Electronic Supplementary Information (ESI)

Switching a Regular Tryptophan *C4*-Prenyltransferase to a Reverse Tryptophan-containing Cyclic Dipeptide *C3*-Prenyltransferase by Sequential Site-directed Mutagenesis

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Author Contributions

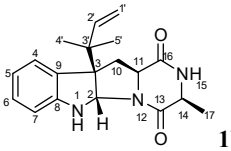
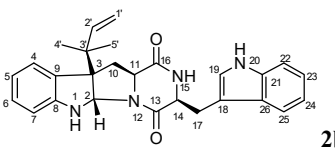
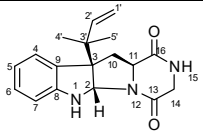
‡ Liujuan Zheng and Peter Mai contributed equally to this work.

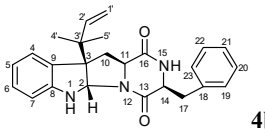
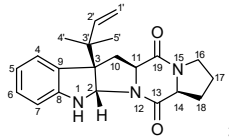
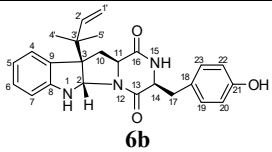
Table S1 Construction of expression plasmids

Mutants	Primer name	Primer sequence for PCR amplification (5'-3')	Plasmid name	Protein yield (mg/L culture)
FgaPT2_K174A	FgaPT2_K174A_f FgaPT2_K174A_r	CAGAACGCACTCGCGCTCGATCTGAAGGATGGCCGCTTTGC GAGCGCGAGTGCGTTCTGCGTCCTGATAGTGCCGCCCA	pLZ1	1.2
FgaPT2_K174F_R244Q	FgaPT2_R244Q_f FgaPT2_R244Q_r	GCCAGTCCCCAACTAGTGTCCTGTGAT GGACACTAGTTGGGGACTGGCAGTGCT	pALF56	2.3
FgaPT2_K174F_R244N	FgaPT2_R244N_f FgaPT2_R244N_r	GCCAGTCCC(A/G)ATCTAGTGTCCTGTGAT GGACACTAGATYGGGACTGGCAGTGCT	pALF55	1.5
FgaPT2_K174F_R244Y	FgaPT2_R244Y_f FgaPT2_R244Y_r	CAGAACTTTCTCGCGCTCGATCTGAAGGATGGCCGCTTTGC GAGCGCGAGAAAGTTCTGCGTCCTGATAGTGCCGCCCA	pPM72	2.0
FgaPT2_K174F_R244L	FgaPT2_R244L_f FgaPT2_R244L_r	CAGAACTTTCTCGCGCTCGATCTGAAGGATGGCCGCTTTGC GAGCGCGAGAAAGTTCTGCGTCCTGATAGTGCCGCCCA	pPM71	2.2

pIU18[1] was used as template for creation of FgaPT2_K174A and pES26 containing the K174F mutation[2] for creation of double mutants

Table S2 ^{1}H NMR (500 MHz) Data for **1b-5b** in $CDCl_3$ and **6b** in DMSO-*d*6

	 **1b**	 **2b**	 **3b**
no	δ_H, mult., (*J* in Hz)	δ_H, mult, (*J* in Hz)	δ_H, mult, (*J* in Hz)
2	5.50, s	5.55, s	5.55, s
4	7.16, dd (7.5, 0.7)	7.14 [a]	7.16, ddd (7.5, 1.2, 0.5)
5	6.77, td (7.5, 1.0)	6.75, td (7.5, 1.0)	6.77, td (7.5, 1.0)
6	7.11, td (7.5, 1.0)	7.11[a]	7.11, td (7.6, 1.0)
7	6.58, d (7.8)	6.61, d (7.8)	6.59, ddd (7.6, 1.0, 0.5)
10	2.47, dd (12.7, 11.0)	2.42, dd (12.5, 11.2)	2.45, dd (12.7, 11.2)
	2.55, dd (12.8, 6.5)	2.52, dd (12.5, 6.2)	2.56, dd (12.7, 6.2)
11	3.96, ddd (10.9, 6.4, 1.7)	3.92, ddd(10.9, 6.3, 1.7)	3.95, ddd (11.2, 6.2, 1.6)
14	4.05, qd (6.9, 1.6)	4.31, ddd (10.9, 3.6, 1.8)	4.04, dd (17.0, 2.0)
	-	-	3.87, dd (17.0, 4.1)
15	-	5.65, s	-
17	1.46, d (6.9)	3.75, ddd (15.2, 3.7, 0.9)	-
	-	2.97, dd (15.1, 11.0)	-
19	-	7.11[a]	-
20	-	8.13, s	-
22	-	7.39, dt (8.3, 0.9)	-
23	-	7.23, td (7.7, 0.9)	-
24	-	7.13 [a]	-
25	-	7.55, d (7.9)	-
1'	5.13, dd (10.8, 1.1)	5.13, dd (10.8, 1.1)	5.13, dd (10.8, 1.1)
	5.08, dd (17.4, 1.1)	5.08, dd (17.4, 1.1)	5.09, dd (17.4, 1.1)
2'	5.98, dd (17.4, 10.8)	5.97, dd (17.4, 10.8)	5.98, dd (17.4, 10.8)
4'	1.12, s	1.11, s	1.12, s
5'	1.01, s	1.01, s	1.01, s

	 **4b**	 **5b**	 **6b**
no.	δ_H, mult., (*J* in Hz)	δ_H, mult., (*J* in Hz)	δ_H, mult., (*J* in Hz)
2	5.54, s	5.46, s	5.32, d (1.1)
4	7.14, d (7.5)	7.16, dd, (7.5, 1.0)	7.09, d (7.0)
5	6.76, d (7.5)	6.77, td, (7.5, 1.0)	6.59, t (7.4)
6	7.11, t (7.7)	7.11, td (7.5,1.0)	6.98, t (7.4)
7	6.59, d (7.7)	6.59, d (7.5)	6.49, d (7.0)
10	2.40, t (1.9)	2.49, dd (12.9, 10.8)	2.50[b]
	2.52,dd (12.8, 6.2)	2.54, dd (12.9, 6.7)	
11	3.93, ddd (11.3, 6.2, 1.8)	3.98, dd (10.8, 6.7)	3.67, ddd (11.6, 5.7, 1.8)
14	4.21, ddd (10.8, 3.6, 1.8)	4.05, t (8.3)	4.07, m
15	5.50, s		-
	-	2.12, m	-
16	-	3.56, m	-
	-	3.50, m	-
17	3.60, dd (14.4, 3.6)	2.04, m	3.06, dd (14.0, 3.8)
	2.78, dd (14.4, 10.8)	1.89, m	2.84, dd (14.0, 5.0)
18		2.32, dtd (9.1, 6.7, 2.5)	
19	7.19, d (7.1)	-	7.01, d (8.4)
20	7.34, t (7.3)	-	6.66, m (8.4)
21	7.29, t (7.4)	-	9.17, s (OH)
22	7.34, t (7.3)	-	6.66, m (8.4)
23	7.19, d (7.1)	-	7.01, d (8.4)
1'	5.13 ,dd (10.8, 1.0)	5.07, dd (17.4, 1.1)	4.97, dd (17.3, 1.4)
	5.08, dd (17.4, 10)	5.05, dd (10.8, 1.1)	5.05, dd (10.8, 1.4)
2'	5.96, dd (17.3, 10.8)	5.99, dd (17.4, 10.8)	5.76, dd (17.3, 10.8)
4'	1.11, s	1.11, s	0.93, s
5'	1.01, s	1.00, s	0.79, s

[a] Signals overlapping with each other. [b] signals overlapping with those of solvent. The data of **1b** – **6b** correspond well to those of published previously.[3,4]

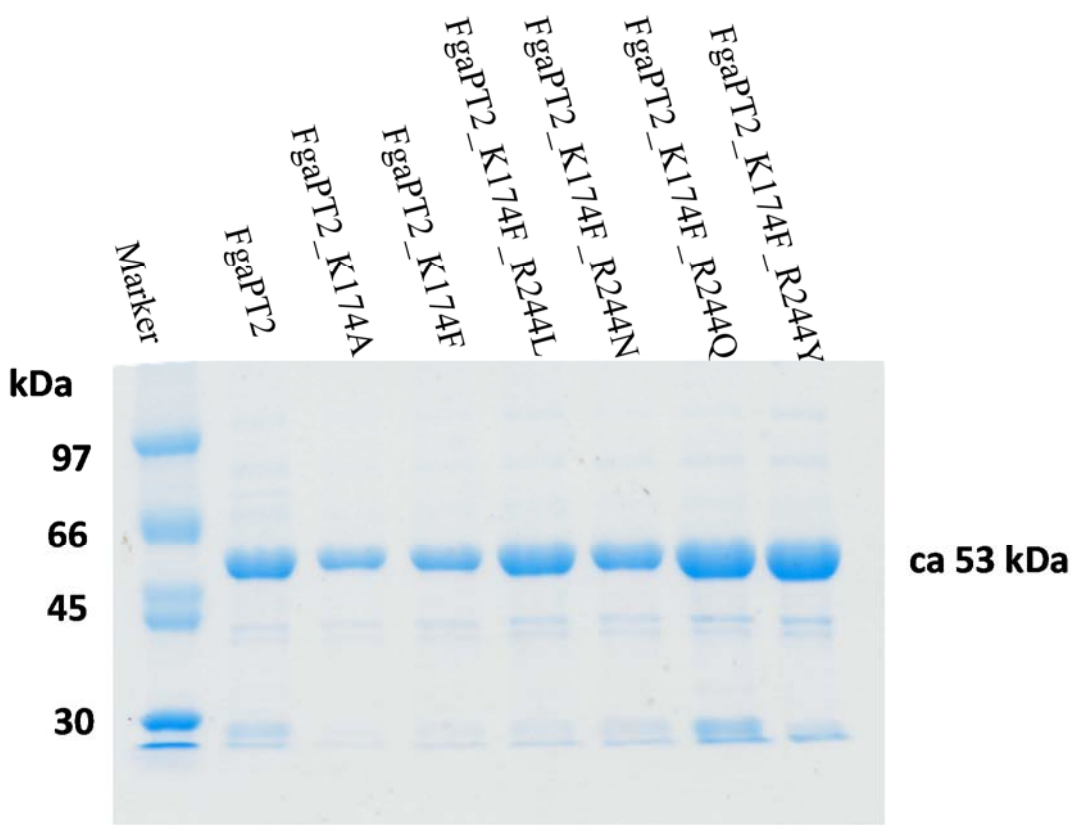


Fig. S1 SDS-PAGE analysis of the purified proteins.

The proteins were separated on a 12% polyacrylamide gel and stained with Coomassie brilliant blue R-250

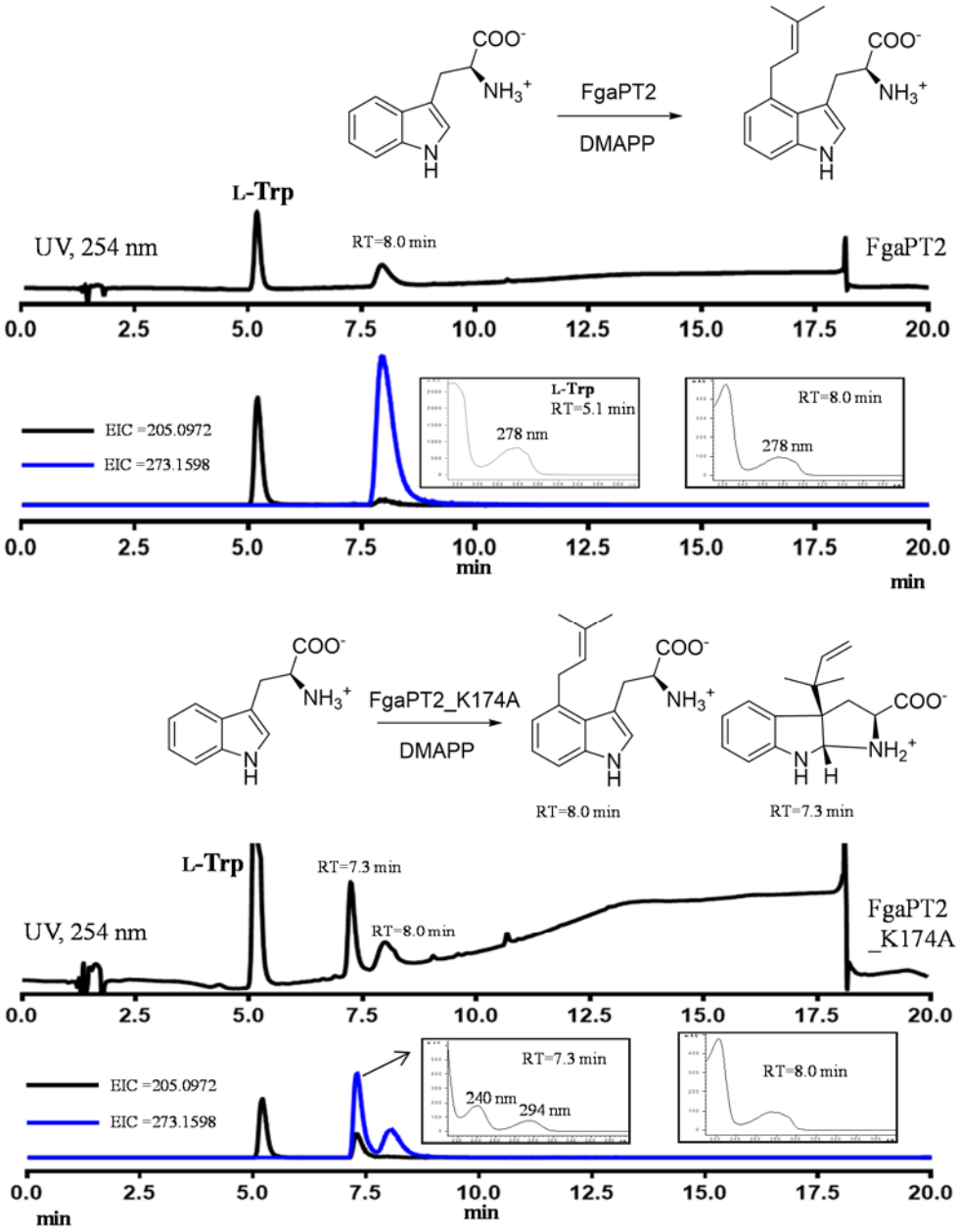


Fig. S2 LC-HRMS analysis of FgaPT2 and FgaPT2_K174A reactions with L-tryptophan in the presence of DMAPP.

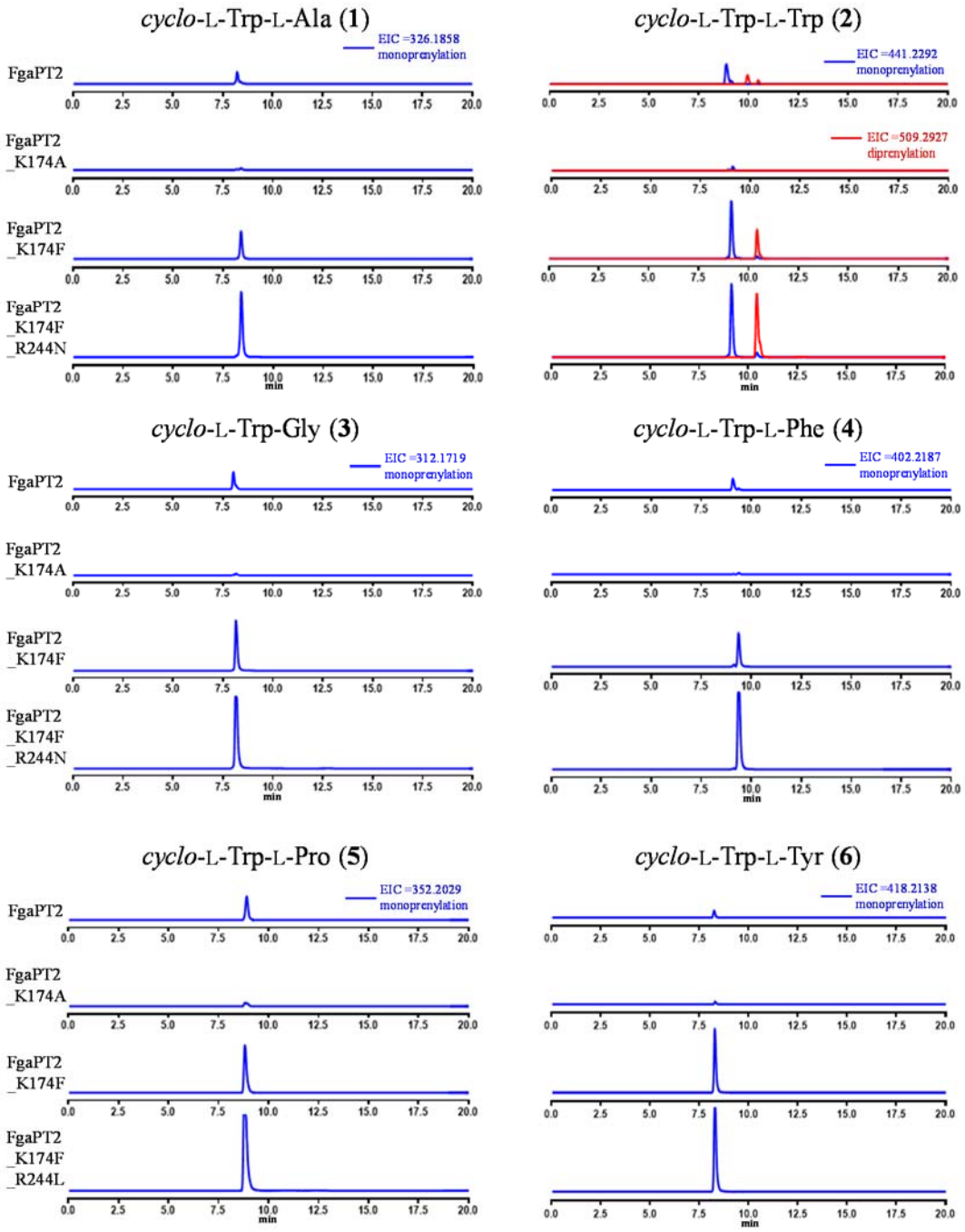


Fig. S3 LC-MS analysis of the incubation mixtures of cyclic dipeptides with FgaPT2 and its mutants

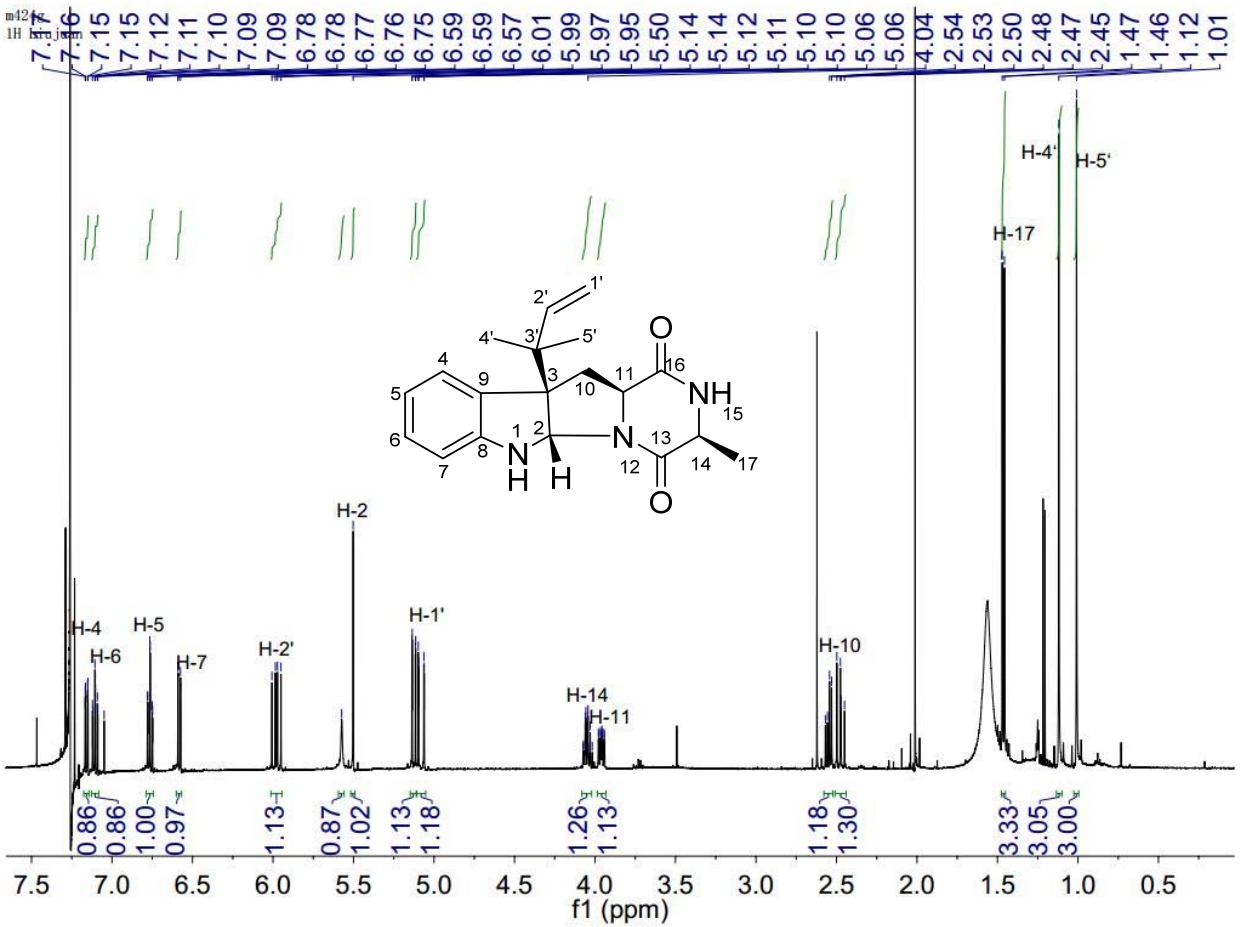


Fig. S4 ^{1}H NMR (500 MHz) spectrum of **1b** in $CDCl_3$

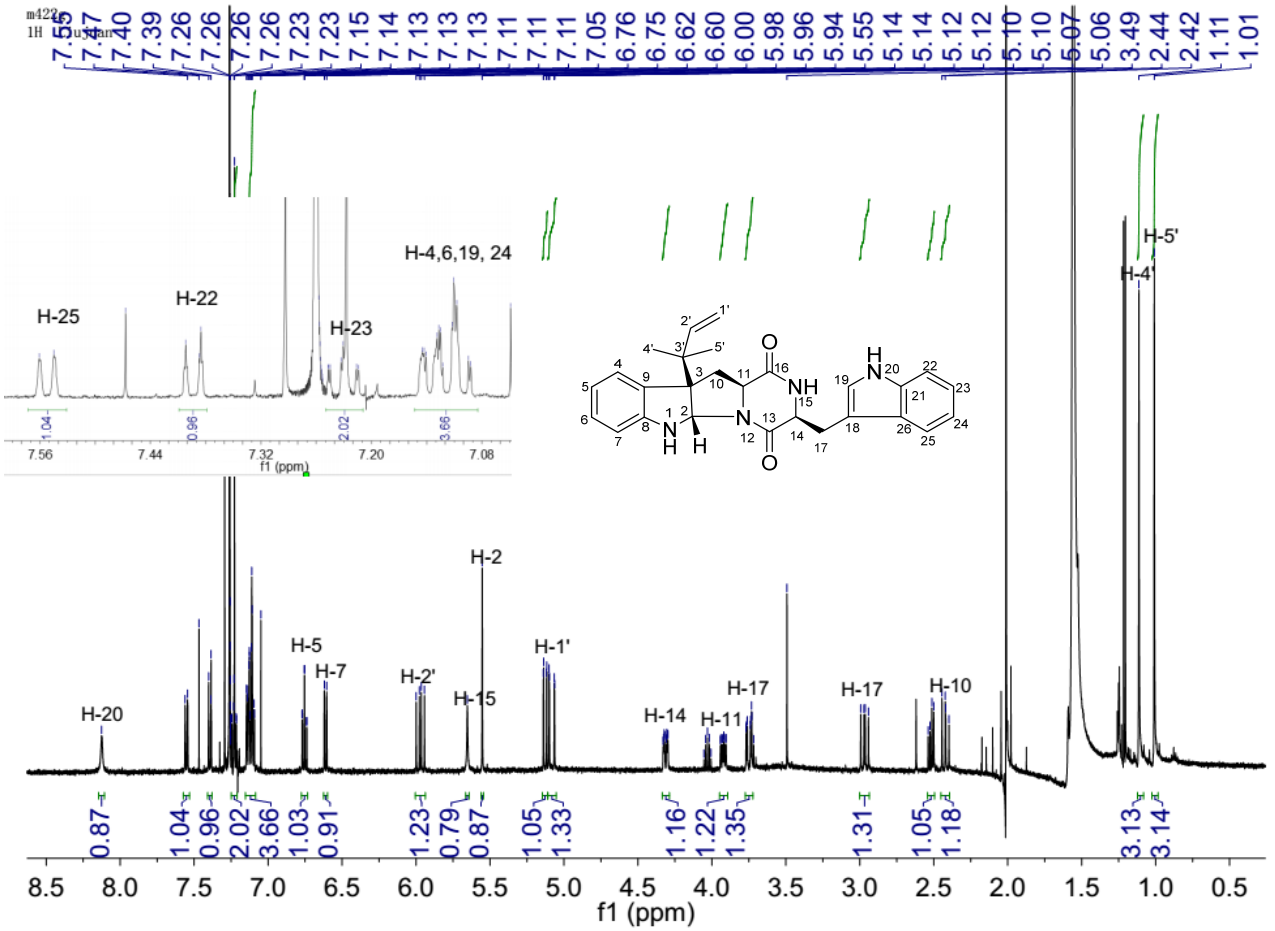


Fig. S5 ^{1}H NMR (500 MHz) spectrum of **2b** in $CDCl_3$

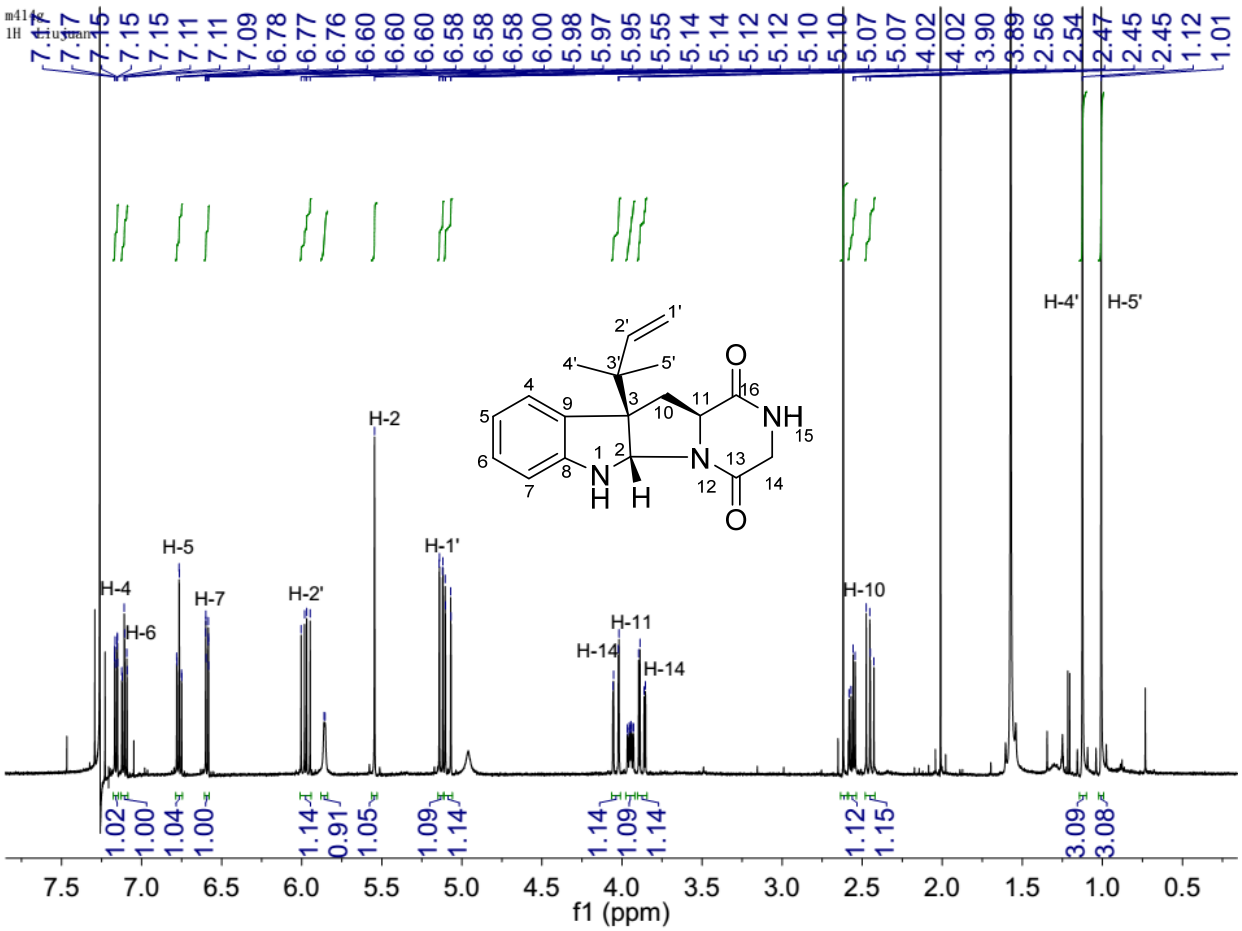


Fig. S6 ^{1}H NMR (500 MHz) spectrum of **3b** in $CDCl_3$

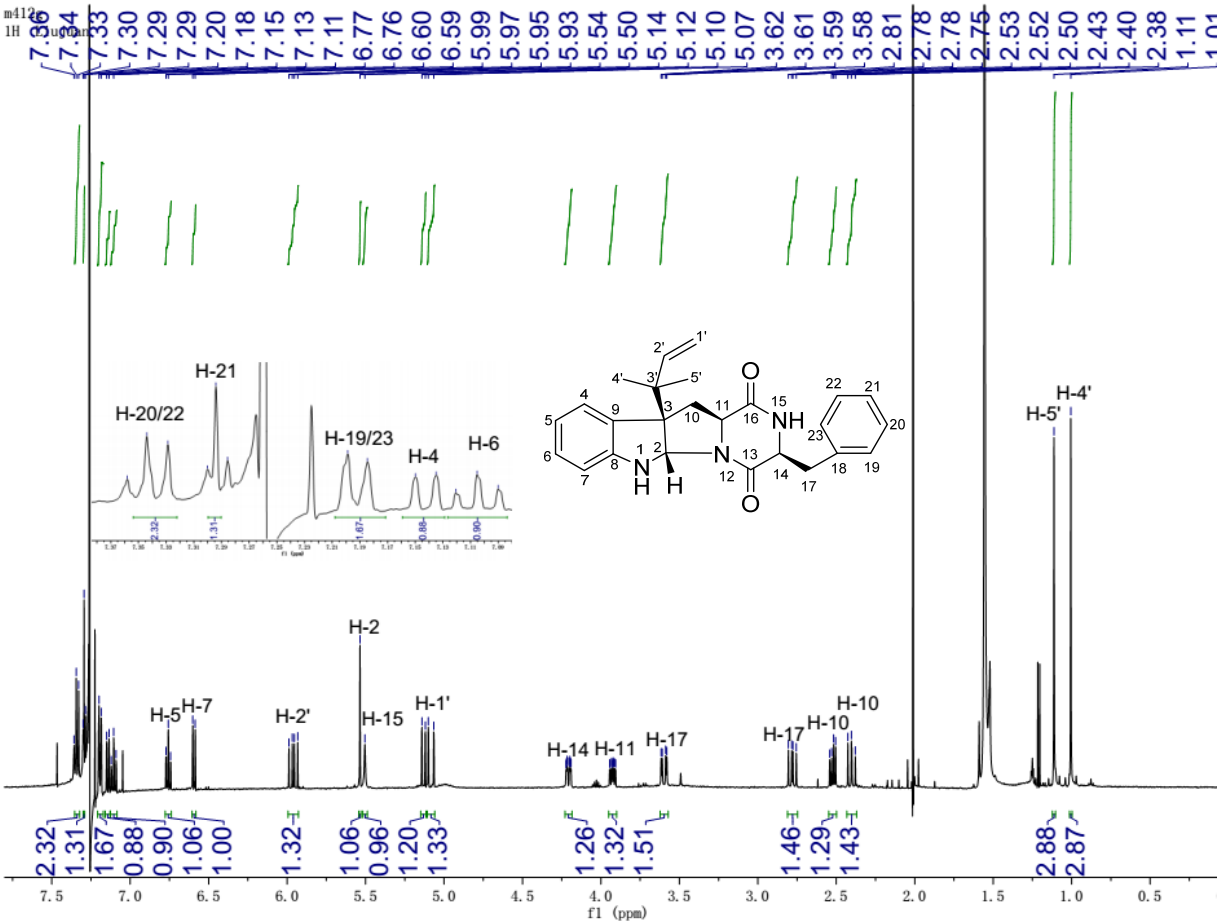


Fig. S7 ^{1}H NMR (500 MHz) spectrum of **4b** in $CDCl_3$

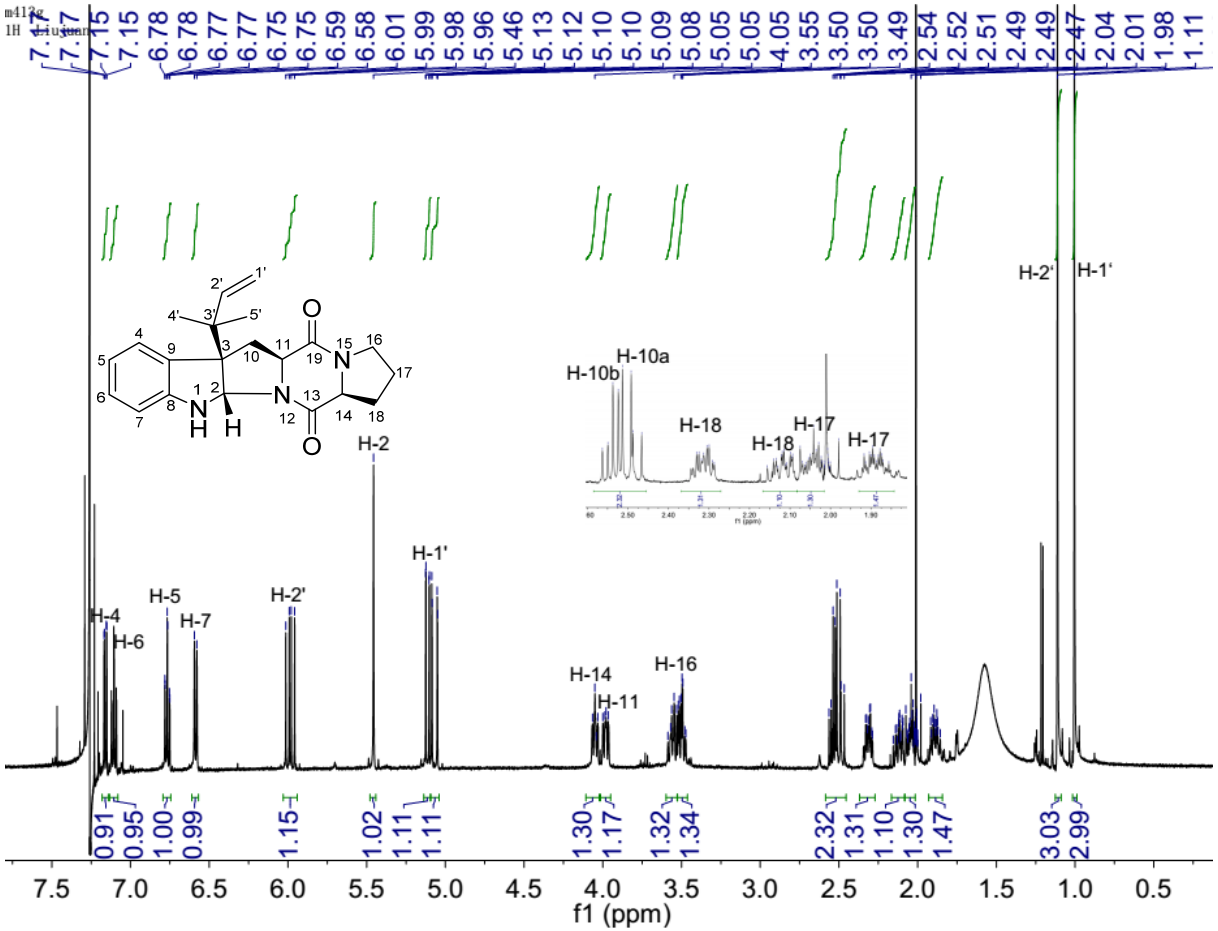


Fig. S8 ^{1}H NMR (500 MHz) spectrum of **5b** in $CDCl_3$

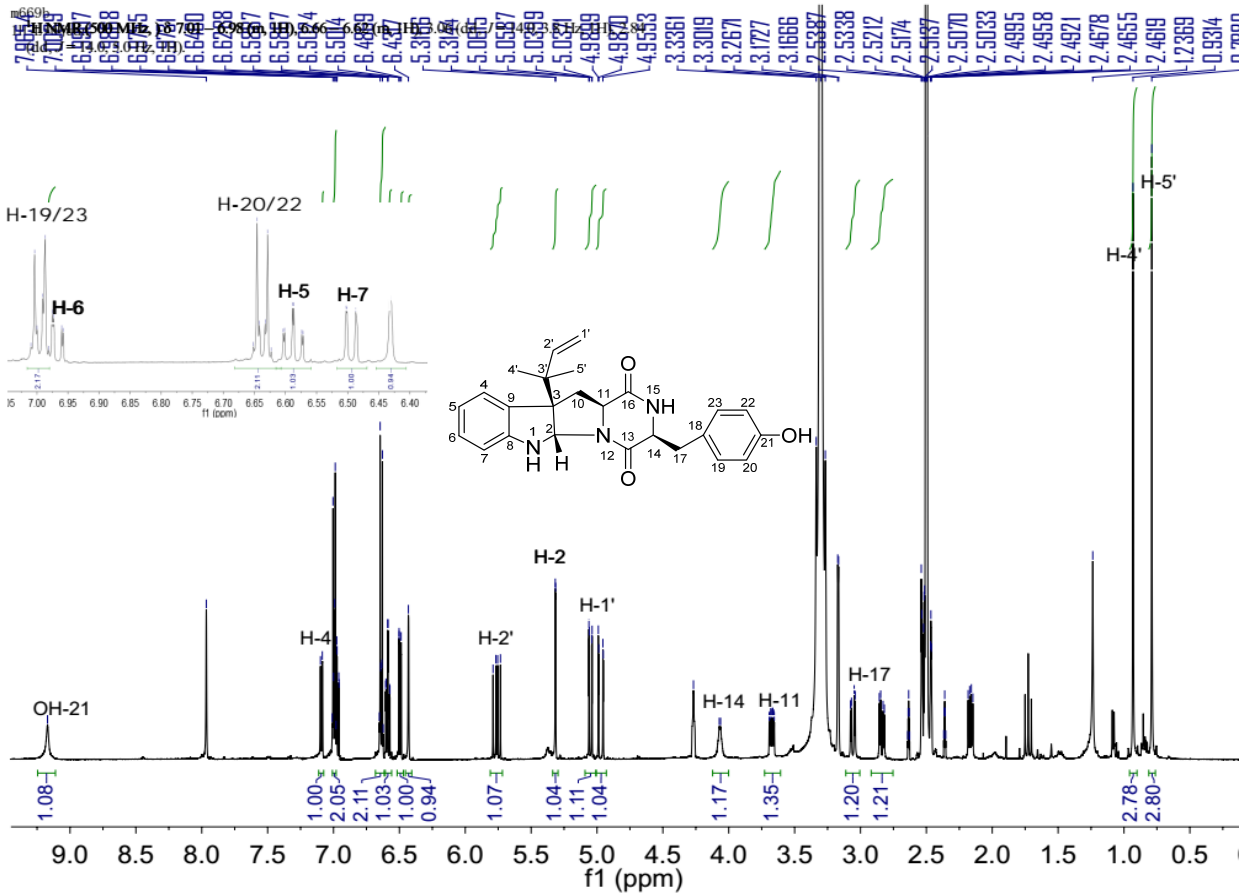


Fig. S9 ^{1}H NMR(500 MHz) spectrum of compound **6b** in DMSO-*d6*

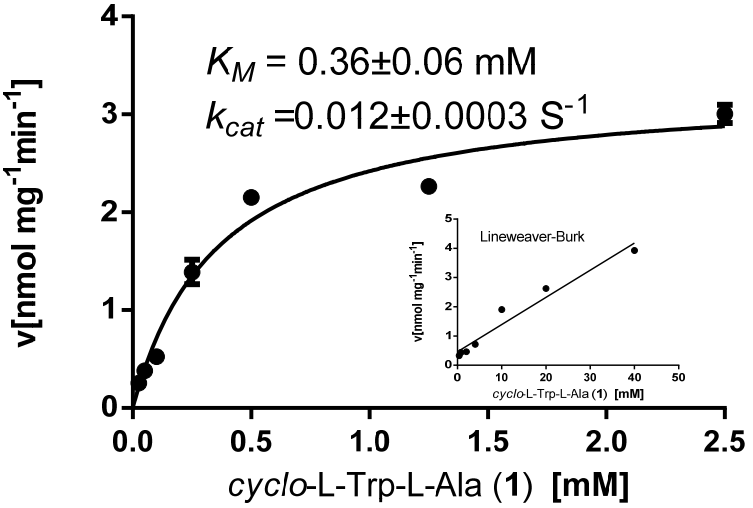


Fig. S10 Determination of the kinetic parameters of the FgaPT2_K174F_R244N reaction toward **1** in the presence of DMAPP.

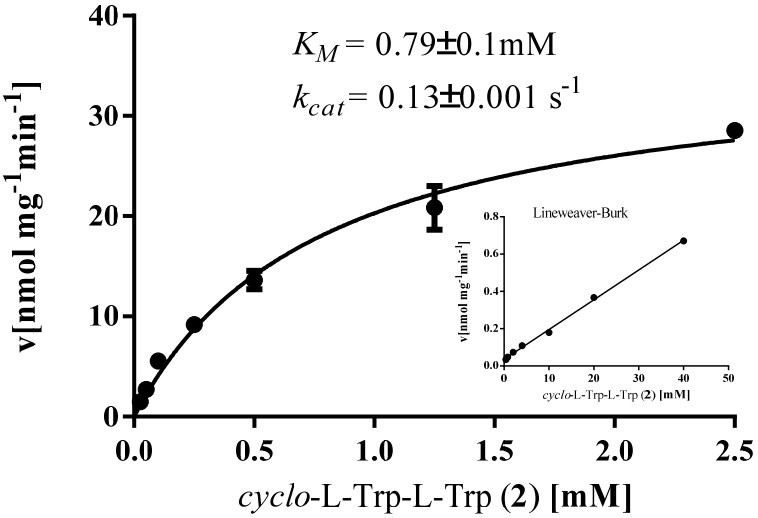


Fig. S11 Determination of the kinetic parameters of the FgaPT2_K174F_R244N reaction toward **2** in the presence of DMAPP.

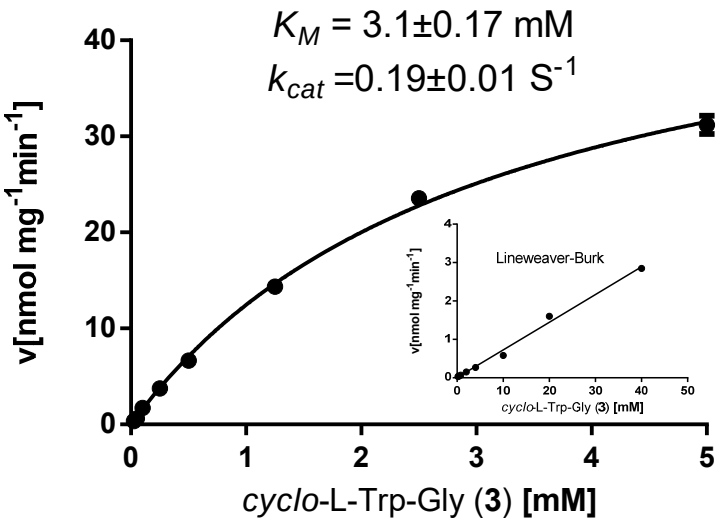


Fig. S12 Determination of the kinetic parameters of the FgaPT2_K174F_R244N reaction toward **3** in the presence of DMAPP.

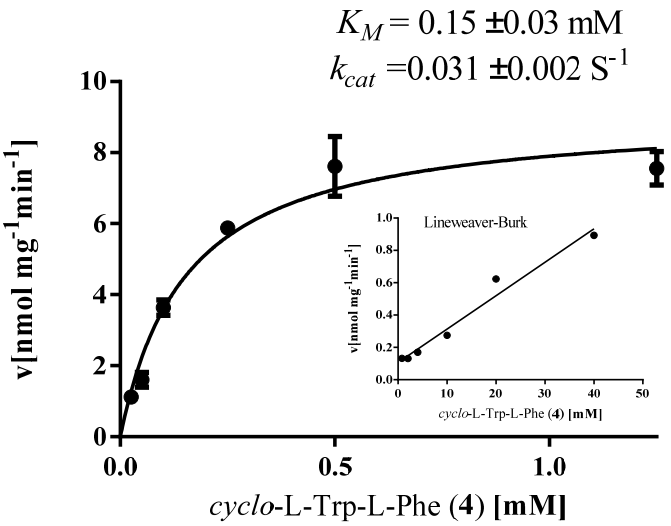


Fig. S13 Determination of the kinetic parameters of the FgaPT2_K174F_R244N reaction toward **4** in the presence of DMAPP.

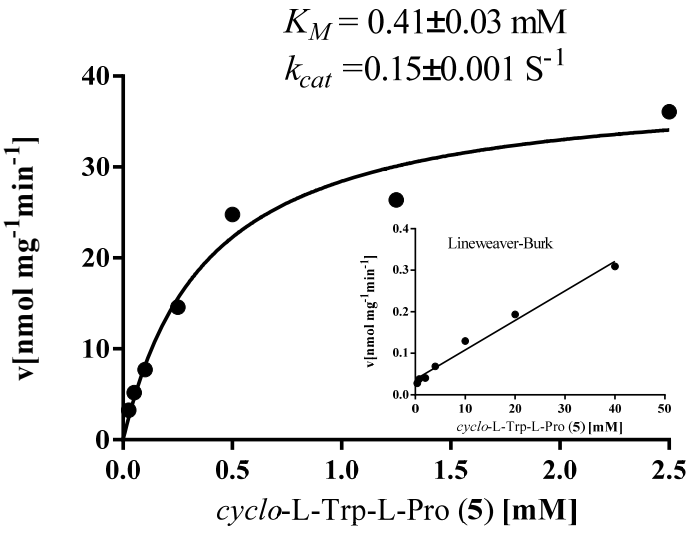


Fig. S14 Determination of the kinetic parameters of the FgaPT2_K174F_R244L reaction toward **5** in the presence of DMAPP.

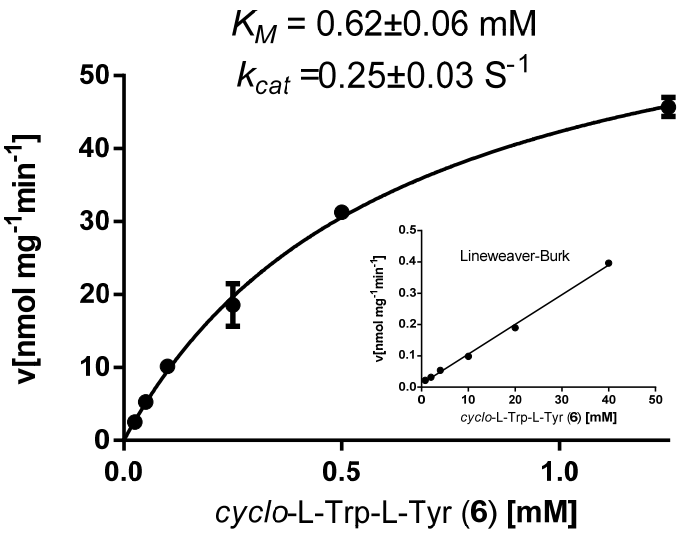


Fig. S15 Determination of the kinetic parameters of the FgaPT2_K174F_R244L reaction toward **6** in the presence of DMAPP.

References and Notes

(1) Steffan, N.; Unsöld, I. A.; Li, S.-M. *Chembiochem* **2007**, *8*, 1298-1307.

(2) Fan, A.; Zocher, G.; Stec, E.; Stehle, T.; Li, S.-M. *J. Biol. Chem.* **2015**, *290*, 1364-1373.

(3) Yin, W.-B.; Yu, X.; Xie, X.-L.; Li, S.-M. *Org. Biomol. Chem.* **2010**, *8*, 2430-2438.

(4) Yu, X.; Zocher, G.; Xie, X.; Liebhold, M.; Schütz, S.; Stehle, T.; Li, S.-M. *Chem. Biol.* **2013**, *20*, 1492-1501.