Supporting Information
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Potent Oligomerization and Macrocyclization Activity of the Thioesterase Domain of Vicenistatin Polyketide Synthase

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Experimental Section

Preparation of thioesterase domains (VinTE, HlsTE, and GfsTE)
The gene for VinP4TE domain (VinTE) was amplified by PCR with previously obtained cosmids as template, which contains the vin gene cluster.1,2 Primers VinTE3-N 5’-GCCcatGgCGGACGACGACGCGGAC-3’ and VinTE-C 5’-TCCGGCGCTCGACaaGCTtATGGGG-3’ were used for the amplification. PCRs were carried out using KOD –Plus– polymerase (Toyobo) according to the manufacture’s protocol. The amplified DNA fragments were ligated into Litmus28 vector. After transformation with E. coli DH5α competent cells and selection of positive colonies, the plasmids were extracted and sequenced to confirm the DNA sequences. Desired plasmids were digested with NcoI-HindIII and ligated to linearized pET30b vector via the corresponding restriction sites.

The recombinant VinTE was expressed in E. coli BL21(DE3) cells transformed with the corresponding plasmid. Cultivation was carried out in 2.4 L of Luria Bertani (LB) medium containing 30 μg/mL kanamycin at 37°C under agitation (200 rpm). The expression was induced at mid-log phase (absorbance at 600 nm = 0.6 ~ 1.0) with 0.1 mM isopropyl β-D-thiogalactopyranoside (IPTG). The culture was continued for 3 hr at 37°C and the cells were collected by centrifugation (7,000 rpm, at 4 °C for 20 min). The cell paste was re-suspended in ten times weight of 100 mM sodium phosphate buffer (pH 7.0) and the cells were disrupted by sonication. The resulting suspension was centrifuged (10,000 rpm at 4°C for 20 min) and the supernatant was loaded on a His·bind resin (Novagen) column. After washing with 100 mM sodium phosphate buffer, the proteins were eluted with the same buffer containing 200 mM imidazole. The collected fractions were analyzed by SDS-PAGE (Figure S1) and the fractions containing the pure proteins were concentrated with Vivaspin 20 centrifugal concentrator (10,000 MWCO, Sartorius stedim biotech). Usually, 18 mg of pure VinTE was obtained from the cells of 1 L culture. Protein concentrations were estimated by Lowry method with BSA as standard.

A TE domain from halstoctacosanolide biosynthetic gene (HlsTE)3 was cloned with primers HlsTE-N 5’-CGGCCCCCAGGCAATATGTCCCAGGCGGTG-3’ and HlsTE-C
5’-GGTGTGGATCCTGCAAAACGGTCTAC, and a TE domain from FD-891 biosynthetic pathway (GfsTE)4 was also cloned with primers GfsTE-N 5’-CGCATATGGGGGGCGGCACC-3’ GfsTE-C and 5’-TCATCTCTCGAGATCAGGACAGGG-3’ using with PrimeStar Polymerase (Takara) according to the manufacture’s protocol. Appropriate cosmid DNAs were used as template for PCR. Desired plasmid clones in pT7Blue vector were digested with appropriate restriction enzymes and the DNA fragments were ligated to linearized pET28a vector via the corresponding restriction sites. HlsTE and GfsTE were expressed and purified in the same manner as VinTE.

Site-directed mutagenesis: Single amino acid mutation in VinTE was introduced by QuikChange (Stratagene) method according to the manufacturer’s protocol with the primer 5’-GTCCTCCTCGCTACgCGCTGGCGGTGTC-3’ and its complemented oligonucleotide. The obtained mutant proteins (S136A) was purified in the same manner as the wild type of VinTE and used for the enzymatic assay.

Enzymatic assay (a general method)
One mL of 10 mM ω-hydroxy fatty acid ethyl esters in DMSO and 9 mL of TE protein (final 60-300 μM) in 100 mM sodium phosphate buffer (pH 7.0) were mixed and the mixture was incubated at 28°C for 24 hr. Enzymatic reaction products were extracted with excess amount of CHCl3 and the dried extract was purified by silica-gel chromatography.
Preparation of ω-hydroxy fatty acid ethyl esters

Ethyl 6-hydroxyhexanoate (compound 1, 6-hydroxyhexanoic acid ethyl ester) was purchased from Aldrich.

Ethyl 8-hydroxyoctanoate 2 (8-hydroxyoctanoic acid ethyl ester)\(^5,6\)

8-Octanolid (86 mg) was treated with 5 mL of ethanol and 0.3 mL of sulfuric acid under refluxing condition for 6 hr. The reaction mixture was neutralized with sat. NaHCO\(_3\) solution. After removal of ethanol, the residue was extracted with CHCl\(_3\) and the extract was dried over MgSO\(_4\). The solvent was removed and the residue was purified by silica-gel chromatography to afford 2 (54 mg, 44%). \(^1\)H-NMR (500 MHz, CDCl\(_3\)): \(\delta\) 4.12 (q, \(J = 7.2\) Hz, 2H), 3.62 (t, \(J = 6.6\) Hz, 2H), 2.28 (t, \(J = 7.5\) Hz, 2H), 1.62 (m, 2H), 1.55 (m, 2H), 1.33 (m, 6H), 1.24 (t, \(J = 7.2\) Hz, 3H); \(^13\)C-NMR (125 MHz, CDCl\(_3\)): \(\delta\) 173.9, 62.9, 60.2, 34.3, 32.7, 29.06, 29.02, 25.5, 24.9, 14.2.

Ethyl (2E)-8-hydroxy-2-octenoate 3 ((2E)-8-hydroxy-2-octenoic acid ethyl ester)\(^6\)

1,6-Hexanediol (TCI, 2.0 g, 17 mmol) was dissolved in 30 mL of CH\(_2\)Cl\(_2\) and followed by addition of \(p\)-toluenesulfonic acid (0.2 g) and 3,4-dihydro-2\(H\)-pyran (1.6 mL) at 0°C. After stirring for 1.5 hr at 0°C, the solution was washed with sat. NaHCO\(_3\) and brine, and then dried over Na\(_2\)SO\(_4\). After removal of the solvent, the residue was purified by silica-gel chromatography to afford mono-THP-ether (1.2 g, 35%).

THP protected compound was then added to a mixture of oxalyl chloride (0.8 mL) and DMSO (0.9 mL) in 40 mL of CH\(_2\)Cl\(_2\) at -78°C. After stirring for 2 hr at -78°C, triethylamine (4.3 mL) was added and the mixture was stirred for 5 min at -78°C. The reaction temperature was gradually raised up to room temperature. The reaction was quenched with water, and the mixture was extracted with CH\(_2\)Cl\(_2\). The organic layer was washed with brine, and then dried over Na\(_2\)SO\(_4\). After removal of the solvent, the aldehyde was used for the next reaction without purification.

NaH (134 mg) was reacted with triethyl phosphonoacetate (789 mg) in THF solution (5 mL) at 0°C. After 0.5 hr at 0°C, the aldehyde (700 mg) in 15 mL THF was added into the solution. After 1 hr, sat. NH\(_4\)Cl solution was added and the product was extracted with CH\(_2\)Cl\(_2\). The organic layer was washed with water and brine, and then dried over Na\(_2\)SO\(_4\). After removal of the solvent, the residue was treated with \(p\)-toluenesulfonic acid in ethanol (10 mL) at room temperature for 1.5 hr. After removal of the solvent, the residue was purified by silica-gel chromatography to afford 3 (443 mg, 37% in 3 steps). \(^1\)H-NMR (500 MHz, CDCl\(_3\)): \(\delta\) 6.95 (dt, \(J = 15.6, 7.1\) Hz, 1H), 5.81 (dt, \(J = 15.6, 1.6\) Hz, 1H), 4.18 (q, \(J = 7.2\) Hz, 2H), 3.64 (t, \(J = 6.3\) Hz, 2H), 2.21 (dq, \(J = 1.6, 7.1\) Hz, 2H), 1.63 (br, 1H), 1.59 (m, 2H), 1.49 (m, 2H), 1.41 (m, 2H), 1.29 (t, \(J = 7.2\) Hz, 3H); \(^13\)C-NMR (125 MHz, CDCl\(_3\)): \(\delta\) 166.7, 149.0, 121.5, 62.8, 60.2, 32.5, 32.1, 27.8, 25.3, 14.3.
Ethyl 12-hydroxylaurate 4 (12-hydroxydodecanoic acid ethyl ester)

To a solution of acetic anhydride (13 mL, 132 mmol) in 16 mL of dichloromethane at 0°C, 10 mL of 30% H₂O₂ and maleic anhydride (10 g, 102 mmol) were added, and the mixture was stirred at 0°C for 2.5 hr and at reflux for 2.5 hr. Cyclododecanone (2.5 g, 13.7 mmol, TCI) was then added into the solution. The mixture was stirred at reflux for 5 hr and at room temperature for 11 hr. The precipitates were filtered and the filtrate was washed with water, 10% aqueous KOH, and aqueous Na₂SO₃. The organic layer was dried over anhydrous MgSO₄ and the solvent was removed by evaporator. The residue was purified by silica-gel chromatography to afford 12-dodecanolide (1.3 g, 48%). ¹H-NMR (300 MHz, CDCl₃): δ 4.16 (m, 2H), 2.36 (m, 2H), 1.67 (m, 4H), 1.35 (brs, 14H).

The obtained 12-dodecanolide (1.3 g, 6.6 mmol) was treated with ethanol solution of sulfuric acid (one drop of Pasteur pipet) in 34 mL of ethanol under reflux for 5 hr. The reaction mixture was neutralized with sat. NaHCO₃ solution. After removal of ethanol, the mixture was extracted with CHCl₃ and the organic layer was dried over MgSO₄. The solvent was removed by evaporator and the residue was purified by silica-gel chromatography to afford 4 (0.32 g, 19%). ¹H-NMR (500 MHz, CDCl₃): δ 4.12 (q, J = 7.2 Hz, 2H), 3.63 (t, J = 6.6 Hz, 2H), 2.28 (t, J = 7.6 Hz, 2H), 1.58 (m, 4H), 1.27 (m, 14H), 1.25 (t, J = 7.2 Hz, 3H); ¹³C-NMR (125 MHz, CDCl₃): δ 173.9, 63.1, 60.1, 34.4, 32.8, 29.54, 29.47, 29.39 (x2), 29.23, 29.12, 25.7, 25.0, 14.3.

Ethyl (9Z)-12-hydroxy-9-dodecenate 5 ((9Z)-12-hydroxy-9-dodecenoic acid ethyl ester)

Ethyl oleate (TCI, 4.2 g, 13.6 mmol) was dissolved in 50 mL of CH₂Cl₂ and ozone was bubbled onto the solution at -60°C for 5 hr. After removal of ozone gas with N₂ gas, PPh₃ (7.0 g, 26.7 mmol) was added to the solution and the mixture was stirred at room temperature for 18 hr. After removal of the solvent, the residue was purified by silica-gel chromatography to afford 4 (0.32 g, 98%). ¹H-NMR (300 MHz, CDCl₃): δ 9.76 (t, 1.7 Hz, 1H), 4.14 (q, J = 7.1 Hz, 2H), 2.42 (dt, J = 1.7, 7.3 Hz, 2H), 2.29 (t, J = 7.5 Hz, 2H), 1.63 (brs, 4H), 1.33 (brs, 6H), 1.25 (t, J = 7.1 Hz, 3H).

THP ether of 3-bromo-1-propanol (4.1 g, 18.3 mmol) in 20 mL of acetonitrile was mixed with PPh₃ (7.5 g, 28.5 mmol) and K₂CO₃ (4.0 g, 28.7 mmol). The mixture was refluxed for 19 hr. The residual solid was filtered and the solvent was removed by evaporator. The residue was dissolved in CHCl₃ and followed by addition of excess amount of diethyl ether to give phosphonium bromide (5.2 g, crude).

The phosphonium bromide (4.5 g) was dissolved in anhydrous THP under argon atmosphere and KHMDS toluene solution (25 mL, 13 mmol) was added at -78°C, followed by ethyl 8-oxooctanoate (2.4 g, 12 mmol). The reaction temperature was gradually raised up to room temperature and the solution was stirred for 10 hr at room temperature. The reaction was quenched by addition of water and the mixture was extracted with diethyl ether and washed with brine. After
removal of the solvent, the residue was purified by silica-gel chromatography to afford THP protected 5 (1.3 g, 32%), which was then treated with PPTS (0.35 g, 1.4 mmol) in ethanol (30 mL) at 55°C for 5 hr. After removal of the solvent, the residue was purified by silica-gel chromatography to afford 5 (0.45 g, 48%). \( ^1\)H-NMR (500 MHz, CDCl\(_3\)): \( \delta \) 5.55 (m, 1H), 5.34 (m, 1H), 4.12 (q, \( J = 7.2 \) Hz, 2H), 3.64 (t, \( J = 6.6 \) Hz, 2H), 2.32 (brq, \( J = 6.5 \) Hz, 2H), 2.28 (t, \( J = 7.5 \) Hz, 2H), 2.05 (brq, \( J = 7.0 \) Hz, 2H), 1.61 (m, 2H), 1.30 (m, 8H), 1.25 (t, \( J = 7.2 \) Hz, 3H); \(^{13}\)C-NMR (125 MHz, CDCl\(_3\)): \( \delta \) 173.9, 133.4, 125.0, 62.3, 60.2, 34.4, 30.8, 29.6, 29.06, 29.03, 27.3, 24.9, 14.3; HR-MS (FAB, glycerol) m/z 243.1975 (M+H), calcd for C\(_{14}\)H\(_{27}\)O\(_3\): 243.1960.

N-Acetylcysteamine thioester of (2E,4E,6S,7S,8E)-6,12-dihydroxy-2,4,6-trimethyldodeca-2,4,8-trienoic acid 6

To a solution of 6-triisopropylsilyl ether of ethyl ester of 6 (100 mg, 0.221 mmol) in THF was added tetrabutylammonium fluoride (531 \( \mu \)L, 0.531 mmol) at 0°C. The mixture was warmed to room temperature, and was stirred for 1 hr. The resulting solution was re-cooled to 0°C, the reaction was quenched by addition of aq. NH\(_4\)Cl solution. The mixture was diluted with ethyl acetate. The organic layer was separated, and the aqueous portion was extracted with ethyl acetate. The combined organic layer was washed with brine and dried over Na\(_2\)SO\(_4\). After evaporation of the solvent, the residue was purified by silica gel column chromatography to afford 80 mg of diol (crude); IR (neat): 3392, 1698 cm\(^{-1}\); \([\alpha]\)\(_{26}\) D +27.5 (c 1.83, CHCl\(_3\)). \(^1\)H-NMR (CDCl\(_3\)): \( \delta \) 7.10 (s, 1H), 5.68 (dt, \( J = 15.2, 6.8 \) Hz, 1H), 5.50 (dd, \( J = 15.2, 6.8 \) Hz, 1H), 5.40 (d, \( J = 10.0 \) Hz, 1H), 4.20 (q, \( J = 7.2 \) Hz, 1H), 3.95 (dd, \( J = 6.8, 6.8 \) Hz, 1H), 3.65 (t, \( J = 6.4 \) Hz, 2H), 2.65 (m, 1H), 2.13 (dt, \( J = 6.8, 6.8 \) Hz, 2H), 1.98 (d, \( J = 1.2 \) Hz, 3H), 1.85 (s, 3H), 1.65 (tt, \( J = 6.8, 6.4 \) Hz, 2H), 1.30 (t, \( J = 7.2 \) Hz, 3H), 1.04 (d, \( J = 8.4 \) Hz, 1H); \(^{13}\)C-NMR (CDCl\(_3\)): \( \delta \) 169.0, 142.6, 137.1, 132.6, 132.4, 131.0, 125.8, 62.3, 60.7, 39.2, 32.0, 28.6, 17.7, 16.5, 14.1, 12.3; Anal. Calcd. For C\(_{17}\)H\(_{20}\)O\(_4\): C, 68.89; H, 9.52. Found: C, 68.61; H, 9.61.

To a solution of the crude ethyl ester (80 mg) in water-dioxane (1 : 2, 4.4 mL) was added LiOH \cdot H\(_2\)O (21 mg, 0.504 mmol). After stirring for 11 hr, the solution was evaporated to remove dioxane. The aqueous portion was neutralized with 1 M citric acid, and extracted with ethyl acetate. The organic layer was separated, and the aqueous portion was extracted with ethyl acetate. The combined organic solution was washed with brine and dried over anhydrous Na\(_2\)SO\(_4\). After evaporation of the solvent, the residue was purified by silica gel column chromatography (EtOAc) to give carboxylate (49 mg, 82% in 2 steps). IR (neat): 3448, 1646 cm\(^{-1}\); \([\alpha]\)\(_{26}\) D +38.4 (c 0.35, CH\(_3\)OH); \(^1\)H-NMR (CDCl\(_3\)): \( \delta \) 7.23 (s, 1H), 5.69 (dt, \( J = 15.2, 6.8 \) Hz, 1H), 5.50 (dd, \( J = 15.2, 6.4 \) Hz, 1H), 5.47 (d, \( J = 10.0 \) Hz, 1H), 3.96 (t, \( J = 6.4 \) Hz, 1H), 3.65 (t, \( J = 6.4 \) Hz, 1H), 2.68 (m, 1H), 2.15 (q, \( J = 6.8 \) Hz, 2H), 2.01 (d, \( J = 1.2 \) Hz, 3H), 1.88 (d, \( J = 1.2 \) Hz, 3H), 1.65 (quint, \( J = 6.8 \) Hz, 2H), 1.05 (d, \( J = 6.8 \) Hz, 3H); \(^{13}\)C-NMR (CDCl\(_3\)): \( \delta \) 173.7, 144.9, 138.6, 132.6, 132.4, 131.0, 124.7,
To a mixture of carboxylate (22 mg, 0.082 mmol), 1,3-dicyclohexylcarbodiimide (24 mg, 0.11 mmol), and 4-dimethylaminopyridine (1.0 mg, 0.0082 mmol) in CH$_2$Cl$_2$ (23 mL) was added N-acetylcysteamine (10 $\mu$L, 0.098 mmol) at 0°C. After stirring for 6 hr at room temperature, phosphate buffer (1.0 M, pH 7.0) was added. The mixture was diluted with ethyl acetate. The organic layer was separated, and the aqueous portion was extracted with ethyl acetate. The combined organic phase was washed with brine and dried over Na$_2$SO$_4$. After evaporation of the solvent, the residue was purified by recycle gel column chromatography to give 6 (11 mg, 37%). IR (neat): 3376, 1652 cm$^{-1}$; $[\alpha]_{26}^D$ +14.6 (c 0.59, CH$_3$OH); $^1$H-NMR (CDCl$_3$): $\delta$ 7.12 (s, 3H), 6.02 (brs, 1H), 5.69 (dt, $J$ = 15.2, 6.8 Hz, 1H), 5.50 (dd, $J$ = 15.2, 6.8 Hz, 1H), 3.96 (dd, $J$ = 6.4, 6.4 Hz, 1H), 3.64 (t, $J$ = 7.2 Hz, 2H) 3.45 (dt, $J$ = 6.4, 6.4 Hz, 2H), 3.08 (t, $J$ = 6.4 Hz, 2H), 2.68 (m, 1H), 2.10 (dt, $J$ = 7.2, 7.2 Hz , 2H), 2.04 (d, $J$ = 1.2 Hz, 3H), 1.96 (s, 3H), 1.89 (d, $J$ = 1.2 Hz, 3H), 1.65 (tt, $J$ = 7.2 Hz, 3H) $^{13}$C-NMR (CDCl$_3$): $\delta$ 194.7, 170.2, 142.3, 139.4, 133.2, 132.4, 132.1, 131.1, 76.6, 62.2, 39.8, 39.3, 32.1, 28.7, 28.6, 23.3, 16.8, 16.4, 14.2.

**Aleuritic acid ethyl ester 7 (DL-threo-9,10,16-trihydroxypalmitic acid ethyl ester)**

Aleuritic acid (Wako, 500 mg, 1.6 mmol) was treated with ethanolic sulfuric acid solution at reflux for 2 hr. The reaction was quenched by addition of sat NaHCO$_3$ solution, and the mixture was extracted with CHCl$_3$. The organic layers were washed with brine and then dried over MgSO$_4$. The solvent was removed by evaporator and the residue was purified by silica-gel chromatography to afford 7 (0.39 g, 71%). $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 4.12 (q, $J$ = 7.2 Hz, 2H), 3.64 (t, $J$ = 6.6 Hz, 2H), 3.39 (br, 2H), 2.28 (t, $J$ = 7.5 Hz, 2H), 2.09 (brd, $J$ = 11.8 Hz, 2H), 1.60 (m, 4H), 1.46 (m, 6H), 1.37 (m, 6H), 1.31 (m, 6H), 1.25 (t, $J$ = 7.2 Hz, 3H); $^{13}$C-NMR (125 MHz, CDCl$_3$): $\delta$ 173.9, 74.47, 74.45, 63.0, 60.2, 34.3, 33.6, 33.5, 32.7, 29.395, 29.386, 29.1, 29.0, 25.67, 25.59, 25.54, 24.9, 14.3.

**Ethyl (9Z)-18-hydroxy-9-octadecenoate 8 ((9Z)-18-hydroxy-9-octadecenoic acid ethyl ester)**

1,9-Nonanediol (Aldrich, 4.1 g, 25.7 mmol) was dissolved in 90 mL of CH$_2$Cl$_2$ and triethylamine (20 mL, 144 mmol) and $p$-toluenesulfonyl chloride (5.1 g, 26.8 mmol) were added. After stirring for 20 min at room temperature, water was added and the mixture was extracted with CHCl$_3$. The organic layer was washed with water and dried over Na$_2$SO$_4$. After removal of the solvent, the residue was purified by silica-gel chromatography to afford monotosylate (3.6 g, 45%). $^1$H-NMR (300 MHz, CDCl$_3$): $\delta$ 7.80 (d, $J$ = 8.4 Hz, 2H), 7.36 (d, $J$ = 8.4 Hz, 2H), 4.02 (t, $J$ = 6.6 Hz, 2H), 3.62 (t, $J$ = 6.9 Hz, 2H), 2.45 (s, 3H), 1.50-1.67 (m, 4H), 1.20-1.35 (brs, 10H).

The monotosylate (3.5 g, 11.3 mmol) was dissolved in DMF (28 mL) and NaBr (7.0 g, 67.6 mmol) was added. After stirring for 3 hr at 50°C, the mixture was extracted with ethyl acetate. The organic layer was washed with water and dried over Na$_2$SO$_4$. After removal of the solvent, the
residue was purified by silica-gel chromatography to afford 9-bromo-1-nonanol (2.0 g, 80%).

\[ ^1H-NMR \ (300 \text{ MHz, CDCl}_3): \delta 3.63 \ (t, J = 6.6 \text{ Hz, 2H}), 3.41 \ (t, J = 6.9 \text{ Hz, 2H}), 1.86 \ (m, 2H), 1.56 \ (m, 2H), 1.32 \ (brs, 10H). \]

9-Bromo-1-nonanol (2.0 g, 9.0 mmol) was dissolved in 18 mL of CH$_2$Cl$_2$, and 3,4-dihydro-2H-pyran (4.0 mL, 44 mmol) and PPTS (0.55 g, 2.2 mmol) were added. After stirring for 20 min at room temperature, the mixture was extracted with CHCl$_3$. The organic layer was washed with water and dried over Na$_2$SO$_4$. After removal of the solvent, the residue was purified by silica-gel chromatography to afford THP-ether of 9-bromo-1-nonanol (2.7 g, 98%).

\[ ^1H-NMR \ (300 \text{ MHz, CDCl}_3): \delta 4.58 \ (t, J = 4.5 \text{ Hz, 1H}), 3.87 \ (m, 1H), 3.71 \ (m, 1H), 3.49 \ (m, 1H), 3.38 \ (m, 3H), 1.85 \ (m, 2H), 1.58 \ (m, 8H) \ 1.32 \ (br, 10H). \]

THP ether of 9-bromo-1-nonanol (2.3 g, 7.5 mmol) was treated in the same manner for the compound 5 to obtain 8 (0.61 g, 25% in 2 steps).

\[ ^1H-NMR \ (500 \text{ MHz, CDCl}_3): \delta 5.34 \ (m, 2H), 4.12 \ (q, J = 7.2 \text{ Hz, 2H}), 3.63 \ (t, J = 6.7 \text{ Hz, 2H}), 2.28 \ (t, J = 7.6 \text{ Hz, 2H}), 2.00 \ (m, 4H), 1.61 \ (m, 2H), 1.56 \ (m, 2H), 1.30 \ (m, 18H), 1.25 \ (t, J = 7.2 \text{ Hz, 3H}), ^{13}C-NMR \ (125 \text{ MHz, CDCl}_3): \delta 173.9, 129.9, 129.8, 63.1, 60.2, 34.4, 32.8, 29.72, 29.68, 29.5, 29.4, 29.21, 29.16, 29.12, 29.09, 27.18, 27.16, 25.7, 25.0, 14.3; \text{HR-MS (FAB, glycerol) m/z} \ 327.2917 \ (M+H), \text{calcd for C}_{20}\text{H}_{39}\text{O}_3: \ 327.2900. \]

**Ethyl 8-aminooctanoate 10 (8-aminooctanoic acid ethyl ester)**

Acetyl chloride (0.1 mL, 1.4 mmol) was added to ethanol (3 mL) at room temperature. After 1 hr, 8-aminocaprylic acid (Aldrich, 50 mg, 0.31 mmol) was added. The reaction mixture was stirred at room temperature for 15 min. After removal of solvent, hydrochloride salt of 10 (59 mg, 85%) was obtained.

\[ ^1H-NMR \ (500 \text{ MHz, CDCl}_3): \delta 8.30 \ (brs, 2H), 4.05 \ (q, J = 7.2 \text{ Hz, 2H}), 2.98 \ (t, J = 7.6 \text{ Hz, 2H}), 2.28 \ (t, J = 7.5 \text{ Hz, 2H}), 1.76 \ (quint, J = 7.6 \text{ Hz, 2H}), 1.61 \ (quint, J = 7.2 \text{ Hz, 2H}), 1.39 \ (m, 2H), 1.34 \ (m, 4H), 1.25 \ (t, J = 7.2 \text{ Hz, 3H}); ^{13}C-NMR \ (125 \text{ MHz, CDCl}_3): \delta 173.8, 60.2, 39.9, 34.2, 28.8, 28.6, 27.6, 26.2, 24.7, 14.3. \]

**Ethyl 12-aminolaurate 11 (12-aminododecanoic acid ethyl ester)**

Acetyl chloride (0.1 mL, 1.4 mmol) was added to ethanol (3 mL) at room temperature. After 1 hr, 12-aminolauric acid (TCI, 58 mg, 0.27 mmol) was added. The reaction mixture was stirred at room temperature for 15 min. After removal of solvent, hydrochloride salt of 11 (71 mg, 94%) was obtained.

\[ ^1H-NMR \ (500 \text{ MHz, CDCl}_3): \delta 8.30 \ (brs, 2H), 4.11 \ (q, J = 7.2 \text{ Hz, 2H}), 2.97 \ (t, J = 7.7 \text{ Hz, 2H}), 2.28 \ (t, J = 7.6 \text{ Hz, 2H}), 1.75 \ (quint, J = 7.6 \text{ Hz, 2H}), 1.60 \ (quint, J = 7.3 \text{ Hz, 2H}), 1.37 \ (m, 2H), 1.28 \ (m, 12H), 1.25 \ (t, J = 7.2 \text{ Hz, 3H}); ^{13}C-NMR \ (125 \text{ MHz, CDCl}_3): \delta 173.9, 60.1, 39.9, 34.4, 29.43, 29.38, 29.33, 29.24, 29.13, 28.95, 27.7, 26.5, 25.0, 14.3. \]
Characterization of VinTE reaction products

**From compound 1 (a mixture of cyclic trimer 12 and cyclic tetramer 13)**

One mL of 1 in DMSO (4.1 mg, final 2.6 mM) and 9 mL of VinTE (final 60 μM) in 100 mM sodium phosphate buffer (pH 7.0) were mixed and incubated at 28°C for 24 hr. Enzymatic reaction products were extracted with chloroform and the extract was purified by silica-gel chromatography (hexane/ethyl acetate, 2:1) to obtain 1.7 mg of cyclic products (12 : 13 = 5 : 1).

Product 12 and 13; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 4.16 (t, $J = 5.2$ Hz, 2H), 2.38 (t, $J = 6.0$ Hz, 2H), 1.62-1.68 (m, 4H), 1.42-1.51 (m, 2H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 173.3, 63.0, 34.6, 28.2, 24.7, 24.7.

FAB-MS (positive, glycerol): 12; m/z 343, [M+H]$^+$ for C$_{18}$H$_{31}$O$_6$; 13; m/z 457, [M+H]$^+$ for C$_{24}$H$_{41}$O$_8$. 

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**Substrate 1**

**Products 12 and 13**
From compound 2 (a mixture of cyclic dimer 14<sup>10-12</sup> and cyclic trimer 15 plus linear polymers)

One mL of 2 in DMSO (4.0 mg, final 2.1 mM) and 9 mL of VinTE (final 60 µM) in 100 mM sodium phosphate buffer (pH 7.0) were mixed and incubated at 28°C for 24 hr. Enzymatic reaction products were extracted with CHCl₃ and the extract was purified by silica-gel chromatography (hexane/ethyl acetate, 3:1) to obtain 1.0 mg of cyclic products (14 : 15 = ca. 4 : 1) and 1.0 mg of linear polyesters (m/z 331, 473, 616, and 758).

Product 14 and 15; <sup>1</sup>H-NMR (400 MHz, CDCl₃): δ 4.12 (t, J = 6.0 Hz, 2H), 2.32 (t, J = 7.2 Hz, 2H), 1.64 (m, 2H), 1.52 (brs, 2H), 1.35 (br, 6H). FAB-MS (positive, glycerol): 14; m/z 285, [M+H]<sup>+</sup> for C₁₆H₂₉O₄; 15; m/z 427, [M+H]<sup>+</sup> for C₂₄H₄₃O₆.
From compound 3 (a mixture of cyclic dimer 16 and cyclic trimer 17, plus linear polymers)\textsuperscript{13}

One mL of 3 in DMSO (4.0 mg, final 2.1 mM) and 9 mL of VinTE (final 60 μM) in 100 mM sodium phosphate buffer (pH 7.0) were mixed and incubated at 28°C for 24 hr. Enzymatic reaction products were extracted with chloroform and the extract was purified by silica-gel chromatography (hexane/ethyl acetate, 2:1) to obtain 1.1 mg of cyclic products (16 and 17) and 0.5 mg of linear polyesters (m/z 467 and 607).

Product 16 and 17; \textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}): \(\delta 6.87 \text{ (dt, } J = 15.6 \text{ Hz, } 7.2 \text{ Hz, } 1 \text{H}), 5.81 \text{ (dt, } J = 15.6 \text{ Hz, } 1.6 \text{ Hz, } 1 \text{H}), 4.20 \text{ (t, } J = 5.6 \text{ Hz, } 2 \text{H}), 2.27 \text{ (m, } 2 \text{H}), 1.64 \text{ (m, } 2 \text{H}), 1.53 \text{ (m, } 2 \text{H}), 1.38 \text{ (m, } 2 \text{H}).

FAB-MS (positive, NBA): 16; m/z 281, [M+H]\textsuperscript{+} for C\textsubscript{16}H\textsubscript{25}O\textsubscript{4}; 17; m/z 421, [M+H]\textsuperscript{+} for C\textsubscript{24}H\textsubscript{37}O\textsubscript{6}. 

Substrate 3

Products 16 and 17

Products linear polyesters

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From compound 4 (cyclic dimer $18^{11,14}$ and linear dimer $19$)

One mL of 4 in DMSO (5.0 mg, final 2 mM) and 9 mL of VinTE (final 60 μM) in 100 mM sodium phosphate buffer (pH 7.0) were mixed and incubated at 28°C for 24 hr. Enzymatic reaction products were extracted with CHCl₃ and the extract was purified by silica-gel chromatography (hexane/ethyl acetate, 9:1) to obtain 2.1 mg of cyclic product $18$ and 1.5 mg of linear dimer $19$.

$^1$H-NMR (400 MHz, CDCl₃): $18$; δ 4.10 (t, $J = 6.0$ Hz, 4H), 2.31 (t, $J = 6.8$ Hz, 4H), 1.52-1.65 (br, 8H), 1.23-1.35 (brs, 28H), $19$; δ 4.13 (q, $J = 7.2$ Hz, 2H), 4.05 (t, $J = 6.4$ Hz, 2H), 3.64 (t, $J = 6.8$ Hz, 2H), 2.28 (t, $J = 7.2$ Hz, 4H), 1.54-1.65 (br, 8H), 1.23-1.35 (31H). $^{13}$C-NMR (125 MHz, CDCl₃): $19$; δ 174.0, 173.9, 64.4, 63.1, 60.1, 34.41, 34.39, 32.8, 29.55, 29.48, 29.41, 29.40, 29.24, 29.14, 28.7, 25.9, 25.7, 25.02, 24.98, 14.3. FAB-MS (positive, NBA): $18$; m/z 397, [M+H]$^+$ for C$_{24}$H$_{45}$O$_4$, $19$; m/z 443, [M+H]$^+$ for C$_{26}$H$_{51}$O$_5$. 

![Substrate 4](image1.png)

![Product 18](image2.png)

![Product 19](image3.png)
From compound 5 (cyclic dimer 20 and linear dimer 21)

One mL of 5 in DMSO (4.8 mg, final 2 mM) and 9 mL of VinTE (final 60 µM) in 100 mM sodium phosphate buffer (pH 7.0) were mixed and incubated at 28°C for 24 hr. Enzymatic reaction products were extracted with CHCl₃ and the extract was purified by silica-gel chromatography (hexane/ethyl acetate, 20:1) to obtain 2.0 mg of cyclic product 20 and 1.3 mg of linear dimer 21.

$^1$H-NMR (400 MHz, CDCl₃): 20; δ 5.49 (m, 2H), 5.34 (m, 2H), 4.11 (t, J = 6.3 Hz, 4H), 2.38 (q, J = 6.6 Hz, 4H), 2.28 (t, J = 7.6 Hz, 4H), 2.03 (q, J = 6.9 Hz, 4H), 1.61 (m, 4H), 1.30 (brs, 16H), 21; 5.54 (m, 1H), 5.48 (m, 1H), 5.35 (m, 2H), 4.13 (q, J = 7.1 Hz, 2H), 4.06 (t, J = 6.9 Hz, 2H), 3.64 (t, J = 6.5 Hz, 2H), 2.35 (m, 4H), 2.28 (t, J = 7.6 Hz, 4H), 2.05 (hex, J = 6.7 Hz, 4H), 1.61 (m, 4H), 1.30 (brs, 16H), 1.25 (t, 7.1 Hz, 3H). $^{13}$C-NMR (125 MHz, CDCl₃): 20; δ 173.8, 132.8, 125.06, 63.6, 34.5, 29.5, 19.17, 19.14 (thick), 27.3, 27.0, 25.1, 21; δ 173.9, 133.4, 132.8, 125.1, 124.4, 63.8, 62.3, 60.2, 34.37, 34.3, 30.8, 29.57, 29.51, 29.16, 29.09, 29.98, 29.04, 27.30, 27.28, 26.9, 24.96, 24.94, 14.3. EI-MS (positive): 20; m/z 392, M⁺ for C₂₄H₄₀O₄, FAB-MS (positive, glycerol), 21; m/z 439, [M+H]⁺ for C₂₆H₄₇O₅.
From compound 6 (cyclic dimer 22)

A reaction mixture (final volume; 2.2 mL of phosphate buffer pH 7.0) containing 6 mg of NAC thioester 6 in 0.2 mL of DMSO, and ca. 30 mg (0.3 mM) of VinTE was incubated at 28°C overnight. The mixture was diluted with ethyl acetate. The organic layer was separated, and the aqueous portion was extracted with ethyl acetate. The combined organic layer was filtered through a pad of Celite. After evaporation of the solvent under reduced pressure, the residue was purified by silica gel column chromatography to afford 2 mg of 22; 1H-NMR (CDCl3): δ 7.08 (s, 2H), 5.67 (dt, J = 15.0, 6.4 Hz, 2H), 5.50 (dd, J = 15.0, 7.4 Hz, 2H), 5.34 (d, J = 10.0 Hz, 2H), 4.15 (dt, J = 10.8, 6.4 Hz, 2H), 4.10 (dt, J = 10.8, 6.8 Hz, 2H), 3.91 (dd, J = 7.4, 6.8 Hz, 2H), 2.69 (m, 2H), 2.15 (dt, J = 6.4, 5.2 Hz, 4H), 1.97 (d, J = 1.2 Hz, 6H), 1.85 (d, J = 1.2 Hz, 6H), 1.75 (m, 4H), 1.06 (d, J = 6.8 Hz, 6H); HR FAB-MS calc. for C30H45O6 (M+H+) 501.3216, found 501.3202.
From compound 7 (cyclic monomer 23\textsuperscript{15,16})

One mL of 7 in DMSO (3.3 mg, final 1 mM) and 9 mL of VinTE (final 60 \(\mu\)M) in 100 mM sodium phosphate buffer (pH 7.0) were mixed and incubated at 28\(^\circ\)C for 24 hr. Enzymatic reaction products were extracted with CHCl\(_3\) and the dried extract was purified by silica-gel chromatography (hexane/ethyl acetate, 1:2) to obtain 1.5 mg of cyclic product 23 and 1.3 mg of hydrolyzed compound.

\(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta\) 4.13 (m, 2H), 3.47 (br, 2H), 2.33 (dd, \(J = 7.5\) and 5.7 Hz, 2H), 2.08 (brd, \(J = 8.5\) Hz, 2H), 1.3-1.65 (m, 22H).

FAB-MS (positive, glycerol); \(m/z\) 287, [M+H]\(^+\) for C\(_{16}\)H\(_{31}\)O\(_4\).
From compound 8 (cyclic monomer 24)\textsuperscript{17,18}

One mL of 8 in DMSO (4.8 mg, final 1.5 mM) and 9 mL of VinTE (final 60 μM) in 100 mM sodium phosphate buffer (pH 7.0) were mixed and incubated at 28°C for 24 hr. Enzymatic reaction products were extracted with CHCl\textsubscript{3} and the dried extract was purified by silica-gel chromatography (hexane/ethyl acetate, 5:1) to obtain 3.4 mg of cyclic product 24 and 0.2 mg of hydrolyzed compound.

![Substrate 8 and Product 24](image)

Compound 24:

\textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}): \( \delta \) 5.31 (m, 2H), 4.10 (t, \( J = 5.5 \) Hz, 2H), 2.31 (t, \( J = 6.8 \) Hz, 2H), 2.03 (m, 4H), 1.63 (m, 4H), 1.20-1.38 (m, 18H). \textsuperscript{13}C-NMR (125 MHz, CDCl\textsubscript{3}): \( \delta \) 174.1, 130.29, 130.16, 64.3, 35.0, 29.70, 29.39, 29.23, 29.13, 29.08, 28.84, 28.82, 27.9, 27.1, 26.4, 26.2, 25.3. EI-MS (Positive): \( m/z \) 280 (M\(^{+}\), C\textsubscript{18}H\textsubscript{32}O\textsubscript{2}).
Figure S1. SDS-PAGE and ESI-MS analysis of VinTE

Estimated size of vinTE: 36 kDa
LC-ESI-MS: An aliquot of VinTE solution was loaded onto a semi-micro HPLC system (Nanospace SI-1/2001 semi-micro pump x2, SI-1/2002 UV-VIS detector (Shiseido), and Sensyu column oven SSC-2300) equipped with Senshu Pak PEGASIL-300 C4P column (1.0 mm x 15 cm, Senshu), which was connected on-line to an electrospray ionization (ESI) mass spectrometer LCQ (Finnigan). The column was first washed with acetonitrile/water (1:9) containing 0.1% HCOOH and 0.01% TFA for 10 min at a flow rate of 100 µL/min. Elution was carried out with a mixture consisting of acetonitrile/water (9:1) containing 0.1% HCOOH and 0.01% TFA. The elution was monitored with a UV detector at 280 nm, and mass spectra for each fraction were simultaneously scanned for a range from 300 to 2000 atomic mass units. The molecular mass was estimated by deconvolution with Bio-Works 1.0 software (ThermoQuest). The observed mass was 35,494 Da, which agreed with the calculated average mass (35,508 Da) for VinTE.
Figure S3. Structural model of VinTE

VinTE structural model was constructed by use of Discovery studio ver.2.0 (Accelrys) based on the crystal structure of PikTE (PDB ID 2H7X) with vicenilactam as a ligand. S120 corresponds to S136 of the sequence of VinTE shown in Figure S4.
VinTE: vicenistatin PKS TE, PikTE: pikromycin PKS TE, Debs TE: erythromycin PKS TE, GfsTE: FD-891 PKS TE, HlsTE: halstoctacosanolide PKS TE. Yellow shows an active site serine residue (S136 in VinTE). The marked S136, D163, and H282 of VinTE resemble a catalytic triad, which is conserved in TE domains.
References


