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Breaking Cyclic Dipeptide Prenyltransferase Regioselectivity by Unnatural Alkyl Donors

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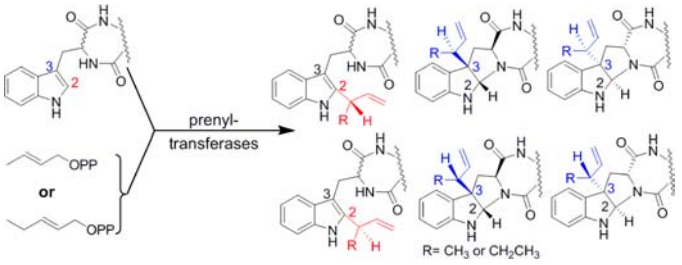
ABSTRACT



The behavior of five cyclic dipeptide prenyltransferases, responsible for C2-regular, C2-reverse, or C3-reverse prenylation, was investigated in the presence of the unnatural alkyl donors monomethylallyl and 2-pentenyl diphosphate. Both substrates were well accepted by the tested enzymes. Interestingly, C2-reverse and C3-reverse monoalkylated derivatives were identified as enzyme products in all of the enzyme assays. These findings indicate their similar reaction characteristics in the presence of unnatural alkyl donors.

Enzymes of the dimethylallyl tryptophan synthase (DMATS) superfamily represent a versatile class of aromatic prenyltransferases. They are involved in the biosynthesis of biologically active natural products in fungi and can be used as biocatalysts for chemoenzymatic synthesis.[1] A large number of these prenyltransferases usually catalyze transfer reactions of a dimethylallyl moiety from dimethylallyl diphosphate (DMAPP) onto tryptophan or tryptophan-containing dipeptides regiospecifically at one of the seven positions of the indole moiety.[1a,c] Furthermore, as in the case of FtmPT1, prenylation can occur in a regular or reverse manner, as for other enzymes illustrated in Scheme 1.[2] Most of these enzymes show significant flexibility for their aromatic substrates but a strict substrate specificity toward their prenyl donors. They usually accept solely DMAPP but not geranyl diphosphate (GPP).[1a,c,2] Only one known enzyme from the DMATS superfamily, i.e., VrtC, utilizes GPP but not DMAPP as prenyl donor.[3] We have recently demonstrated that two L-tryptophan prenyltransferases, FgaPT2 and 5-DMATS, are able to use the unnatural alkyl donors monomethylallyl diphosphate (MAPP, **1**, Figure 1) and 2-pentenyl diphosphate (2-pen-PP, **2**, Figure 1), which contain a double bond in the β-position to pyrophosphate (PPi).[4] Partial or total shift of the alkylation positions was observed in that study. The acceptance of **1** and **2** by FgaPT2 and 5-DMATS as well as alkylation at different positions significantly expands the potential uses of these enzymes as biocatalysts for chemoenzymatic synthesis. The results obtained in that study encouraged us to learn more about the acceptance of these compounds by other

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members of the DMATS superfamily, e.g., by cyclic dipeptide prenyltransferases.

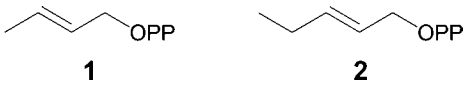


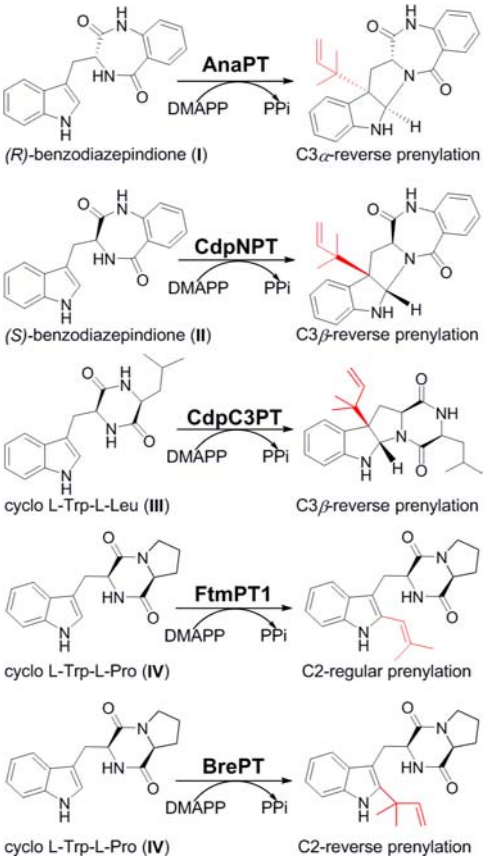
Figure 1. Unnatural DMAPP analogues used as alkyl donors.

For this purpose, we first chose the three C3-prenyltransferases, AnaPT, CdpNPT, and CdpC3PT (Scheme 1), and tested the acceptance of **1** and **2** in the presence of their natural or best accepted aromatic substrates, i.e., (*R*)-benzodiazepindione (**I**), (*S*)-benzodiazepindione (**II**), and *cyclo*-L-Trp-L-Leu (**III**), respectively.[2b−d] HPLC chromatography on both silical gel and reverse-phase C-18 (RP18) columns of the incubation mixtures with 20 μg (AnaPT) or 50 μg of protein (CdpNPT and CdpC3PT) for 16 h showed that both alkyl donors were well accepted by the three tested enzymes. Up to six product peaks, with two to three dominant ones, were detected in the HPLC chromatograms. Total product yields from 32.3% to 98.8% and from 35.5% to 96.5% were calculated for **1** and **2**, respectively. For structure elucidation, we carried out large-scale enzyme incubations and isolated the enzyme products by repeated chromatography, if necessary, on HPLC with RP18 and silical gel columns. The structures of the purified products were elucidated by NMR and MS analyses. For better understanding and comparison, we named the enzyme products by using the number of aromatic substrate (**I**, **II**, or **III**) and alkyl donor (**1** or **2**) as well as the alkylation positions (**a** and **b** for C2- and **c** and **d** for C3-alkylated products). The letters **a** and **b** as well as **c** and **d** denote also the different configurations at C3′ of the alkyl moieties.

In the presence of the natural prenyl donor DMAPP, AnaPT catalyzes α-prenylation at C3 of **I** and other tryptophan-containing cyclic dipeptides.[2b,5] In contrast, CdpNPT and CdpC3PT catalyze β-prenylations at the same position of these compounds.[2c,d]

From incubation mixtures of AnaPT with **I** and **1**, three products, **I-1a**, **I-1c**, and **I-1d**, were isolated and determined as C2-reversely and C3-reversely alkylated derivatives. Both **I-1c** and **I-1d** have an α-alkylation at C3, i.e., *syn-cis* configuration of the fused indoline−diketopiperazine system, as in the case of the product with DMAPP. They differ from each other by their configurations at C3′ of the alkyl moiety, which were determined by HSQC, HMBC, and NOESY experiments. Due to the free rotation of multiple C−C bonds, the configuration at C3′ in **I-1a** cannot be determined in this study. Interestingly, the C2-alkylated **I-1a** was the dominant enzyme product (65.2%) of the C3-prenyltransferase AnaPT, at nearly 2-fold the combined yield of **I-1c** (14.4%) and **I-1d** (19.4%). Obviously, the regioselectivity of AnaPT was disrupted by the unnatural alkyl donor **1**. A similar phenomenon was also observed in the presence of the larger alkyl donor **2**. Two products were identified in the enzyme assay of **I** with **2**. This is consistent with the observed higher flexibility of FgaPT2 and 5-DMATS toward **1** than **2** regarding the attack possibility.[4] In comparison to the assay with **1**, the C3-alkylated derivative **I-2d** is the main product in the assay with **2**, and the yield of C2-alkylated **I-2a** was approximately 42% of that of **I-2d**.

Scheme 1. Enzyme Reactions with the Natural Alkyl Donor



From enzyme assays of CdpNPT with **II** in the presence of **1** and **2**, two product peaks each were obtained after isolation on HPLC. Structure elucidation of the isolated peaks revealed that they contained two C2- and two C3-alkylated derivatives, respectively. Unfortunately, the two C2-alkylated derivatives **II-1a** and **II-1b**, or **II-2a** and **II-2b**, cannot be separated from each other, and their structures were elucidated as a mixture. The ratios of the product pairs given in Scheme 2 were calculated from integrals in their ^{1}H NMR spectra.

By comparing their ^{1}H NMR spectra, **II-1a** was unequivocally elucidated as the enantiomer of **I-1a**, and **II-1b** was proven to be a diastereomer of **II-1a**. The ^{1}H NMR spectra of **II-1c** and **II-1d** overlapped almost completely with those of **I-1c** and **I-1d**, respectively, proving that **II-1c** and **II-1d** are enantiomers of **I-1c** and **I-1d**, respectively, and

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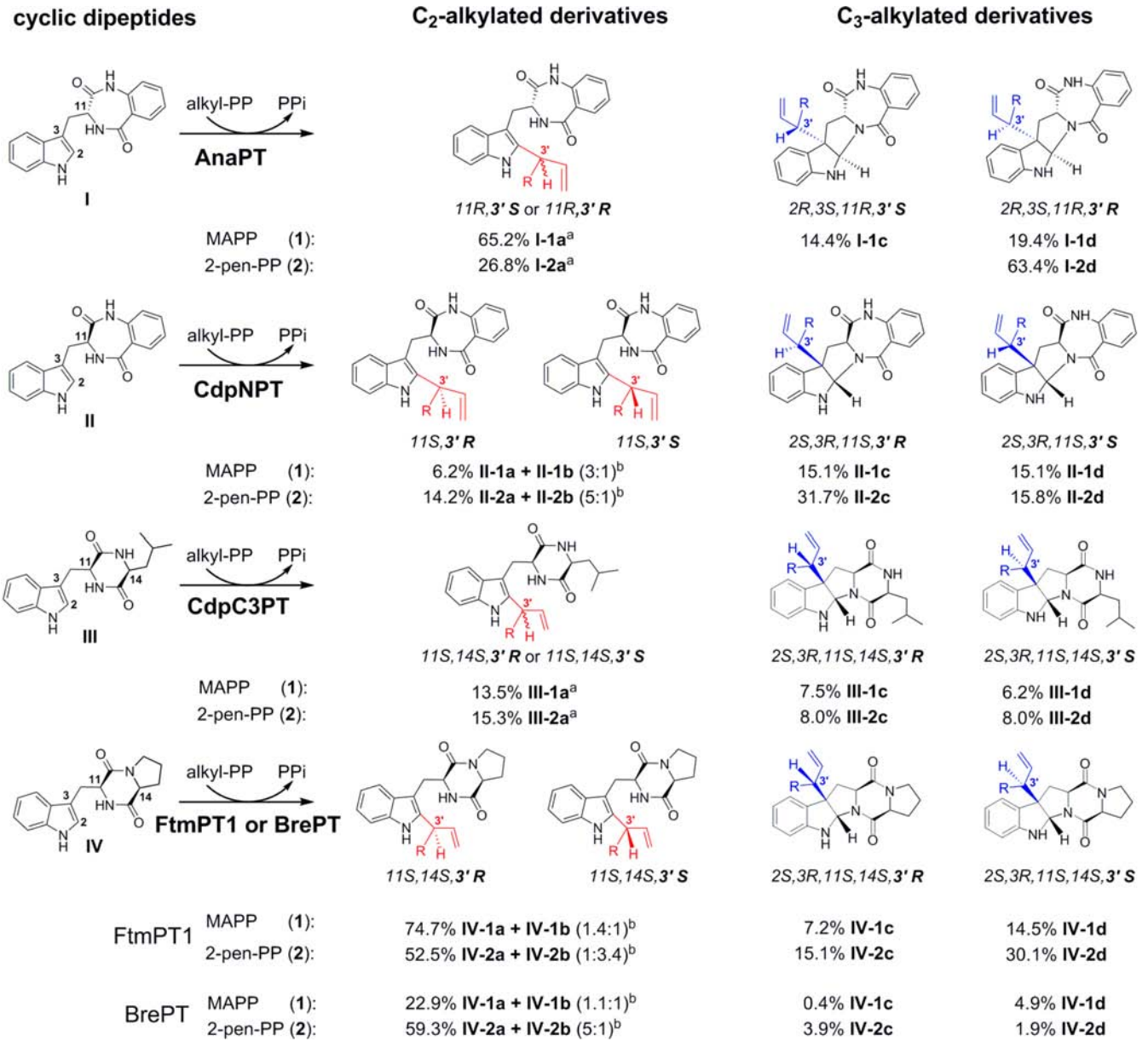
Scheme 2. C2- and C3-Alkylated Derivatives from Enzyme Assays of AnaPT, CdpNPT, CdpC3PT, FtmPT1, and BrePT with **1** and **2** (R = CH_3, MAPP; CH_2CH_3, 2-pen-PP)



[a] Configuration at C3′ not determinable. [b] Assignment of stereoisomer **a** or **b** to the structures not possible.

therefore carry an alkyl moiety at the β-position. The structures of **II-2a**–**II-2d** were elucidated by comparison of the NMR spectra with those of **I-2a** and **I-2d**, respectively. In the assay of CdpNPT with **II** and **1**, the product yield of C2-alkylated derivatives is about 20% of that of C3-alkylated ones (Scheme 2). Using **2** instead of **1** as alkyl donor led to higher conversion yields, especially for C2-alkalyted derivatives, which reached 30% of that of C3-alkylated derivatives.

The third C3-prenyltransferase, CdpC3PT, like CdpNPT, also catalyzed β-prenylation of tryptophan-containing cyclic dipeptides in the presence of its natural alkyl donor, DMAPP (Scheme 1).[2d] Using **1** and **2** as alkyl donors, CdpC3PT was assayed in the presence of its best reported aromatic substrate, *cyclo*-L-Trp-L-Leu (**III**). One C2-reversely alkylated derivative each, **III-1a** or **III-2a**, and two C3-reversely β-alkylated derivatives, **III-1c**/**III-1d** or **III-2c**/**III-2d**, were identified in the reaction mixtures of **1** and **2**, respectively. In both assays, the product yield for the C2-alkylated derivative is nearly identical to that of the C3-alkylated derivatives together.

Taken together, AnaPT, CdpNPT, and CdpC3PT usually catalyze, in the presence of DMAPP, regioselective reverse prenylations at C3 of tryptophan-containing cyclic dipeptides.[2b–d] However, in the presence of the unnatural alkyl donors **1** and **2**, they catalyze both C2- and C3-reverse alkylations. The ratio of C2- to C3-alkylated derivatives was strongly dependent on the enzyme and substrates used

and varied from 1:5 in the reaction mixture of CdpNPT with **II** and **1** to 2:1 in that of AnaPT with **I** and **1**.

Identification of C2- and C3-alkylated products by three C3-prenyltransferases with **1** and **2** raised a challenge to investigate the behavior of C2-prenyltransferases. For this purpose, we used FtmPT1 and BrePT, which catalyze transfer reactions of a regular and a reverse prenyl moiety onto C2 of *cyclo*-L-Trp-L-Pro (**IV**; Scheme 1), respectively. HPLC analysis revealed the presence of the same product peaks in the reaction mixtures of FtmPT1 and BrePT with **1**, although the peak intensities differed. This phenomenon was also observed for the two enzyme assays with **2**. Product yields of 96.4% and 97.7% were calculated from enzyme assays with 20 μg of FtmPT1 for 16 h with **1** and **2**. From BrePT assays (50 μg, 16 h), the values were determined to be 69.7% for **1** and 32.2% for **2**.

Interpretation and comparison of ^{1}H NMR spectra with those of C2- and C3-prenylated derivatives[2d,e,6] proved indeed the presence of the same products in the corresponding enzyme assays of FtmPT1 and BrePT, i.e., two reversely C2- and two reversely C3-alkylated derivatives in each assay. This means that both C2-prenyltransferases catalyzed also C3-alkylation in the presence of the unnatural alkyl donors **1** and **2**. Interestingly, only reversely C2-alkylated derivatives were identified, even in the assays of the regular C2-prenyltransferase FtmPT1. It seems that the unnatural alkyl donors are less bulky and therefore more flexible than DMAPP. Thus, they can be easily reached by C2 and C3 of the indole ring. The reverse alkylations are likely the favored reactions. The total yield of C3-alkylated derivatives **IV-1c** and **IV-1d** in the assay of FtmPT1 with **1** reached nearly 30% of that of the C2-alkylated **IV-1a** and **IV-1b**. In the assay of FtmPT1 with **2**, the product yield of **IV-2c** and **IV-2d** was even 86% of that of **IV-2a** and **IV-2b**. The ratios of C3-alkylated to C2-alkylated derivatives were found to be 1:4 for BrePT with **1**, and 1:10 for BrePT with **2**.

Parallel alkylation at both the C2 and C3 positions of the indole ring by five prenyltransferases in the presence of unnatural alkyl donors breaks the regioselectivity of these enzymes. It seems that **1** and **2** were placed in the active sites of the tested enzymes in such positions, which allowed alkylations at both positions C2 and C3. Even in the presence of DMAPP, both C2 and C3 of **IV** are within a possible distance for prenylation. However, a regularly C2-prenylated intermediate would be more stable.[6] Breaking FtmPT1 regioselectivity was also observed for DMAPP with poorly accepted cyclic dipeptides.[7]

To learn more about the behavior of the five prenyltransferases in the presence of **1** and **2**, kinetic parameters were determined and calculated from Hanes–Woolf and Eadie–Hofstee transformations (Table 1). The observed reactions apparently followed Michaelis–Menten kinetics. In comparison to DMAPP, both **1** and **2** were comparably or even better accepted by the tested enzymes. However, the turnover numbers for **1** and **2** were much lower than those of DMAPP. For a given enzyme, no significant difference was observed between turnover numbers of **1** and **2**.

Table 1. Kinetic Parameters of the Tested Enzymes toward Their Alkyl Donors[a]

	DMAPP		MAPP (**1**)		2-pen-PP (**2**)	
	K_M	k_{cat}	K_M	k_{cat}	K_M	k_{cat}
AnaPT	156	1.5	52	0.020	80	0.011
CdpNPT	–	–	446	0.004	339	0.009
CdpC3PT	1400	0.098	1432	0.002	997	0.004
FtmPT1	74	5.57	49	0.007	63	0.015
BrePT	98	0.276	68	0.001	72	0.002

[a] K_M and k_{cat} values are in μM and s^{-1}, respectively. Data of DMAPP are from refs 2a,2b,2d,2e.

In conclusion, cyclic dipeptide prenyltransferases of the DMATS superfamily also accept unnatural DMAPP analogues and catalyze Friedel–Crafts alkylation. In comparison to the partial or total shift of alkylation position observed for the tryptophan prenyltransferases FgaPT2 and 5-DMATS with **1** and **2** in a previous study,[4] the regioselectivity was lost completely in the reactions of the cyclic dipeptide prenyltransferases. Regardless of the prenyltransferase, both C2- and C3-alkylated products were detected. The ratios of the two C2- to the two C3-stereoisomers and those of the C2- to C3-alkylated products strongly depend on the enzyme and alkyl donor used. Moreover, all main products exhibit substitution patterns of a reverse alkylation. Finally, the alkyl moieties at C3 have the same orientation as in the products with DMAPP.

The results presented in this study provide evidence for the fact that the members of the DMATS superfamily share not only structural similarity[2c,6] but also the ability to catalyze the same reactions, at least for the cyclic dipeptide prenyltransferases tested in this study. This feature can be considered as an important prerequisite for convenient creation of desired enzyme derivatives by site-directed mutagenesis.

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Supporting Information Available. Experimental procedures, determination of kinetic parameters, HR-MS, and detailed NMR data as well as NMR spectra. This material is available free of charge via Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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