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Tryptophan prenyltransferases showing higher catalytic activities for Friedel–Crafts alkylation of *o*- and *m*-tyrosines than tyrosine prenyltransferases†

Aili Fan,[a] Xiulan Xie[b] and Shu-Ming Li*[a]

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Tryptophan prenyltransferases FgaPT2, 5-DMATS, 6-DMATS$_{Sv}$ and 7-DMATS catalyse regiospecific *C*-prenylations on the indole ring, while tyrosine prenyltransferases SirD and TyrPT catalyse the *O*-prenylation of the phenolic hydroxyl group. In this study, we report the Friedel–Crafts alkylation of L-*o*-tyrosine by these enzymes. Surprisingly, no conversion was detected with SirD and three tryptophan prenyltransferases showed significantly higher activity than another tyrosine prenyltransferase TyrPT. *C*5-prenylated L-*o*-tyrosine was identified as a unique product of these enzymes. Using L-*m*-tyrosine as the prenylation substrate, product formation was only observed with the tryptophan prenyltransferases FgaPT2 and 7-DMATS. *C*4- and *C*6-prenylated derivatives were identified in the reaction mixture of FgaPT2. These results provided additional evidence for the similarities and differences between these two subgroups within the DMATS superfamily in their catalytic behaviours.

Introduction

Prenylated secondary metabolites are widely distributed in nature. The biological and pharmacological activities of prenylated products are usually distinct from their non-prenylated precursors and therefore contribute largely to drug discovery and development programmes.[1–3] Prenyltransferases are involved in the biosynthesis of these natural products and catalyse the regiospecific, in most cases Friedel–Crafts alkylations by transferring prenyl moieties from different prenyl donors to various acceptors. The prenyl moieties with different carbon chain lengths (C5, C10, C15 or C20 units) can be attached in the reverse or regular pattern and further modified by cyclization, oxidation and more. Therefore, prenyltransferases play an important role in the structural diversity of such products.[4–6] These features have drawn considerable attention from scientists in different disciplines to prenyltransferases and prenylated derivatives. Based on their primary sequences, biochemical properties and structures, prenyltransferases can be divided into different subgroups including protein prenyltransferases, prenyl diphosphate synthases and aromatic prenyltransferases.[4,7] In the last few years, significant progress has been achieved for aromatic prenyltransferases, especially for those from the dimethylallyl tryptophan synthase (DMATS) superfamily.[6,8,9] So far, more than 40 such prenyltransferases have been identified and characterised biochemically from microorganisms, especially from fungi and bacteria.[6,8,9] The majority of the DMATS superfamily uses indole derivatives including tryptophan and tryptophan-containing cyclic dipeptides as substrates. In the presence of dimethylallyl diphosphate (DMAPP), for example, FgaPT2,[10] 5-DMATS,[11] 6-DMATS$_{Sv}$ [8] and 7-DMATS[12] from this family catalyse regiospecific prenylations of L-tryptophan (**1a**) at C-4, C-5, C-6 and C-7 of the indole ring, respectively (Fig. 1). FgaPT2 and 7-DMATS from *Aspergillus fumigatus* are involved in the biosynthesis of fumigaclavine C[13] and astechrome,[14] respectively. 6-DMATS$_{Sv}$ is likely to be involved in the biosynthesis of 6-dimethylallylindole-carbaldehyde in *Streptomyces violaceusniger*.[8] A few members of the DMATS superfamily are responsible for the prenylation of non-indole substances. For example, SirD from *Leptosphaeria maculans* catalyses an *O*-prenylation of tyrosine (**2a**) (Fig. 1),[15] the first specific step in the biosynthesis of sirodesmin PL.[16] Last year, a new tyrosine *O*-prenyltransferase TyrPT (Fig. 1) was identified in *Aspergillus niger*, which was demonstrated to have similar functions to SirD.[17] Further study showed that SirD and TyrPT also accepted L-tryptophan (**1a**) as a substrate *in vitro* and catalyse the same *C*7-prenylation as 7-DMATS (Fig. 1).[15,17] Correspondingly, 7-DMATS catalyses

[a]*Institut für Pharmazeutische Biologie und Biotechnologie, Philipps-Universität Marburg, Deutschhausstrasse 17A, 35037 Marburg, Germany. E-mail: shuming.li@staff.uni-marburg.de*
[b]*Fachbereich Chemie, Philipps-Universität Marburg, Hans-Meerwein-Strasse, 35032 Marburg, Germany*

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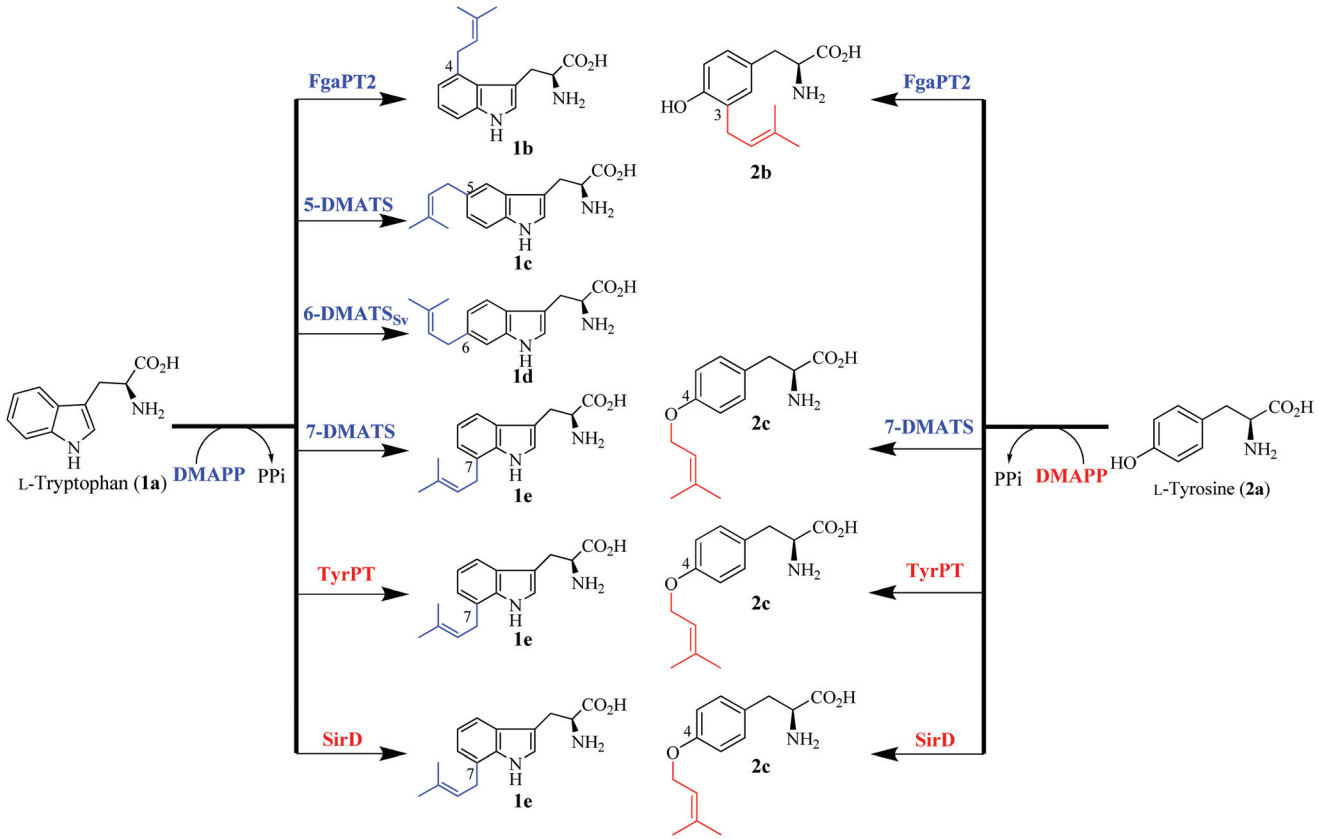


Fig. 1 Selected examples of prenyl transfer reactions catalysed by tryptophan and tyrosine prenyltransferases.

the same *O*-prenylation of tyrosine as SirD and TyrPT (Fig. 1). Very recently, we demonstrated the *C*3-prenylation of L-tyrosine (**2a**) by the tryptophan *C*4-prenyltransferase FgaPT2 (Fig. 1).[18] These results demonstrated the close relationship between tryptophan *C*-prenyltransferases and tyrosine *O*-prenyltransferases. The different regioselectivities of the mentioned enzymes with **2a** encouraged us to test the acceptance of the **2a** isomers, L-*o*-tyrosine (**3a**) and L-*m*-tyrosine (**4a**), by tryptophan and tyrosine prenyltransferases. **3a** and **4a** are important amino acid analogues found in metabolic pathways of human beings and have shown potential for treatments of different diseases.[19–21] Prenylations of these two compounds have not yet been reported and their prenylated derivatives could be interesting candidates for further biological and pharmacological investigations.

Results and discussion

Comparison of the enzyme activities of tryptophan and tyrosine prenyltransferases towards tryptophan (1a) and tyrosine (2a)

For better comparison of their catalytic activities, we carried out incubation of **1a** and **2a** with FgaPT2, 5-DMATS, 6-DMATS$_{Sv}$, 7-DMATS, SirD and TyrPT under the same conditions, *i.e.* 2 μg enzyme for **1a** and 20 μg for **2a**, 1 mM **1a** or **2a**, 5 mM $CaCl_2$ and 1 mM DMAPP in 100 μL reaction mixtures. With the exception of **2a** with 5-DMATS and 6-DMATS$_{Sv}$, HPLC analysis of the reaction mixtures (Fig. 2A–L) revealed the formation of one product each, confirming the results published previously.[17,18,22] Product yields of these reactions are summarized in Table 1. It is obvious that tryptophan prenyltransferases FgaPT2, 5-DMATS, 6-DMATS$_{Sv}$ and 7-DMATS accepted **1a** much better than **2a**. Over 50% conversion yields were observed with **1a** as the substrate by tryptophan prenyltransferases after incubation at 37 °C for 1.5 h (Table 1; Fig. 2A–D). With **2a** as the substrate, product formation was detected only in the reaction mixtures of FgaPT2 and 7-DMATS for tryptophan prenyltransferases, with product yields of 6.2 ± 1.0 and 13.8 ± 1.3%, respectively (Table 1). As expected, **2a** was much better accepted by tyrosine than by tryptophan prenyltransferases. Almost total conversion of **2a** was observed for TyrPT and SirD (Table 1; Fig. 2K and L). Product yields of **1a** with TyrPT and SirD were found to be 5.2 ± 0.40 and 5.9 ± 1.0% (Table 1, Fig. 2), respectively.

Acceptance of L-*o*-tyrosine (3a) by all the tested tryptophan prenyltransferases, but not by the tyrosine prenyltransferase SirD

In the presence of 1 mM DMAPP, L-*o*-tyrosine (**3a**) was incubated with the six prenyltransferases (20 μg in 100 μL assay)

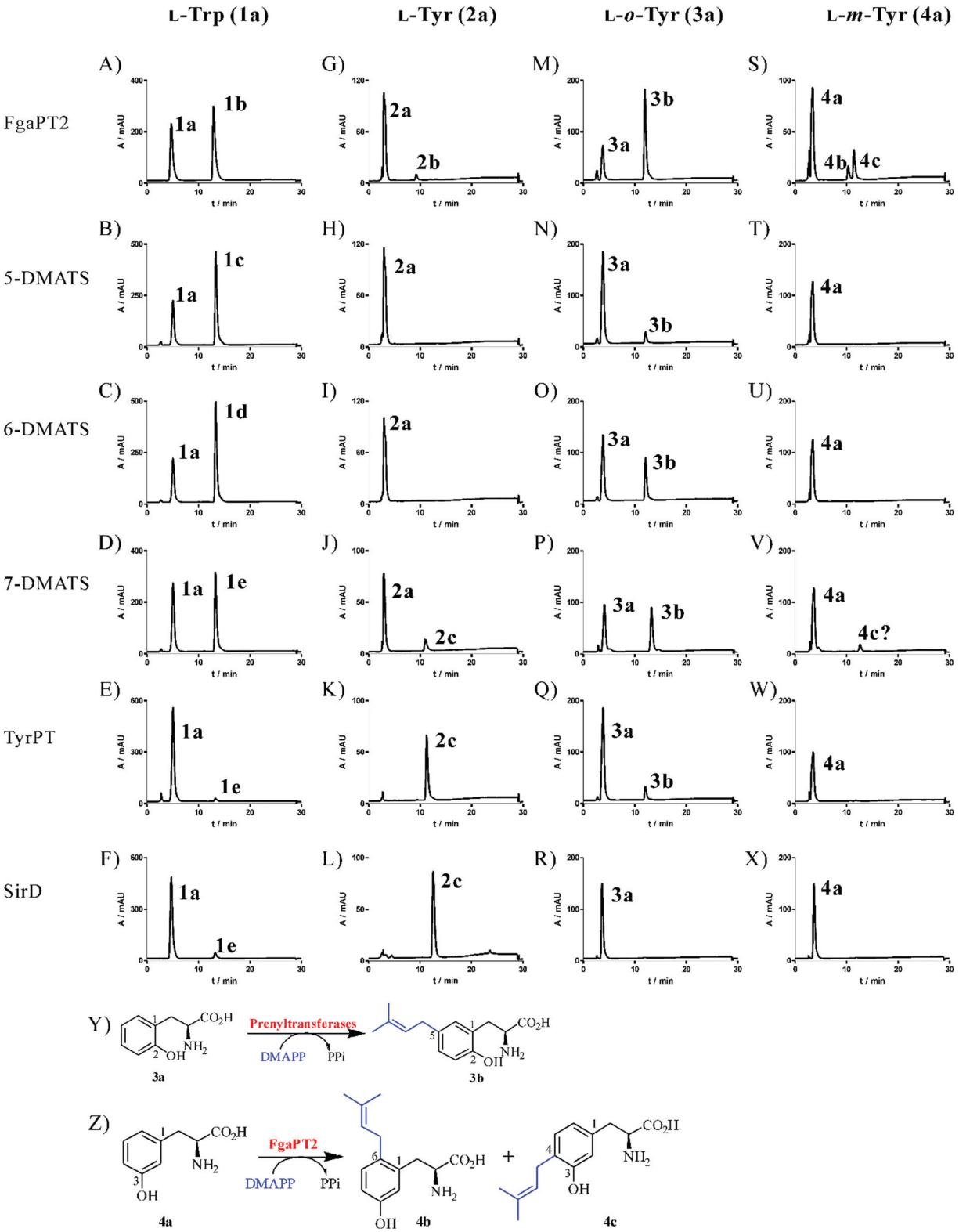


Fig. 2 HPLC analysis of the reaction mixtures of **1a**–**4a** with tryptophan and tyrosine prenyltransferases and the enzyme reactions of **3a** and **4a**. The assays contained 1 mM aromatic substrate, 5 mM $CaCl_2$, 1 mM DMAPP, and 2 (for **1a**) or 20 (for **2a**–**4a**) μg purified recombinant protein and were incubated at 37 °C for 1.5 h. Results are illustrated for absorption at 277 nm.

Table 1 Prenylation positions and product yields [%] of enzyme reactions

	1a		2a		3a		4a	
	Prenylated position[a]	Product yield [%]	Prenylated position[a]	Product yield [%]	Prenylated position[a]	Product yield [%]	Prenylated position[a]	Product yield [%]
FgaPT2	C-4	51.2 ± 4.6	C-3	6.2 ± 1.0	C-5	73.6 ± 3.6	C-4; C-6	33.4 ± 1.4
5-DMATS	C-5	62.1 ± 2.8	—	≤0.5	C-5	10.9 ± 2.5	—	≤0.5
6-DMATS$_{Sv}$	C-6	63.6 ± 2.9	—	≤0.5	C-5	29.9 ± 2.5	—	≤0.5
7-DMATS	C-7	52.1 ± 1.9	O-4	13.8 ± 1.3	C-5	40.2 ± 3.8	—	4.8 ± 1.6
TyrPT	C-7	5.2 ± 0.40	O-4	100	C-5	10.8 ± 0.7	—	≤0.5
SirD	C-7	5.9 ± 1.0	O-4	100	C-5	≤0.5	—	≤0.5

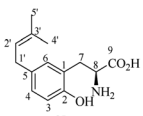
[a] For structures see Fig. 1; "—" not determined.

mentioned in Fig. 2 at 37 °C for 1.5 h. Subsequent HPLC analysis revealed product formation in five reaction mixtures (Fig. 2M–Q). Surprisingly, **3a** was accepted by all the tested tryptophan prenyltransferases, but not by the tyrosine prenyltransferase SirD (Fig. 2R), although TyrPT and SirD shared nearly the same behaviour towards the diverse substrates tested before.[17] Furthermore, the tryptophan prenyltransferases FgaPT2, 6-DMATS$_{Sv}$ and 7-DMATS with product yields of 73.6 ± 3.6, 29.9 ± 2.5 and 40.2 ± 3.8%, respectively, showed significantly higher activities than TyrPT with a product yield of 10.8 ± 0.7%, which is comparable to that of 5-DMATS. The product peaks detected in these assays display the same retention time, indicating the formation of an identical enzyme product by different prenyltransferases.

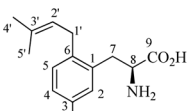
*C*5-prenylated *o*-tyrosine (3b) as the unique product of tyrosine and tryptophan prenyltransferases

To elucidate their structures, the enzyme products of **3a** with FgaPT2, 5-DMATS, 6-DMATS$_{Sv}$, 7-DMATS and TyrPT were isolated on HPLC from 5–10 ml of incubation mixtures and subjected to NMR (Table 2, Fig. S1 in the ESI†) and HR-MS analyses (Experimental section). HR-MS data confirmed the monoprenylation of the isolated products by detection of molecular masses, which are 68 Da larger than that of **3a**. ^{1}H-NMR data proved that FgaPT2, 5-DMATS, 6-DMATS$_{Sv}$, 7-DMATS and TyrPT converted indeed **3a** to the same product **3b**. Inspection of the ^{1}H-NMR spectra (Fig. S1 in the ESI†) of **3b** revealed the presence of signals for a regular prenyl moiety at δ_H 3.21 (d, H-1′), 5.25 (tsept, H-2′), 1.70 (d, H-4′) and 1.69 ppm (br s, H-5′). Furthermore, signals for three aromatic protons indicated that the prenylation took place on the aromatic ring, rather than at the hydroxyl group. The signals of the aromatic protons at δ_H 6.76 (d, 8.0 Hz), 6.91 (dd, 8.0, 2.0 Hz) and 6.99 (d, 2.0 Hz) of **3b** proved the prenylation at C-4 or C-5 of **3a** (Table 2). For the *C*5-prenylated **3a**, it is expected that the signal of H-3 should be found in the high-field with a coupling constant of approximately 8.0 Hz and the signal of H-6 in the low-field with a coupling constant of 2.0 Hz. The obtained data corresponded very well to this prediction and therefore confirmed **3b** to be the *C*5-prenylated product (Fig. 2Y). These results sound somewhat surprising, because an *O*-prenylated derivative was identified in the reaction mixtures of L-tyrosine (**2a**) with 7-DMATS and TyrPT.[17,22] However, the *C*3-prenylated derivative was the enzyme product of FgaPT2 with **2a** (Fig. 1).[18]

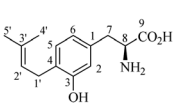
Table 2 ^{1}H-NMR and ^{13}C-NMR data of the enzyme products (500 MHz)



3b



4b



4c

Pos	δ_H, multi, *J*	δ_H, multi, *J*	δ_C	δ_H, multi, *J*
1	—	—	134.9	—
2	—	6.69, d, 2.5	116.4	6.69, s
3	6.76, d, 8.0	—	155.4	—
4	6.91, dd, 8.0, 2.0	6.64, dd, 8.0, 2.5	114.1	—
5	—	7.00, d, 8.0	130.6	7.00, d, 7.5
6	6.99, d, 2.0	—	131.2	6.67, d, 7.5
7	Approx. 3.30[a]	3.42, dd, 15.0, 4.5	34.4	3.19, dd, 14.0, 3.0
	2.95, dd, 14.3, 8.5	2.80, dd, 15.0, 10.5		2.86, dd, 14.0, 9.3
8	3.85, dd, 8.5, 4.0	3.72, dd, 10.5, 4.5	55.5	3.69, m
9	—	—	172.6	—
1′	3.21, d, 7.0	3.33, d, 7.0	30.0	3.24, d, 7.5
2′	5.25, tsept, 7.5, 1.5	5.19, br t, 7.0	123.4	5.28, br t, 7.5
3′	—	—	131.3	—
4′	1.70, d, 1.0	1.73, s	16.6	1.70, d, 1.0
5′	1.69, br s	1.72, s	24.4	1.69, s
	MeOH-d_4	MeOH-d_4	MeOH-d_4	MeOH-d_4

[a] Signals overlapping with those of solvent.

Acceptance of L-*m*-tyrosine (4a) by two tryptophan prenyltransferases and identification of *C*4- and *C*6-prenylated derivatives as products of the FgaPT2 reaction

After the identification of the *C*5-prenylated **3a** from reaction mixtures of tyrosine and tryptophan prenyltransferases, we tested the behaviour of these enzymes towards L-*m*-tyrosine

(**4a**) under the same conditions as for **3a**. As shown in Fig. 2S–X, product formation was only observed in the reaction mixtures of FgaPT2 and 7-DMATS, with product yields of 33.4 ± 1.4 and 4.8 ± 1.6% (Table 1), respectively. Unexpectedly, **4a** was not accepted by the two tyrosine prenyltransferases SirD and TyrPT (Fig. 2W and X). Another surprise is the much better acceptance of **4a** by FgaPT2 than by 7-DMATS (Fig. 2S and V), which differs clearly from the preference of **2a** towards these two enzymes (Fig. 2G and J). It seems that the positions of the hydroxyl groups at the benzene ring of phenylalanine have critical influence on their acceptance by tryptophan and tyrosine prenyltransferases.

Inspection of the HPLC chromatograms in Fig. 2S and V revealed the presence of two product peaks **4b** and **4c** with a ratio of 1 : 2 in the reaction mixture of FgaPT2 at 10.3 and 11.4 min and only one in that of 7-DMATS at 11.4 min. The UV maxima of **4b** at 220 and 283 nm differed slightly from those of **4c** at 220 and 278 nm. For structure elucidation, **4b** and **4c** were isolated on HPLC from the incubation mixture of **4a** with FgaPT2 and subjected to NMR (Table 2; Fig. S2–S5 in the ESI†) and HR-MS analyses (Experimental section). HR-MS data confirmed the monoprenylation of the isolated products. Inspection of the ^{1}H-NMR spectra (Table 2) of **4b** and **4c** revealed the presence of signals for a regular prenyl moiety each at δ_H 3.33 or 3.24 (d, H-1′), 5.19 or 5.28 (br t H-2′), 1.73 or 1.70 (s H-4′) and 1.72 or 1.69 ppm (s, H-5′). The signals of the aromatic protons at 6.69 (d, 2.5 Hz, H-2), 6.64 (dd, 8.0, 2.5 Hz, H-4), and 7.00 (d, 8.0 Hz, H-5) of **4b** and 6.69 (s, H-2), 7.00 (d, 7.5 Hz, H-5), and 6.67 (d, 7.5 Hz, H-6) of **4c** indicated the prenylation at C-4 in one case and C-6 in another case. To determine the structures of **4b** and **4c**, we recorded HSQC and HMBC spectra for **4b** (Fig. 3, Fig. S3 and S4 in the ESI†). The important correlation between H-1′ and C-1 prove unequivocally that **4b** is the *C*6-prenylated derivative, *i.e.* prenylation at the *para*-position to the phenolic hydroxyl group (Fig. 2Z). Consequently, **4c** is the *C*4-prenylated product (Fig. 2Z). Due to the low quality, the structure of the enzyme product of **4a** with 7-DMATS could not be elucidated in this study. However, from its same retention time and UV absorption maxima with those of **4c**, it could be speculated that this substance has the same structure as **4c**.

Our results mentioned above suggest that the acceptance of a substrate by tryptophan or tyrosine prenyltransferases would not strictly depend on the chemical category of the aromatic nucleus, but strongly rely on the position of the functional groups on the aromatic nucleus, which perform the direct interaction with the enzyme active sites. Furthermore, these results reveal that tryptophan and tyrosine prenyltransferases possess very likely similar active sites and strengthen their close relationship in the evolution.

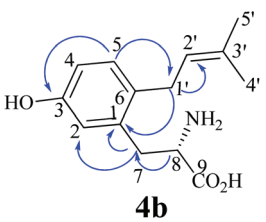


Fig. 3 Important HMBC correlations of **4b**.

Kinetic study of the enzymatic Friedel–Crafts reactions

To get insights into the catalytic efficiency, kinetic parameters were determined for FgaPT2, 5-DMATS, 6-DMATS$_{Sv}$, 7-DMATS and TyrPT towards **3a** and FgaPT2 towards **4a** (Table 3; Fig. S6–S11 in the ESI†). As expected for unnatural substrates, K_M values between 0.17 ± 0.0076 and 0.58 ± 0.081 mM were determined for **3a**, much higher than for their natural substrates.[8,10–12,17] The K_M value of FgaPT2 towards **4a** was found to be 0.90 ± 0.013 mM, higher than that with **3a** (Table 3). In agreement with the product yields given in Table 1, FgaPT2 showed the highest turnover number and catalytic efficiency toward **3a**, followed by 7-DMATS and 6-DMATS$_{Sv}$. TyrPT and 5-DMATS demonstrated comparable kinetic parameters for their reactions with **3a** (Table 3).

Table 3 Kinetic parameters of the tested enzymes for **3a** and **4a**

	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M (s^{-1} M^{-1})
L-*o*-Tyrosine (**3a**)			
FgaPT2	0.47 ± 0.0024	0.29 ± 0.029	600 ± 58
5-DMATS	0.49 ± 0.0031	0.013 ± 0.00011	27 ± 0.40
6-DMATS$_{Sv}$	0.58 ± 0.081	0.034 ± 0.00038	59 ± 3.6
7-DMATS	0.17 ± 0.0076	0.045 ± 0.036	270 ± 8.9
TyrPT	0.58 ± 0.014	0.014 ± 0.00066	24 ± 1.7
L-*m*-Tyrosine (**4a**)			
FgaPT2	0.90 ± 0.013	0.0065 ± 0.00044	6.8 ± 0.093

Proposed reaction mechanisms of Friedel–Crafts alkylation of *o*- and *m*-tyrosine

Previous studies on structures of several prenyltransferases and mutagenesis experiments have shown that the prenyl transfer reactions catalysed by the prenyltransferases of the DMATS superfamily are initiated by the formation of a dimethylallyl carbocation.[23–26] Nucleophilic attack of this ion by an electron-rich aromatic ring leads to the formation of non-aromatic intermediates, which will be rearomatized by elimination of a proton and result in the formation of the prenylated derivatives. A similar mechanism could also be proposed for reactions observed in this study. As shown in Fig. 4, attack of the dimethylallyl carbocation by C-5 of **3a** would result in the formation of the intermediate I, which would undergo proton elimination to form the final product **3b**. For the FgaPT2 reaction with **4a**, attack from two positions, C-6 and C-4, would be possible. It can be expected that two different intermediates II and III will be formed *via* routes A and B, respectively, and then converted to **4b** and **4c** after proton elimination (Fig. 4).

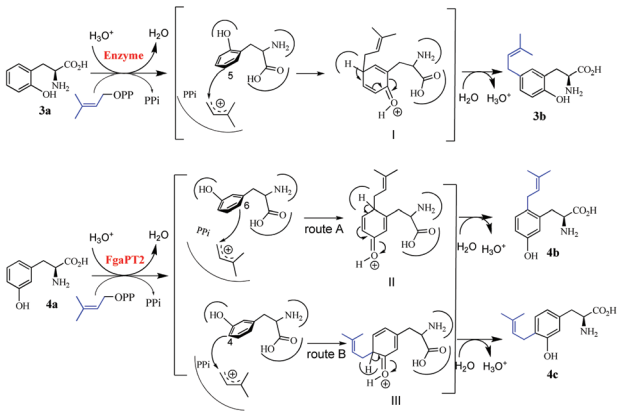


Fig. 4 Proposed reaction mechanisms for the formation of **3b** from **3a** as well as **4b** and **4c** from **4a**.

Experimental section

Chemicals

DMAPP was synthesized according to the method described for geranyl diphosphate (GPP) reported previously.[27] L-*o*-Tyrosine and L-*m*-tyrosine used for the enzyme assays were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and Alfa Aesar (Karlsruhe, Germany), respectively.

Overexpression and purification of recombinant proteins

Protein overproduction and purification were carried out for FgaPT2,[10] 5-DMATS,[11] 6-DMATS$_{Sv}$,[8] 7-DMATS,[12] SirD[15] and TyrPT[17] as described previously.

Assays for determination of enzyme activities

Reaction mixtures (100 μl) for determination of the enzyme activities contained an aromatic substrate (1 mM), $CaCl_2$ (5 mM), DMAPP (1 mM), glycerol (1.0–6.0% v/v), dimethyl sulfoxide (DMSO, 0–5.0% v/v), 50 mM Tris-HCl (pH 7.5) and a purified recombinant protein (2 or 20 μg). The reaction mixtures were incubated at 37 °C for 1.5 h and then terminated by the addition of 100 μl MeOH. Protein was removed by centrifugation at 17 000 rpm for 20 min.

Enzyme assays for product isolation and structure elucidation

Assays for isolation of the enzyme products were carried out in large scales (5–10 ml) containing an aromatic substrate (1 mM), DMAPP (1.5 mM), $CaCl_2$ (5 mM), glycerol (1.0–9.9% v/v), DMSO (0–5.0% v/v), 50 mM Tris-HCl (pH 7.5) and a recombinant protein (0.2–0.6 mg per ml assay). After incubation for 16 h at 37 °C, the reaction mixtures were terminated by the addition of 5–10 ml methanol. After removal of the precipitated protein by centrifugation at 6000 rpm for 30 min, the reaction mixtures were concentrated in a rotating vacuum evaporator at 35 °C to a final volume of 1 ml before injection into HPLC.

Enzyme assays for determination of the kinetic parameters

Assays for determination of the kinetic parameters contained $CaCl_2$ (5 mM), glycerol (1.0–9.9% v/v), DMSO (0–5.0% v/v), 50 mM Tris HCl (pH 7.5), DMAPP (1 mM) and an aromatic substrate at final concentrations up to 2.0 mM. The reactions were then terminated with 100 μl MeOH. Protein was removed by centrifugation at 17 000 rpm for 20 min.

HPLC analysis and isolation of enzyme products for structure elucidation

The enzyme products were routinely analysed by HPLC on an Agilent series 1200 by using a Multospher 120 RP-18 column (250 × 4 mm, 5 μm C + S Chromatographie Service, Langerwehe, Germany) at a flow rate of 1 ml min^{-1}. Water (solvent A) and methanol (solvent B) were used as solvents for analysis and isolation of the enzyme products. A linear gradient of 30–100% (v/v) solvent B in 20 min was used for analysis of the enzymatic products. The column was then washed with 100% solvent B for 5 min and equilibrated with 30% solvent B for another 5 min. Detection was carried out on a photodiode array detector.

For isolation of the enzyme products, the same HPLC equipment with a Multospher 120 RP-18 column (250 × 10 mm, 5 μm, C + S Chromatographie Service, Langerwehe, Germany) was used. A linear gradient was run with 50–100% (v/v) of methanol (solvent B) in water (solvent A) in 50–80 min and a flow rate at 2.5 ml min^{-1}. The column was then washed with 100% (v/v) solvent B for 10 min and equilibrated with 50% (v/v) solvent B for 10 min.

HR-ESI-MS and NMR analysis of the enzyme products

The obtained products were analysed by NMR (in CD_3OD) and MS analyses. NMR spectra were recorded at room temperature on a Bruker Avance 600 MHz or a JEOL ECA-500 spectrometer. The heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) spectra were recorded with standard methods[28] and processed with MestReNova.5.2.2. The positive HR-ESI-MS was carried out on an AutoSpec instrument (Micromass Co. UK Ltd) with the following results:

3b: *m*/*z* 249.1374 $[M]^+$ ($C_{14}H_{19}NO_3$, cal.: 249.1365); **4b**: *m*/*z* 249.1373 $[M]^+$ ($C_{14}H_{19}NO_3$, cal.: 249.1365); **4c**: *m*/*z* 249.1348 $[M]^+$ ($C_{14}H_{19}NO_3$, cal.: 249.1365).

Conclusions

Prenyltransferases of the DMATS superfamily are known for their broad aromatic substrates.[6] As shown in Fig. 2, tyrosine prenyltransferases TyrPT and SirD also accepted tryptophan as the substrate, but with lower activity than with tyrosine. Correspondingly, tryptophan prenyltransferases FgaPT2 and 7-DMATS also prenylated tyrosine, but with lower activity than for tryptophan. In this study, we demonstrated surprisingly that L-*o*-tyrosine (**3a**) was accepted by all of the tested tryptophan prenyltransferases for prenylations at C-4, C-5, C-6 and

C-7 of the indole ring, but not by the tyrosine prenyltransferase SirD. The unique product *C*5-prenylated *o*-tyrosine was identified in all the reaction mixtures of FgaPT2, 5-DMATS, 6-DMATS$_{Sv}$, 7-DMATS and TyrPT, although these enzymes utilized different natural substrates and catalysed diverse regioselective reactions.

L-*m*-Tyrosine (**4a**) was only converted by two tryptophan prenyltransferases, but by none of the tyrosine prenyltransferases. Structure elucidation of the enzyme products of **4a** with FgaPT2 revealed a low regioselectivity. *C*4- and *C*6-prenylated *m*-tyrosines with a ratio of approximately 2 : 1 were identified as the reaction products.

Comparison studies in the future on the structure level of these two groups of prenyltransferases could provide detailed insights into the difference and similarity of their substrate and catalytic promiscuities. Meanwhile, it could even provide suggestions for protein engineering to create new biocatalysts in order to meet a given target, such as broader substrate spectra and/or different regioselectivity.[29,30]

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