Amino Acids and Peptides; 55[1]. Synthesis of Biologically Active Cyclopeptides; 7[1]. Total Synthesis of Chlamydocin

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Methods for the synthesis of cyclopeptides containing non-essential amino acids are described. The total synthesis of the highly cancerostatic Chlamydocin is published.

1. Introduction

In the last few years a number of cyclopeptides and cyclodepsipeptides have been isolated from plants, bacteria, molds, and lower animals. These compounds are not synthesized at a ribosomal level by protein biosynthesis but rather by multi-enzyme complexes which activate the amino acids as thiol esters. Since biosynthesis proceeds without RNA and DNA, non-essential amino acids are often included as well. Salient compounds of these cyclopeptides are amino acids of "exotic" structure, *R*-amino acids, dehydroamino acids, aminoalkylthiazole-carboxylic acids, and aminoalkyloxazoline-carboxylic acids. There are many reasons to believe that the latter are formed from cysteine or threonine peptides, respectively. These cyclopeptides are not opened by the proteolytic enzymes of the metabolism in mammals. They are often biologically active, especially cancerostatic and/or cytostatic.

Apart from the fact that the untypical structure of these cyclopeptides fascinates the organic chemist, their syntheses give him a chance to vary the structure. Often only very small amounts have been obtained from sea animals. In that case only the synthesis makes it possible to check the substances in biological and medical screenings and to confirm the structures. Prerequisites for an economic preparation of such cyclopeptides are a suitable synthesis of the untypical non-ribosomal amino acids and an efficient method of ring closure under very mild conditions.

We have described a synthesis of complex and non-essential amino acids[2] and an effective method for the construction of medium and large lactams[3]. In this publication we give a further example for the synthesis of a very complex amino acid and variations of the pentafluorophenyl ester ring closure reaction. These methods were used in the stereoselective total synthesis of Chlamydocin, a powerful cancerostatic compound. In a separate paper we will report on the synthesis of optically active aminoalkyl thiazole-carboxylic acids, oxazoline-carboxylic acids and cyclopeptides containing these components.

2. Synthesis of Unnormal Amino Acids

We described an effective condensation of acyl-2-(dialkoxyphosphinyl)-glycine esters and aldehydes to form Z-dehydroamino acid esters with a diastereoselectivity of 90–100%[2], which, in turn, can be easily hydrogenated to *R*- or *S*-amino acid esters. Especially from complex dehydroamino acids and with the Monsanto catalyst[4] $[Rh(cod)(diPAMP)]^{\oplus}BF_4^{\ominus}$, high enantioselectivity is observed.

The configuration of the amino acid formed and the optical yield only depend on the structure and configuration of the phosphorus ligand on the rhodium. The configuration of the double bond is only of minor account, at least by operations with the Monsanto catalyst. Hydrogenation of the (*E,Z*)-mixture **8** produced the amino acid **9** with a diastereomeric purity of at least 99%.

Because of the very mild reaction conditions the condensation of dialkoxyphosphinyl glycine derivatives can be applied to the synthesis of di- and tripeptides containing a C=C double bond. For this purpose a peptide containing a phosphoryl glycinate unit is formed and condensed with an aldehyde. This sequence is illustrated by the synthesis of hexaacetylcelenamide A[5], a linear peptide alkaloid from pacific sponge *Cliona celata* with the characteristic components of Z-triacetoxydehydrophenylalanine and an additional enamide function.

3. Ring Closure to form Cyclopeptides

Facts contributing to stability and yield in formation of carbocyclic rings are of minor account in the field of ring compounds containing several O, N, or S atoms as endocyclic components. The difference is that in medium heterocyclic rings Pitzer strain does not appear to be chemically significant. The most important factor in the synthesis of cyclopeptides is the conformation of the amide groups. Cyclotetrapeptides for example with at least one amino acid of the *R*-configuration and therefore with four *s-trans*-amide bonds, have been prepared in 96% yield; these 12-membered ring compounds formally are medium rings.

To date ring closure giving rise to cyclopeptides has been performed by nearly all the methods which are used to form an amide bond in linear peptides. But for the most part the activation of the carboxylic groups as the nitrophenyl ester, the hydroxysuccinimide ester, by the phosphite method, or by reaction with diphenylphosphoryl azide was preferred. Scope and limitations of the cyclopeptide synthesis have been characterized by Jones[6]: "The yields in peptide cyclisations are never 'good' in the usual sense of that term when used by organic chemists, but 30% is generally regarded as a satisfactory outcome; sometimes the yields are very low."

We have developed a new cyclisation method for converting *o*-Z-peptide-pentafluorophenyl esters to medium and large ring cyclopeptides[3]. They are injected slowly to a rapidly

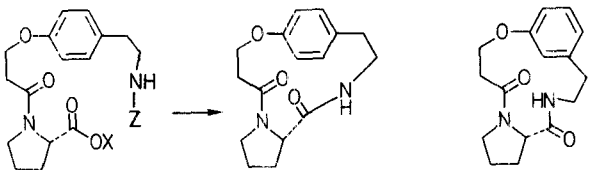
Table. Ring Closure of Linear Ester **1** under Dilution Conditions[a,b]

Z^1	X^2	Cleavage of Protecting Group	Alcohol (% based on dioxane)	Reaction Time	Yield [%] of **2**
$C_6H_5CH_2OCO$	C_6F_5	H_2/Pd	ethanol (2)	7 h	50
Boc	C_6F_5	CF_3COOH	ethanol (2)	7 h	45
Boc	C_6F_5	CF_3COOH	t-butanol (50)	7 h	50
Boc	C_6F_5	CF_3COOH	t-butanol (5)	1 h	50
Boc	4-O_2N—C_6H_4	CF_3COOH	ethanol (3.5)	7 h	45
Boc	4-O_2N—C_6H_4	CF_3COOH	t-butanol (5)	1 h	< 5

[a] Ester **1** (1 mmol), 4-pyrrolidinopyridine (1 mmol) at 95 °C in dioxane/alcohol (450 ml).

[b] The method of choice for the synthesis of non-rigid cyclopeptides is the dropwise addition of the pentafluorophenyl ester to trifluoroacetic acid in acetonitrile/base (room temperature; 5 min.); 85% yield of **3**. Details will be given soon.

stirred suspension of palladium/charcoal in dioxane at 95 °C through which hydrogen is bubbled. The solution contains 1 mol 4-pyrrolidino pyridine per mol synthon and 2% ethanol (with respect to the solvent). The benzyloxycarbonyl group is first removed by hydrogenolysis, then ring closure occurs. Under conditions of dilution, 50% of the rigid ring compound **2** was formed. The flexible 13-membered ring **3** was obtained in up to 80% yield.



1 **2** **3**

When preparing cyclopeptides with sulfur-containing components, for example thiazole ring-containing cyclopeptides, catalytic hydrogenation must be avoided. We therefore examined other protecting and activating groups and found that the application of *t*-butoxy-carbonylpeptide-pentafluorophenyl esters is the method of choice. After acidolytic deblocking of the protecting group with trifluoroacetic acid and evaporation of the excess reagent, the dioxane solution of the peptide-pentafluorophenyl ester trifluoroacetate is added very slowly at 95 °C to dioxane which contains 4-pyrrolidinopyridine and alcohol. The *p*-nitrophenyl esters function as well but the separation of the *p*-nitrophenol is much more difficult than of pentafluorophenol. The Table compares yields and reaction conditions of the ring closure to form the rigid *p*-ansa compound **2**.

4. Chlamydocin

4.1. Introduction

Five biologically high active cyclotetrapeptides of similar structure[7–11] but isolated from rather different species all contain (*S*)-2-amino-(*S*)-9,10-epoxy-8-oxo decanoic acid. One (*R*)-amino acid in the cyclopeptide ring makes possible the *trans*-configuration of the four amide bonds and favours the ring closure.

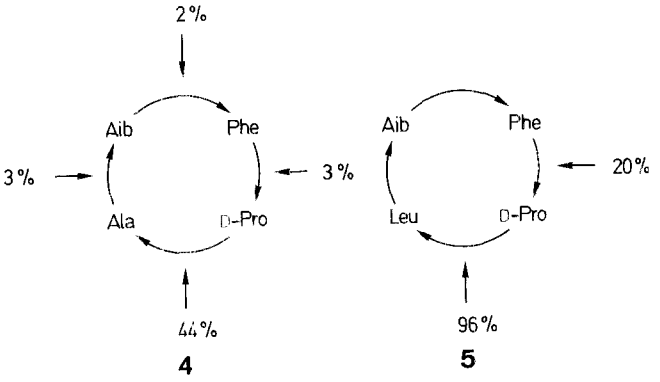
Chlamydocin **(15)**[7] – the first isolated member of this group – was isolated together with dihydrochlamydocin **(14)** from culture filtrates of *Diheterospora chlamydosporia*. Its structure and conformation were elucidated in 1976. In cultures of P-815 mouse mastocytoma cells, it has higher cytostatic activity than actinomycine D, amethopterine, colchicine, and vincristine. However *in vivo* the drug is inactivated rapidly in blood[12]. Two non-stereoselective synthesis of a mixture of chlamydocin and epi-chlamydocin have been reported, one by Rich[13] and the other by us[14], which reflects the fact that epoxidation of the corresponding vinyl ketone proceeds without optical induction by the peptide ring.

The stereoselective total synthesis of **15** and **14** will be described in the following[15]. The correct configurations at C-8 and C-9 of the substituted decanoic acid was achieved with the aid of (*R,R*)-tartaric acid and the *S*-configuration at C-2 was formed by enantioselective hydrogenation.

There are many reasons to believe that the epoxy-keto group represents the cytostatic dagger and the cyclopeptide functions as a carrier. We therefore developed a synthesis of derivatives of (*S*)-2-amino-(*S*)-9,10-epoxy-8-oxo decanoic acid which will enable us to combine it with other carriers to prevent the inactivation of the sensitive epoxy-keto group. Besides tentoxine – which contains dehydrophenylalanine as ring component – only the five cyclopeptides with (*S*)-2-amino-(*S*)-9,10-epoxy-8-oxo decanoic acid represents cyclotetrapeptides isolated from a natural source.

4.2. Studies for the Synthesis of Model Cyclopeptides

First model experiments to form cyclotetrapeptides of a chlamydocin-like structure were performed by Rich[16]. Comparison of the yield of cyclo[Aib-Phe-(*R*)-Pro-Ala] **(4)** by ring closure at the four amide bonds unequivocally demonstrates that the formation of the Pro-Ala bond in the ring closure step is the method of choice. We examined the ring formation of the model cyclo[Aib-Phe-(*R*)-Pro-Leu] **(5)** by catalytic hydrogenation of the corresponding ω-Z-pentafluorophenyl esters. The very high yield in the ring closure reaction (95%) by formation of the (*R*)-Pro-Leu bond reflects the superiority of this method.



Ring closure with hydroxy-succinimide esters

Ring closure with pentafluorophenyl esters

4.3. Synthesis of Chlamydocin and Dihydrochlamydocin

Condensation of the aldehyde **6**[17] – which is readily accessible from (*R,R*)-tartaric acid[18] – with triphenyl-(4-trimethylsilyloxybutylidene)-phosphorane and subsequent oxidation led to the aldehyde **7** (*E*/*Z* mixture. $E < 3\%$) which was reacted with methyl *N*-benzyloxycarbonyl-2-(dimethoxyphosphoryl)-glycinate to give the dehydroamino acid ester **8** (diastereomeric mixture). Enantioselective homogeneous hydrogenation of the *E*/*Z*-mixture with the Monsanto catalyst[4] [Rh(1,5-cod)diPAMP]$^{\oplus}BF_4$[23] led with high enantioselectivity (e.e. > 99%) to the (*S*)-amino acid. Subsequent heterogeneous hydrogenation of the C-6/C-7 double bond, hydrogenolysis of the benzyl ether and the benzyloxycarbonyl protecting group, followed by reincorporation of the protecting group furnished the amino acid ester **9**. The hydroxy group was replaced by chlorine to form **10**. After saponification, the carboxylic acid was combined with the tripeptide H—Aib—(*S*)-Phe—(*R*)-Pro—OCH_3 by the dicyclohexylcarbodiimide method to form the tetrapeptide methyl ester **11**, which was transformed by conventional methods into the pentafluorophenyl ester **12**.

Ring closure by catalytic hydrogenation under high dilution conditions afforded the cyclopeptide **13** in high yield. Absolutely no racemisation at the proline was observed, for this would have led to a cyclotetrapeptide with three (*S*)-amino acids. In this series, such isomers are easy to recognise on chromatographic separation. Hydrolysis of the acetal and treatment with base yielded dihydrochlamydocin (**14**), identical in every respect with the natural compound.

The oxidation of dihydrochlamydocin with pyridinium chlorochromate gave a product which was identical with chlamydocin with regard to the N.M.R. spectrum, M.S., and R_f in H.P.L.C. The value of the optical rotation (100°) however was less than that of the natural product (147°). This problem could be solved by the C.D. spectra (Figure). In the natural product (—) absorptions appear in different regions for the peptide ring (220–275 nm) and the epoxyketone group (250–340 nm)[19].

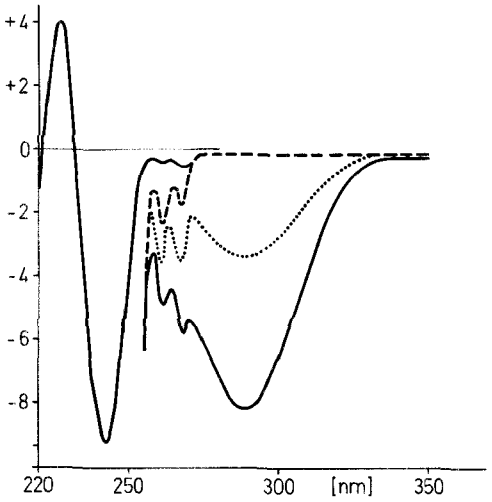
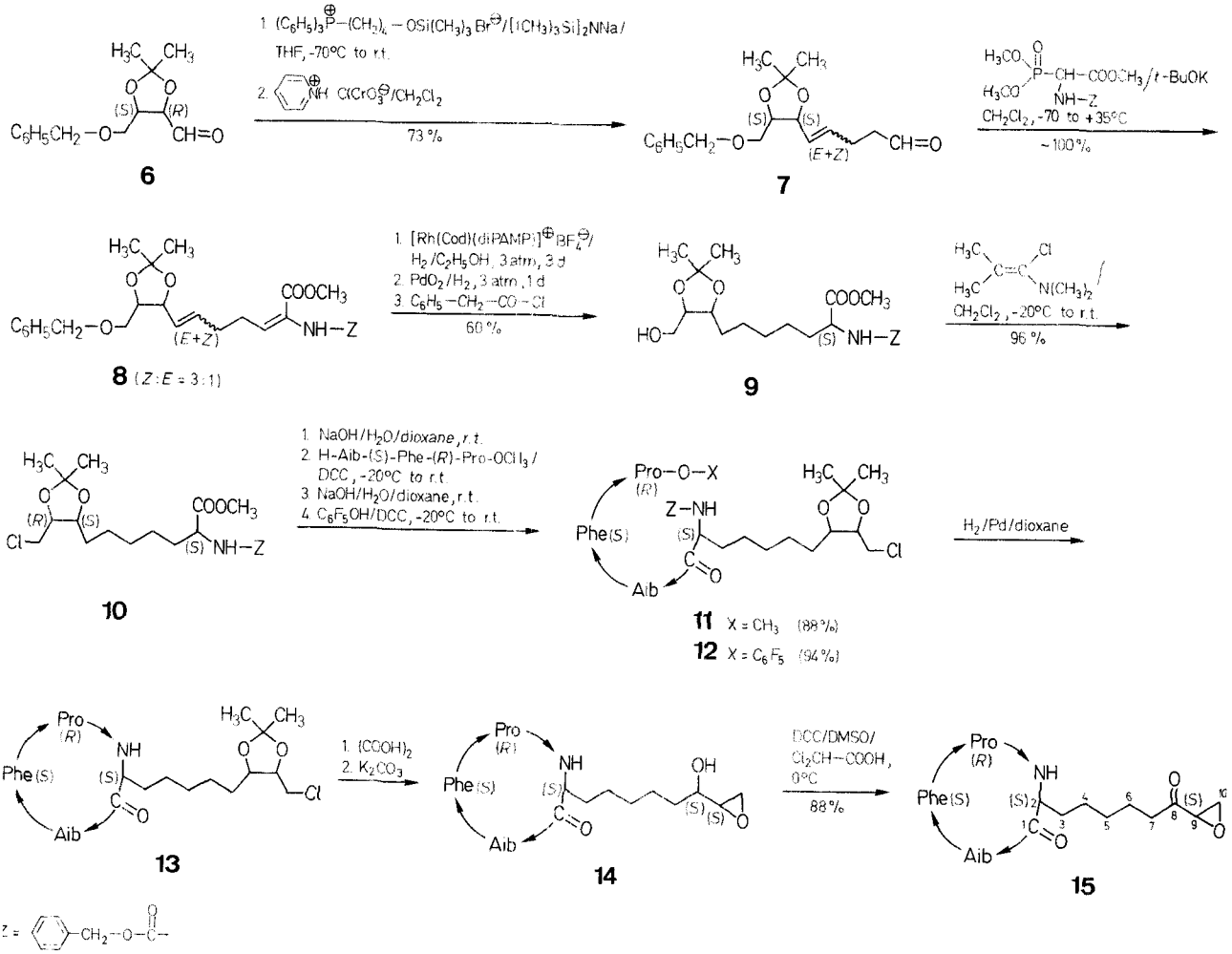


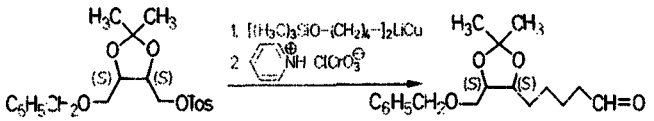
Figure. C. D. Spectra of Chlamydocin and Chlamydocin/*epi*-Chlamydocin mixtures



A much weaker absorption in the region of the epoxyketone group is registrated in the spectrum of the product obtained by oxidation of dihydrochlamydocin with pyridinium chlorochromate (...) compared with the spectrum of natural chlamydocin. This points to the fact that in the oxidation step nearly 30% inversion at C-9 occurred.

Oxidation of dihydrochlamydocin with dicyclohexylcarbodiimide/dimethyl sulfoxide/dichloroacetic acid yielded chlamydocin (**15**), identical in every respect with the natural product.

An alternative route involved the tartaric derivative **16**[18] and the 4-trimethylsiloxybutylcuprate readily prepared from 1-iodo-4-trimethylsiloxybutane[21]. The coupling product was oxidised to the aldehyde **17** which reacted analogously to the dehydro compound **7** to give the amino acid ester **10**.



16 **17**

^{1}H-N.M.R. spectra were recorded on a Bruker Spectrospin 80-MHz spectrometer and Bruker CXP-300-MHz spectrometer. An MAT 711 was used for determining mass spectra. Optical rotations were determined with a Perkin-Elmer 241-polarimeter. Circular dichroism (C.D.) was recorded with a YASCO J-500 A spectropolarimeter. T.L.C. was done on silica (Merck silica 60 F_{254} sheets) and medium pressure column chromatography used Merck LiChroprep Si 60 (15–25 μm). The dioxan for the ring closure reaction was filtered through basic aluminium oxide and distilled from sodium benzophenone ketyl.

***cyclo*-[*N*-[3-[4-(β-Aminoethyl)phenyl]oxy]propanoyl]-L-prolyl] (2):** The pentafluorophenyl ester of *N*-[3-[[3-[[β-[(*t*-butoxycarbonyl)-amino]ethyl]phenyl]oxy]propanoyl]-L-proline[20] (166 mg, 0.29 mmol) is reacted with trifluoroacetic acid (10 ml) for 30 min. After evaporation of the solvent (0.001 torr), the solution of the residue in dioxane (20 ml) is injected to a rapidly stirred solution of dioxane (450 ml) at 95 °C containing *t*-butanol (27 ml) and 4-pyrrolidinopyridine (40 mg) over a period of 1 h. Stirring is continued for 1 h. The cold reaction solution is filtered and evaporated in vacuo. Chromatography of the residue on silica gel with dichloromethane/methanol (98/2) affords compound **2**[3]; yield: 43 mg (50%). The yields are identical with those determined by gas chromatography. Cyclopeptide **2**[3] is obtained as a solid, optically pure material.

(*E*/*Z*)-8-Benzyloxy-6(*S*),7(*S*)-*O*-isopropylidene-4-octenal (7): Triphenylphosphine hydrobromide (from 38 g triphenylphosphine, 143 mmol), tetrahydrofuran (125 ml) and acetonitrile (75 ml) are heated at 90 °C overnight in a glass autoclave. The solution is cooled with ice to give white crystals, which are filtered and washed with cold acetonitrile to afford 1,1,1-triphenyl-4-hydroxybutylphosphonium bromide; yields: 31 g (52%).

A mixture of 1,1,1 triphenyl-4-hydroxybutylphosphonium bromide (4.18 g, 10 mmol) and hexamethyldisilazane (4.1 g, 25 mmol) in absolute dichloromethane (30 ml) is refluxed for 5 h. After evaporation of the solvent, to the solution of the residue in tetrahydrofuran (10 ml) (−70 °C) is added lithium hexamethyldisilazane [from hexamethyldisilazane (2 g, 12.2 mmol) and 1.6 normal *n*-butyllithium (6.3 ml, 10 mmol) at −70 °C] in absolute tetrahydrofuran (10 ml). The reaction mixture is stirred for 0.5 h at −70 °C, then aldehyde **6** (1.8 g, 7.2 mmol) in absolute tetrahydrofuran (10 ml) is added and the mixture is allowed to warm to room temperature overnight. After heating at 50 °C for 0.5 h, the solution is cooled, evaporated in vacuo, and the residue filtrated on silica gel (petroleum ether/ethyl acetate, 7/3). After evaporation, the resulting oil is directly oxidized using pyridinium chlorochromate (2.15 g, 10 mmol) in absolute dichloromethane (20 ml). Workup consisted of sucking off, filtration on silica gel (petroleum ether/ethyl acetate, 7/3), and Kugelrohr distillation (140 °C/10^{-3} torr) to give aldehyde **7**; yield: 1.6 g (73%); R_f = 0.53 (petroleum ether/ethyl acetate, 7/3); $[\alpha]_D^{20}$: 7.3° (*c* 0.59, chloroform).

$C_{18}H_{24}O_4$	calc.	C 71.02	H 7.95
(304.4)	found	71.15	7.98

^{1}H-N.M.R. ($CDCl_3$, TMS, 300 MHz): δ = 9.66 (s, 1 H); 7.29 (m, 5 H); 5.58 (dt, J_1 = 6.3 Hz, J_2 = 10.6 Hz, 1 H); 5.47 (t, *J* = 10.2 Hz, 1 H); 4.66 (t, *J* = 8.7 Hz, 1 H); 4.57 (s, 2 H); 3.84 (m, 1 H); 3.58 (dd, J_1 = 3 Hz, J_2 = 4.8 Hz, 2 H); 2.4 (m, 4 H); 1.44 (s, 3 H); 1.43 ppm (s, 3 H).

Methyl 10-Benzyloxy-2-(*S*)-benzyloxycarbonylamino-8(*S*),9(*S*)-*O*-isopropylidene-2(*E*/*Z*),6(*E*/*Z*)-decadienoate (8): To a suspension of potassium t-butoxide (685 mg, 6.10 mmol) in absolute dichloromethane (10 ml) at −70 °C is added methyl 2-benzyloxycarbonylamino-2-dimethoxyphosphinyl)-acetate (2.02 g, 6.10 mmol) in absolute dichloromethane (10 ml). After 10 min aldehyde **7** (1.55 g, 5.09 mmol) in absolute dichloromethane (5 ml) is added. The mixture is kept for 0.5 h at −70 °C, then slowly warmed up to +35 °C, and washed with ice/water. The organic phase is dried, filtered, evaporated, and the residue is filtered on silica gel (petroleum ether/ethyl acetate, 7/3) to give the dehydroamino acid **8** in a (*E*/*Z*)-ratio of 25/75; yield: 2.6 g (~100%). The (*E*)- and (*Z*)-isomers of **8** can be separated by medium pressure chromatography on silica gel (petroleum ether/ethyl acetate, 7/3); (*E*)-**8**, R_f = 0.58, (*Z*)-**8**, R_f = 0.43 (petroleum ether/ethyl acetate, 7/3). Configuration of the 6-double bond in the mixture of (*E*)-**8** and (*Z*)-**8**: > 97% *Z*.

$C_{29}H_{35}NO_7$	calc.	C 68.35	H 6.92	N 2.75
(509.6)	(*E*) found	68.42	6.77	2.52
	(*Z*) found	68.31	6.95	2.51

^{1}H-N.M.R. (*E*) ($CDCl_3$/TMS, 80 MHz): δ = 7.37 (s, 5 H); 7.34 (s, 5 H); 6.94 (br. s, 1 H); 6.74 (t, *J* = 7.5 Hz, 1 H); 5.58 (m, 2 H); 5.14 (s, 2 H); 4.68 (dd, J_1 = 7.5 Hz, J_2 = 8 Hz, 1 H); 4.60 (s, 2 H); 3.93 (m, 1 H); 3.75 (s, 3 H); 3.61 (m, 2 H); 2.58 (br. t, *J* = 7 Hz, 2 H); 2.25 (m, 2 H); 1.42 ppm (s, 6 H).

^{1}H-N.M.R. (*Z*) ($CDCl_3$/TMS, 80 MHz): δ = 7.36 (s, 5 H); 7.33 (s, 5 H); 6.59 (t, *J* = 7 Hz, 1 H); 6.46 (br. s, 1 H); 5.55 (m, 2 H); 5.14 (s, 2 H); 4.68 (dd, J_1 = 7.5 Hz, J_2 = 8 Hz, 1 H); 4.60 (s, 2 H); 3.91 (m, 1 H); 3.73 (s, 3 H); 3.60 (m, 2 H); 2.25 (m, 4 H); 1.42 ppm (s, 6 H).

Methyl 2(*S*)-Benzyloxycarbonylamino-10-hydroxy-8(*S*),9(*S*)-*O*-isopropylidenedecanoate (10): The (*E*/*Z*)-mixture of **8** (2.30 g, 4.5 mmol) in absolute ethanol (50 ml) is hydrogenated in the presence of the Monsanto catalyst[4] [Rh(cod)(diPAMP)]$^{\oplus}BF_4^{\ominus}$ (30 mg) at 3 atm for 3 days. After filtration and evaporation, the residue is filtrated on silica gel (ethyl acetate). The eluent is concentrated in vacuo and the solution of the resulting oil in absolute methanol (40 ml) is hydrogenated using palladium oxide (300 mg) at 3 atm overnight. After filtration and evaporation, the residue is dissolved in dioxane (30 ml). Under stirring at 0 °C were added first benzyl carbonochloridate (0.71 ml, 5.0 mmol) next 1 normal aqueous potassium hydrogen carbonate (10 ml). The mixture is stirred for 4 h at room temperature dioxane is evaporated and the water phase is extracted with chloroform (3 × 20 ml). The combined organic layers are dried with magnesium sulfate, filtrated, and evaporated. Purification of the residue by medium pressure chromatography (petroleum ether/ethyl acetate, 1/1) affords the amino acid ester **9**; yield: 1.15 g (60%); R_f = 0.16 (petroleum ether/ethyl acetate, 7/3); $[\alpha]_D^{20}$: −8.3° (*c* 1.73, chloroform); e.e. > 99%.

$C_{22}H_{33}NO_7$	calc.	C 62.39	H 7.86	N 3.31
(423.5)	found			

^{1}H-N.M.R. ($CDCl_3$/TMS, 80 MHz): δ = 7.36 (s, 5 H); 5.45 (d, *J* = 8 Hz, 1 H); 5.14 (s, 2 H); 4.39 (m, 1 H); 4.10–3.40 (m, 4 H); 3.74 (s, 3 H); 2.40 (br. s, 1 H); 2.15–1.15 (m, 10 H); 1.40 ppm (s, 6 H).

Methyl 2(*S*)-Benzyloxycarbonylamino-10-chloro-8(*S*),9(*R*)-*O*-isopropylidenedecanoate (10): To a solution of the amino acid ester **9** (1 g, 2.36 mmol) in absolute dichloromethane (10 ml) containing pyridine (0.1 ml) at −20 °C

under argon is added 1-chloro-1-dimethylamino-2-methyl-1-propene (0.4 ml, 3 mmol). The reaction mixture is allowed to warm to room temperature overnight, refluxed for 0.5 h, and washed with saturated potassium hydrogen sulfate at 0 °C. The water layer is extracted with dichloromethane (3 × 20 ml). The organic layers are washed with 1 normal aqueous potassium hydrogen carbonate, dried, filtrated and evaporated. Medium pressure chromatography (petroleum ether/ethyl acetate, 7.5/2.5) of the residue gives the amino acid ester **10**; yield: 1 g (96%); $R_f = 0.66$ (petroleum ether/ethyl acetate, 7/3); $[\alpha]_D^{20}$: −3.0° (*c* 2.08, chloroform).

$C_{22}H_{32}ClNO_6$	calc.	C 59.79	H 7.30	N 3.17	Cl 8.02
(442.0)	found	59.62	7.14	3.18	8.27

^{1}H-N.M.R. ($CDCl_3$/TMS, 80 MHz): $\delta = 7.37$ (s, 5H); 5.45 (d, $J = 8$ Hz, 1H); 5.14 (s, 2H); 4.39 (m, 1H); 3.88 (m, 2H); 3.73 (s, 3H); 3.60 (m, 2H); 2.20–1.15 (m, 10H); 1.41 ppm (s, 6H).

Methyl Ester of *N*-[2(*S*)-Benzyloxycarbonylamino-10-chloro-8(*S*),9(*R*)-*O*-isopropylidenedecanoyl]-dimethylglycyl-(*S*)-phenylalanyl-(*R*)-proline (11):

Amino acid ester **10** (900 mg, 2.04 mmol) in dioxane (30 ml) at room temperature is treated over 4 h with sodium hydroxide (82 mg, 2.05 mmol) in water (10 ml). After evaporation of dioxane, the aqueous layer is washed with diethyl ether, acidified with 2 normal hydrochloric acid at 0 °C, and extracted with ethyl acetate (3 × 20 ml). The organic layers are dried, filtered, and evaporated in vacuo. To the solution of the residue in absolute ethyl acetate (−20 °C) are added the methylester of *N*-(dimethylglycyl)-(*S*)-phenylalanyl-(*R*)-proline (832 mg, 2.3 mmol; prepared by catalytical hydrogenation of the corresponding *N*-*p*-methoxybenzyloxycarbonyl-AIB-(*S*)-Phe-(*R*)-Pro-OCH_3[16]) and *N*,*N*′-dicyclohexylcarbodiimide (422 mg, 2.05 mmol). The mixture is allowed to warm to room temperature overnight, filtrated, concentrated, and the residue is purified by medium pressure chromatography (ethyl acetate) to give **11**; yield: 1.38 g (88%); $R_f = 0.57$ (ethyl acetate); $[\alpha]_D^{20}$: +18° (*c* 1.93, chloroform).

$C_{40}H_{55}ClN_4O_9$	calc.	C 62.29	H 7.19	N 7.26	Cl 4.60
(771.4)	found	61.79	7.09	7.13	4.62

^{1}H-N.M.R. ($CDCl_3$/TMS, 300 MHz): $\delta = 7.33$ (s, 5H); 7.22 (m, 5H); 6.99 (br. d, $J = 7.9$ Hz, 1H); 6.77 (s, 1H); 5.45 (d, $J = 7.9$ Hz, 1H); 5.12 (m, 2H); 4.90 (d t, $J_1 = 5.4$ Hz, $J_2 = 8.0$ Hz, 1H); 4.30 (d d, $J_1 = 4.0$ Hz, $J_2 = 8.0$ Hz, 1H); 4.10 (m, 1H); 3.85 (m, 2H); 3.67 (s, 3H); 3.59 (d, $J = 4.9$ Hz, 2H); 3.55 (m, 1H); 3.01 (m, 2H); 2.72 (m, 1H); 1.87 (m, 4H); 1.65–1.25 (m, 10H); 1.55 (s, 3H); 1.48 (s, 3H); 1.41 (s, 3H); 1.40 ppm (s, 3H).

Pentafluorophenyl Ester of *N*-[2(*S*)-Benzyloxycarbonylamino-10-chloro-8(*S*),9(*R*)-*O*-isopropylidenedecanoyl]-dimethylglycyl-(*S*)-phenylalanyl-(*R*)-proline (12):

Tetrapeptide ester **11** (1 g, 1.3 mmol) in dioxane (20 ml) is hydrolyzed with sodium hydroxide (52 mg, 1.3 mmol) in (10 ml) water at room temperature. After 3 h dioxane is removed in vacuo. The aqueous layer (diluted with 1 normal potassium hydrogen carbonate is washed with diethyl ether, acidified with cold 2 normal hydrochloric acid and immediately extracted with ethyl acetate (3 × 20 ml). The organic layers are dried, filtered, and evaporated. To the solution of the residue in absolute ethyl acetate (−20 °C) are added pentafluorophenol (240 mg, 1.3 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (268 mg, 1.3 mmol) and the mixture is allowed to warm to room temperature overnight. After filtration and evaporation, the residue is filtered through silica gel (petroleum ether/ethyl acetate, 3/7) affording the pentafluorophenyl ester **12**; yield: 1.13 g (94%); $R_f = 0.46$ (petroleum ether/ethyl acetate, 1/1); $[\alpha]_D^{20}$: +18.1° (*c* 1.43, chloroform).

$C_{45}H_{52}ClF_5N_4O_9$	calc.	C 58.53	H 5.68	N 6.07	Cl 3.84
(923.4)	found	58.32	5.71	6.02	3.76

^{1}H-N.M.R. ($CDCl_3$/TMS, 300 MHz): $\delta = 7.40$ (br. d, $J = 8.0$ Hz, 1H); 7.31 (s, 5H); 7.23 (m, 5H); 6.92 (s, 1H); 5.58 (br. d, $J = 7.5$ Hz, 1H); 5.10 (m, 2H); 4.88 (d t, $J = 6.5$ Hz, $J = 8.0$ Hz, 1H); 4.31 (d d, $J = 3.5$ Hz, $J = 8.0$ Hz, 1H); 4.05 (d, t, $J = 6.0$ Hz, $J = 7.5$ Hz, 1H); 3.85 (m, 2H); 3.61 (m, 1H); 3.58 (d, $J = 4.9$ Hz, 2H); 3.01 (m, 2H); 2.65 (m, 1H); 2.07 (m, 1H); 1.80 (m, 3H); 1.70–1.30 (m, 10H); 1.51 (s, 3H); 1.43 (s, 3H); 1.41 (s, 3H); 1.40 ppm (s, 3H).

***cyclo*-[(2(*S*)-Amino-10-chloro-8(*S*),9(*R*)-*O*-isopropylidenedecanoyl)-dimethylglycyl-(*S*)-phenylalanyl-(*R*)-prolyl] (13):**

To a rapidly stirred solution of absolute dioxane (550 ml, 95 °C) containing absolute ethanole (14 ml), 4-pyrrolidinopyridine (160 mg, 1.08 mmol) and palladium on charcoal (5%, 1 g) is injected a solution of pentafluorophenyl ester **12** (1 g, 1.08 mmol) in absolute dioxane (55 ml) continuously over a period of 5 h. At the same time hydrogen is passed through the reaction solution. The cold reaction solution is filtered and evaporated. Medium pressure chromatography (ethyl acetate/petroleum ether, 6/4) of the residue affords pure cyclopeptide **13**; yield: 615 mg (95%); $R_f = 0.44$ (petroleum ether/ethyl acetate, 1/1); $[\alpha]_D^{20}$: −90.4° (*c* 1.79, chloroform).

$C_{31}H_{45}ClN_4O_6$	calc.	C 61.53	H 7.50	N 9.26	Cl 5.85
(605.2)	found	61.30	7.46	9.18	5.75

M.S. (20 eV): *m*/*e* = 604 (M^+, 98%); 589 ($M^+ - CH_3$); 485 (100%).

^{1}H-N.M.R. ($CDCl_3$/TMS, 300 MHz): $\delta = 7.51$ (d, $J = 10.2$ Hz, 1H); 7.25 (m, 5H); 7.09 (d, $J = 10.3$ Hz, 1H); 5.90 (s, 1H); 5.16 (d t, $J_1 = 5.8$ Hz, $J_2 = 10.2$ Hz, 1H); 4.66 (dd, $J_1 = 2.3$ Hz, $J_2 = 7.7$ Hz, 1H); 4.18 (d t, $J_1 = 7.7$ Hz, $J_2 = 10.3$ Hz, 1H); 3.86 (m, 3H); 3.60 (d, $J = 4.9$ Hz, 2H); 3.26 (dd, $J_1 = 10.1$ Hz, $J_2 = 13.5$ Hz, 1H); 3.22 (m, 1H); 2.94 (dd, $J_1 = 5.8$ Hz, $J_2 = 13.5$ Hz, 1H); 2.32 (m, 1H); 2.19 (m, 1H); 1.85–1.25 (m, 12H); 1.77 (s, 3H); 1.42 (s, 3H); 1.41 (s, 3H); 1.34 ppm (s, 3H).

Dihydrochlamydocin (14):

To a solution of cyclopeptide **13** (500 mg, 0.83 mmol) in dioxane (10 ml) is added oxalic acid (50 mg) and water (1 ml). After heating overnight at 80 °C, dioxane is evaporated and the water phase is extracted with chloroform (3 × 10 ml). The organic layers are dried, filtered, and evaporated. Filtration on silica gel (ethyl acetate) gives cyclo[(2(δ)-amino-10-chloro-8(*S*),9(*R*)-dihydroxydecanoyl)-dimethylglycyl-(*S*)-phenylalanyl-(*R*)-prolyl]; yield: 422 mg (90%); $R_f = 0.48$ (ethyl acetate).

^{1}H-N.M.R. ($CDCl_3$/TMS, 300 MHz): $\delta = 7.51$ (d, $J = 10.2$ Hz, 1H); 7.25 (m, 5H); 7.14 (d, $J = 10.3$ Hz, 1H); 6.06 (s, 1H); 5.16 (d t, $J_1 = 5.8$ Hz, $J_2 = 10.2$ Hz, 1H); 4.67 (dd, $J_1 = 2.3$ Hz, $J_2 = 7.8$ Hz, 1H); 4.19 (d t, $J_1 = 7.7$ Hz, $J_2 = 10.3$ Hz, 1H); 3.86 (m, 1H); 3.73–3.58 (m, 4H); 3.26 (dd, $J_1 = 10.0$ Hz, $J_2 = 13.5$ Hz, 1H); 3.21 (m, 1H); 2.95 (dd, $J_1 = 5.8$ Hz, $J_2 = 13.5$ Hz, 1H); 2.74 (br. s, 1H); 2.35 (br. s, 1H); 2.31 (m, 1H); 2.17 (m, 1H); 1.88–1.25 (m, 12H); 1.77 (s, 3H); 1.34 ppm (s, 3H).

A solution of the above obtained chlorodiol (422 mg) in methanol (20 ml) is treated with potassium carbonate (150 mg, dried) at room temperature for 5 h. After evaporation, the suspension of the residue in water (20 ml) is extracted with chloroform (4 × 20 ml). The organic layers are dried, filtered, evaporated, and the residue is purified by medium pressure chromatography (ethyl acetate) to yield dihydrochlamydocin (**14**; yield: 325 mg (75% from **13**); $R_f = 0.2$ (ethyl acetate/petroleum ether, 7/3); $[\alpha]_D^{20}$: −96.1° (*c* 0.53, chloroform), Lit.[7] $[\alpha]_D^{20}$: −96.8° (*c* 0.5, chloroform).

$C_{28}H_{40}N_4O_6$	calc.	C 63.62	H 7.63	N 10.60	
(528.7)	found	63.40	7.69	10.40	

^{1}H-N.M.R. ($CDCl_3$/TMS, 300 MHz): $\delta = 7.51$ (d, $J = 10.3$ Hz, 1H); 7.25 (m, 5H); 7.10 (d, $J = 10.4$ Hz, 1H); 5.92 (s, 1H); 5.16 (d t, $J_1 = 5.8$ Hz, $J_2 = 10.3$ Hz, 1H); 4.66 (dd, $J_1 = 2.4$ Hz, $J_2 = 5.6$ Hz, 1H); 4.19 (d t, $J_1 = 7.6$ Hz, $J_2 = 10.4$ Hz, 1H); 3.86 (m, 1H); 3.44 (m, 1H); 3.26 (dd, $J_1 = 10.2$ Hz, $J_2 = 13.6$ Hz, 1H); 3.22 (m, 1H); 2.98 (m, 1H); 2.94 (dd, $J_1 = 5.8$ Hz, $J_2 = 13.7$ Hz, 1H); 2.82 (dd, $J_1 = 4.1$ Hz, $J_2 = 4.9$ Hz, 1H); 2.73 (dd, $J_1 = 2.7$ Hz, $J_2 = 4.9$ Hz, 1H); 2.29 (m, 1H); 2.19 (m, 1H); 1.89 (d, $J = 6$ Hz, 1H); 1.80–1.25 (m, 12H); 1.77 (s, 3H); 1.34 ppm (s, 3H).

Chlamydocin (15):

Dihydrochlamydocin (**14**; 250 mg, 0.47 mmol) and *N*,*N*′ dicyclohexylcarbodiimide (114 mg, 0.55 mmol) are dissolved in a mixture of absolute dimethyl sulfoxide (1.5 ml) and absolute benzene (10 ml), cooled (0 °C), and dichloroacetic acid (31 mg, 0.24 mmol) is added. After stirring for 1 h at room temperature, the reaction mixture is poured into cold water containing potassium hydrogen carbonate (100 mg). After extraction with diethyl ether (5 × 10 ml), the organic layers are washed with water (2 × 5 ml), dried, and evaporated.

Medium pressure chromatography (ethyl acetate/petroleum ether, 7/3) gives chlamydocin **(15)**; yield: 218 mg (88%); $R_f = 0.31$ (petroleum ether/ethyl acetate, 3/7). The C.D. values of the natural and the synthetic products were identical in every respect.

$C_{28}H_{38}N_4O_6$	calc.	C 63.86	H 7.27	N 10.64
(526.6)	found	63.58	7.22	10.54

H.R.M.S.: $m/e = 526.2792$; calc. for M^+: 526.2790.

M.S. (20 eV): $m/e = 526$ (M^+, 31%); 426 (100%).

^{1}H-N.M.R. ($CDCl_3$/TMS, 300 MHz): $\delta = 7.49$ (d, $J = 10.3$ Hz, 1 H); 7.25 (m, 5 H); 7.08 (d, $J = 10.3$ Hz, 1 H); 5.85 (s, 1 H); 5.16 (d t, $J_1 = 5.8$ Hz, $J_2 = 10.3$ Hz, 1 H); 4.66 (dd, $J_1 = 2.2$ Hz, $J_2 = 7.7$ Hz, 1 H); 4.17 (d t, $J_1 = 7.6$ Hz, $J_2 = 10.3$ Hz, 1 H); 3.86 (m, 1 H); 3.42 (dd, $J_1 = 2.5$ Hz, $J_2 = 4.6$ Hz, 1 H); 3.26 (dd, $J_1 = 10.1$ Hz, $J_2 = 13.5$ Hz, 1 H); 3.22 (m, 1 H); 2.99 (dd, $J_1 = 4.6$ Hz, $J_2 = 5.8$ Hz, 1 H); 2.94 (dd, $J_1 = 5.8$ Hz, $J_2 = 13.5$ Hz, 1 H); 2.86 (dd, $J_1 = 2.5$ Hz, $J_2 = 5.8$ Hz, 1 H); 2.49–2.16 (m, 4 H); 1.85–1.25 (m, 10 H); 1.77 (s, 3 H); 1.34 ppm (s, 3 H).

8-Benzyloxy-6(*S*),7(*S*)-*O*-isopropylidene-1-octanal (17):

To 1-iodo-4-trimethylsiloxybutan[21] (2.68 g, 9.85 mmol) in diethyl ether (10 ml), a 1.4 molar solution of *t*-butyllithium in pentane (14.1 ml, 19.74 mmol) is slowly added at −70 °C. After stirring for 15 min, this solution is added dropwise at −70 °C to a suspension of copper(I) iodide (0.94 g, 4.95 mmol) in diethyl ether (5 ml) and stirring is continued for 0.5 h. 4-Benzyloxy-2(*S*),3(*S*)-*O*-isopropylidene-1-butyl *p*-toluenesulfonate[18] (16; 1.1 g, 2.7 mmol) is added at −70 °C and the reaction mixture is allowed to warm to room temperature overnight and saturated aqueous ammonium chloride is added. The water phase is extracted with diethyl ether, the combined extracts are washed with water, dried, and concentrated in vacuo. After filtration on silica gel (petroleum ether/ethyl acetate, 9/1) and evaporation, the residue is oxidized with pyridinium chlorochromate (1.5 g, 7 mmol) in dichloromethane (20 ml); work-up consists of sucking off, evaporation, and medium pressure column chromatography on silica gel (petroleum ether/ethyl acetate, 7/3) to give **17**; yield: 0.495 g (60%); $R_f = 0.51$ (petroleum ether/ethyl acetate, 7/3); $[\alpha]_D^{20}$: −8.40° (*c* 1.0, benzene).

$C_{18}H_{26}O_4$	calc.	C 70.56	H 8.55
(306.4)	found	70.29	8.44

^{1}H-N.M.R. ($CDCl_3$/TMS, 80 MHz): $\delta = 9.73$ (s, 1 H); 7.3 (s, 5 H); 4.58 (s, 2 H); 3.8 (m, 2 H); 3.56 (m, 2 H); 2.38 (m, 2 H); 1.55 (m, 6 H); 1.4 ppm (s, 6 H).

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