Supporting Information

Specificity of the Ester Bond-Forming Condensation Enzyme SgcC5 in C-1027 Biosynthesis

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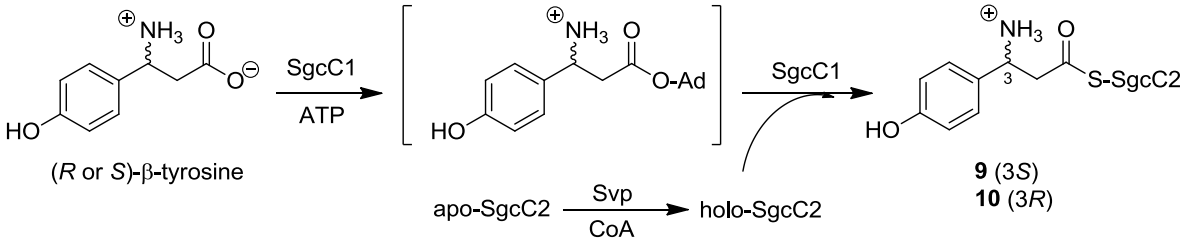
General experimental procedures

Adenosine triphosphate disodium salt (ATP), coenzyme A (CoA), flavin adenine dinucleotide disodium salt (FAD), β-nicotinamide adenine dinucleotide reduced disodium salt (NADH), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), (*S*)-1-phenyl-1,2-ethanediol (**11**), (*R*)-1-phenyl-1,2-ethanediol (**3**), (*R*)-2-phenylglycinol (**18**), (*R*)-2-phenyl-1-propanol (**19**), 2-phenylethanol (**20**), 3-phenyl-1-propanol (**21**), 4-phenyl-1-butanol (**22**), phenol (**23**), benzyl alcohol (**24**), *sec*-phenethyl alcohol (**25**), (*R*)-1-(2-naphthyl)-1,2-ethanediol (**26**), cyclohexylmethanol (**27**), cyclohexylethanol (**28**) and isobutanol (**29**) were purchased from Sigma-Aldrich (St. Louis, MO). The 3-substituted β-amino acid analogues were chemically synthesized as described previously.[S1,S2] (*S*)-3-Amino-3-(4-hydroxyphenyl)-propionic acid [(*S*)-β-tyrosine] and (*R*)-3-Amino-3-(4-hydroxyphenyl)-propionic acid [(*R*)-β-tyrosine] were from PepTech Corporation (Burlington, MA). High resolution electrospray ionization-mass spectroscopy (HR-ESI-MS) was performed with an Agilent 1100 HPLC-MSD SL ion trap mass spectrometer (Agilent Technologies, Inc., Santa Clara, CA), and high resolution matrix-assisted laser desorption-ionization mass spectroscopy (HR-MALDI-MS) was measured with an Agilent 1100 VL APCI Mass Spectrometer. ^{1}H and ^{13}C NMR data were recorded at 25 °C on Bruker AM 700 instruments operating at 700 MHz for ^{1}H and 175 MHz for ^{13}C nuclei. High performance liquid chromatography (HPLC) analyses were carried out on a Varian HPLC system equipped with Prostar 210 pumps, a photodiode array (PDA) detector, and an Apollo C18 reverse phase column (5 μm, 4.6 × 250 mm, Alltech Associates Inc.), using the following mobile phase system that consisted of 0.1% trifluoroacetic acid (TFA) in milli-Q water (A) and 0.1% TFA in 90% acetonitrile/water (B).

Enantiospecificity of SgcC5-catalyzed ester-bond formation between 2, 8–10 and 3, 11

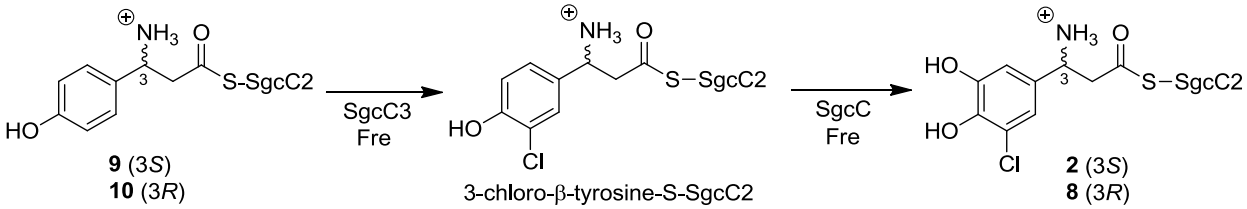
*Preparation of **9** and **10***



Prior to preparation of the SgcC2-tethered substrates, recombinant Svp,[S3] SgcC1, [S4] and SgcC2 [S1] were prepared as described in our previous work. The (*S*)-β-tyrosyl-S-SgcC2 (**9**) was generated as described previously.[S1,S5] To make (*R*)-β-tyrosyl-S-SgcC2 (**10**), two enzymatic reactions were performed together in one pot: i) the phosphopantetheinylation of SgcC2 (*i.e.* apo-SgcC2 was converted to holo-SgcC2 with CoA and the phosphopantetheinyl transferase Svp), and ii) the loading of holo-SgcC2 with (*R*)-β-tyrosine by the ATP-dependent SgcC1 enzyme to afford **10**. Apo-SgcC2 (200 μM) was incubated with 1 mM CoA, 12.5 mM $MgCl_2$, 2 mM TCEP, commercial (*R*)-β-tyrosine (7.5 mM), 7.5 mM ATP, 10 μM Svp and 10 μM SgcC1 in a 4-mL reaction mixture with 100 mM Tris-HCl as buffer (pH 7.5). After incubation at 25 ^{o}C for 45 min, the proteins were removed from the loading mixture by ultrafiltration using a 4-mL centrifugal filter device

(5,000 MWCO, GE Healthcare) to remove the trace amount of (*S*)-β-tyrosine contained in (*R*)-β-tyrosine. The resulting 3.0 mL of filtrate was incubated with apo-SgcC2 (200 μM), 10 μM Svp and 20 μM SgcC1 at 25 °C for an additional 45 min. The purification of **10** was performed following the procedure used for the purification of **9** as described previously. [S1]

*Preparation of **2** and **8***



The generation of (*S*)-3-chloro-5-hydroxy-β-tyrosyl-S-SgcC2 (**2**) was performed as described previously, [S1, S5] and (*R*)-3-chloro-5-hydroxy-β-tyrosyl-S-SgcC2 (**8**) was prepared in a similar manner. The purified **10** was incubated with 100 μM SgcC3, 10 μM flavin reductase from *E. coli* (Fre), [S1] 0.3 M NaCl, 10 mM NADH (added fresh every second hour), 100 μM FAD, 1mM TCEP, and 100 mM phosphate buffer (pH 6.0) at 30 °C for 3 h. The reaction mixtures were first diluted 5 times with water before they were loaded on a HiTrap Q anion exchange column for purification. After SgcC3-generated (*R*)-3-chloro-β-tyrosyl-S-SgcC2 was purified and concentrated using a centrifugal filter device, it was hydroxylated at C-5 in a reaction containing 10 μM SgcC, 2 μM Fre, 10 mM NADH (added fresh every hour), 20 μM FAD, 1 mM TCEP, 0.1 M NaCl, and 20 μM SgcC3 in 100 mM phosphate buffer (pH 6.0). After this reaction was incubated at 25 °C for 2 h, the product **8** was also purified by anion-exchange column chromatography.

*Confirmation of the stereochemistry of **8***

After purification, **8** was subjected to alkaline hydrolysis in 0.1 M KOH containing 50 mM DTT by incubating at 50 °C for 15 min. After work-up as described previously, [S1] the resulting solution containing the free 3-chloro-5-hydroxy-β-tyrosine was injected onto an Apollo C18 column using a 20 min linear gradient from 0 to 25 % B at a flow rate of 1 mL min^{-1} and UV-Vis detection at 283 nm for purification. The peak corresponding to the 3-chloro-5-hydroxy-β-tyrosine was collected and the solvent was removed using a speed-vac. The resultant residue was re-dissolved in perchloric acid buffer (pH 1.5) and analyzed using a chiral CrownPak CR (+) column (5 μm, 150 × 4.0 mm, Chiral Technologies Inc., West Chester, PA) to determine the stereochemistry. The column was developed under isocratic aqueous perchloric acid buffer (pH 1.5) at a flow rate of 1 mL min^{-1} as recommended by the manufacturer. The racemic 3-chloro-5-hydroxy-β-tyrosine standard was synthesized as described previously. [S2] The (*S*)-3-chloro-5-hydroxy-β-tyrosine standard **2** was made following the identical procedure to that used for the preparation of **8**, except that **9** was used as the starting material (Figure S4).

*SgcC5-catalyzed coupling of **2** with **3***

The SgcC5 assay solution contained 200 μM apo-SgcC2 (final concentration), 1 mM CoA, 5 mM ATP, 2 mM TCEP, 12.5 mM $MgCl_2$, 5 mM (*S*)-3-chloro-5-hydroxy-β-tyrosine, 10 μM Svp, and 10

μM SgcC1 in 75 mM Tris-HCl buffer (pH 7.5) and was incubated at 25 ^{o}C for 45 min. To the above solution, 5 mM (*R*)-1-phenyl-1,2-ethanediol (**3**) and 1 μM SgcC5 were added to initiate the coupling reaction and then incubated at 25 ^{o}C for 10 min. The reactions were quenched by the addition of trifluoroacetic acid (TFA) to a final concentration of 16%, and the resulting solution was directly subjected to HPLC analysis (Table S1). The control reactions were carried out under identical conditions using SgcC5 that had been boiled for 5 min.

*SgcC5-catalyzed coupling of **8** with **3***

A solution of 200 μM **8** and 5 mM **3** were incubated with 150 μM SgcC5 in 75 mM Tris-HCl buffer (pH 7.5) for 3 hrs. After the reaction was quenched by the addition of trifluoroacetic acid (TFA) to a final concentration of 16% and centrifuged to remove the protein, the resulting supernatant was directly subjected to HPLC analysis (Table S1).

*Kinetic analysis of SgcC5-catalyzed coupling of **9** and **10** with **3***

Steady-state kinetic parameters for SgcC5 with **9** or **10** as the donor substrate and **3** as the acceptor substrate were obtained as follows. Assays containing **9** or **10** (0-250 μM), 5 mM **3**, and SgcC5 [10 μM for **9** and 100 μM for **10**] in 50 mM phosphate buffer (pH 7.5) were incubated at 30 ^{o}C for 30 min for **9** and 60 min for **10**. The assays were carried out in duplicate. The reactions were quenched by addition of TFA (to a final concentration of 16%) to precipitate protein, followed by centrifugation to remove the protein. The resulting solution was analyzed by HPLC with UV detection at 275 nm. To quantify the amount of products formed from each reaction, a standard curve based on the HPLC peak area correlated with the known amount of the authentic β-tyrosine was first generated. The concentrations of the donor substrates were determined based on the above standard curve correlated with HPLC peaks of the free β-tyrosine released from SgcC2 by alkaline hydrolysis as described previously (Table S1).[S1] The Michaelis-Menten equation was least-squares fitted to plots of the initial formation rate of products to extract the K_m and k_{cat} parameters (Figure S1).

*Kinetic analysis of SgcC5-catalyzed coupling of **2** with **3** and **11***

The kinetic analyses of SgcC5 for the acceptor substrates were performed with **2** used as the donor substrate in a 200-μL reaction mixture. The 190-μL loading reaction contained 200 μM apo-SgcC2 (final concentration), 1 mM CoA, 5 mM ATP, 2 mM TCEP, 12.5 mM $MgCl_2$, 5 mM 3-chloro-5-hydroxy-β-tyrosine, 10 μM Svp, and 10 μM SgcC1 in 75 mM Tris-HCl buffer (pH 7.5) and incubated at 25 ^{o}C for 45 min. To each loading reaction, the acceptor substrate **3** (0.2-6.4 mM) or **11** (0.5-16 mM) was added to generate the assays for kinetic analysis which was initiated by the addition of SgcC5 (1 μM for **3** and 100 μM for **11**) and incubated at 25 ^{o}C for 5 min and carried out in triplicate. The reactions were quenched by addition of TFA (to a final concentration of 16%) to precipitate protein, followed by centrifugation to remove the protein. The standard curve to quantify the amount of product formed was generated based on the HPLC peak area correlated with the known amount of the authentic 3-chloro-5-hydroxy-β-tyrosine standard (Table S1). The Michaelis-Menten equation was fitted to plots of the initial formation rate of products to extract the K_m and k_{cat} parameters (Figure S2).

*Kinetic resolution of SgcC5-catalyzed coupling of racemic **3** with **11***

A reaction containing 200 μM apo-SgcC2 (final concentration), 1 mM CoA, 5 mM ATP, 2 mM TCEP, 12.5 mM $MgCl_2$, 5 mM **2**, 10 μM Svp, and 10 μM SgcC1 in 75 mM Tris-HCl buffer (pH 7.5) and was incubated at 25 ^{o}C for 45 min. To the above solution, 5 mM racemic 1-phenyl-1,2-ethanediol and 10 μM SgcC5 were added to initiate the coupling reaction and then incubated at 25 ^{o}C for 10 min. The reactions were quenched using trifluoroacetic acid (TFA) at a final concentration of 16%, and the resulting solution was subjected to HPLC analysis (Figure S3).

Donor substrate promiscuity of SgcC5-catalyzed ester-bond formation between 2, 9, 12–17 and 3

*Assays of SgcC5-catalyzed coupling of **12–17** with **3***

Prior to assays of **12**–**17** with **3**, the free 3-substituted β-tyrosine analogues were synthesized from the corresponding aldehyde described as previously.[S1, S2] In order to test the substrate specificity of SgcC5 with respect to the donor substrates **12**–**17**, assays were performed in two steps. The first step for loading free 3-substituted β-tyrosine analogues to SgcC2 was carried out in 190 μL reaction mixtures containing 200 μM apo-SgcC2 (final concentration), 1 mM CoA, 5 mM ATP, 2 mM TCEP, 12.5 mM $MgCl_2$, 5 mM of the appropriate 3-substituted β-tyrosine analogue, 10 μM Svp, and 10 μM SgcC1 in 75 mM Tris-HCl buffer (pH 7.5) and incubated at 25 ^{o}C for 45 min. To each loading reaction, the acceptor substrate **3** (5 mM) and SgcC5 (20 μM) were added to bring the volume to 200 μL for the SgcC5 assay step, and the resulting solutions were incubated at 25 ^{o}C for 30 min and then quenched with TFA to a final concentration of 16%.

HPLC and high resolution analysis of the ester products

After the reactions were quenched by TFA, the product formation was examined by HPLC analysis as described in the *General Experimental Procedures* section. The peaks corresponding to the products were collected and subjected to HR-ESI-MS or HR-MALDI-MS analyses (described in the *General experimental procedures* section) after the complete removal of TFA (Table S2). To determine the relative activity of SgcC5 for each putative donor substrate, the amount of products formed from each reaction was calculated based on the standard curves that were generated from the HPLC peak area correlated with the known amount of each β-tyrosine analogue,[S2] based on the assumption that the formed esters have similar UV-absorption with each β-tyrosine analogue.

Acceptor substrate promiscuity of SgcC5-catalyzed ester-bond formation between 2 and 3, 18–29

*Assay of SgcC5-catalyzed coupling of **2** with **3**, **18**–**29***

To explore the substrate specificity of SgcC5 for the acceptor substrates **3** and **18**–**29**, the assays were also performed by two-step reactions. The generation of **2** was carried out under the identical condition to that used for the kinetic assay for acceptor substrate: a 190-μL loading

reaction contained 200 μM apo-SgcC2 (final concentration), 1 mM CoA, 5 mM ATP, 2 mM TCEP, 12.5 mM $MgCl_2$, 5 mM 3-chloro-5-hydroxy-β-tyrosine, 10 μM Svp, and 10 μM SgcC1 in 75 mM Tris-HCl buffer (pH 7.5) and incubated at 25 ^oC for 45 min. Subsequently, the assays for different acceptor substrates were carried out with 5 mM of an acceptor alcohol (one of **3**, **18**–**29**) and 50 μM SgcC5. After incubation at 25 ^oC for 30 min, the reactions were quenched by TFA to a final concentration of 16% and analyzed by HPLC as described below.

HPLC and high resolution analysis of the ester products

After the reactions were quenched by TFA, the product formation was examined by HPLC analysis as described in the *General experimental procedures* section. The peaks corresponding to the products were collected and subjected to HR-ESI-MS or HR-MALDI-MS analyses (described in the *General experimental procedures* section) after the complete removal of TFA (Table S3). To determine the relative activity of SgcC5 for each putative acceptor substrate, the amount of products formed from each reaction was calculated based on the standard curves that were generated from the HPLC peak area correlated with the known amount of 3-chloro-5-hydroxy-β-tyrosine, based on the assumption that the coupling ester has the similar UV-absorption with 3-chloro-5-hydroxy-β-tyrosine.

*Scaled-up reaction of **2** and (R)-2-phenyl-1-propanol (**19**)*

A large scale reaction of **2** and (*R*)-2-phenyl-1-propanol was performed in 10-mL reaction mixture containing 200 μM apo-SgcC2, 1 mM CoA, 15 mM ATP, 2 mM TCEP, 12.5 mM $MgCl_2$, 20 mM 3-chloro-5-hydroxy-β-tyrosine, 10 μM Svp, and 10 μM SgcC1, 20 mM (*R*)-2-phenyl-1-propanol (**19**), and 50 μM SgcC5 in 100 mM Tris-HCl buffer (pH 7.5). Every the third hour, 10 μM SgcC1 was added to the reaction mixture. After being incubated at 25 ^oC for 8 h, the reaction was quenched by TFA. Ethyl acetate (3 x 10 mL) was used to extract the products from the reaction mixture. The organic fractions were combined and dried with anhydrous sodium sulfate for 3 h. The solvent was evaporated under reduced pressure and the resulting residue was re-dissolved in acetonitrile and purified using a semipreparative HPLC column following a gradient from 0 to 75% B (0.1% TFA in 90% acetonitrile in water) to yield 1.2 mg white powder after lyophilization. The complete ^1H and ^{13}C NMR analyses identified it as the condensation product. ^1H NMR (700 MHz, CD_3OD): δ (ppm) 7.18 (t, J = 7.0 Hz, 2H), 7.09 (overlapped，1H), 7.08 (d, J = 7.0 Hz, 2H), 6.75 (s, 1H), 6.66 (s, 1H), 4.24 (t like, J = 6.8 Hz, 1H), 4.13 (dd, J = 10.5, 7.0 Hz, 1H), 4.03 (dd, J = 10.5, 7.0 Hz, 1H), 2.91 (m, 1H), 2.70-2.80 (m, 2H), 1.12 (d, J = 7.0 Hz, 3H). ^{13}C NMR (175 MHz, CD_3OD): δ (ppm) 171.4, 148.4, 144.4, 143.9, 130.5, 129.6, 128.3, 127.8, 122.2, 119.9, 113.7, 71.1, 52.7, 40.2, 38.3, 18.4.

Table S1. Identification of the ester products formed by SgcC5-catalyzed coupling between **2**, **8–10** and **3**, **11**

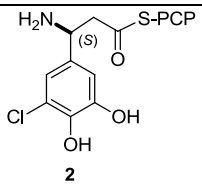
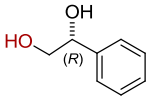
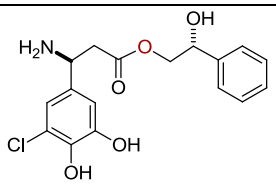
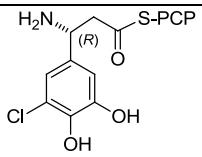
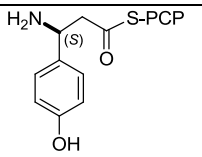
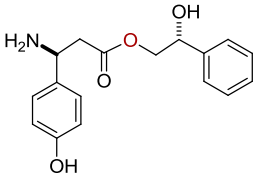
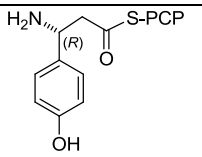
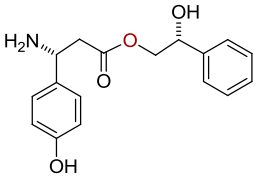
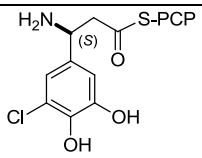
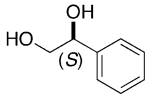
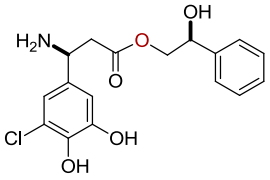
Donor substrate	Acceptor substrate	Predicted ester products			
		Ester product	Molecular formula	Molecular ion found (*m/z*)	Molecular ion calcd (*m/z*)
 **2**	 **3**		$C_{17}H_{18}ClNO_5$	$[M+H]^+$ 352.0950	352.0946
 **8**		No reaction			
 **9**			$C_{17}H_{19}NO_4$	$[M+H]^+$ 302.1391	302.1386
 **10**			$C_{17}H_{19}NO_4$	$[M+H]^+$ 302.1391	302.1386
 **2**	 **11**		$C_{17}H_{18}ClNO_5$	$[M+H]^+$ 352.0946	352.0946

Table S2. Identification of the ester products formed by SgcC5-catalyzed coupling between **2**, **9**, **12–17** and **3**

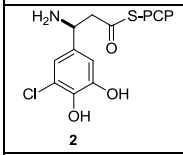
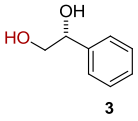
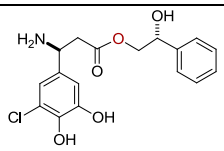
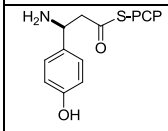
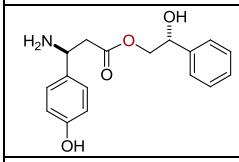
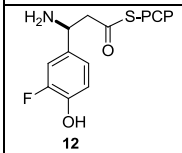
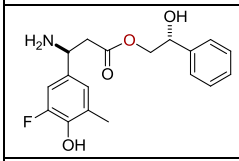
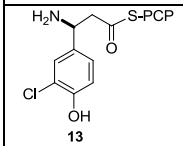
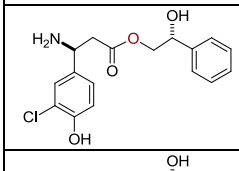
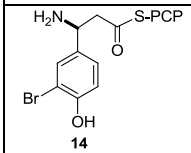
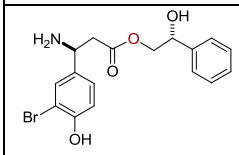
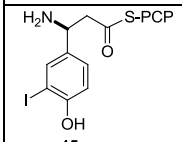
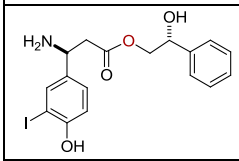
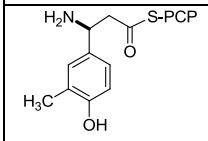
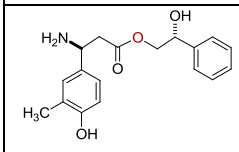
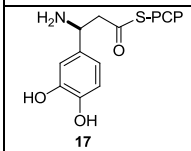
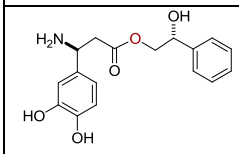
Donor substrate	Acceptor substrate	Predicted ester products			
		Ester product	Molecular formula	Molecular ion found (*m/z*)	Molecular ion calcd (*m/z*)
 **2**	 **3**		$C_{17}H_{18}ClNO_5$	$[M+H]^+$ 352.0950	352.0946
 **9**			$C_{17}H_{19}NO_4$	$[M+H]^+$ 302.1391	302.1386
 **12**			$C_{17}H_{18}FNO_4$	$[M+H]^+$ 320.1293	320.1298
 **13**			$C_{17}H_{18}ClNO_4$	$[M+H]^+$ 336.0998	336.1003
 **14**			$C_{17}H_{18}BrNO_4$	$[M+H]^+$ 380.0487	380.0497
 **15**			$C_{17}H_{18}INO_4$	$[M+H]^+$ 428.0357	428.0359
 **16**			$C_{18}H_{21}NO_4$	$[M+H]^+$ 316.1541	316.1549
 **17**			$C_{17}H_{19}NO_5$	$[M+H]^+$ 318.1336	318.1341

Table S3. Identification of the ester products formed by SgcC5-catalyzed coupling between **2** and **3**, **18–29**

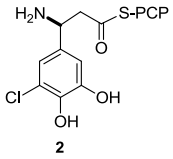
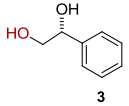
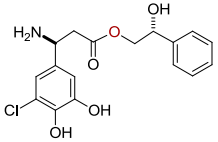
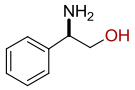
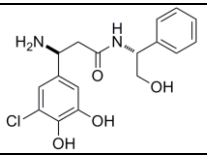
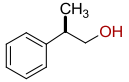
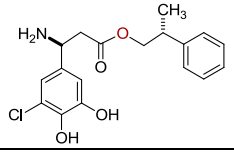
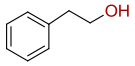
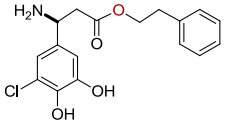
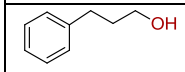
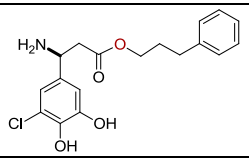
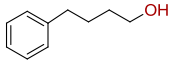
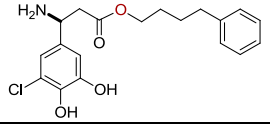
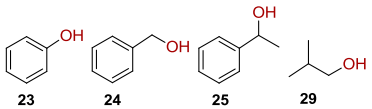
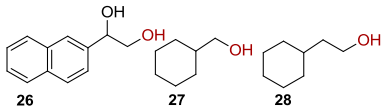
Donor substrate	Acceptor substrate	Predicted ester products			
		Ester product	Molecular formula	Molecular ion found (*m/z*)	Molecular ion calcd (*m/z*)
 **2**	 **3**		$C_{17}H_{18}ClNO_5$	$[M+H]^+$ 352.0950	352.0946
	 **18**		$C_{17}H_{19}ClN_2O_4$	$[M+H]^+$ 351.1112	351.1106
	 **19**		$C_{18}H_{20}ClNO_4$	$[M+H]^+$ 350.1155	350.1154
	 **20**		$C_{17}H_{18}ClNO_4$	$[M+H]^+$ 336.1003	336.0997
	 **21**		$C_{18}H_{20}ClNO_4$	$[M+H]^+$ 350.1160	350.1154
	 **22**		$C_{19}H_{22}ClNO_4$	$[M+H]^+$ 364.1317	364.1310
	 **23** **24** **25** **29**  **26** **27** **28**	No products			

Figure S1. Pseudo first-order kinetic analyses of SgcC5-catalyzed coupling between **9** or **10** and **3**: (A) the reaction equation and (B) the rate plots.

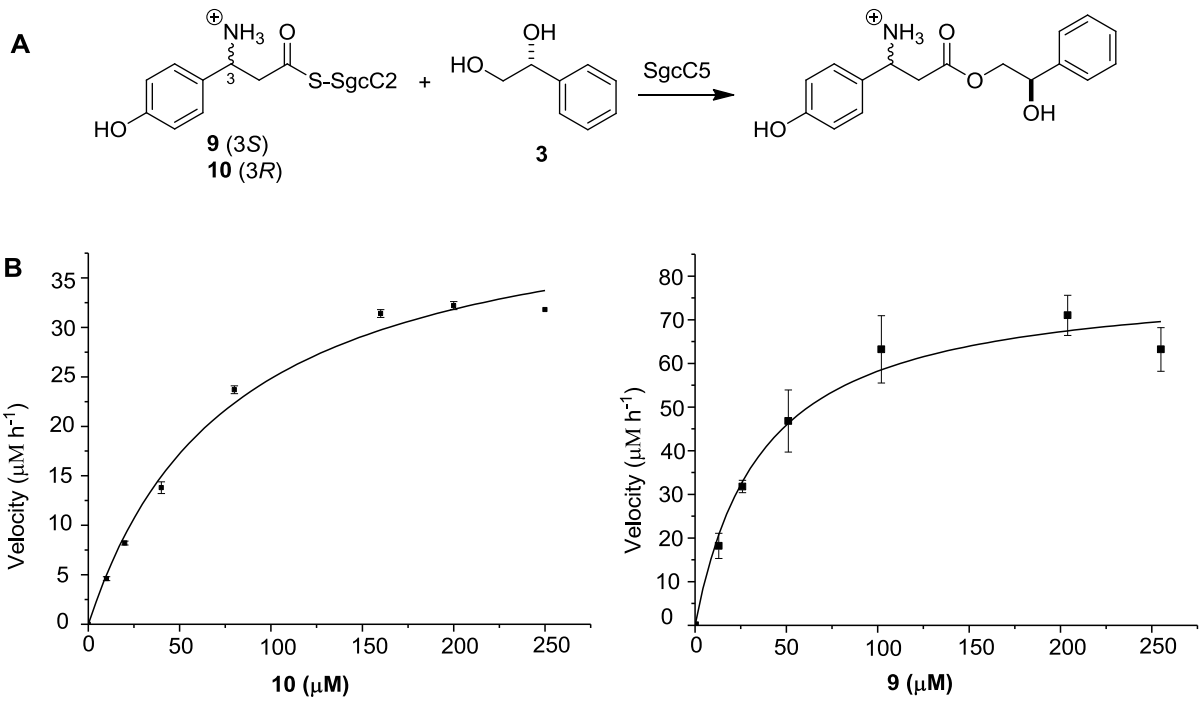


Figure S2. Pseudo first-order kinetic analyses of SgcC5-catalyzed coupling between **2** and **3** or **11**: (A) the reaction equation and (B) the rate plots.

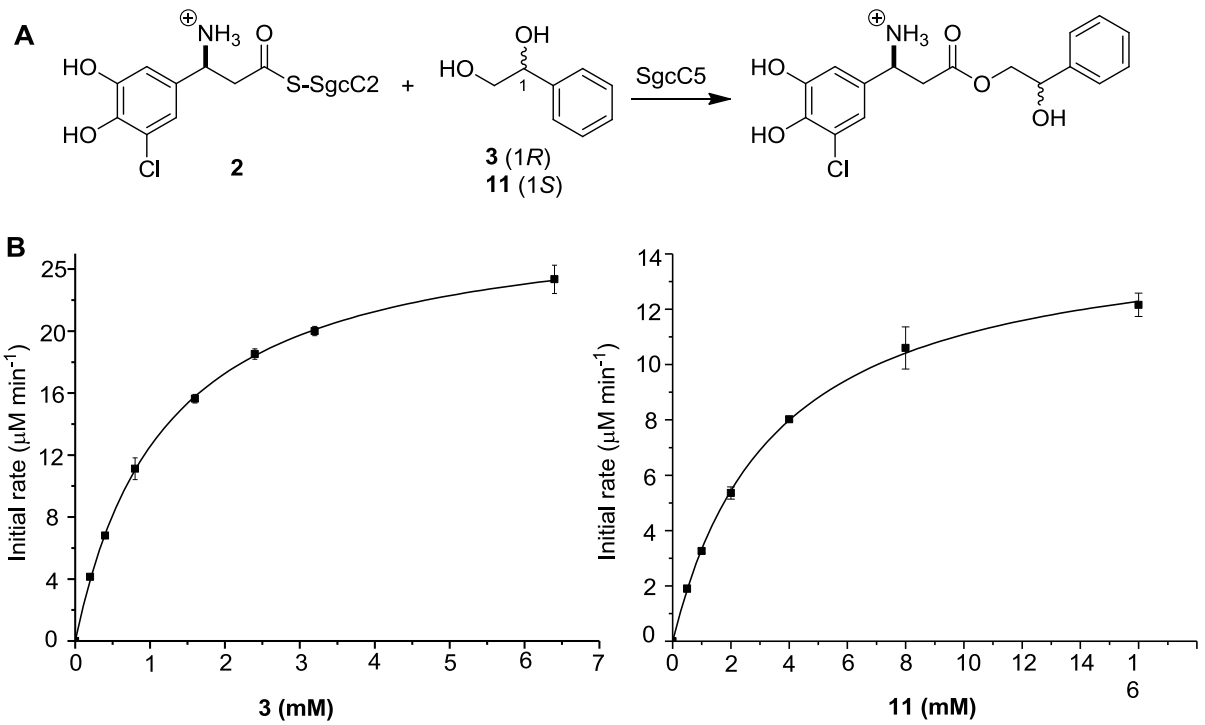
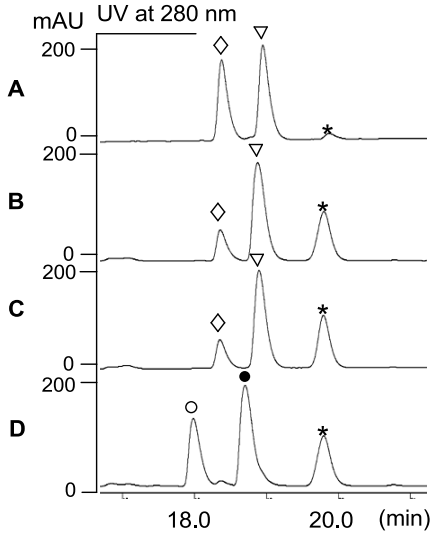


Figure S3. HPLC analysis of kinetic resolution of a racemic mixture of (*R*)-1-phenyl-1,2-ethanediol **(3)** and (*S*)-1-phenyl-1,2-ethanediol **(11)** by SgcC5: (A) SgcC5-catalyzed condensation reaction between **2** and **3** with 10 μM SgcC5 for 1 hr to form the coupling products **2a** (▽) and **2b** (◊); SgcC5-catalyzed condensation between **2** and a racemic mixture of **3** and **11** incubated with (B) 10 μM SgcC5 and (C) 5 μM SgcC5 for 10 min; (D) SgcC5-catalyzed condensation between **2** and **11** with 100 μM SgcC5 incubation for 2 hrs to form **2c** (●) and **2d** (○). The other peak (*) at retention time 19.7 min is 4,5-dihydroxy-1,2-dithiane presented in the assay. [S2]



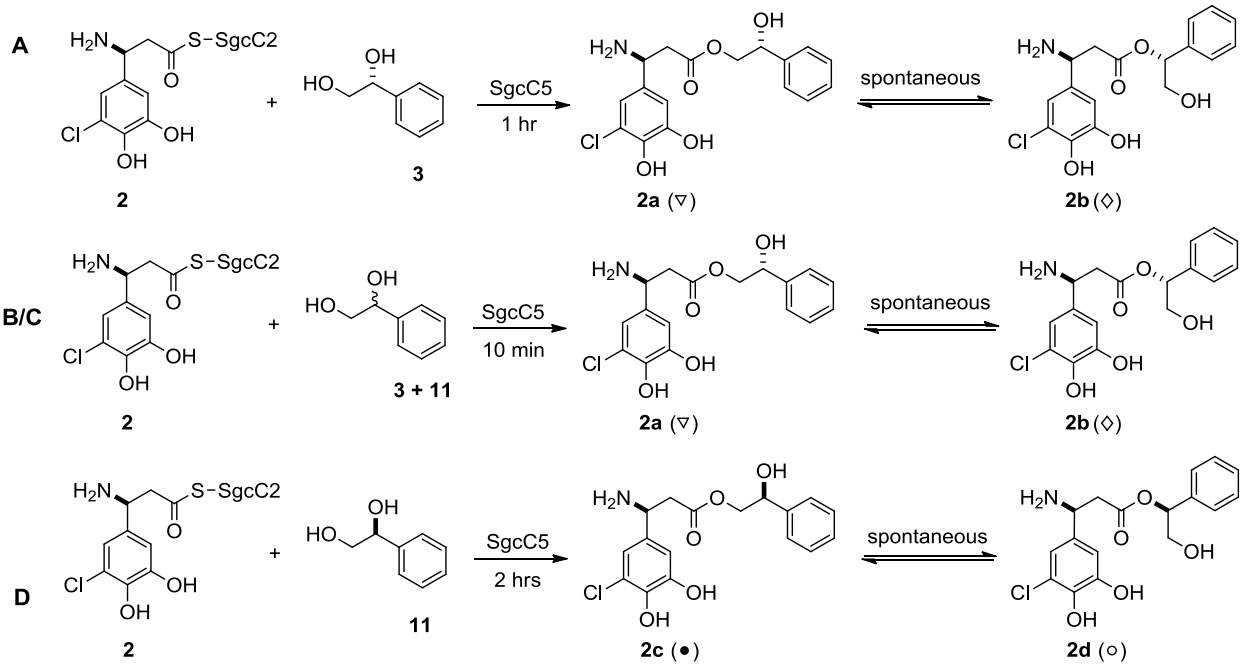


Figure S4. Chiral HPLC analyses to determine the stereochemistry of SgcC2-tethered (*R*)- or (*S*)-3-chloro-5-hydroxy-β-tyrosine: (I) synthetic (±)-3-chloro-5-hydroxy-β-tyrosine standard, (II) (*S*)-3-chloro-5-hydroxy-β-tyrosine released from the (*S*)-3-chloro-5-hydroxy-β-tyrosyl-S-SgcC2 prepared by SgcC1-catalyzed loading (*S*)-3-chloro-5-hydroxy-β-tyrosine from (±)-3-chloro-5-hydroxy-β-tyrosine pool onto SgcC2 used as the donor substrate for SgcC5, (III) (*S*)-3-chloro-5-hydroxy-β-tyrosine prepared from the enzymatic C-5 hydroxylation and C-3 chlorination of (*S*)-β-tyrosyl-S-SgcC2, and (IV) (*R*)-3-choro-5-hydroxy-β-tyrosine prepared from the enzymatic C-5 hydroxylation and C-3 chlorination of (*R*)-β-tyrosyl-S-SgcC2. (*S*)-3-chloro-5-hydroxy-β-tyrosine (•); (*R*)-3-chloro-5-hydroxy-β-tyrosine (♦).

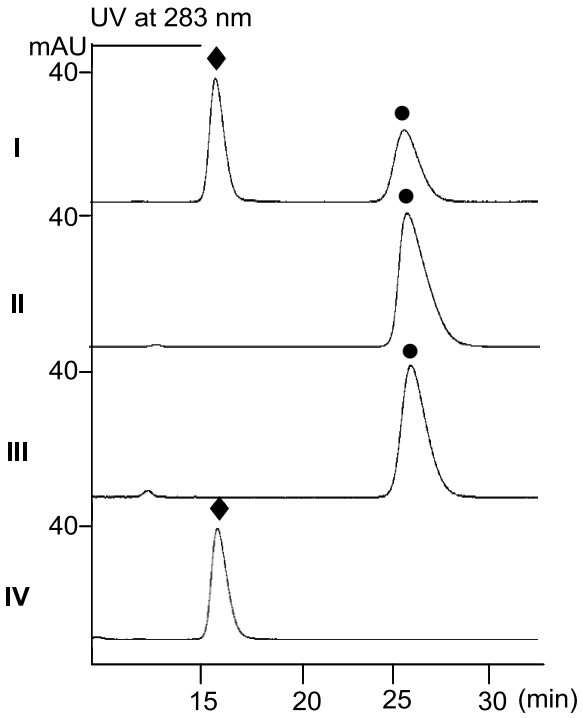
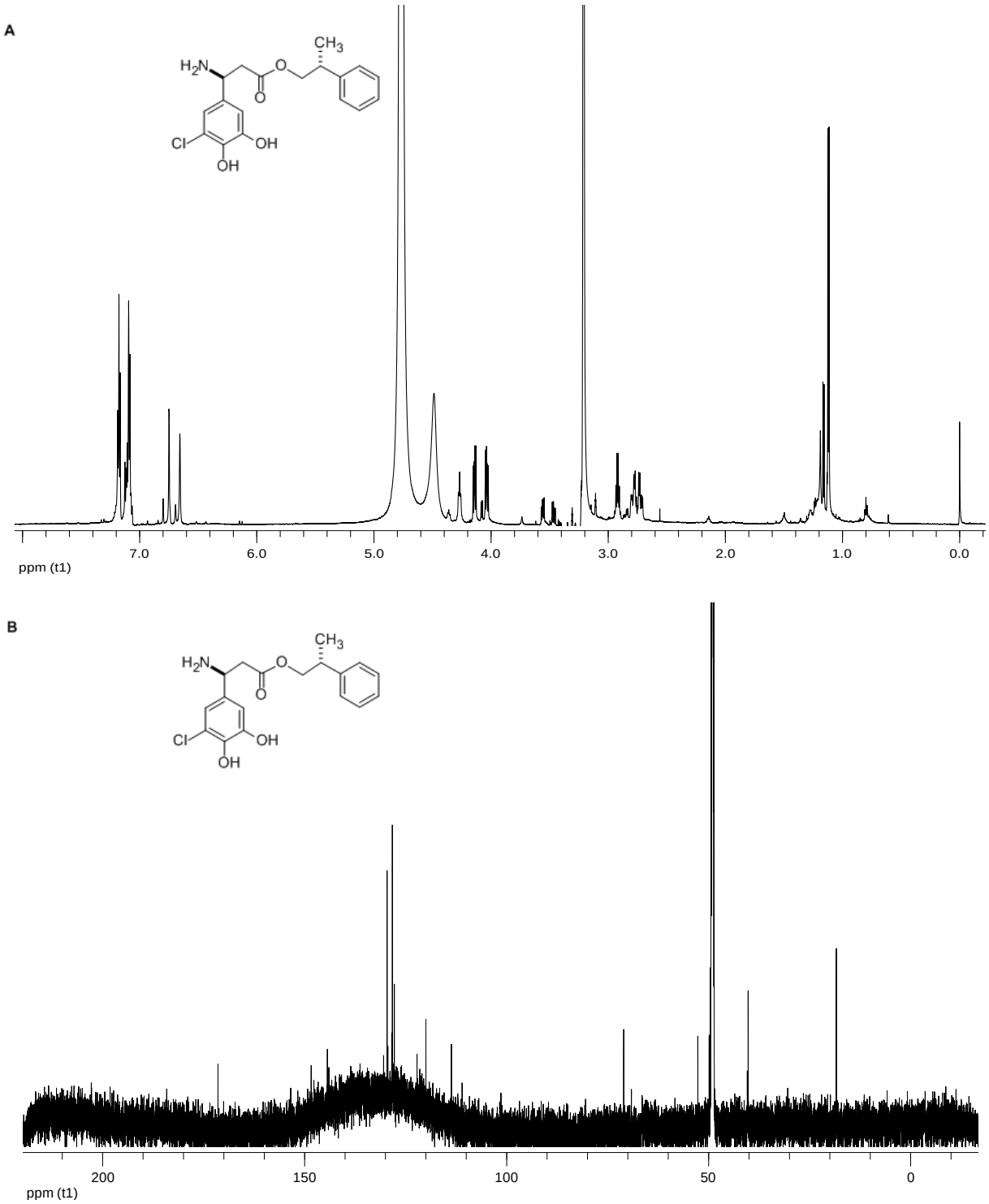


Figure S5. ^{1}H (A) and ^{13}C (B) NMR spectra of the coupling product between **2** and **19**



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(S1) Lin, S.; Van Lanen, S. G.; Shen, B. *J. Am. Chem. Soc.* **2007**, *129*, 12432-12438.

(S2) Lin, S.; Van Lanen, S. G.; Shen, B. *J. Am. Chem. Soc.* **2008**, *130*, 6616-6623.

(S3) Sanchez, C.; Du, L.-C.; Edwards, D. J.; Toney, M. D.; Shen, B. *Chem. Biol.* **2001**, *8*, 725-738.

(S4) Van Lanen, S. G.; Dorrestein, P. C.; Christenson, S. D.; Liu, W.; Ju, J. H.; Kelleher, N. L.; Shen, B. *J. Am. Chem. Soc.* **2005**, *127*, 11594-11595.

(S5) Lin, S.; Van Lanen, S. G.; Shen, B. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 4183-4188.