Biocatalytic C3-Indole Methylation—A Useful Tool for the Natural-Product-Inspired Stereoselective Synthesis of Pyrroloindoles

Pascal Schneider, Birgit Henßen, Beatrix Paschold, Benjamin P. Chapple, Marcel Schatton, Florian P. Seebeck, Thomas Classen, and Jörg Pietruszka*

In memory of Volker Jäger

Abstract: *Enantioselective synthesis of bioactive compounds bearing a pyrroloindole framework is often laborious. In contrast, there are several S-adenosyl methionine (SAM)-dependent methyl transferases known for stereo- and regioselective methylation at the C3 position of various indoles, directly leading to the formation of the desired pyrroloindole moiety. Herein, the SAM-dependent methyl transferase PsmD from Streptomyces griseofuscus, a key enzyme in the biosynthesis of physostigmine, is characterized in detail. The biochemical properties of PsmD and its substrate scope were demonstrated. Preparative scale enzymatic methylation including SAM regeneration was achieved for three selected substrates after a design-of-experiment optimization.*

Introduction

In the past twenty years, biocatalysis was shown to be a valuable tool within the toolbox of an organic chemist.[1] Besides organocatalysts and metal catalysts, biocatalysts are state of the art in medicinal chemistry:[2] They are not only employed in the synthesis of dedicated (chiral) building blocks, but in particular in late-stage functionalization of active agents. After overcoming initial limitations, e.g., with respect to cofactor recycling systems or scaling, the proven versatility of enzymes even in industrial processes increased

the demand for new biocatalysts in recent years.[3,4] Also with regard to natural product synthesis, enzymes exhibit great potential for challenging synthetic tasks such as stereoselective synthesis and late-stage functionalization under mild conditions.[5]

One particular class of natural products and derivatives thereof, namely drugs based on pyrroloindolines **1**–**5**,[6] involve methyl transferases as key enzymes within their biosynthesis (Figure 1, A).[7] The quaternary stereogenic carbon center is formed by C-alkylation of indoles **6** or **7**, a reaction that has attracted considerable attention recently.[6c,q] The major synthetic strategies towards the tricyclic scaffold **8** can be divided into two groups, either following a biomimetic approach—electrophilic C3-alkylation followed by cyclization—or utilizing oxindoles **9** (Figure 1, B).[8,9] Biosynthesis itself is an alternative for the natural product, as has been demonstrated for acetylcholinesterase inhibitor physostigmine (**1**) that is produced by a *Streptomyces griseo-*

[*] P. Schneider, B. Paschold, B. P. Chapple, M. Schatton,
Prof. Dr. J. Pietruszka
Institut für Bioorganische Chemie
Heinrich-Heine-Universität Düsseldorf im Forschungszentrum Jülich
and Bioeconomy Science Center (BioSC)
Stetternicher Forst, Geb. 15.8, 52426 Jülich (Germany)
E-mail: j.pietruszka@fz-juelich.de

B. Henßen, Dr. T. Classen, Prof. Dr. J. Pietruszka
Institut für Bio- und Geowissenschaften: Biotechnologie (IBG-1)
Forschungszentrum Jülich GmbH
52428 Jülich (Germany)

Prof. Dr. F. P. Seebeck
Department of Chemistry, University of Basel
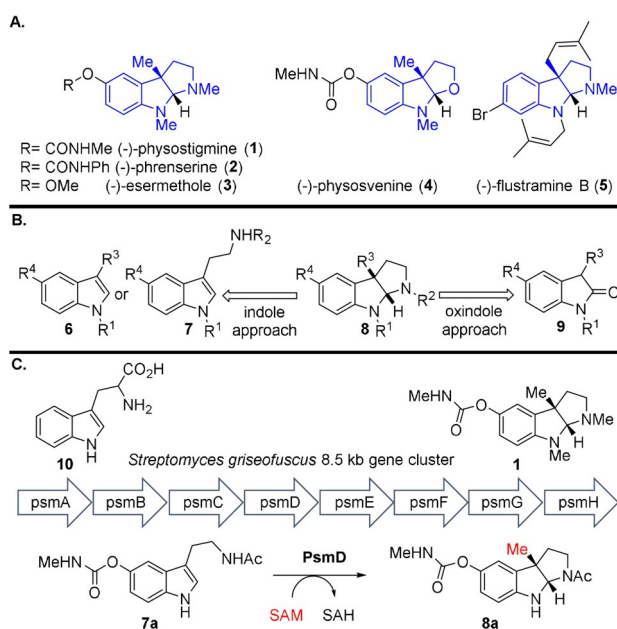Mattenstrasse 24a, CH-4058 Basel (Switzerland)

Figure 1. A. Selected examples of (natural) drugs bearing the pyrroloindoline framework (blue). B. Common retrosynthetic approaches towards pyrroloindolines. C. The methyl transferase (MT) PsmD investigated in this work represents the key enzyme in the biosynthesis of physostigmine (**1**). For a proposed mechanism of the PsmD reaction see Supporting Information (Figure S1). SAM: *S*-adenosyl methionine; SAH: *S*-adenosyl homocysteine.

fuscus strain from tryptophan (**10**) with titers of up to 790 mg L^{-1}.[10]

The corresponding gene cluster encoding for the eight enzymes was resolved in 2014, also suggesting the central role of PsmD for the stereoselective methylation (Figure 1, C).[6c] While fermentation proved to be versatile for physostigmine (**1**) production, derivatives hereof and of the corresponding biosynthetic intermediates are less readily available and the stereoselective access remains challenging. Thus, there is still a demand for efficient, robust and mild methods to access the pyrroloindole framework in an enantiomerically pure fashion and we anticipated that the methyl transferase PsmD might serve as the required tool to overcome these limitations.
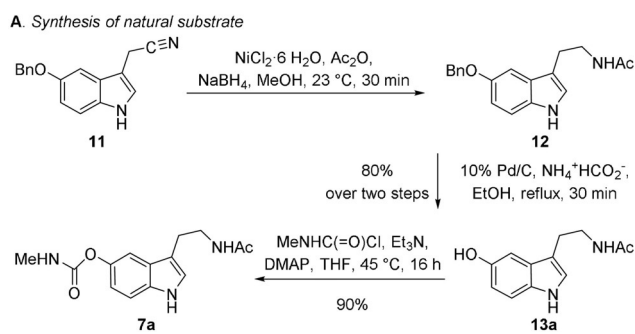
Six classes of methyl transferases have been identified, the most common representatives belonging to the family of the *S*-adenosyl methionine (SAM)-dependent methyl transferases, including N-, O-, S- as well as C-methyl transferases.[11–13] They are involved in many different processes including DNA and RNA regulation,[14,15] posttranslational protein modification,[16] and biosynthesis of several bioactive natural compounds.[7,17,18] Despite their undeniable potential, their application in organic synthesis was hampered by the lack of a scalable recycling system for SAM, one of the most expensive cofactors available.[19] Only recently, promising protocols have been established: Apart from utilizing the complete SAM regeneration cycle,[20] mainly two approaches are used to address this limitation. On the one hand, there is the so called "supply chain", which uses methionine adenosyl transferases (MAT) to form SAM from methionine and adenosine triphosphate (ATP).[20] Even though this reaction requires stoichiometric amounts of ATP and methionine, these chemicals are comparably cheap and available in large quantities. On the other hand, there is the direct recycling by using halide methyl transferases (HMT), which consume methyl iodide to convert *S*-adenosyl homocysteine (SAH) back to SAM directly.[21]

Herein, we report the application of the C3-methyl transferase PsmD with regard to potential application for total synthesis of physostigmine derivatives on an enzymatic preparative scale applying only catalytic amounts of the cofactor by direct SAM recycling.[21]
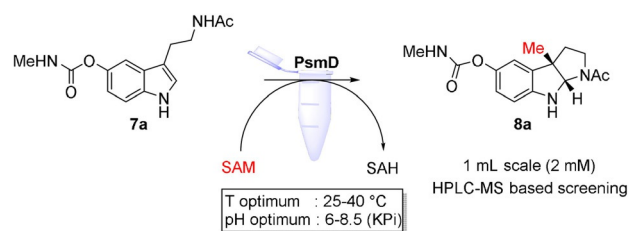
Results and Discussion

Optimized conditions for PsmD reaction. To evaluate the proposed reaction of the methyl transferase PsmD (Figure 1 C), the enzyme was expressed and purified as described by Liu et al.[6] The natural substrate was synthesized starting from the commercially available 5-benzyloxyindole-3-acetonitrile (**11**, Scheme 1 A): reductive acylation provided amide **12** that could be de-O-benzylated furnishing phenol **13a**.[22] The consecutive carbamoylation led to methyl carbamate **7a** in high yield (90%; 65% yield over three steps).

Next, the proposed enzymatic transformation of indole **7a** was performed in vitro and formation of product **8a** was confirmed by HPLC–MS (Scheme 1 B). Based on the initial results, the optimal reaction conditions for the biocatalytic methylation were systematically tested. Thus, the temper-

Scheme 1. A. Substrate synthesis; DMAP: 4-dimethylaminopyridine. B. Experimental setup used for the reaction characterization (for details see Supporting Information). C. Summary of the parameters of PsmD. [a] Calculation based on the amino acid sequence. [b] Kinetic parameters were obtained by application of the Mtase-glo Assay (Promega; for details see Supporting Information).

ature optimum was determined by comparing relative conversion from compound **7a** to compound **8a** after 4 hours by HPLC–MS. The tested temperature ranged from 25°C to 40°C; the enzyme showed best conversion at 35°C. The pH optimum was shown to be at 7.5 in KPi buffer (100 mM, see Supporting Information for details), even though only slight differences were observed in the tested pH range (6.0–8.5, 100 mM KPi buffer). In the following, the optimized parameters were used to elucidate the kinetics of the PsmD-catalyzed methylation. For this, the commercially available bioluminescence-based Mtase-glo Assay (Promega) was used for the SAM-dependent methyl transferase activity screening.[23]

For determining the kinetic parameters, substrate **7a** was tested with concentrations ranging from 0.1 µM to 100 µM in an activity screening in 96-well format. The SAM concentration was fixed to 60 µM and 1 µg of enzyme (or 0.1 µg for low substrate concentrations from 0.1 µM to 1 µM) was used in total for each well (20 µL reaction volume). The reaction was stopped after 5, 7, and 10 minutes by addition of 0.5% trifluoroacetic acid. After addition of the Mtase reagent and Mtase detection solution the bioluminescent signal based on

the formed SAH concentration was measured. Since this is an indirect method, it is assumed that SAH formation is exclusively based on the consumption of SAM by the methyl transferase reaction. To rule out autodemethylation of SAM, a negative control with 60 µM SAM and 1 µg enzyme was used for each run. Reaction rate was calculated and plotted against the tested concentration. Applying a least-square-fit with the Michaelis–Menten equation, the maximal velocity is 18.3 µmol min^{-1} g^{-1} with a K_M value of 11.3 µM (see Supporting Information for details; the validity of the method was independently confirmed by HPLC reaction control). The biochemical data obtained for PsmD are summarized in Scheme 1 C.

Substrate scope. In order to test the scope of PsmD beyond the natural substrate **7a**, a set of 16 selected substrates **7a–p** was synthesized first (see Supporting Information for details) and then evaluated in the enzymatic transformation towards compounds **8a–p**. Enzyme activities were measured in triplicates as described for the determination of kinetic parameters using the Mtase-glo Assay. The substrate concentration was set to 20 µM. To validate that conversion always resulted from the respective reaction from substrate to product, all samples were also monitored by HPLC–MS. First, the influence of the carbamate group was evaluated (Scheme 2). While the convenient formation of compounds **8a–d** could be confirmed, it was apparent that increasing the steric bulk resulted in a decrease in activity, ultimately leading to no (*tert*-butyl product **8e**) or almost no (phenyl carbamate **8f**) product formation. Similarly, an analogous trend was observed when varying the amide group (products **8a,g–k**): However, while a decrease in activity from the acetyl group (in compound **8a**) to the pivalate **8j** or benzoate **8k** can be observed, the remaining activity should still qualify for preparative scale transformation, thus allowing to test the limits of the enzymatic approach towards physostigmine derivatives. Surprisingly, omitting the carbamate completely (products **8l–p**, see box in Scheme 2) was feasible and transformation was detectable, albeit with low residual relative activity (for **8l–n**). Since the latter examples are still important with respect to further application of the methyl transferase in organic syntheses, we decided to include the target compound **8m** in the set of experiments for enzymatic preparative conversion as well. While the carbamate group is crucial for high activity, the volume of the substrates is another important parameter as can be seen from the correlation of the PsmD activity with the Connolly solvent-excluded volume (Figure 2).

PsmD—stereoselectivity. Since the natural compound physostigmine (**1**) is enantiomerically pure, it can be assumed that the PsmD-mediated methylation is also enantioselective. In order to validate this, three representatives **8a,j,m** had been selected for the synthesis of the respective racemic chromatographic standard (Scheme 3 A): For the key step, the dearomative cyclization of the tryptamine, an adapted protocol by Yi et al. was used forming **8m** as well as **15a,b** in good yield (67–84%).[6g] It should be noted that under these reaction conditions the direct transformation of carbamates **7** led to decomposition of the starting material. Next, deprotection and reprotection allowed the selective phenol carba-
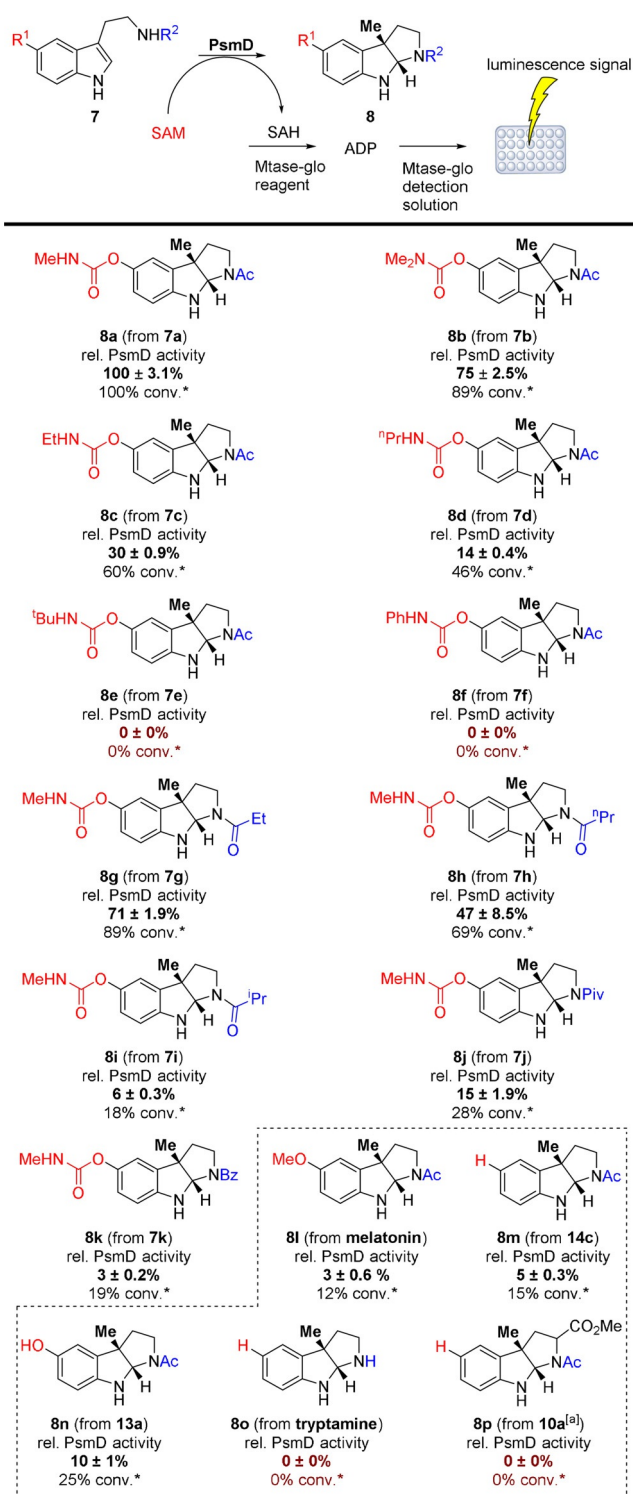
Scheme 2. Substrate scope and schematic procedure for evaluation of enzyme promiscuity. Relative PsmD activity is displayed in % (relative to the natural reaction **7a** to **8a**). The results represent the mean of three experiments and details are shown in the Supporting Information. * Conversion is displayed as relative conversion based on the substrate-to-product ratio and was determined by HPLC–MS as described in the Supporting Information. Reactions were stopped after 16 hours and performed as described for pH and T screening. [a] *N*-acetyl-L-tryptophan methyl ester (**10a**, not shown).
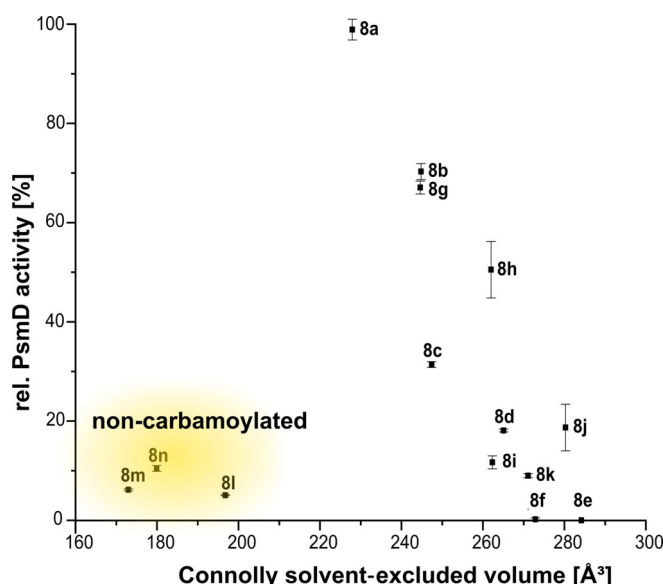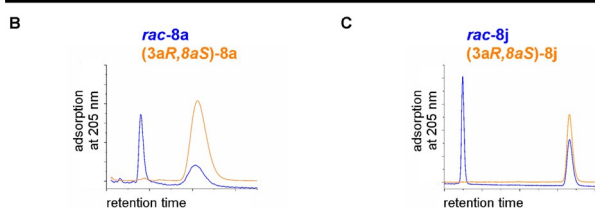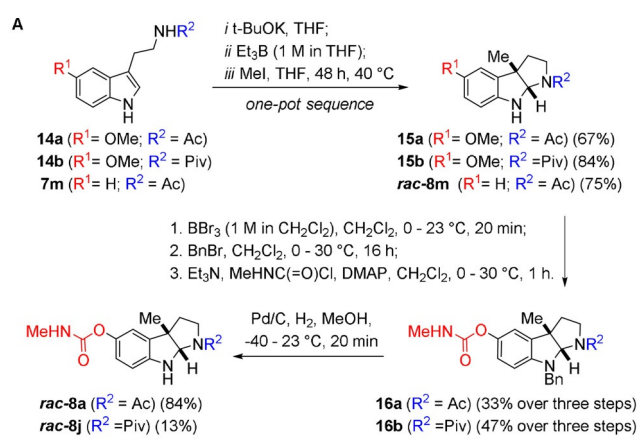
Figure 2. Correlation between the volume of the tested compounds and relative PsmD activity [%] (compounds **8a–n**). The volume was calculated using Chem3D Connolly solvent-excluded volume (probing radius 1.4 Å).[24] The non-carbamoylated compounds have been high-lighted in yellow.


Scheme 3. A. Synthesis of the racemic standard compounds **8a,j,m**. B,C. HPLC traces for the *ee* analytics of compounds **8a,j** (in blue—racemic standard, in orange—compounds obtained after enzymatic methylation with > 99% *ee*; for details see Supporting Information).

moylation leading to intermediates **16a,b**. As the N-unsubstituted hexahydropyrroloindoles **8a,j** are prone to ring-opening of the heterocycle, the benzyl deprotection has to be performed under mild conditions (−40–23 °C) to suppress this side reaction. Although the non-optimized reaction sequence mimics the biosynthesis in terms of the dearomative cyclization, it lacks both the elegance of a one-step procedure and

the efficiency of the enzyme-mediated reaction. However, with the racemic standards **8a,j,m** at hand, it was unambiguously proven that the corresponding enzymatic in vitro conversions shown in Scheme 2 were indeed highly enantioselective (> 99% *ee*; Scheme 3B,C as well as Supporting Information).

Preparative scale enzymatic methylation. While the PsmD-catalyzed methylation proved to be mild, protecting-group-free, and highly selective, providing a short-cut in the synthesis of pyrroloindolines **8**, its synthetic utility remains unclear until the stoichiometric demand for SAM can be overcome. Here, we based the recycling on the halide methyl transferase from *Chloracidobacterium thermophilum* (*Ct*HMT) described previously.[21] This enzyme transfers a methyl group from methyl iodide onto SAH to form SAM needed for the PsmD reaction (Scheme 4 A). The plasmid containing the gene encoding for the His-tagged *Ct*HMT and the SAH-nucleosidase-deficient strain *E. coli* Δmtn (DE3), which was shown to be superior to the commercially available strains for the cofactor recycling, were in general used as described previously.[21] However, the reaction conditions had to be adapted for the chosen substrate as well as PsmD, and hence a systematic optimization was performed to find the best parameters for the conversion, keeping the enzyme load as low as possible (Figure 3). First, a factorial design was
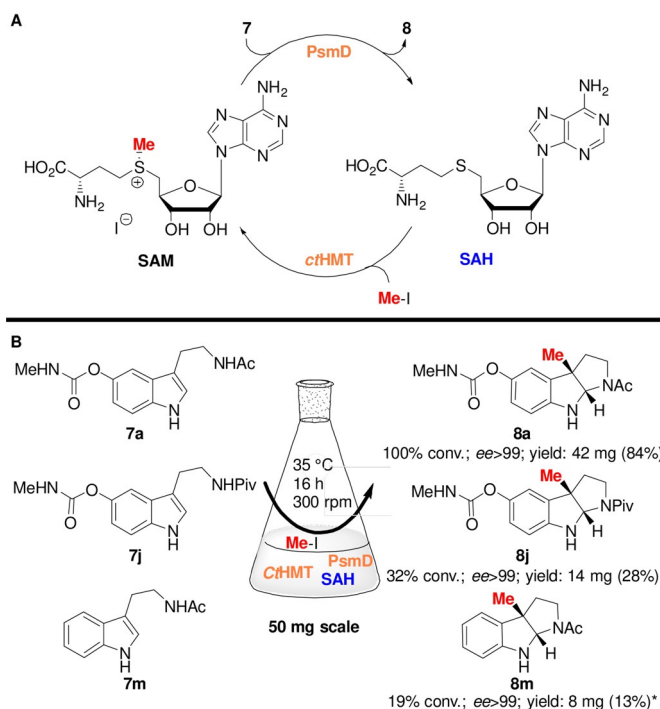
Scheme 4. A. SAM cofactor recycling as applied in the enzymatic preparative scale synthesis of methylated compounds **8**. B. Enzymatic preparative scale methylation of substrates **7a,j,m**. All reactions were carried out with 50 mg of the respective substrate utilizing the optimized conditions: 35 °C, pH 7.5, 1 mM EDTA, 2 mM substrate, 13 mM MeI, 20 mM SAH 6.7 μM PsmD (16 U g^{-1}), 262 μL *Ct*HMT lysate (0.09 U mL^{-1}) in a closed Schott flask. Yield is given as yield of isolated pure product and conversion is displayed as relative conversion based on the substrate-to-product ratio determined by HPLC. * A 10-fold excess of PsmD was used.
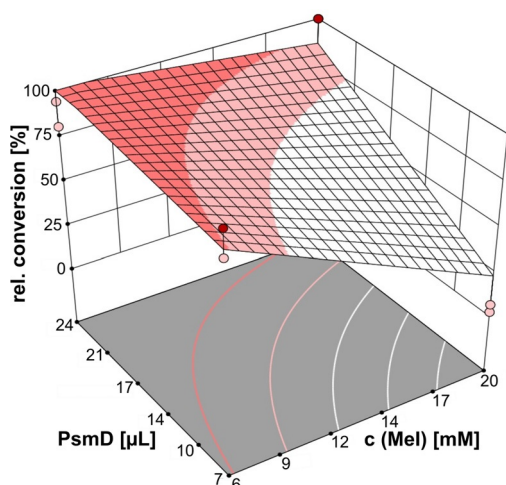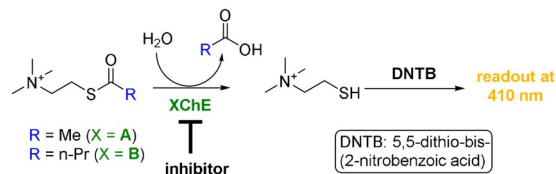
Figure 3. The diagram shows a 3D cut of the hypersurface for the 4D optimization. The red maximum corresponds to 100% conversion. For detailed information on the tested conditions for recycling optimization, see Supporting Information. The hypersurface is described by the equation:

$$\text{conversion} = 125.5\% - 7.5898\ \%\mu\text{M}^{-1} \times c(\text{MeI}) + 0.5684\ \%\mu\text{L}^{-1} \times V(\text{PsmD}) - 0.0184\ \%\mu\text{L}^{-1} \times V(Ct\text{HMT}) + 0.2074\ \%\mu\text{M}^{-1}\mu\text{g}^{-1}\ c(\text{MeI}) \times V(\text{PsmD}) + 0.0058\ \%\mu\text{M}^{-1}\mu\text{L}^{-1} \times c(\text{MeI}) \times V(Ct\text{HMT}) - 0.00418\ \%\mu\text{L}^{-1}\mu\text{L}^{-1}\ V(\text{PsmD}) \times V(Ct\text{HMT}).$$

performed testing the following factors: SAH (20 μM & 50 μM), methyl iodide (2 mM & 10 mM), EDTA (0 mM & 1 mM), temperature (25°C & 35°C), pH (7.5 & 8.0). Three parameters turned out to influence the reaction outcome dominantly and were optimized in a second round using a response surface design-of-experiment. The parameters were: the methyl iodide concentration, the PsmD amount, and the *Ct*HMT concentration. The conversion of compound **7a** to **8a** can be modelled by the hypersurface shown in Figure 3. By this, we were not only able to define ideal parameters for the conversion [35°C, pH 7.5, 1 mM EDTA, 2 mM substrate, 13 mM MeI, 20 mM SAH, 6.7 μM PsmD (16 U g^{-1}), 262 μL *Ct*HMT lysate (0.09 U mL^{-1})], but furthermore were able to even reduce the demand for SAH to 20 μM.

After successful screening on 2 mM scale, we decided to use the best conditions for preparative scale enzymatic methylation (50 mg scale; see Scheme 4B). While the focus was on the natural substrate **7a**, we also decided to test the scope with two indoles **7j,m** that proved to be poor substrates in the initial tests (see Scheme 2). Pleasingly, the key intermediate **8a** within the physostigmine (**1**) biosynthesis was isolated after quantitative conversion of tryptamine **7a** in 84% yield of isolated pure product. We were surprised to find that also the non-natural products **8j,m** could be obtained, albeit, as expected, in poor yield of pure isolated product (28% and 13%, respectively): With the same amount of PsmD (6.7 M; 16 U g^{-1}) and PsmD showing the lowest residual activity for compound **7m,** only 4% conversion was observed after 16 hours (reaction was monitored by TLC, after 16 hours no further conversion was observed). A tenfold excess of PsmD was necessary to increase the conversion (19%), also making enantiomerically pure product **8m** accessible (8 mg; 13%).

Bioactivity testing. The compounds **8a**, **8j**, and **8m** were tested for their inhibition of the acetylcholinesterase and butyrylcholinesterase. Even though physostigmine (**1**) is a known and potent inhibitor of these medicinally important enzymes, it has poor pharmacokinetics and is therefore no longer considered for Alzheimer's disease treatment.[25] Nevertheless, because of the rising importance and thus demand for Alzheimer-targeting drugs and the known potential of the hexahydropyrrolo indole scaffold, we decided to test the three obtained compounds with the Ellman method (Scheme 5).[26] We were able to determine the respective IC$_{50}$ values by applying a nonlinear dose–response curve (for detailed information and assay procedure see Supporting

Compound	AChE IC$_{50}$	BChE IC$_{50}$
physostigmine	0.13 ± 0.01 μM	0.12 ± 0.02 μM
rivastigmine	36.1± 2.7 μM	1.0 ± 0.06 μM
8a	0.09 ± 0.01 μM	0.01 ± 0.002 μM
8j	90.0 ± 1.8 μM	20.0 ± 0.9 μM
8m	17.0 ± 0.6 μM	400 ± 45 μM

Scheme 5. Bioactivity assay used for in vitro activity profiling of the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) determined with the Ellman method.[26] General assay used in this study (for detailed description, see Supporting Information) and summary of the results obtained in this study. Values are displayed as IC$_{50}$ [μM] and represent the mean of three experiments.

Information). As a reference compound, commercially available physostigmine (**1**) and its analogue rivastigmine were used. Compound **8a** proved its potential as an inhibitor for the AChE as well as for the BChE: At least in the in vitro setting, superior values relative to the reference compounds were measured. Furthermore, for compound **8j** and **8m**, IC$_{50}$ values in the μM range were determined, showing on the one hand that increasing the bulk at the amide residue leads to a sterical clash within the target enzyme and on the other hand that the carbamoyl moiety seems to be necessary for proper binding to the enzyme. In summary, we were able to apply the methyl transferase PsmD in the direct and scalable synthesis of enantiomerically pure and bioactive physostigmine derivatives.

Conclusion

In the present study, PsmD, the key enzyme in the biosynthesis of physostigmine (**1**) that is responsible for the enantioselective dearomative C3-methylation of tryptamine **7a**, was heterologously expressed, and biochemically characterized for the first time. It was demonstrated that it shows remarkable substrate promiscuity by converting twelve out of

sixteen selected tryptamine derivatives with SAM as cofactor. Compared to conventional synthesis, the reaction conditions proved to be much milder, also providing products with excellent enantioselectivity (> 99 % as determined by HPLC analysis). The objective of the study could be reached by successfully implementing cofactor recycling of SAM in an enzymatic preparative scale transformation by using the halide methyl transferase from *Chloracidobacterium thermophilum* (*Ct*HMT): Methyl iodide was the stoichiometric reagent in the methylation of SAH. Optimal reaction conditions were obtained upon a design-of-experiments investigation proving at the same time that an excess of methyl iodide does have a negative influence on the overall conversion. Thus, PsmD was demonstrated to be a versatile tool in the enantioselective synthesis of pyrroloindolines **8**, compounds that were shown to display remarkable bioactivity as inhibitors of the acetylcholinesterase and butyrylcholinesterase.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

Keywords: biocatalysis · methyl transferase · natural products · physostigmine · SAM recycling

[1] A. Fryszkowska, P. N. Devine, *Curr. Opin. Chem. Biol.* **2020**, *55*, 151–160.

[2] a) S. D. Dreher, *React. Chem. Eng.* **2019**, *4*, 1530–1535; b) S. Wu, R. Snajdrova, J. C. Moore, K. Baldenius, U. T. Bornscheuer, *Angew. Chem. Int. Ed.* **2021**, *60*, 88–119; *Angew. Chem.* **2021**, *133*, 89–123; c) E. Romero, B. S. Jones, B. N. Hogg, A. Rue Casamajo, M. A. Hayes, S. L. Flitsch, N. J. Turner, C. Schnepel, *Angew. Chem. Int. Ed.* **2021**, *60*, 16824–16855; *Angew. Chem.* **2021**, *133*, 16962–16993; d) A. S. Klein, T. Classen, J. Pietruszka in *Pharmaceutical Biocatalysis—Fundamentals, Enzyme Inhibitors, and Enzymes in Health and Diseases* (Ed.: P. Grunwald), Jenny Stanford Publishing, Singapore, **2019**, pp. 713–738.

[3] S. Mordhorst, J. N. Andexer, *Nat. Prod. Rep.* **2020**, *37*, 1316–1333.

[4] J. Micklefield, *Nat. Catal.* **2019**, *2*, 644–645.

[5] L. E. Zetzsche, A. R. H. Narayan, *Nat. Rev. Chem.* **2020**, *4*, 334–346.

[6] Recent review: a) A. Roy, A. Maity, S. S. Mk, R. Giri, A. Bisai, *Arkivoc* **2020**, *2020*, 437–471; selected publications on physostigmine (**1**), phenserine (**2**) (a synthetic drug), esermethol (**3**), physosvenine (**4**), and related alkaloids: b) G. E.-S. Batiha, L. M. Alkazmi, E. H. Nadwa, E. K. Rashwan, A. M. Beshbishy, H. Shaheen, L. Wasef, *J. Drug Delivery Ther.* **2020**, *10*, 187–190; c) J. Liu, T. Ng, Z. Rui, O. Ad, W. Zhang, *Angew. Chem. Int. Ed.* **2014**, *53*, 136–139; *Angew. Chem.* **2014**, *126*, 140–143; d) N. H. Greig, X.-F. Pei, T. T. Soncrant, D. K. Ingram, A. Brossi, *Med. Res. Rev.* **1995**, *15*, 3–31; e) Z.-J. Zhan, H.-L. Bian, J.-W. Wang, W.-G. Shan, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1532–1534; f) M. Shinada, F. Narumi, Y. Osada, K. Matsumoto, T. Yoshida, K. Higuchi, T. Kawasaki, H. Tanaka, M. Satoh, *Bioorg. Med. Chem.* **2012**, *20*, 4901–4914; g) J. C. Yi, C. Liu, L. X. Dai, S. L. You, *Chem. Asian J.* **2017**, *12*, 2975–2979; selected publications on flustramine (**5**) and related prenylated compounds: h) J. S. Carle, C. Christophersen, *J. Am. Chem. Soc.* **1979**, *101*, 4012–4013; i) J. S. Carle, C. Christophersen, *J. Org. Chem.* **1980**, *45*, 1586–1589; j) T. Lindel, L. Bräuchle, G. Golz, P. Böhrer, *Org. Lett.* **2007**, *9*, 283–286; k) C. Bunders, J. Cavanagh, C. Melander, *Org. Biomol. Chem.* **2011**, *9*, 5476–5481; l) S. K. Adla, F. Sasse, G. Kelter, H.-H. Fiebig, T. Lindel, *Org. Biomol. Chem.* **2013**, *11*, 6119–6130; m) J. M. Müller, C. B. W. Stark, *Angew. Chem. Int. Ed.* **2016**, *55*, 4798–4802; *Angew. Chem.* **2016**, *128*, 4877–4881; n) H.-F. Tu, X. Zhang, C. Zheng, M. Zhu, S.-L. You, *Nat. Catal.* **2018**, *1*, 601–608; diketopiperazine-based natural products: o) A. D. Borthwick, *Chem. Rev.* **2012**, *112*, 3641–3716; p) H. Wang, S. H. Reisman, *Angew. Chem. Int. Ed.* **2014**, *53*, 6206–6210; *Angew. Chem.* **2014**, *126*, 6320–6324; q) H. Li, Y. Qiu, C. Guo, M. Han, Y. Zhou, Y. Feng, S. Luo, Y. Tong, G. Zheng, S. Zhu, *Chem. Commun.* **2019**, *55*, 8390–8393.

[7] a) H. Schönherr, T. Cernak, *Angew. Chem. Int. Ed.* **2013**, *52*, 12256–12267; *Angew. Chem.* **2013**, *125*, 12480–12492; b) C. Sommer-Kamann, A. Fries, S. Mordhorst, J. N. Andexer, M. Müller, *Angew. Chem. Int. Ed.* **2017**, *56*, 4033–4036; *Angew. Chem.* **2017**, *129*, 4091–4094.

[8] Selected examples (see also ref. [6]): a) D. Liu, G. Zhao, L. Xiang, *Eur. J. Org. Chem.* **2010**, 3975–3984; b) S. Lucarini, F. Bartoccini, F. Battistoni, G. Diamantini, G. Piersanti, M. Righi, G. Spadoni, *Org. Lett.* **2010**, *12*, 3844–3847.

[9] Selected examples (see also ref. [6]): a) M. Kawahara, A. Nishida, M. Nakagawa, *Org. Lett.* **2000**, *2*, 675–678; b) T. Bui, S. Syed, C. F. Barbas III, *J. Am. Chem. Soc.* **2009**, *131*, 8758–8759; c) Y. Zhang, W. Wang, *Catal. Sci. Technol.* **2012**, *2*, 42–53; d) Y. Li, Z. Ding, A. Lei, W. Kong, *Org. Chem. Front.* **2019**, *6*, 3305–3309.

[10] a) J. Zhang, C. Martin, M. A. Shifflet, P. Salmon, T. Brix, R. Greasham, B. Buckland, M. Chartrain, *Appl. Microbiol. Biotechnol.* **1996**, *44*, 568–575; for a recent mutasynthetic approach, see: b) L. Winand, P. Schneider, S. Kruth, N.-J. Greven, W. Hiller, M. Kaiser, J. Pietruszka, M. Nett, *Org. Lett.* **2021**, *23*, 6563–6567.

[11] M. R. Bennett, S. A. Shepherd, V. A. Cronin, J. Micklefield, *Curr. Opin. Chem. Biol.* **2017**, *37*, 97–106.

[12] Q. Sun, M. Huang, Y. Wei, *Acta Pharm. Sin. B* **2021**, *11*, 632–650.

[13] D. G. Fujimori, *Curr. Opin. Chem. Biol.* **2013**, *17*, 597–604.

[14] M. V. C. Greenberg, D. Bourc'his, *Nat. Rev. Mol. Cell. Biol.* **2019**, *20*, 590–607.

[15] Y. Dor, H. Cedar, *Lancet* **2018**, *392*, 777–786.

[16] M. Zhang, J.-Y. Xu, H. Hu, B.-C. Ye, M. Tan, *Proteomics* **2018**, *18*, 1700300.

[17] D. K. Liscombe, G. V. Louie, J. P. Noel, *Nat. Prod. Rep.* **2012**, *29*, 1238–1250.

[18] J. Zhang, J. P. Klinman, *J. Am. Chem. Soc.* **2016**, *138*, 9158–9165.

[19] A.-W. Struck, M. L. Thompson, L. S. Wong, J. Micklefield, *ChemBioChem* **2012**, *13*, 2642–2655.

[20] a) S. Mordhorst, J. Siegrist, M. Müller, M. Richter, J. N. Andexer, *Angew. Chem. Int. Ed.* **2017**, *56*, 4037–4041; *Angew. Chem.* **2017**, *129*, 4095–4099; b) D. Popadić, D. Mhaindarkar, M. H. N. D.

Thai, H. C. Hailes, S. Mordhorst, J. N. Andexer, *RSC Chem. Biol.* **2021**, *2*, 883–891.

[21] C. Liao, F. P. Seebeck, *Nat. Catal.* **2019**, *2*, 696–701.

[22] C. Markl, W. P. Clafshenkel, M. I. Attia, S. Sethi, P. A. Witt-Enderby, D. P. Zlotos, *Arch. Pharm. Chem. Life Sci.* **2011**, *344*, 666–674.

[23] K. Hsiao, H. Zegzouti, S. A. Goueli, *Epigenomics* **2016**, *8*, 321–339.

[24] M. L. Connolly, *J. Am. Chem. Soc.* **1985**, *107*, 1118–1124.

[25] B. David, P. Schneider, P. Schäfer, J. Pietruszka, H. Gohlke, *J. Enzyme Inhib. Med. Chem.* **2021**, *36*, 491–496.

[26] G. L. Ellman, K. D. Courtney, V. Andres, Jr., R. M. Feather-Stone, *Biochem. Pharmacol.* **1961**, *7*, 88–95.