Fungal Metabolites. IV.[1,2] Synthesis of an Antibiotic Peptide, Trichosporin B-V, from *Trichoderma polysporum*

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The antibiotic icosapeptide trichosporin B-V, which was isolated from *Trichoderma polysporum*, was synthesized by assembling five peptide fragments *via* the *N,N'*-dicyclohexylcarbodiimide method. The synthesized peptide was identical with the natural one.

Keywords *Trichoderma polysporum*; peptaibol; α-aminoisobutyric acid; phenylalaninol; uncoupling activity; trichosporin B-V

Trichosporin B-V[3] was isolated from a culture broth of *Trichoderma polysporum*. This peptide contains a high proportion of an abnormal amino acid, α-aminoisobutyric acid (Aib). The N-terminal amino acid of this peptide is protected by an acetyl group and the C-terminal amino acid is linked with an amino alcohol. Thus, trichosporin B-V belongs to the class of peptaibols, like alamethicin,[4] suzukacillin[5] and hypelcin.[6a,b] The primary structure of this peptide is as follows: Ac–Aib–Ala–Ala–Ala–Aib–Aib–Gln–Aib–Ile–Aib–Gly–Leu–Aib–Pro–Val–Aib–Aib–Gln–Gln–Pheol (Pheol: phenylalaninol). Trichosporin B-V has uncoupling activity in mitochondria of rat liver, as do hypelcin[6c] and alamethicin.[7] Taking account of the fact that alamethicin forms voltage-dependent ion channels in lipid bilayers,[8] this activity can be deduced to have some relation to the formation of ion channels in mitochondrial membranes. Therefore, trichosporin B-V is expected to be useful for the study of biomembrane functions. Since this peptide is found in extremely low abundance, we tried to synthesize it by the solution-phase method in order to obtain a sufficient amount for examination of the biological activities and for structural confirmation.

In the case of the synthesis of peptaibols, steric hinderance of Aib seems to decrease the total yields considerably. However, the preliminary condensation of Z–X–OH (X=Aib, Gln, Ile and Leu) with H–Aib–OMe by the convenient DCC method gave relatively good yields and, thus, we expected that this method would be useful to synthesize trichosporin B-V. The synthetic scheme for trichosporin B-V, designed by taking into consideration the syntheses of other trichosporin Bs (Table I),[1,9] is shown in Fig. 1. Of the five fragments, fragments **[1]** and **[3]** can be used commonly in the synthesis of all trichosporin Bs. Each of **[2]**—**[5]** has an Aib residue, which does not have an α-proton, at the C-terminus, and this avoids racemization[10] both in the alkaline hydrolysis of esters and in the activation of carboxyl components. The purity of the fully protected peptides was ascertained by thin layer chromatography (TLC) and elemental analyses. Although amino acid analysis was only carried out for the final product because of the extremely low reactivity of Aib to ninhydrin reagent, the structures of these peptides were confirmed by ^{1}H- and/or ^{13}C-nuclear magnetic resonance (^{1}H- and/or ^{13}C-NMR), and electron impact (EI) or positive ion fast atom bombardment mass spectrometry (FAB-MS).

The C-terminal fragment, Z–Gln–Gln–Pheol **[1]** (positions 18—20), was prepared by the active ester procedure with *N*-hydroxysuccimide in a stepwise manner as shown in Fig. 2. The N^{α}-protecting group, Z, of the tripeptide was removed with 30% HBr in AcOH (usual catalytic hydrogenation in MeOH did not proceed because of the very poor solubility of the substrate in MeOH). The deprotected tripeptide, which is very hygroscopic, was treated with Amberlite IRA-400.

Fragment **[2]**, Z–Pro–Val–Aib–Aib–OMe (positions 14—17), was prepared by the DCC–HOBt procedure in a stepwise manner as shown in Fig. 3. The ^{1}H- and ^{13}C-NMR spectra of this tetrapeptide measured in CD_3OD revealed

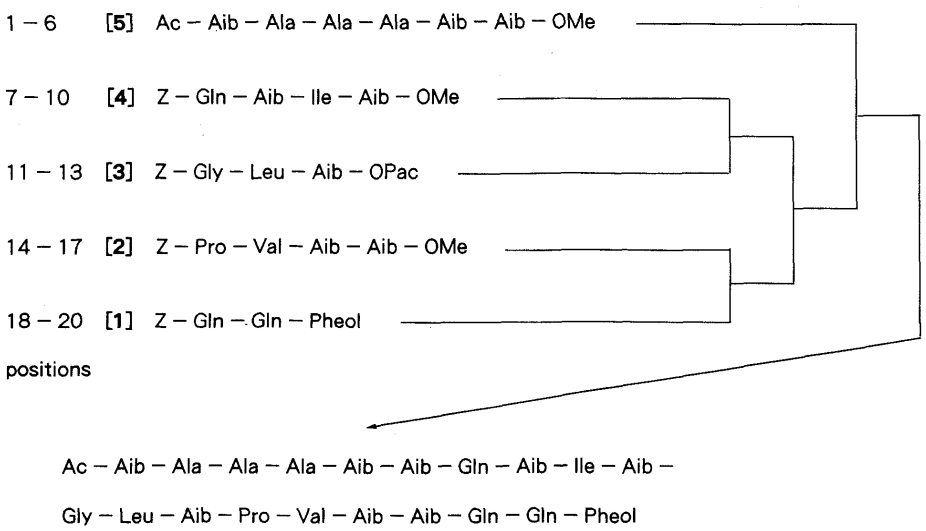

Fig. 1. Synthetic Route to Trichosporin B-V

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the presence of *cis–trans* isomers at Pro[11] based on the chemical shifts of the C^{β} resonances (*trans* = δ, 31.1; *cis* = δ, 32.4).

Fragment [3], Z–Gly–Leu–Aib–OPac (positions 11—13), was obtained by ester-exchange from Z–Gly–Leu–Aib–OMe. The methyl ester was synthesized by the DCC–HOBt method in a stepwise manner followed by alkaline hydrolysis and treatment with phenacyl bromide to give the tripeptide phenacyl ester, as shown in Fig. 4. This ester-exchange was important for the total synthesis of trichosporin B-V as described later. The Z group of the protected tripeptide was removed in the same manner as in the case of fragment [1].

Fragment [4], Z–Gln–Aib–Ile–Aib–OMe (positions 7—10) was prepared by the condensation of the two dipeptides as shown in Fig. 5. Unexpectedly, the target tetrapeptide showed considerable hydrophilicity. On the other hand, the acid obtained by saponification did not show hydrophilicity.

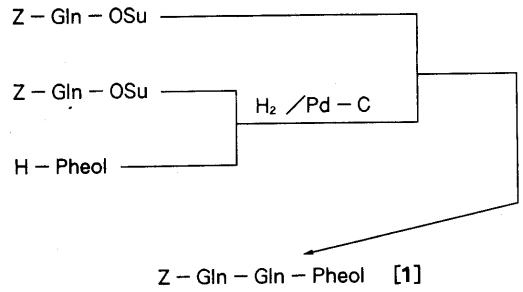

Fig. 2. Synthetic Scheme for the C-Terminal Tripeptide [1] (Positions 18—20)

Fragment [5], Ac–Aib–Ala–Ala–Ala–Aib–Aib–OMe (positions 1—6), was synthesized according to the route shown in Fig. 6. The condensation step of the Z–Ala–Ala–OH (positions 2—3) with H–Ala–Aib–Aib–OMe (positions 4—6) could involve racemization at Ala. However, the product, obtained in an acceptable yield, was found to be uniform from its ^{1}H- and ^{13}C-NMR spectra. The hexapeptide, fragment [5] obtained by coupling of Ac–Aib–OH with the amine component (positions 2—6) and the corresponding peptide acid were readily soluble in water.

Thus, five peptide fragments were obtained. They were condensed successively according to the route shown in Fig. 1. All fragment condensation steps were carried out by the DCC–HOBt procedure in DMF at room temperature. The condensation of the amine component from fragment [1] with the peptide acid from fragment [2] gave the C-terminal heptapeptide (positions 14—20). The product was purified by Sephadex LH-20 column chromatography, followed by recrystallization from MeOH and ether. The amino protecting group, Z, was removed by hydrogenation over palladium-on-charcoal to give the deprotected heptapeptide.

The heptapeptide (positions 7—13) located in the middle of the molecule of trichosporin B-V was obtained by the condensation of the peptide derivatives from fragment [3] (positions 11—13) and [4] (positions 7—10). The phenacyl ester was purified by chromatography on silica gel. The

TABLE I. Primary Structures[a] of Trichosporin Bs

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Trichisporin B V	Ac	Aib	Ala	Ala	Ala	Aib	Aib	Gln	Aib	Ile	Aib	Gly	Leu	Aib	Pro	Val	Aib	Aib	Gln	Gln–Pheol
	(5)							(4)				(3)			(2)				(1)	
Ia	Ac	Aib	Ala	Ser	Ala	Aib	Aib	Gln	Aib	Leu	Aib	(3)			(2)				(1)	
IIIa	(5)							Gln	Aib	Leu	Aib	(3)			(2)				(1)	
IIIb	(5)							(4)				(3)		Pro	Val	Aib	Ala		(1)	
IIIc	Ac	Aib	Ala	Ala	Ala	Ala	Aib	(4)				(3)			(2)				(1)	
IIId	(5)							Gln	Aib	Val	Aib	(3)			(2)				(1)	
IVb	(5)							Gln	Aib	Leu	Aib	(3)		Pro	Val	Aib	Iva		(1)	
IVc	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Val	Aib	(3)			(2)				(1)	
IVd	(5)							Gln	Aib	Val	Aib	(3)		Pro	Val	Aib	Iva		(1)	
VIa	Ac	Aib	Ala	Aib	Ala	Aib	Aib	(4)				(3)			(2)				(1)	
VIb	(5)							(4)				(3)		Pro	Val	Aib	Iva		(1)	

a) (1), Gln–Gln–Pheol; (2), Pro–Val–Aib–Aib; (3), Gly–Leu–Aib; (4), Gln–Aib–Ile–Aib; (5), Ac–Aib–Ala–Ala–Ala–Aib–Aib. (1)—(5) correspond to fragments [1]—[5], respectively.

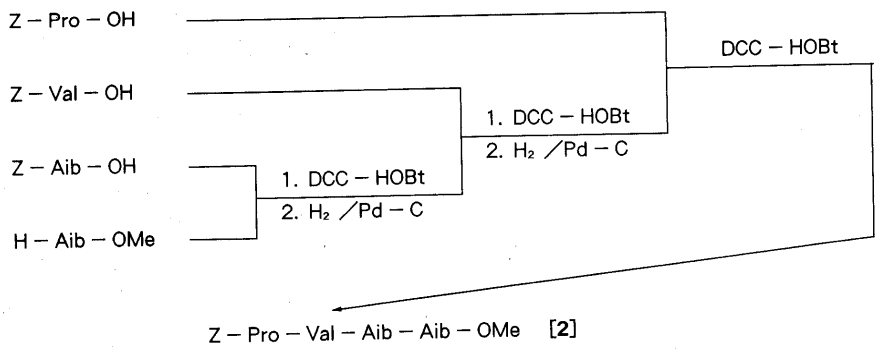

Fig. 3. Synthetic Scheme for the Protected Tetrapeptide [2] (Positions 14—17)

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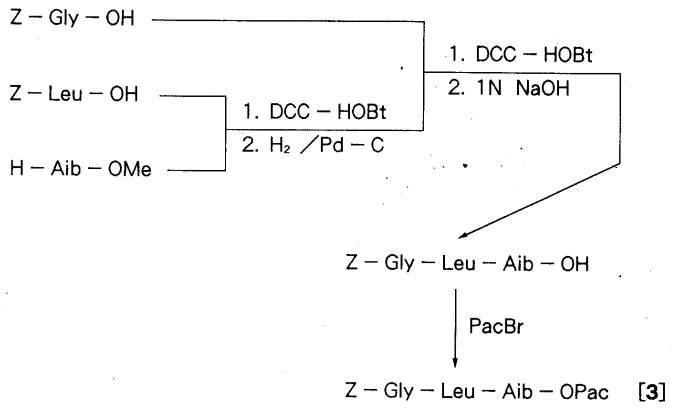

Fig. 4. Synthetic Scheme for the Tripeptide Derivative [3] (Positions 11—13)

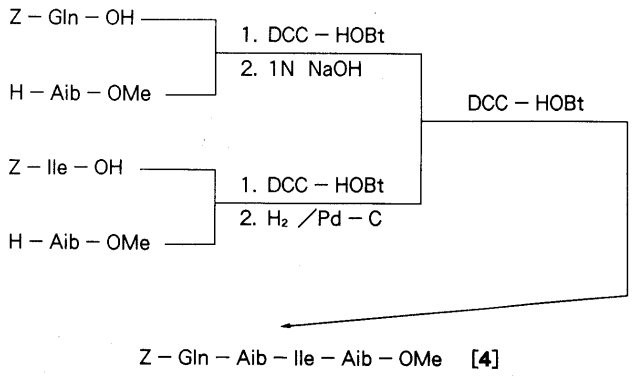

Fig. 5. Synthetic Scheme for the Protected Tetrapeptide [4] (Positions 7—10)

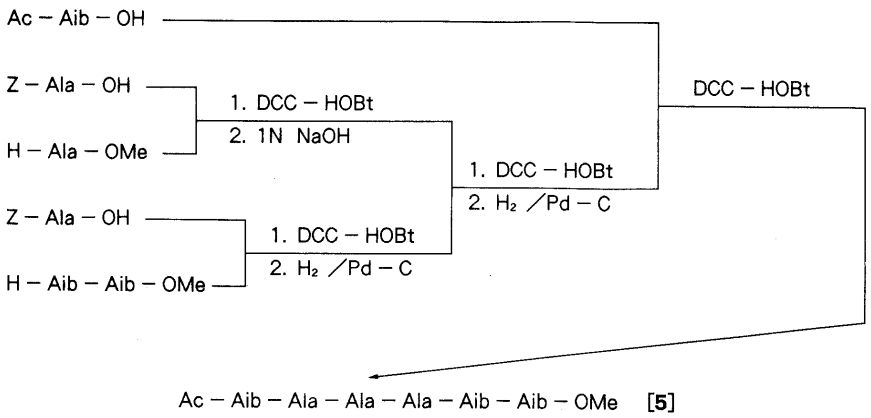

Fig. 6. Synthetic Scheme for the N-Terminal Hexapeptide [5] (Positions 1—6)

phenacyl group was easily removed by Zn powder in AcOH to give the pure heptapeptide acid in a satisfactory yield. The intermediate methyl ester derivative was synthesized, but alkaline hydrolysis of the methyl ester afforded many spots on TLC.

The heptapeptide acid (positions 7—13) was condensed with the C-terminal amine component (positions 14—20) by the DCC–HOBt method. However, the yield of the coupling reaction was low because of the insufficient activation of the acid component owing to steric hindrance. Since the tetradecapeptide (positions 7—20) has an acid-labile Aib–Pro peptide bond, the use of acid during purification should be avoided. Thus, the peptide was purified by gel-filtration on Sephadex LH-20, followed by silica gel chromatography. The Z group of the product was cleaved in a routine manner. The deprotected peptide (amine component, positions 7—20) was pure on TLC.

The synthetic trichosporin B-V was prepared from the N-terminal hexapeptide acid (positions 1—6) and the above amine component (positions 7—20). For purification of the crude trichosporin B-V, gel-filtration on Sephadex LH-20 was carried out. Each fraction was checked by TLC and the fractions containing the target compound were combined and evaporated. Then, the residue was purified by reversed-phase preparative high performance liquid chromatography (HPLC). The purified synthetic trichosporin B-V was homogeneous on analytical HPLC chromatograms with two types of reversed-phase columns and revealed the same retention time as the natural peptide.

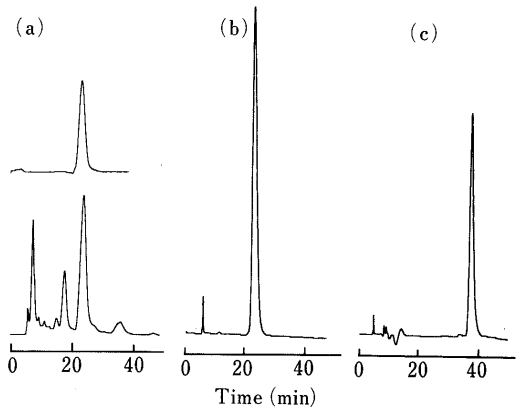

Fig. 7. Analytical HPLC Chromatograms of the Synthetic Trichosporin B-V

(a) The natural trichosporin B-V (upper) and the crude sample after gel-filtration (lower). (b) and (c) The purified sample. Conditions: mobile phase, MeOH–H_2O (85 : 15, v/v); flow rate, 1.0 ml/min for (a) and (b) and 1.2 ml/min for (c); detector, UV (220 nm); column, YMC AM-313 (6 mm i.d. × 250 mm) for (a) and (b), Nacalai Cosmosil 5 Ph (8 mm i.d. × 250 mm) for (c); column temperature, 40°C.

Figure 7 shows analytical HPLC chromatograms of the purified compound.

The synthetic trichosporin B-V was identified by FAB-MS, and ^1H- and ^{13}C-NMR. Figure 8(a) and (b) shows FAB-MS of the synthetic and the natural trichosporin B-V[3]; the fragmentation patterns are identical. The ^1H and ^{13}C resonances of the synthetic product were in good agreement with those of the natural one. Parts of the spectra

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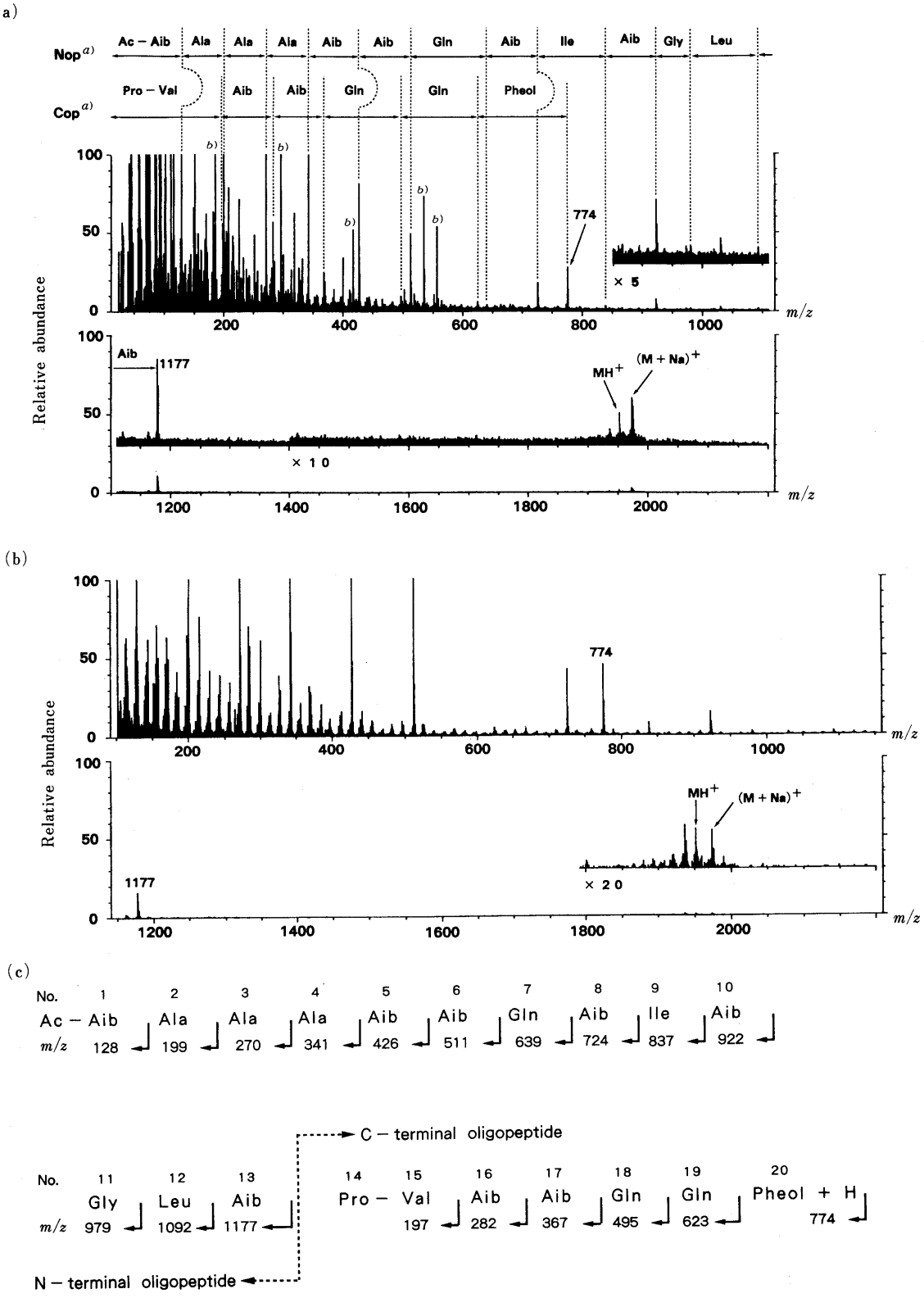

Fig. 8. FAB-MS of the Synthetic (a) and the Natural Trichosporin B-V (b), and Sequence-Characteristic Ions Observed in Both Spectra (c)

a) Nop and Cop represent the N- and the C-terminal oligopeptide, respectively.
b) Ion peaks arising from the matrix.

are shown in Fig. 9. The slight difference of the line shapes is considered to exist because the chemical shifts of the ^{1}H and ^{13}C resonances of trichosporin B-V are affected by the sample concentrations and measuring temperature. The physico-chemical constants (melting points and $[\alpha]_D$) were also in good agreement with those of the natural trichosporin B-V.

Experimental

General Methods All melting points are uncorrected. Optical rotations

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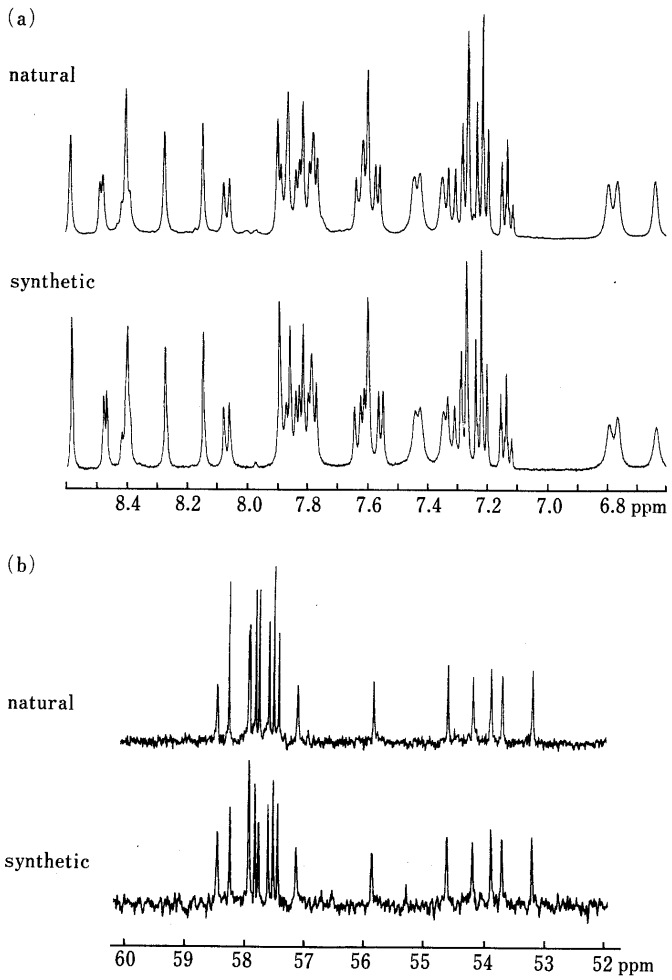

Fig. 9. Parts of the ^{1}H- and ^{13}C-Spectra of the Synthetic Trichosporin B-V Measured in CD_3OH at 26°C (4.8 mM)

(a) Amide regions of the ^{1}H-NMR spectra of the natural (upper) and the synthetic trichosporin B-V (lower). (b) Parts of the α-carbon regions of the ^{13}C-NMR spectra of the natural (upper) and the synthetic trichosporin B-V (lower). The assignments of individual resonances will be reported elsewhere.

were measured with a JASCO DIP-181 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-FX200 and a Bruker AM-400. Samples were dissolved in $CDCl_3$, CD_3OD or CD_3OH containing tetramethylsilane as an internal standard. For brevity, assignments of individual resonances are omitted. FAB-MS were taken on a JEOL JMS-HX100. Samples were bombarded with 8 keV xenon atoms and glycerol–thiogrycerol was used as a matrix. TLC was performed on silica gel (Kieselgel $60F_{254}$, 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 (7 : 3), Rf_5 $CHCl_3$–MeOH–H_2O (6 : 5 : 2). For column chromatography on silica gel, Silica gel 60 (70—230 mesh, Merck) was used. The desired fractions (checked by TLC) were combined and the solvent was removed *in vacuo*. For gel-filtration, Sephadex LH-20 (Pharmacia) was employed. Samples were dissolved in MeOH and eluted with the same solvent. The desired fractions (checked by TLC) were combined and the solvent was removed *in vacuo*. Analytical and preparative HPLC were performed on a Shimadzu LC-6A system. As the solvent system for both, MeOH–H_2O (85 : 15) was used.

Coupling Reactions Unless otherwise stated, coupling reactions were mainly performed by the DCC–HOBt method at room temperature for 24—72 h and the mixtures were worked up according to procedure A or B after removal of DCU and the solvent.

Procedure A: In the case of EtOAc-soluble protected peptides, the extract was washed 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: In the case of EtOAc-insoluble protected peptides, the crude product was purified by gel-filtration on Sephadex LH-20 in MeOH. Fractions containing the product were collected and concentrated. The residue was usually recrystallized or precipitated from appropriate solvents.

Hydrolysis of Z-Peptide Methyl Esters Unless otherwise described, Z-peptide methyl esters were hydrolyzed in MeOH with 1 N NaOH (2—3 eq) below 35 °C. After complete saponification, neutralization with 1 N HCl and evaporation of the 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 (procedure C).

Catalytic Hydogenation The benzyloxy carbonyl 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. The residue was usually employed in the following step without further purification (procedure D).

Z-Gln-Pheol Z–Gln–OSu[12] (5.89 g, 15.6 mmol) was added to a solution of Pheol[13] (2.36 g, 1 eq) in DMF (60 ml) and the solution was stirred at room temperature for 36 h and concentrated. The residue was triturated with ether, washed with EtOAc and dried to give Z–Gln–Pheol; yield 4.60 g (71%), mp 189—191 °C, $[\alpha]_D^{26}$ −37.2° (c=1.0, MeOH), Rf_3 0.40. EI-MS *m/z*: 413 (M^+), 263 (M^+ −Pheol). *Anal.* Calcd for $C_{22}H_{27}N_3O_5$: C, 63.90; H, 6.58; N, 10.16. Found: C, 63.83; H, 6.72; N, 10.15.

H-Gln-Pheol Z–Gln–Pheol (5.22 g, 12.6 mmol) in MeOH (150 ml) was hydrogenated for 5 h according to procedure D to give H–Gln–Pheol; yield 3.37 g (95%), mp 128—131 °C, Rf_5 0.41.

Z-Gln-Gln-Pheol [1] Z–Gln–OSu (2.70 g, 7.16 mmol) was added to a solution of H–Gln–Pheol (2.00 g, 1 eq) in DMF (90 ml) and the solution was stirred at room temperature for 72 h. After evaporation of the solvent, the residue was triturated with EtOAc–MeOH (1 : 1), washed with the same solvent and filtered off to afford Z–Gln–Gln–Pheol [**1**]; yield 2.83 g (73%), mp 225—227 °C, Rf_5 0.60. *Anal.* Calcd for $C_{27}H_{35}N_5O_7$: C, 59.88; H, 6.51; N, 12.93. Found: C, 59.82; H, 6.60; N, 12.65.

H-Gln-Gln-Pheol Z–Gln–Gln–Pheol (2.81 g, 5.19 mmol) was suspended in AcOH (5 ml). Then 30% HBr in AcOH (20 ml) was added with stirring. After 2 h, dry ether was added. The precipitate was collected on a filter, washed with dry ether as soon as possible, dissolved in MeOH and treated with Amberlite IRA-400. The solvent was removed to give H–Gln–Gln–Pheol; yield 1.76 g (83%), Rf_5 0.39.

Z-Aib-Aib-OMe Z–Aib–OH (22.53 g, 95.0 mmol), HOBt (12.83 g, 1 eq) and DCC (19.60 g, 1 eq) were added successivey to a solution of HCl·H–Aib–OMe (14.59 g, 1 eq) containing TEA (13.30 ml, 1 eq) in DMF (100 ml) with stirring. After 24 h, the solution was worked up as described in procedure A. The residue was recrystallized from EtOAc to afford Z–Aib–Aib–OMe; yield 23.06 g (72%), mp 107—109 °C, Rf_2 0.56. EI-MS *m/z*: 336 (M^+), 305 (M^+ −CO). *Anal.* Calcd for $C_{17}H_{24}N_2O_5$: C, 60.70; H, 7.19; N, 8.33. Found: C, 60.58; H, 7.17; N, 8.33.

HCl·H-Aib-Aib-OMe Z–Aib–Aib–OMe (3.00 g, 8.92 mmol) in 90% aqueous MeOH (30 ml) was hydrogenated according to procedure D. After filtration of the catalyst, 1 N HCl was added to the filtrate and the solvent was removed to give HCl·H–Aib–Aib–OMe; yield 1.99 g (93%), Rf_2 0.28.

Z-Val-Aib-Aib-OMe Z–Val–OH (3.16 g, 12.6 mmol), HOBt (1.70 g, 1 eq) and DCC (2.60 g, 1 eq) were added successively to a stirred solution of HCl·H–Aib–Aib–OMe (3.00 g, 1 eq) in DMF (20 ml) containing TEA (1.76 ml, 1 eq). After 48 h, the solution was worked up according to procedure A. The residue was recrystallized from EtOAc–petroleum ether to afford Z–Val–Aib–Aib–OMe; yield 3.35 g (61%), mp 100—102 °C, $[\alpha]_D^{26}$ −3.2° (c=1.0, MeOH), Rf_2 0.54. EI-MS *m/z*: 435 (M^+), 404 (M^+ −OCH_3), 319 (404 − Aib), 291 (319 − CO). *Anal.* Calcd for $C_{22}H_{33}N_3O_6$: C, 60.67; H, 7.63; N, 9.64. Found: C, 60.59; H, 7.86; N, 9.64.

H-Val-Aib-Aib-OMe Z–Val–Aib–Aib–OMe (2.50 g, 5.74 mmol) in MeOH (10 ml) was hydrogenated as described in procedure D to give H–Val–Aib–Aib–OMe as a syrup; yield 1.70 g (98%), Rf_2 0.25.

Z-Pro-Val-Aib-Aib-OMe [2] Z–Pro–OH (2.60 g, 10.4 mmol), HOBt (1.41 g, 1 eq) and DCC (2.15 g, 1 eq) were added successively to a stirred solution of H–Val–Aib–Aib–OMe (3.14 g, 1 eq) in DMF (24 ml). After 40 h, the solution was worked up according to procedure A to afford the chromatographically pure protected tetrapeptide [**2**]. Further purification was not carried out; yield 5.28 g (95%), mp 56—58 °C, $[\alpha]_D^{26}$ −47.8° (c=1.0, MeOH), Rf_2 0.60. EI-MS *m/z*: 532 (M^+), 501 (M^+ −OCH_3), 416 (501 − Aib), 331 (416 − Aib), 232 (331 − Val). *Anal.* Calcd for $C_{27}H_{40}N_4O_7$: C, 60.89; H, 7.57; N, 10.52. Found: C, 60.58; H, 7.88; N, 10.52.

Z-Pro-Val-Aib-Aib-OH The above protected tetrapeptide [**2**] (1.32 g, 2.48 mmol) in MeOH (10 ml) was saponified as described in procedure C to give the pure tetrapeptide acid; yield 0.98 g (99%), mp 108—112 °C, Rf_5 0.36.

Z-Leu-Aib-OMe Z–Leu–OH (6.46 g, 24.4 mmol), HOBt (3.29 g, 1 eq)

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and DCC (5.02 g, 1 eq) were added successively to a solution of HCl·H–Aib–OMe (3.74 g, 1 eq) in DMF (30 ml) containing TEA (3.41 ml, 1 eq) with stirring. After 40 h, the solution was treated according to procedure A. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH = 95 : 5) to yield Z–Leu–Aib–OMe; yield 7.28 g (82%), mp 71—72 °C, $[\alpha]_D^{26}$ −22.4° (c = 1.0, MeOH), Rf_1 0.76. EI-MS m/z: 364 (M^+). *Anal.* Calcd for $C_{19}H_{28}N_2O_5$: C, 62.62; H, 7.74; N, 7.69. Found: C, 62.51; H, 7.47; N, 7.78.

HCl·H–Leu–Aib–OMe Z–Leu–Aib–OMe (6.00 g, 16.5 mol) in 90% aqueous MeOH (15 ml) was hydrogenated as described in procedure D. After removal of the catalyst, 1 N HCl (17 ml) was added to the filtrate and the solution was concentrated to give HCl·H–Leu–Aib–OMe; yield 4.07 g (95%), Rf_3 0.48.

Z–Gly–Leu–Aib–OMe Z–Gly–OH (3.78 g, 18.1 mmol), HOBt (2.44 g, 1 eq) and DCC (3.73 g, 1 eq) were added successively to a solution of HCl·H–Leu–Aib–OMe (4.82 g, 1 eq) in DMF (20 ml) containing TEA (2.53 ml, 1 eq) with stirring. After 40 h, the solution was worked up according to procedure A. The residue was recrystallized from EtOAc and *n*-hexane to afford Z–Gly–Leu–Aib–OMe; yield 5.94 g (78%), mp 149—151 °C, $[\alpha]_D^{26}$ −34.2° (c = 1.0, MeOH), Rf_1 0.45. EI-MS m/z: 421 (M^+), 390 ($M^+ - OCH_3$), 277 (390 − Aib − CO). *Anal.* Calcd for $C_{21}H_{31}N_3O_6$: C, 59.84; H, 7.41; N, 9.97. Found: C, 59.83; H, 7.41; N, 10.15.

Z–Gly–Leu–Aib–OH Z–Gly–Leu–Aib–OMe (3.00 g, 7.12 mmol) in MeOH (20 ml) was saponified as described in procedure C. The residue was recrystallized from EtOAc and *n*-hexane to give Z–Gly–Leu–Aib–OH; yield 1.97 g (68%), mp 152—154 °C, Rf_5 0.56.

Z–Gly–Leu–Aib–OPac [3] The above tripeptide acid (1.25 g, 3.07 mmol) and phenacyl bromide (0.61 g, 1 eq) were dissolved in DMF (6 ml). TEA (0.43 ml, 1 eq) was added to the stirred solution. After 24 h, the solvent was evaporated off and the residue was extracted with EtOAc. The extract was washed with 5% $NaHCO_3$ and saturated NaCl, and dried over Na_2SO_4. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH = 98 : 2) to afford Z–Gly–Leu–Aib–OPac [**3**]; yield 1.37 g (82%), mp 53—55 °C, $[\alpha]_D^{26}$ −30.9° (c = 1.0, MeOH), Rf_1 0.52. Positive FAB-MS m/z: 526 (MH^+), 390 (MH^+ − OPac − H), 305 (390 − Aib), 222 (MH^+ − [Z − Gly − Leu] + H), 335 (MH^+ − [Z − Gly] + H), 392 (MH^+ − Z + H). *Anal.* Calcd for $C_{28}H_{35}N_3O_7 \cdot 1/4H_2O$: C, 63.44; H, 6.75; N, 7.93. Found: C, 63.47; H, 6.70; N, 7.85.

HBr·H–Gly–Leu–Aib–OPac A solution of the above tripeptide [**3**] (1.20 g, 2.28 mmol) in AcOH (5 ml) was treated with 30% HBr in AcOH (10 ml) under stirring. After 2 h, dry ether was added and the resulting precipitate was filtered off, washed with dry ether and dried over KOH *in vacuo* to give HBr·H–Gly–Leu–Aib–OPac: yield 1.02 g (95%), Rf_4 0.24.

Z–Ile–Aib–OMe Z–Ile–OH (5.00 g, 18.9 mmol), HOBt (2.55 g, 1 eq) and DCC (3.89 g, 1 eq) were added successively to a solution of HCl·H–Aib–OMe (2.89 g, 1 eq) in DMF (20 ml) containing TEA (2.74 ml, 1 eq). After 40 h, the solution was treated according to procedure A. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH = 95 : 5) to give Z–Ile–Aib–OMe; yield 3.74 g (54%), mp 82.5—85 °C, $[\alpha]_D^{26}$ −24.0° (c = 1.0, MeOH), Rf_1 0.63. EI-MS m/z: 364 (M^+), 333 ($M^+ - OCH_3$), 248 (333 − Aib), 220 (248 − CO). *Anal.* Calcd for $C_{19}H_{28}N_2O_5$: C, 62.62; H, 7.74; N, 7.69. Found: C, 62.63; H, 7.82; N, 7.69.

HCl·H–Ile–Aib–OMe Z–Ile–Aib–OMe (3.00 g, 8.23 mmol) in 90% aqueous MeOH (20 ml)) containing several drops of AcOH was hydrogenated (procedure D). After removal of the catalyst, 1 N HCl (10 ml) was added to the filtrate and the solution was concentrated to give HCl·H–Ile–Aib–OMe; yield 2.10 g (95%), Rf_3 0.39.

Z–Gln–Aib–OMe Z–Gln–OH (5.48 g, 19.6 mmol), HOBt (2.64 g, 1 eq) and DCC (4.03 g, 1 eq) were dissolved successively in a stirred solution of HCl·H–Aib–OMe (3.00 g, 1 eq) in DMF (20 ml) containing TEA (2.74 ml, 1 eq) with stirring. After 44 h, the solution was worked up according to procedure A. The residue was recrystallized from EtOH to yield Z–Gln–Aib–OMe; yield 5.27 g (71%), mp 134—136 °C, $[\alpha]_D^{26}$ −15.8° (c = 1.0, MeOH), Rf_3 0.57. EI-MS m/z: 379 (M^+), 348 ($M^+ - OCH_3$), 320 (348 − CO), 263 (348 − Aib), 235 (268 − CO). *Anal.* Calcd for $C_{18}H_{25}N_3O_6$: C, 56.98; H, 6.64; N, 11.08. Found: C, 57.13; H, 6.78; N, 10.99.

Z–Gln–Aib–OH Z–Gln–Aib–OMe (2.61 g, 6.88 mmol) in MeOH (20 ml) was saponified according to procedure C to afford pure Z–Gln–Aib–OH; yield 2.16 g (86%), mp 141—144 °C, Rf_5 0.18.

Z–Gln–Aib–Ile–Aib–OMe [4] Z–Gln–Aib–OH (1.39 g, 3.80 mmol), HOBt (513 mg, 1 eq) and DCC (784 mg, 1 eq) were added successively to a solution of HCl·H–Ile–Aib–OMe (1.02 g, 1 eq) in DMF (10 ml) containing TEA (0.53 ml, 1 eq) with stirring. The solution was stirred for 72 h. After filtration to remove DCU and evaporation of the solvent, the residue was chromatographed on silica gel ($CHCl_3$: MeOH = 9 : 1) to give the protected tetrapeptide [**4**]; yield 1.82 g (82%), mp 83.5—85.5 °C, $[\alpha]_D^{26}$ −17.6° (c = 1.0, MeOH), Rf_3 0.39. EI-MS m/z: 577 (M^+), 546 ($M^+ - OCH_3$), 461 (546 − Aib), 433 (461 − CO), 348 (461 − Ile), 320 (348 − CO), 263 (348 − Aib). *Anal.* Calcd for $C_{28}H_{43}N_5O_8 \cdot 1/2H_2O$: C, 57.32; H, 7.56; N, 11.94. Found: C, 57.02; H, 7.50; N, 11.91.

Z–Gln–Aib–Ile–Aib–OH Fragment [**4**] (1.01 g, 1.75 mmol) was saponified according to procedure C to give the chromatographically homogeneous tetrapeptide acid; yield 0.78 g (79%), mp 102—104 °C, Rf_5 0.49.

Z–Ala–Aib–Aib–OMe Z–Ala–OH (2.72 g, 12.2 mmol), HOBt (1.65 g, 1 eq) and DCC (2.51 g, 1 eq) were dissolved successively in a solution of HCl·H–Aib–Aib–OMe (2.91 g, 1 eq) in DMF (30 ml) containing TEA (1.71 ml, 1 eq) with stirring. After 24 h, the solution was worked up according to procedure A. Recrystallization of the residue from EtOAc afforded Z–Ala–Aib–Aib–OMe; yield 4.05 g (82%), mp 125—126 °C, $[\alpha]_D^{26}$ −7.0° (c = 2.27, MeOH), Rf_3 0.63. EI-MS m/z: 376 ($M^+ - OCH_3$), 263 (376 − Aib − CO). *Anal.* Calcd for $C_{20}H_{29}N_3O_6$: C, 58.95; H, 7.17; N, 10.31. Found: C, 58.99; H, 7.36; N, 10.18.

H–Ala–Aib–Aib–OMe The above tripeptide (5.87 g, 14.4 mmol) in 90% aqueous MeOH (40 ml) containing some drops of AcOH was hydrogenated according to procedure D to give pure H–Ala–Aib–Aib–OMe; yield 3.74 g (95%), Rf_5 0.39.

Z–Ala–Ala–OMe Z–Ala–OH (11.48 g, 51.4 mmol) and DCC (10.61 g, 1 eq) were dissolved in a solution of HCl·H–Ala–OMe (7.18 g, 1 eq) in CH_2Cl_2 (250 ml) containing TEA (7.20 ml, 1 eq) with stirring. After 24 h, the solution was worked up as described in procedure A and the residue was recrystallized from EtOAc and *n*-hexane to afford Z–Ala–Ala–OMe; yield 11.78 g (74%), mp 109—112 °C, $[\alpha]_D^{26}$ −46.0° (c = 1.0, MeOH), Rf_3 0.75. EI-MS m/z: 308 (M^+), 277 ($M^+ - OCH_3$), 206 (277 − Aib). *Anal.* Calcd for $C_{15}H_{20}N_2O_5$: C, 58.43; H, 6.54; N, 9.09. Found: C, 58.54; H, 6.56; N, 9.38.

Z–Ala–Ala–OH Z–Ala–Ala–OMe (11.78 g, 38.2 mmol) was saponified according to procedure C except that the reaction temperature was kept below 5 °C, to give Z–Ala–Ala–OH. The dipeptide was recrystallized from EtOAc and *n*-hexane; yield 7.82 g (70%), mp 149—151 °C, Rf_5 0.23.

Z–Ala–Ala–Ala–Aib–Aib–OMe Z–Ala–Ala–OH (2.58 g, 8.77 mmol), HOBt (1.18 g, 1 eq) and DCC (1.81 g, 1 eq) were added successively to a stirred solution of H–Ala–Aib–Aib–OMe (2.40 g, 1 eq) in DMF (30 ml). After 48 h, the solution was worked up according to procedure A. The residue was precipitated from EtOAc with ether to give the protected pentapeptide; yield 3.56 g (74%), mp 196—198 °C, $[\alpha]_D^{26}$ −34.0° (c = 1.0, MeOH), Rf_2 0.24. EI-MS m/z: 549 (M^+), 518 ($M^+ - OCH_3$), 433 (518 − Aib), 406 (433 − CO), 348 (433 − Aib), 277 (348 − Ala), 206 (277 − Ala). *Anal.* Calcd for $C_{26}H_{39}N_5O_8$: C, 56.82; H, 7.15; N, 12.74. Found: C, 56.92; H, 7.22; N, 12.57.

HCl·H–Ala–Ala–Ala–Aib–Aib–OMe The above pentapeptide (1.43 g, 2.60 mmol) in MeOH (15 ml) containing several drops of AcOH was hydrogenated according to procedure D. The catalyst was filtered off and 1 N HCl (2.6 ml) was added to the filtrate. The solvent was evaporated off to afford the title compound; yield 1.14 g (97%), Rf_4 0.29.

Ac–Aib–Ala–Ala–Ala–Aib–Aib–OMe [5] Ac–Aib–OH was prepared by refluxing H–Aib–OH and Ac_2O in AcOH. Ac–Aib–OH (373 mg, 2.57 mmol), HOBt (347 mg, 1 eq) and DCC (529 mg, 1 eq) were added successively to a solution of HCl·H–Ala–Ala–Ala–Aib–Aib–OMe (1.16 g, 1 eq) in DMF (10 ml) containing TEA (0.36 ml, 1 eq) with stirring. After 48 h, DCU and the solvent were removed and the residue was extracted with EtOAc. The aqueous layer was treated with Amberlite IR-120 and IRA-400 successively. The water was evaporated off and the residue was recrystallized from MeOH and ether; yield 1.07 g (77%), mp 108—111 °C, $[\alpha]_D^{26}$ −3.4° (c = 0.9, MeOH), Rf_3 0.35. Positive FAB-MS m/z: 543 (MH^+), 511 ($MH^+ - OCH_3 - H$), 426 (511 − Aib), 341 (426 − Aib), 270 (341 − Ala), 199 (270 − Ala), 128 (199 − Ala). *Anal.* Calcd for $C_{24}H_{42}N_6O_8 \cdot 3/2H_2O$: C, 50.60; H, 7.96; N, 14.75. Found: C, 50.57; H, 7.77; N, 14.71.

Ac–Aib–Ala–Ala–Ala–Aib–Aib–OH The above protected hexapeptide [**5**] (0.55 g, 1.01 mmol) was saponified and the solution was neutralized with Amberlite IR-120. The solvent was evaporated off *in vacuo* to give the pure hexapeptide acid; yield 0.51 g (95%), mp 125—128 °C, Rf_5 0.21.

Z–Pro–Val–Aib–Aib–Gln–Gln–Pheol [Positions 14—20] The tetrapeptide acid (717 mg, 1.38 mmol) derived from [**2**], HOBt (187 mg, 1 eq) and DCC (285 mg, 1 eq) were dissolved successively in a solution of H–Gln–Gln–Pheol (563 mg, 1 eq) in DMF (5 ml). After 48 h, the solution was worked up according to procedure B. The residue was precipitated from MeOH with ether to give the heptapeptide (positions 14—20); yield 657 mg (52%), mp 110—113 °C, $[\alpha]_D^{26}$ −33.9° (c = 1.0, MeOH), Rf_3 0.20. Positive FAB-MS m/z: 908 (MH^+), 629 (MH^+ − Pheol − Gln − H), 501

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(629—Gln), 416 (501—Aib), 331 (416—Aib), 232 (331—Val). *Anal.* Calcd for $C_{45}H_{65}N_9O_{11}\cdot H_2O$: C, 58.36; H, 7.29; N, 13.61. Found: C, 58.68; H, 7.27; N, 13.51.

H-Pro-Val-Aib-Aib-Gln-Gln-Pheol The above heptapeptide (1.25 g, 1.38 mmol) in MeOH (15 ml) was hydrogenated according to procedure D to give the title compound; yield 0.96 g (90%), Rf_5 0.33.

Z-Gln-Aib-Ile-Aib-Gly-Leu-Aib-OPac [Positions 7—13] The tetrapeptide acid (600 mg, 1.06 mmol) derived from [**4**], HOBt (144 mg, 1 eq) and DCC (219 mg, 1 eq) were added successively to a stirred solution of HBr·H-Gly-Leu-Aib-OPac (500 mg, 1 eq) in DMF (7 ml) containing TEA (148 μl, 1 eq). After 48 h, the solution was worked up according to procedure A. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH = 9 : 1) to give the heptapeptide (positions 7—13); yield 519 mg (52%). mp 103—106 °C. $[\alpha]_D^{26}$ −7.8° (*c* = 1.0, MeOH), Rf_3 0.51. Positive FAB-MS *m/z*: 937 (MH^+), 801 (MH^+ − OPac − H), 716 (801 − Aib), 603 (716 − Leu), 546 (603 − Gly), 461 (546 − Aib), 348 (461 − Ile), 263 (348 − Aib), 803 (MH^+ − Z + H). *Anal.* Calcd for $C_{47}H_{68}N_8O_{12}\cdot H_2O$: C, 59.10; H, 7.39; N, 11.73. Found: C, 59.30; H, 7.48; N, 11.78.

Z-Gln-Aib-Ile-Aib-Gly-Leu-Aib-OH The above heptapeptide (positions 7—13) (328 mg, 0.35 mmol) was dissolved in 90% AcOH (10 ml), and Zn powder (1.03 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 the Zn powder, the solvent was evaporated off *in vacuo*. 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 *in vacuo*. The residue was triturated with ether and washed with the same solvent to afford the heptapeptide acid (positions 7—10); yield 241 mg (84%), mp 123—126 °C, Rf_5 0.38.

Z-Gln-Aib-Ile-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Pheol [Positions 7—20] The above heptapeptide acid (positions 7—13) (295 mg, 0.36 mmol), HOBt (48.6 mg, 1 eq) and DCC (74 mg, 1 eq) were added successively to a solution of the deprotected heptapeptide (positions 14—20) (278 mg, 1 eq) in DMF (5 ml) with stirring. The solution was stirred for 72 h and worked up according to procedure B. The residue was purified by silica gel chromatography (CH_2Cl_2 : MeOH = 8 : 2); yield 255 mg (45%), mp 152—154 °C, $[\alpha]_D^{26}$ −14.9° (*c* = 0.4, MeOH), Rf_4 0.50. Positive FAB-MS *m/z*: 1574 (MH^+), 1295 (MH^+ − Pheol − Gln − H), 1167 (1295 − Gln), 1082 (1167 − Aib), 997 (1082 − Aib), 898 (997 − Val), 801 (898 − Pro), 716 (801 − Aib), 603 (716 − Leu), 546 (603 − Gly), 461 (546 − Aib), 348 (461 − Ile), 263 (348 − Aib). *Anal.* Calcd for $C_{76}H_{119}N_{17}O_{19}\cdot H_2O$: C, 56.67; H, 7.70; N, 14.78. Found: C, 56.42; H, 7.71; N, 14.81.

H-Gln-Aib-Ile-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Pheol The above tetradecapeptide (124 mg, 78.70 μmol) in MeOH (4 ml) was hydrogenated according to procedure D; yield 108 mg (96%), Rf_5 0.74.

Ac-Aib-Ala-Ala-Ala-Aib-Aib-Gln-Aib-Ile-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Pheol [Trichosporin B-V] The hexapeptide acid (37.3 mg, 2 eq) derived from [**5**], HOBt (9.5 mg, 2 eq) and DCC (14.6 mg, 2 eq) were added successively to a solution of the above amine component (positions 7—20) (51 mg, 35.3 μmol) in DMF (5 ml) with stirring. The solution was stirred for 48 h and treated according to procedure B. The residue (62.5 mg) was purified by preparative HPLC [conditions: mobile phase, MeOH–H_2O (85 : 15, v/v); flow rate, 5 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-V; yield 23.3 mg (34%), mp 267—270 °C,[3] $[\alpha]_D^{26}$ −17.7° (*c* = 0.3, MeOH),[3] Rf_4 0.31. Positive FAB-MS: see Fig. 8. Amino acid ratios (6 N HCl, 24 h): Glu 3.33 (3), Ala 2.88 (3), Val 1.00 (1), Gly 1.00 (1), Ile 0.94 (1), Leu 0.98 (1) and Pro 0.91 (1).

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