Fungal Metabolites. XVII.1,2) Synthesis and NMR Study of Ion Channel-Forming Peptides, Trichosporin B-VIa and Its Derivative

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A membrane-modifying peptide antibiotic, trichosporin B-VIa, having catecholamine secretion-inducing activity on bovine adrenal chromaffin cells has been synthesized. Aib1-2-Trichosporin B-VIa, in which Pro14 was replaced by Aib, has also been synthesized to modify the secondary structure of trichosporin B-VIa. Sequence-specific 1H-NMR assignments of both peptides in methanol were achieved by using two-dimensional NMR techniques.

Keywords Trichoderma polysporum; peptabiol; trichosporin; z-aminosuberic acid; catecholamine

Trichosporin Bs from Trichoderma polysporum3,4) are peptide antibiotics containing a high proportion of an unusual amino acid, z-aminosuberic acid (Aib). Their N- and C-terminal amino acids are protected by an acetyl group and phenylalaninol, respectively. These characteristics indicate that the peptides belong to the class of membrane-modifying peptides named peptabiotics, such as alamethicin.5) Trichosporin Bs have a catecholamine secretion-inducing activity on bovine adrenal chromaffin cells6) and act as uncouplers in rat liver mitochondria.7) Trichosporin B-VIa, which has a low abundance in nature, is especially potent. Recently, we have reported that trichosporin B-VIa forms a voltage-gated ion channel in lipid bilayers.8) Accordingly, the above two biological activities are likely to be due to channel formation in the biomembranes. The properties of voltage-gated ion channels formed by alamethicins have been well investigated.9) 11) Alamethicins are Pro14-containing icosapeptabiotics, and their primary structures are similar to that of trichosporin B-VIa. Voltage-dependent ion channels formed by alamethicins are considered to be aggregates of several molecules having a bent structure around the Pro14 residue, which may govern the voltage dependence in the channel-forming mechanism.12) Trichosporin B-VIa also contains Pro14 in its structure. Thus, it seemed interesting to investigate the effects of the replacement of Pro to Aib at position 14 on the structure and function of trichosporin B-VIa. In this paper, we describe the synthesis and NMR assignments of trichosporin B-VIa and its Aib14 derivative.


Results and Discussion

Synthesis of Trichosporin B-VIa and the Aib14 Derivative
Peptabiotics have generally been synthesized by the solution-phase method,13) and we chose this method for the syntheses of trichosporin B-VIa and Aib14-trichosporin B-VIa. The synthetic strategy is based on our previous work on the synthesis of trichosporin B-V14) and hylpepin A-III.15) The optically active amino acids are all of l-form. Fragments were designed such that Aib was placed at the C-terminal in order to avoid racemization during activation and deprotection. All fragment condensation steps were carried out by the DCC-HOBt procedure in DMF at room temperature. The Z group was used for protection of N-terminals of fragments.

The N-terminal fragment (fragment [4]; positions 1—6)

(A)

Ac-Aib-OH

Z-Ala-OH

H-Aib-OMe

H-Ala-Aib-Aib-OMe

(b, c)

(b, a)

Fig. 1. Synthetic Route to Trichosporin B-VIa and Aib14-Trichosporin B-VIa
(A)—(D) Synthetic schemes for fragment [4], trichosporin B-VIa, fragment [2], and Aib14-trichosporin B-VIa, respectively. Reagents: a, H2/Pd-C; b, DCC-HOBt; c, NaNH; d, PdcBr; e, HBr/AcOH; f, Zn/AcOH.
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Fig. 2. ES-MS and MS/MS of Synthetic Trichosporin B-Vla and FAB-MS of Alb^{14} Trichosporin B-Vla

(a) Two- and 3- fold charged molecular ions of trichosporin B-Vla were observed at m/z 983.5 and m/z 655.9 when the inlet voltage (orifice voltage) was set at 50 V. The monoisotopic mass, 1964, estimated from these ions was in agreement with that of the natural product. (b), (c) and (d) MS/MS spectra of m/z 284.0, 1191.8 and 774.5 found at high orifice voltages (110, 120 and 150 V, respectively). (e) FAB-MS of Alb^{14}-trichosporin B-Vla.
for trichosporin B-VIa and its Aib\(^{14}\) derivative was synthesized by the DCC–HOBt method as shown in Fig. 1a. The N-terminal hexapeptide acid (fragment [4]) and the amine component (positions 7–20), which was used to synthesize trichosporin B-V,\(^{14}\) were coupled to give trichosporin B-VIa (Fig. 1b). The synthetic trichosporin B-VIa was homogeneous and identical with the natural peptide on analytical HPLC chromatography (see Experimental). The synthetic trichosporin B-VIa was characterized by mass spectrometry (MS) (Fig. 2a) and MS/MS (Fig. 2b–d). The spectral and physical data of the synthetic trichosporin B-VIa were in good agreement.

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Fig. 3. Parts of the 600 MHz NOESY Spectra of Trichosporin B-VIa and Aib\(^{14}\)-Trichosporin B-VIa at 20°C in CD\(_3\)OH (20%aq)

Sequential NH–NH and acetyl C\(_{\text{H}}\)–NH cross-peaks [\(\delta_{\text{NH}}(i, i+1)\) and \(\delta_{\text{NH}}(i, i+1)\)] of trichosporin B-VIa (a) and Aib\(^{14}\)-trichosporin B-VIa (b). The NH proton which has a cross peak with the acetyl C\(_{\text{H}}\) proton was assigned as the Aib\(^{1}\) NH proton. The other NH protons were assigned from the NH–NH connectivities [\(\delta_{\text{NH}}(i, i+1)\)] extended from the Aib\(^{1}\) NH proton. The signals of the Aib\(^{1}\) and Ala\(^{2}\) NHs of trichosporin B-VIa (a) were suppressed because of the transfer of solvent saturation.
with those of the natural trichosporin B-VIa.

Syntheses of fragments [1] and [3] for Aib\textsuperscript{14}-trichosporin B-VIa in Fig. 1d were described in our previous report.\textsuperscript{14} In the synthesis of the tetrapeptide acid (positions 14–17; fragment [2]) for Aib\textsuperscript{14}-trichosporin B-VIa, alkaline hydrolysis of the methyl ester, Z-(14–17)-OMe, did not give a good result. Therefore, the tetrapeptide phenacyl ester was synthesized according to the route shown in Fig. 1c. The phenacyl group of the tetrapeptide ester was easily removed by Zn powder in AcOH to give the tetrapeptide acid (fragment [2]). Fragments [1]–[4] were condensed successively according to the route shown in Fig. 1d to give Aib\textsuperscript{14}-trichosporin B-VIa.

The synthetic Aib\textsuperscript{14}-trichosporin B-VIa was homogeneous on the analytical HPLC chromagram. It was characterized by FAB-MS (Fig. 2e).

\textbf{\textsuperscript{1}H-NMR Study of Trichosporin B-VIa and the Aib\textsuperscript{14} Derivative} The \textsuperscript{1}H-NMR signals of trichosporin B-VIa and Aib\textsuperscript{14}-trichosporin B-VIa in CD\textsubscript{3}OD were assigned by DQF-COSY\textsuperscript{16} and NOESY.\textsuperscript{17} The spin systems of amino acids and phenol were identified from the DQF-COSY spectra. The Aib C\textsubscript{6}H\textsubscript{3}SO\textsubscript{3} signals, which were unassignable by DQF-COSY owing to the lack of \(\gamma\)-protons, were assigned on the basis of the NOEs between NH and C\textsubscript{6}H\textsubscript{3}SO\textsubscript{3} protons in the NOESY spectra. The backbone NH signals were sequence-specifically assigned on the basis of inter-residue NOE connectivities \([d_{NN}(i, i+1)]\) according to the procedures described by Wagner and Wüthrich\textsuperscript{18} (Fig. 3).

The chemical shifts and coupling constant values of the \textsuperscript{1}H-NMR signals are summarized in Table I.

The \(\textsuperscript{1}H\) chemical shifts of the N-terminal parts (positions 1 to 10) in both peptides are almost identical to each other, and the \(J_{NH\textsuperscript{\text{H}}\text{H}}\) values are less than 7 Hz, indicating that both peptides have similar helical structure in this region.\textsuperscript{19} On the other hand, the NH signals of Aib\textsuperscript{14}-trichosporin B-VIa in the C-terminal part (positions 11 to 19, except for position 13) were shifted to lower field than those of trichosporin B-VIa. The \(J_{NH\textsuperscript{\text{H}}\text{H}}\) values of this region in Aib\textsuperscript{14}-trichosporin B-VIa are small and indicative of a helical structure but the values for Leu\textsuperscript{12} and Val\textsuperscript{15} of trichosporin B-VIa are larger than those for Aib\textsuperscript{14}-trichosporin B-VIa. These results indicated that the inter-residual hydrogen-bond connectivities around position 14 were changed by replacing Pro with Aib, and suggested a straight helical structure for the Aib\textsuperscript{14} derivative.

Some carbonyl oxygen atoms of trichosporin B-VIa do not participate in the backbone hydrogen bonding owing to the lack of an amide proton of Pro and disruption of hydrogen bonding, as was observed in Pro-containing peptaidols, such as trichosporin B-V,\textsuperscript{20} alamethicins,\textsuperscript{12,21}

\begin{table}[h]
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\renewcommand{\arraystretch}{1.3}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
\textbf{Residue} & \textbf{NH\textsuperscript{a}} & \textbf{Trichosporin B-VIa} & \textbf{Others\textsuperscript{a}} & \textbf{Residue} & \textbf{NH\textsuperscript{a}} & \textbf{Aib\textsuperscript{14}-trichosporin B-VIa} & \textbf{Others\textsuperscript{a}} \\
\hline
Ac & \(\alpha = 2.020\) & & & Ac & \(\alpha = 2.026\) & & \\
Aib\textsuperscript{1} & 8.461 & & & Aib\textsuperscript{1} & 8.460 & & \\
Ala\textsuperscript{2} & 8.302 (d, 4.1) & \(\alpha = 4.01\) & \(\beta = 1.425\) & (7.3) & Ala\textsuperscript{2} & 8.321 (d, 4.2) & \(\alpha = 4.01\) & \(\beta = 1.430\) & (7.3) \\
Aib\textsuperscript{3} & 7.629 & & & Aib\textsuperscript{3} & 7.646 & & \\
Ala\textsuperscript{4} & 7.624 (d, 5.7) & \(\alpha = 4.061\) & \(\beta = 1.463\) & (7.1) & Ala\textsuperscript{4} & 7.652 (d, 5.7) & \(\alpha = 4.04\) & \(\beta = 1.463\) & (7.5) \\
Aib\textsuperscript{5} & 8.013 & & & Aib\textsuperscript{5} & 8.030 & & \\
Gln\textsuperscript{6} & 7.991 & & & Gln\textsuperscript{6} & 8.018 & & \\
Glv\textsuperscript{7} & 7.812 (d, 5.5) & \(\beta = 3.87\) & \(\gamma = 2.26\) & (2.16, m, 12.4) & (5.6, 9.3, 15.2, 2.40 m) & & \\
& & & & & & & \\
Aib\textsuperscript{8} & 8.192 & & & Aib\textsuperscript{8} & 8.229 & & \\
Ile\textsuperscript{9} & 7.556 (d, 6.0) & \(\beta = 1.585\) & \(\gamma = 2.08\) & (2.16, m, 13.4) & (2.5) & & \\
& & & & & & & \\
Gly\textsuperscript{10} & 8.412 (d, 5.0, 6.5) & \(\alpha = 3.934\) & \(\beta = 1.620\) & (6.5, 1.545) & (7.3) & & \\
& & & & & & & \\
Leu\textsuperscript{11} & 8.070 (d, 7.7) & \(\alpha = 4.45\) & \(\beta = 1.95\) & (1.60, m, 1.95) & (5.995) & 0.918 & (6.5) & & \\
& & & & & & & \\
Aib\textsuperscript{13} & 8.397 & & & Aib\textsuperscript{13} & 8.211 & & \\
Pro\textsuperscript{14} & 8.388 (d, 6.3, 8.7) & \(\beta = 2.32\) & \(\gamma = 2.181\) & (2.17) & (2.23) & (1.97) & 0.388 & (3.76 m) & & \\
& & & & & & & \\
Val\textsuperscript{15} & 7.631 (d, 8.2) & \(\alpha = 3.73\) & \(\beta = 2.33\) & (1.978) & (6.8) & (1.071) & (6.5) & & \\
& & & & & & & \\
Aib\textsuperscript{16} & 7.602 & & & Aib\textsuperscript{16} & 8.219 & & \\
Aib\textsuperscript{17} & 7.829 & & & Aib\textsuperscript{17} & 7.936 & & \\
Gln\textsuperscript{18} & 7.802 (d, 6.2) & \(\alpha = 4.00\) & \(\beta = 2.24\) & (2.16, m, 13.4) & (2.5) & & \\
& & & & & & & \\
& & & & & & & \\
Gln\textsuperscript{19} & 7.890 (d, 7.4) & \(\alpha = 4.16\) & \(\beta = 2.13\) & (2.31, m, 2.19 m) & & & \\
& & & & & & & \\
Phc0\textsuperscript{20} & 7.331 (d, 9.2) & \(\alpha = 4.14\) & \(\beta = 2.399\) & (5.5, 13.7) & (2.729) & (8.9, 13.7) & (2.617) & (5.1) & & \\
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\textsuperscript{a} Chemical shifts (ppm) were measured either from the one-dimensional spectra \((\delta = \pm 0.01 \text{ ppm})\) or from the two-dimensional spectra \((\delta = \pm 0.01 \text{ ppm})\). Coupling constant values (Hz) were measured from the one-dimensional spectra.
and saturnsporins. These carbonyl oxygen atoms are considered to increase the polarity of the peptides. It is, therefore, clear that the longer retention time of Aib-trichosporin B-VIa ($\tau_g = 50.34$) on HPLC in comparison with that of trichosporin B-VIa ($\tau_g = 20.23$) is due to the increase of lipophilicity caused by the lack of hydrogen bond-free carbonyl oxygen atoms.

**Experimental**

**General Methods** All melting points are uncorrected. Optical rotations were measured with a JASCO DIP-181 digital polarimeter at room temperature. All NMR experiments were performed on a Bruker AM-600 spectrometer. Samples were dissolved in CD$_3$OD containing tetramethylsilane as an internal standard. The details of NMR measurements have already been described in the previous paper. The EM-MS was performed on a JEOL JMS-A XA- QMG Auto-Tec Spectrometer. Samples were bombarded with 3 keV xenon atoms and glycerol–thioglycerol or m-nitrobenzyl alcohol–glycerol was used as the matrix. Pneumatically assisted electrostatic mass spectrometry (ES-MS) was performed on an API III (Perkin Elmer Sciex). Samples were dissolved in CH$_3$CN–H$_2$O (1:1) containing 0.1% TFA. MS/MS experiments were carried out by collision-induced dissociation. Argon atoms were used as the collision gas for MS/MS. TLC was performed on silica gel (Kieselgel 60 F254, Merck). The R$_f$ values refer to the following solvent systems (v/v): RF$_f$ = CHCl$_3$–MeOH (95:5), RF$_f$ = CHCl$_3$–MeOH (9:1), RF$_f$ = CHCl$_3$–MeOH (8:2), RF$_f$ = CHCl$_3$–MeOH (5:4:1). For column chromatography, silica gel 60 (70–230 mesh, Merck) and Sephadex LH-20 (Pharmacia) were used. Analytical and preparative HPLC were performed on a Shimadzu LC-6A system, using a MeOH–H$_2$O solvent system. Amino acid analyses were done with a Hitachi Model 835 amino acid analyzer.

**Coupling Reactions** Unless otherwise stated, coupling reactions were performed on the DCC–HOBr method at room temperature for 12–72 h and the mixtures were worked up according to procedure A or B after removal of DCC and the solvent.

**Procedure A:** EtOAc-soluble protected peptides were each dissolved in EtOAc and the solution was evaporated with 1 N HCl, 5% NaHCO$_3$ and saturated NaCl, dried over Na$_2$SO$_4$ and concentrated. The residue was usually recrystallized or precipitated from appropriate solvents.

**Procedure B:** EtOAc-insoluble protected peptides were purified by gel-filtration on Sephadex LH-20 in MeOH.

**Hydrolysis of Z-Peptide Methyl Esters (Procedure C)** Z-Peptide methyl esters were hydrolyzed with 1 N NaOH (2–3 eq) in MeOH below 35°C. After complete saponification, the reaction was neutralized with 1 N HCl and evaporated to remove MeOH. The residue solution was acidified to pH 3 and extracted with EtOAc. The extract was washed with saturated NaCl, dried over Na$_2$SO$_4$ and concentrated. The residue was usually employed in the following step without further purification.

**Catalytic Hydrogenation (Procedure D)** The benzyloxycarbonyl group, Z, was removed by the use of H$_2$ gas over 10% palladium-on-charcoal with stirring. After removal of the catalyst by filtration, the filtrate was concentrated and used in the next step without further purification.

**Synthesis of Trichosporin B-VIa (A)** Z-Ala–Aib–OMe Z-Ala–OH (3.46 g, 15.5 mmol) and DCC (3.20 g, 1 eq) were dissolved in a solution of HCl in H$_2$O–OMe (2.38 eq) in DMF (250 ml) containing TEA (2.15 ml, 1 eq) with stirring. After 24 h, the solution was worked up as described in procedure A and the residue purified by silica gel chromatography (CHCl$_3$–MeOH = 95:5) to afford a syrup product, Z-Ala–Aib–OMe; yield 3.60 g (72%), $\tau_g = 30.3^\circ$ (c = 1, MeOH), RF$_f$ 0.54, EI–MS m/z: 563 (M$^+$), 447 (M$^+$–Aib–OMe), 362 (447–Aib), 291 (447–Ala), 206 (291–Aib). Anal. Calcd for C$_{21}$H$_{31}$N$_3$O$_6$: C, 57.54; H, 7.33; N, 12.42. Found: C, 57.72; H, 7.22; N, 12.55.

**H-Ala-Ala–Ala–Aib–OMe** The above pentapeptide (1.60 g, 3.72 mmol) was dissolved in MeOH (40 ml) and treated with DCC (1 eq) in DMF (7 ml) with stirring. After 48 h, the solvent was removed and the residue was dissolved in MeOH. The solution was treated with Amberlite IR-120 and IRA–400 successively. The solvent was evaporated off and the residue was recrystallized from MeOH and ether. yield: 1.43 g (73%), mp 174–176°C ($\tau_g = 7.2^\circ$ (c = 0.3, MeOH), RF$_f$ 0.46. EI–MS m/z: 557 (M$^+$), 525 (M$^+$–Aib–OMe), 450 (291–Aib), 355 (440–Aib), 284 (355–Ala), 199 (284–Ala), 128 (199–Ala). Anal. Calcd for C$_{32}$H$_{37}$N$_5$O$_{12}$: C, 53.18; H, 8.02; N, 14.86. Found: C, 53.13; H, 8.09; N, 14.78.

**Ac-Ala–Ala–Ala–Ala–Aib–OMe (B)** The above protected hexapeptide (49.6 mm, 0.089 mmol) was saponified in 6 N NaOH and the solution was evaporated to dryness. The solution was evaporated at 120°C. The residue was dissolved in MeOH and evaporated to give the free hexapeptide acid; yield 46.0 mg (95%), mp 210–212°C, RF$_f$ 0.73.

**Ac-Ala–Ala–Ala–Ala–Aib–Ala–BzGly–Leu–Ala–Pro–Val–Ala–Gln–Gln–Phenyl (Trichosporin B-VIa)** Ac-Ala–Ala–Ala–Ala–Aib–OMe (positions 1–6) (46.0 g, 1.5 eq), HOBr (11.5 mm, 1.5 eq) and DCC (17.5 mm, 1.5 eq) were added successively to a solution of H-Ala–Ala–Ala–Aib–OMe (441 mg, 1 eq) in DMF (7 ml) with stirring. After 48 h, the solvent was removed and the residue was dissolved in MeOH. The solution was treated with Amberlite IR-120 and IRA–400 successively. The solvent was evaporated off and the residue was recrystallized from MeOH and ether, yield: 418 mg (73%), mp 174–176°C ($\tau_g = 6.2^\circ$ (c = 0.3, MeOH), RF$_f$ 0.46. EI–MS m/z: 557 (M$^+$), 525 (M$^+$–Aib–OMe), 450 (291–Aib), 355 (440–Aib), 284 (355–Ala), 199 (284–Ala), 128 (199–Ala). Anal. Calcd for C$_{32}$H$_{37}$N$_5$O$_{12}$: C, 53.18; H, 8.02; N, 14.86. Found: C, 53.13; H, 8.09; N, 14.78.

**Synthesis of Aib–BzGly–Leu–Ala–Pro–Val–Ala–Gln–Gln–Phenyl (Trichosporin B-VIa) A** Z-Ala–Val–Aib–OMe Z-Ala–OH (3.46 g, 15.5 mmol) and DCC (3.20 g, 1 eq) were dissolved in a solution of HCl in H$_2$O–OMe (2.38 eq) in DMF (250 ml) containing TEA (2.15 ml, 1 eq) with stirring. After 24 h, the solution was worked up as described in procedure A and the residue purified by silica gel chromatography (CHCl$_3$–MeOH = 95:5) to afford a syrup product, Z-Ala–Aib–OMe; yield 3.60 g (72%), $\tau_g = 30.3^\circ$ (c = 1, MeOH), RF$_f$ 0.54, EI–MS m/z: 563 (M$^+$), 291 (M$^+$–OMe), 206 (291–Aib). DCC (1 eq). Z-Ala–OH Z-Ala–Aib–OMe (9.45 g, 29.33 mmol) was saponified according to procedure C to give Z-Ala–Aib–OH. The dipeptide was recrystallized from EtOAc and n-hexane; yield 7.60 g (84%), mp 181–183°C, RF$_f$ 0.67.

**C** Z-Ala–Aib–Ala–Aib–OMe Z-Ala–OH (1.00 g, 3.23 mmol), HOBr (440 mg, 1 eq) and DCC (670 mg, 1 eq) were added successively to a stirred solution of HCl in H$_2$O–OMe (2.38 eq) in DMF (9 ml) containing TEA (0.45 ml, 1 eq). After 72 h, the solution was worked up according to procedure A. The residue was precipitated from EtOAc with ether to give the protected pentapeptide.
0.256 mmol) was dissolved in 90% AcOH (5 ml), and Zn powder (0.5 g) was added to the solution at 0 °C. Stirring was continued at the same temperature for 1 h and at room temperature for 2 h. After filtration to remove Zn powder, the solvent was evaporated off. The residue was taken up in 5% citric acid and the aqueous layer was extracted with EtOAc. The extract was washed with saturated NaCl, dried over Na2SO4, and concentrated. The residue was recrystallized from MeOH and ether to afford the tetracetepicide acid; yield 92 mg (79%), mp 85–89 °C, Rf = 0.46.

F) Z-Alb-Val-Alb-Alb-Gln-Gln-Pheol The above tetracetepicide acid (90 mg, 0.178 mmol), HOBr (24 mg, 1 eq.), and DCC (37 mg, 1 eq.) were added successively to a stirred solution of H-Gln-Gln-Pheol [1]141 (73 mg, 1 eq) in DMF. After 12 h, the solution was worked up according to procedure B. Fractions containing the product were collected and concentrated. The residue was recrystallized from MeOH and ether to give the tetracetepicide; yield 125 mg (78%), mp 112–113 °C, [α]D 190 -18.0 (c = 0.3, MeOH), Rf 0.64, FAB-MS m/z: 896 (M+H), 745 (M+H - Pheol-H), 487 (745-Gln-Gln), 404 (487-Alb), 220 (404-Alb-Val). Anal. Caled for C31H14N2O9: 1/2 H2O: C, 58.39; H, 7.35; N, 13.93. Found: C, 58.40; H, 7.29; N, 13.72.

G) H-Alb-Val-Alb-Alb-Gln-Gln-Pheol The above tetracetepicide (92 mg, 0.103 mmol) in MeOH was hydrogenated according to procedure D. After removal of the catalyst by filtration, the filtrate was concentrated to give the title compound; yield 71 mg (90%), Rf 0.38.

H) Z-Gln-Alb-Ile-Alb-Gly-Leu-Alb-Alb-Val-Alb-Alb-Gln-Gln-Pheol Z-Gln-Alb-Ile-Alb-Gly-Leu-Alb-Val-Alb-Alb-Gln-Gln-Pheol (70 mg, 0.018 mmol), HOBr (14 mg, 1 eq.), and DCC (21 mg, 1 eq.) were added successively to a stirred solution of H-Alb-Val-Alb-Alb-Alb-Gln-Gln-Pheol (70 mg, 1 eq) in DMF. After 24 h, the solution was worked up according to procedure B. Fractions containing the product were collected and concentrated. The residue was recrystallized from MeOH and ether to give the tetracetepicide acid; yield 58 mg (40%), mp 145–147 °C, [α]D 190 -23.8 (c = 0.8, MeOH), Rf 0.54, FAB-MS m/z: 1562 (M+H), 1411 (M+H - Pheol-H), 1155 (1411-Gln-Gln), 1070 (1155-Alb), 886 (985-Val), 801 (886-Alb), 716 (801-Alb), 603 (716-Leu), 546 (603-Gly), 461 (546-Alb), 348 (461-Ile). Anal. Caled for C51H19N2O14: C, 56.8; H, 7.71; N, 15.06. Found: C, 56.64; H, 7.48; N, 15.23.

I) H-Gln-Alb-Ile-Alb-Gly-Leu-Alb-Alb-Val-Alb-Alb-Gln-Gln-Pheol The above tetracetepicide (33 mg, 0.021 mmol) in MeOH was hydrogenated according to procedure D. After removal of the catalyst by filtration, the filtrate was concentrated to give the title compound; yield 28 mg (93%), Rf 0.58.

J) Ac-Alb-Ala-Ala-Alb-Alb-Alb-Gly-Leu-Alb-Alb-Alb-Alb-Alb-Gln-Gln-Pheol [Alb14-Trichosporin B-Vla] Ac-Alb-Ala-Ala-Alb-Ala-Alb-Val-Alb-Alb-Alb-Alb-Alb-Alb-Alb-Gln-Gln-Pheol (28 mg, 0.021 mmol) in DMF. After 48 h, the solution was treated according to procedure B. The residue (30 mg) was purified by preparative HPLC (conditions: mobile phase, MeOH-H2O (87:13, v/v); flow rate, 7 ml/min detector, UV (220 nm); column, ZMC X-5 120A ODS (20 mm i.d. x 250 mm); column temperature, 40 °C) to give Alb14-trichosporin B-Vla; yield 13 mg (33%), mp 257–260 °C, [α]D 190 -24.5 (c = 0.2, MeOH), amino acid ratios (6 N HCl, 24 h): Observed (Caled); Gly 1.0 (1); Gin 3.0 (3); Ala 2.0 (2); Val 0.49 (1); Ile 0.94 (1); Leu 0.99 (1). FAB-MS: see Fig. 3. HR-FAB-MS, Caled for C51H14N2O14: 1953.154. Found: 1953.152.

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References and Notes
2) The following abbreviations are used: Ac = acetyl group, AcOH = acetic acid, DCC = N,N'-dicyclohexylcarbodiimide, DCCU = N,N'-dicyclohexylurea, DMF = dimethylformamide, Pac = phenacetyl, TEA = triethylamine, Z = benzylxycarbonyl, DQF-COSY = double quantum filtered correlation spectroscopy, NOESY = nuclear Overhauser enhancement spectroscopy, NOE = nuclear Overhauser effect, TFA = trifluoroacetic acid.