doi:10.2533/chimia.2025.797 Chimia 79 (2025) 797–802

Bio-Based Approaches for Selective Cyclization

Eléonore Moore and Amy E. Fraley*

Abstract: Cyclic natural products offer important, unique scaffolds and functionalities for the pharmaceutical industry. They are produced by enzymes catalyzing a wide range of cyclization reactions. This large family of enzymes creates distinctive cyclic structures *via* a variety of mechanisms naturally evolved for selectivity. In this review, we aim to present an overview of these natural catalysts, the therapeutic compounds for which they are involved in the production, as well as engineering efforts to tune them for anthropogenic needs in human medicine. Biochemical methodologies commonly used for the discovery and engineering of enzymes will also be highlighted, with an emphasis on enzymatic terpene cyclization and Pictet-Spengler-type cyclization.

Keywords: Biocatalysis · Biosynthesis · Cyclization · Natural products

Eléonore Moore received both her Bachelor's and Master's degrees from ETH Zürich. She achieved her Master's thesis in the group of Jörn Piel, where she worked on the establishment of a reporter system for enzymatic activity. She then joined the Medicinal Chemistry laboratory of Prof. Amy E. Fraley as a PhD student studying biocatalysis and enzyme engineering.

Amy E. Fraley earned her PhD in Medicinal Chemistry in 2019 from the University of Michigan working in the laboratories of David Sherman and Janet Smith where she studied fungal indole alkaloid biosynthesis. She then moved to the ETH Zürich Institute of Microbiology for postdoctoral studies in the laboratory of Jörn Piel, with a focus on the biosynthesis of anticancer polyketides by marine symbiotic bacteria. Currently, she is an Assistant Professor of Medicinal Chemistry in the Institute of Pharmaceutical Sciences and the Department of Chemistry and Applied Biosciences at ETH Zürich. Her research group is developing new bio-based methods for sustainable chemistry.

1. Introduction

Cyclic natural products (cNPs) have had a profound impact on the pharmaceutical industry, whether through their direct use as treatments or by their diverse scaffolds which have inspired and enabled the development of novel classes of therapeutics. This is perfectly exemplified by the β-lactam containing fungal natural product penicillin, which is the most widely used antibiotic in the world, and is estimated to have saved over 500 million lives.[1]

Natural products are generally impactful due to their impressive diversity in structure and complex molecular architectures, both increasing chemical space[2,3] and driving drug discovery. These molecules are rich in chiral centers that are selectively installed by biosynthetic enzymes, dictating the activity of the final compounds.[4] However, they are often difficult to synthesize, requiring arduous processes for their production. Furthermore, synthetic chemistry often requires harsh solvents and reaction conditions that are not environmentally sustainable. The aforementioned pitfalls can be mitigated by employing the naturally producing biological systems, and the encompassed enzymes, since they are stereo-, regio-, and chemoselective[3,5] and operate under mild aqueous conditions.[6]

This review will focus on various classes of enzymes that generate chemical complexity in natural products *via* cyclization reactions. An overview of their reactivity, biocatalytic properties, as well as the methods used to study them will be presented. We will also discuss engineering efforts and expand on two classes of enzymatic cyclization under study in our research group: terpene cyclases (TCs) and Pictet-Spenglerases (PSs). We will highlight respective cNPs from these classes, their biosynthetic enzymes, and their relevance in therapeutic production.

1.1 *Traditional Terminal Cyclization Mechanisms in Polyketide and Nonribosomal Peptide Biosynthesis*

Polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs) are large megasynthases comprising multiple modules with distinct domains, each involved in the chain-like elongation and modification of polyketide or peptide chains, respectively. These enzymes are found across various kingdoms of life and have evolved to produce molecules with unique cyclic structures, functional groups, and functions (Fig. 1, red and brown). For example, erythromycin A (**1**) is a member of the macrolide antibiotic family[7,8] and the cyclization step catalyzed by the terminal thioesterase domain (TE) greatly contributes to its bioactivity.[9,10] Daptomycin[11,12] (**2**) is a lipopeptide made by an NRPS and is active against methicillin-resistant *Staphylococcus aureus* (MRSA) by disrupting multiple areas of the bacterial cell membrane. Cyclosporine (**3**) is an immunosuppressant cyclic peptide produced by a fungal host.[13] The rigid structure of the macrocycle enables the key interaction necessary for its functional activity.[14]

1.2 *Pictet-Spengler Cyclization*

Pictet-Spenglerases (PSs) are crucial in both plants and bacteria, playing a key role in the biosynthesis of indole alkaloids (Fig. 1, green).[15] PSs mainly accept aldehyde moieties, although a few examples of ketone-accepting enzymes have been characterized. They have been shown to catalyze the cyclization of various rings, which are then further derivatized by tailoring enzymes.[15,16] Alkaloids are often found in plants, with the toxic indole alkaloid

*Correspondence: Prof. Dr. A. E. Fraley, E-mail: afraley@ethz.ch
Department of Chemistry and Applied Biosciences, ETH Zürich, Zürich, CH-8050

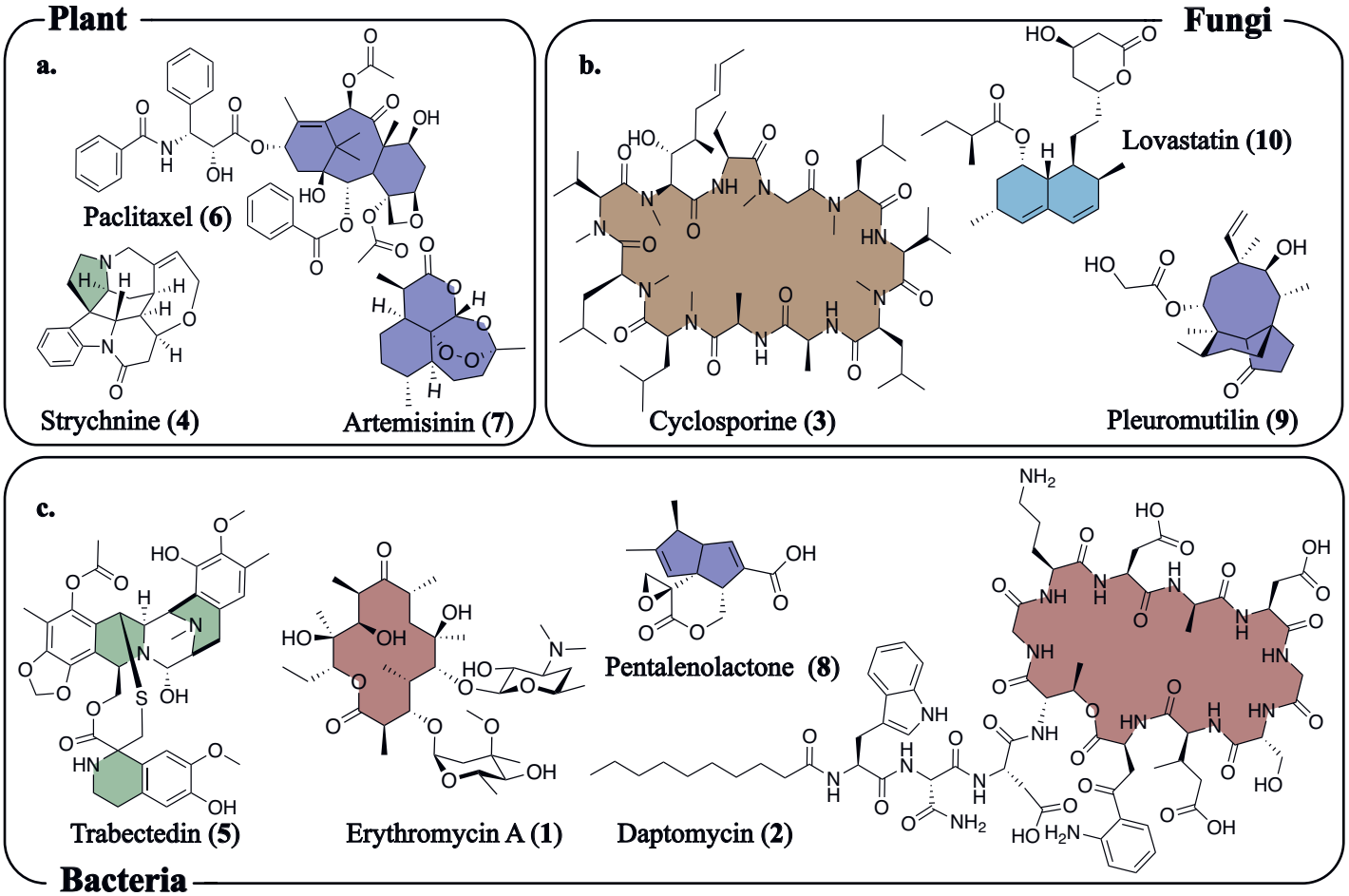

Fig. 1. Overview of select biomedically relevant cyclic natural products. The cyclized cores are highlighted by the type of enzymatic cyclization reaction and are indicated as follows: green, Pictet-Spenglerase; violet, terpene cyclase; blue, Diels-Alderase; red, nonribosomal peptide synthetase terminal domain; brown, polyketide synthase terminal domain.

strychnine (**4**) having a long-lasting impact on society due to its activity as an antagonist of glycine receptors. The cyclized core provides the necessary rigidity for effective interaction with these receptors.[17] PS cyclized alkaloid natural products are not restricted to plants and can also be found in bacteria. For example, the tetrahydroisoquinoline natural product ET-743 (**5**, also known as Yondelis® or Trabectedin) which is an anticancer compound produced by an NRPS that has evolved the ability to catalyze PS reactions. ET-743 acts by blocking the DNA repair mechanism and generating DNA breaks through the alkylation of guanine.[18] This DNA-binding ability is mediated by the formation of the first tetrahydroisoquinoline core by the biosynthetic PS reaction.

1.3 *Terpene Cyclases*

Terpenes are a large class of compounds with high structural diversity and multiple functions across different kingdoms of life (Fig. 1, violet). They all originate from the same two C5 precursors: isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are coupled *via* an isomerization reaction and cyclized by terpene cyclases (TCs) to form core scaffolds. A series of modifications incorporated by tailoring enzymes serves to further diversify the terpenoid natural products. There are several pharmaceutically relevant terpenoids, with the plant-based paclitaxel (Taxol®, **6**), a member of the diterpenoid family, serving as a prime example. It functions as an anticancer agent, whose ring system is essential for its mechanism of action and tubulin binding properties that halt cell proliferation.[19,20] Another plant terpenoid is artemisinin (**7**), discovered by Tu Youyou, who was awarded a share of the 2015 Nobel Prize for its discovery.[21] Artemisinin is widely used against malaria, with the TC-mediated trioxane ring being essential for releasing the cyclic peroxide required for its antiparasitic activity.[22] The bacterial pentalenolactones (**8**) are a family of gram-positive antibacterial therapeutics whose stereospecifically generated tricyclic ring is critical for their activity.[23,24] Lastly, pleuromutilin (**9**) is a fungal terpene that has activity against *Staphylococcus aureus* by acting on the ribosome.[25,26]

1.4 *Diels-Alderases*

Diels-Alder reactions are key chemical transformations for the [4+2] cycloaddition between a conjugated diene and a dienophile that were long sought after in the enzymatic realm.[27,28] They are challenging to define due to the difficulty in validating enzyme involvement over spontaneous ring formation. However, advances in the field have revealed the existence of numerous Diels-Alderases (DAs) catalyzing both intra- and inter-molecular [4+2] cycloaddition reactions.[29–31] One of the earliest cases of DA in cNP biosynthesis was LovB (Fig. 1, blue).[32,33] LovB is involved in the biosynthesis of the HMG-CoA reductase inhibitor Lovastatin (**10**), and generates the bicyclic scaffold responsible for its cholesterol-lowering activity.

2. Methods for Studying Enzymatic Cyclization

Understanding the basic biochemical underpinnings of natural product production enables the ability to tune the respective enzymes for incorporation into synthetic schemes. The development of these engineered enzymes as biocatalysts requires methodologies spanning multiple fields. (Fig. 2)

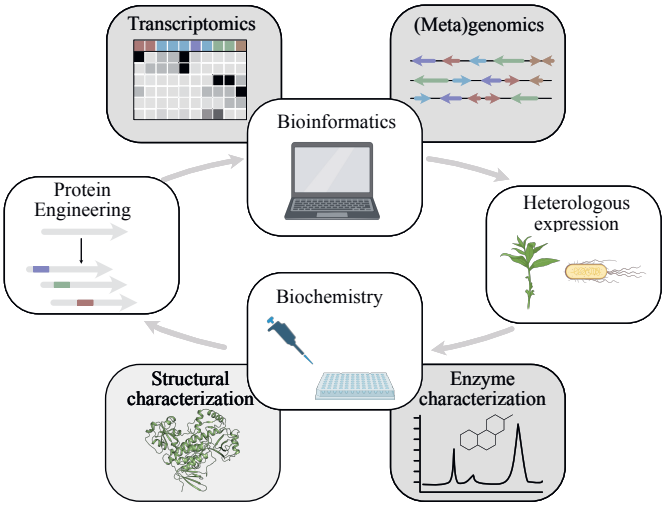

Fig. 2. Overview of the experimental workflow for biosynthetic discovery and biocatalytic development. Created in BioRender. Moore, E. (2025), https://BioRender.com/8iwx88v.

The workflow often begins with bioinformatic approaches, which can be applied in various scenarios, including searching for genes encoding known bioactive compound production or searching for homologous genes to a known biosynthesis in new producers. Many times the proximity of the genes, or encoding in biosynthetic gene clusters (BGCs) which mainly occurs in microbes, can inform on the functionality and likely final product. Given the limited ability to cultivate many natural product producers, genes of interest are typically transformed into a heterologous host such as the widely applicable *Escherichia coli* (*E. coli*). Other systems, such as *Saccharomyces cerevisiae* and *Nicotiana benthamiana*, are often used to study eukaryotic systems that require additional considerations for protein production, such as glycosylation and splicing machinery.

In general, characterization of the wild-type enzyme is completed with the purified protein, enabling kinetic studies to investigate how reaction rates change in response to varying conditions. The final cyclized product is typically structurally elucidated *via* a series of analytical experiments, such as gas chromatography or liquid chromatography coupled to high-resolution mass spectrometry, or nuclear magnetic resonance spectroscopy (NMR) for compounds that can be isolated in the pure form. The substrate scope of an enzyme can also be studied, as greater promiscuity is frequently desired for its broader applications in the pharmaceutical industry. For mechanistic insight, the enzyme structure is often required and can be acquired through various techniques, including X-ray crystallography, cryo-electron microscopy (for larger complexes), and NMR (for small to moderately sized proteins). Experimental structural data also enables rational, structure-based protein engineering.[34,35]

Another powerful tool is AI-based structural prediction, which is becoming increasingly accurate.[36] These structural tools enable the visualization of a reliable model of the catalytic pocket and surrounding amino acids, as well as highlighting the various interactions involved in substrate binding and catalysis. Pairing experimental or predicted models with docking studies and computational approaches (*e.g.* molecular dynamic simulations) can provide a further basis for future engineering endeavours. With both experimental and predicted protein structures, one can perform mutagenesis experiments to understand the role of each essential amino acid around the active site. In contrast, directed evolution[37,38] involves the random, nearly all-encompassing, integration of mutations and the selection of the relevant mutant, which can then be subjected to multiple rounds of evolution until the desired reactivity is achieved. Both techniques have had great success in building more efficient and stable enzymes. One of the key aspects in bringing biocatalysts to the forefront is an efficient readout for analysis of mutants to ensure the intended change in their reactivity, substrate scope, specificity, or stability, *etc.*

3. Terpene Cyclases

There are over 95,000 known terpenoids, for which the TC class of enzymes is responsible for forming the core architecture.[39] These cores can be broken down into the classes of monoterpenes (C10), sesquiterpenes (C15 as in pentalenolactone (**8**)), diterpenes (C20 as in pleuromutilin (**9**)), sesterterpenes (C25), and triterpenes (C30), and these are built by two mechanistically distinct types of TC: type I and type II. Current knowledge in the field indicates that monoterpene and sesquiterpene units are uniquely cyclized by type I TCs, whereas diterpene and sesterterpenes can be cyclized by both type I and II TCs, and triterpenes are only cyclized by type II TCs.[40] The type I and II TC activities can be encompassed in monofunctional enzymes as well as structurally similar bifunctional proteins encompassing both type I and type II activities. It was also observed that bacterial TCs often possess the bifunctional protein structure, however, with only one functional active site. The TC family is biosynthetically complex, and each class has exceptions in which general rules cannot be applied. In this review, we will highlight the class of diterpene cyclases (DTCs) that are of interest in our group.

3.1 *Type II Terpene Cyclases*

Type II TCs mediate acid-base cyclization without requiring the elimination of the diphosphate group of the linear terpene precursor.[39] In diterpene production, this is typically the first step in a series of core-forming TC-catalyzed reactions. In most cases, the active site of the enzyme contains a DxDD motif that, by acting as a general acid, allows for the protonation of the terminal alkenes (**11**) or epoxide of the acyclic terpene. Variations of this motif are common among type II TCs, especially in those protonating an epoxide. The formed reactive carbocation intermediate (not shown) is stabilized through interaction with hydrophobic or aromatic amino acids. The carbocation can be attacked by the nearby double bond, forming a bicycle and a new carbocation (**12**), which can then further react *via* a cyclization cascade. The carbocation is finally quenched by a water molecule or basic residue within the active site (steps **12** to **13** in Fig. 3)[39]

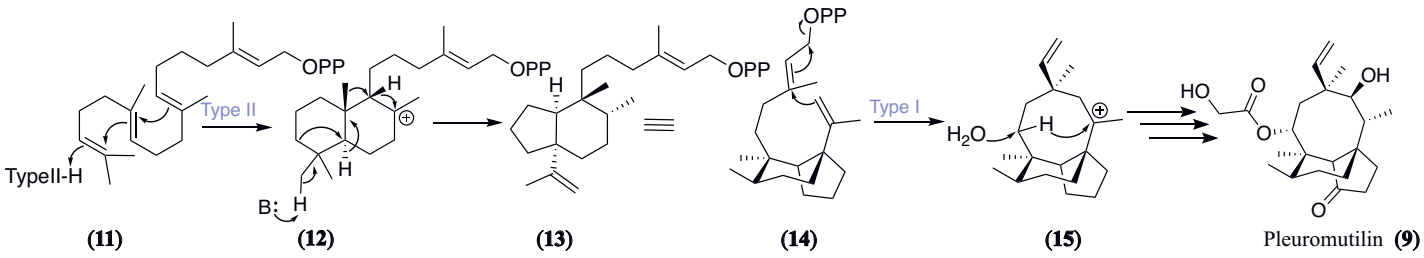

Fig. 3. Part of the pleuromutilin (**9**) diterpene cyclase mechanism (PI-cyc), which contains both type II and type I, is depicted.[26]

In diterpene biosynthesis, it is often observed that after a type II cyclization, a type I cyclization occurs, accompanied by the elimination of the diphosphate group, resulting in highly diverse molecular structures.

3.2 *Type I Terpene Cyclases*

A type I TC is initiated by the ionization of the diphosphate group to form an allylic cation intermediate (not shown).[40] The aspartate-rich DDxxD motif binds to the bound divalent catons, which are essential for the abstraction of the diphosphate moiety. The neighboring double bond then attacks the carbocation (not shown) and is precisely delocalized through a series of cyclization reactions to form the carbocation **15** (Fig. 3). This carbocation **15** is stabilized by interactions with aromatic residues located near the active site and is ultimately quenched by water (steps after **15**).[41] Type I TCs often produce the corresponding fully cyclized monoterpenes, sesquiterpenes, and diterpenes.[39,40,42]

3.3 *Engineering of Diterpene Cyclases*

The increasing knowledge about terpene cyclase mechanisms and substrate promiscuity has led to the advent of TC engineering for broadening the chemical space of these complex skeletons. One avenue used for DTC engineering is to create novel skeletons by prematurely stopping the catalytic chain of reactions. As described in sections 3.1 and 3.2, the aromatic regions seem to be responsible for carbocation stabilization in both type I and type II DTCs, indicating that they heavily dictate the final terpene structure.[39] There has also been a lot of effort made in targeting the carbocation quenching steps. Recent work in DTC rational engineering has shown that by diverting or preventing the carbocation quenching step, novel scaffolds or a cyclized alcohol-containing intermediate could be obtained.[43] This semi-rational methodology used structural studies and docking experiments to localize possible catalytic bases and mutate them. Another avenue of engineering focuses on the DTC active site since even a single amino acid substitution in the active site can reprogram the enzyme.[39,40,44,45] The aforementioned engineering efforts have built a higher knowledge of DTC enzymatic mechanisms and potential for greater structural diversity spanning all TCs.

4. Pictet-Spenglerases

Pictet-Spenglerases (PSs) catalyze the condensation and ring formation between an β-arylethylamine moiety and a carbonyl-containing compound. The PS mechanism is as follows: the amine of the β-arylethylamine moiety attacks the carbonyl partner (aldehyde or ketone) (step **16** to **17** in Fig. 4). The resultant hydroxyl group (**17**) is protonated and subsequently eliminated, forming the key iminium intermediate (step **18** to **19** in Fig. 4). This intermediate is then intramolecularly attacked by the nearby aryl group (step **19** to **20** in Fig. 4), resulting in the formation of the bicyclic architecture, followed by restoration of the aromaticity of the aryl group through deprotonation (step **20** to **21** in Fig. 4). The cyclized alkaloid core is often further derivatized to create a variety of compounds. PSs mainly occur as standalone enzymes, but this type of cyclization has also been found to be catalyzed by the condensation domain of some NRPSs in bacterial systems.

4.1 *Pictet-Spenglerases as Standalone Enzymes*

Standalone Pictet-Spenglerases are frequently employed in the biosynthetic pathways of plant and bacterial natural products. For example, strictosidine synthase (STR) is responsible for the formation of the β-carboline moiety in strychnine (**4**) biosynthesis.[47,48] The mechanism of STR was elucidated by the O'Connor group, who demonstrated that product formation proceeds through a sequence of acid–base catalytic steps. Specifically, the formation of the iminium ion intermediate is acid-

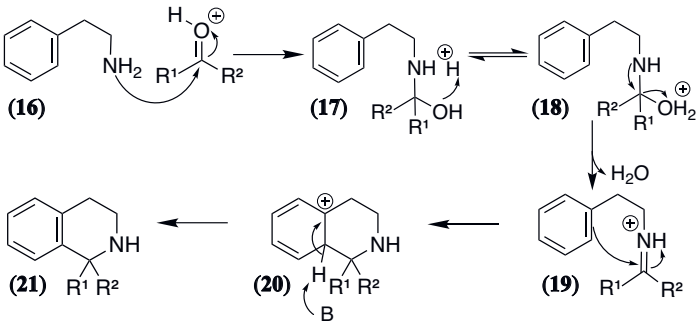

Fig. 4. The general mechanism of the Pictet-Spengler reaction.

catalyzed (step **18** to **19** in Fig. 4), while restoration of aromaticity is facilitated by base catalysis (step **20** to **21** in Fig. 4). Structural analysis identified a key glutamate residue (Glu309) responsible for mediating the acid–base transformations.

4.2 *Pictet-Spenglerases in NRPS Systems*

To date, NRPS Pictet-Spenglerases have only been found in bacteria, specifically in the biosynthesis of some members of the large tetrahydroisoquinoline (THIQ)[49] family, such as ET-743 (**5**).[50–52] The mechanism of this particular PS is noteworthy due to its ability to catalyze two or even three successive Pictet-Spengler reactions, enabling the stepwise construction of the pentacyclic core shared within the THIQ family.[53] *Via* deletion of the C domain of the structurally similar saframycin A PS, the Oguri lab found that this is indeed the domain responsible for the PS cyclization reaction.[54] Furthermore, the fatty acyl chain attached to the modified alanine (R^2 in Fig. 4) appears to be essential for substrate recognition in the saframycin pathway.[55] A fatty acyl chain length of C14 led to the expected product formation, while the activity of C12 was significantly reduced, and activity was completely abolished with shorter chain lengths. The specific mechanism or interaction responsible for recognizing the substrate remains unknown.

4.3 *Engineering of Pictet-Spenglerases*

Pictet-Spenglerase engineering has focused on expanding the enzyme's substrate scope to include aldehydes, ketones, or functionalized β-arylethylamine moieties.[56–58] The O'Connor group engineered STR to accept halogenated tryptophan, which could serve as a chemical handle for further functionalization.[56,59,60]

The modular architecture of NRPSs makes them highly attractive for engineering efforts. Substitution of entire modules or the adenylation domain responsible for the amino acid selectivity allows for rapid diversification. These cut-and-paste methods have become relatively popular in the field, with numerous successful reprogrammed NRPSs existing.[61–63] However, this swapping tactic is not always robust and often requires donor and acceptor NRPSs to have similar characteristics such as GC content.[64] Moreover, the disruption of interdomain connectivity and changes in the overall structure and folding can lead to a reduction or complete inhibition of activity. Engineering efforts have often focused on the A domain, which is responsible for the specificity of the amino acid; however, engineering can also occur at the C domain.[65,66] This foundation provides an intriguing proposition to apply these principles to the engineering of PS-catalyzing NRPSs. However, it must be considered that the condensation domain sometimes fails at recognizing amino acids supplied by the recombinant A domain.

5. Outlook for the Use of Cyclization Biocatalysts in the Drug Development Process

The use of enzymes in the pharmaceutical industry is still limited to relatively simple enzymatic reactions, such as hydrolysis, transamination,[67] ketoreductase, alcohol dehydrogenase and recently, imine reduction, which Novartis utilized to produce ZPL389.[68] The challenges associated with using more non-standard enzymes involve issues with yield, throughput,[3,69,70] and adherence to industry standards.[71] The pharmaceutical sector requires high turnover rates for production-level biosynthesis. Only a few examples of large-scale production have been achieved using enzymes, for instance the use of hydrolases and lyases.[3,69]

Incorporating a broader range of enzyme types into synthetic schemes in the pharmaceutical industry is also vital for accessing novel scaffolds that were previously challenging to obtain through traditional synthetic chemistry. Additionally, using more diverse enzymes could help lower reaction scale-up costs and promote sustainability in certain processes.

One of the initial steps in the drug discovery process is screening libraries. It has been shown that 83% of the unique ring systems in natural products are absent from libraries due to our inability to synthesize them,[3,72] and therefore, incorporating more ring-forming or cyclization biocatalysts in our toolbox could expand those libraries and allow for facile and rapid analog generation.

To the best of our knowledge, cyclases are yet to be used on an industrial scale in their purified form for production of therapeutics. Nevertheless, they have been applied in whole cell biocatalysis or semi-synthetic methods[50,73,74] for the production of building blocks for drug-like molecules. The biochemical understanding of cyclization-catalyzing enzymes and their mechanisms paves the way for their use in the construction of complex chemical architectures that were previously inaccessible *via* traditional methods.

6. Development and Use of Cyclization Biocatalysts in the Fraley Lab

The Fraley Lab at ETH Zürich has a central theme of using chemistry to understand the molecular complexity of living systems. A main line of research within the group involves understanding the biosynthetic development of complex chemical reactions, such as that for terpene and THIQ natural products, and deciphering the molecular-level intricacies that facilitate the selectivity with which they are catalyzed. This work requires a versatile work environment with specialties spanning biology and chemistry with a pipeline from microbial and plant cultivation, genetic manipulation, isolation and characterization of metabolites, enzymology, and structural biology. With the breadth of these expertises present in the lab, we pursue a common goal of elucidating natural product biosyntheses and tuning the responsible enzymes toward selective chemical transformations.

The methodologies described in this review represent the overarching skillset applied in former work by the principal investigator to characterize, for example, indole alkaloid and polyketide biosynthetic pathways.[75–78] This includes contributions to the field of natural product biosynthesis and structural biology. The classes of enzymes highlighted in this manuscript represent the trajectory of the new natural products research group led by Amy Fraley in the ETH Zürich Department of Chemistry and Applied Biosciences and the Institute of Pharmaceutical Sciences.

Acknowledgements

The authors acknowledge ETH Zürich for their funding and Anita Berg for her helpful feedback during the writing process.

Received: September 15, 2025

[1] Our History, https://www.path.ox.ac.uk/centenary/our-history/, accessed July 18, 2025.
[2] E. Romero, B. S. Jones, B. N. Hogg, A. Rué Casamajo, M. A. Hayes, S. L. Flitsch, N. J. Turner, C. Schnepel, *Angew. Chem., Int. Ed.* **2021**, *60*, 16824, https://doi.org/10.1002/anie.202014931.
[3] E. N. Kissman, M. B. Sosa, D. C. Millar, E. J. Koleski, K. Thevasundaram, M. C. Y. Chang, *Nature* **2024**, *631*, 37, https://doi.org/10.1038/s41586-024-07506-w.
[4] J. McConathy, M. J. Owens, *Prim. Care Companion J. Clin. Psychiatry* **2003**, *5*, 70, https://doi.org/10.4088/pcc.v05n0202.
[5] M. Hönig, P. Sondermann, N. J. Turner, E. M. Carreira, *Angew. Chem., Int. Ed*. **2017**, *56*, 8942, https://doi.org/10.1002/anie.201612462.
[6] K. M. Koeller, C.-H. Wong, *Nature* **2001**, *409*, 232, https://doi.org/10.1038/35051706.
[7] P. F. Wiley, K. Gerzon, E. H. Flynn, M. V. Sigal, O. Weaver, U. C. Quarck, R. R. Chauvette, R. Monahan, *J. Am. Chem. Soc.* **1957**, *79*, 6062, https://doi.org/10.1021/ja01579a059.
[8] J. R. Martin, W. Rosenbrook, *Biochemistry* **1967**, *6*, 435, https://doi.org/10.1021/bi00854a010.
[9] M. L. Adrover-Castellano, J. J. Schmidt, D. H. Sherman, *ChemCatChem* **2021**, *13*, 2095, https://doi.org/10.1002/cctc.202001886.
[10] B. Beckert, E. C. Leroy, S. Sothiselvam, L. V. Bock, M. S. Svetlov, M. Graf, S. Arenz, M. Abdelshahid, B. Seip, H. Grubmüller, A. S. Mankin, C. A. Innis, N. Vázquez-Laslop, D. N. Wilson, *Nat. Commun*. **2021**, *12*, 4466, https://doi.org/10.1038/s41467-021-24674-9.
[11] V. Miao, M.-F. Coëffet-LeGal, P. Brian, R. Brost, J. Penn, A. Whiting, S. Martin, R. Ford, I. Parr, M. Bouchard, C. J. Silva, S. K. Wrigley, R. H. Baltz, *Microbiology* **2005**, *151*, 1507, https://doi.org/10.1099/mic.0.27757-0.
[12] S. Patel, S. Saw, in 'StatPearls', StatPearls Publishing, Treasure Island (FL), **2025**.
[13] C. J. Dunn, A. J. Wagstaff, C. M. Perry, G. L. Plosker, K. L. Goa, *Drugs* **2001**, *61*, 1957, https://doi.org/10.2165/00003495-200161130-00006.
[14] H. Fliri, G. Baumann, A. Enz, J. Kallen, M. Luyten, V. Mikol, R. Movva, V. Quesniaux, M. Schreier, M. Walkinshaw, *Ann. N. Y. Acad. Sci.* **1993**, *696*, 47, https://doi.org/10.1111/j.1749-6632.1993.tb17141.x.
[15] J. Stöckigt, A. P. Antonchick, F. Wu, H. Waldmann, *Angew. Chem., Int. Ed*. **2011**, *50*, 8538, https://doi.org/10.1002/anie.201008071.
[16] R. Roddan, J. M. Ward, N. H. Keep, H. C. Hailes, *Curr. Opin. Chem. Biol*. **2020**, *55*, 69, https://doi.org/10.1016/j.cbpa.2019.12.003.
[17] D. P. Zlotos, Y. M. Mandour, A. A. Jensen, *Nat. Prod. Rep*. **2022**, *39*, 1910, https://doi.org/10.1039/D1NP00079A.
[18] M. D'Incalci, C. M. Galmarini, *Mol. Cancer Ther*. **2010**, *9*, 2157, https://doi.org/10.1158/1535-7163.MCT-10-0263.
[19] S. B. Horwitz, *Ann. Oncol*. **1994**, *5* Suppl 6, S3.
[20] P. T. Lim, B. H. Goh, W.-L. Lee, in 'Paclitaxel', Elsevier, **2022**, pp. 47, https://doi.org/10.1016/B978-0-323-90951-8.00007-2.
[21] The Nobel Prize in Physiology or Medicine **2015**, https://www.nobelprize.org/prizes/medicine/2015/summary/, accessed September 5, 2025.
[22] M. Rudrapal, D. Chetia, *Drug Des., Dev. Ther*. **2016**, *10*, 3575, https://doi.org/10.2147/DDDT.S118116.
[23] K. Mann, D. Mecke, *Nature* **1979**, *282*, 535, https://doi.org/10.1038/282535a0.
[24] T. A. Pemberton, D. W. Christianson, *J. Antibiot*. **2016**, *69*, 486, https://doi.org/10.1038/ja.2016.39.
[25] Z. Eyal, D. Matzov, M. Krupkin, S. Paukner, R. Riedl, H. Rozenberg, E. Zimmerman, A. Bashan, A. Yonath, *Sci. Rep.* **2016**, *6*, 39004, https://doi.org/10.1038/srep39004.
[26] F. Alberti, K. Khairudin, E. R. Venegas, J. A. Davies, P. M. Hayes, C. L. Willis, A. M. Bailey, G. D. Foster, *Nat. Commun*. **2017**, *8*, 1831, https://doi.org/10.1038/s41467-017-01659-1.
[27] W. L. Kelly, *Org. Biomol. Chem*. **2008**, *6*, 4483, https://doi.org/10.1039/B814552K.
[28] H. J. Kim, M. W. Ruszczycky, H. Liu, *Curr. Opin. Chem. Biol*. **2012**, *16*, 124, https://doi.org/10.1016/j.cbpa.2011.12.017.
[29] B. Jeon, S.-A. Wang, M. W. Ruszczycky, H. Liu, *Chem. Rev*. **2017**, *117*, 5367, https://doi.org/10.1021/acs.chemrev.6b00578.
[30] L. Gao, Y. Zou, X. Liu, J. Yang, X. Du, J. Wang, X. Yu, J. Fan, M. Jiang, Y. Li, K. N. Houk, X. Lei, *Nat. Catal*. **2021**, *4*, 1059, https://doi.org/10.1038/s41929-021-00717-8.
[31] L. Gao, Q. Ding, X. Lei, *Acc. Chem. Res*. **2024**, *57*, 2166, https://doi.org/10.1021/acs.accounts.4c00315.
[32] E. M. Stocking, R. M. Williams, *Angew. Chem. Int. Ed.* **2003**, *42*, 3078, https://doi.org/10.1002/anie.200200534.
[33] J. Kennedy, K. Auclair, S. G. Kendrew, C. Park, J. C. Vederas, C. Richard Hutchinson, *Science* **1999**, *284*, 1368, https://doi.org/10.1126/science.284.5418.1368.
[34] K. Steiner, H. Schwab, *Comput. Struct. Biotechnol. J.* **2012**, *2*, e201209010, https://doi.org/10.5936/csbj.201209010.
[35] P. Ferreira, P. A. Fernandes, M. J. Ramos, *Chem Catal*. **2022**, *2*, 2481, https://doi.org/10.1016/j.checat.2022.09.036.

[36] J. Abramson, J. Adler, J. Dunger, R. Evans, T. Green, A. Pritzel, O. Ronneberger, L. Willmore, A. J. Ballard, J. Bambrick, S. W. Bodenstein, D. A. Evans, C.-C. Hung, M. O'Neill, D. Reiman, K. Tunyasuvunakool, Z. Wu, A. Žemgulyté, E. Arvaniti, C. Beattie, O. Bertolli, A. Bridgland, A. Cherepanov, M. Congreve, A. I. Cowen-Rivers, A. Cowie, M. Figurnov, F. B. Fuchs, H. Gladman, R. Jain, Y. A. Khan, C. M. R. Low, K. Perlin, A. Potapenko, P. Savy, S. Singh, A. Stecula, A. Thillaisundaram, C. Tong, S. Yakneen, E. D. Zhong, M. Zielinski, A. Žídek, V. Bapst, P. Kohli, M. Jaderberg, D. Hassabis, J. M. Jumper, *Nature* **2024**, *630*, 493, https://doi.org/10.1038/s41586-024-07487-w.
[37] R. J. McLure, S. E. Radford, D. J. Brockwell, *Trends Chem*. **2022**, *4*, 378, https://doi.org/10.1016/j.trechm.2022.02.004.
[38] L. Sellés Vidal, M. Isalan, J. T. Heap, R. Ledesma-Amaro, *RSC Chem. Biol*. **2023**, *4*, 271, https://doi.org/10.1039/d2cb00231k.
[39] X. Pan, J. D. Rudolf, L.-B. Dong, *Nat. Prod. Rep*. **2024**, *41*, 402, https://doi.org/10.1039/D3NP00033H.
[40] D. W. Christianson, *Chem Rev.* **2017**, *117*, 11570, https://doi.org/10.1021/acs.chemrev.7b00287.
[41] P. Baer, P. Rabe, K. Fischer, C. A. Citron, T. A. Klapschinski, M. Groll, J. S. Dickschat, *Angew. Chem., Int. Ed.* **2014**, *53*, 7652, https://doi.org/10.1002/anie.201403648.
[42] C. A. Lesburg, G. Zhai, D. E. Cane, D. W. Christianson, *Science* **1997**, *277*, 1820, https://doi.org/10.1126/science.277.5333.1820.
[43] P. Zerbe, A. Chiang, M. Yuen, B. Hamberger, B. Hamberger, J. A. Draper, R. Britton, J. Bohlmann, *J. Biol. Chem*. **2012**, *287*, 12121, https://doi.org/10.1074/jbc.M111.317669.
[44] T. Tomita, S.-Y. Kim, K. Teramoto, A. Meguro, T. Ozaki, A. Yoshida, Y. Motoyoshi, N. Mori, K. Ishigami, H. Watanabe, M. Nishiyama, T. Kuzuyama, *ACS Chem. Biol*. **2017**, *12*, 1621, https://doi.org/10.1021/acschembio.7b00154.
[45] A. M. A. A. Raslan, R. J. Peters, *Phytochemistry* **2025**, *235*, 114459, https://doi.org/10.1016/j.phytochem.2025.114459.
[46] B. R. Lichman, J. Zhao, H. C. Hailes, J. M. Ward, *Nat. Commun*. **2017**, *8*, 14883, https://doi.org/10.1038/ncomms14883.
[47] J. J. Maresh, L.-A. Giddings, A. Friedrich, E. A. Loris, S. Panjikar, B. L. Trout, J. Stöckigt, B. Peters, S. E. O'Connor, *J. Am. Chem. Soc*. **2008**, *130*, 710, https://doi.org/10.1021/ja077190z.
[48] B. Hong, D. Grzech, L. Caputi, P. Sonawane, C. E. R. López, M. O. Kamileen, N. J. Hernández Lozada, V. Grabe, S. E. O'Connor, *Nature* **2022**, *607*, 617, https://doi.org/10.1038/s41586-022-04950-4.
[49] A. N. Kim, A. Ngamnithiporn, E. Du, B. M. Stoltz, *Chem. Rev.* **2023**, *123*, 9447, https://doi.org/10.1021/acs.chemrev.3c00054.
[50] C. Cuevas, A. Francesch, *Nat. Prod. Rep*. **2009**, *26*, 322, https://doi.org/10.1039/B808331M.
[51] C. M. Rath, B. Janto, J. Earl, A. Ahmed, F. Z. Hu, L. Hiller, M. Dahlgren, R. Kreft, F. Yu, J. J. Wolff, H. K. Kweon, M. A. Christiansen, K. Håkansson, R. M. Williams, G. D. Ehrlich, D. H. Sherman, *ACS Chem. Biol*. **2011**, *6*, 1244, https://doi.org/10.1021/cb200244t.
[52] M. M. Schofield, S. Jain, D. Porat, G. J. Dick, D. H. Sherman, *Environ. Microbiol*. **2015**, *17*, 3964, https://doi.org/10.1111/1462-2920.12908.
[53] A. E. Fraley, S. L. Robinson, J. Piel, *Aldrichimica Acta* **2022**, *55*, 55.
[54] K. Koketsu, K. Watanabe, H. Suda, H. Oguri, H. Oikawa, *Nat. Chem. Biol*. **2010**, *6*, 408, https://doi.org/10.1038/nchembio.365.
[55] R. Tanifuji, N. Haraguchi, H. Oguri, *Tetrahedron Chem* **2022**, *1*, 100010, https://doi.org/10.1016/j.tchem.2022.100010.
[56] H. Kries, S. E. O'Connor, *Curr. Opin. Chem. Biol*. **2016**, *31*, 22, https://doi.org/10.1016/j.cbpa.2015.12.006.
[57] H. Liu, S. Panjikar, X. Sheng, Y. Futamura, C. Zhang, N. Shao, H. Osada, H. Zou, *ACS Chem. Biol*. **2022**, *17*, 187, https://doi.org/10.1021/acschembio.1c00844.
[58] H. Liu, W. Jiang, J. Li, Y. Futamura, H. Osada, H. Zou, *ACS Chem. Biol*. **2025**, https://doi.org/10.1021/acschembio.5c00455.
[59] W. Runguphan, X. Qu, S. E. O'Connor, *Nature* **2010**, *468*, 461, https://doi.org/10.1038/nature09524.
[60] W. Runguphan, S. E. O'Connor, *Org. Lett*. **2013**, *15*, 2850, https://doi.org/10.1021/ol401179k.
[61] A. Schneider, T. Stachelhaus, M. A. Marahiel, *Mol. Genet. Genomics* **1998**, *257*, 308, https://doi.org/10.1007/s004380050652.
[62] R. H. Baltz, *ACS Synth. Biol.* **2014**, *3*, 748, https://doi.org/10.1021/sb3000673.
[63] H. Kries, D. L. Niquille, D. Hilvert, *Chem. Biol*. **2015**, *22*, 640, https://doi.org/10.1016/j.chembiol.2015.04.015.
[64] K. A. J. Bozhüyük, L. Präve, C. Kegler, L. Schenk, S. Kaiser, C. Schelhas, Y.-N. Shi, W. Kuttenlochner, M. Schreiber, J. Kandler, M. Alanjary, T. M. Mohiuddin, M. Groll, G. K. A. Hochberg, H. B. Bode, *Science* **2024**, *383*, eadg4320, https://doi.org/10.1126/science.adg4320.
[65] I. B. Folger, N. F. Frota, A. Pistofidis, D. L. Niquille, D. A. Hansen, T. M. Schmeing, D. Hilvert, *Nat. Chem. Biol*. **2024**, *20*, 761, https://doi.org/10.1038/s41589-023-01532-x.
[66] H. Peng, J. Schmiederer, X. Chen, G. Panagiotou, H. Kries, *ACS Chem. Biol.* **2024**, *19*, 599, https://doi.org/10.1021/acschembio.3c00678.
[67] C. K. Savile, J. M. Janey, E. C. Mundorff, J. C. Moore, S. Tam, W. R. Jarvis, J. C. Colbeck, A. Krebber, F. J. Fleitz, J. Brands, P. N. Devine, G. W. Huisman, G. J. Hughes, *Science* **2010**, *329*, 305, https://doi.org/10.1126/science.1188934.
[68] E. Siirola, F. Eggimann, C. Moore, K. Schroer, A. Vargas, T. Peschke, T. Schlama, R. Snajdrova, *CHIMIA* **2023**, *77*, 376, https://doi.org/10.2533/chimia.2023.376.
[69] A. Schmid, J. S. Dordick, B. Hauer, A. Kiener, M. Wubbolts, B. Witholt, *Nature* **2001**, *409*, 258, https://doi.org/10.1038/35051736.
[70] S. Wu, R. Snajdrova, J. C. Moore, K. Baldenius, U. T. Bornscheuer, *Angew. Chem., Int. Ed*. **2021**, *60*, 88, https://doi.org/10.1002/anie.202006648.
[71] U. T. Bornscheuer, G. W. Huisman, R. J. Kazlauskas, S. Lutz, J. C. Moore, K. Robins, *Nature* **2012**, *485*, 185, https://doi.org/10.1038/nature11117.
[72] J. Hert, J. J. Irwin, C. Laggner, M. J. Keiser, B. K. Shoichet, *Nat. Chem. Biol*. **2009**, *5*, 479, https://doi.org/10.1038/nchembio.180.
[73] P. A. Ceballos, M. Pérez, C. Cuevas, A. Francesch, I. Manzanares, A. M. Echavarren, *Eur. J. Org. Chem*. **2006**, *2006*, 1926, https://doi.org/10.1002/ejoc.200500882.
[74] R. Tanifuji, H. Oguri, *Beilstein J. Org. Chem*. **2024**, *20*, 1693, https://doi.org/10.3762/bjoc.20.151.
[75] A. E. Fraley, M. Garcia-Borràs, A. Tripathi, D. Khare, E. V. Mercado-Marin, H. Tran, Q. Dan, G. P. Webb, K. R. Watts, P. Crews, R. Sarpong, R. M. Williams, J. L. Smith, K. N. Houk, D. H. Sherman, *J. Am. Chem. Soc*. **2017**, *139*, 12060, https://doi.org/10.1021/jacs.7b06773.
[76] A. E. Fraley, K. Caddell Haatveit, Y. Ye, S. P. Kelly, S. A. Newmister, F. Yu, R. M. Williams, J. L. Smith, K. N. Houk, D. H. Sherman, *J. Am. Chem. Soc*. **2020**, *142*, 2244, https://doi.org/10.1021/jacs.9b09070.
[77] A. E. Fraley, C. L. Dieterich, M. F. J. Mabesoone, H. A. Minas, R. A. Meoded, F. Hemmerling, J. Piel, *Angew. Chem., Int. Ed.* **2022**, *61*, e202206385, https://doi.org/10.1002/anie.202206385.
[78] A. E. Fraley, M. Dell, M. Schmalhofer, R. A. Meoded, C. Bergande, M. Groll, J. Piel, *Structure* **2023**, *31*, 565, https://doi.org/10.1016/j.str.2023.02.010.

The definitive version of this article is the electronic one that can be found at https://doi.org/10.2533/chimia.2025.797