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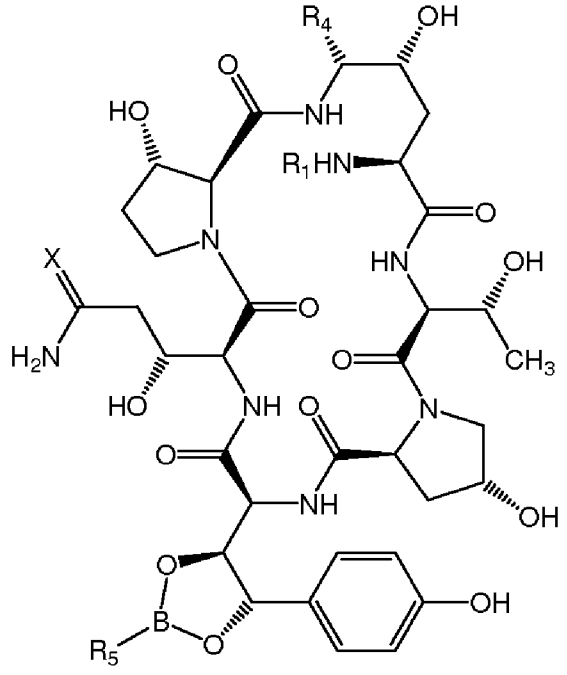
(54) **Title**: METHOD FOR THE PREPARATION OF CYCLOPEPTIDES



(8)

(57) **Abstract**: The present invention relates to a method for preparing cyclopeptides by means of protection with a substituted boronic acid. The present invention also discloses novel boronate esters of cyclopeptides of general formula (8).

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METHOD FOR THE PREPARATION OF CYCLOPEPTIDES

Field of the invention

The present invention relates to novel cyclopeptides and to a method for preparing cyclopeptides using substituted boronic acids.

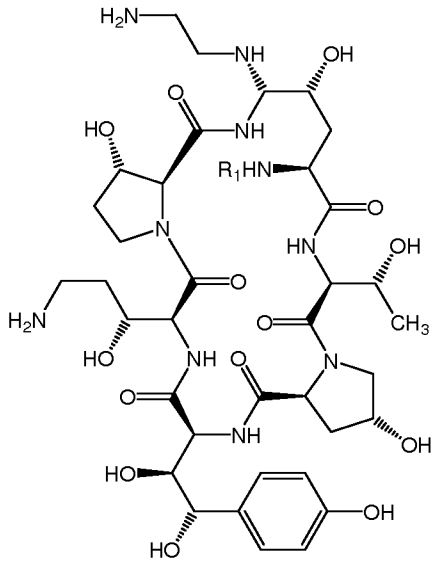
Background of the invention

Cyclopeptides are polypeptides in which the terminal amine and carboxyl groups form an internal peptide bond. Several cyclopeptides are known for their advantageous medicinal properties. An excellent example of this is the class of echinocandins which are potent antifungals. Cyclopeptides can be naturally occurring compounds but may also be obtained by total synthesis or by synthetic or genetic modification of naturally occurring or produced precursors; the latter class is referred to as semi synthetic cyclopeptides. Examples of medicinally useful echinocandins are the cyclic hexapeptides anidulafungin, caspofungin, cilofungin and micafungin which are useful in treating fungal infections especially those caused by *Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides* and *Histoplasma*. Anidulafungin, caspofungin and micafungin are all semi synthetic cyclopeptides derivable from naturally occurring echinocandins such as for instance echinocandin B, pneumocandin A_0 or pneumocandin B_0.

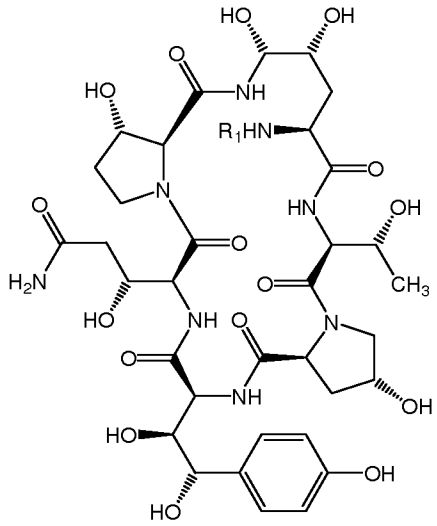
Although nature can provide a substantive part of the complex chemical structure of semi synthetic cyclopeptides, and in many cases having all chiral centers in the required configuration, the subsequent chemical conversions into the therapeutically active derivatives nevertheless often require unprecedented approaches. Usually the structures in question are chemically unstable and/or prone to racemization and simply do not allow for otherwise obvious synthetic manipulation taught in synthetic organic chemical textbooks. This chemical instability is even more pronounced in anidulafungin, caspofungin and micafungin due to the presence of the notoriously fragile hemiaminal moiety.

The preparation of caspofungin (**1**) from fermentatively obtained pneumocandin B_0 (**2**), with $R_1 = C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$) in both

compounds, may serve as an example of the complexity in cyclopeptide chemistry described above.



(**1**)



(**2**)

Initially, in US 5,378,804 a process was disclosed requiring five steps and having major drawbacks in lack of stereo selectivity and an overall yield of less than 10%. The conversion of the amide functionality in (**2**) into the amine as present in (**1**) required two steps, namely dehydration of the primary amide to the nitrile followed by reduction to the amine. Introduction of the ethylenediamine moiety at the hemiaminal position required three steps. An improved procedure was disclosed in US 5,552,521 requiring three steps in total, namely reduction of the amide followed by activation with thiophenol and stereoselective displacement of the thiophenol function to introduce the ethylenediamine moiety. Still this process suffers from a low overall yield of no more than 25%. A further improvement in yield was realized in US 5,936,062 describing intermediate protection of, amongst others, the vicinal hydroxyl groups of the homotyrosine moiety using phenylboronic acid. Two synthetic approaches were suggested, the first one starting with phenylboronic acid protection followed by reduction with borane and activation with thiophenol and the second one starting with thiophenol activation followed by phenylboronic acid protection and reduction with borane. Both approaches were completed by introduction of the ethylenediamine moiety and overall yields ranging from 25-36% were reported. In the first approach the claimed sequence of steps involved the

presence of a diboronate ester intermediate. An alternative approach to this was described by W.R. Leonard *et al.* (J. Org. Chem. **2007**, *72*, 2335-2343) involving the initial formation of a mono-phenylboronate ester protection of the vicinal hydroxyl groups allowing for immediate introduction of the thiophenol activating group. This latter approach resulted in a 45% overall yield.

Today there are no convenient alternatives to the above approaches so there remains a challenge for finding alternative chemical approaches that allow for conversion of naturally occurring cyclopeptides into semi synthetic cyclopeptides. These approaches can be used as alternative to prior art methods, or preferably to achieve a higher yield, higher chemical purity, higher optical purity, less waste streams or any or all of the above.

Detailed description of the invention

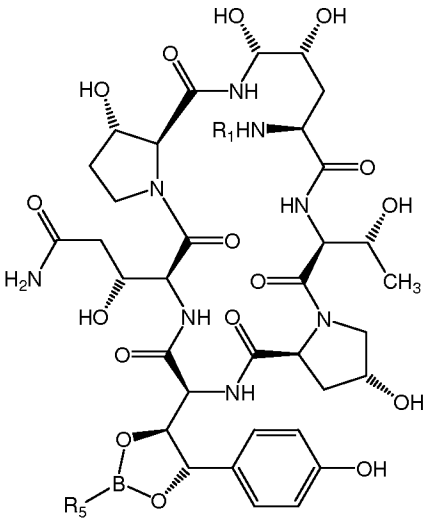
In a first aspect of the present invention there is provided a method for the preparation of a first cyclopeptide comprising a vicinal diol from a second cyclopeptide comprising a vicinal diol wherein the vicinal diol is protected with a boronic acid derivative. In the context of the present invention, a vicinal diol is a compound bearing at least two hydroxyl functional groups that are attached to adjacent carbon atoms. The use of ethyl- and phenylboronic acid as a protecting group for 1,2-diols is well known, for instance from Greene, T.W. and Wuts, P.G.M., Protective Groups in Organic Synthesis (John Wiley & Sons, Inc., New York/Chichester/Brisbane/Toronto/Singapore, 2nd Ed., 1991, ISBN 0-471-62301-6). In addition, the use of phenylboronic acid in cyclopeptide chemistry has been described in US 5,936,062. In the present invention, approaches alternative to the use of phenylboronic acid were investigated in order to solve several problems associated with phenylboronic acid such as sub-optimal yields and toxicity of the phenylboronic acid which is eventually released in the waste stream. It was found that substituted boronic acids other than phenylboronic acid or naphthylboronic acid are suitable protecting groups for vicinal diols in cyclopeptides. Notably cyclohexylboronic acid and 4-*tert*-butylphenylboronic acid, both unmentioned in the major textbook in the art by Greene and Wuts, described above.

The suitability of cyclohexylboronic acid is particularly surprising as the skilled person would preferably look for alternate compounds bearing a chromophore (such as tolyl, naphthyl or phenyl) as chromophoric compounds greatly facilitate analysis during

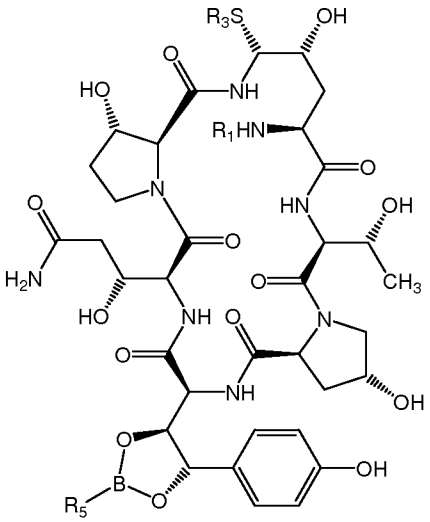
research and production activities. Cyclohexyl boronic acid does not have such a chromophore thereby making it a non-obvious choice. Moreover, to the best of our knowledge cyclohexylboronic acid is not suggested in any means in the art in question.

In a first embodiment there is disclosed a method for the preparation of a compound of general formula (**1**) or a salt thereof comprising the steps of treating a compound of general formula (**2**) wherein R_1 in compounds (**1**) and (**2**) is $C(O)R_2$ wherein R_2 is C_9-C_{21} alkyl, C_9-C_{21} alkenyl, C_1-C_{10} alkoxyphenyl, C_1-C_{10} alkoxynaphthyl or C_1-C_{10} alkoxyterphenyl, with a substituted boronic acid, an activating agent and a reducing agent. Preferably R_1 is $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$. The steps mentioned above may be carried out in various sequences using various activating agents and reducing agents as known to the skilled person. Preferred activating agents are thiols with general formula R_3-SH. More preferably said activating agents are 2-mercaptobenzimidazole, 2-mercaptobenzothiazole, 2-mercapto-1-methylimidazole, 2-mercapto-4-methoxyphenol or thiophenol. The various preferred sequence of steps are outlined in the following embodiments.

In a second embodiment said compound of general formula (**2**) is first reacted with a substituted boronic acid R_5-$B(OH)_2$ to afford a compound of general formula (**3**) wherein R_5 is cyclohexyl, substituted cyclohexyl or substituted phenyl. In a next step, compound (**3**) is reacted with a compound of general formula R_3-SH to afford a compound of general formula (**4**) with R_5 as mentioned above.

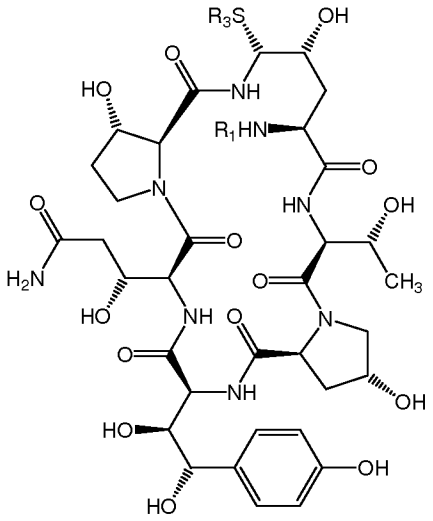


(**3**)

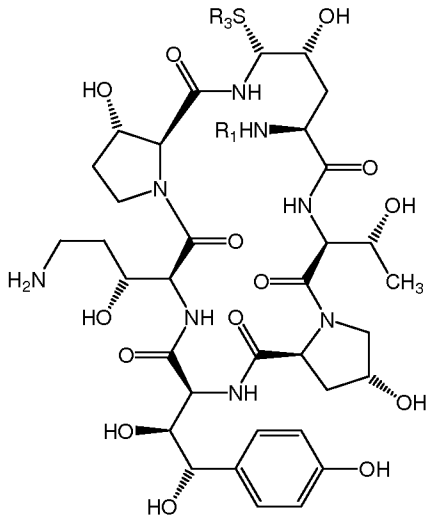


(**4**)

The above conversion may be carried out in a variety of solvents that are inert to the reaction conditions such as (substituted) alkanes, ethers and substituted benzenes, for instance acetonitrile, dichloromethane, diethyl ether, tetrahydrofuran, toluene and the like. Preferably the substituted boronic acid R_5-$B(OH)_2$ is cyclohexylboronic acid or 4-*tert*-butylphenylboronic acid. Preferred temperatures are from -100ºC to 30ºC, more preferably from -50ºC to 0ºC, most preferably from -20ºC to -5ºC Subsequently, compound (**4**) is hydrolyzed to afford a compound of general formula (**5**) which is then reduced to afford a compound of general formula (**6**);



(**5**)



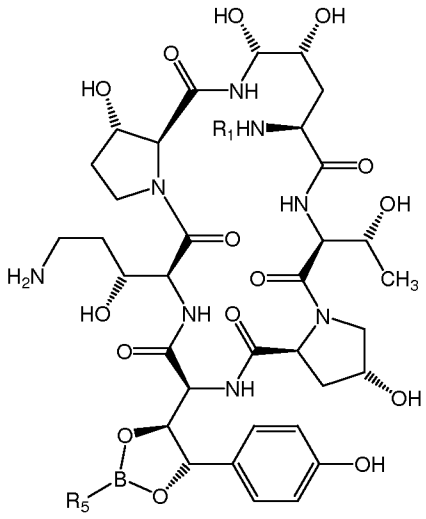
(**6**)

Finally, compound (**6**) is converted to said compound of general formula (**1**) by reaction with ethylenediamine. In W.R. Leonard *et al.* (J. Org. Chem. **2007**, *72*, 2335-2343) the formation of the mono-phenylboronate ester of the vicinal hydroxyl groups required, as evidenced from the experimental details, two equivalents of phenylboronic acid. Although it was established that this excess could also be applied in the present invention with a substituted boronic acid, it was surprisingly established that lower amounts of substituted boronic acid were equally or even better suitable thereby reducing the amount of waste. Thus, the preferred ratio, on a molecular basis, of substituted boronic acid to the compound of general formula (**1**) is from 1.01 to 3, more preferably from 1.05 to 2 and most preferably from 1.1 to 1.5.

In a third embodiment a compound of general formula (**2**) is first reacted with a substituted boronic acid to afford a compound of general formula (**3**), which is

subsequently reacted with a compound of general formula R_3-SH to afford a compound of general formula (**4**) with R_5 as mentioned above. Compound (**4**) is then reduced and hydrolyzed to afford a compound of general formula (**6**) which is converted to said compound of general formula (**1**) by reaction with ethylenediamine.

In a fourth embodiment a compound of general formula (**2**) is first reacted with a substituted boronic acid to afford a compound of general formula (**3**) which is subsequently reduced to afford a compound of general formula (**7**) with R_5 as mentioned above. Compound (**7**) is then reacted with a compound of general formula R_3-SH and hydrolyzed to afford a compound of general formula (**6**) which is converted to said compound of general formula (**1**) by reaction with ethylenediamine.

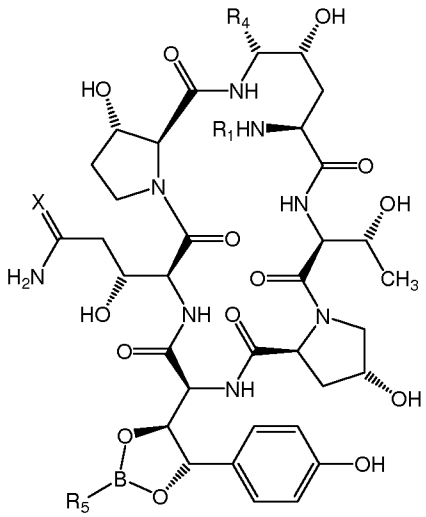


(**7**)

In a fifth embodiment, the compound of general formula (**4**) described above is first reacted with a silylating agent prior to further conversions. Suitable silylating agents are bis(trimethylsilyl)trifluoroacetamide, tert-butyldimethylsilyl chloride, trimethylsilyl chloride and the like.

In a sixth embodiment said reaction with a substituted boronic acid R_5-$B(OH)_2$ that is not phenylboronic acid or naphthylboronic acid is succeeded by and/or combined with reaction with phenylboronic acid. It was surprisingly found that such combination of protecting groups can lead to still more favourable results. Preferably said reaction with phenylboronic acid is carried out after reaction with from 0.8 to 1.2 equivalents of a substituted boronic acid R_5-$B(OH)_2$ that is not phenylboronic acid or naphthylboronic acid

In a second aspect of the present invention there is provided a compound of general formula (**8**)



(**8**)

wherein R_1 is C(O)R_2 wherein R_2 is C_9-C_{21} alkyl, C_9-C_{21} alkenyl, C_1-C_{10} alkoxyphenyl, C_1-C_{10} alkoxynaphthyl or C_1-C_{10} alkoxyterphenyl, wherein R_4 is OH or –SR3 wherein R_3 is benzimidazol-2-yl, benzothiazol-2-yl, 1-methylimidazol-2-yl, 4-methoxyphenyl or phenyl, wherein R_5 is cyclohexyl, substituted cyclohexyl or substituted phenyl and wherein X is O or H,H.

In one embodiment the preferred substituent R_1 is $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$ which is the substituent present in the antifungal agent caspofungin.

In another embodiment the preferred substituent R_4 is OH or –S-phenyl. In yet another embodiment the preferred substituent R_5 is cyclohexyl or 4-*tert*-butylphenyl.

In a third aspect of the invention there is provided the use of a substituted boronic acid in the preparation of a cyclopeptide bearing a vicinal diol. Protection of diols with substituted boronic acids is not limited to the compound of the first embodiment of the first aspect of the present invention but can also be applied to similar cyclopeptides containing a vicinal diol system. In a preferred embodiment the cyclopeptide is anidulafungin, caspofungin, cilofungin or micafungin.

EXAMPLES

General

Pneumocandin was obtained by fermentation of *Glarea Lozoyensis* (*Zalerion arboricola*) as described in WO 2000/008197. Commercially available reagents were used as received unless mentioned otherwise. Solvents were dried over 3Å molecular sieves. HPLC analysis was carried out using a Waters XBridge C18 column, 3.5 µm, 150 mm x 2.1 mm under the following conditions:

- Injection volume: 5 µL
- Detection: UV (210 and 270 nm)
- Flow: 0.40 ml/min
- Column temp: 25ºC
- Mobile phase A: 50 mM K_2HPO_4 + acetonitrile (6:4); pH 6.0
- Mobile phase B: 75% acetonitrile
- Gradient:

Time (min)	0	1.5	5.0	7.0	7.5	11
%A	100	100	0	0	100	100
%B	0	0	100	100	0	0

Retention times (all with R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$): **1**: 2.5 min; **2**: 5.8 min; **6** (R_3 = phenyl): 6.4 min; **5** (R_3 = phenyl): 7.3 min.

Example 1

Pneumocandin cyclohexylboronate ester using 2.0 equiv. cyclohexylboronic acid

(8; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_4 = OH; R_5 = cyclohexyl; X = O)

Under N_2 finely divided pneumocandin B_0 (0.68 g, assay total pneumocandins 95%, assay pneumocandin B_0 and C_0 81%; 0.61 mmol pneumocandins) and cyclohexylboronic acid (156 mg, 1.22 mmol) were added to acetonitrile (20 ml, pre-dried on molecular sieves of 3Å).

Example 2

Pneumocandin phenylthioaminal cyclohexylboronate ester

(8; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_4 = S-phenyl; R_5 = cyclohexyl; X = O)

To the suspension obtained in Example 1 thiophenol (190 µl, 1.86 mmol) was added. The suspension was cooled and maintained at -15°C and trifluoromethanesulfonic acid (163 µL, 1.83 mmol) was added and the reaction mixture was maintained at -15°C for 20 h under nitrogen. The conversion was followed by HPLC: sample after 3 h (50 µl reaction mixture + 20 µl 0.85 M sodium acetate + 0.88 ml methanol): conversion was 79%; after 20 h the conversion was 97%.

Example 3

Pneumocandin phenylthioaminal

(5; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_3 = phenyl)

The reaction mixture obtained in Example 2 was quenched with 0.844 M sodium acetate trihydrate (2.2 ml; 1.86 mmol). The suspension was warmed to 17°C, maintained for 2 h, and cooled to 0°C and stirred at 0°C overnight, during which the concentration of the title compound in the mother-liquor decreased from 2.1 to 1.6 g/l. The precipitate was filtered off, washed with 90% acetonitrile (3 x 10 ml), and dried under vacuum at 30°C, giving 0.53 g of the title compound as an off-white powder with an HPLC-assay of 87%. Isolated yield (over B_0 and C_0): 77%. Loss to mother liquor: 44 ml; 1.4 g/l; 10%.

Example 4

Pneumocandin cyclohexylboronate ester using 1.1 equiv. cyclohexylboronic acid

(8; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_4 = OH; R_5 = cyclohexyl; X = O)

Under nitrogen finely divided pneumocandin B_0 (0.68 g, assay total pneumocandins 95%, assay pneumocandin (B_0 and C_0) 81%; 0.61 mmol pneumocandins) and cyclohexylboronic acid (86 mg, 0.67 mmol) were added to acetonitrile (20 ml, pre-dried on molecular sieves of 3Å).

Example 5

Pneumocandin phenylthioaminal cyclohexylboronate ester

(8; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_4 = S-phenyl; R_5 = cyclohexyl; X = O)

To the suspension obtained in Example 4 thiophenol (190 µl, 1.86 mmol) was added. The suspension was cooled and maintained at -15°C and trifluoromethanesulfonic acid (163 µL, 1.83 mmol) was added and the reaction mixture was maintained at -15°C for 20 h under nitrogen after which the conversion was determined with HPLC (50 µl reaction mixture + 20 µl 0.85 M sodium acetate + 0.88 ml methanol) to be 99%.

Example 6

Pneumocandin phenylthioaminal

(5; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_3 = phenyl)

The reaction mixture obtained in Example 5 was quenched with 0.844 M sodium acetate trihydrate (2.2 ml; 1.86 mmol). The suspension was warmed to 17°C, maintained overnight, and cooled to 0°C and stirred at 0°C for 3 h. The precipitate was filtered off, washed with 90% acetonitrile of 0ºC (3 x 8 ml), and dried under vacuum at 30°C, giving 0.51 g of the title compound as an off-white powder with an HPLC-assay of 90%. Isolated yield (over B_0 and C_0): 77%.

Example 7

Pneumocandin phenylthioaminal amine

(6; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_3 = phenyl) using BSTFA (3 equiv.)

Under nitrogen phenylthioaminal (**5**; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_3 = phenyl; 0.56 g; 0.32 mmol; assay 67% by NMR) was suspended in dry THF (30 ml). At 20°C bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.26 ml; 0.97 mmol) was added and the mixture was stirred for 2 h at 20°C under nitrogen. The solution was cooled to -2°C and 1 M BH_3.THF (2.25 ml; 2.25 mmol) was added. The solution was stirred at ~-2°C overnight. A sample of 3 ml was taken which was quenched with 2 M HCl (200 µl): conversion 24%. Another portion of 1 M BH_3.THF (0.75 ml; 0.75 mmol) was added and stirring at -2°C was continued for 24 h. The reaction mixture was quenched with 2 M HCl (2 ml; 4 mmol). Hydrogen gas evolved from the mixture. This solution was stirred at 0°C for 2 h and analyzed by HPLC: conversion 34%.

Example 8

Pneumocandin phenylthioaminal amine

(6; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_3 = phenyl) using BSTFA

(4 equiv.)

Under nitrogen phenylthioaminal (**5**; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_3 = phenyl; 0.56 g; 0.32 mmol; assay 67% by NMR) was suspended in dry THF (30 ml). At 20°C BSTFA (0.34 ml; 1.27 mmol) was added and the mixture was stirred for 2 h at 20°C under nitrogen. The solution was cooled to -2°C and 1 M BH_3.THF (2.25 ml; 2.25 mmol) was added. The solution was stirred at ~-2°C overnight. A sample of 3 ml was taken which was quenched with 2 M HCl (200 µl): conversion 28%. Another portion of 1 M BH_3.THF (0.75 ml; 0.75 mmol) was added and stirring at -2°C was continued for 24 h. The reaction mixture was quenched with 2 M HCl (2 ml; 4 mmol). Hydrogen gas evolved from the mixture. This solution was stirred at 0°C for 2 h and analyzed by HPLC: conversion 41%.

Example 9

Pneumocandin phenylthioaminal amine

(6; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_3 = phenyl) using BSTFA

(5 equiv.)

Under nitrogen phenylthioaminal (**5**; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_3 = phenyl; 0.56 g; 0.32 mmol; assay 67% by NMR) was suspended in dry THF (30 ml). At 20°C BSTFA (0.43 ml; 1.60 mmol) was added and the mixture was stirred for 1 h at 20°C under nitrogen. The solution was cooled to -3.5°C and 1 M BH_3.THF (2.24 ml; 2.24 mmol) was added. The temperature rose to -3ºC and the solution was stirred at ~-3°C overnight. It was quenched with 2 M HCl (2 ml; 4 mmol). Hydrogen gas evolved from the mixture. This solution was stirred at 0°C for 2.5 h and analyzed by HPLC: conversion 32%.

Example 10

Pneumocandin phenylthioaminal amine cyclohexylboronate ester

(8; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_4 = S-phenyl; R_5 = cyclohexyl; X = H, H) using cyclohexylboronic acid and BSTFA

Under nitrogen phenylthioaminal (**5**; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_3 = phenyl; 0.40 g; 0.23 mmol; assay 66% by NMR) was suspended in dry THF (25 ml). Cyclohexylboronic acid (33 mg; 0.26 mmol) was added. The mixture was heated to reflux. THF was distilled off and the volume was maintained by replenishment with dry THF. The temperature of the reaction mixture rose from 65.4 to 66.3°C. After 1.5 h the mixture (20 ml) was cooled to 20°C in 1 h. BSTFA (183 µl; 0.68 mmol) was added and the mixture was stirred for 1 h at 20°C under nitrogen. The solution was cooled to -2°C in 2 h and 1 M BH_3.THF (1.6 ml; 1.6 mmol) was added. The solution was stirred at ~-2°C overnight. A sample of 1 ml was taken which was quenched with 2 M HCl (67 µl): conversion 83%. Another portion of 1 M BH_3.THF (0.45 ml; 0.45 mmol) was added and stirring at -2°C was continued for 3 hours.

Example 11

Pneumocandin phenylthioaminal amine

(6; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_3 = phenyl)

The reaction mixture obtained in Example 10 was quenched with 2 M HCl (1.4 ml; 2.8 mmol). Hydrogen gas evolved from the mixture. This solution was stirred at 0°C for 2 h and analyzed by HPLC: conversion 83%.

Example 12

Comparison of cyclohexylboronic acid (CHBA), phenylboronic acid (PBA) and 4-*tert*-butylphenylboronic acid (TBPBA) in the synthesis of pneumocandin phenylthioaminal

(5; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_3 = phenyl)

Several combinations of cyclohexylboronic acid (CHBA), phenylboronic acid (PBA) and 4-*tert*-butylphenylboronic acid (TBPBA) were investigated in time on conversion, yield and formation of so-called bis-adducts (products having a second thiophenol moiety).

12.1: 2 equiv. PBA

Under nitrogen finely divided **2** (CAS0902/187; 1 g, assay total pneumocandins 100 %, 0.94 mmol pneumocandins) and phenylboronic acid (PBA; 115 mg, 0.94 mmol), were added to 30 ml dry acetonitrile (< 30 ppm water) and the mixture was stirred at RT for 60 min. PBA (115 mg; 0.94 mmol) was added, followed by thiophenol (290 µl, 2.84 mmol) and the suspension was cooled to -15°C. At -15°C triflic acid (250 µl, 2.82 mmol) was added and the reaction mixture was stirred at -15°C for 26 h.

Time (h)	Conversion (%)	Bis-adducts/5	Yield (%)
0.5	50.4	0.99	50.2
1	62.2	0.96	61.8
1.5	71.1	0.97	70.6
2	76.5	1.05	75.9
3	83.4	1.20	82.5
4	88.3	1.25	87.3
6	95.3	1.44	94.0
9	98.6	1.72	96.9
19	99.9	2.58	97.3
26	100	3.38	96.6

12.2: 1 equiv. PBA and 1 equiv. CHBA

Under nitrogen finely divided **2** (1 g, 0.94 mmol pneumocandins) and CHBA (120 mg, 0.94 mmol), were added to 30 ml dry acetonitrile (< 30 ppm water) and the mixture was stirred at RT for 60 min. PBA (115 mg; 0.94 mmol) was added, followed by thiophenol (290 µl, 2.84 mmol) and the suspension was cooled to -15°C. At -15°C triflic acid (250 µl, 2.82 mmol) was added and the reaction mixture was stirred at -15°C for 26 h.

Time (h)	Conversion (%)	Bis-adducts/5	Yield (%)
0.5	44.5	1.71	44.2
1	52.7	1.71	52.2
1.5	59.4	1.63	58.8
2	63.5	1.63	62.8
3	69.8	1.58	69.0
4	75.0	1.60	74.1
6	81.8	1.66	80.7
9	87.1	1.79	85.7
19	93.4	2.31	91.4
26	95.5	2.57	93.2

12.3: 1 equiv. PBA and 1 equiv. TBPBA

Under nitrogen finely divided **2** (1 g, 0.94 mmol pneumocandins) and TBPBA (167 mg, 0.94 mmol), were added to 30 ml dry acetonitrile (< 30 ppm water) and the mixture was

stirred at RT for 60 min. PBA (115 mg; 0.94 mmol) was added, followed by thiophenol (290 µl, 2.84 mmol) and the suspension was cooled to -15°C. At -15°C triflic acid (250 µl, 2.82 mmol) was added and the reaction mixture was stirred at -15°C for 26 h.

Time (h)	Conversion (%)	Bis-adducts/5	Yield (%)
0.5	48.8	1.38	48.5
1	59.1	1.25	58.7
1.5	66.5	1.26	65.9
2	71.4	1.28	70.7
3	76.4	1.45	75.6
4	80.8	1.45	79.8
6	87.3	1.57	86.1
9	91.2	1.82	89.7
19	97.2	2.59	94.8
26	98.3	2.80	95.6

12.4: 1.2 equiv. CHBA

Under nitrogen finely divided **2** (1 g, 0.94 mmol pneumocandins) and CHBA (144 mg, 1.13 mmol), were added to 30 ml dry acetonitrile (< 30 ppm water) and the mixture was stirred at RT for 60 min. Thiophenol (290 µl, 2.84 mmol) was added and the suspension was cooled to -15°C. At -15°C triflic acid (250 µl, 2.82 mmol) was added and the reaction mixture was stirred at -15°C for 26 h.

Time (h)	Conversion (%)	Bis-adducts/5	Yield (%)
0.5	62.4	1.67	61.7
1	68.0	1.85	67.1
1.5	74.7	1.91	73.6
2	78.3	1.95	77.1
3	83.5	2.10	82.0
4	87.7	2.37	85.8
6	92.9	2.57	90.6
9	95.9	2.99	93.2
19	97.9	4.70	93.4
26	98.0	5.70	92.6

12.5: 1.2 equiv. TBPBA

Under nitrogen finely divided **2** (1 g, 0.94 mmol pneumocandins) and TBPBA (200 mg, 1.13 mmol), were added to 30 ml dry acetonitrile (< 30 ppm water) and the mixture was stirred at RT for 60 min. Thiophenol (290 µl, 2.84 mmol) was added and the suspension was cooled to -15°C. At -15°C triflic acid (250 µl, 2.82 mmol) was added and the reaction mixture was stirred at -15°C for 26 h.

Time (h)	Conversion (%)	Bis-adducts/5	Yield (%)

0.5	70.6	1.92	69.6
1	76.2	2.11	75.0
1.5	78.1	2.34	76.6
2	79.4	2.51	77.8
3	80.4	2.77	78.6
4	81.9	2.98	79.9
6	85.2	3.40	82.7
9	91.3	3.86	88.1
19	96.9	5.11	92.1
26	98.1	5.82	92.5

12.6: 2 equiv. CHBA

Under nitrogen finely divided **2** (1 g, 0.94 mmol pneumocandins) and cyclohexylboronic acid (CHBA; 86 mg, 0.67 mmol), were added to 20 ml dry acetonitrile and the mixture was for 60 min. Next thiophenol (190 µl, 1.86 mmol) was added and the suspension was cooled to -15°C. At -15°C triflic acid (163 µl, 1.84 mmol) was added and the reaction mixture was stirred at -15°C for 20 h.

Time (h)	Conversion (%)	Bis-adducts/5	Yield (%)
3	54.2	0.24	54.1
20	86.2	2.27	84.5

12.7: 2 equiv. TBPBA

Under nitrogen finely divided **2** (1 g, 0.94 mmol pneumocandins) and cyclohexylboronic acid (CHBA; 120 mg, 0.67 mmol), were added to 20 ml dry acetonitrile and the mixture was for 60 min. Next thiophenol (190 µl, 1.86 mmol) was added and the suspension was cooled to -15°C. At -15°C triflic acid (163 µl, 1.84 mmol) was added and the reaction mixture was stirred at -15°C for 20 h.

Time (h)	Conversion (%)	Bis-adducts/5	Yield (%)
3	76.7	1.27	75.9
20	94.1	3.04	91.4

Example 13

Pneumocandin phenylthioaminal

(5; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_3 = phenyl)

using a mixture of cyclohexylboronic acid and phenylboronic acid

Under nitrogen finely divided **2** (1.0 g, assay total pneumocandins 100 %, 0.94 mmol pneumocandins) and cyclohexylboronic acid (120 mg, 0.94 mmol) were added to 30 ml dry acetonitrile. The mixture was stirred at 35-40°C for 1 h. After cooling to 20°C

phenylboronic acid (115 mg; 0.94 mmol) and thiophenol (290 µl, 2.84 mmol) were added and the suspension was cooled to -15°C and triflic acid (250 µL, 2.82 mmol) was added and the reaction mixture was maintained at -15°C for 20 h. The reaction mixture was quenched with 0.85 M sodium acetate.trihydrate (3.32 ml; 2.8 mmol) and the suspension was maintained at 17°C for 2 h, cooled to 0°C and stirred overnight. The precipitate was filtered off, washed three times with 10 ml 90% acetonitrile, and dried under vacuum at 30°C, giving 0.87 g of the title product with a purity of 72.8% (HPLC).

Example 14

Pneumocandin phenylthioaminal

(5; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_3 = phenyl)

using a mixture of 4-*tert*-butylphenylboronic acid and phenylboronic acid

Under nitrogen finely divided **2** (1.0 g, assay total pneumocandins 100 %, 0.94 mmol pneumocandins) and 4-*tert*-butylphenylboronic acid (167 mg, 0.94 mmol) were added to 30 ml dry acetonitrile. The mixture was stirred at 35-40°C for 1 h. After cooling to 20°C phenylboronic acid (115 mg; 0.94 mmol) and thiophenol (290 µl, 2.84 mmol) were added and the suspension was cooled to -15°C and triflic acid (250 µL, 2.82 mmol) was added and the reaction mixture was maintained at -15°C for 20 h. The reaction mixture was quenched with 0.85 M sodium acetate.trihydrate (3.32 ml; 2.8 mmol) and the suspension was maintained at 17°C for 2 h, cooled to 0°C and stirred overnight. The precipitate was filtered off, washed three times with 10 ml 90% acetonitrile, and dried under vacuum at 30°C, giving 0.86 g of the title product with a purity of 74.7% (HPLC).

Example 15

Pneumocandin phenylthioaminal amine

(6; R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_3 = phenyl) using 4-*tert*-butylphenylboronic acid and BSTFA (3 equiv.)

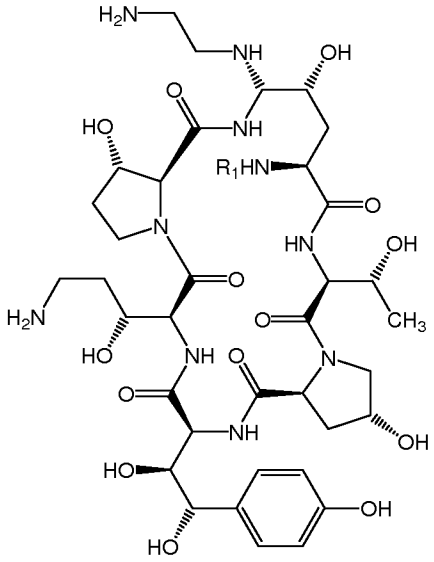
Under nitrogen **5** (R_1 = $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$; R_3 = phenyl; 3.0 g; 71.7 %) was suspended in dry THF (120 ml). 4-*tert*-Butylphenylboronic acid (0.464 g; 2.6 mmol) was added and the mixture was heated to reflux and azeotropically dried by passing the refluxate through a bed of molecular sieves of 0.3 nm (400 g) until the reaction temperature remained constant (T increased from 67.2 to 67.5°C). After 4 h the mixture was cooled to 21°C and BSTFA (1.87 ml; 7.08 mmol) was added and the

mixture was stirred for 1 h at 20°C under nitrogen. The solution was cooled to -10°C and 1 M BH_3.THF (10.65 ml; 10.65 mmol) was added between -12 and -10°C. The solution was stirred at ~-10°C overnight. The reaction mixture was sampled (0.5 ml reaction mixture + 50 µl 2M HCl; diluted with 10 ml methanol) and the conversion was determined with HPLC to be 66%.

CLAIMS

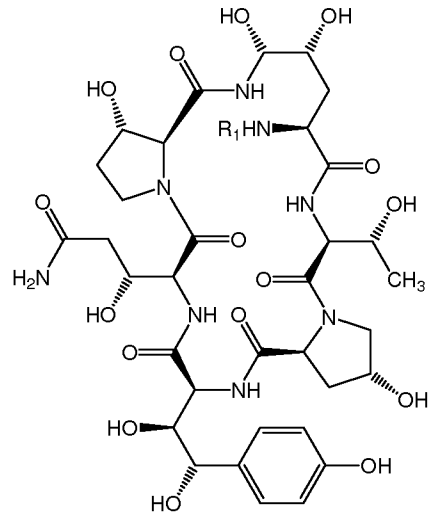
1. Method for the preparation of a first cyclopeptide comprising a vicinal diol from a second cyclopeptide comprising a vicinal diol characterized in that said vicinal diol in said second cyclopeptide is protected by reaction with a substituted boronic acid R_5-$B(OH)_2$ that is not phenylboronic acid or naphthylboronic acid.

2. Method according to claim 1 wherein said first cyclopeptide is a compound of general formula (**1**)



(**1**)

or a salt thereof and said second cyclopeptide is a compound of general formula (**2**)

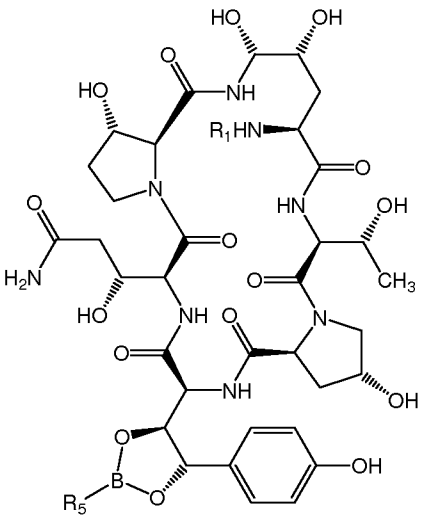


(**2**)

wherein R_1 in compounds (**1**) and (**2**) is $C(O)R_2$ wherein R_2 is C_9-C_{21} alkyl, C_9-C_{21} alkenyl, C_1-C_{10} alkoxyphenyl, C_1-C_{10} alkoxynaphthyl or C_1-C_{10} alkoxyterphenyl, comprising the steps of treating (**2**) with a substituted boronic acid R_5-$B(OH)_2$, an activating agent and a reducing agent.

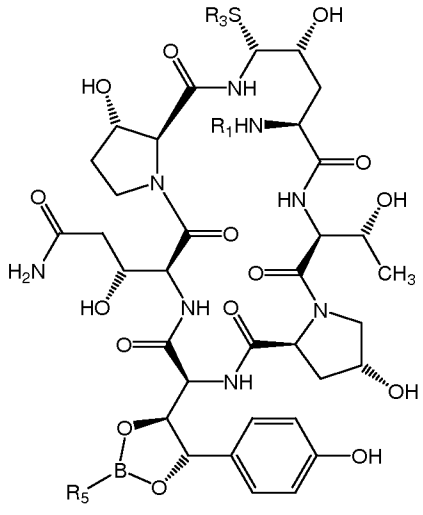
3. Method according to claim 2 comprising the steps of:

(a) reacting said second cyclopeptide of general formula (**2**) with a substituted boronic acid R_5-$B(OH)_2$ to afford a compound of general formula (**3**);



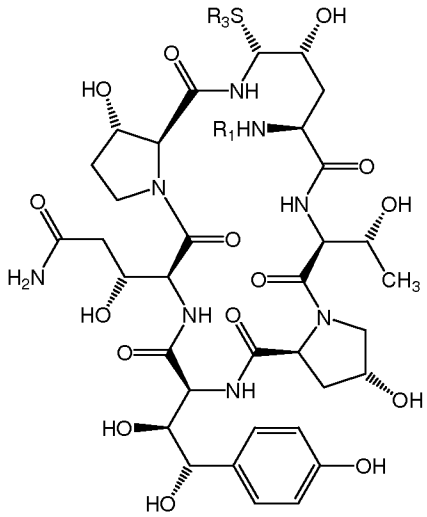
(**3**)

(b) which is subsequently reacted with a compound of general formula R_3-SH wherein R_3 is benzimidazol-2-yl, benzothiazol-2-yl, 1-methylimidazol-2-yl, 4-methoxyphenyl or phenyl to afford a compound of general formula (**4**);



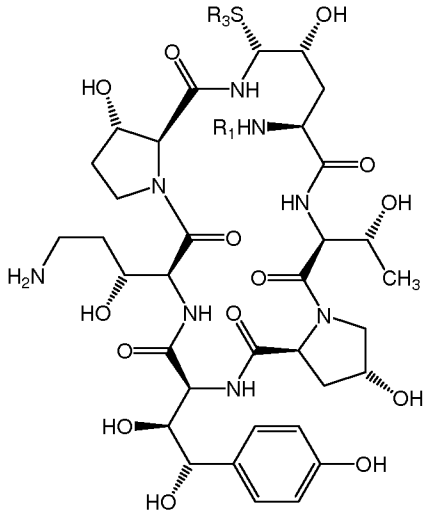
(**4**)

(c) which is subsequently hydrolyzed to afford a compound of general formula (**5**);



(**5**)

(d) which is subsequently reduced to afford a compound of general formula (**6**);

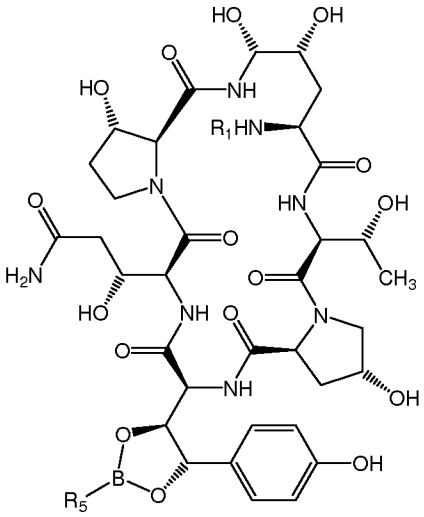


(**6**)

(e) which is subsequently converted to said first cyclopeptide of general formula (**1**) by reaction with ethylenediamine.

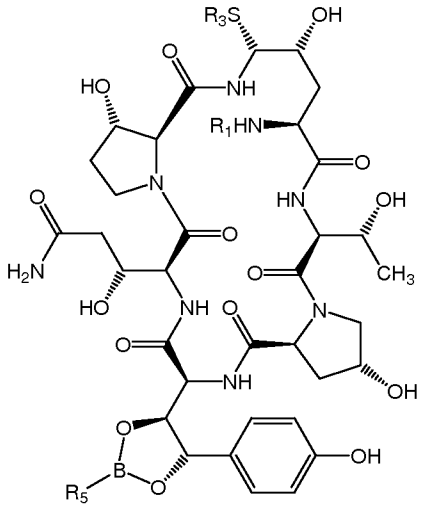
4. Method according to claim 2 comprising the steps of:

(a) reacting said second cyclopeptide of general formula (**2**) with a substituted boronic acid R_5-$B(OH)_2$ to afford a compound of general formula (**3**);



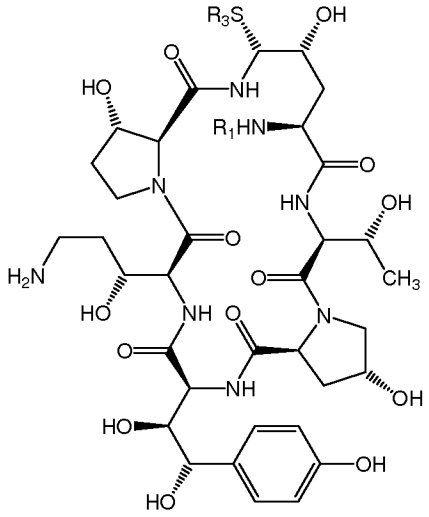
(**3**)

(b) which is subsequently reacted with a compound of general formula R_3-SH wherein R_3 is benzimidazol-2-yl, benzothiazol-2-yl, 1-methylimidazol-2-yl, 4-methoxyphenyl or phenyl to afford a compound of general formula (**4**);



(**4**)

(c) which is subsequently reduced and hydrolyzed to afford a compound of general formula (**6**);

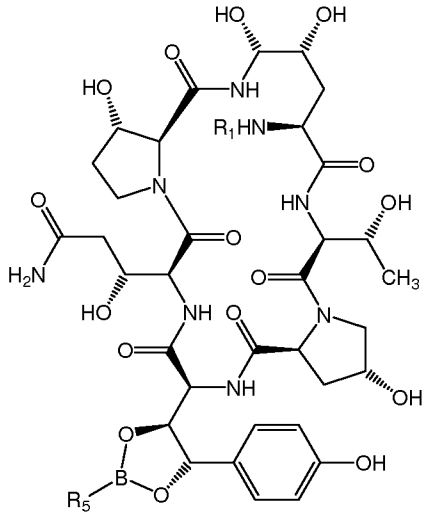


(**6**)

(d) which is subsequently converted to said first cyclopeptide of general formula (**1**) by reaction with ethylenediamine.

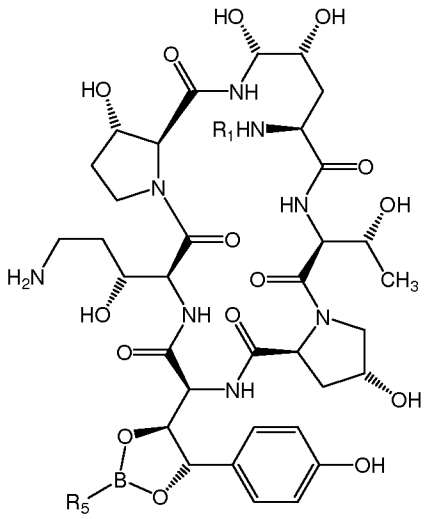
5. Method according to claim 2 comprising the steps of

(a) reacting said second cyclopeptide of general formula (**2**) with a substituted boronic acid R_5-$B(OH)_2$ to afford a compound of general formula (**3**);



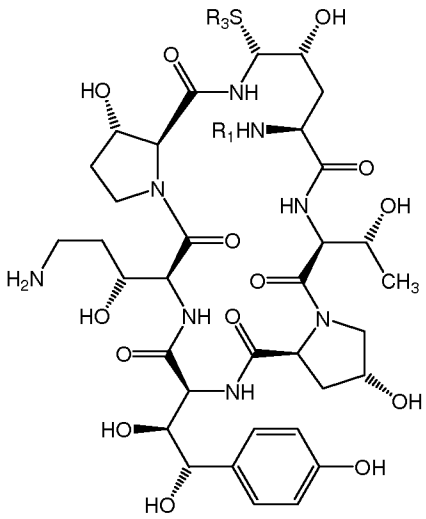
(**3**)

(b) which is subsequently reduced to afford a compound of general formula (**7**);



(7)

(c) which is subsequently reacted with a compound of general formula R_3-SH wherein R_3 is benzimidazol-2-yl, benzothiazol-2-yl, 1-methylimidazol-2-yl, 4-methoxyphenyl or phenyl and hydrolyzed to afford a compound of general formula (**6**);



(6)

(d) which is subsequently converted to said first cyclopeptide of general formula (**1**) by reaction with ethylenediamine.

6. Method according to any one of claims 2 to 5 wherein R_1 is $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH_3)CH_2CH_3$.

7. Method according to any one of claims 2 to 6 wherein said compound of general formula R_3-SH is thiophenol.

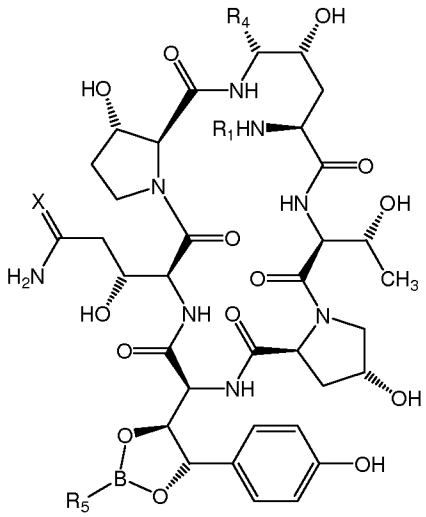
8. Method according to any one of claims 2 to 7 wherein said reduction is carried out by reaction with borane.

9. Method according to any one of claims 2 to 8 wherein the ratio, on a molecular basis, of substituted boronic acid R_5-$B(OH)_2$ to said compound of general formula (**1**) is from 1.1 to 1.5.

10. Method according to any one of claims 2 to 9 wherein said substituted boronic acid R_5-$B(OH)_2$ is cyclohexylboronic acid or 4-*tert*-butylphenylboronic acid.

11. Method according to any one of claims 1 to 10 wherein said reaction with a substituted boronic acid R_5-$B(OH)_2$ that is not phenylboronic acid or naphthylboronic acid is succeeded by and/or combined with reaction with phenylboronic acid.

12. A compound of the general formula (**8**)



(**8**)

wherein R_1 is C(O)R_2 wherein R_2 is C_9-C_{21} alkyl, C_9-C_{21} alkenyl, C_1-C_{10} alkoxyphenyl, C_1-C_{10} alkoxynaphthyl or C_1-C_{10} alkoxyterphenyl, wherein R_4 is OH or –SR3 wherein R_3 is benzimidazol-2-yl, benzothiazol-2-yl, 1-methylimidazol-2-yl, 4-methoxyphenyl or phenyl, wherein R_5 is cyclohexyl, substituted cyclohexyl or substituted phenyl and wherein X is O or H,H.

13. Compound according to claim 12 wherein R_1 is $C(O)(CH_2)_8CH(CH_3)CH_2CH(CH)CH_2CH_3$. and wherein R_5 is cyclohexyl or 4-*tert*-butylphenyl.

14. Compound according to any one of claims 12 to 13 wherein R_4 is OH or -S-phenyl.

15. Use of cyclohexylboronic acid or 4-*tert*-butylphenylboronic acid in the preparation of a cyclopeptide bearing a vicinal diol.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/056147

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K7/56
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, CHEM ABS Data, WPI Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEONARD WILLIAM R JR ET AL: "Synthesis of the antifungal beta-1,3-glucan synthase inhibitor CANCIDAS (caspofungin acetate) from pneumocandin B-0" JOURNAL OF ORGANIC CHEMISTRY, vol. 72, no. 7, March 2007 (2007-03), pages 2335-2343, XP002535918 ISSN: 0022-3263 cited in the application Abstract; Schemes, p. 2340, col. 2, par. 3, last sentence.	1-15
X	US 5 936 062 A (LEONARD WILLIAM [US] ET AL) 10 August 1999 (1999-08-10) cited in the application Abstract; Reaction schemes, claims.	1-15

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 May 2010	02/07/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	López García, F

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GREENE T W ED - GREENE T W ET AL: "CHAPTER 2 - PASSAGE: CYCLIC BONORATES" PROTECTIVE GROUPS IN ORGANIC SYNTHESIS ED. 4, NEW YORK, WILEY & SONS, US, 2007, pages 363-366, XP002535919 cited in the application the whole document	1-15
Y	LI G ET AL: "Application of 1,2:5,6-di-O-cyclohexylidene-d-mannitol as the chiral director in Matteson's asymmetric homologation" JOURNAL OF ORGANOMETALLIC CHEMISTRY, ELSEVIER-SEQUOIA S.A. LAUSANNE, CH, vol. 581, no. 1-2, 5 June 1999 (1999-06-05), pages 66-69, XP004172712 ISSN: 0022-328X Abstract; Experimental Section, in particular, 4.1, 4.2 and 4.6.	1-15
Y	BROOKS ET AL.: "THE MASS SPECTRA OF SOME CORTICOSTEROID BORONATES" ORGANIC MASS SPECTROMETRY, vol. 5, 1971, pages 1429-1453, XP002535920 Abstract; p. 1446 "Boronates of 20,21-diols".	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2010/056147

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5936062 A	10-08-1999	NONE	