1,3-dipole as the elimination occurs much faster than a competing cycloaddition of the Boc-nitronate intermediate.”

With a satisfying solution towards the hydroxy alkyne **9**, the group’s initial plan was to convert this intermediate into a hydroxylactone of type **14**, which they believed, after debenzilation, would transactonize and form the dioxo-adamantane core of TTX spontaneously. “Silver-based π -acids initiated the desired 5-*endo*-dig cyclization towards an enol ether, which after Boc-protection of the isoxazolidine could be converted into hydroxylactone **14** (Scheme 4), whose configuration at C9 we were unable to firmly establish,” said Professor Trauner. He continued: “Deprotection of the C5 and C7 benzyl ethers was possible, yet all attempts to isomerize the five-membered lactone to the desired dioxo-adamantane core of TTX proved futile.”

These results forced the group to adopt a new strategy, which sought to establish a hydroxylactone bridge to the C5



Scheme 3 The glucose-derived iodoglycoside **11** and its cascade transformations towards isoxazoline **13**

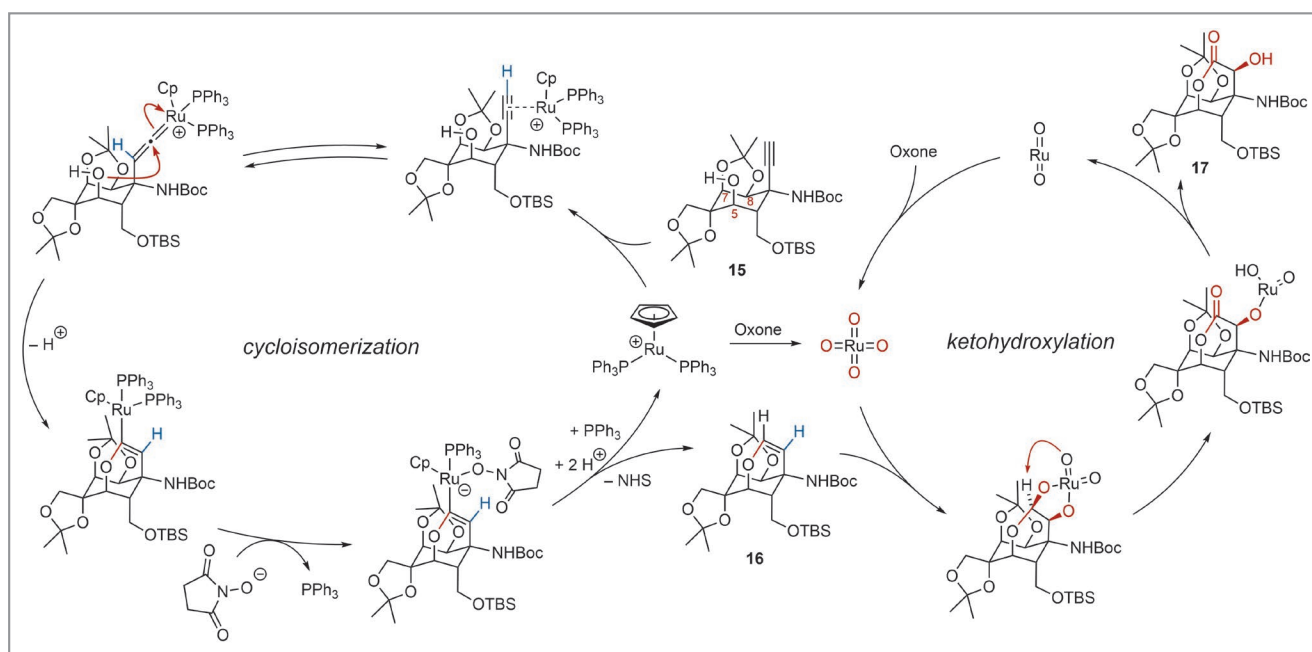


Scheme 4 Initial attempts to establish a hydroxylactone of type **14**

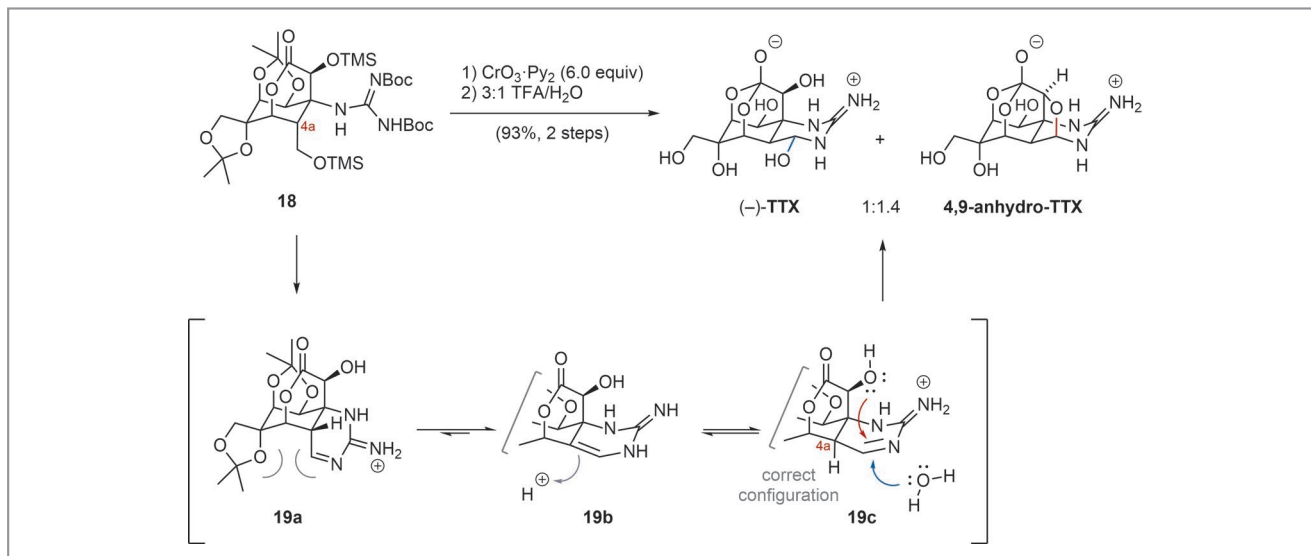
hydroxy group. “Such a maneuver would avoid a risky and unprecedented transactonization step,” Professor Trauner explained, adding: “To this end, intermediate **15** was synthesized from **9** in 6 steps, which took advantage of a novel photochemical debenzoylation methodology (*Org. Lett.* **2021**, *23*, 514–518). Initially, gold or silver π -acids exclusively yielded 5-*exo*-dig cyclization products of **15**. The breakthrough came with the identification of a Ru(II) catalyst that cleanly converted hydroxy alkyne **15** through a 6-*endo*-dig cyclization into enol ether **16** (Scheme 5), presumably through the intermediacy of a metallovinylidene carbene. Based on scarce literature precedence, Bryan then reasoned that addition of Oxone™ to this reaction mixture would oxidize our Ru(II) catalyst in situ to RuO₄ and dihydropyran **16** to the desired hydroxylactone **17**. After several rounds of optimization, we found that this transformation indeed was possible, requiring careful moni-

toring of the reaction mixture in order to avoid overoxidation to the ketolactone side product. I believe that this reaction is a nice example for on-demand methodology development in the spirit of Sam Danishefsky’s saying: “Sometimes you have to be a bit desperate to do something really interesting.” We currently are investigating this chemistry further and plan to apply it towards other natural products.”

Deprotection of **17**, followed by silylation/guanidinylation, yielded the penultimate intermediate **18** (Scheme 6). This compound bears all of the atoms and stereocenters of TTX, albeit with the wrong configuration at C4a. “Our final transformation required the chemoselective oxidation of the primary silyl ether at C4, epimerization of the C4a stereocenter, global deprotection of the remaining protecting groups, and spontaneous formation of the cyclic guanidine and *ortho*-acid,” said Professor Trauner. He continued: “This was achieved



Scheme 5 Proposed mechanism for the hydroxylactonization



Scheme 6 Global deprotection and epimerization of the C4a center afforded TTX and 4,9-anhydro-TTX

by exposure to Collins reagent followed by treatment with aqueous TFA, affording the desired product as a mixture of TTX and its naturally occurring 4,9-anhydro-TTX isomer. The handling and purification of this toxic mixture required much expertise, awareness, and patience. Belinda Hetzler and Peter Rühmann investigated the purification of the crude mixture using different HPLC columns and ultimately found conditions that could separate all impurities, affording a clean mixture of TTX and 4,9-anhydro-TTX. Since these interconvert in aqueous solution, we refrained from separating them.”

“Finally, as another, albeit imprecise, measure for the identity of our compound, we have tested the ability of our synthetic TTX/anhydro-TTX mixture to block action potentials in mouse brain slices (Figure 1),” said Professor Trauner. He added: “These measurements were done in collaboration with Dr. Jingjing Liu and Prof. Richard Tsien (NYU Neuroscience Institute), with whom we will pursue selective NaV1.7 (and NaV1.8) inhibitors as next-generation analgesics in the future.” Professor Trauner concluded: “We believe that our efficient approach provides a good platform for the synthesis of simplified TTX analogues and could even be used to procure TTX itself should supply ever become a concern.”

Mattes Fank

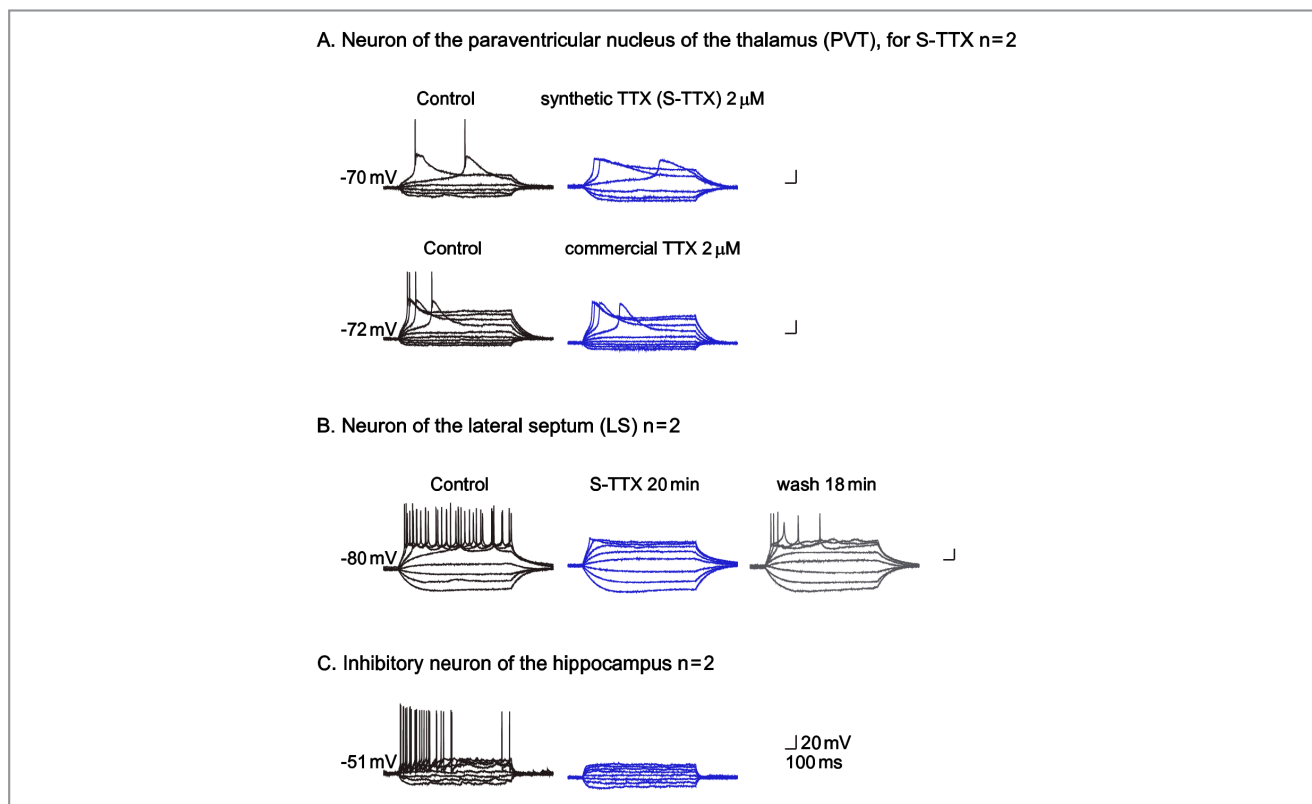


Figure 1 Electrophysiology traces of different neurons from mouse brain slices demonstrate successful inhibition of action potentials

Lucretia Fenske

About the authors



Dr. D. B. Konrad

David B. Konrad is a Liebig fellow and research group leader at the Department of Pharmacy of the Ludwig Maximilian University of Munich (LMU Munich, Germany). He obtained his M.Sc. degree in chemistry and biochemistry at the LMU in 2013 which included research stays in the group of Prof. Dean Toste at the University of California, Berkeley (USA) and the group of Prof. Benjamin List at the Max Planck Institute for Coal Research in Mülheim an der Ruhr (Germany). In 2014, he joined the group of Prof. Dirk Trauner for his graduate studies to work on an asymmetric synthesis of tetrodotoxin and methods to red-shift

photopharmaceuticals. After graduating in 2018, he moved to the Scripps Research Institute in La Jolla (USA) as a postdoctoral fellow focusing on (chemo)proteomics-guided fragment-based ligand discovery using cysteine-targeted and photoaffinity probes under the guidance of Prof. Benjamin Cravatt. As part of the second phase of his fellowship, he returned to LMU Munich to join the group of Prof. Ivan Huc to analyze interactions of foldameric B-DNA mimics with the drosophila proteome before transitioning to an independent group leader in 2021. His research is focused on the development of covalent drugs that target advanced non-small cell lung cancers by working at the interface of chemical biology, (chemo)proteomics, medicinal chemistry, and synthetic methodology development.

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K.-P. Rühmann

Klaus-Peter Rühmann obtained his Bachelor's and Master's degrees at LMU Munich (Germany) in chemistry and biochemistry. He completed his Master's thesis research in the group of Prof. Andrew G. Myers at Harvard University (USA) before he joined the graduate program at New York University (USA) where he is conducting synthetic studies towards different medicinally relevant natural products under the guidance of Prof. Dirk Trauner.



Dr. B. E. Hetzler

Belinda E. Hetzler received her B.Sc. in chemistry from Heidelberg University (Germany). She then moved to LMU Munich (Germany) and joined Prof. Dirk Trauner's lab, where she completed her M.Sc. She then continued as a MacCracken Graduate Fellow at New York University (NY, USA), also in the group of Prof. Dirk Trauner. There, she worked on synthetic methodology for complex natural product synthesis and the design of novel molecular switches for the control of protein function. After receiving her Ph.D. in 2022, Belinda joined Vir Biotechnology (USA), where she is currently working in the infectious disease space as a Scientist in Medicinal Chemistry.



N. Strassner

Nina Strassner received her B.Sc. degree in chemistry from the University of Erlangen-Nuremberg (Germany) in 2021. She then spent seven months in Prof. Kendall N. Houk's lab at the University of California, Los Angeles (USA) as a Visiting Graduate Researcher doing computational studies of stereoselective (bio-)organic mechanisms. Supported by a scholarship of the Studienstiftung des deutschen Volkes, she is currently working toward her M.Sc. degree in Erlangen.



Prof. K. N. Houk

K. N. Houk received his A.B., M.S., and Ph.D. degrees at Harvard University (USA), working with R. B. Woodward on experimental tests of orbital symmetry selection rules. In 1968, he joined the faculty at Louisiana State University (USA), moved to the University of Pittsburgh (USA) in 1980, and to UCLA (USA) in 1986. From 1988–1990, he was Director of the Chemistry Division of the National Science Foundation (USA). He was the UCLA Saul Winstein Chair in Organic Chemistry from 2009–2021 and is now a Distinguished Research Professor. Beginning as an experimental organic chemist, he evolved over his career into a theoretical and computational organic chemist, involved in collaborations with groups all over the world. He has been elected as a member of both the US and Chinese Academies of Science. He has published almost 1500 refereed publications.



Dr. B. S. Matsuura

Bryan S. Matsuura received his B.A. from Boston University (USA) in 2009, after which he went on to the University of Michigan (USA) to study under the guidance of Prof. Corey R. J. Stephenson. He received his Ph.D. in 2015 which focused on photoredox catalysis, natural products total synthesis, biomass processing, and methodology development. He then joined the group of Prof. Dirk Trauner as an Alexander von Humboldt postdoctoral research fellow at LMU Munich (Germany), where he studied natural product total synthesis and photopharmacology. In 2021, Bryan joined Merck South San Francisco (USA) as a Senior Scientist, where he is currently working in the cardiometabolic disease area.



Prof. D. Trauner

Dirk Trauner was born and raised in Linz, Austria, studied biology and chemistry at the University of Vienna (Austria) and received his undergraduate degree in chemistry from the Free University of Berlin (Germany). He then pursued graduate studies in chemistry under the direction of Prof. Johann Mulzer and became a postdoctoral fellow with Prof. Samuel

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J. Danishefsky at the Memorial Sloan-Kettering Cancer Center (USA). Subsequently Dirk joined the Department of Chemistry at the University of California, Berkeley (USA), where he rose through the ranks to become an Associate Professor of chemistry (with tenure) and a member of the Lawrence Berkeley National Laboratory. In 2008, he moved to LMU Munich (Germany) as a Professor of Chemistry and Chemical Genetics. In the Spring of 2017, he returned to the United States as the Janice Cutler Chair in Chemistry at New York University. In July 2022, Prof. Trauner moved to the University of Pennsylvania (USA) as a Penn Integrates Knowledge Professor, holding joint appointments in the Department of Chemistry at the School of Arts and Sciences and the Department of Systems Pharmacology and Translational Therapeutics in the Perelman School of Medicine.