Kutznerides 1−4, Depsipeptides from the Actinomycete *Kutzneria* sp. 744 Inhabiting Mycorrhizal Roots of *Picea abies* Seedlings

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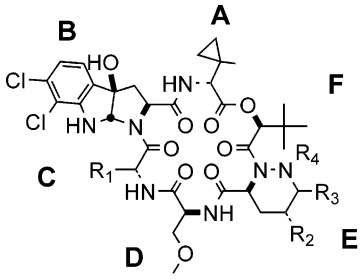
Bioassay-guided fractionation of culture supernatants of the actinomycete *Kutzneria* sp. 744 resulted in the isolation of four new depsipeptides (**1**−**4**). Structure analysis revealed the general structure: cyclo[2-(1-methylcyclopropyl)-D-glycine-(2*S*,3a*R*,8a*S*)-6,7-dichloro-3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-2-carboxylic acid-3-hydroxy-D-glutamic acid-*O*-methyl-L-serine-L-piperazic acid-(*S*)-2-hydroxy-3,3-dimethylbutanoic acid]. The 3-hydroxy-D-glutamic acid was present as its *threo*-isomer in **1** and **2** and as its *erythro*-isomer in **3** and **4**. The piperazic acid was modified to its (*R*)-4-chloro analogue in **2** and to its C-5/N unsaturated analogue in **4**. Compounds **1**−**4** displayed moderate spore germination inhibiting activity against several common root-rotting fungi.

Pathogenic fungi cause significant economic losses annually in agriculture and forestry. During recent decades, various chemical fungicides have been extensively used to control these fungi, a method that, however, may be hazardous in the long run, since the pathogenic fungi may evolve resistance to the chemicals in use or the fungicides may be accumulated in biological systems. An alternate approach is to use antagonistic microorganisms to control pathogenic fungi.[1] The mechanisms behind this type of microbial antagonism may be of many different types, e.g., competition for nutrients or ecological niche, or the production of biologically active metabolites.[1−3] At present, there are a few examples of commercial products, based on living fungi or bacteria, designed to control pathogenic fungi in forestry and agriculture.[1] There is, however, still a need for research leading to the isolation and characterization of new microorganisms suitable for control of pathogenic fungi.

The present report is part of a larger study where microorganisms antagonistic to fungi that cause root-rot in north-temperate and boreal forests have been isolated and characterized. An important part of this characterization is the investigation of the biologically active metabolites produced by the organisms, since these metabolites may be crucial to the biological activity of the antagonistic organisms. Cyclic depsipeptides are a frequently occurring type of secondary metabolites displaying antimicrobial properties. Depsipeptides have been isolated from many types of organisms, including mollusks, sponges, fungi, and bacteria, and the biological activity ascribed to different depsipeptides has been quite varied, e.g., anticancer, antibacterial, antiviral, antifungal, anti-inflammatory, and cardiovascular activity.[4,5] Examples include the dolastatins,[6] the luzopeptins,[7] and globomycin.[8]

The aim of this study was to investigate the secondary metabolites produced by the actinomycete *Kutzneria* sp. 744, demonstrated to have antagonistic effects on the growth of several common root-rotting fungi. In this paper, the isolation and characterization of novel depsipeptides, kutznerides 1, 2, 3, and 4 (**1**−**4**), are described. These depsipeptides display moderate antifungal effects against several root-rotting fungi.



1: R_1=  ; R_2, R_3, R_4 = H

2: R_1=  ; R_2= Cl; R_3, R_4 = H

3: R_1=  ; R_2, R_3, R_4 = H

4: R_1=  ; R_2 = H; R_3, R_4 = π-bond

Results and Discussion

The actinomycete *Kutzneria* sp. 744 inhibited the growth of the root pathogens *Pythium undulatum*, *Ceratobasidium bicorne*, and *Fusarium avenaceum* in dual cultures on agar. In addition, the conidial germination of *F. avenaceum* was completely inhibited in a culture filtrate of *Kutzneria* sp. 744. These findings indicated that *Kutzneria* sp. 744 would make an interesting candidate for studies of antifungal compounds.

Consequently, cell-free supernatants from liquid cultures of *Kutzneria* sp. 744 were extracted by reversed-phase SPE. The resulting 95% CH_3CN fraction displayed conidiospore germination inhibiting activity against *F. avenaceum*, whereas the H_2O fraction did not show any activity. Gradient HPLC fractionation of the 95% CH_3CN fraction, followed by spore germination inhibition bioassay, resulted in the isolation of several fractions with antifungal activity (Figure 1). ^1H NMR data indicated that two antifungal fractions contained single compounds (**1** and **2**), whereas two further compounds (**3** and **4**) were isolated after one additional chromatographic step (at 61% aqueous CH_3CN). The production of **1**−**4** in cultures of *Kutzneria* sp. 744 varied significantly between batches, from several milligrams to less than a milligram of each compound.

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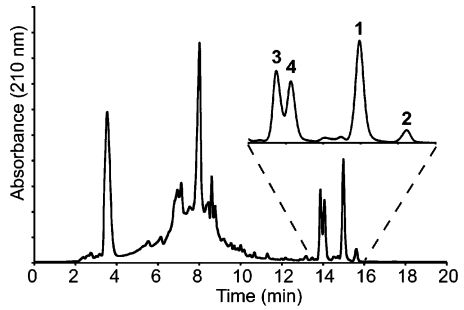

Figure 1. Chromatogram from isolation of compounds **1**−**4** with gradient reversed-phase HPLC. Compounds **3** and **4** were further purified by isocratic reversed-phase HPLC.

Compounds **1**−**4** were determined by NMR, ESIMS, and HR-FABMS analysis to be four new depsipeptides (**1**−**4**), named kutzneride 1, kutzneride 2, kutzneride 3, and kutzneride 4, respectively. The absolute configuration was investigated by acidic hydrolysis followed by GC-MS after chemical modification, as well as by molecular modeling.

Analysis of compound **1** by ESIMS in negative mode (*m*/*z* 852.4 $[M - H]^-$) and positive mode (*m*/*z* 854.3 $[M + H]^+$ and 876.7 $[M + Na]^+$) indicated a molecular mass of 853 Da. The isotope distribution pattern of the pseudomolecular ions, 100/67/13 for M/M+2/M+4, pointed to the presence of two chlorine atoms in the molecule. This was corroborated by HRFABMS, which resulted in a calculated molecular formula of $C_{37}H_{49}N_7O_{12}Cl_2$. Compound **1** gave ^{1}H NMR signals evenly dispersed over a wide range. Data from ^{1}H−^{1}H COSY, ^{1}H−^{13}C HSQC, ^{1}H−^{13}C HSQC-DEPT, and ^{1}H−^{13}C HMBC experiments enabled the reconstruction of the skeletons of the subunits of the peptide, as described below.

Subunit A of **1** contained two spin-systems. One signal at δ_H 7.66 (NH) was coupled to a CH signal (δ_H 4.02), and there was a spin-system consisting of upfield-shifted signals from two CH_2 groups (δ_H 1.02/0.67 and 0.71/0.39). The CH group was assigned by HMBC to be linked to a carbonyl at δ_C 173.4, as well as to the two CH_2 groups through a quaternary carbon with a signal at δ_C 19.3. Furthermore, a CH_3 group (δ_H 1.04) was linked to the quaternary carbon. These findings indicated this residue to be 2-(1-methylcyclopropyl)glycine (MecPGly), in accordance with the upfield-shifted CH_2 signals. This was later corroborated by acidic hydrolysis of the peptide followed by catalytic hydrogenation (H_2/PtO_2/HOAc), which afforded *tert*-leucine (Table 1), a compound previously demonstrated to be formed from MecPGly by catalytic hydrogenation under these conditions.[9]

Subunit B was found to contain several spin-systems. A signal at δ_H 5.29 (CH) was coupled to signals from two CH_2 protons at δ_H 2.79 and 2.17, and a signal at δ_H 5.25 (CH) was coupled to a signal at δ_H 6.42 (NH). Additionally, there were signals from two vicinal aromatic protons at δ_H 6.97 and 7.16, as well as a singlet signal at δ_H 5.88 (OH). HMBC experiments afforded data enabling the identification of this residue as a tryptophan derivative similar to 3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-2-carboxylic acid (PIC, Figure 2), and the NMR data for subunit B were in good agreement with literature data for PIC residues in peptides.[10,11] Subunit B, however, displayed signals from only two aromatic protons; that is, two positions on the aromatic ring were carrying unknown substituents. An HMBC correlation between H-4 (δ_H 7.16) and C-3a (δ_C 91.7), together with ROESY correlations between H-4 and the H-3 signals (δ_H 2.79 and 2.17, respectively), indicated the vicinal aromatic protons to be on positions 4 and 5. HRFABMS data indicated the presence of two chlorine atoms in **1**, and the chemical shifts of C-6 and C-7 (δ_C 133.4 and 115.8, respectively) were compatible with chlorine substituents on these positions. Thus, the aromatic ring was assumed to be substituted with chlorine atoms on C-6 and C-7. This was later verified by hydrolysis experiments analyzed by LCMS (Figure 3). During acidic hydrolysis, PIC is almost quantitatively transformed to 2-hydroxytryptophan,[12] and most likely, the PIC derivative constituting subunit B of compound **1** reacts in the same way. The LCMS data showed the presence of a compound with *m*/*z* 289 $[M + H]^+$, displaying the expected isotope distribution pattern for a compound containing two chlorine atoms. The molecular mass 288 Da is the mass of a PIC molecule carrying two chlorine substituents, as well as the mass of dichloro-2-hydroxytryptophan. Thus, regardless of the stability of the subunit during acidic hydrolysis, the LCMS data verified that subunit B carried two chlorine substituents. Consequently,

Table 1. Data from GC-MS Analysis for Determination of Absolute Configuration of the Subunits Constituting **1**−**4**[a]

		elution time (min) for samples				
		reference	**1**	**2**	**3**	**4**
2OHdiMeBu	*R*	24.87	*b*	*b*	*b*	*b*
	S	25.21	25.23	25.22	25.23	25.23
tert-Leu[c]	D	39.47	39.50	39.50	39.51	39.50
	L	40.81	*b*	*b*	*b*	*b*
Ser[d]	D	39.77	*b*	*b*	*b*	*b*
	L	40.16	40.12	40.20	40.23	40.20
Orn[e]	D	57.14	*b*	*f*	*b*	*f*
	L	57.29	57.26	*f*	57.33	*f*
threo-3OHGlu	D	56.33	56.31	56.36	*b*	*b*
	L	56.41	*b*	*b*	*b*	*b*
erythro-3OHGlu	D	57.89	*b*	*b*	57.90	57.92
	L	57.99	*b*	*b*	*b*	*b*

[a] Following acidic hydrolysis, the samples were hydrogenated and treated with (*S*)-2-BuOH/AcCl (10:1) followed by perfluoropropanoic anhydride, and finally analyzed by GC-MS [b] The corresponding diastereomeric compounds were detected at approximately 10% of the main peaks. These compounds were formed during the hydrolysis and esterification steps. [c] Formed from 2-(1-methylcyclopropyl)glycine during hydrogenation. [d] Formed from *O*-methylserine during acidic hydrolysis. [e] Formed from piperazic acid during hydrogenation. [f] Not detected.

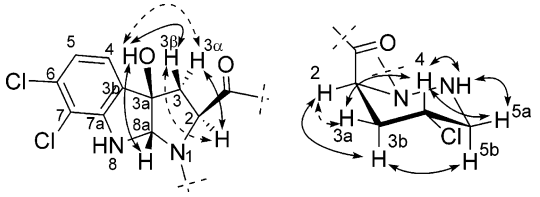

Figure 2. Left: ROESY correlations indicating the relative configuration of diClPIC in **1**−**4**. Right: ROESY correlations indicating the relative configuration of the chlorinated Pip in **2**. Solid arrows indicate strong ROESY correlations, whereas dashed arrows indicate weak ones.

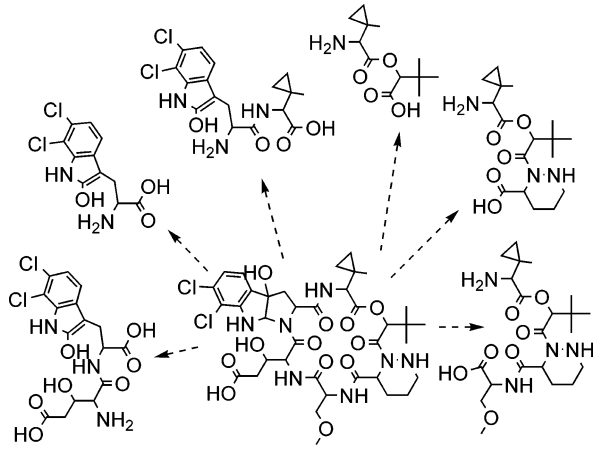

Figure 3. Fragments obtained from compound **1** by partial hydrolysis (6 M HCl, 110 °C, 0.5 to 5 h). The fragments were identified by LCMS.

subunit B was identified as 6,7-dichloro-3a-hydroxy-1,2,3,3a,8,-8a-hexahydropyrrolo[2,3-*b*]indole-2-carboxylic acid (diClPIC). The relative configuration of this residue was investigated by ROESY experiments, which afforded cross-peaks between the signals of H-8a and OH, OH and H-3β, OH and H-3α (weak), H-3α and H-2, and H-3β and H-2 (weak) (Figure 2). No correlation was observed between H-2 and H-8a. The ROESY data indicated that the OH group was on the same side of the ring system as the carbonyl group and H-8a (Figure 2). The same relative configuration has been reported for a PIC residue in a peptide,[10] with NMR data in good agreement with the data obtained for the diClPIC residue in **1**. A different relative configuration, i.e., H-2, H-8a, and OH on the same face of the ring system, was reported for a PIC residue in a peptide.[11] This finding was based on ROESY data including a cross-peak between H-2 and H-8a, a correlation absent in the ROESY data for **1**.

Subunit C showed two separate spin-systems. A signal at δ_H 7.20 (NH) was coupled to a signal at δ_H 5.31 (CH), whereas a signal at δ_H 4.76 (CH) was coupled to the signals of a CH_2 group (δ_H 2.62 and 2.54), as well as to a broad signal at δ_H 3.96 (OH, coupling only observable in COSY experiments). HMBC experiments gave correlations between the CH_2 protons and the carbons of both CH groups, as well as to a carbonyl group (δ_C 174.7). The presence of this carbonyl was also indicated by the large geminal coupling constant of the CH_2 group (15.6 Hz). Furthermore, HMBC gave a cross-peak between the signal of the CH group at δ_H 5.31 and another carbonyl group (δ_C 171.4). The data pointed to the existence of a 3-hydroxyglutamic acid or 3-hydroxyglutamine residue in **1**, and since no ^{1}H NMR signal was detected from a NH_2 group linked to the side chain carbonyl, subunit C was identified as 3-hydroxyglutamic acid (OHGlu).

Subunit D contained one spin-system. One signal at δ_H 7.62 (NH) was connected via a CH group (δ_H 4.36) to a CH_2 group (δ_H 3.78 and 3.41). HMBC experiments showed the presence of a carbonyl linked to the CH group, as well as a CH_3O group (δ_H 3.25, δ_C 59.9) bound to the CH_2 group, all in accordance with an *O*-methylserine (MeSer) residue.

Subunit E also contained one single spin-system. A CH group (δ_H 5.01) was linked to three consecutive CH_2 groups (δ_H 2.34/1.61, δ_H 2.08/1.56, and δ_H 3.10/2.80) and finally to a NH (δ_H 4.70). The marked differences between the chemical shifts within the geminal proton pairs indicated a ring-closed structure, possibly a six-membered ring in a chairlike conformation. Comparison with literature data showed good agreement with piperazic acid (Pip) in peptides, e.g., ref 13. This finding was later confirmed by catalytic hydrogenation (H_2/PtO_2/HOAc), which afforded the amino acid ornithine[13] as identified by GC-MS (Table 1).

Subunit F gave only two singlet signals in the ^{1}H NMR spectrum (δ_H 5.85 and 1.12), corresponding to one CH and three CH_3 groups, respectively. HMBC experiments proved the CH group to be linked to a carbonyl (δ_C 172.8) as well as to a quaternary carbon (δ_C 33.9), which also carried the three CH_3 groups. The downfield-shifted CH signal (δ_C 78.6) indicated that an oxygen atom was linked to this group and that the oxygen was part of an ester linkage to one of the amino acid residues in the peptide. Thus, this subunit was assigned as 2-hydroxy-3,3-dimethylbutanoic acid (OHdiMeBu).

The molecular formula $C_{37}H_{49}N_7O_{12}Cl_2$, obtained by HRFABMS, and the identification of the subunits made it apparent that the peptide was ring-closed by five amide bonds and one ester linkage. The sequence of amino and hydroxy acids was determined by ROESY, HMBC, and partial hydrolysis experiments. Diagnostic ROESY cross-peaks were obtained between the signals of A-NH and B-2, B-NH and C-2, B-8a and C-NH, C-NH and D-2, C-NH and D-NH, D-NH and E-2, E-2 and F-4, E-NH and F-2, E-5 and F-4, and F-2 and A-2, suggesting the sequence of **1** to be cyclo-(MecPGly-diClPIC-OHGlu-MeSer-Pip-OHdiMeBu). HMBC experiments failed to yield data determining the whole sequence of the depsipeptide. However, correlations were found between both H-2 and NH of residue A and the carbonyl group of residue B, as well as between NH of residue C and the carbonyl group of residue D. These findings were in agreement with the sequence proposed by ROESY experiments. This sequence was further corroborated by partial hydrolysis experiments, by treatment of **1** with 6 M HCl at 110 °C, between 0.5 and 5 h, and subsequent analysis by LCMS. These experiments showed fragments all in agreement with the suggested sequence (Figure 3).

Acidic hydrolysis, followed by catalytic hydrogenation, esterification with (*S*)-2-BuOH, treatment with perfluoropropanoic anhydride, and subsequent analysis with GC-MS, identified (*S*)-2-hydroxy-3,3-dimethylbutanoic acid, *threo*-3-hydroxy-D-glutamic acid, and *O*-methyl-L-serine (identified as L-serine formed during the acidic hydrolysis), as well as 2-(1-methylcyclopropyl)-D-glycine (identified as D-*tert*-leucine formed by catalytic hydrogenation[9]) and L-piperazic acid (identified as L-ornithine formed by catalytic hydrogenation[13]) (Table 1). To obtain information about the absolute configuration of the diClPIC residue, molecular modeling using the MM2 force field was employed. Compound **1** was constructed of the above configurationally determined subunits, together with the D- or L-isomer of diClPIC with the relative configuration as indicated by ROESY experiments (Figure 2). Subsequently, starting from different initial conformations, energetically favorable conformers of **1** were calculated, and selected interatomic distances were measured. In **1**, containing the L-isomer of diClPIC, the average MecPGly NH/diClPIC H-2 and MecPGly NH/diClPIC H-8a distances were 2.5 and 4.6 Å, respectively, whereas the corresponding distances were 3.4 and 2.6 Å, respectively, for **1** containing the D-isomer. In ROESY experiments on **1**, using mixing times between 100 and 300 ms, strong correlations were observed between MecPGly NH and diClPIC H-2, whereas no cross-peaks were detected between MecPGly NH and diClPIC H-8a. Thus, the data from ROESY experiments were in accordance with the molecular modeling data for **1** containing L-diClPIC. Taken together with the relative configuration of the diClPIC residue (Figure 2), this suggests the residue to be (2*S*,3a*R*,8a*S*)-6,7-dichloro-3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-2-carboxylic acid. According to these findings, the structure of **1** was cyclo(D-MecPGly-(2*S*,3a*R*,8a*S*)-diClPIC-*threo*-D-OHGlu-L-MeSer-L-Pip-(*S*)-OHdiMeBu), which is a novel depsipeptide.

Compounds **2**, **3**, and **4** were also subjected to extensive spectroscopic analysis. NMR data indicated that these compounds all were similar to **1**. The differences between the compounds were confined to two of the amino acids: the Pip and the OHGlu residues. The Pip residues of **2** and **4** were modified relative to **1** and **3**, whereas the relative configuration of the OHGlu residues of **3** and **4** was different compared to **1** and **2**. All compounds were found to contain L-MeSer, D-MecPGly, and (*S*)-OHdiMeBu (Table 1), and the absolute configuration of the diClPIC residues was assumed to be (2*S*, 3a*R*, 8a*S*) for all compounds, as supported by molecular modeling (data not shown) and ROESY experiments. Moreover, the general sequence of subunits was determined by ROESY and HMBC experiments to be the same for **2**−**4** as for **1**. The structure analysis of these compounds will not be described in full detail, since it was performed in very similar manners as for **1**. Thus, only important differences will be highlighted.

Compound **2** was identical to **1** except for a modification of the Pip residue, which was found to carry a chlorine substituent at C-4. This was manifested as changes in the corresponding NMR spectra, as this Pip residue contained only two CH_2 groups with signals at δ_H 2.77/1.81 and δ_H 3.36/2.85 and one additional CH group at δ_H 4.66 (δ_C 50.5). COSY experiments showed this CH group to be positioned between the two CH_2 groups and thus that an unknown substituent was linked to C-4 of the Pip residue. HRFABMS pointed to the molecular formula $C_{37}H_{48}N_7O_{12}Cl_3$ for **2**, which taken together with the NMR data was in good agreement with the

presence of a chlorine substituent at C-4 in the Pip residue. The relative configuration of the chlorinated Pip residue was investigated by ROESY experiments, which yielded diagnostic cross-peaks between the signals of H-2 and H-3a (weak), H-2 and H-3b, H-3a and H-4, H-3b and H-5b, H-4 and H-5a, and H-4 and NH (Figure 2). These data indicated a *trans*-relation between the carbonyl group and the chlorine atom and an axial position for the H-4, assuming a chairlike conformation of the residue. The axial position for H-4 was further supported by the coupling patterns of the H-3b and H-5b signals, which both displayed large couplings to H-4, indicating a *trans*-diaxial relation to H-4 for both protons. The Pip residues of **1** and **3** were both determined to be of L-configuration (Table 1), making it likely that the chlorinated Pip residue of **2** also was of L-configuration. Assuming this, the chirality of C-4 was deduced to be *R*; that is, the residue was (*R*)-4-chloro-L-piperazic acid.

When analyzing compound **3** by HRFABMS the data pointed to the same molecular formula ($C_{37}H_{49}N_7O_{12}Cl_2$) for **3** as for **1**. NMR data indicated that the only difference between **1** and **3** was the configuration of the OHGlu residues, which was manifested by the coupling pattern of the corresponding H-2 signals. In **1**, no coupling was observed between the H-2 and H-3 signals of the OHGlu residue, whereas in **3** the H-2 signal was a triplet (10 Hz) due to vicinal couplings to the NH signal as well as to the H-3 signal. Subsequent component analysis proved the OHGlu residue of **3** to be *erythro*-3-hydroxy-D-glutamic acid (Table 1).

The difference between compounds **3** and **4** was shown by NMR to be in the Pip residues. This was demonstrated by the absence of the C-5 CH_2 group as well as the NH, in the Pip residue of **4**. Instead, a signal at δ_H 7.17 (δ_C 146.9) was present, which was coupled to the signals of the C-4 CH_2 group, suggesting a double bond between C-5 and the neighboring nitrogen atom. The NMR data were in good agreement with literature data describing this modified Pip residue (Pip*) in aurantimycins.[14] Additionally, data from HRFABMS supported this conclusion, as the measured molecular mass was two mass units lower than for **1** and **3**, giving a calculated molecular formula of $C_{37}H_{47}N_7O_{12}Cl_2$. The absolute configuration of the Pip* residue was assumed to be the same as for Pip residues in **1** and **3**, i.e., the L-configuration.

Compounds **1**−**4** were all constructed of several unusual building blocks, of which diClPIC and the chlorine-substituted Pip have not been described before. The non-chlorinated analogue of diClPIC, i.e., PIC, has been shown to occur in peptides and proteins as a product of oxidation of tryptophan residues[12] as well as a product of oxidation of free tryptophan,[15] but also as a constituent of peptides produced by bacteria and fungi.[10,11] The other subunits of these depsipeptides have previously all been described, either as free molecules or as parts of peptides or other natural products. The synthesis and optical resolution of the enantiomers of OHdiMe-Bu has been described,[16] but to our knowledge, this is the first report of this α-hydroxy acid as part of a natural product. MecPGly has been isolated from *Micromonospora miyakonensis*[9] as a free amino acid, whereas OHGlu has been reported from different sources, e.g., in the peptidoglycan of *Microbacterium lacticum*.[17] MeSer, as well as Pip, has been found to occur in several depsipeptides, isolated from, for example, sponges[18] or strains of *Streptomyces*.[13]

On the basis of phenotypic and genomic differences, three species of the genus *Streptosporangium* were previously transferred to the new genus *Kutzneria*,[19] and these three species have been described with respect to secondary metabolites only in a few reports. *K. albida* and *K. viridogrisea* (formerly *S. albidum* and *S. viridogriseum*[19]) have both been found to produce the antibiotic macrocyclic lactone sporaviridin,[20] whereas aculeximycin,[21] also a macrocyclic lactone, has been isolated from *K. albida*. However, nothing similar to compounds **1**−**4** has been described from this genus earlier, or from any other source.

Compounds **1**−**4** were tested for growth inhibition of four common root-rotting fungi, *Cylindrocladium canadense*, *F. avenaceum*, *F. oxysporum*, and *Nectria radicicola*. The growth inhibition was studied in the concentration range 0.5 μg/mL to 1.0 mg/mL, and in no case did the growth inhibition at the highest concentration, 1.0 mg/mL, exceed 60−99%. At this concentration of **1**−**4**, *C. canadense* displayed the highest sensitivity with 60−99% inhibition for all tested compounds, whereas *F. oxysporum* was the least sensitive, with observable inhibition only for **2** (30−59% inhibition). *N. radicicola* and *F. avenaceum* were inhibited 60−99% by **1**−**3** and **1**−**2**, respectively, and 30−59% by **4**. *F. avenaceum* was not tested with **3** at this concentration. Due to the low water solubility of the compounds, the growth inhibition was not tested at higher concentrations.

Experimental Section

General Experimental Procedures. Optical rotation was measured on a Model 341 polarimeter (Perkin-Elmer) using CH_3OH as solvent, in a 1 mL cell (path 10.0 cm, λ 589 nm, 20 °C). UV data were acquired at 20 °C with a U-2100 spectrophotometer (Hitachi) using CH_3OH as solvent. NMR data were obtained using Bruker DRX400 and DRX600 NMR spectrometers operated at 400 and 600 MHz, respectively, for ^1H, and 100 and 150 MHz, respectively, for ^{13}C. The Bruker DRX400 was equipped with 5 mm QNP or BBO probe heads, whereas the DRX600 was equipped with a 5 mm QXI probe head. NMR experiments were performed at 30 or 20 °C, with $CDCl_3$ as solvent, using pulse programs for one-dimensional ^1H and ^{13}C, and two-dimensional $^1H-^1H$ COSY, $^1H-^{13}C$ HSQC-DEPT, $^1H-^{13}C$ HMBC (65 ms), and $^1H-^1H$ ROESY (100, 200, or 300 ms) experiments, supplied by Bruker. Chemical shifts were determined relative to $CHCl_3$ (δ_H 7.27) and $CDCl_3$ (δ_C 77.23). ESIMS data were obtained using a Bruker Esquire mass spectrometer in positive and negative mode, using CH_3OH as solvent. HRFABMS data were obtained using a four-sector instrument (JEOL SX102/SX102) using glycerol as matrix and PEG as internal standard. LCMS was performed on a HP1100 LC system (Hewlett-Packard) connected to a Bruker Esquire mass spectrometer, and GCMS on a HP5890/5970 GCMS (Hewlett-Packard).

Isolation and Identification of Microorganisms. The actinomycetous strain 744 was isolated on Melin Norkrans medium (MMN)[22] from surface-sterilized mycorrhizal root tips of bare root cultivated *Picea abies* seedlings in a forest nursery (54°58′ N, 23°38′ E) in Lithuania.[23] The isolated culture was maintained on MMN media in 9 cm diameter Petri dishes at 20 ± 1 °C. For long-term storage it was maintained at 4 ± 1 °C.

Morphological studies (the Central Bureau of Fungal Cultures in Utrecht, The Netherlands) determined the isolate to be *Streptomyces*-like aerobic actinomycete. Molecular studies, involving extraction of DNA, amplification of 16S rDNA with primers 27F and 1492R,[24] and sequencing, as previously described,[25] resulted in a nucleotide sequence, 1425 bp in length, that was searched against the 16S rDNA sequences available at the GenBank database.[26] The total length of the sequence was analyzed by nucleotide-nucleotide BLAST (blastn) and pairwise two sequence alignments (bl2seq). Identification-based comparison showed that the sequence was the most similar (98%) to the sequence of the actinomycete *Kutzneria kofuensis*, differing by 18 nucleotides as another two nucleotides in the reference sequence were provided as unknown. Interspecific 16S rDNA sequence comparison of the type species *K. viridogrisea* and *K. kofuensis* originating from a phylogenetic study[27] showed that nucleotide homology within the genus *Kutzneria* is at least 97%; therefore it was concluded that the actinomycetous strain 744 was eligible for assignment to this particular genus. The isolate, defined as *Kutzneria* sp. 744, has been deposited at the culture collection of the Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences (SLU), Uppsala, and the 16S rDNA sequence at the GenBank under the accession number DQ181633.

Assay of Antifungal Activity. To estimate the antifungal activity of the isolate, the actinomycete *Kutzneria* sp. 744 was confronted on MMN agar media in 9 cm diameter Petri dishes with the root pathogens *Pythium undulatum* strain SN1, *Ceratobasidium bicorne* strain 248, and *Fusarium avenaceum* strain D2, originating from the culture collection of the Department of Forest Mycology and Pathology, SLU.

The plates were incubated at 20 ± 1 °C for 30 days, and the outcome was studied by ocular observations.

Conidiospores of the root pathogen *F. avenaceum* were used to guide the isolation of antifungal compounds. Conidiospores were produced on MMN agar media in 9 cm diameter Petri dishes at 20 ± 1 °C in the dark, and the spores were harvested from 15- to 30-day-old cultures by flooding mycelium with liquid MMN medium and gently rubbing the culture colony with a glass rod. The resulting suspensions were filtered through a 50 μm mesh to separate mycelial fragments. A haemacytometer was used to determine the conidial concentration. Bioassays were performed as previously described.[28] Aliquots of SPE or HPLC fractions were transferred to microtiter plates and the solvents evaporated overnight in a fume hood. Subsequently, 100 μL of MMN media containing 10^5 conidiospores/mL of *F. avenaceum* was added to each well, and the microtiter plates were incubated at 20 ± 1 °C for 48 h. Control wells comprised conidiospores suspended in liquid MMN medium. The extent of germination of conidiospores was estimated by ocular observation of the microtiter plates with a stereomicroscope as well as by an automated plate reader (Labsystems Multiscan RC, Helsinki, Finland) operated at 620 nm.

The growth inhibition caused by the isolated compounds was tested for conidiospores of *F. avenaceum* as well as for the root-rotting fungi *Cylindrocladium canadense* strain aurim1149, *F. oxysporum* strain aurim1101-10, and *Nectria radicicola* strain aurim1148-1. Solutions of the peptides were transferred to wells in microtiter plates, and the solvent (CH_3OH) was allowed to evaporate overnight in a fume hood. To the dried wells were added suspensions of conidiospores of the different fungal species (all conidiospores prepared as described above), in liquid MMN media (total volume 100 μL per well). The growth inhibition studies were performed in triplicate and covered the concentration range 1.0 mg/mL to 0.5 μg/mL. The extent of germination of conidiospores was estimated by ocular observation of the microtiter plates with a stereomicroscope as well as by an automated plate reader (Labsystems Multiscan RC) at 620 nm.

Isolation of Compounds 1, 2, 3, and 4. Twenty Erlenmeyer flasks (500 mL) with 300 mL of liquid MMN medium were each inoculated with three MMN agar plugs (diameter ca. 3 mm) colonized by *Kutzneria* sp. 744. Inoculated flasks were incubated for 14 days at 21 ± 2 °C on a Unitwist 400 rotary shaker (Uniequip, Martinsried, Germany) at 120 rpm. The liquid cultures were filtered (50 μm), and the pooled filtrates were subsequently extracted by SPE. For each batch a 150 g SPE column (C-18, International Sorbent Technology, UK) was packed and activated in CH_3CN. Following equilibration with H_2O, the culture filtrate was passed through the column. Nonbound substances were eluted from the column with H_2O (1 L), whereas compounds adsorbed to the SPE column were eluted with aqueous 95% CH_3CN (1 L). The H_2O phase as well as the CH_3CN phase were analyzed for antifungal activity as described above. The 95% CH_3CN fraction from SPE was dried under reduced pressure (yield typically 450 mg) and the resulting material subsequently dissolved in 6 mL of aqueous 40% CH_3CN. This sample was filtered (0.45 μm) and further fractionated (6 × 1 mL injected) by preparative reversed-phase HPLC (C-18, 3 μm, 20 × 100 mm and 20 × 30 mm guard column, Dr. A. Maisch High Performance LC GmbH, Germany) using a gradient of CH_3CN in H_2O (10−100% in 10 min at 10 mL/min, followed by a 10 min hold at 100% CH_3CN). The eluate was monitored by UV detection at 210 nm, and fractions (12 s) were collected in 96-well plates (2.2 mL wells) by a fraction collector. Aliquots (200 μL) were transferred to microtiter plates for bioassay as described above. Isocratic elution at 61% aqueous CH_3CN, 10 mL/min, using the same column, was used to further purify compounds **3** and **4**.

Compound 1: 6.8 mg; white powder; $[\alpha]^{20}_D$ −16.7 (*c* 0.33, CH_3OH); ^{1}H NMR ($CDCl_3$, 600 MHz) δ MecPGly 7.66 (1H, d, *J* = 9.4 Hz, N*H*), 4.02 (1H, d, *J* = 9.4 Hz, H-2), 1.04 (3H, s, CH_3), 1.02 (1H, m, H-4a), 0.71 (1H, m, H-5a), 0.67 (1H, m, H-4b), 0.39 (1H, m, H-5b); diClPIC 7.16 (1H, d, *J* = 8.1 Hz, H-4), 6.97 (1H, d, *J* = 8.1 Hz, H-5), 6.42 (1H, d, *J* = 5.5 Hz, N*H*), 5.88 (1H, s, O*H*), 5.29 (1H, d, *J* = 7.7 Hz, H-2), 5.25 (1H, d, *J* = 5.5 Hz, H-8a), 2.79 (1H, d, *J* = 14.3 Hz, H-3β), 2.17 (1H, dd, *J* = 14.3, 7.7 Hz, H-3α); OHGlu 7.20 (1H, d, *J* = 10.8 Hz, N*H*), 5.31 (1H, d, *J* = 10.8 Hz, H-2), 4.76 (1H, dd, *J* = 8.8, 5.0 Hz, H-3), 3.96 (1H, s, O*H*), 2.62 (1H, dd, *J* = 15.6, 8.8 Hz, H-4a), 2.54 (1H, dd, *J* = 15.6, 5.0 Hz, H-4b); MeSer 7.62 (1H, d, *J* = 5.9 Hz, N*H*), 4.36 (1H, dt, *J* = 5.8, 2.8 Hz, H-2), 3.78 (1H, dd, *J* = 9.5, 2.2 Hz, H-3a), 3.41 (1H, dd, *J* = 9.5, 2.9 Hz, H-3b), 3.25 (3H, s, CH_3); Pip 5.01 (1H, d, *J* = 5.5 Hz, H-2), 4.70 (1H, d, *J* = 12.3 Hz, N*H*), 3.10 (1H, d, *J* = 13 Hz, H-5a), 2.80 (1H, q, *J* = 12 Hz, H-5b), 2.34 (1H, d, *J* = 13.2 Hz, H-3a), 2.08 (1H, m, H-4a), 1.61 (1H, m, H-3b), 1.56 (1H, m, H-4b); OHdiMeBu 5.85 (1H, s, H-2), 1.12 (9H, s, H-4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ MecPGly 173.4 (CO, C-1), 60.2 (CH, C-2), 19.3 (C, C-3), 18.8 (CH_3, *C*H_3), 14.4 (CH_2, C-4), 12.0 (CH_2, C-5); diClPIC 172.0 (CO, CH*C*ONH), 147.0 (C, C-7a), 133.4 (C, C-6), 131.2 (C, C-3b), 122.3 (CH, C-5), 122.0 (CH, C-4), 115.8 (C, C-7), 91.7 (C, C-3a), 85.5 (CH, C-8a), 60.7 (CH, C-2), 39.3 (CH_2, C-3); OHGlu 174.7 (CO, C-5), 171.4 (CO, C-1), 68.0 (CH, C-3), 52.6 (CH, C-2), 36.7 (CH_2, C-4); MeSer 172.6 (CO, C-1), 71.5 (CH_2, C-3), 59.9 (CH_3, *C*H_3), 55.9 (CH, C-2); Pip 171.4 (CO, C-1), 50.8 (CH, C-2), 46.6 (CH_2, C-5), 23.7 (CH_2, C-3), 20.8 (CH_2, C-4); OHdiMeBu 172.8 (CO, C-1), 78.6 (CH, C-2), 33.9 (C, C-3), 26.6 (CH_3, C-4); HRFABMS *m/z* 854.2888 $(M + H)^+$ (calcd for $C_{37}H_{50}N_7O_{12}Cl_2$ 854.2895).

Compound 2: 3.7 mg; white powder; $[\alpha]^{20}_D$ −19.4 (*c* 0.29, CH_3OH); ^{1}H NMR ($CDCl_3$, 600 MHz) δ MecPGly 7.61 (1H, d, *J* = 9.4 Hz, N*H*), 4.03 (1H, d, *J* = 9.4 Hz, H-2), 1.05 (3H, s, CH_3), 1.03 (1H, m, H-4a), 0.71 (1H, m, H-5a), 0.69 (1H, m, H-4b), 0.41 (1H, m, H-5b); diClPIC 7.17 (1H, d, *J* = 8.1 Hz, H-4), 6.99 (1H, d, *J* = 8.1 Hz, H-5), 6.37 (1H, d, *J* = 5.3 Hz, N*H*), 5.84 (1H, s, O*H*), 5.28 (1H, d, *J* = 7.9 Hz, H-2), 5.25 (1H, d, *J* = 5.3 Hz, H-8a), 2.79 (1H, d, *J* = 14.1 Hz, H-3β), 2.17 (1H, dd, *J* = 14.1, 7.9 Hz, H-3α); OHGlu 7.13 (1H, d, *J* = 11.0 Hz, N*H*), 5.30 (1H, d, *J* = 11.0 Hz, H-2), 4.75 (1H, dd, *J* = 9.2, 4.6 Hz, H-3), 4.0 (1H, s, O*H*), 2.65 (1H, dd, *J* = 14.8, 9.2 Hz, H-4a), 2.55 (1H, dd, *J* = 14.8, 4.6 Hz, H-4b); MeSer 7.52 (1H, d, *J* = 4.6 Hz, N*H*), 4.35 (1H, m, H-2), 3.79 (1H, dd, *J* = 8.6 Hz, H-3a), 3.41 (1H, dd, *J* = 8.6, 2.2 Hz, H-3b), 3.26 (3H, s, CH_3); Pip 5.21 (1H, d, *J* = 13.0 Hz, N*H*), 5.18 (1H, d, *J* = 5.1 Hz, H-2), 4.66 (1H, m, H-4), 3.36 (1H, d, *J* = 12.6 Hz, H-5a), 2.85 (1H, q, *J* = 12.0 Hz, H-5b), 2.77 (1H, m, H-3a), 1.81 (1H, ddd, *J* = 13, 12, 5.9 Hz, H-3b); OHdiMeBu 5.75 (1H, s, H-2), 1.13 (9H, s, H-4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ MecPGly 173.8 (CO, C-1), 60.2 (CH, C-2), 19.2 (C, C-3), 18.8 (CH_3, *C*H_3), 14.5 (CH_2, C-4), 12.0 (CH_2, C-5); diClPIC 171.9 (CO, CH*C*ONH), 147.0 (C, C-7a), 133.5 (C, C-6), 131.1 (C, C-3b), 122.3 (CH, C-5), 122.0 (CH, C-4), 115.9 (C, C-7), 91.7 (C, C-3a), 85.6 (CH, C-8a), 60.7 (CH, C-2), 39.3 (CH_2, C-3); OHGlu 173.6 (CO, C-5), 171.4 (CO, C-1), 68.2 (CH, C-3), 52.6 (CH, C-2), 36.5 (CH_2, C-4); MeSer 172.3 (CO, C-1), 71.3 (CH_2, C-3), 59.9 (CH_3, *C*H_3), 56.1 (CH, C-2); Pip 170.5 (CO, C-1), 53.5 (CH_2, C-5), 53.4 (CH, C-2), 50.5 (CH, C-4), 34.2 (CH_2, C-3); OHdiMeBu 172.6 (CO, C-1), 78.5 (CH, C-2), 33.9 (C, C-3), 26.5 (CH_3, C-4); HRFABMS *m/z* 888.2534 $(M + H)^+$ (calcd for $C_{37}H_{49}N_7O_{12}Cl_3$ 888.2505).

Compound 3: 3.0 mg; white powder; $[\alpha]^{20}_D$ −30.9 (*c* 0.16, CH_3OH); ^{1}H NMR ($CDCl_3$, 600 MHz) δ MecPGly 7.88 (1H, d, *J* = 9.4 Hz, N*H*), 4.11 (1H, d, *J* = 9.4 Hz, H-2), 1.08 (1H, m, H-4a), 1.07 (3H, s, CH_3), 0.71 (1H, m, H-5a), 0.69 (1H, m, H-4b), 0.38 (1H, m, H-5b); diClPIC 7.15 (1H, d, *J* = 8.0 Hz, H-4), 6.97 (1H, d, *J* = 8.0 Hz, H-5), 6.38 (1H, d, *J* = 5.5 Hz, N*H*), 5.68 (1H, s, O*H*), 5.33 (1H, d, *J* = 7.7 Hz, H-2), 5.28 (1H, d, *J* = 5.5 Hz, H-8a), 2.82 (1H, d, *J* = 14.2 Hz, H-3β), 2.16 (1H, dd, *J* = 14.2, 8.1 Hz, H-3α); OHGlu 6.81 (1H, d, *J* = 10.5 Hz, N*H*), 5.24 (1H, t, *J* = ∼10 Hz, H-2), 4.74 (1H, m, H-3), 3.25 (1H, s, O*H*), 2.74 (1H, dd, *J* = 14.5, 3.7 Hz, H-4a), 2.69 (1H, dd, *J* = 14.5, 10.5 Hz, H-4b); MeSer 7.53 (1H, d, *J* = 7.0 Hz, N*H*), 4.60 (1H, d, *J* = 7.0 Hz, H-2), 3.88 (1H, d, *J* = 8.9 Hz, H-3a), 3.27 (1H, dd, *J* = 8.9, 3.1 Hz, H-3b), 3.24 (3H, s, CH_3); Pip 5.10 (1H, m, N*H*), 5.08 (1H, d, *J* = 5.0 Hz, H-2), 3.11 (1H, d, *J* = 12.7 Hz, H-5a), 2.87 (1H, q, *J* = 12.7 Hz, H-5b), 2.40 (1H, d, *J* = 13.6 Hz, H-3a), 2.12 (1H, m, H-4a), 1.64 (1H, m, H-3b), 1.54 (1H, m, H-4b); OHdiMeBu 5.85 (1H, s, H-2), 1.15 (9H, s, H-4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ MecPGly 172.7 (CO, C-1), 59.9 (CH, C-2), 19.9 (C, C-3), 18.8 (CH_3, *C*H_3), 15.0 (CH_2, C-4), 11.9 (CH_2, C-5); diClPIC 172.0 (CO, CH*C*ONH), 147.0 (C, C-7a), 133.4 (C, C-6), 131.0 (C, C-3b), 122.1 (CH, C-5), 121.9 (CH, C-4), 115.7 (C, C-7), 91.8 (C, C-3a), 85.2 (CH, C-8a), 60.7 (CH, C-2), 39.0 (CH_2, C-3); OHGlu 174.2 (CO, C-5), 171.2 (CO, C-1), 69.3 (CH, C-3), 55.8 (CH, C-2), 38.6 (CH_2, C-4); MeSer 172.2 (CO, C-1), 73.1 (CH_2, C-3), 60.0 (CH_3, *C*H_3), 55.8 (CH, C-2); Pip 175.1 (CO, C-1), 51.1 (CH, C-2), 46.5 (CH_2, C-5), 23.5 (CH_2, C-3), 20.1 (CH_2, C-4); OHdiMeBu 172.8 (CO, C-1), 78.3 (CH, C-2), 34.2 (C, C-3), 26.8 (CH_3, C-4); HRFABMS *m/z* 854.2889 $(M + H)^+$ (calcd for $C_{37}H_{50}N_7O_{12}Cl_2$ 854.2895).

Compound 4: 1.1 mg; white powder; $[\alpha]^{20}_D$ −17.9 (*c* 0.067, CH_3OH); ^{1}H NMR ($CDCl_3$, 600 MHz) δ MecPGly 7.84 (1H, d, *J* = 9.4 Hz, N*H*), 4.12 (1H, d, *J* = 9.4 Hz, H-2), 1.08 (1H, m, H-4a), 1.07 (3H, s, CH_3), 0.71 (1H, m, H-5a), 0.71 (1H, m, H-4b), 0.40 (1H, m, H-5b); diClPIC 7.15 (1H, d, *J* = 8.1 Hz, H-4), 6.97 (1H, d, *J* = 8.1

Hz, H-5), 6.36 (1H, s, N*H*), 5.76 (1H, s, O*H*), 5.31 (1H, d, $J = 7.9$ Hz, H-2), 5.28 (1H, d, $J = 5.3$ Hz, H-8a), 2.80 (1H, d, $J = 14.2$ Hz, H-3β), 2.16 (1H, dd, $J = 14.2$, 7.9 Hz, H-3α); OHGlu 6.72 (1H, d, $J = 10.5$ Hz, N*H*), 5.20 (1H, dd, $J = 10$, 9 Hz, H-2), 4.58 (1H, q, $J = 8$ Hz, H-3), 3.62 (1H, s, O*H*), 2.62 (2H, m, H-4ab); MeSer 7.91 (1H, d, $J = 7.4$ Hz, N*H*), 4.51 (1H, m, H-2), 3.84 (1H, d, $J = 7.2$ Hz, H-3a), 3.32 (1H, dd, $J = 7.2$, 2.6 Hz, H-3b), 3.27 (3H, s, CH_3); Pip 7.17 (1H, d, $J = 4$ Hz, H-5), 5.23 (1H, d, $J = 3.7$ Hz, H-2), 2.78 (1H, m, H-4a), 2.46 (1H, dd, $J = 13.4$, 6.6 Hz, H-3a), 2.21 (1H, m, H-4b), 1.57 (1H, m, H-3b); OHdiMeBu 5.91 (1H, s, H-2), 1.11 (9H, s, H-4); ^{13}C NMR ($CDCl_3$, 150 MHz) δ MecPGly 172.8 (CO, C-1), 60.0 (CH, C-2), 19.9 (C, C-3), 19.0 (CH_3, CH_3), 14.9 (CH_2, C-4), 12.1 (CH_2, C-5); diClPIC 172.2 (CO, CHCONH), 147.3 (C, C-7a), 133.3 (C, C-6), 131.1 (C, C-3b), 122.2 (CH, C-5), 122.0 (CH, C-4), 115.7 (C, C-7), 91.8 (C, C-3a), 85.3 (CH, C-8a), 60.8 (CH, C-2), 39.1 (CH_2, C-3); OHGlu 173.2 (CO, C-5), 171.4 (CO, C-1), 69.1 (CH, C-3), 54.9 (CH, C-2), 38.0 (CH_2, C-4); MeSer 172.1 (CO, C-1), 72.6 (CH_2, C-3), 60.1 (CH_3, CH_3), 55.7 (CH, C-2); Pip 172.4 (CO, C-1), 146.9 (CH, C-5), 50.5 (CH, C-2), 21.0 (CH_2, C-4), 17.3 (CH_2, C-3); OHdiMeBu 172.5 (CO, C-1), 79.0 (CH, C-2), 35.2 (C, C-3), 26.7 (CH_3, C-4); HRFABMS *m*/*z* 852.2688 $(M + H)^+$ (calcd for $C_{37}H_{48}N_7O_{12}Cl_2$ 852.2738).

Partial Hydrolysis of 1. Compound **1** (approximately 50 μg for each experiment) was hydrolyzed for 0.5, 1, 2, and 5 h, in 0.5 mL of 6 M HCl at 110 °C, in evacuated glass ampules. The samples were dried under a stream of N_2, redissolved in 1 mL of H_2O, and dried under vacuum. Each sample was dissolved in 1 mL of H_2O for analysis by LCMS. Samples (10 μL) were injected on a reversed-phase column (C-18, 2 × 150 mm, 5 μm, Dr. A. Maisch High Performance LC GmbH), eluted with a gradient of CH_3OH in H_2O (45−60% in 10 min at 0.2 mL/min, followed by 60% CH_3OH for 10 min). MS data were acquired in the positive ion mode.

Absolute Configuration of Subunits A, C, D, E, and F of 1−4. Compounds **1**−**4** (25−50 μg of each) were hydrolyzed overnight in 0.5 mL of 6 M HCl at 110 °C, in evacuated glass ampules. The samples were dried under a stream of N_2 and hydrogenated overnight, H_2 (1 atm)/PtO_2/AcOH, at room temperature, followed by filtration and evaporation under a stream of nitrogen. (*S*)-2-Butyl esters were formed by treatment with 200 μL of (*S*)-2-BuOH/AcCl (10:1) at 100 °C for 40 min in sealed test tubes. Following evaporation under a stream of nitrogen, 200 μL of perfluoropropanoic anhydride was added and the mixtures were kept at 100 °C for 40 min in sealed test tubes. Each reaction mixture was dried under a stream of N_2 and dissolved in EtOAc (100 μL). Analysis by GC-MS was performed on a fused silica column (HP-5MS, 0.25 mm × 30 m, Agilent Technologies) using He as carrier gas at 1 mL/min. The injector was held at 240 °C and the oven at 60 °C for 5 min, followed by a gradient to 100 °C at 1 °C/min, to 150 °C at 5 °C/min, and finally to 240 °C at 3 °C/min. The transfer line to the MS was kept at 260 °C. Samples of D- and L-serine, (*S*)-2-hydroxy-3,3-dimethylbutanoic acid (Sigma-Aldrich), D- and L-*tert*-leucine (Sigma-Aldrich), D- and L-ornithine, and *threo*-3-hydroxy-D-glutamic acid, *threo*-3-hydroxy-L-glutamic acid, *erythro*-3-hydroxy-D-glutamic acid, and *erythro*-3-hydroxy-L-glutamic acid (synthesized as described in Supporting Information) were derivatized as above (or with racemic 2-BuOH) and used as reference compounds.

Absolute Configuration of Subunit B (diClPIC) of 1−4. Starting from several different conformations, the energetically most favorable conformers of **1**−**4**, constructed of the configurationally determined subunits A, C, D, E, and F (as above), together with the D- or L-isomer of diClPIC, were calculated by molecular modeling employing the MM2 force field using the Chem3D Ultra ver. 8.0.3 software (CambridgeSoft Corp.). Subsequently, the relevant interatomic distances were measured using the same software.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra and UV spectrum for **1**, as well as NMR data for **1**, **2**, **3**, and **4**, together with experimental procedures for the synthesis of 3-hydroxyglutamic acid, and associated NMR and MS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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