Alkaloid Synthesis

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The Pictet–Spengler Reaction in Nature and in Organic Chemistry

Joachim Stöckigt, Andrey P. Antonchick, Fangrui Wu, and Herbert Waldmann**

Keywords:
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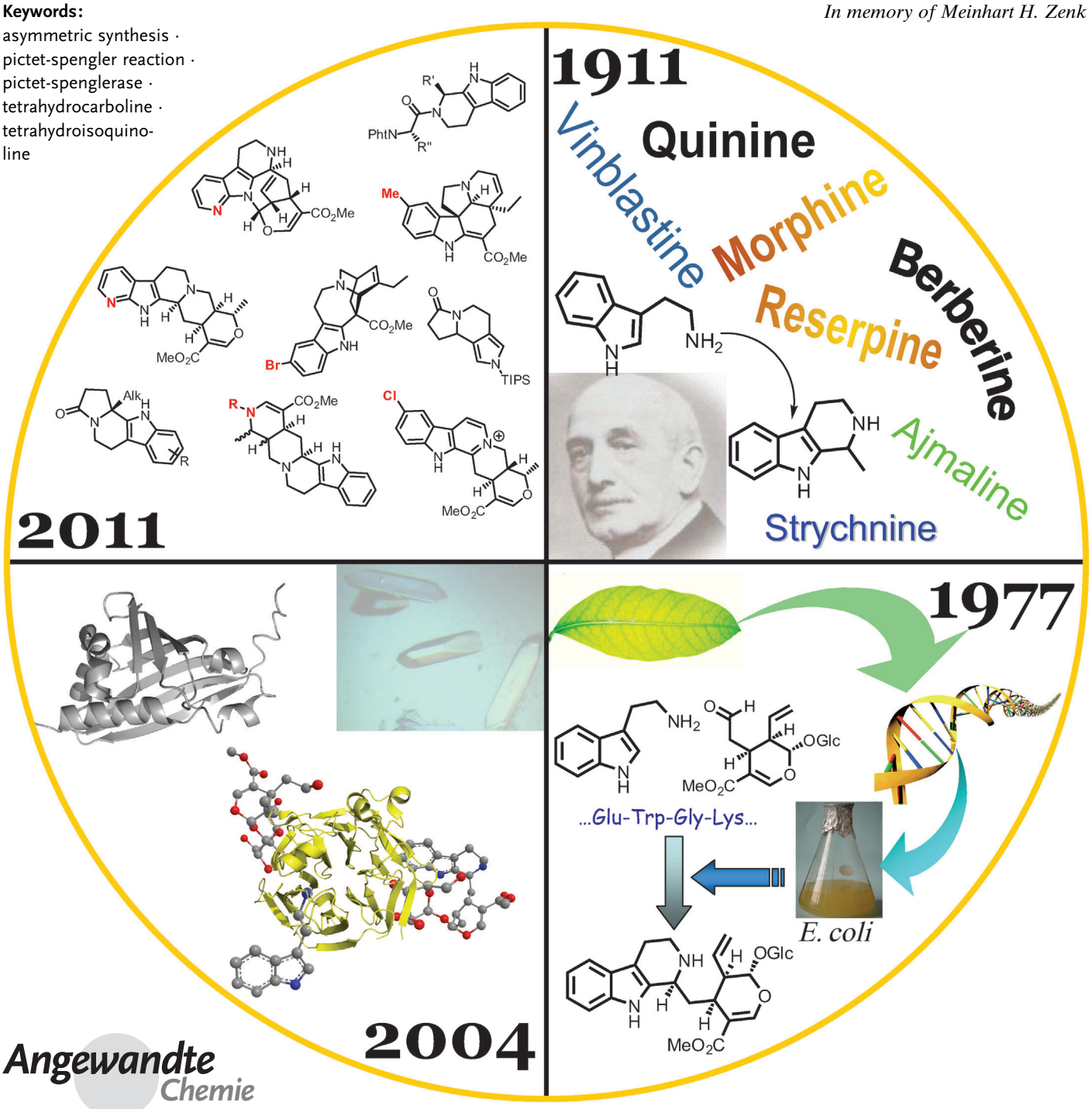
In memory of Meinhart H. Zenk



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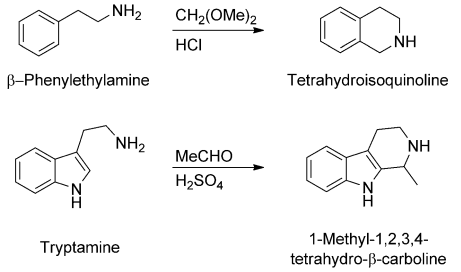
Alkaloids are an important class of natural products that are widely distributed in nature and produced by a large variety of organisms. They have a wide spectrum of biological activity and for many years were used in folk medicine. These days, alkaloids also have numerous applications in medicine as therapeutic agents. The importance of these natural products in inspiring drug discovery programs is proven and, therefore, their continued synthesis is of significant interest. The condensation discovered by Pictet and Spengler is the most important method for the synthesis of alkaloid scaffolds. The power of this synthesis method has been convincingly proven in the construction of stereochemicaly and structurally complex alkaloids.

From the Contents

1. Discovery of the Pictet–Spengler Reaction (PSR)

When, in their laboratories at the University of Geneva in 1911, the chemists Amè Pictet and Theodor Spengler heated β-phenylethylamine and formaldehyde dimethylacetal in the presence of hydrochloric acid, the two compounds underwent a cycloaddition reaction.[1] The new product formed was the alkaloid 1,2,3,4-tetrahydroisoquinoline (THIQ). Was it already at this time Pictet's intention to find a simple way of achieving the chemical synthesis of alkaloids? His previously written "La constitution chimique des alcaloides végétaux" may suggest as much. The reaction was named the Pictet–Spengler reaction (PSR), and is now a reaction used for the synthesis of a large variety of heterocyclic compounds. In 2011, the PSR celebrates 100 years of success in the chemical laboratory. During this long period, and up to the present day, the reaction has become one of the most successful synthetic strategies particularly directed towards the synthesis of the isoquinoline and indole alkaloid frameworks. The reaction (Scheme 1) has undergone continuous modification and found broader application in converting, for example, N-alkylated, N-acylated, or N-sulfonylated derivatives of phenylethylamine.

After the discovery of the PSR it took nearly 20 years before Tatsui used tryptamine as the amine component, which paved the way for the first synthetic creation of the 1,2,3,4-tetrahydro-β-carboline (THBC) skeleton (Scheme 1).[2] Not only do THIQ and THBC represent the structural key elements of an immense range of structurally complex synthetic products, they are key elements of thousands of naturally occurring isoquinoline and indole alkaloids, several of them being of enormous physiological and therapeutic significance.[3] Just as the amine component can be varied



Scheme 1. The first Pictet–Spengler reactions (PSRs), carried out in the synthesis of the tetrahydroisoquinoline (THIQ) and tetrahydro-β-carboline (THBC) alkaloid skeletons.[1,2]

[*] Prof. Dr. J. Stöckigt, F. Wu
Institute of Materia Medica
College of Pharmaceutical Sciences, Zhejiang University
866 Yuhangtang Road, Hangzhou, 310058 (China)
E-mail: joesto2000@yahoo.com
Dr. A. P. Antonchick, Prof. Dr. H. Waldmann
Max-Planck-Institut für Molekulare Physiologie
Abteilung Chemische Biologie
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany)
Fax: (+49) 231-133-2499
and
Technische Universität Dortmund, Fakultät Chemie
Chemische Biologie
Otto-Hahn-Strasse 6, 44221 Dortmund (Germany)
E-mail: herbert.waldmann@mpi-dortmund.mpg.de

structurally, the same is true for the aldehyde component, including masked aldehydes (such as acetals, enol ethers, hemiaminals, aminonitrils) as well as ketones.

Although the synthetic power of the PSR has been realized for many decades, an expanded application has clearly been restricted by the lack of asymmetric methods. However, during the last two decades there has been increasing interest and remarkable progress in developing new and highly enantioselective methods for the synthesis of THIQs and THBCs.[4,5] Such developments followed the discovery in 1977 of the first enzyme which catalyzes a Pictet–Spengler condensation with complete stereoselectivity (see Section 2). Enzymes of this type are known as Pictet–Spenglerases.

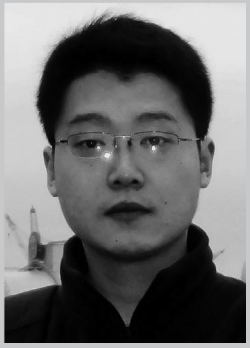
One of the most famous THIQ alkaloids is morphine, the major constituent of opium. Opium has possibly been used by humans since the beginning of civilization. Sumerians probably consumed opium 6000 years ago, and it was described as a tonic in Egyptian medical texts dating back 3500 years.[6a,b]

An enzyme-catalyzed Pictet–Spengler condensation is the key step for the biosynthesis of morphine. Whereas this reaction was developed thousands of years ago in nature, chemists have only applied it for a comparatively short time. As described in this Review, we are at the dawn of exploring and developing the detailed understanding required for the rational modulation of the enzymes responsible for the biological Pictet–Spengler reaction.

2. Enzyme-Catalyzed PSR: Strictosidine Synthases (STRs)

2.1. Detection of the First Pictet–Spenglerase and Structure of its Reaction Product

The first enzymatic experiments carried out to characterize the biosynthesis of the heteroyohimbine-type monoterpenoid indole alkaloid ajmalicine (raubasine) were performed about 35 years ago by employing the biosynthetically distant precursors tryptamine and secologanin with cell-free extracts from seedlings, callus, and cell suspension cultures of *Catharanthus roseus* (Apocynaceae).[7,8] The ajmalicine pathway became the first example of an alkaloid biosynthetic pathway elucidated in detail through the participating enzymes.[9–13] Several suggestions had already been made at that time relating to the nature and participation of the first intermediate in indole alkaloid biosynthesis that was generated by condensation between the amine and the aldehyde secologanin.[14,15] The *Catharanthus* cell suspension cultures were optimized for efficient alkaloid production,[16,17] which turned out to be an excellent system to prove these suggestions. Interrupting the cell-free synthesis of ajmalicine after the first reaction step led to the accumulation of the glucoalkaloid strictosidine, formed by an enzyme-catalyzed Pictet–Spengler reaction.



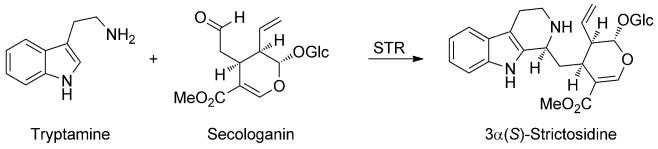
Fangrui Wu studied biochemistry at Hefei University of Technology and received a masters degree at Biomass Research Center, Ministry of Education (Hefei, China) in the field of isolation and analysis (LC-MS/MS) of gingkolides and natural glucosidase inhibitors from Morus albus. He is currently carrying out PhD research at Zhejiang University on the biochemistry and structural biology of key enzymes of monoterpenoid indole alkaloid biosynthesis.

Herbert Waldmann studied chemistry at the University of Mainz and completed his PhD in 1985 with Prof. Horst Kunz. After postdoctoral research with Prof. George Whitesides at Harvard University, he completed his habilitation at the University of Mainz in 1991. After professorships at the Universities of Bonn (1991) and Karlsruhe (1993), in 1999 he was appointed Director at the Max Planck Institute of Molecular Physiology Dortmund and Professor of Organic Chemistry at the University of Dortmund. His research concerns the synthesis of signal transduction modulators and natural product derived compound libraries, lipidated peptides and proteins, as well as protein microarray technology. His prizes include the Otto Bayer Award, the Max Bergmann Medal, and the GSK Award. He is a Member of "Deutsche Akademie der Naturforscher Leopoldina".

Joachim Stoeckigt received a PhD in organic chemistry from Muenster University (Germany) with Professor Burchard Franck. He has worked with Professor Meinhart H. Zenk at the Faculty of Biology (Bochum University, Germany) and the Faculty of Chemistry and Pharmacy (Munich University, Germany). Currently he is Professor at College of Pharmaceutical Sciences, Zhejiang University (Hangzhou, China) and retired from the Institute of Pharmacy (Mainz University, Germany). His research interests include natural products biosynthesis (phytochemistry, enzymology, as well as molecular and structural biology).

Andrey P. Antonchick studied chemistry at Belarussian State University and received a PhD in bioorganic chemistry from the Institute of Bioorganic Chemistry of the National Academy of Sciences of Belarus (Minsk, Belarus) and the Max Planck Institute for Chemical Ecology (Jena, Germany) under the guidance of Prof. V. A. Khripach and Priv. Doz. Dr. B. Schneider. After postdoctoral research with Prof. M. Rueping (Frankfurt University), he joined the group of Prof. H. Waldmann at the Max Planck Institute of Molecular Physiology (Dortmund, Germany), where in 2010 he was appointed group leader.

More than six decades after the discovery of the chemical Pictet–Spengler reaction,[1,2] the first Pictet–Spenglerase, named strictosidine synthase (STR; EC 4.3.3.2), was detected in nature.[18–20] As a consequence of the various suggestions as to the C3 configuration of the glucoalkaloid, a rigorous proof of its structure was necessary. A comparison of an authentic sample of 3α(*S*)-strictosidine with some of its acetylated derivatives by chromatography, circular dichroism, NMR spectroscopy, and isotope dilution analysis revealed that the enzyme produced 3α(*S*)-strictosidine stereoselectively.[18–20] Formation of the 3β epimer (vincoside), which is generated in an approximate 1:1 mixture with the 3α epimer by the chemical PSR, was not observed. Detailed information provided by Hutchinson (USA), Brown (UK), and Snatzke (Germany) ultimately helped to determine unambiguously the structure of the enzymatically formed strictosidine (Scheme 2).



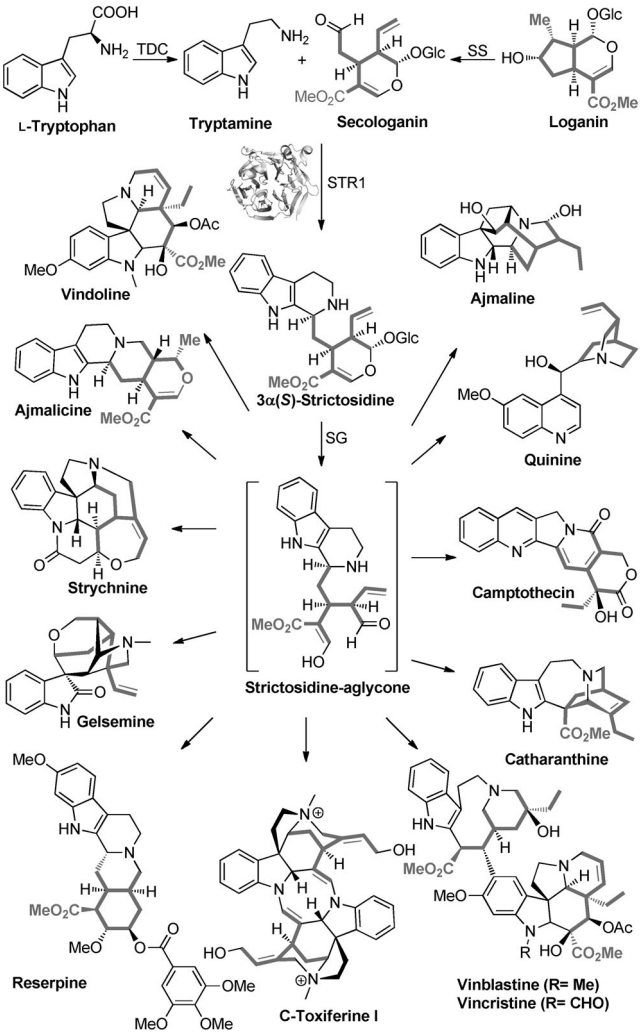
Scheme 2. Enzymatic condensation of tryptamine and the monoterpenoid aldehyde secologanin catalyzed by the Pictet–Spenglerase strictosidine synthase from *Catharanthus* (STR) or *Rauvolfia* (STR1).

2.2. Biosynthetic Significance of Strictosidine and STRs

Feeding experiments with isotope-labeled strictosidine to a range of plants clearly established the compound as the central and sole biosynthetic progenitor of probably all monoterpenoid indole alkaloids, including Corynanthè/Strychnos, Aspidosperma, and Iboga alkaloids, as well as alkaloids displaying a 3β instead of the 3α configuration (Scheme 3).[21,22] Confirmation that strictosidine is the first biosynthetic intermediate in pathways that deliver the structurally diverse monoterpenoid indole alkaloids soon followed.[23–25] A taxonomic survey of STR synthase distribution was performed, and the enzyme was shown to occur in plants producing solely indole alkaloids, and its absence in indole alkaloid-unproductive plants.[26] Despite in vivo feeding experiments,[21] it is this co-occurrence of enzyme and alkaloids, which provided further proof for the key biosynthetic role of STR and its product.

2.3. Biochemical Characterization and Purification of STR

It was the divergent synthetic and later biosynthetically useful nature of strictosidine as a gateway to a diverse range of pathways that led to the synthesis of therapeutically established drugs and pharmacological lead structures (such as ajmalicine, ajmaline, camptothecin, vinblastine, vincristine, strychnine, and toxiferines), which made the requirement of a detailed characterization and knowledge of the mechanism of this enzyme reaction so appealing.



Scheme 3. The biosynthetic significance of strictosidine as a central precursor of diverse types of the monoterpenoid indole alkaloid family, which consists of about 2000 structurally different members from higher plants, including the quinoline alkaloid quinine. The secologanin carbon skeleton in gray highlights the major structural changes arising by skeleton rearrangements during biosynthesis. TDC = tryptophan decarboxylase; SS = secologanin synthase; STR1 = strictosidine synthase; SG = strictosidine glucosidase.

The first purification protocols of *Catharanthus* STR were reported in 1979—two years after the detection of the synthase—and resulted in partial purification of the enzyme.[27,28] In subsequent studies, homogeneous STR was obtained from *Catharanthus* cell suspensions, but consisted of isozymes with similar physical and kinetic properties.[29] This made further biochemical studies on the enzyme inappropriate. Fortunately, the isolation and purification of the STR1 enzyme from cell cultures of the Indian medicinal plant *Rauvolfia serpentina* Benth. ex Kurz resulted in a single, pure STR1 isoform.[30] To differentiate from the *Catharanthus* STR, "STR1" hereafter refers to the Rauvolfia enzyme and "*str1*" to the gene. To produce the synthetically valuable strictosidine, and in particular to develop biomimetic alkaloid syntheses, the enzyme was immobilized on CNBr-activated sepharose; this simple approach allowed the straightforward

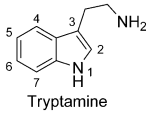
isolation of pure strictosidine on a multigram scale. Moreover, the immobilized form of the synthase showed higher stability than the solubilized STR.[31,32]

Despite its high degree of substrate specificity, potentially limiting its use in the generation of new alkaloid libraries, STR1 became the enzyme of choice to explore biotechnological applications of chemoenzymatic alkaloid synthesis. A list of the substrates accepted by STR1 is presented in Section 2.4.

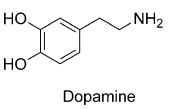
2.4. Substrate Specificity of STR1

The next major task in exploring the applicability of the Pictet–Spengler product to divergent biomimetic alkaloid syntheses was to test a wide range of both amines and aldehydes as putative substrates. Such studies were performed several times at different stages of STR purity obtained from *C. roseus*, *R. serpentina*, and *Cinchona robusta* cell cultures, as well as heterologously expressed STR and STR1.[27,33–36] Around 30 tryptamine derivatives were studied as potential substrates, of which about one third were accepted by the STRs in the presence of secologanin, although with differing conversion rates (mostly $<10\%$, compared to tryptamine; Table 1).[27,33–36]

Table 1: Selection of putative tryptamine derivatives with altered substituents at the indole unit which were tested for the enzymatic synthesis of various strictosidines by wild-type His_6-STR1.[a]



Tryptamine



Dopamine

Substrate	STR1 activity [%]
tryptamine	100
5-fluorotryptamine	9.9
5-hydroxytryptamine	9.5
7-methyltryptamine	8.9
6-methyltryptamine	8.9
5,6-dihydroxytryptamine	7.0
6-fluorotryptamine	6.6
6-methoxytryptamine	2.1
5-methyltryptamine	0
5-methoxytryptamine	0
dopamine	0

[a] Data are taken in part from Ref. [34] (relative enzyme activities in %; notice that 5-methyltryptamine and 5-methoxytryptamine are not converted).

These studies also demonstrated that the indole moiety is required for recognition and conversion by, for example, STR1. Tryptamines substituted with small groups (F, OH) in the aromatic ring showed enzyme-catalyzed turnover. Larger groups (CH_3O, CH_3) resulted in lower conversion rates or complete prevention of the reaction. Changes at other tryptamine positions were not accepted by the enzyme. When non-indolic compounds with the ethylamino side chain of tryptamine were tested with STR, only two of ten were converted, namely 3-(2-aminoethyl)benzofuran and its corresponding thiophene derivative.[35] The new strictosidines would be excellent synthons for the biomimetic synthesis of novel oxygen- or thio-analogous indole alkaloids, most likely displaying significantly altered biological and pharmacological activities.

STRs seem to be significantly less tolerant for the second Pictet–Spengler component—the secologanin aldehyde derivatives. Conversion of the secologanic acid or the aldehyde iridotrial into the corresponding strictosidine derivatives could not be detected.[27,30] However, 1) hydrogenation of the vinyl function, 2) methylation (2- and 3-OH group) of the glucose part of secologanin, and 3) alteration of its ester group resulted in derivatives that are accepted for the condensation reaction.[27,30,36] In addition, O′Connor and coworkers recently reported more detailed studies on the substrate specificity of a known[37,38] strictosidine synthase with a broader specificity from the plant *Ophiorhiza pumila* (Rubiaceae family). Notably, a range of achiral aldehydes are accepted by the enzyme, which can now be utilized to form highly enantioenriched tetrahydro-β-carbolines (*ee* $>98\%$).[39]

Table 1 summarizes the amine components tested for conversion into strictosidines in the presence of N-terminal His_6-tagged STR1, thus illustrating the variety of strictosidines that are now enzymatically available by using the Pictet–Spenglerase. These strictosidines appear to be applicable in further synthetic or chemoenzymatic approaches. Moreover, the problems relating to the inefficient conversion rates of STR1, its selective substrate acceptance, and relatively low yield from plant sources were overcome by using molecular genetic approaches developed by Kutchan and coworkers (see below) at the end of the 1980s. This study also paved the way for the first crystallization and X-ray analysis of the enzyme.

3. First 3D Structure of a Pictet–Spenglerase (STR1)

3.1. Cloning, Expression, and Crystallization of STR1

The availability of homogeneous and partially sequenced STR1 allowed for the isolation of cDNA from *Rauvolfia* cell cultures and the expression of the active Pictet–Spenglerase in *E. coli*, thereby confirming the isolation of the correct clone.[40,41] Heterologous and functional expression of *Rauvolfia* strictosidine synthase was achieved in different microbial hosts such as yeast (*Saccharomyces cerevisiae*), *E. coli*, and insect cells (*Spodoptera frugiperda*),[42] thereby allowing selection of the most powerful expression system. Optimal expression conditions were achieved in *S. frugiperda* and resulted in 10–15 mg L of pure STR1.

The *Rauvolfia* Pictet–Spenglerase was the first enzyme of all the plant alkaloid biosynthetic pathways whose cDNA had been cloned. A decade after its initial detection, this milestone represented a further breakthrough for STR1 research, and provided a significant boost to research into indole alkaloids.

To date, a total of six cDNAs encoding strictosidine synthase has been detected in plants of the Apocynaceae

family: three from the *Rauvolfia* species (*R. serpentina*,[40–42] *R. mannii*,[43] *R. verticillata*[44]) which exhibit 100% sequence identity, one from *C. roseus*,[40,41] and two from the *Ophiorhiza* species (*O. pumila* and *O. japonica*; Rubiaceae).[37,38,45] Although three of these (from *C. roseus* (*Cs-str*), *R. serpentina* (*Rs-str1*), and *O. pumila*[37]) have been functionally expressed in *E. coli*, *Rauvolfia* STR1 has been the most intensively studied and consequently the best known enzyme of the *Rauvolfia* alkaloid metabolism, as well as the biosynthesis of monoterpenoid indole alkaloids in general.

STR1 is an excellent and highly promising system which has to date allowed development of in-depth knowledge of this unique enzyme and the reaction it catalyzes, especially by using structural biology. The determination of its 3D structure has allowed elucidation of the overall enzyme architecture, has provided an insight into the active center, and the knowledge required to unravel the reaction mechanism so as to ultimately identify structurally and catalytically important amino acids which can be used as a basis for the rational redesign of its limited substrate acceptance. A His_6-tag STR1 has been expressed in *E. coli* and purified by affinity (Ni-NTA) and ion-exchange chromatography to allow crystallization of the native STR1 (Figure 1a).[46,47] Extensive screening established the necessary conditions for the successful crystallization of the first Pictet–Spenglerase.

3.2. The β-Propeller Fold of STR1 and Ligand Complexes with Substrates and the Enzyme Product

The X-ray analysis has shown that the overall architecture of STR1 represents a new member of the six-bladed β-propeller family, a specific fold detected for the first time in the plant kingdom (Figure 1b).[34,46,47] Each blade of the propeller consists of four-stranded antiparallel β sheets A–D. They are arranged around one axis to form the binding pocket in which the Pictet–Spengler reaction is catalyzed. The structure of the complex obtained by cocrystallization of STR1 with the substrate tryptamine allowed further insight into the nature of the reaction center (Figure 1b).[34] The substrate is located deep in the pocket with the amine group coordinated to Glu309. The indole part is sandwiched between the aromatic rings of Tyr151 and Phe226. This orientation most probably helps to keep the tryptamine in a productive position for the condensation reaction with the second substrate, secologanin (Figure 1c). The structure of the complex with secologanin shows that 1) the hydrophilic glucose part points out of the catalytic center towards the solvent, and 2) the aldehyde group points towards the nearest residue Glu309, which is close to the amino group of tryptamine. Clearly this is the favored position of both substrates for the primary reaction of the Pictet–Spengler condensation. The position of the substrates was illustrated by the structure of STR1 with its reaction product strictosidine. As shown in Figure 1d, the location of the enzyme product is nearly identical to that of both substrates.

The superimposition of all three ligands bound in the active center (Figure 1e) shows they are in nearly identical locations, with only relatively small differences (ca. 1.5 Å). The residues which bind to the sugar component of secologanin and strictosidine (but see Scheme 4), which may have indirect influence on the reaction pathway, are not shown.

Site-directed mutations of polar residues at the binding site (Table 2) reveal a sharp decrease in catalytic efficiency to about 0.1% (compared to the wild-type enzyme) when Glu309 is replaced by Ala. Since His307 binds to the glucose unit, the activity of His307Ala decreases down to about 2.3%, and when Tyr151 is exchanged for Phe, the k_{cat} value decreases by a mere 26%. Structural analysis and site-directed mutagenesis experiments currently suggest that Glu309 may be the catalytic amino acid responsible for the synthesis of strictosidine by STR1.

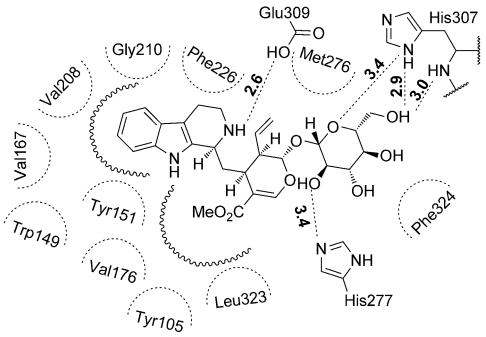
Table 2: Catalytic efficiency of wild-type STR1 and its mutants of hydrophilic residues of the active center.[34]

Enzyme	K_{cat} [min^{-1}]	%
wild-type	78.2	100
Glu309Ala	0.089	0.11
Tyr151Phe	57.7	73.8
His307Ala	1.8	2.3

3.3. Binding Pocket, Catalytic Residues, and Mechanistic Aspects of STR1

Three groups of amino acid residues form the shape of the binding pocket (Scheme 4):

- The hydrophobic residues Trp149, Tyr151, Val167, Val208, and Phe226, as well as Gly210; all are located less than 4 Å from strictosidine and interact with the tryptamine part.
- The four residues Tyr105, Val176, Leu323, and Met276 in proximity to the terpenoid skeleton of the secologanin part.
- The three amino acids His277, His307, and Phe324 close to the glucose moiety. Both polar histidine residues are



Scheme 4. Simplified representation of STR1 complexed with the reaction product strictosidine to illustrate the binding pocket and the interacting residues at a distance of under 4 Å. The space for structural modification of the substrates tryptamine and secologanin is indicated by wavy lines. Those regions are appropriate for enzyme engineering by site-directed mutagenesis to expand the substrate acceptance of the enzyme.

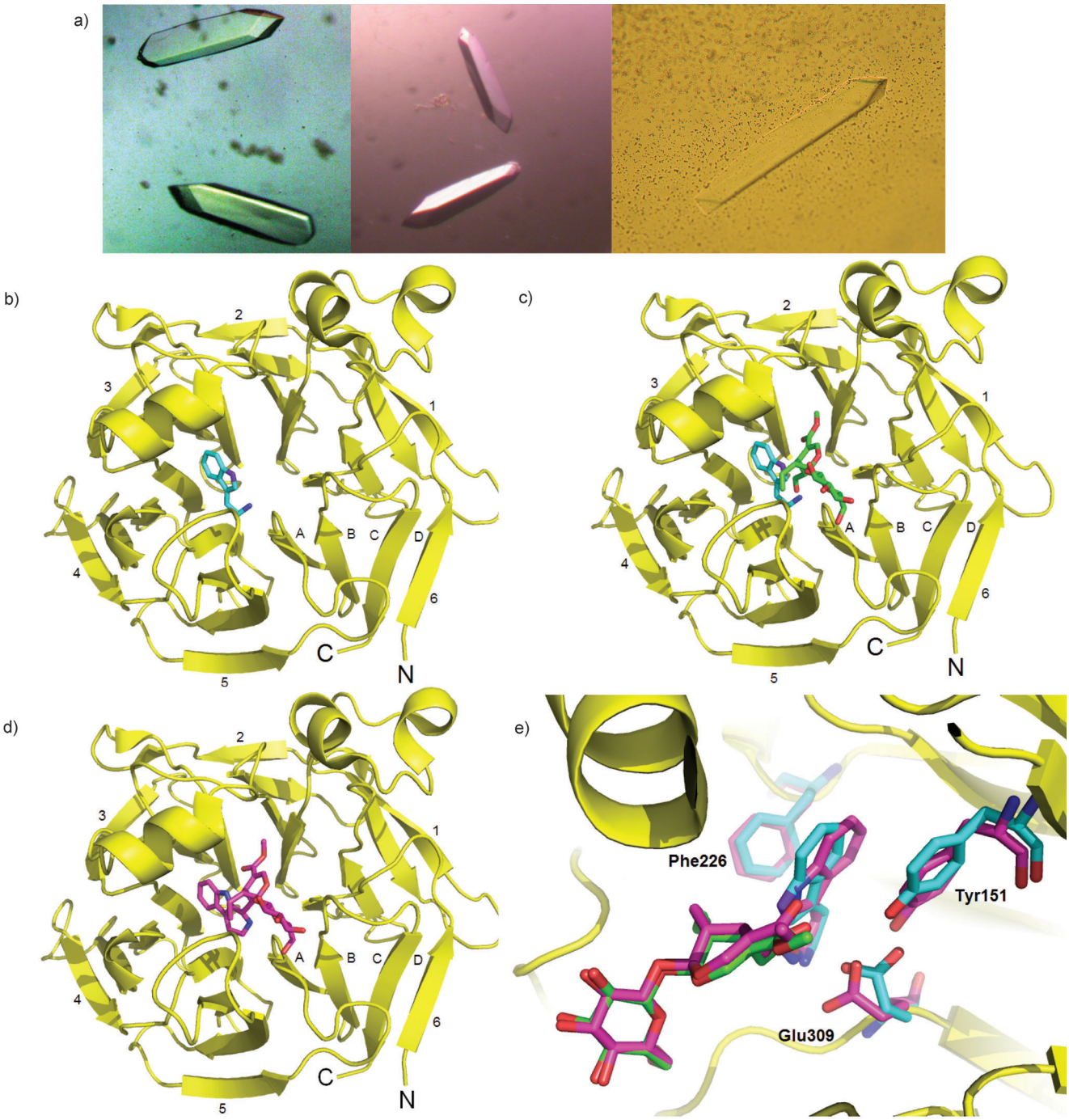
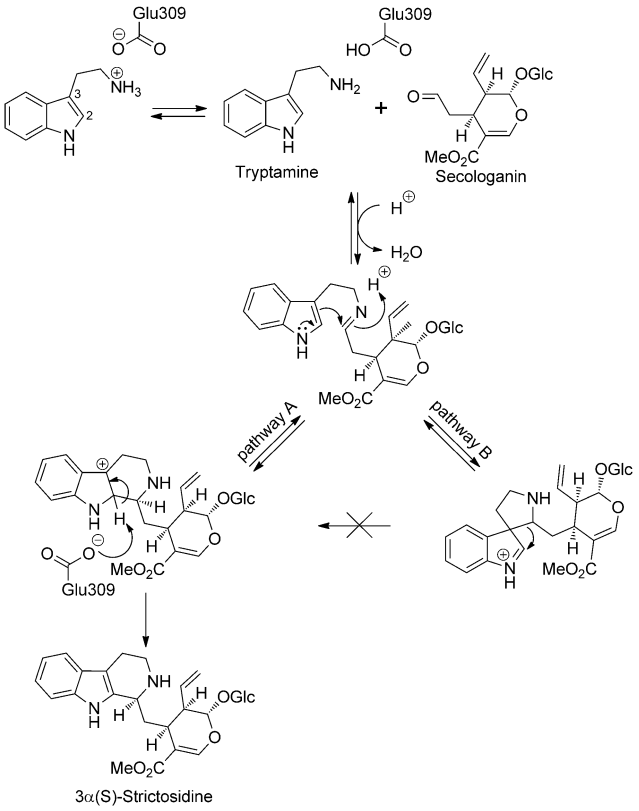


Figure 1. a) Single crystals of the Pictet–Spenglerase STR1 from *Rauvolfia* for the initial X-ray analysis. The crystals were grown by the "hanging-drop" technique (pictures provided by E. Loris). b) Overall architecture of STR1 in a complex with the substrate tryptamine (blue). The six-bladed β-propeller fold of the enzyme, with blades 1–6 and antiparallel β sheets A–D, as well as the position of tryptamine can be seen.[34,46] c) The tryptamine–Str1 complex (b) superimposed on the complex of bound secologanin (green); N and C are the N and C termini of the enzyme. d) Structure of STR1 in a complex with the bound enzyme product strictosidine (pink).[48,49] e) Enlarged image of the catalytic center of the three superimposed complexes (b–d) of STR1, shown with Glu309 as the catalytic residue. Tyr151 and Phe226 maintain the tryptamine in a favorable position for the Pictet–Spengler reaction by hydrophobic interaction (for simplicity, the interactions of the amino acids with secologanin are omitted).

located at the entrance of the binding pocket, most likely to accommodate the secologanin part and especially its glucose unit, which can face the solvent region located on the enzyme surface. These residues also keep the secologanin molecule in an optimal orientation for condensation with the amine. The arrangement of the residues is shown in Scheme 4.

Following elucidation of the 3D structure of STR1, a significant milestone in the history of research on this reaction could be made: the first detailed suggestions for the mechanism of the enzyme-catalyzed Pictet–Spengler reaction and redesign of the enzyme. Similar to the chemical reaction, formation of a Schiff base (iminium ion) between the amine function of tryptamine and the aldehyde group of secologanin follows formation of the carbinol, which can be defined as the first reaction step (Scheme 5). It has been suggested on the



Scheme 5. Suggested mechanism of the strictosidine synthase catalyzed Pictet–Spengler reaction between tryptamine and secologanin, which generates the biosynthetic monoterpenoid indole alkaloid precursor 3α(*S*)-strictosidine enantioselectively.[50,51]

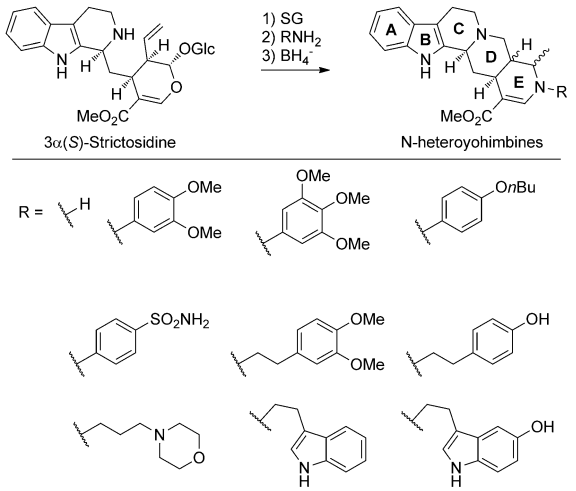
basis of the 3D structures of STR1 and its complexes with the substrates and the enzyme product that the Glu309 residue is instrumental to the enzymatic Pictet–Spengler reaction.[34] Glu309 is indeed very close to the amine at position 2, thereby ensuring that it stays neutral and nucleophilic for the PSR and for generation of the iminium intermediate. The C2 atom of the indole part of tryptamine then attacks the iminium species in an electrophilic substitution reaction via formation of a six-membered ring system and a tertiary carbocation. Glu309 may then deprotonate the cation at the C2-position by rearomatization and a final, exclusive creation of the 3α(*S*)-strictosidine. As demonstrated by a primary kinetic isotope effect, the rate-controling step of the reaction appears to be this rearomatization process. This is also suggested for the non-enzymatic PSR in solution. The isotope effect measured indicates that all the reaction steps before rearomatization by deprotonation are reversible.[50,51]

In principal, the nucleophilic C3 atom, instead of C2, could also attack the iminium carbon atom to perform the ring closure, but this would then lead to a spirocyclic intermediate followed by a rearrangement and formation of a six-membered ring. Ab initio calculations on the transition-state energy of such a spiro intermediate and rearrangement from a five- to a six-membered ring system show the process to be energetically unfavorable. Both results support a reaction sequence through pathway A and not pathway B.[49–51]

4. New Approaches Towards Novel Indole Alkaloids

4.1. STR-Based, Biomimetic Approach to Heteroyohimbines

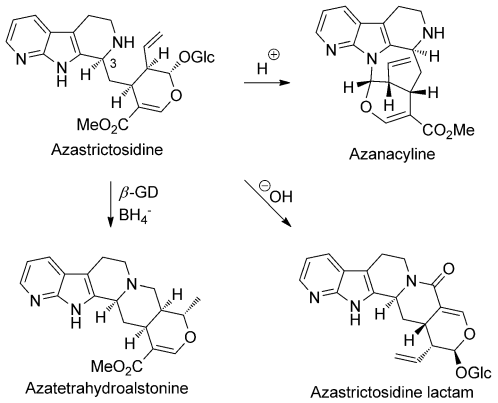
A biomimetic approach was developed on the basis of the first known enzyme-catalyzed pathway leading to ajmalicine,[9–12] and is similar to a related chemoenzymatic route described earlier.[52,53] Starting with strictosidine, removal of the glucosyl group is the next reaction step catalyzed by strictosidine glucosidase (SG), obtained from crude or pre-purified protein extracts of *C. roseus* or *R. serpentina* cell suspension cultures, or, if necessary, with the heterologously expressed 3D-structure-based modulated SG (Scheme 6).[54,55] The highly reactive strictosidine aglycon



Scheme 6. Biomimetic, chemoenzymatic approach directed towards novel N-analogues of heteroyohimbines from strictosidine (SG = strictosidine glucosidase).[48,56]

(progenitor of all the indole alkaloid pathways, as shown in Scheme 3) is formed, which is transformed into the well-known intermediates dehydrogeissoschizine and cathenamine in the biosynthetic pathway of ajmalicine (for details see Ref. [12]). In the likely presence of an excess of any primary amine (other nucleophiles have not yet been applied so far) closure of the E ring by a Michael addition takes place to deliver, after borohydride reduction, a diastereomeric mixture of each of the four N-analogous heteroyohimbines.[48,56]

This "one-pot" chemoenzymatic approach has recently been simplified and extended for the generation of the new alkaloid aza-tetrahydroalstonine[56] by using recombinant and His_6-tagged immobilized STR1 obtained from expression of its cDNA in *E. coli*. Following optimized overexpression,[57] STR1 was used for the synthesis of azastrictosidine from 7-azatryptamine (Scheme 7).[56,58] Moreover, azastrictosidine



Scheme 7. One-step chemical and chemoenzymatic generation of novel azaindole alkaloids from azastrictosidine (β-GD = β-glucosidase).[56]

has been used for the simple one-step chemical synthesis of a previously unknown lactam of azastrictosidine and for azanacycline under basic and acidic conditions, respectively.[56]

Modification of the tryptamine unit by introducing a nitrogen atom in different positions should, therefore, ultimately result in a range of diverse and novel azaindole alkaloids.

In an additional strategy, expression of both STR1 and SG (strictosidine glucosidase) cDNAs by a newly constructed dual vector in *E. coli* allowed chemoenzymatic synthesis of the heteroyohimbine alkaloid tetrahydroalstonine by using bacteria.[59]

4.2. Alkaloid Libraries by Structure-Based Re-Engineering of STR1

Information relating to the 3D structure of STR1 complexed with strictosidine was successfully used to analyze the possibility of broadening the substrate acceptance of the enzyme for various tryptamines, with a particular focus on modification of the aromatic region. Substrate studies showed that tryptamines, in particular those with bulky groups at position C5 and C6, are poor substrates or not accepted by STR1 at all (see Section 2.4). Analysis of residues in the active center revealed that residues Try149, Val167, and especially Val208 sterically shield the aforementioned tryptamine positions, and may not allow binding of, for example, 5-methyl- and 5-methoxytryptamine to STR1. Notably, both of these compounds are not converted by the STR1 wild-type enzyme.

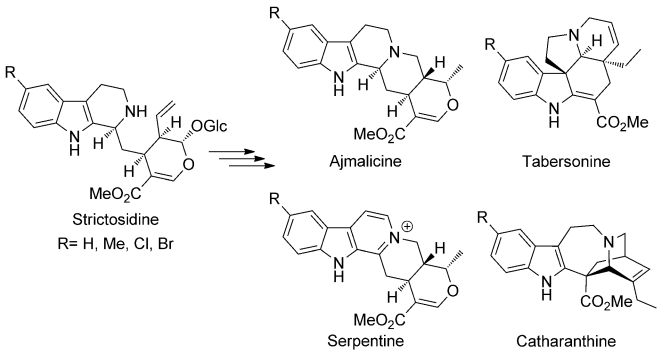
Mutation of Val208 (at a distance of 4 Å from the 5-position) to the smaller amino acid Ala results in a Spenglerase mutant (Val208Ala), which for the first time is able to generate the 5- methyl- and 5-methoxystrictosidines. Moreover, the same STR1 mutant (Val208Ala) converted the 6-methyl- and 6-methoxytryptamines into the corresponding strictosidines more efficiently than the wild-type enzyme.[48] The 3α(*S*) configuration was retained in the new strictosidines obtained by this structure-based approach.[48] It should also be emphasized that the methyl ester group and the vinyl side chain of secologanin can, to some extent, be modified, in particular to flexible, longer chains. The analogous strictosidine derived can also clearly be converted into new heteroyohimbine alkaloids, such as serpentine derivatives.[36,60]

It appears from the 3D structural understanding of the enzyme that the substrate selectivity of STR or STR1 may be easy to manipulate. This may finally lead to significant expansion of alkaloid diversity for the approaches described here, but may also be very helpful in enhancing our understanding of this Pictet–Spenglerase. Out of seven similar STR1 mutants prepared, five exhibited broadened substrate specificity (Stöckigt, et al. unpublished results), thereby highlighting that a structure-based modulation of STR1 can be achieved. The 3D structure-based approach can likely be significantly extended by generation of additional STR1 and SG mutants from *R. serpentina* or other plants. When extended to a whole range of commercially available primary amines, a combination of the biomimetic approach (see Section 4.1) with the structure-based approach will be possible. Previously unacceptable tryptamine derivatives (see Section 2.4), including a range of azatryptamines, can now be additionally applied.[48,56] Such a combinatorial strategy could lead to a chemoenzymatic concept for the generation of large and diverse libraries containing thousands of heteroyohimbine-type alkaloids, potentially harboring pharmacological activities. The heteroyohimbine ajmalicine, for example, exhibits positive effects on postischemic hypoxia and cerebral protection, but there remains a therapeutic need for new drugs with better activity.[61–63]

4.3. Metabolic Reprogramming of Alkaloid Biosynthesis

Re-engineering of STR from *C. roseus* also revealed the potential for a broader application of structure-based redesign. In this way, substrate analogues for the synthesis of novel strictosidines could be applied in in vivo transformation studies or for the generation of novel non-natural indole alkaloids.[64,65] The 3D structure of STR1 has not only enabled generation of novel alkaloids by chemoenzymatic strategies, but has also allowed the first directed metabolic programming of alkaloid biosynthesis in *C. roseus* roots. The transgenic roots produced various new *Catharanthus* alkaloids substituted at the C5-position (C10 in strictosidines) in vivo when cells were re-engineered to produce the STR mutant Val214Met. Val214 of the *Catharanthus* STR corresponds to Val208 of the *Rauvolfia* STR1, and changes in this residue result in structural modification of this region. Feeding the transgenic roots that express the cDNA of a non-natural STR

mutant with 5-substituted tryptamine analogues resulted in the production of a variety of substituted alkaloid types (Scheme 8).[58]



Scheme 8. In vivo formation of a variety of novel, monoterpenoid indole alkaloids substituted at the 5-position by reprogramming in vivo pathways to ajmalicine, tabersonine, serpentine, and catharanthine in *C. roseus*. The plant cells were transformed with the cDNA of strictosidine synthase re-engineered by structure-based mutation to accept 5-substituted tryptamine analogues as the substrate, which were then transformed into the substituted alkaloids.[64, 65a,b]

In a related approach, RNA silencing of tryptamine biosynthesis was used to block alkaloid production in *C. roseus* roots. Tryptophan decarboxylase, which generates tryptamine, was blocked. Application of tryptamine derivatives to the roots resulted in the biosynthetic formation of non-natural 5-fluoro-substituted alkaloids, such as 5-fluoroajmalicine, 5-fluorocatharanthine, and 5-fluorotabersonine.[65a]

In an even more elegant strategy, *C. roseus* roots were transformed with bacterial genes of the tryptophan-chlorinating flavin halogenase together with the cDNA coding for the STR mutant, which resulted in the ability to convert 5- or 7-chlorotryptamine into chlorinated strictosidines. Halogenase cDNAs led to production of 5- and 7-chlorotryptophanes, which are decarboxylated in vivo to the corresponding tryptamines. These tryptamines are in turn biotransformed by the STR mutant to a variety of alkaloids, such as chlorinated ajmalicine, tabersonine, catharanthine, and dihydroakuammicine.[65b] These results provide an impressive demonstration of the power of rational metabolic engineering of biosynthetic pathways, originally known from prokaryotic pathways, but now applicable to more metabolically complex systems such as those found in plants.[66]

Modified natural plant products derived from long and complex pathways—such as those of monoterpenoid indole alkaloids—with pharmacologically interesting activities are now becoming accessible. One significant advantage is that the industrial, large-scale production of plant cell and organ cultures (roots) by fermenter technology for the production of berberine[67] or taxol,[68, 69a,b] for example, has been in place for decades and would not require additional development should larger amounts of new indole alkaloids need to be produced.

The second Pictet–Spenglerase, norcoclaurine synthase (NCS), which participates in the biosynthesis of isoquinoline alkaloids and has been the subject of recent in-depth investigation, will be discussed in the next section.

5. Norcoclaurine Synthase

Norcolaurine synthase (NCS; EC 4.2.1.78), which has now been identified and very well characterized as a Pictet–Spenglerase, was originally discovered 30 years ago,[70] only a few years after STR.[18,19] The enzyme catalyzes the stereospecific condensation of the substrates dopamine and 4-hydroxyphenylacetaldehyde (4-HPAA), both originating from L-tyrosine.[70] (*S*)-[1-^{13}C]-Norcoclaurine was incorporated in exceptionally high yields (2.5–36%) into diverse alkaloids belonging to the aporphine, protoberberine, benzophenanthridine, and pavine alkaloid types by in vivo feeding experiments.[70] NCS delivers (*S*)-norcoclaurine, which occupies a role as the central biosynthetic precursor at the root of various and most likely all benzylisoquinoline alkaloid pathways.[70,71]

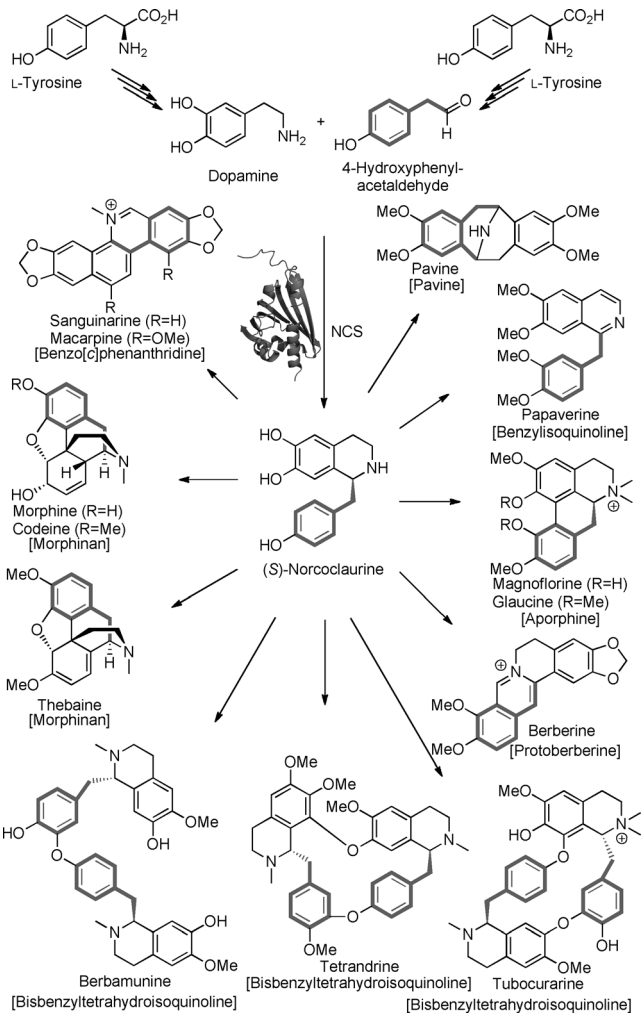
5.1. Functional Importance of NCS, its Product Norcoclaurine, and derived Alkaloids

The functional significance of NCS lies in delivering the key biosynthetic progenitor of probably all alkaloids of the benzylisoquinoline family in plants. The number of these natural alkaloids is estimated, amazingly, to be about 3000 different chemical structures with diverse structural types, such as simple benzylisoquinolines, bisbenzylisoquinolines, protoberberines, morphinans, pavines, benzo[*a*]phenanthridines, and others. Many examples of these types exhibit a broad range of pharmacological activities and as such have been developed as therapeutically useful drugs.

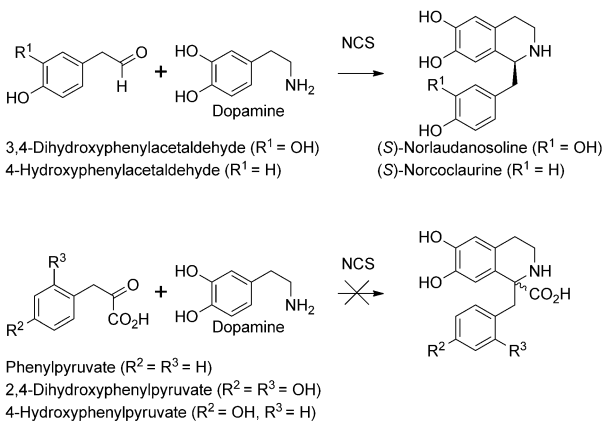
Since the discovery of the first alkaloid of the benzylisoquinolines (morphine) 200 years ago, alkaloid research has become a great challenge for synthetic, analytical, and natural product chemists. An important aim of natural product chemistry is to elucidate the role of plant cells in biosynthetic pathways that lead to the synthesis of structurally complex alkaloids. As shown in Scheme 9, the Pictet–Spenglerase norcoclaurine synthase (NCS) condenses dopamine and 4-hydroxyphenylacetaldehyde (gray) stereospecifically.

This biosynthetic entry reaction leads through the central intermediate (*S*)-norcoclaurine and, through various biosynthetic pathways, to alkaloid skeletons of high diversity. Examples of common benzylisoquinoline alkaloids are presented in Scheme 9.

Thus, the central biosynthetic function of norcoclaurine is comparable to the biosynthetic significance of the previously discussed glucoalkaloid strictosidine in the biosynthesis of monoterpenoid indole alkaloids (see Section 2.1, Scheme 3). Both Pictet–Spenglerases STR1 and NCS occupy a key position at the beginning of enzyme-catalyzed pathways that lead to indole and isoquinoline alkaloid families, which consist of a total of about 5000 structurally distinct members.



Scheme 9. The key biosynthetic function of NCS and its enzymatic product (*S*)-norcoclaurine together with derived famous alkaloid examples of the large benzylisoquinoline family are summarized (NCS = norcoclaurine synthase).



Scheme 10. In contrast to earlier suggestions, NCS does not accept α-ketoacids such as phenyl pyruvate and its derivatives, but uses 4-hydroxyphenylacetaldehyde and its 3,4-dihydroxyderivative as substrates for the Pictet–Spengler condensation with dopamine.[70]

5.2. Detection and Characterization of NCS

The activity of NCS synthase—with 3,4-dihydroxyphenylacetaldehyde as a substrate (hence, the original name of the enzyme was (*S*)-laudanosoline synthase)—was first detected by Zenk and co-workers in cell material of ten plant genera belonging to three plant families (Berberidaceae, Papaveraceae, and Ranunculaceae).[70] All these families are unique for their production of benzylisoquinoline-type alkaloids. Importantly, the experiments showed that phenylpyruvate and its 4-hydroxy and 2.4-dihydroxy derivatives do not serve as substrates for NCS (Scheme 10).[70]

These findings were in agreement with suggestions made by Winterstein and Trier in 1910,[72] just one year before the Pictet–Spengler reaction was discovered.[1] The in vivo experiments ruled out the previously suggested intermediacy of norlaudanosoline-1-carboxylic acid.[73] Again, as for the STR enzyme, cultures of plant cell suspensions were a critical prerequisite for the isolation and partial purification of NCS and other enzymes involved in the biosynthesis of benzylisoquinoline alkaloids including early steps of the biosynthesis at the amino acid stage.[70]

About a decade later, Facchini and co-workers carried out extensive research on NCS, which led to the enzyme being described in greater detail.[74,75] The application of cultured plant cells (*Thalictrum flavum*; Meadow rue) was instrumental in providing a sufficient yield of enzyme for meaningful biochemical characterization.[75] At this stage of the investigation, the data clearly showed that the enzyme exists in four isoforms, and kinetic analyses of the catalyzed reaction suggested that NCS follows a mechanism in which there is prior binding of the aldehyde to dopamine. The results finally provided the basis for further molecular studies of the enzyme, and for heterologous expression of the cDNA of *Thalictrum* NCS in *E. coli*.[75]

5.3. Heterologous Expression of NCS cDNA

The isolation of a clone encoding the enzyme resulted in a very low heterologous expression. The expression rate was, however, sufficient to obtain enzyme amounts necessary to repeat the kinetic analysis.[75a] A highly productive *E. coli* expression of truncated NCS (in which the first 19 amino acids were deleted at the N terminus) was later established, and successfully optimized to allow expression of up to 15 mg of active enzyme per liter of bacteria.[75b] Addition of a C-terminal His_6 tag combined with a common purification method (metal ion affinity chromatography, nickel/nitrilotriacetic acid) yielded a homogeneous synthase for the first time; the NCS expression protocol later applied to support structural studies of the crystallized enzyme was similar to this one. Kinetic analysis of the reaction catalyzed by His_6-NCS was again carried out by using an assay based on circular dichroism spectroscopy (CD). The advantage of this assay is that the non-enzymatically formed racemic norcoclaurine ("so-called background") does not interfere with the conversion rates of the enzymatically formed reaction product.[75b]

This His-tagged NCS shows normal Michaelis–Menten kinetics with a hyperbolic profile for 4-hydroxyphenylacetaldehyde but a sigmoid profile for dopamine. This observation was rationalized as being due to NCS existing as a homodimer in solution and that binding of the first dopamine molecule results in affinity of NCS for a second dopamine molecule (which could not be confirmed by NMR analysis, see Section 5.4). Moreover, substrate studies of NCS revealed that this enzyme does not display particularly high substrate specificity. Although a very limited number of putative substrates have been assayed, it is now evident that the amine components 4-deoxy- and 4-methoxydopamine as well as 3,4-dihydroxyphenylacetaldehyde are enzymatically converted by NCS. This property of the enzyme should allow future generation of new benzylisoquinoline alkaloids from the appropriate norcoclaurine analogues, as has been recently reported for novel strictosidines and their application in generating novel indole alkaloids.[48,56]

Similarly, as described for STR1,[50] a primary isotope effect was also observed for the NCS reaction. It was suggested that rearomatization might be the first irreversible and rate-determining step in the enzyme-catalyzed formation of norcoclaurine (Scheme 9). Based on the biochemical data, two mechanisms were originally discussed for the NCS reaction (Section 5.5).[75b]

Sequence analysis of the cDNA of *Thalictrum* NCS revealed moderate identity (ca. 30%) as well as some structural homology to the Bet v 1 protein family. Bet v 1 is a well-known allergen of birch-tree pollen and belongs to a subfamily of pathogenesis-related proteins (PR-10 proteins) with undefined function in plants.[76]

Another plant source, cell suspension cultures of *Coptis japonica* (Japanese Goldthread), yielded an NCS cDNA distinct from *Thalictrum* NCS as well as a PR-10 homologous protein. The two enzymes are completely different and both exhibit NCS activity. The *Coptis* NCS requires ferrous ions for activity and has an Fe^{2+}-binding motif. In contrast, the PR-10 protein is not Fe^{2+}-dependent and, more strikingly, exhibits broader substrate specificity, accepting, in addition to 4-hydroxyphenylacetaldehyde, phenyl- and 3,4-dihydroxyphenylacetaldehyde, as well as pyruvate and 4-hydroxyphenylpyruvate.[77] Based on these findings, and published data, it is likely that several different proteins may catalyze the Pictet–Spengler reaction between dopamine and various structurally similar aldehydes in higher plants.

Milligram amounts of pure, isotopically labeled protein are indispensible for more advanced studies on NCS (e.g. investigation of its 3D structure in solution by NMR spectroscopy). Such quantities of enzyme can usually be obtained by optimized high expression rates of the corresponding cDNA in heterologous systems such as bacteria (*E. coli*), which are most popular for the generation of His-tagged enzymes relating to the biosynthesis of natural products from plants. Two efficient and similar *E. coli* expression systems have been developed for NCS which generate sufficient amounts of protein.[78] One approach results in a truncated (first 19 and 29 amino acids, respectively) His_6-tagged NCS, the other NCS contains a His tag at the C terminus and an additional nucleotide sequence at the N terminus.

5.4. Solution and Crystal Structure of NCS

2D and 3D NMR spectroscopy as well as CD experiments provided some initial insights into the 3D structure of NCS in solution. An assignment (86%) of the backbone amino acids of deuterated ^{15}N,^{13}C-labeled truncated NCS could be made, thus indicating high homology to the fold of Bet v 1, a birch pollen allergenic protein. A homology structure of NCS has been constructed based on the known X-ray structure of Bet v 1 as a template.[76]

This model consists of seven antiparallel β sheets. A long α helix at the C terminal is below two short α helices. Notably, both of these short helices are the only structural elements that are not observed in the Bet v 1 structure. The model generated supports not only the close structural relationships between NCS and Bet v 1, but also demonstrates for the first time that, from a structural point of view, *Thalicturm*-derived NCS is a novel member of the Bet v 1 enzyme family. This was later convincingly confirmed by X-ray analysis of crystallized NCS (see Section 5.5).

The homology model described has also been somewhat useful for partially mapping the substrate binding site of NCS. Together with NMR titration experiments with dopamine and analogues of the second substrate (aldehyde), the model suggests that dopamine binds to amino acids Phe71 and Met155. However, since the aldehyde derivatives lead to significant side-chain rearrangements, the interaction site of the substrate could not be clearly identified. Kinetic studies previously performed on *T. flavum* NCS suggest a dopamine-induced dimerization of NCS with cooperative dopamine binding.[74,75a,b] Cooperativity of substrate binding is, in general, expected to have an important regulatory function in biosynthetic pathways; in this case, it means regulation at the beginning of the biosynthetic pathway of the *Thalictrum* alkaloids or even for all NCS-derived alkaloids. However, NMR relaxation data, as well as-size exclusion experiments, favor a monomeric, not a dimeric, state of NCS at relatively low enzyme concentrations (10 μM–1000 μM). These data are fully in line with results of a non-cooperative dopamine binding reported much earlier on with NCS preparations from several plant species, including another *Thalictrum* species (*T. tuberosum*).[70a,b] It must, however, be pointed out that real enzyme concentrations at the site of biosynthesis are normally not known in vivo.

Most of the features of NCS discussed up to this point have recently been confirmed and further expanded upon by an impressive study, which for the first time describes the crystallization and X-ray analysis of the enzyme.[78b,79a,b] In fact, after STR1, NCS is only the second Pictet–Spenglerase to be characterized by crystallization and X-ray analysis to date. Although NCS shows no sequence or structural relationship to the β propeller fold of STR1, both enzymes catalyze a Pictet–Spengler condensation reaction. Structural data of the His_6-tagged truncated NCS construct conclusively reconfirmed that the *Thalictrum* NCS belongs to the Bet v 1

protein family of the PR-10 protein super family. Comparison of the 3D structure of Bet v1 and the 3D of NCS structure illustrates the close relationship of the two proteins (Figure 2).

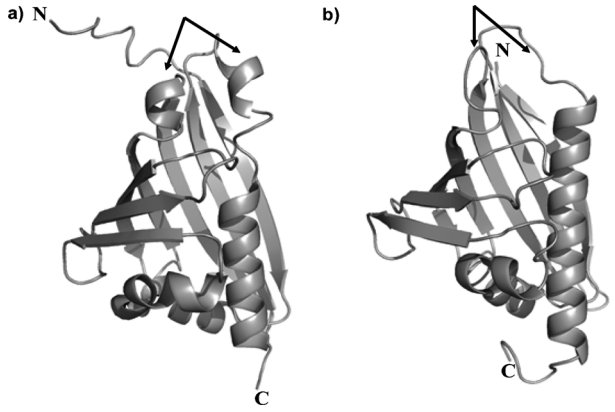


Figure 2. Comparison of the overall 3D structure of a) the Pictet–Spenglerase NCS from *Thalictrum* plants and b) the birch-tree allergen Bet v1. The same fold and the close structural relationship of the two proteins can be seen (data are from PDB: NCS, 2VNE and Bet v1 1BV1; NCS has been truncated for expression in *E. coli* by 19 amino acids at the N terminus).

Generation of the complex structure of the synthase with the substrate dopamine and the nonproductive aldehyde analogue 4-hydroxybenzaldehyde (4-HBA) provided important and detailed insights into the catalytic tunnel, in which both ligands adopt a stacked orientation as a result of hydrophobic interactions of their aromatic residues. The active site of NCS is shaped by the hydrogen-bond donor Tyr108 together with the three strong polar residues Glu110, Lys122, and Asp141, which are all likely to be involved in the catalytic formation of norcoclaurine (Figure 3). Of these amino acids, Lys122 is likely to be the key catalytic residue, since a Lys122Ala mutant is completely inactive. In contrast, exchange of Tyr108 and Glu110 residues for Phe or Ala, respectively, only results in a decrease in the enzyme activity. No clear information is available, for example, by mutation experiments, on the role of Asp141. Notably, Asp141 is closer (2.4 Å) to the hydroxy group of 4-HBA compared to the distance between the catalytic Lys122 and 4-HBA (2.6 Å). The arrangement of these four residues suggests the mechanism discussed in the next section (Section 5.5). For details of the proposed mechanism, which is different to strictosidine synthase, see also Refs. [51, 79a,b].

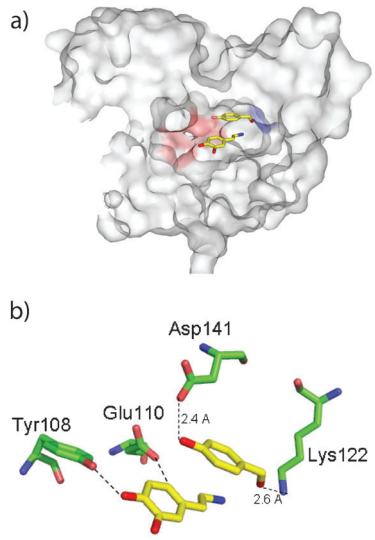
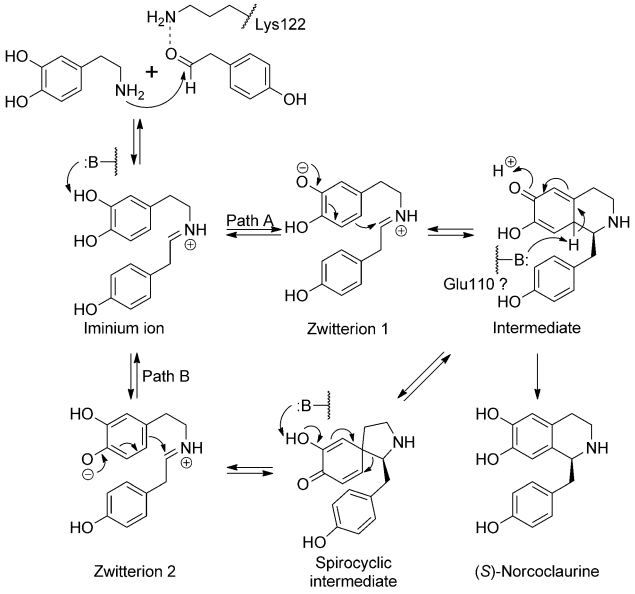


Figure 3. a) Transparent representation of the active site of norcoclaurine synthase from *Thalictrum* to illustrate the inner surface of the protein with its substrate dopamine and the unproductive 4-hydroxybenzaldehyde analogue. b) Close-up view of the catalytic site of NSC and the residues interacting with the substrate and analogue. Data are from PDB: 2VQ5; NCS in a complex with dopamine and 4-HBA.[79a]



Scheme 11. The NCS-catalyzed reaction is proposed to proceed by a typical bifunctional acid/base catalytic process, which is similar to the two-step chemical Pictet–Spengler reaction, often described in the literature.[51, 75b, 79a,b]

5.5. Mechanistic Aspects of the NCS Reaction

As a consequence of the results obtained during the last five years—including both biochemical and kinetic investigations,[75a,b] data for NCS in solution derived from NMR measurements,[76] and very recent X-ray analysis[78b, 79a,b]—two reaction sequences involving two-step mechanisms have been suggested for the NCS reaction (pathways A and B; Scheme 11).

Pathway B proceeds through a total of four proposed intermediates including a spiro intermediate. This pathway can most probably be ruled out, as it was already excluded when the mechanism of the STR1 enzyme was investigated.[50] Kinetic data for the transformation of 4-methoxydopamine and *m*-tyramine (4-deoxydopamine) indicate that both compounds are excellent NCS substrates, with activity comparable to that of dopamine. A substitution of both compounds at

the 4-positions would not allow formation of the intermediate spiro compound.

This conclusion is further supported by additional substrate studies: in contrast to the 4-substituted substrates, 3-deoxydopamine and 3-methoxydopamine are not accepted by the enzyme, most probably because they lack the acidic hydroxy group at C3, which would be necessary for the ring-closure reaction leading to the spiro intermediate. The behaviors of these substrates with NCS again favor pathway A.

Moreover, the X-ray structures suggest that the positively charged Lys122 (the key catalytic residue) may polarize the carbonyl group of the aldehydic substrate of NCS for the formation of a Schiff base with dopamine. In addition, Lys122 might also assist in loss of H_2O from an intermediate carbinolamine (structure not shown). Cyclization might then be achieved under assistance of Glu110, which could additionally help in the aromatization process, thereby resulting in the final formation of norcoclaurine. The detailed function of amino acid residues responsible for these late steps of pathway A need, however, be more conclusively characterized.

It is expected that, similar to the broadly and successfully applied STR1 for in vivo transformations and in chemoenzymatic syntheses to generate new monoterpenoid indole alkaloid libraries, NCS might also have great potential for the future production of novel benzylisoquinoline alkaloids, potentially including compounds with interesting therapeutic properties.[79b]

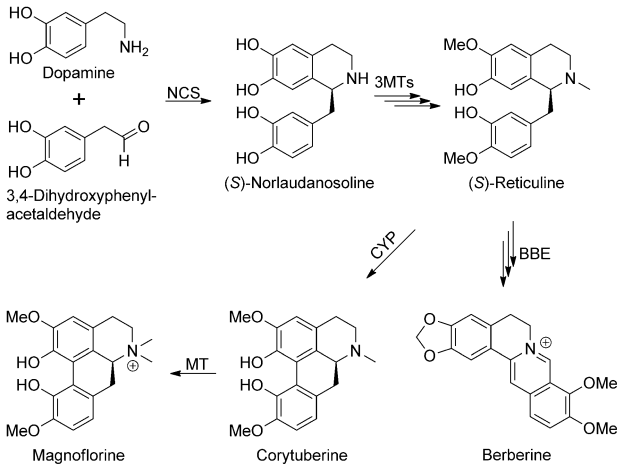
6. Transfer and Re-Engineering of THIQ Alkaloid Biosynthesis: From Plant Cells to Microbial Hosts

Plant cells, compared to microbial cells, are large in size with a slower product turnover, especially in the transformation and biosynthesis of secondary metabolites, including alkaloids. The accumulation and storage of alkaloids in plant organs (such as roots, root bark, leaves) occurs over a period of years, and in plant cell suspension cultures, production might take weeks. In contrast, the production of, for example, antibiotics in microbial cells may take a matter of days, thus demonstrating the advantage of microbial hosts versus plant systems.

Successful research on antibiotic biosynthetic pathways and their re-engineering in bacteria can be easily compared with that of plant cells. In contrast to bacteria, in which gene clusters regulate the biosynthesis, the biosynthetic genes of plants are usually broadly distributed in the whole genome. Plant genomes are much larger than bacteria, and it can be a long, time-consuming, and challenging process to isolate single enzymes and cDNAs of a biosynthetic pathway from plant systems. However, exploring and designing efficient production lines for valuable plant products of structural diversity in re-engineered microbial systems might pave the way for a future supply of natural plant products and their analogues.[80,81a,b]

The latest achievement in the alkaloid field concerns the remarkable production rate of taxadiene, the first intermediate in the biosynthesis of the anticancer drug taxol. Up to one gram of taxadiene was formed per liter of engineered *E. coli* strain.[81] This is a 1000-fold increase in yield compared to previously reported experiments.[82,83] These results highlight the preparative possibilities of such a strategy, which might even become interesting from an industrial perspective.

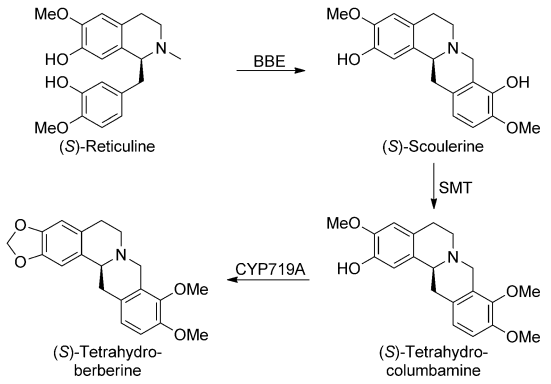
Since 2008, remarkable progress has been made in the microbial production of plant- and NCS-derived benzylisoquinoline alkaloids, two of which are worth mentioning: 1) the synthesis of the aporphine-type alkaloid magnoflorine and the protoberberine alkaloid berberine using engineered *E. coli* and yeast strains[84,85] and 2) the first microbial synthesis of the alkaloids reticuline and scoulerine through the intermediacy of the NCS reaction by using the transgenic *E. coli* strain modified with five genes (Scheme 12).[86]



Scheme 12. Genetically reconstructed plant isoquinoline alkaloid pathway in microbes (*E. coli* and *Saccharomyces cerevisiae*) involving an NCS reaction (MTs = methyltransferases; BBE = berberine bridge enzyme; CYP = cytochrome P450).[86]

At the same time, Hawkins and Smolke reported on a multipurpose transgenic yeast-based production platform, for which a range of yeast strains was developed that carried several selected plant cDNAs as well as human P450.[87] With these systems—similar to above—reticuline, scoulerine, salutaridine, and tetrahydroberberine could be produced in yields ranging from 2 to 10% (up to 0.15 $g L^{-1}$; Scheme 13). The strains described can now be applied in the further construction and design of various heterologous hosts for modulation of the biosynthetic generation of natural and in the future of non-natural alkaloids. If the substrate specificity of NCS can be modified in a broad manner, for example, by structure-based mutations, one important prerequisite for the biological synthesis of novel benzylisoquinoline alkaloids in microbial systems may be fulfilled in the near future. The strategies mentioned and the results from these NCS studies are an important step in the direction of synthetic biology approaches.

Although the efficiency of these strains is not yet high enough for practical and industrial purposes, and needs, in general, to be optimized for benzylisoquinolines, both



Scheme 13. Microbial synthesis of plant-derived benzylisoquinoline alkaloids after re-engineering yeast strains by transformation with appropriate cDNAs of plant and human origin.[87]

approaches can be regarded as a breakthrough for the expression of multistep alkaloid biosynthetic routes from higher plants in microorganisms. The major challenge remaining is that of increasing production rates. However, recent years have clearly shown a rapid development in the field of metabolic engineering, and in the area of isoquinoline alkaloids.

Further development will be accelerated by increased success in making available cDNAs from genomic sequencing projects of alkaloid-containing medicinal plants. Substantial input in the development of metabolomic and transcriptomic profiling of a variety of medicinal plants has very recently been realized. Three leading consortia in collaboration with a large number of interdisciplinary-oriented research institutions should be mentioned, namely NapGen (Natural Products Genomics, National Research Council, Canada),[88,89] the USA-based MPGR (Medicinal Plant Consortium at the University of Kentucky, Michigan State University, and MIT),[90] the Medicinal Plant Genomics Resource at Washington State University.[91] The design and use of new module-directed approaches for more-efficient synthetic biology strategies are already on the way, as was reported for the production of the biosynthetic taxol precursor taxadiene in 2010.[81a]

7. Conclusions and Future Aspects of the Pictet–Spenglerase Family

The enzyme family catalyzing the Pictet–Spengler condensation is slowly growing. The 3D structure of only one member of the STR family, from *Rauvolfia*, has been characterized. Other members, such as those from the genus *Catharanthus* or from two *Ophiorhiza* species, which produce the anticancer compound camptothecin, have also been characterized to various degrees. Their cDNAs are known and, with the exception of the enzyme from *O. japonica*, have been heterologously expressed. It is just a matter of time until crystallization as well as structural and deeper functional elucidation of these STRs will be successfully achieved, thereby shedding more light onto the molecular mechanisms of the biological STR reaction.

It appears that a similar family of genes has evolved in plants, namely the "STR-like" or "SSL" genes. A family consisting of 15 of these genes has been detected in the model plant *Arabidopsis*.[92,93] However, both protein extracts and heterologously expressed versions of these cDNAs were devoid of STR activity, which suggests that these proteins have a distinct and unknown function.[93,94]

Knowledge of the NCS-catalyzed Pictet–Spengler reaction has very recently emerged, and a biochemical, mechanistic, and structural understanding of the enzyme based on its X-ray structure is now at an advanced stage. Utilization of NCS to generate novel alkaloids, as reported for STRs, has not been realized experimentally to date. The close phylogenetic relationship between NCS and proteins of the allergens of birch-tree pollen and the relationship to the pathogen-related (PR-10) protein family on the basis of function is interesting and is in need of future exploration. In this context, a wider search for this particular type of Pictet–Spenglerase would be helpful.

There is probably a third type of Pictet–Spenglerases in plant alkaloid biosynthesis. In the South-American plant *Psychotria* the "NCS amine" dopamine is condensed with the "STR aldehyde" secologanin on the biosynthetic route to monoterpenoid tetrahydroisoquinoline alkaloids. One example is the emetic emetine. A large number of the enzymes involved in the biosynthesis of the ipecacuanha alkaloids has recently been described.[95,96] Knowledge of the relevant Pictet—Spenglerase, named deacetylipecoside synthase (DIS; EC 4.3.3.4), is still limited, although it has been pre-purified and partially characterized.[97,98] Comparison with the NCS and STR enzymes should significantly extend the knowledge.

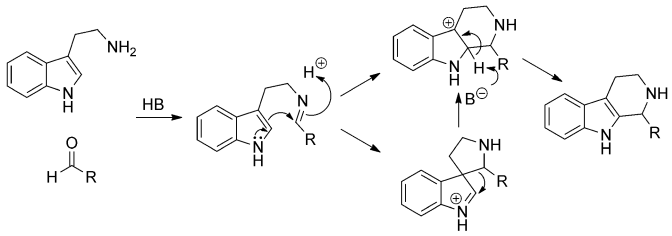
Salsolinol (SAL; 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) is the Pictet–Spengler product of dopamine and acetaldehyde, and has been detected in foods such as banana and port wine[99] as well as in human brain tissue.[100,101] SAL has been speculated to play a role in the etiology of alcoholism, a somewhat controversial finding.[102]

Naoi et al. previously detected exclusively the (*R*)-SAL enantiomer as well as the corresponding enzyme (*R*)-salsonilol synthase in human brain.[103] The synthase has been partially purified and characterized,[104] but not further investigated; this leaves open the question on the type or fold of this interesting human Pictet–Spenglerase.

In summary, after one century of research, the Pictet–Spengler reaction—and nowadays the Pictet–Spenglerases—continue to offer great and challenging opportunities from a chemical, biochemical, genetic, and very recently from a structural perspective.

8. The Mechanism of the Non-Enzymatic Pictet–Spengler Reaction

The Pictet–Spengler condensation is initialized by the formation of an iminium ion followed by nucleophilic attack by the aryl group and cyclization (Scheme 14). Attack on the



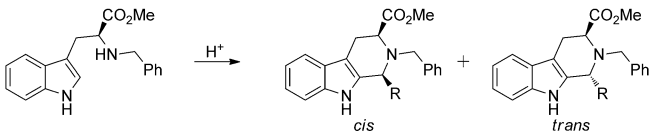
Scheme 14. The mechanism of the Pictet–Spengler reaction.

iminium ion can occur either directly at position 2 or at position 3 of the indole to form a spiroindolenine.[105] Evidence for the involvement of the spiro intermediate was obtained by employing isotopic labeling.[106] The study demonstrated that the formation of the spiroindolenine is fast and reversible, and that the formation of the pentahydro-β-carboline carbonium ion is the rate-limiting step of the reaction. However, it is still unclear whether, in general, the carbonium ion is formed by rearrangement of the spiroindolenine or by direct attack at position 2 of the indole.[4b,107,108]

9. Diastereoselective Pictet–Spengler Reactions

9.1. Application of Tryptophan Derivatives

The classical Pictet–Spengler reaction under aqueous conditions usually led to limited diastereocontrol. In 1979, Cook and co-workers introduced aprotic conditions to improve the reaction rate and product yield (Scheme 15).[109] Detailed examination of the reaction conditions allowed thermodynamic *trans* stereocontrol by application of the N_β-benzylalkyl esters of tryptophan.[108,110–112]

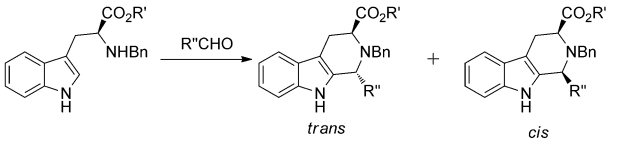


Scheme 15. Synthesis of 1,2,3-trisubstituted tetrahydro-β-carbolines.

A mixture of isomers of 1,2,3-trisubstituted tetrahydro-β-carbolines was formed when small aliphatic aldehydes were used under acidic and non-acidic aprotic conditions (Table 3). Selective formation of the *trans* diastereomer was observed for cyclohexanecarboxaldehyde. Cook and co-workers showed that the steric bulk of the carbonyl compounds, the substituents at the N_β nitrogen atom, and the ester group played major roles in the diastereoselective formation of *trans*-1,2,3-trisubstituted tetrahydro-β-carbolines.[4b,108,111,112]

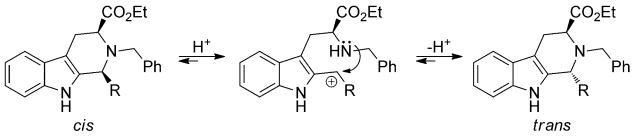
The diastereomers can usually be separated by flash chromatography. The thermodynamically more-stable *trans* isomer could be obtained exclusively from the *cis*/*trans* mixture by epimerization of the N_β-substituted *cis* isomer under acidic conditions (trifluoroacetic acid; Scheme 16).[113,114] The epimerization proceeded by protonation of the N_β nitrogen atom followed by cleavage of the

Table 3: *trans*/*cis* ratio in the synthesis of 1,2,3-trisubstituted tetrahydro-β-carbolines under thermodynamic reaction control.[a]



R′	R″	Non-acidic cond. *trans*/*cis*	Acidic cond. *trans*/*cis*
Me	Me	74:26	88:12
Me	*n*Pr	77:23	89:11
Me	c-hexyl	100:0	100:0
*i*Pr	Me	77:23	87:13
*i*Pr	*n*Pr	87:13	88:12
*i*Pr	c-hexyl	100:0	100:0

[a] Bn = benzyl.



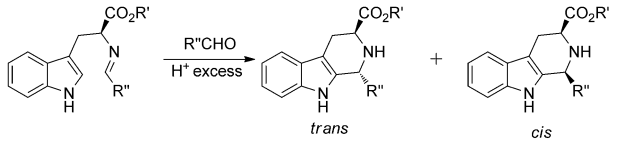
Scheme 16. *Cis* to *trans* epimerization of *N*-benzyl adducts of the Pictet–Spengler reaction.

C(1)–N(2) bond to generate a carbocation, which can be recyclized to provide the thermodynamically more-stable *trans* diastereomer. This epimerization afforded efficient control over the formation of the *trans* isomer under acidic conditions. Importantly, no incorporation of deuterium in the epimerized product was detected on treatment with CF_3CO_2D.[4b,5a,111,114]

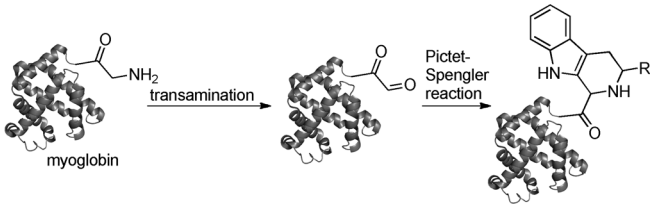
The *trans*-configured tryptophan derivatives found wide application in total syntheses of numerous alkaloids[115] as well as in the biology-oriented synthesis[116] of focused compound libraries with diverse biological properties. A main drawback of this approach is the possible racemization of the products under the reaction conditions.[117] Additionally, the synthesis of natural products requires unnatural D-tryptophan as the source of chirality. To overcome these problems, Bailey et al. developed a Pictet–Spengler reaction under kinetic reaction control.[118,119] Excellent *cis* stereocontrol was achieved by using tryptophan allyl ester and benzaldehyde, preformation of imine, and an excess of trifluoroacetic acid at low temperature (Table 4). A wide range of electron-withdrawing and electron-donating substituents in the *o*-, *m*-, and *p*-positions are tolerated on the benzaldehyde under these conditions to give the products in high yields and with excellent *cis* stereocontrol.[119] Reactions with aliphatic aldehydes provided mixtures of diastereomers with predominantly the *cis* isomer.[119b]

Recently, the Pictet–Spengler reaction was used to label horse heart myoglobin (Scheme 17).[120] A terminal amino group was converted into an aldehyde by a transamination reaction, and tryptophan methyl ester and tryptamine were used for the coupling in phosphate buffer. The tertiary structure of myoglobin was not altered under the Pictet–Spengler reaction conditions.

Table 4: *cis/trans* ratio in the synthesis of 1,3-disubstituted tetrahydro-β-carbolines under kinetic reaction control.



R′	R″	Yield [%]	*cis*/*trans* ratio
Me	Ph	74	4.6:1
Me	c-hexyl	71	2.5:1
Me	*n*Pr	75	4.9:1
allyl	Ph	57	>20:1
allyl	c-hexyl	39	3.0:1
allyl	*n*Pr	67	3.5:1



Scheme 17. N-terminal labeling of myoglobin by the Pictet–Spengler reaction.

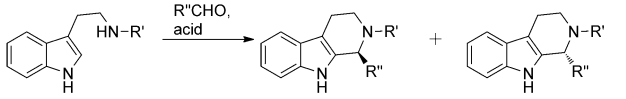
9.2. Application of Chiral Auxiliary Groups

The success in the development of diastereoselective Pictet–Spengler reactions with tryptophan derivatives stimulated studies on the stereoselective transformation of tetrahydro-β-carbolines by using a removable auxiliary. The first attempt was made using α-methylbenzylamine as the chiral source and 5,6-dimethoxytryptamine, which provided a diastereomer ratio of 3:2.[121] Further studies focused on optimization of the reaction conditions using a variety of Brønsted and Lewis acids. The best results were obtained using trifluoroacetic acid in benzene at reflux. A diastereomeric excess (*de*) up to 72% was determined for aromatic aldehydes and up to 38% for aliphatic aldehydes (Table 5).[122] An exchange of the auxiliary group to 1-naphthyl-1-ethylamine led to a slightly better control of the diastereoselectivity.[123] However, decreased yields were observed for electron-rich aldehydes.

The auxiliaries benzyl- and 1-naphthyl-1-ethylamine provide low diastereocontrol for aliphatic aldehydes, which are more relevant for the synthesis of alkaloids and their analogues. Recently, in an effort to improve the selective formation of 1-alkyltetrahydro-β-carbolines, chiral sulfoxides were used (Table 5).[124] The application of *N*-sulfinyliminium ions in the Pictet–Spengler reaction of tryptamine provided the desired products with up to 76% *de* and good yields.

High levels of diastereoselectivity were obtained in Pictet–Spengler reactions using *N*,*N*-phthaloylamino acids as the chiral auxiliary.[125] Preformed imines were treated with amino acid chlorides in the presence of titanium alkoxides at room temperature (Table 6). The desired products were obtained in good yields and with a diastereoselectivity of up to >98% *de*. The reaction conditions are general for the Pictet–Spengler reaction and suitable for a variety of aliphatic aldehydes as well as for electron-rich and electron-poor aromatic aldehydes.

Table 5: Selected auxiliaries for the diastereoselective Pictet-Spengler reaction.



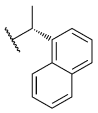
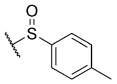
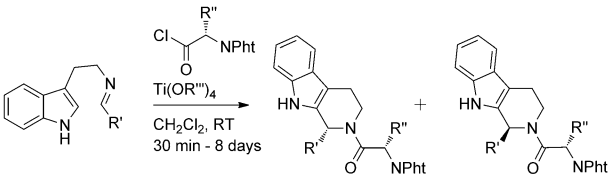
R′	R″	Yield [%]	*de* [%]
	Ar	39—86	34—72
	Alk	56—93	18—38
	Ar	9—90	60—84
	Me	33	40
	Alk	69—84	44—76

Table 6: Pictet–Spengler reactions with *N*,*N*-phthaloylamino acid as the auxiliary.[a]



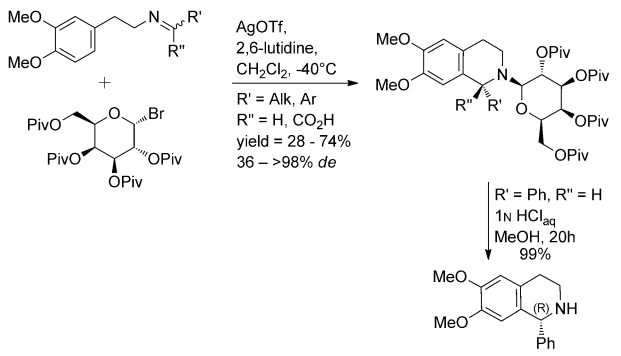
R′	R″	R‴	Yield [%]	*de* [%]
Ph	*t*Bu	*n*Pr	60	>98
4-$NO_2C_6H_4$	*t*Bu	*n*Pr	54	86
4-ClC_6H_4	*t*Bu	*i*Pr	60	92
Me	*t*Bu	*n*Pr	66	92
Et	*t*Bu	*n*Pr	59	90
*i*Pr	*i*Pr	*n*Pr	99	>98

[a] Pht = phthaloyl.

Allef and Kunz demonstrated a diastereoselective Pictet–Spengler reaction for the synthesis of 1-substituted tetrahydroisoquinolines (Scheme 18).[126] By using galactosyl bromide as the chiral auxiliary and imines derived from 2-(3,4-dimethoxyphenyl)ethylamine, the corresponding products were obtained with a diastereoselectivity up to >98% *de*. The chiral auxiliary can be removed in the presence of hydrochloric acid at room temptrerature without racemization.

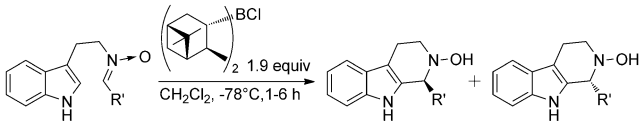
9.3. Application of Stoichiometric Lewis Acids

Enantioselective Pictet–Spengler reactions using chiral Lewis acid were studied by Nakagawa and co-workers.[127] The chiral Lewis acid was employed as a stoichiometric reagent and reacted with nitrones, which were prepared from N_β-hydroxytryptamine and aldehydes (Table 7). The correspond-



Scheme 18. Synthesis 1-substituted tetrahydroisoquinolines by a Pictet–Spengler reaction.

Table 7: Chiral Lewis acid mediated Pictet–Spengler reactions.

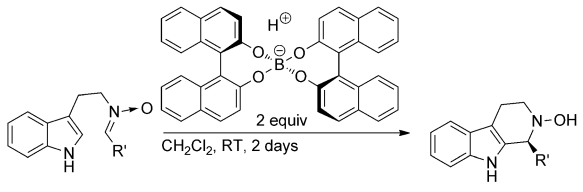


R′	Yield [%]	*ee* [%]
Ph	92	75
4-$MeOC_6H_4$	65	90
4-$NO_2C_6H_4$	81	*rac*
1-naphthyl	94	86
Me	91	43
*i*Bu	75	35

ing 2-hydroxytetrahydro-β-carbolines were obtained in good yields and with *ee* values up to 90% at low temperature. Unfortunately, the reaction with electron-poor aromatic aldehydes produced racemic products. Nitrones which had been prepared from aliphatic aldehydes gave β-carbolines in high yields, but with modest enantioselectivity.

Similar results were obtained by employing nitrones for the synthesis of 2-hydroxytetrahydro-β-carbolines mediated by Brønsted acid assisted Lewis acid catalysis (Table 8).[127] The use of 2 equivalents of the 2,2′-binaphthol-derived Brønsted acid at room temperature led to products being isolated in 39–94% yield and 15–91% *ee*.

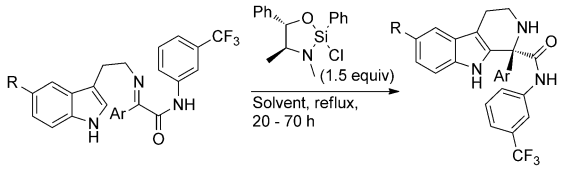
Table 8: Pictet–Spengler reactions mediated by Brønsted acid assisted Lewis acid catalysis.



R′	Yield [%]	*ee* [%]
Ph	81	73
4-$MeOC_6H_4$	39	91
4-$NO_2C_6H_4$	75	74
1-naphthyl	59	31
Me	94	15
*i*Bu	68	50

Very recently, Bou-Hamdan and Leighton developed a Pictet–Spengler reaction with α-ketoamide-derived ketimines to access a class of quaternary α-amino amides.[128] It was found that electron-withdrawing groups in the aniline part dramatically enhanced the reaction rate. Of the aryl amides screened, *N*-3-(trifluoromethyl)phenylamide gave the best results. A series of imines were subjected to the reaction with 1.5 equivalents of a chiral chorosilane (Table 9). Substitution

Table 9: Asymmetric Pictet–Spengler reactions of ketimines.

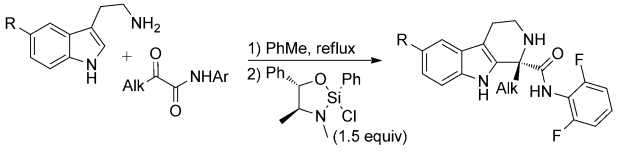


R	Ar	Solvent	*t* [h]	Yield [%]	*ee* [%]
H	Ph	CH_2Cl_2	48	93	93
Br	Ph	$CHCl_3$	70	68	89
OMe	Ph	CH_2Cl_2	27	93	82
H	4-$CF_3C_6H_4$	CH_2Cl_2	48	89	90
H	4-$MeOC_6H_4$	$CHCl_3$	20	94	87
H	3-pyridyl	CH_2Cl_2	42	77	87

of the aryl group was well tolerated, as was a heteroaromatic (3-pyridyl) group. While *ortho* substitution on the aryl group was tolerated, the reactions were sluggish, and required increased loading of the chiral Lewis acid and higher reaction temperatures. However, a variety of products was obtained in 50–94% yield and with 82–93% *ee*.

To determine the generality and scope of the transformation, different imines of alkyl ketones were examined. A brief survey of aryl amides led to the discovery that the 2,6-difluorophenyl group provided good results. A one-pot procedure was developed wherein tryptamine and ketones were condensed and 1.5 equivalents of a chiral chorosilane were added upon completion of the imine formation (Table 10). A variety of tryptamines and ketones were investigated under these conditions. The corresponding products were obtained in 67–86% yield and with 81–94% *ee*. Importantly, the reaction is readily scalable to the 5 mmol scale and the pseudoephedrine can be quantitatively recovered.

Table 10: One-pot Pictet–Spengler reactions of tryptamines and alkyl-ketoamides.



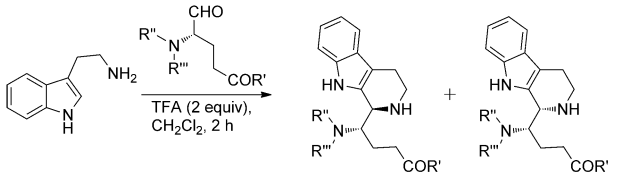
R	Alk	*T* [°C]	*t* [h]	Yield [%]	*ee* [%]
H	Me	50	36	78	89
Br	Me	75	48	67	86
OMe	Me	50	48	86	81
H	*i*Pr	50	26	81	94
H	*i*Bu	55	25	83	90

9.4. Application of Chiral Carbonyl Compounds

The critical step in the development of an asymmetric Pictet–Spengler reaction is the generation of a chiral iminium intermediate. Besides using chiral derivatives of tryptamine, such as tryptophan and different auxiliary groups, various chiral carbonyl compounds have found application in asymmetric transformations. Different strategies were followed for the use of chiral carbonyl components in intermolecular Pictet–Spengler reactions and for the transfer of chirality to the newly generated center of tetrahydro-β-carbolines. Selected recent examples from the numerous reported approaches are described below.

Tetrahydro-β-carbolines from tryptamine and protected α-aminoaldehydes derived from L-glutamic acid were studied.[129] The aldehydes were condensed with tryptamine under different conditions to form the products diastereoselectively (Table 11). A series of carbamate-protected aldehydes led to

Table 11: Diastereoselective Pictet–Spengler reactions with chiral α-aminoaldehydes.

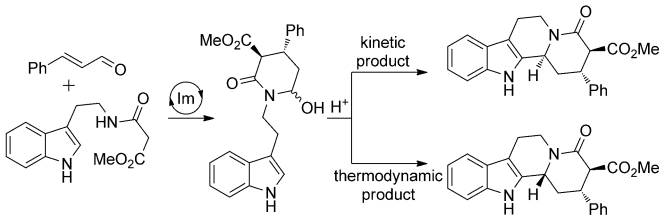


R′	R′′[a]	R′′′	*T* [°C]	Yield [%]	*trans* [%]	*cis* [%]
*t*BuO	Cbz	H	−40	81	0	100
*i*BuO	Cbz	H	−40	77	0	100
*t*BuO	Boc	H	−40	71	10	90
*t*BuO	CO_2Me	H	−40	73	9	91
*i*BuO	Troc	H	−40	74	14	86
*t*BuO	pyrrole		−50	62	100	0
Et_2N	Pht		RT	68	93	7

[a] Troc = trichloroethoxycarbonyl.

the preferred formation of the *cis* diastereomer. The size of the protecting group has only a minor influence on the stereoselective course of the reaction. Selective formation of the *cis* diastereomer was observed with benzyl carbamate (Cbz). Exchange of the protecting group with pyrrole or phthalimide led to the reverse diastereoselectivity. The *trans* diastereomer was obtained exclusively from pyrrole-protected aminoaldehydes.

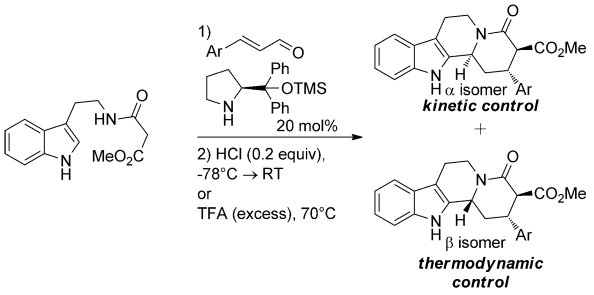
A variety of new methods for the synthesis of chiral carbonyl compounds, especially aldehydes, by using organocatalysis has been developed during the last decade. These approaches also found application in the efficient construction of complex scaffolds by using iminium catalysis. The organocatalytic conjugate addition of a nucleophile derived from tryptamine amide to cinnamic aldehyde yielded a hemiaminal with two stereogenic centers (Scheme 19).[130] An acyliminium cyclization was subsequently initiated under acidic conditions. A Pictet–Spengler cyclization was performed under kinetic or thermodynamic control. This epimeric switch provided an efficient protocol for the diastereo- and enantioselective synthesis of an alkaloid scaffold.



Scheme 19. Enantioseletive synthesis of indolo[2,3*a*]quinolizidines.

The enantioselective addition of tryptamine amides to α,β-unsaturated aldehydes was catalyzed by a chiral pyrrolidine. In the second step of the one-pot sequence, the addition of a catalytic amount of hydrochloric acid at −78 °C led to the formation of the kinetically favored diastereomer. Alternatively, trifluoroacetic acid at 70 °C gave the thermodynamic products (Table 12). A variety of β-aryl-substituted acroleins

Table 12: Organocatalyzed one-pot synthesis of indoloquinolizidines.[a]

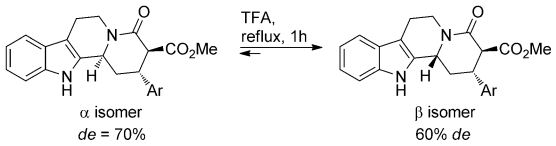


Ar	*t* [days]	*T* [°C]	Acid	Yield [%]	*ee* [%]	α [%]	β [%]
Ph	3	3	HCl	69	94	85	15
Ph	3	3	TFA	64	94	18	82
2-$NO_2C_6H_4$	5	RT	HCl	53	95	90	10
2-$NO_2C_6H_4$	5	RT	TFA	36	94	17	83
4-$MeOC_6H_4$	1	3	HCl	71	89	83	17
4-$MeOC_6H_4$	1	3	TFA	38	88	18	82
4-$NO_2C_6H_4$	3	40	HCl	74	96	72	28

[a] TMS = trimethylsilyl.

with electron-withdrawing or -donating groups were well tolerated, as was a heteroaromatic (2-furyl) group. In contrast, the use of β-alkyl-substituted acrolein did not result in the corresponding product. By using kinetic or thermodynamic reaction conditions, both epimers were selectively obtained in 38–74% yield and 89–96% *ee*. The epimer ratio ranged from 90:10 to 17:83.

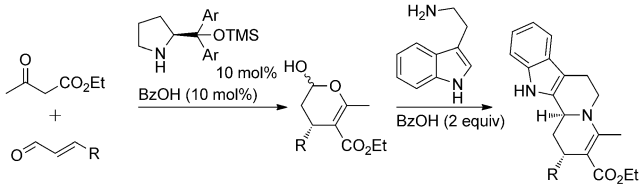
Different 2-aryl ethylamines such as phenyl-, 3-benzofuryl-, and 2-furylethylamine were successfully employed. Variation of the reaction time and temperature led to different diastereomer ratios in the formation of the β epimer under thermodynamic control. This observation led to the assumption that the α epimer is formed initially, and this is epimerized to the thermodynamically more-stable β epimer. This notion was supported by epimerization of the kinetic into the thermodynamic product in trifuoroacetic acid at reflux (Scheme 20).



Scheme 20. Epimerization of indolo[2,3*a*]quinolizidines.

Recently an enantioselective one-pot Michael addition and Pictet–Spengler sequence was developed.[131] This method is based on the synthesis of a hemiaminal from α,β-unsaturated aldehydes and β-ketoesters, which is followed by treatment with tryptamine in the presence of acid. The initial organocatalytic conjugate addition of β-ketoesters to cinnamic aldehyde in the presence of a chiral pyrrolidine and benzoic acid in toluene was followed by addition of tryptamine and a stoichometric amount of benzoic acid. Incubation at 50 °C for 1 day led to the selective formation of one indoloquinolizidine diastereomer in moderate yield and with good enantioselectivity. Investigation of the scope of the cascade one-pot reaction (Table 13) revealed that β-aryl- and

Table 13: Organocatalyzed one-pot sequence of Michael addition and Pictet–Spengler reactions.



R	Ar	Yield [%]	*ee* [%]
Ph	Ph	81	92
4-BrC_6H_4	Ph	76	93
4-$NO_2C_6H_4$	3,5-$(CF_3)_2C_6H_3$	91	93
3-furanyl	3,5-$(CF_3)_2C_6H_3$	93	88
Me	3,5-$(CF_3)_2C_6H_3$	56	81
n-Pr	3,5-$(CF_3)_2C_6H_3$	68	85

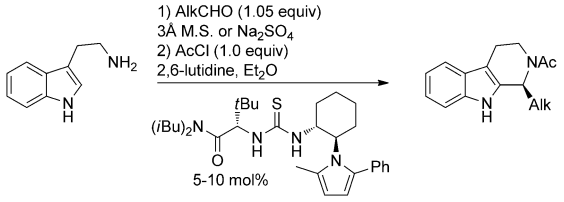
β-alkyl-substituted α,β-unsaturated aldehydes can be employed. Aldehydes with aromatic or heteroaromatic substituents provided the products in 76–93 % yield and with 88–95 % *ee*. Notably, indoloquinolizidines were obtained from β-alkyl acroleins in moderate yield and with 81–85 % *ee*. The stereochemistry of the products is defined in the Michael addition step and the diastereoselectivity of the Pictet–Spengler reaction is controlled by the kinetically controlled reaction conditions.

10. Brønsted Acid Catalyzed Enantioselective Pictet–Spengler Reactions

10.1. Thiourea-Catalyzed Transformations

The first enantioselective catalytic Pictet–Spengler reaction was reported by Taylor and Jacobsen in 2004.[132] The challenge in the development of the asymmetric catalytic Pictet–Spengler reaction was to overcome the low reactivity of the imine substrate. Initial screening of potential catalysts for this cyclization did not lead to useful results. Therefore, a general strategy for reactivity enhancement by generation of *N*-acyliminium ions was applied. Preliminary screening of the acyl-Pictet–Spengler reaction resulted in formation of N_β-acetyltetrahydro-β-carboline in 59 % *ee* by using 2-ethylbutanal and a chiral thiourea catalyst. The enantioselectivity of the cyclization depended on the structure of the acylation reagent, solvent, and temperature. As a consequence of its modular nature, the catalyst could be optimized by variation of the individual building blocks. These studies enabled the development of a new chiral thiourea catalyst and optimal reaction conditions for a wide range of substrates (Table 14).

Table 14: Asymmetric acyl-Pictet–Spengler reactions catalyzed by a chiral thiourea catalyst.



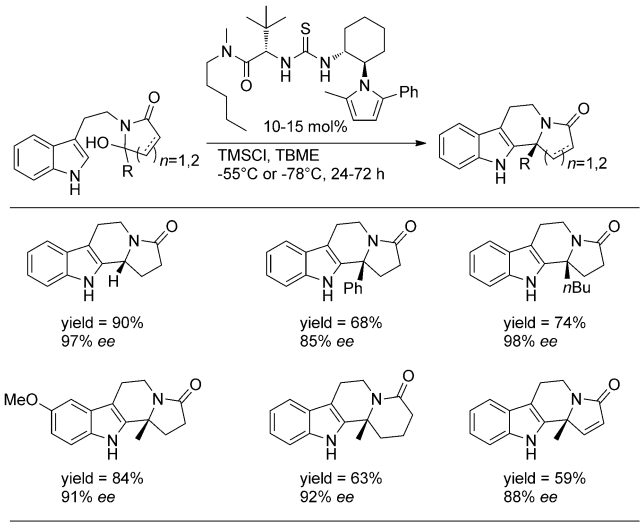
Alk	Catalyst loading [%]	Yield [%]	*ee* [%]
$CH(CH_2CH_3)_2$	5	65	93
*i*Pr	10	67	85
n-pentyl	10	65	95
*i*Bu	10	75	93
CH_2CH_2OTBDPS[a]	10	77	90

[a] TBDMS = *tert*-butyldimethylsilyl.

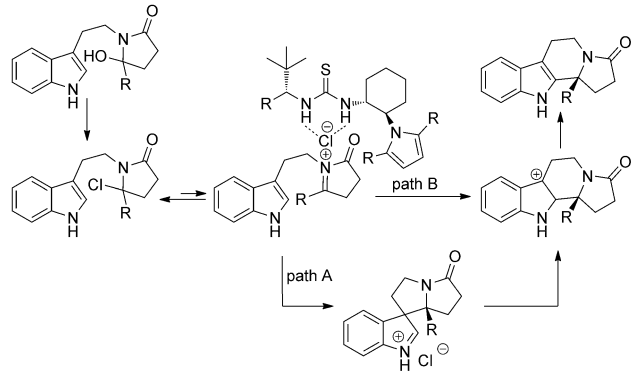
Imines obtained by condensation of aliphatic aldehydes and tryptamine were used without further purification. A range of N_β-acetyltetrahydro-β-carbolines were obtained in 65–81 % yield and with 85–93 % *ee* by using this method. 5-Methoxy- and 6-methoxytryptamines were tolerated. Imines derived from aromatic aldehydes and pivalic aldehyde were, however, not transformed.

The possibility of activating weakly Lewis basic *N*-acyliminium ions in enantioselective acyl-Pictet–Spengler reactions was applied in the cyclization of hydroxyalkyllactams (Scheme 21).[133] The lactams were obtained either by reduction of the imides with $NaBH_4$ or by alkylation of the imide with organolithium reagents. A broad set of crucial parameters, such as catalyst structure, solvent, temperature, concentration, and additives, were investigated in a model reaction. It was found that the best acidic additives, which are crucial for the formation of the *N*-acyliminium ions, are either chlorotrimethylsilane or a combination of hydrochloric acid and molecular sieves. Water had a deleterious effect on the catalytic activity and substantially improved yields were recorded at lower concentrations. Under optimal reactions conditions, cyclization of hydroxyalkyllactams derived from a variety of succinimides and glutarimides led to the desired products in 51–94 % yield and with 81–99 % *ee* (Scheme 21).

^{1}H NMR spectroscopic studies at various temperatures showed the fast and reversible formation of chlorolactams from hydroxylactams (Scheme 22). Since the enantioselectiv-



Scheme 21. Asymmetric cyclization with a chiral thiourea catalyst.

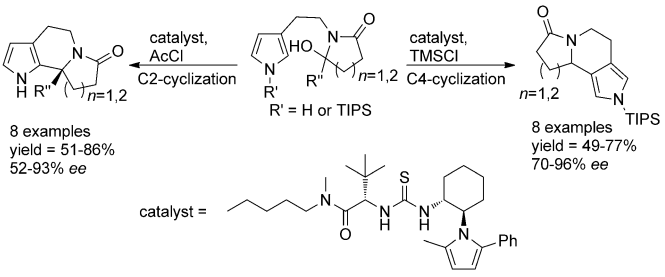


Scheme 22. Proposed mechanism for the cyclization of hydroxylactams.

ity-determining step is the cyclization of the *N*-acyliminim ion, it has to interact with the catalyst. However, the intermediate contains no viable Lewis basic site for catalyst binding. Therefore, the authors proposed that the thiourea catalyst promotes the cyclization by induction of the dissociation of the chloride counterion and formation of a chiral complex. The catalysis and enantioinduction may thus result from initial abstraction of the chloride ion from the chlorolactam and subsequent cyclization mediated by the ion-bound thiourea. The enantioselectivity of the obtained products strongly depended on the size of the halogen ion.

Jacobsen and co-workers also discovered an enantioselective catalytic acyl-Pictet–Spengler reaction by using hydroxylactams derived from 2-(pyrrol-3-yl)ethanamines (Scheme 23).[134] Successful regiocontrol for the C_4-cyclization instead of the C_2-cyclization was achived by using sterically demanding protecting groups such as *N*-triisopropylsilyl. This enabled pyrroloindolizidinones and pyrroloquinolizidinones to be obtained with modest to excellent *ee* values and yields.

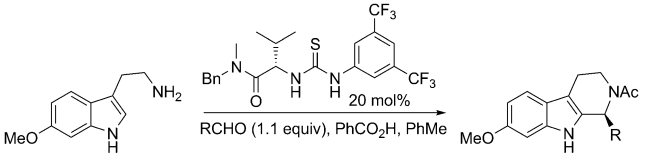
Very recently, an enantioselective catalytic Pictet–Spengler reaction with a broad substrate scope for tryptamine derivatives, which afforded unprotected tetrahydro-β-carbo-



Scheme 23. Regio- and enantioselective catalytic cyclization of pyrroles. TIPS = triisopropylsilyl.

lines, were reported.[135] In initial experiments, an imine derived from 6-methoxytryptamine and 4-chlorobenzaldehyde was screened against a variety of thiourea catalysts and achiral Brønsted acids. Under the best conditions, namely in the presence of acetic acid as co-catalyst at room temperature, tetrahydro-β-carboline was obtained in 85% yield and with 87% *ee*. Notably, the corresponding product was not observed in the absence of acetic acid. Optimization led to the discovery of a new simple thiourea-based catalyst which can be prepared in 69% yield in three steps from commercially available chemicals and requires a single chromatographic purification. It was found that benzoic acid increased the reaction rate and enantioselectivity. Under the optimized reaction conditions, a series of substituted benzaldehydes (Table 15) yielded unprotected tetrahydro-β-carbolines in

Table 15: Pictet–Spengler reactions catalyzed by a thiourea and benzoic acid.



R	$PhCO_2H$ [%]	*t* [h]	Yield [%]	*ee* [%]
Ph	20	70	94	86
4-FC_6H_4	20	78	81	92
4-$MeOC_6H_4$	20	91	78	85
2-BrC_6H_4	20	11	74	95
*i*Pr	0	88	90	94
n-pentyl	0	18	74	86

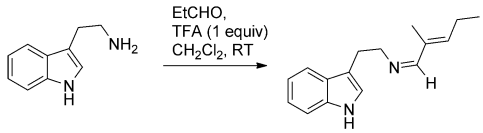
good yields and with excellent *ee* values. Higher reaction rates were observed for *ortho*- and *meta*-substituted benzaldehydes. Importantly, the reaction of tryptamine with *ortho*-bromobenzaldehyde required a longer reaction time (10 days) compared to 6-methoxytryptamine (11 h) and led to a lower yield with a similar *ee* value.

The generality of the cyclization was examined with reactions of aliphatic aldehydes with 6-methoxytryptamine. In contrast to benzaldehydes, the Pictet–Spengler reaction occurred in the absence of benzoic acid with increased enantioselectivity; however, a longer reaction time was needed. Linear and branched aldehydes were tolerated

(Table 15). The corresponding products were obtained in 74–90% yield and 86–94% *ee*. However, reactions with less nucleophilic tryptamines under neutral conditions were not successful and acidic additives were necessary.

10.2. Strong Brønsted Acid Catalyzed Transformations

Since the Pictet–Spengler reaction is an acid-catalyzed reaction and strong chiral Brønsted acids had been successfully employed for iminium ion mediated transformations, List and co-workers focused on a direct enantioselective catalytic Pictet–Spengler reaction of aryl ethylamines and aldehydes.[136] Attempts to carry out the reaction on unsubstituted substrates were not successful because of the formation of undesired by-products (Scheme 24).

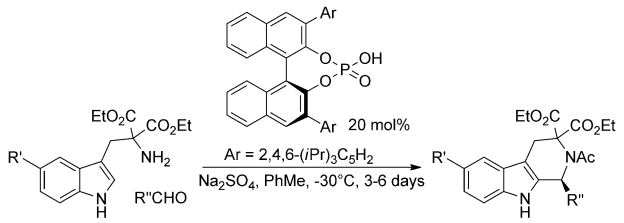


Scheme 24. Tryptamine-mediated homoaldol condensation.

However, application of geminally disubstituted tryptamines led to the formation of the desired cyclization products. A screen of chiral phosphoric acid diesters, solvents, and reaction temperatures was performed to find the optimal reaction conditions. The best results were obtained with bulky chiral phosphoric acid diesters in toluene at low temperature, and a protocol for reactions of geminally disubstituted tryptamines with a variety of aliphatic aldehydes in the presence of 20 mol% of the catalyst was developed, which yielded the products in 40–98% yield and 72–96% *ee* (Table 16). Electron-poor aromatic aldehydes gave excellent results. The need for geminally disubstituted tryptamines limits this method.

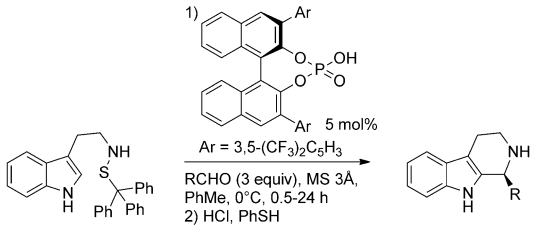
Hiemstra and co-workers suggested using *N*-sulfenyl intermediates in the catalytic Pictet–Spengler condensation.[137] *N*-Sulfenyltryptamines were obtained from tryptamine and commercially available sulfenyl chloride in the presence of base. Initial screening using *N*-(2-nitrophenyl)-sulfenyltryptamine as the substrate in the presence of chiral phosphoric acid diesters resulted in excellent yields (92–98%) of tetrahydro-β-carbolines, although the enantioselectivity of the products was low (up to 31% *ee*). A subsequent screening using the bulkier *N*-tritylsulfenyltryptamine led to a substantial improvement in the enantioselectivity of the cyclization. However, the obtained product was unstable, possibly because of cleavage of the trityl–sulfur bond. The addition of 3,5-di(*tert*-butyl)-4-hydroxytoluene to the reaction mixture as a radical scavenger prevented the decomposition of the product. Further studies included application of toluene as solvent, lower temperatures, and the addition of molecular sieves to remove water, which had a deleterious effect on the yield and enantioselectivity of the product. A two-step synthesis procedure for the generation of unprotected tetrahydro-β-carbolines was developed to avoid isolation of the unstable cyclization product (Table 17). Examination of a series of aldehydes under the optimized reaction conditions revealed that linear and branched aliphatic and aromatic aldehydes gave the desired products in 77–90% yield and with 72–87% *ee*. An exception is the tetrahydro-β-carboline obtained from acetaldehyde, which was isolated in 88% yield but only with 30% *ee*, possibly because of poor steric discrimination by the chiral Brønsted acid catalyst. Simple deprotection of the obtained tetrahydro-β-carbolines and the scalability of the procedure make this method attractive.

Table 16: Brønsted acid catalyzed enantioselective Pictet–Spengler reactions.



R′	R″	Yield [%]	*ee* [%]
H	Et	76	88
MeO	Et	96	90
H	*n*Bu	91	87
H	Bn	58	76
H	4-$NO_2C_6H_4$	60	88
OMe	4-$NO_2C_6H_4$	98	96
OMe	Ph	82	62

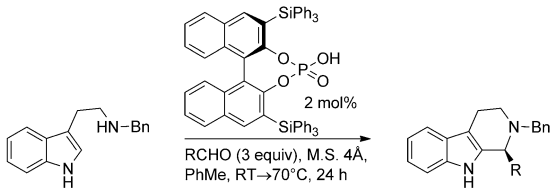
Table 17: Catalytic Pictet–Spengler reactions with *N*-sulfenyltryptamine.



R	*t* [h]	Yield [%]	*ee* [%]
Me	1	88	30
*i*Pr	24	77	78
n-pentyl	2	87	84
Bn	4	90	87
Ph	24	77	82
4-$NO_2C_6H_4$	24	78	82

Further studies on chiral phosphoric acids diesters was carried out using N_β-benzyltryptamine as the starting material of choice for pharmaceutically relevant compounds.[138] The optimal reaction conditions include the use of a bulky Brønsted acid catalyst in toluene at high temperature in the presence of molecular sieves. A series of aliphatic and aromatic aldehydes were investigated in the catalytic Pictet–Spengler reaction under these conditions, (Table 18). All the reactions proceeded smoothly to give the corresponding N_β-benzyltetrahydro-β-carbolines in 77–97% yield with moderate to good enantioselectivity (61–87%). However, low

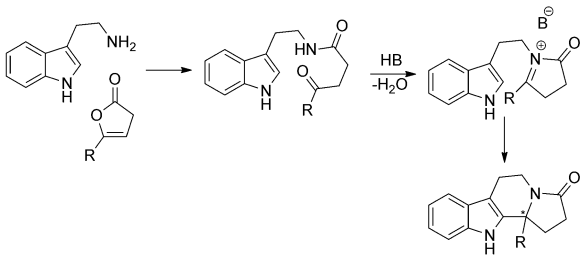
Table 18: Catalytic Pictet–Spengler reactions with *N*-alkyl tryptamine.



R	Yield [%]	*ee* [%]
*i*Pr	90	81
n-pentyl	77	68
Ph	95	72
4-$MeOC_6H_4$	84	80
4-$NO_2C_6H_4$	95	87
3-ClC_6H_4	92	20

ee values were observed for *meta*-substituted benzaldehydes and 3-phenylproponal, while no product was detected with phenylacetaldehyde.

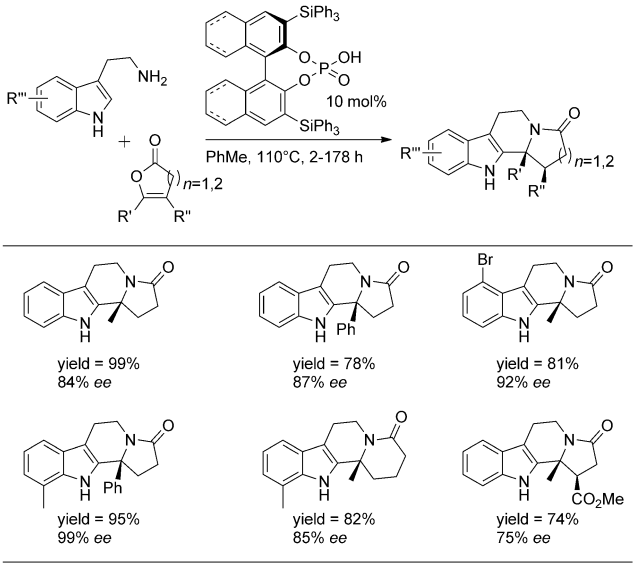
Recently Dixon and co-workers developed enantioselective Brønsted acid catalyzed cyclization cascades[139] with a Pictet–Spengler reaction to arrive at a powerful one-pot strategy for the synthesis of polycyclic compounds. Ring opening of enol lactones followed by formation of *N*-acyliminium intermediates and cyclization leads to the formation of tetracyclic products (Scheme 25). In this transformation, the chiral Brønsted acids provide enantiocontrol during the ring-formation step through ion paring with the chiral counterion. A study of the chiral Brønsted acid catalyst, solvent, temperature, and concentration in a model reaction revealed the optimal reaction conditions. The best results were obtained using 10 mol% of a chiral phosphoric acid diester at high dilution (7 mM) in toluene at reflux. Various enol lactones and derivatives of tryptamine were investigated in the cyclization cascade under these optimized conditions (Scheme 26).



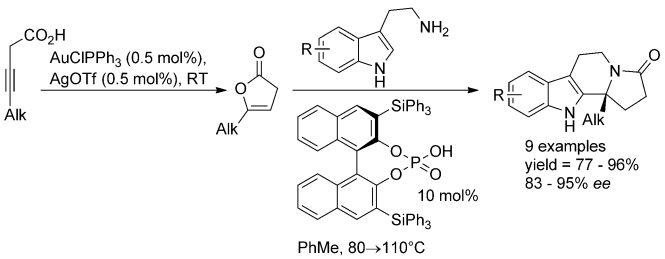
Scheme 25. Concept of a Brønsted acid catalyzed cyclization cascade.

A range of substituted tryptamines with electron-withdrawing and -donating groups at positions 4, 5, 6, and 7 are tolerated and afford the corresponding products in good yields and 83–99% *ee*. Besides monosubstituted enol lactones carrying an aryl or alkyl group, disubstituted enol lactones may be used to give selective formation of one diastereomer.

A gold(III)-catalyzed Pictet–Spengler reaction was demonstrated by Youn.[140] Interestingly, the Brønsted acid catalyzed cyclization cascade was compatible with gold(I)-catalyzed cycloisomerization of alkynoic acids (Scheme 27). Alkynoic acids were treated with a gold(I) catalyst and the tryptamines and chiral Brønsted acid were added after formation of enol lactones. The products of the cascade reaction, which proceeds by dual catalysis, were isolated in good yields and with high *ee* values. Mechanistically, the developed cascade reaction proceeds through a gold(I)-catalyzed formation of enol lactones, which can be opened by trypamine to give the amide of the ketoacid. The following cyclization to an acylenamine yields an *N*-acyliminium intermediate. A counterion-controlled Pictet–Spengler cyclization leads to the diastereo- and enantioselective formation of the product. This strategy was very recently applied in a cascade reaction using tryptamines and ketoacids, which are readily assessible compared to enol lactones.[141]



Scheme 26. A Brønsted acid catalyzed enantioselective cyclization cascade.



Scheme 27. Au^I and chiral Brønsted acid catalyzed cyclization.

11. Conclusions and Outlook on the Development of the Asymmetric Pictet–Spengler Reaction

The discovery of the Pictet–Spengler reaction provided a powerful method for the synthesis of natural biologically active compounds. Impressive results have been obtained during the 100 years since its discovery, and the scope of the reaction has been greatly extended. The Pictet–Spengler

reaction is particularly important in the total synthesis of alkaloids and in nature. Notable results were obtained in the asymmetric construction of natural products, for example, norsuaveoline, ajmaline, alkaloid G, suaveoline, affinisine, vellosimine, talpinine, talcarpine, alstonerine, anhydromacrosalhine-methine, raumacline, 9-methoxygeissoschizol, 9-methoxy-N_b-methylgeissoschizol, phalarine, jorumycin, renieramycin G, and corynantheidine.[115] The Pictet–Spengler reaction has been successfully applied in the construction of compound libraries on solid supports and used in multicomponent reactions.[142]

Very recently, the first highly enatioselective catalytic Pictet–Spengler reaction with a chiral thiourea as the catalyst was developed. During the last five years, a number of chiral thioureas and bulky chiral phosphoric acid diesters have found application in the enantioselective catalytic cyclization of tryptamines. However, there is high demand for the development of new efficient catalytic systems with broad substrate scope and not limited to tryptamines.

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