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. Aib^{14}-Trichosporin B-VIa, in which Pro^{14} was replaced by Aib, has also been synthesized to modify the secondary structure of trichosporin B-VIa. Sequence-specific ^{1}H-NMR assignments of both peptides in methanol were achieved by using two-dimensional NMR techniques.

Keywords *Trichoderma polysporum*; peptaibol; trichosporin; α-aminoisobutyric acid; catecholamine

Trichosporin Bs from *Trichoderma polysporum*[3,4] are peptide antibiotics containing a high proportion of an unusual amino acid, α-aminoisobutyric 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 peptaibols, such as alamethicin.[5] Trichosporin Bs have a catecholamine secretion-inducing activity on bovine adrenal chromaffin cells[6] 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 Pro^{14}-containing icosapeptaibols, 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 Pro^{14} residue, which may govern the voltage dependence in the channel-forming mechanism.[12] Trichosporin B-VIa also contains Pro^{14} 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 Aib^{14} derivative.

Trichosporin B-VIa and Aib^{14}-trichosporin B-VIa have the following primary structures: trichosporin B-VIa, Ac–Aib–Ala–Aib–Ala–Aib–Aib–Gln–Aib–Ile–Aib–Gly–Leu–Aib–Pro–Val–Aib–Aib–Gln–Gln–Pheol; Aib^{14}-trichosporin B-VIa, Ac–Aib–Ala–Aib–Ala–Aib–Aib–Gln–Aib–Ile–Aib–Gly–Leu–Aib–Aib–Val–Aib–Aib–Gln–Gln–Pheol.

Results and Discussion

Synthesis of Trichosporin B-VIa and the Aib^{14} Derivative

Peptaibols have generally been synthesized by the solution-phase method,[13] and we chose this method for the syntheses of trichosporin B-VIa and Aib^{14}-trichosporin B-VIa. The synthetic strategy is based on our previous work on the synthesis of trichosporin B-V[14] and hypelcin 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)

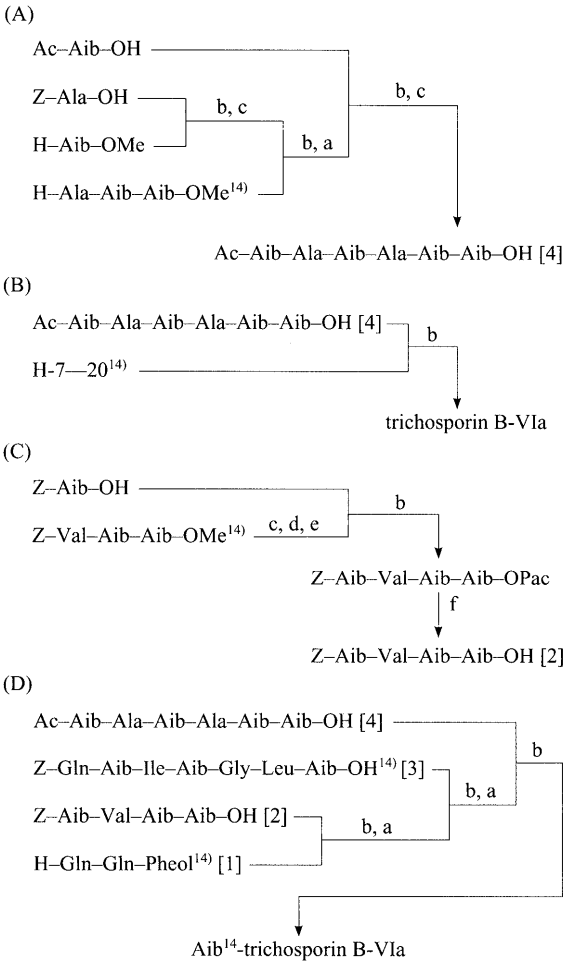

Fig. 1. Synthetic Route to Trichosporin B-VIa and Aib^{14}-Trichosporin B-VIa

(A)—(D) Synthetic schemes for fragment [4], trichosporin B-VIa, fragment [2], and Aib^{14}-trichosporin B-VIa, respectively. Reagents: a, H_2/Pd–C; b, DCC–HOBt; c, 1 N NaOH; d, PacBr; e, HBr/AcOH; f, Zn/AcOH.

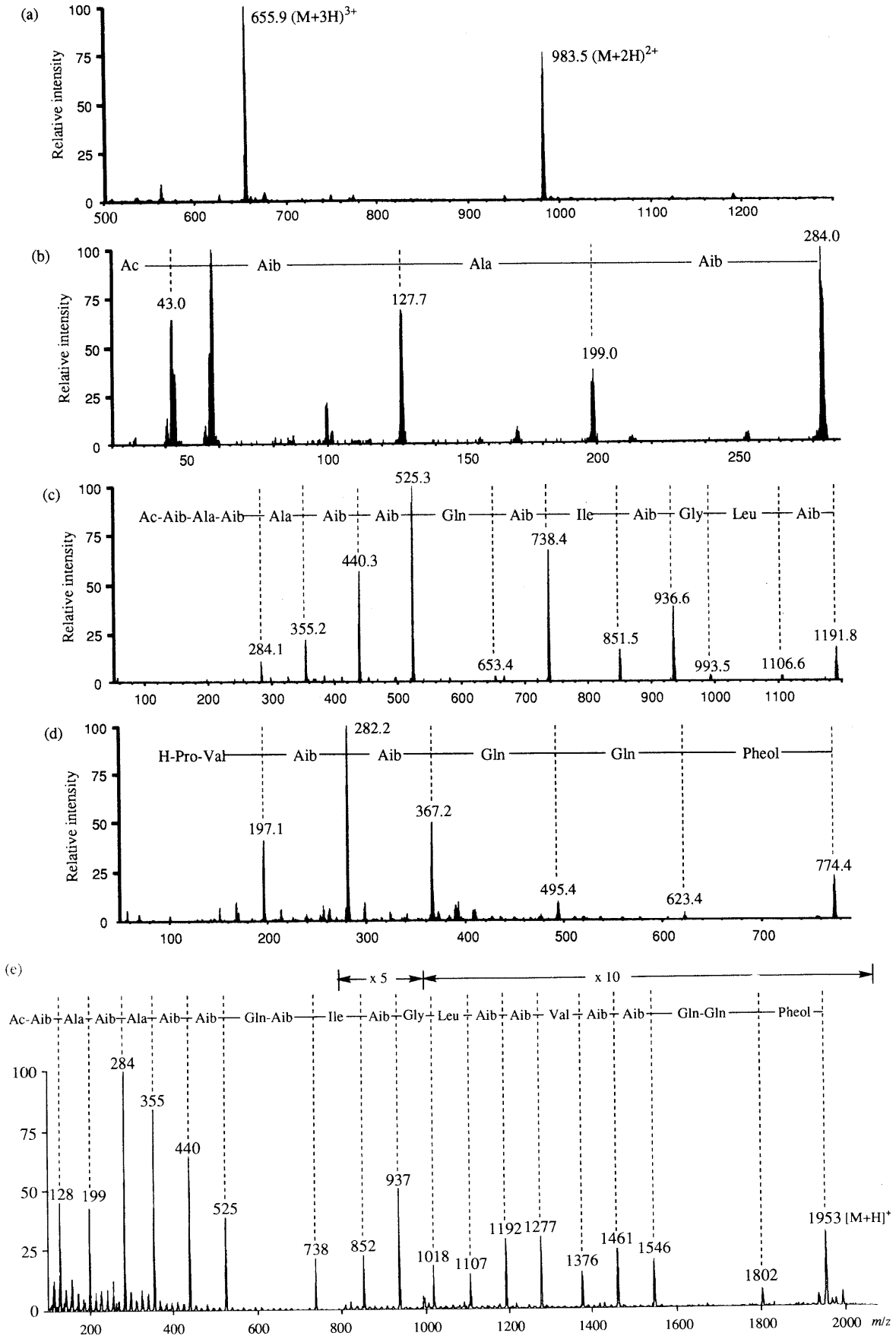

Fig. 2. ES-MS and MS/MS of Synthetic Trichosporin B-IVa and FAB-MS of Aib[14]-Trichosporin B-VIa

(a) Two- and 3- fold charged molecular ions of trichosporin B-VIa 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 Aib[14]-trichosporin B-VIa.

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

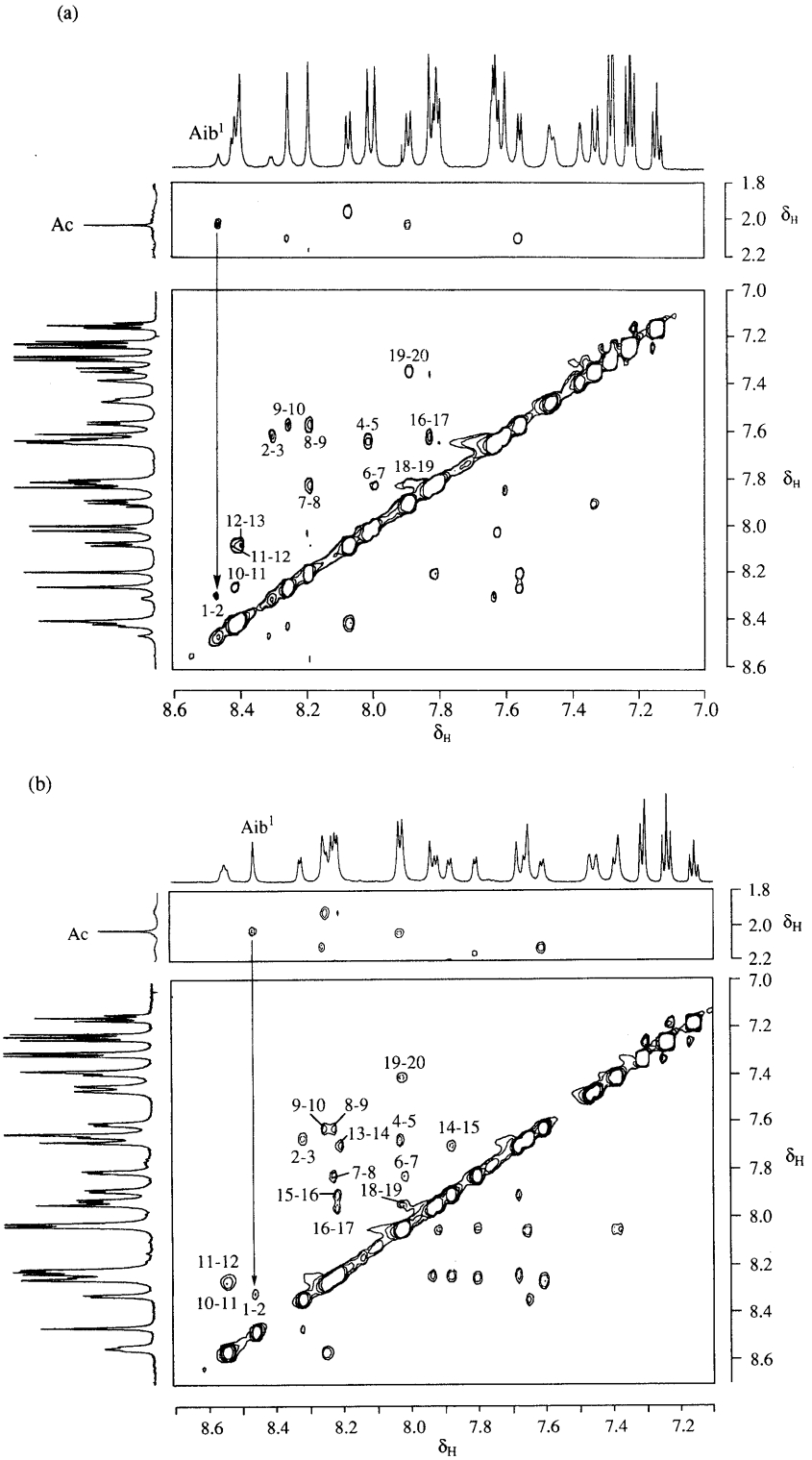

Fig. 3. Parts of the 600 MHz NOESY Spectra of Trichosporin B-VIa and Aib[14]-Trichosporin B-VIa at 20°C in CD_3OH (20 mM)

Sequential NH–NH and acetyl $C^{\alpha}H$–NH cross-peaks [d_{NN} (i, $i+1$) and $d_{\alpha N}$ (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 $C^{\beta}H_3$ proton was assigned as the Aib[1] NH proton. The other NH protons were assigned from the NH-NH connectivities [d_{NN} (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^{14}-trichosporin B-VIa in Fig. 1d were described in our previous report.[14] In the synthesis of the tetrapeptide acid (positions 14—17; fragment [2]) for Aib^{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 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^{14}-trichosporin B-VIa.

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

^{1}H-NMR Study of Trichosporin B-VIa and the Aib^{14} Derivative The ^{1}H-NMR signals of trichosporin B-VIa and Aib^{14}-trichosporin B-VIa in CD_3OH were assigned by DQF-COSY[16] and NOESY.[17] The spin systems of amino acids and Pheol were identified from the DQF-COSY spectra. The Aib C^{β}-H_3 signals, which were unassignable by DQF-COSY owing to the lack of α-protons, were assigned on the basis of the NOEs between NH and C^{β}-H_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[18] (Fig. 3).

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

The NH chemical shifts of the N-terminal parts (positions 1 to 10) in both peptides are almost identical to each other, and the $^3J_{NH\text{-}\alpha H}$ values are less than 7 Hz, indicating that both peptides have similar helical structure in this region.[19] On the other hand, the NH signals of Aib^{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 $^3J_{NH\text{-}\alpha H}$ values of this region in Aib^{14}-trichosporin B-VIa are small and indicative of a helical structure but the values for Leu^{12} and Val^{15} of trichosporin B-VIa are larger than those for Aib^{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^{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 peptaibols, such as trichosporin B-V,[20] alamethicins,[12,21]

TABLE I. Proton Chemical Shifts and Coupling Constant Values of Trichosporin B-VIa and Aib^{14}-Trichosporin B-VIa in CD_3OH at 20 °C (20 mM)

Trichosporin B-VIa			Aib^{14}-trichosporin B-VIa		
Residue	NH[a]	Others[a]	Residue	NH[a]	Others[a]
Ac		α=2.020 s	Ac		α=2.026 s
Aib^1	8.461 s		Aib^1	8.460 s	β=1.47 s
Ala^2	8.302 d (4.1)	α=4.01 m, β=1.425 d (7.3)	Ala^2	8.321 d (4.2)	α=4.01 m, β=1.430 d (7.3)
Aib^3	7.629 s	β=1.524 s	Aib^3	7.646 s	β=1.526 s
Ala^4	7.624 d (5.7)	α=4.061 dq (5.7, 7.1), β=1.463 d (7.1)	Ala^4	7.652 d (5.7)	α=4.04 m, β=1.463 d (7.5)
Aib^5	8.013 s	β=1.535 s, 1.462 s	Aib^5	8.030 s	β=1.546 s
Aib^6	7.991 s	β=1.571 s, 1.520 s	Aib^5	8.018 s	β=1.58 s, 1.511 s
Gln^7	7.812 d (5.5)	α=3.87 m, β=2.26 m, 2.16 m, γ=2.562 ddd (5.6, 9.3, 15.2), 2.40 m	Gln^7	7.802 d (4.7)	α=3.88 m, β=2.25 m, 2.15 m, γ=2.576 ddd (5.5, 9.4, 15.1), 2.381 ddd (6.6, 9.2, 15.1)
Aib^8	8.192 s	β=1.585 s, 1.52 s	Aib^8	8.229 s	β=1.605 s, 1.546 s
Ile^9	7.556 d (6.0)	α=3.71 m, β=2.08 m, γ=1.75 m, 1.35 m, γ^2=0.959 d (7.2), δ=0.870 t (7.3)	Ile^9	7.604 d (5.5)	α=3.677 dd (5.5, 10.6), β=2.13 m, γ=1.76 m, 1.38 m, γ^2=0.967 d (6.8), δ=0.869 t (7.4)
Aib^{10}	8.254 s	β=1.550 s	Aib^{10}	8.254 s	β=1.58 s, 1.533 s
Gly^{11}	8.412 dd (5.0, 6.5)	α=3.934 dd (5.0, 16.5), 3.675 dd (6.5, 16.5)	Gly^{11}	8.546 dd (4.5, 6.6)	α=3.801 dd (4.5, 15.7), 3.691 dd (6.6, 15.7)
Leu^{12}	8.070 d (7.7)	α=4.45 m, β=1.95 m, 1.60 m, γ=1.95 m, δ=0.959 d (6.7), 0.918 d (6.5)	Leu^{12}	8.247 d (5.6)	α=4.06 m, β=1.91 m, 1.66 m, γ=1.89 m, δ=0.952 d (6.1), 0.942 d (5.7)
Aib^{13}	8.397 s	β=1.620 s, 1.545 s	Aib^{13}	8.211 s	β=1.58 s, 1.494 s
Pro^{14}		α=4.388 dd (6.3, 8.7), β=2.32 m, 1.81 m, γ=2.07 m, 1.97 m, δ=3.88 m, 3.76 m	Aib^{14}	7.676 s	β=1.633 s
Val^{15}	7.631 d (8.2)	α=3.73 m, β=2.33 m, γ=1.978 d (6.8), 1.071 d (6.5)	Val^{15}	7.878 d (5.1)	α=3.615 dd (5.1, 9.6), β=2.22 m, γ=1.138 d (6.5), 0.992 d (6.7)
Aib^{16}	7.602 s	β=1.545 s	Aib^{16}	8.219 s	β=1.502 s
Aib^{17}	7.829 s	β=1.535 s	Aib^{17}	7.936 s	β=1.608 s
Gln^{18}	7.802 d (6.2)	α=4.00 m, β=2.24 m, γ=2.62 ddd (6.4, 8.8, 15.2), 2.44 m	Gln^{18}	7.918 d (5.5)	α=4.02 m, β=2.28 m, γ=2.673 ddd (5.5, 9.6, 14.8), 2.465 dt (8.4, 14.8)
Gln^{19}	7.890 d (7.4)	α=4.16 m, β=2.03 m, γ=2.31 m, 2.19 m	Gln^{19}	8.024 d (7.0)	α=4.16 m, β=2.03 m, γ=2.338 dt (15.0, 7.2), 2.24 m
Pheol^{20}	7.331 d (9.2)	α=4.14 m, β=2.939 dd (5.5, 13.7), 2.729 dd (8.9, 13.7), β^2=3.617 d (5.1)	Pheol^{20}	7.385 d (8.8)	α=4.15 m, β=2.942 dd (5.5, 13.8), β=2.750 dd (9.0, 13.8), β^2=3.63

a) Chemical shifts (ppm) were measured either from the one-dimensional spectra ($\Delta\delta$=±0.001 ppm) or from the two-dimensional spectra ($\Delta\delta$=±0.01 ppm). Coupling constant values (Hz) were measured from the one-dimensional spectra.

and saturnisporins.[22] These carbonyl oxygen atoms are considered to increase the polarity of the peptides.[12] It is, therefore, clear that the longer retention time of Aib[14]-trichosporin B-VIa ($t_R = 50.34$) on HPLC in comparison with that of trichosporin B-VIa ($t_R = 20.23$) 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_3OH containing tetramethylsilane as an internal standard. The details of NMR measurements have already been described in the previous paper.[23] EI-MS was performed on a JEOL 01-SG. FAB-MS was performed on a JEOL HX-110 or VG AutoSpec-T. Samples were bombarded with 3 keV xenon atoms and glycerol–thioglycerol or *m*-nitrobenzyl alcohol–glycerol was used as the matrix. Pneumatically assisted electrospray mass spectroscopy (ES-MS) was performed on an API III (Perkin Elmer Sciex). Samples were dissolved in CH_3CN–H_2O (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 60F254, Merck). The *Rf* values refer to the following solvent systems (v/v): Rf_1 = $CHCl_3$–MeOH (95 : 5), Rf_2 = $CHCl_3$–MeOH (9 : 1), Rf_3 = $CHCl_3$–MeOH (8 : 2), Rf_4 = $CHCl_3$–MeOH–H_2O (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_2O solvent system. Amino acid analyses were done with a Hitachi Model 835 amino acid analyzer.

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

Procedure A: EtOAc-soluble protected peptides were each dissolved in EtOAc and the solution was washed successively with 1 N HCl, 5% $NaHCO_3$ and saturated NaCl, dried over Na_2SO_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 solution was neutralized with 1 N HCl and evaporated to remove MeOH. The residual solution was acidified to pH 3 and extracted with EtOAc. The extract was washed with saturated NaCl, dried over Na_2SO_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·H–Aib–OMe (2.38 g, 1 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 was purified by silica gel chromatography ($CHCl_3$: MeOH = 95 : 5) to afford a syrupy product, Z–Ala–Aib–OMe; yield 3.60 g (72%), $[\alpha]_D$ −30.3° (c = 1.0, MeOH), Rf_3 0.54, EI-MS *m/z*: 322 (M^+), 291 ($M^+ - OCH_3$), 206 (291 − Aib).

B) Z–Ala–Aib–OH Z–Ala–Aib–OMe (9.45 g, 29.3 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_5 0.67.

C) Z–Ala–Aib–Ala–Aib–Aib–OMe Z–Ala–Aib–OH (1.00 g, 3.23 mmol), HOBt (440 mg, 1 eq) and DCC (670 mg, 1 eq) were added successively to a stirred solution of HCl·H–Ala–Aib–Aib–OMe[14] (1.00 g, 1 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; yield 1.31 g (72%), mp 220—221 °C, $[\alpha]_D$ −2.4° (c = 1.0, MeOH), Rf_2 0.32, EI-MS *m/z*: 563 (M^+), 447 ($M^+ - Aib - OCH_3$), 362 (447 − Aib), 291 (447 − Ala), 206 (291 − Aib). *Anal.* Calcd for $C_{27}H_{41}N_5O_8$: C, 57.54; H, 7.33; N, 12.42. Found: C, 57.72; H, 7.22; N, 12.55.

H–Ala–Aib–Ala–Aib–Aib–OMe The above pentapeptide (1.60 g, 3.72 mmol) in MeOH (40 ml) was hydrogenated according to procedure D. The catalyst was removed by filtration and the solvent was evaporated off to afford the title compound; yield 1.37 g (86%), Rf_5 0.69.

E) Ac–Aib–Ala–Aib–Ala–Aib–Aib–OMe Ac–Aib–OH was prepared by refluxing H–Aib–OH and Ac_2O in AcOH. Ac–Aib–OH (150 mg, 1.03 mmol), HOBt (139 mg, 1 eq) and DCC (211 mg, 1 eq) were added successively to a solution of H–Ala–Aib–Ala–Aib–Aib–OMe (441 mg, 1 eq) in DMF (7 ml) with stirring. After 48 h, DCU and the solvent were 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, $[\alpha]_D$ +6.2° (c = 0.3, MeOH), Rf_1 0.46. ES-MS *m/z*: 557 ($M+H^+$), 525 ($M+H^+ - OCH_3 - H$), 440 (525 − Aib), 355 (440 − Aib), 284 (355 − Ala), 199 (284 − Aib), 128 (199 − Ala). *Anal.* Calcd for $C_{25}H_{44}N_6O_8 \cdot 1/2H_2O$: C, 53.08; H, 8.02; N, 14.86. Found: C, 53.13; H, 8.09; N, 14.78.

F) Ac–Aib–Ala–Aib–Ala–Aib–Aib–OH [4] The above protected hexapeptide (49.6 mg, 0.089 mmol) was saponified and the solution was neutralized with Amberlite IR-120. The solvent was evaporated off to give the pure hexapeptide acid; yield 46.0 mg (95 %), mp 210—212 °C, Rf_5 0.73.

G) Ac–Aib–Ala–Aib–Ala–Aib–Aib–Gln–Aib–Ile–Aib–Gly–Leu–Aib–Pro–Val–Aib–Aib–Gln–Gln–Pheol [Trichosporin B-VIa] Ac–Aib–Ala–Aib–Ala–Aib–Aib–OH (positions 1—6) (46.0 mg, 1.5 eq), HOBt (11.5 mg, 1.5 eq) and DCC (17.5 mg, 1.5 eq) were added successively to a solution of H–Gln–Aib–Ile–Aib–Gly–Leu–Aib–Pro–Val–Aib–Aib–Gln–Gln–Pheol[14] (positions 7—20) (82.2 mg, 56.5 μmol) in DMF (1.2 ml) with stirring. The solution was stirred for 48 h and treated according to procedure B. The residue (70.0 mg) was purified by preparative HPLC [conditions: mobile phase, MeOH–H_2O (85 : 15, v/v); flow rate, 7 ml/min; detector, UV (220 nm); column, YMC S-5 120A ODS (20 mm i.d. × 250 mm); column temperature, 40 °C] to give trichosporin B-VIa; yield 37.7 mg (34%), mp 232—234 °C (nat. 239—242 °C[4]), $[\alpha]_D$ −16.0° (c = 0.2, MeOH), Rf_4 0.36, ES-MS: see Fig. 3. Amino acid ratios (6 N HCl, 24 h): Observed (Calcd); Glu 3.10 (3), Ala 1.94 (2), Val 0.99 (1), Gly 1.00 (1), Ile 0.94 (1), Leu 0.99 (1), Pro 0.93 (1). HR-FAB-MS, Calcd for $C_{92}H_{153}N_{23}O_{24}Na$: 1987.136. Found: 1987.148.

Synthesis of Aib[14]-Trichosporin B-VIa **A) Z–Val–Aib–Aib–OH** Z–Val–Aib–Aib–OMe[14] (3.0 g, 7.12 mmol) in MeOH (70 ml) was saponified according to procedure C to give Z–Val–Aib–Aib–OH. Further purification was not carried out; yield 3.0 g (99%), mp 91—93°C, Rf_5 0.24.

B) Z–Val–Aib–Aib–OPac The above tripeptide acid (800 mg, 1.90 mmol) and phenacyl bromide (415 mg, 1.1 eq) were dissolved in DMF (4 ml) containing TEA (0.29 ml, 1.1 eq). After 12 h, the solvent was evaporated off and the residue was dissolved in EtOAc. The solution was washed with 5% $NaHCO_3$ and saturated NaCl, dried over Na_2SO_4 and evaporated to give 894 mg of a crude residue. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH = 95 : 5) to give the tripeptide phenacyl ester; yield 662 mg (65%), mp 51—53°C, $[\alpha]_D$ +5.5° (c = 0.4, MeOH), Rf_1 0.29, ES-MS *m/z*: 540 ($M+H^+$); 404 ($M+H^+ - OPac$); 319 (404 − Aib); 234 (319 − Aib), *Anal.* Calcd for $C_{29}H_{37}N_3O_7$: C, 64.55; H, 6.91; N, 7.79. Found: C, 64.48; H, 7.12; N, 8.02.

C) HBr·Val–Aib–Aib–OPac Z–Val–Aib–Aib–OPac (300 mg, 0.556 mmol) was treated with 30% HBr in AcOH (2 ml) under stirring. After 1.5 h, dry ether was added and the resulting precipitate was collected by filtration, washed with dry ether and dried over NaOH *in vacuo* to give HBr·Val–Aib–Aib–OPac; yield 256 mg (95%), Rf_4 0.60.

D) Z–Aib–Val–Aib–Aib–OPac Z–Aib–OH (126 mg, 0.530 mmol), HOBt (72 mg, 1 eq), and DCC (109 mg, 1 eq) were added successively to a stirred solution of HBr·H–Val–Aib–Aib–OPac (250 mg, 1 eq) with TEA (7.3 μl, 1 eq) in DMF. After 48 h, the solution was worked up according to procedure A. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH = 95 : 5) to give the tetrapeptide phenacyl ester; yield 209 mg (63%), mp 44—46 °C, $[\alpha]_D$ +3.1° (c = 0.3, MeOH), Rf_2 0.39, ES-MS *m/z*: 625 ($M+H^+$); 489 ($M+H^+ - OPac$); 404 (489 − Aib); 319 (404 − Aib). *Anal.* Calcd for $C_{33}H_{44}N_4O_8 \cdot 1/2\ H_2O$: C, 62.54; H, 7.16; N, 8.84. Found: C, 62.28; H, 7.23; N, 8.93.

E) Z–Aib–Val–Aib–Aib–OH [2] The above tetrapeptide (160 mg,

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 Na_2SO_4 and concentrated. The residue was recrystallized from MeOH and ether to afford the tetrapeptide acid; yield 92 mg (79%), mp 85—89 °C, $Rf_4 = 0.46$.

F) Z-Aib-Val-Aib-Aib-Gln-Gln-Pheol The above tetrapeptide acid (90 mg, 0.178 mmol), HOBt (24 mg, 1 eq), and DCC (37 mg, 1 eq) were added successively to a stirred solution of H-Gln-Gln-Pheol [1][14] (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 reprecipitated from MeOH and ether to give the heptapeptide; yield 125 mg (78%), mp 112—113 °C, $[\alpha]_D$ −18.0° ($c = 0.3$, MeOH), Rf_4 0.64, FAB-MS m/z: 896 ($M+H^+$), 745 ($M+H^+$ − Pheol − H), 489 (745 − Gln − Gln), 404 (489 − Aib), 220 (404 − Aib − Val). *Anal.* Calcd for $C_{44}H_{65}N_9O_{11} \cdot 1/2\ H_2O$: C, 58.39; H, 7.35; N, 13.93. Found: C, 58.40; H, 7.29; N, 13.72.

G) H-Aib-Val-Aib-Aib-Gln-Gln-Pheol The above heptapeptide (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_5 0.58.

H) Z-Gln-Aib-Ile-Aib-Gly-Leu-Aib-Aib-Val-Aib-Aib-Gln-Gln-Pheol Z-Gln-Aib-Ile-Aib-Gly-Leu-Aib-OH [3][14] (76 mg, 0.093 mmol), HOBt (14 mg, 1.1 eq), and DCC (21 mg, 1.1 eq) were added successively to a stirred solution of H-Aib-Val-Aib-Aib-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 reprecipitated from MeOH and ether to give the tetradecapeptide; yield 58 mg (40%), mp 145—147 °C, $[\alpha]_D$ −23.8° ($c = 0.8$, MeOH), Rf_4 0.54, FAB-MS m/z: 1562 ($M+H^+$), 1411 ($M+H^+$ − Pheol − H), 1155 (1411 − Gln − Gln), 1070 (1155 − Aib), 985 (1070 − Aib), 886 (985 − Val), 801 (886 − Aib), 716 (801 − Aib), 603 (716 − Leu), 546 (603 − Gly), 461 (546 − Aib), 348 (461 − Ile). *Anal.* Calcd for $C_{75}H_{119}N_{17}O_{19} \cdot H_2O$: C, 56.98; H, 7.71; N, 15.06. Found: C, 56.64; H, 7.48; N, 15.23.

I) H-Gln-Aib-Ile-Aib-Gly-Leu-Aib-Aib-Val-Aib-Aib-Gln-Gln-Pheol The above tetradecapeptide (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_5 0.58.

J) Ac-Aib-Ala-Aib-Ala-Aib-Aib-Gln-Aib-Ile-Aib-Gly-Leu-Aib-Aib-Val-Aib-Aib-Gln-Gln-Pheol [Aib^{14}-Trichosporin B-VIa] Ac-Aib-Ala-Aib-Ala-Aib-Aib-OH (16 mg, 0.03 mmol), HOBt (4 mg, 1.5 eq), and DCC (6.2 mg, 1.5 eq) were added successively to a stirred solution of H-Gln-Aib-Ile-Aib-Gly-Leu-Aib-Aib-Val-Aib-Aib-Gln-Gln-Pheol (28 mg, 0.02 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–H_2O (87 : 13, v/v); flow rate, 7 ml/min detector, UV (220 nm); column, YMC S-5 120A ODS (20 mm i.d. × 250 mm); column temperature, 40 °C] to give Aib^{14}-trichosporin B-VIa; yield 13 mg (33%), mp 257—260 °C, $[\alpha]_D$ −24.2° ($c = 0.2$, MeOH), amino acid ratios (6 N HCl, 24 h): Observed (Calcd); Gly 1.0 (1); Gln 3.0 (3); Ala 2.0 (2); Val 0.94 (1); Ile 0.94 (1); Leu 0.99 (1). FAB-MS: see Fig. 3. HR-FAB-MS, Calcd for $C_{91}H_{154}N_{23}O_{24}$: 1953.154. Found: 1953.152.

Analytical HPLC Chromatography of the Synthetic Trichosporin B-VIa and Aib^{14}-Trichosporin B-VIa Synthetic trichosporin B-VIa: t_R 20.23 (identical to natural trichosporin B-VIa), Aib^{14}-trichosporin B-VIa: t_R 50.34 [conditions: mobile phase, MeOH–H_2O (87 : 13, v/v); flow rate, 0.8 ml/min; detector, UV (220 nm); column, YMC AM-313 (6 mm i.d. × 250 mm); column temperature, 40 °C].

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References and Notes

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2) The following abbreviations are used: Ac = acetyl group, AcOH = acetic acid, DCC = *N*,*N*′-dicyclohexylcarbodiimide, DCU = *N*,*N*′-dicyclohexylurea, DMF = dimethylformamide, Pac = phenacyl, TEA = triethylamine, Z = benzyloxycarbonyl, DQF-COSY = double quantum filtered correlation spectroscopy, NOESY = nuclear Overhauser enhancement spectroscopy, NOE = nuclear Overhauser effect, TFA = trifluoroacetic acid.
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