Regiospecific Chlorination of (*S*)-β-Tyrosyl-S-Carrier Protein Catalyzed by SgcC3 in the Biosynthesis of the Enediyne Antitumor Antibiotic C-1027

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Supporting Information

General information. Coenzyme A (CoA), adenosine triphosphate disodium salt (ATP), flavin adenine dinucleotide disodium salt (FAD), flavin adenine mononucleotide sodium salt dihydrate (FMN), β-nicotinamide adenine dinuceotide reduced disodium salt (NADH), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), were purchased from Sigma-Aldrich (St. Louis, MO). IPTG and dithiothreitol was purchased from Research Products International Corp. (Mt. Prospect, IL). The starting materials for the synthesis of β-amino acid analogues including 3-chloro-4-hydroxybenzaldehyde, 3-chloro-4-hydroxy-5-methoxybenzaldehyde, 3-bromo-4-hydroxybenzaldehyde, 3,4-dibenzyloxybenzaldehyde, boron tribromide and palladium (10 wt. %) on activated carbon were purchased from Sigma-Aldrich and used without further purification. (*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). Media components and all other chemicals were from Fisher Scientific (Fairlawn, NJ). Electrospray ionization-mass spectrometry (ESI-MS) was measured with an Agilent 1100 HPLC-MSD SL ion trap mass spectrometer (Agilent Technologies, Inc., Santa Clara, CA). NMR was recorded on a Varian Unity Inova 400 or 500 MHz NMR Spectrometer (Varian, Inc., Palo Alto, CA). PCR was performed with a PerkinElmer GeneAmp 2400 (PerkinElmer Life And Analytical Sciences, Inc., Waltham, MA).

Synthesis of 3-chloro-β-tyrosine. Synthesis of 3-chloro-β-tyrosine was achieved using the method reported by Weaver.[1] 3-Chloro-4-hydroxybenzaldehyde (76 mg) was refluxed with 1 equivalent of malonic acid (51 mg) and 2 equivalents of ammonium acetate (76 mg) in 5 mL of ethanol for 7 hrs under an argon atmosphere. The reaction mixture was adjusted to pH 4 and separated on a strongly acidic cation-exchange column (Dowex® 50W-X8). The product, 3-chloro-β-tyrosine, was eluted with 1% ammonium hydroxide. Solvent was removed through evaporation under reduced pressure, and the residue was dissolved in distilled water and further purified by C18-reverse phase chromatography. ^{1}H NMR (D_2O - CF_3CO_2D, 400 MHz): δ 7.08 (d, J = 2.4 Hz, 1H), 6.87 (dd, J = 8.8, 2.4 Hz, 1H), 6.65 (d, J = 8.4 Hz, 1H), 4.30 (t, J = 7.2 Hz,

Hβ, 1H), 2.77 (dd, *J* = 17.2, 7.6 Hz, Hα, 1H), 2.66 (dd, *J* = 17.2, 6.4 Hz, Hα, 1H). ^{13}C NMR (D_2O - CF_3CO_2D, 100 MHz): δ 173.1 (C=O), 152.8 (ArC), 129.2 (ArC), 128.0 (ArC), 127.1 (ArC), 120.7 (ArC), 117.6 (ArC), 50.7 (C_β), 37.5 (C_α). ESI-MS *m/z* 216.0 and 218.0 for $[M + H]^+$ and *m/z* 213.9 and 216.0 for $[M - H]^-$; calculated for $C_9H_{10}NO_3Cl$, 215.0 and 217.0. HRMS-ESI-MS *m/z* 214.0263 for $[M - H]^-$; calculated $[M - H]^-$ for $C_9H_{10}NO_3Cl$, 214.0271.

Synthesis of 3-bromo-β-tyrosine. 3-Bromo-β-tyrosine was prepared as described above using 3-bromo-4-hydroxybenzaldehyde as starting material. ^{1}H NMR (D_2O - CF_3CO_2D, 400 MHz): δ 7.60 (d, *J* = 2.4 Hz, 1H), 7.27 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 4.65 (t, *J* = 7.2 Hz, 1H), 3.11 (dd, *J* = 17.2, 8.0 Hz, 1H), 3.00 (dd, *J* = 17.6, 6.8 Hz, 1H). ^{13}C NMR (D_2O - CF_3CO_2D, 100 MHz): δ 173.2 (C=O), 153.8 (ArC), 132.3 (ArC), 128.7 (ArC), 128.1 (ArC), 117.2 (ArC), 110.1 (ArC), 50.8 (C_β), 37.8 (C_α). ESI-MS *m/z* 260.0 and 262.0 for $[M + H]^+$ and *m/z* 243.0 and 245.0 for $[M + H - NH_3]^+$; calculated for $C_9H_{10}NO_3Br$, 259.0 and 261.0. HRMS-ESI-MS *m/z* 257.9757 for $[M - H]^-$; calculated $[M - H]^-$ for $C_9H_{10}NO_3Br$, 257.9766.

Synthesis of 3-hydroxy-β-tyrosine. 3-Hydroxyl-β-tyrosine was prepared as described above using 3,4-dibenzyloxybenzaldehyde as a starting material. After purification with silica chromatography, the protecting groups were removed by catalytic hydrogenation with 10% Pd/C. Benzyl alcohol was removed using C18-reverse phase chromatography to yield a light brown powder. ^{1}H NMR (D_2O, 400MHz): δ 6.74-6.85 (m, 3H), 4.42 (t, *J* = 6.8 Hz, Hβ, 1H), 2.79 (dd, *J* = 15.6, 8.0 Hz, Hα, 1H), 2.69 (dd, *J* = 16.0, 6.4 Hz, Hα, 1H). ^{13}C NMR (D_2O 125 MHz): δ 177.9 (C=O) 145.5 (ArC), 145.6 (ArC), 128.7 (ArC), 119.4 (ArC), 116.6 (ArC), 115.0 (ArC), 52.7 (C_β), 40.9 (C_α). ESI-MS *m/z* 198.0 for $[M + H]^+$ and *m/z* 195.9 for $[M - H]^-$, calculated for $C_9H_{10}NO_4$, 197.0. HRMS-ESI-MS *m/z* 196.0610 for $[M - H]^-$; calculated $[M - H]^-$ for $C_9H_{10}NO_4$, 196.0603.

Synthesis of 3-chloro-5-hydroxy-β-tyrosine. 3-Chloro-4,5-dihydroxybenzaldehyde was prepared by following the procedure reported by Hua and co-workers[2]. To a solution containing 2.0 g of 3-chloro-4-hydroxy-5-methoxybenzaldehyde in 20 mL of dried dichoromethane was added 1.2 mL of boron tribromide. The resulting solution was stirred at 0 ^{o}C for 30 min, heated to room temperature, and incubated at room temperature for an additional 3 hrs. After removal of a trace amount of unreacted 3-chloro-4-hydroxy-5-methoxybenzaldehyde by flash silica column chromatography, the 3-chloro-4,5-dihydroxybenzaldehyde product was added to 40 mL anhydrous ethanol under argon, and was refluxed with ammonium acetate and malonic acid until all 3-chloro-4,5-dihydroxy-benzaldehyde reacted as judged by thin layer chromatography (TLC). 3-Chloro-5-hydroxy-β-tyrosine was purified by C18-reverse phase chromatography using standard conditions. ^{1}H NMR (D_2O, 500 MHz): δ 7.04 (d, *J* = 2.5 Hz, 1H), 6.92 (d, *J* = 2.5 Hz, 1H), 4.60 (t, *J* = 7.5 Hz, 1H), 3.04 (dd, *J* = 17.0, 8.0 Hz, 1H), 2.95 (dd, *J* = 17.0, 6.5 Hz, 1H). ^{13}C NMR (D_2O, 125 MHz): δ 175.1 (C=O), 146.1 (ArC), 141.8 (ArC), 128.6 (ArC), 121.8 (ArC), 120.2 (ArC), 113.6 (ArC), 51.6 (C_β), 38.8 (C_α). ESI-MS *m/z* 232.1 and 234.1 for $[M+H]^+$ and *m/z* 230.1 and 232.1 for $[M-H]^-$; calculated for $C_9H_{10}NO_4Cl$, 231.0 and 233.0. HRMS-ESI-MS *m/z* 230.0211 for $[M - H]^-$; calculated $[M - H]^-$ for $C_9H_{10}NO_4Cl$, 230.0220.

Table S1. Primers used for amplification of the sgcC2, sgcC3, sgcE6, and E. coli fre genes for heterologous expression.

Name of primer	Sequence[a]
SgcC2 forward	5'-GACGACGACAAGATGTCCACCGTTTCCGAC-3'
SgcC2 reverse	5'-GAGGAGAAGCCCGGTCACTGCGTTCCGGAGCC-3'
SgcC3 forward	5'-GGTATTGAGGGTCGCATGGACGTGTCAGCGCAGTAC-3'
SgcC3 reverse	5'-AGAGGAGAGTTAGAGTCAGGACCGCGCACCGGG-3'
SgcE6 forward	5'-GGTATTGAGGGTCGCATGAGTCCGATCATCGCTCC-3'
SgcE6 reverse	5'-AGAGGAGAGTTAGAGTCATGCCGCCCTTCCTTCG-3'
E.Coli Fre forward	5'-GGTATTGAGGGTCGCATGACAACCTTAAGCTGTAAAG-3'
E.Coli Fre reverse	5'-AGAGGAGAGTTAGAGTCAGATAAATGCAAACGCATCG-3'

[a]The start (ATG) and stop (TGA) codons are underlined.

Figure S1. HPLC chromatograms for determination of the FAD cofactor non-covalently bound to SgcC3: (**I**) FAD (♦) standard; (**II**) supernatant from denatured SgcC3 by heat; (**III**) FMN (●) standard.

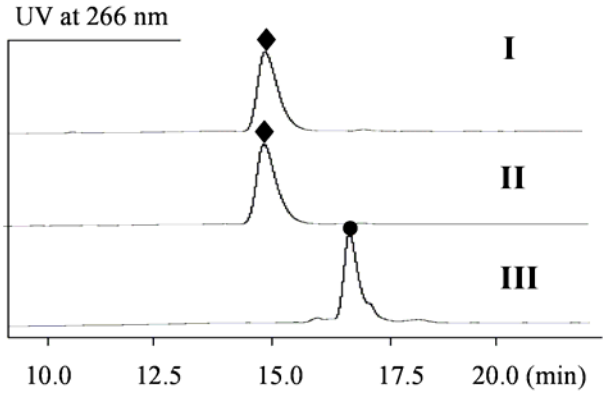


Figure S2. ESI-MS spectra of (*S*)-3-chloro-β-tyrosine (**13**) released from hydrolysis of (*S*)-3-chloro-β-tyrosyl-S-SgcC2 (**10**): (**A**) in positive mode showing $[M + H]^+$ ions at *m/z* 216 and 218 and $[M + H - NH_3]^+$ ions at *m/z* 199 and 201 and (B) in negative mode showing $[M - H]^-$ ions at *m/z* 214 and 216.

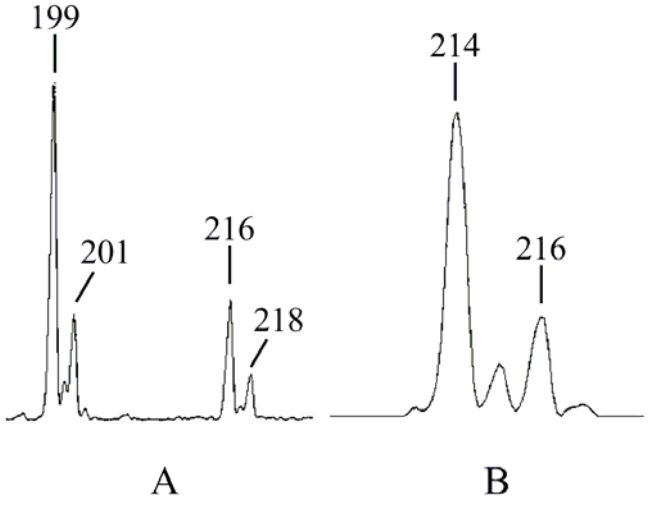
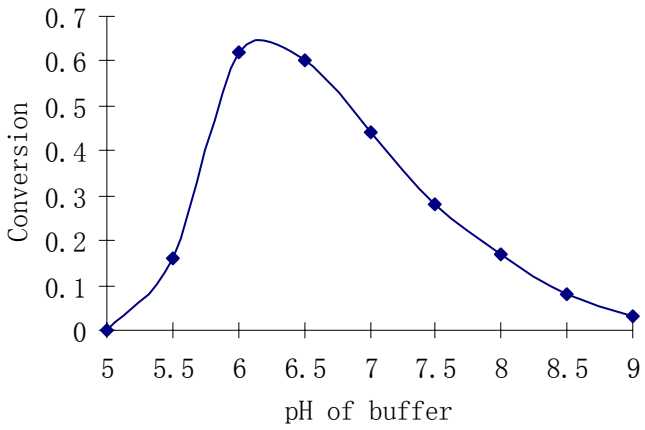


Figure S3. Relative activity of SgcC3 as a halogenase determined in 50 mM sodium acetate, pH 5.0 - 5.5, 50 mM sodium phosphate, pH 6.0 - 8.0, and Tris-HCl, pH 8.5 - 9.0.



References

(1) Tan, C. Y. K.; Weaver, D. F. *Tetrahedro*n **2002**, *58*, 7449-7461.
(2) Hua, T. C.; Huang, X.; Chen, Y.; Battina, S. K.; Tamura, M.; Noh, S. K.; Koo, S. I.; Namatame, I.; Tomoda, H.; Perchellet, E. M.; Perchellet, J.-P. *J. Org. Chem.* **2004**, *69*, 6065-6078.