Final Elucidation of the Absolute Configuration of Hormaomycin
—
Total Synthesis of Hormaomycin and Analogs

Dissertation
zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultäten
der Georg-August-Universität zu Göttingen

vorgelegt von
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Göttingen 2003

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Tag der mündlichen Prüfung: 05.11.2003

Meinem Lehrer, Herrn Prof. Dr. A. de Meijere, danke ich herzlich für die interessante Themenstellung, die während dieser Arbeit erwiesene Unterstützung und stete Diskussionsbereitschaft zu Fragen dieser Arbeit.

Lisa und Anna

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A. INTRODUCTION

The exploration of microorganisms as sources of medicinally relevant compounds has a much shorter and less well-known history than the use of substances of the plant or animal origin. Nevertheless from the time of the discovery of the antibacterial effect of penicillin by A. Flemming in 1928 and beginning of its widespread application and manufacturing during Second World War, the substances, which are produced by these simplest forms of life, attract more and more attention of scientists. Among other substances, which are “manufactured” on these scarcely small “pharmaceutical factories” the special place is occupied by secondary metabolites, which can be defined as the naturally produced substances, which do not play an explicit role in the internal economy of the organisms that produce them. Microorganisms may have evolved the ability to produce such compounds because of the selection advantages conferred upon them as a result of the interactions of the compounds with specific receptors in other organisms. Although almost 20 000 microbial metabolites and approximately 100 000 plant products have been described so far, secondary metabolites still appear to be an inexhaustible source of lead structures for new antimicrobials, antiviral, antitumor and immunosuppressive drugs, and agricultural and pharmacological agents. In addition, numerous secondary metabolites, such as benzylpenicillin, cephalosporins, erythromycin, strobilurin, etc. were lead structures that later became the basis for synthetic and semisynthetic derivatives with improved pharmacological properties.^[1] Some of these compounds play the key role not only in defense mechanisms of microorganisms, but can be used as signal substances for intercellular communication between the cells, executing the function similar to those, that are performed in higher organisms by hormones and pheromones.^[2] There are a lot of processes during the life cycle of bacteria, which are regulated by these substances. They regulate, for example, metabolic potential and quorum

sensing in Gram-negative pathogenic bacteria, competence and sporulation in *Bacillus*, sporulation, multicellular differentiation and motility in *Myxococcus*, antibiotic production, morphological differentiation and sporulation in *Streptomyces* and *Erwinia*, and gene transfer mechanisms in *Enterococcus*.^[3] It seems to be very attractive to use knowledge about these substances either in terms of controlling cellular proliferation or conversely to increase a production of a particular natural products. The latter possibility was first realized in 1960s, when it was shown, that several substances of actinomycetes could control the production of antibiotics and morphological differentiation (aerial mycelium formation) already in nanomolar concentrations. All these compounds were structurally very similar 2,3-disubstituted γ -butyrolactones, which nevertheless showed a remarkably different spectrum of the action: A-factor **1** (Khokhlov factor) stimulated the production of streptomycin, IM-type regulators stimulated the production of staphylomycin and VB-type regulators stimulated virginiamycin production in different *Streptomyces* species.^[4] Also it worth to mention the modified homoserine lactones, i.e. *N*-(β -ketocaproyl)-(*S*)-homoserine lactone (KHL) of *V. fischeri*, which can stimulate the carbapenem antibiotic biosynthesis in *E. carovora* and B-factor of *A. mediterranei* **2**, an adenosine derivative, which induces rifamycin B synthesis in nocardia species.^[5]

Hormaomycin **3** was isolated from *Streptomyces griseoflavus*, strain W-384, during the screening of intermolecular signal substances by Anders et al. in 1989.^[67] It was the first substance with peptide structure, which induced the antibiotic production and aerial mycelium formation not only in own producers but also in another *Streptomyces* species: hydroxistreptomycin in *S. flaveolus*, streptolin in *S. fridae*, Tirandamycin in *S. griseoflavus*, strain 1306, and bafilomycin in *S. griseus*. The significant increase in the antibiotic production was observed already at 0.05 µg/L concentration of hormaomycin. This substance showed also strong antibiotic activity against coryneform bacteria (MIC = 0.0005 µg/mL for *Arthrobacter oxydans*).^[8]

Figure 1. Structure and absolute configuration of Hormaomycin **3**. The stereocenters with unknown before absolute configuration are marked with asterisk (*). **I** (S)-Ile; **II** (2S,3R)-(β Me)Phe; **III** (2R)- α -Thr; **IV** (1'R, 2'R)-(3-Ncp)Ala; **V** (2S,4R)-4-(Z)-(4-PE)Pro; **VI** Chpca.

The constitution of this cyclic depsipeptide showed features unusual even for this structurally flexible class of compounds. Initial structural investigation made by E. Rössner, A. Zeeck et al.^[7,8,9] disclosed that besides one residue of the proteinogenic (S)-isoleucine [Ile], hormaomycin contains two units of 3-(2S,3R)-methylphenylalanine [(β Me)Phe], one of (2R)-*allo*-threonine [α -

Thr] as well as two moieties of 3-(*trans*-2'-nitrocyclopropyl)alanine [(3-Ncp)Ala] and one of 4-(*Z*)-propenylproline [(4-PE)Pro]. The side chain of **3** is terminated with the residue of 5-chloro-1-hydroxypyrrole-2-carboxylic acid [Chpca] (Figure 1). The latter three elements have never been found in any natural product before. A partial assignment of the configuration of (3-Ncp)Ala residues in **3** was made later by Zindel and de Meijere.^[10] The retention times of the derivatized synthetically prepared enantiomerically pure mixtures of the diastereomers of 3-(*trans*-2'-nitrocyclopropyl)alanine were compared with the derivatized total hydrolyzate of natural hormaomycin. These experiments unambiguously proved that both (3-Ncp)Ala residues in the cyclic depsipeptide **3** have the same (1'*R*,2'*R*) configuration at the nitrocyclopropyl ring and the opposite configuration at the α -carbons. Nevertheless the orientation of the substituents at the α -carbons of each (3-Ncp)Ala moiety in **3** left unclear. To clarify the situation, the feeding experiments with enantiomerically pure deuterium labelled 3-(*trans*-2'-nitrocyclopropyl)alanine should be carried out. Recently (2*S*,1'*RS*,2'*RS*)-3,3-dideutero-3-(*trans*-2'-nitrocyclopropyl)alanine was synthesized by K. Loscha^[11] and the deuterium labelled hormaomycin was successfully obtained after the appropriate feeding experiments, which was carried out by P. Alvermann.^[12] ¹H, ²H NMR and MS-ESI spectra of this substance undoubtedly showed that the double insertion of the labelled amino acid took place. The possible explanation for this fact is that the (2*S*)-isomer initially synthesized in cell can later be fully epimerized by the specific epimerase, before the assembling of the peptide chain of hormaomycin started, or already during this process after binding of the amino acid with multienzyme complex to allow access to the (2*R*)-isomer necessary to complete the biosynthesis of **3**. The relative and absolute configuration of 4-(*Z*)-propenylproline moiety stayed unclear and no attempts to elucidate it had been made.

While several synthetic routes for preparation of D-*allo*-threonine and (2*S*,3*R*)-3-phenylmethylalanine were described in the literature, the enantio- and diastereoselective

synthesis of 3-(*trans*-2'-nitrocyclopropyl)alanine, which was not supposed before, was intensely investigated in our group. At first, (2*RS*,1'*S*,2'*S*)-, (2*RS*,1'*R*,2'*R*)- and (2*S*,1'*RS*,2'*RS*)-3-(*trans*-2'-nitrocyclopropyl)alanines were successfully synthesized.^[10,13] Unfortunately, a great number of steps and relatively low overall yield strongly decreased the preparative value of this synthetic route. In fact, this procedure even did not allow to obtain any enantiomerically pure diastereomer of 3-(*trans*-2'-nitrocyclopropyl)alanine. The improvement of the originally reported procedure was connected with the progress in the preparation of the enantiomerically pure (1'*R*,2'*R*)-(2-nitrocyclopropyl)methanol **10** which was the key intermediate for this synthesis and proposed by M. Brandl, A. de Meijere, et al.^[14] The optical resolution of *trans*-(2-nitrocyclopropyl)carboxylic acid via crystallization of the corresponding acyl 4-isopropyl-1,3-oxazolidin-2-ones **9** allowed to prepare the target alcohol only in 5 steps. It was previously obtained via a tedious twelve-step route starting from enantiomerically pure protected glycerine aldehyde. A large accessible quantity of **10** allowed K. Loscha to synthesize (2*S*,1'*R*,2'*R*)-3-(2'-nitrocyclopropyl)alanine successfully, although only 50 mg of this amino acid was prepared.^[11]

Scheme 1

The significant progress was also achieved in the preparation of 4-(*Z*)-propenylproline. This compound was obtained from 5-(2-dimethylaminopropyl)-piperidone-2 already in 1958 but only as a mixture of all four possible isomers of this amino acid together with all possible isomers 4-allylproline.^[15] The procedure recently proposed by E. Melotto^[16] allowed to prepare 4-(*Z*)-propenylproline as an individual compound starting from *N,O*-diprotected pyrrolutamic acid.

No procedures for the synthesis of *N*-hydroxypyrrolcarboxylic acids or *N*-hydroxypyrrolcarboxamides are described in the literature. An attempt to synthesize 5-chloro-1-hydroxypyrrole-2-carboxylic acid, made by Ritzau appeared unsuccessful.^[17]

Structure-activity relationships for hormaomycin were partially investigated using substances obtained via modification of the natural compound and also several hormaomycin analogs which were isolated after feeding experiments with synthetic amino acids or NaBr. The cleavage of the lactone ring of hormaomycin with K_2CO_3 in methanol, which was carried out by Rössner,^[8] gave

only biologically inactive material. The same author performed the hydrogenation of the natural depsipeptide with Pd/C catalyst in methanol, caused the reduction of the double bond of 4-(*Z*)-propenylproline moiety together with the water-elimination and the reductive dehalogenation of Chpca fragment and the partial reduction of the nitro-groups in both (3-Ncp)Ala residues. The resultant mixture which contained hormaomycin-like substances did not show any antibiotic activity. Later the fine attenuation conditions of hydrogenation of native depsipeptide allowed Ritzau^[17] to successfully prepare the hormaomycin analog containing fragment of 4-propylproline instead of original 4-(*Z*)-propenylproline moiety. This compound initiated aerial mycelium formation in *Streptomyces* species even stronger than native hormaomycin and showed antibiotic activity against coryneform bacteria, although it was noticeably lower than those of unmodified depsipeptide. Same author prepared analog of hormaomycin, which contained bromine instead of chlorine substituent in Chpca fragment. This substitution caused only little loss of the capability to induce the formation of aerial mycelium and drastic decrease of the antibiotic activity. The feeding experiments with synthetically prepared 2-(*trans*-2'-nitrocyclopropyl)glycine **10**^[18] and 3-(*trans*-2'-carbomethoxycyclopropyl)alanine **11**^[19] allowed Alvermann^[12] to obtain both possible hormaomycin analogs containing 2-(*trans*-2'-nitrocyclopropyl)glycine residue instead of one (3-Ncp)Ala moiety and depsipeptides with both (3-Ncp)Ala fragments substituted either on 2-(*trans*-2'-nitrocyclopropyl)glycine residues, or on 3-(*trans*-2'-carbomethoxycyclopropyl)alanine moieties. All these analogs did not display any hormaomycin-like activity.

As it was already mentioned, hormaomycin contains two moieties of 3-(*trans*-2'-nitrocyclopropyl)alanine. The aliphatic nitro-compounds occur in nature very seldom. In fact, less than thirty such compounds were isolated till now and among them dipeptide nitropeptine **13**, isolated from *S. xanthochromogenus*, which displayed noticeable antifungal activity.^[20] It would be reasonable to suppose that the unique biological activity of hormaomycin is connected with its nitro-group containing fragments. 3-(*trans*-2'-Nitrocyclopropyl)alanine itself (at least as a mixture of all possible isomers) was already tested and appeared to be inactive, but the inactivity of this amino acid could be connected just with the low capability of many amino acids to permeate across cell wall in the absence of the special transport mechanism because of their low hydrophobicity. Also it was known, that one of the new potent inhibitor of influenza neuraminidase A-315675 **14** contains 4-(*Z*)-propenylproline fragment.²¹ Therefore a more detailed study of the role of this fragment for biological activity of hormaomycin was necessary.

The previously achieved progress in the synthesis of the structural units of hormaomycin as well as the investigation of the structure-activity relationships for this compound which was briefly described above allowed formulating the following goals for the presenting work:

- Final elucidation of the absolute configuration of all stereogenic centers of hormaomycin.
- Total synthesis of this depsipeptide.
- Synthesis of analogs of hormaomycin including, possibly, shortened ones, to determine minimal structure units which are responsible for different kinds of activity of hormaomycin.

B. MAIN PART

1. Elucidation of the absolute configuration of 4-(Z)-propenylproline residue in hormaomycin. Synthesis of (2S,4R)-4-(Z)-propenylproline and (2S,4R)-4-(Z)-(4,1'-dideutero)propenylproline

1.1. *Determination of the relative configuration of 4-(Z)-propenylproline moiety in hormaomycin. Attempts to prepare pure diastereomers of 4-(Z)-propenylproline via alkylation with (Z)-propenyllithium cuprate*

Before the synthesis of 4-(Z)-propenylproline could be initiated, it was necessary to determine which from four possible isomers of this amino acid contained the target molecule and therefore should be synthesized. This problem was solved in two steps: first the relative configuration and then the absolute configuration of (4-PE)Pro moiety in hormaomycin were determined.

To solve the first problem, fast and simple access to the propenylproline even as a mixture of isomers was desired. Taking into account a lack of any reasonably effective routes for the preparation H-(4-PE)Pro-OH, the procedure of Thottathil and Moniot^[22] (used by authors for the large scale preparation of *N*-Boc protected *trans*-4-phenylproline) was chosen first starting point (Scheme 2). The tosylate (2S,4R)-**15**, prepared according to known procedure,^[22,23] was successfully alkylated with 3 equivalents of (Z)-propenyllithium cuprate²⁴ in THF/Et₂O 1:2 mixture to give the inseparable by conventional methods mixture of *N*-Boc protected *cis*- and *trans*-4-propenyl-*N*-prolines, *cis*-**16** and *trans*-**16** in 1:2.2 ratio according to ¹³C NMR spectrum and in 84% yield. The isomerization of the double bond was insignificant and didn't exceed 4–10%, according to ¹³C NMR spectrum (determined via comparison of the intensities of signals of

the methyl groups: $\delta = 13.1$ ppm for (*Z*)-isomer and 17.8 ppm for (*E*)-isomer). A portion of this mixture was separated by preparative HPLC to give desired diastereomers.^[25]

The relative configuration of each isomer was unambiguously established by NOESY experiments (Scheme 2). Although NMR spectra of these substances showed the presence of two rotamers, the signals of 2-H, 3-H_a, 3-H_b and 4-H were cleanly separated from each other. For the *trans*-isomer the strong correlations between 2-H ($\delta = 4.40$ ppm) and 3-H_a ($\delta = 1.82$ ppm), and between 4-H ($\delta = 3.18$ – 3.33) and 3-H_b ($\delta = 2.41$), while no correlations between either 2-H and 3-H_b, or 4-H and 3-H_a, or between 2-H and 4-H were observed. On the contrary, for the *cis*-isomer the direct correlation between 2-H ($\delta = 4.22, 4.28$ ppm) and 4-H ($\delta = 2.99$ – 3.16 ppm) and also strong correlations between 2-H and 3-H_b ($\delta = 2.38, 2.44$), and 4-H and 3-H_b were seen, while no correlation between 2-H and 3-H_a ($\delta = 1.75, 1.88$ ppm) and only weak correlation between 4-H and 3-H_a were observed. Both isomers were deprotected with trifluoroacetic acid for 20 min and the appropriate amino acids – *cis*-**17** and *trans*-**17** – were obtained. Whereas *trans*-**16** was deprotected smoothly to give corresponding trifluoroacetate in quantitative yield, the desired amino acid *cis*-**17** was obtained after several recrystallizations of the hydrochloride in only 25% yield. Two samples of each amino acid were taken. One sample of each amino acid was derivatized with (*S*)-FDVA [*N*_α-2,4-dinitro-5-fluorophenyl-(*S*)-valine amide] reagent^[26] and the enantiomeric purity of each isomer was determined according to Marfey method.^[27] This experiment showed *ee* = 69% for the *trans*-isomer and almost full racemization in case of the *cis*-isomer. These data were in good agreement with the data on enantiomeric purities of isomeric 4-phenylprolines obtained according to this procedure.^[22] The another samples of 4-(*Z*)-propenylprolines were transformed into dabsyl (4-dimethylaminoazobenzene-4'-sulfonyl) derivatives.^[28] That were compared by HPLC with the sample of DABS-(4-PE)Pro-OH obtained after the HPLC separation from the DABS-derivatised total hydrolyzate of natural hormaomycin.

MS/MS technique was used for the determination of the necessary fraction. These experiments showed that **3** contains *cis*-(4-PE)Pro moiety.^[12]

Scheme 2

To explain a significant degree of the retention of the C-4 configuration, it was supposed that this reaction could mainly proceed through intermediate lactone **(2*S*,4*R*)-18**.^[29] The enolate of this lactone can not epimerize at C-2 for sterical reasons (Bredt's rule). The tosylate **(2*S*,4*R*)-15** was treated with 2 equivalents of dry Na₂CO₃ in boiling butanone-2 to give this known substance^[30]

in 91% yield.^[31] However, an attempted reaction of (2*S*,4*R*)-**18** with (*Z*)-propenyllithium cuprate gave a complex mixture, which didn't contain desired products (Scheme 3).

Scheme 3

Although the introduction of the (*Z*)-propenyl substituent at forth position of the proline ring via reaction of the tosylate (2*S*,4*R*)-**15** with the corresponding lithium cuprate proceeded smoothly, it gave mainly the undesirable *trans*-isomer, and *ee* for the *cis*-isomer which was obtained as only minor component was unacceptably low. On the other hand, it is known that tosylates and mesylates secondary alcohols (but not iodides) usually give preferentially S_N2 products in reactions with homocuprates. To study the possibility, if reaction in our case can proceed by S_N2 mechanism, and at the same time to exclude epimerization at C-2, the compound (2*S*)-**22** was prepared according to protocol of Wuts et al.^[32] (Scheme 4).

(2*S*,4*R*)-**19** was first acylated with phenylchloroformate under Schotten-Baumann conditions using NaHCO₃ as a base and, the derivative (2*S*,4*R*)-**20** was reduced with borane in THF to give the *N*-acylprolinol (2*S*,4*R*)-**21** in 74% yield. Then was (2*S*,4*R*)-**21** cyclized with *t*BuOK in THF at 0 °C to the desired bicyclic oxazolidinone (2*S*,4*R*)-**22** in 83% yield. Treatment of this compound with tosyl chloride in pyridine gave the corresponding tosylate (2*S*,4*R*)-**23** in 82% yield, which was further treated with 3 equivalent of (*Z*)-propenyllithium cuprate in THF/Et₂O 1:2 to give the diastereo- and enantiomerically pure product (2*S*,4*S*)-**24** with partially isomerized double bond but only in 5% (9% taking conversion into account) yield. All attempts to improve the yield failed.

Scheme 4

1.2. Elucidation of the absolute configuration of 4-(Z)-propenylproline moiety in hormaomycin

Once the relative configuration of (4-PE)Pro residue in hormaomycin was elucidated, the absolute configuration of this fragment should be established. At first, several experiments failed, as it was impossible to isolate this amino acid (or its derivatives) from the total hydrolyzate of natural depsipeptide due to instability of 4-(Z)-propenylproline in the presence of strong acids routinely applied for the total hydrolysis of peptides. At the same time, it was known for a long time that the catalytic hydrogenation of **3** with palladium or platinum catalysts transforms the (4-PE)Pro moiety into the 4-propylproline residue (and also reduces other functionalities of molecule, see introduction for details). Therefore, both enantiomers of *cis*-propylproline, which was necessary for the determination of the absolute configuration, were prepared (Scheme 5).

Scheme 5

The double bond of the enantiomerically pure *N*-Boc protected 4-(*Z*)-propenylproline (2*S*,4*R*)-**16** was hydrogenated on 10% Pd/C in ethyl acetate, and then *N*-Boc protection was removed with 2 M HCl in EtOAc to give the known (2*S*,4*S*)-**29**^[33] as a hydrochloride in 79% yield. The (2*R*,4*R*)-isomer was obtained after the deprotection of (2*R*,4*R*)-**28**. The latter was prepared from the *cis*-allyl derivative (2*R*,4*R*)-**26** which was in turn obtained by alkylation with allyl bromide of the enolate of the *N*-Boc protected *tert*-butyl-(*R*)-pyroglutamate (*R*)-**25**^[34] in the same way as it was described for (2*S*,4*S*)-isomer (*R*)-**26**.^[35] Hydrogenation of the double bond in (*R*)-**26** on 10% Pd/C, followed by two step reduction of the lactam cycle of the intermediate *N,O*-protected 4-propylpyroglutamic acid (2*R*,4*R*)-**27** according to procedure of J. Ezquerra et al. completed this synthesis.^[36] Both enantiomers were transformed to (*S*)-FDVA derivatives, which were compared with the derivatized hydrolyzate of the hydrogenated **3**^[37] using HPLC/MS technique according

to the advanced Marfey method.^[38] These experiments showed that hormaomycin contains (2*S*,4*R*)-(4-PE)Pro residue.

1.3. Attempts to prepare 4-(*Z*)-propenylproline starting from pyroglutamic acid

As it was already mentioned, when this work was started, the only reasonable route for the preparation of this amino acid was proposed by E. Melotto who prepared *N*-Boc protected (2*S*,4*RS*)-4-(*Z*)-propenylproline (2*S*,4*RS*)-**16** starting from pyroglutamic acid with the construction of the double bond via the Wittig reaction.^[16] It seemed to be possible further use this scheme for the preparation of the desired compound having in mind further improvement of its diastereoselectivity and/or a separation of diastereomeric mixture. First, it was necessary just to reproduce the already described synthesis of the 4-carbonylproline derivative **36**. Unexpectedly, the preparation of the dimethyl acetals (*S*)-**32** from the aldehyde (*S*)-**31** which was in turn prepared from the known enamine (*S*)-**30**^[39] already required an optimization.

The protection of the aldehyde group should be carried out at 20 °C instead of initially proposed 60 °C in order to obtain the desired mixture of the diastereomeric acetals with reproducibly high yield (Scheme 6). This mixture was separated by conventional flash chromatography to give the individual epimers (2*S*,4*S*)-**32** (53% yield) and (2*S*,4*R*)-**32** (22% yield). The reduction of the lactam ring of the acetals **32** with borane in THF according to the proposed procedure was unselective and repeatedly gave the 4-substituted proline (2*S*,4*S*)-**33** only in low yield together with significant quantity of the “half amidal” (2*S*,4*S*)-**34** and variable quantities of prolinol (2*S*,4*S*)-**35**.

Scheme 6

After several unsuccessful attempts to increase the yield of the target product by varying temperature, order of addition or excess of $\text{BH}_3\cdot\text{THF}$, it was found that the separation of the mixture of the “half amidal” (2*S*,4*S*)-**34** and (2*S*,4*S*)-**33** and further reduction of (2*S*,4*S*)-**34** in (2*S*,4*S*)-**33** by treatment with $\text{BF}_3\cdot\text{Et}_2\text{O}$ and triethylsilane in CH_2Cl_2 at $-78\text{ }^\circ\text{C}$ allowed to obtain the required (2*S*,4*S*)-**33** in 48% overall yield over two steps.

The application of the original protocol of J. Ezquerro et al.^[36] gave this substance in 38% yield. The hydrolysis of the dimethyl acetal (2*S*,4*S*)-**33** with TMSI in CH_2Cl_2 at $20\text{ }^\circ\text{C}$, as it was originally described by E. Melotto, gave the aldehyde **36** in 35% (55% taking into account only the reacted material). Alternatively, treatment of (2*S*,4*S*)-**33** with PPTS in refluxing acetone gave the target compound in 40% (59%) yield. Its ^{13}C NMR spectra showed that in both cases significant (up to 30%) epimerization at C-4 took place.^[40] As the acidity of PPTS is very low and TMSI doesn't have any, it was supposed that the configurational lability the aldehyde **36** could cause epimerization already during column chromatography on silica gel. Application of eluents containing different concentration of triethylamine didn't improve anything. As the stereochemical efficiency of this route was unacceptably low, other synthetic pathways should be tested.

Scheme 7

A direct introduction of the (Z)-propenyl fragment at the fourth position of the lactam ring of the pyroglutamic acid via Pd-catalyzed coupling reaction looked as a very attractive way. As it is

known, reactions of this kind are the most powerful, versatile and popular tools for the formation of the carbon-carbon bonds. In our case the palladium-catalyzed Negishi cross-coupling reaction^[41] (reaction of aryl and vinyl halides/triflates with organozinc reagents) seemed to be a method of choice because of the high functional group compatibility of organozinc compounds, that was essentially important in our case. Unfortunately, several attempts to carry out reaction between the organozinc compound (*S*)-**37**, which was obtained from (*S*)-**25** via the corresponding lithium enolate and propenyl bromide, were unsuccessful and gave only complex mixtures (Scheme 7).

Another possibility would be the deconjugative isomerisation of the protected 4-propylidenpyroglutamic acid into the corresponding 4-propenylpyroglutamic acid. At least for simple objects studied before, this reaction often proceeded in such a way that the (*E*)- α,β -unsaturated esters or amides give the (*Z*)-deconjugated products, and ,on the contrary (*Z*)-conjugated double bond transforms into (*E*)-double bond with good selectivity.^[42] The *N*-Boc protected *tert*-butyl (*S*)-4-(*E*)-propilidenepyroglutamate (*S*)-**39**^[33b] was prepared, as it was described by J. Ezquerro et al.^[43] for the corresponding ethyl ester: (*S*)-**25** (Scheme 5) was first treated with LiHMDS in THF at $-78\text{ }^{\circ}\text{C}$, and then the reaction with propionic aldehyde in the presence of 1.15 equivalent of $\text{BF}_3\cdot\text{Et}_2\text{O}$ at the same temperature gave the known alcohol (*S*)-**38**^[44] as a partially separable mixture of three from four possible diastereomers (configuration at C-2 retained) in much more better yield (78% against 28%) than it was obtained before (Scheme 8).

Scheme 8

The latter was treated with an excess of mesyl chloride and triethylamine in CH_2Cl_2 , and the mixture of isomeric 4-propylidene derivatives was separated by column chromatography to give the required (*E*)-isomer (S)-39 (60%) together with small amount of (*Z*)-isomer (4%). The configuration of the double bond in the main product was proved by NOESY experiment. The *N,O*-protected 4-(*Z*)-propylidenepyroglutamic acid (S)-39 was treated with slight excess of LiHMDS in THF/HMPT mixture at -78°C for 1 h, and the resulting enolate was quenched with 2,6-di-*tert*-butylphenol as a sterically hindered proton source. This method of enolates quenching is known to give preferentially *cis*-isomers because of the protonation from the least hindered side.^[45] The formed mixture of diastereomers was separated by column chromatography to give the *cis*-isomer (2S,3S)-40 (37%) and the *trans*-isomer (2S,3R)-40 (19%). Attempt to remove the last traces of the *trans*-epimer from (2S,3S)-40 showed the conformational instability of the latter: ca. 30–40% conversion of this product in the thermodynamically more stable *trans*-isomer was observed after usual flash chromatography. Surprisingly, ^{13}C NMR spectra of these products showed that 100% retention of configuration of the double bond took place. Such kind of deconjugative isomerisation which has not yet been described for such complicated objects makes possible to prepare still unknown 4-alkenyl substituted pyroglutamic acids (and

consequently alkenyl substituted glutamic acids, prolines and leucines). Nevertheless the wrong configuration of the double bond and the conformational instability of (2*S*,3*S*)-**40** precluded the use of this approach for the synthesis of 4-(*Z*)-propenylproline.

1.4. Synthesis of *N*-Boc protected 4-(*Z*)-propenylproline and dideutero-4-(*Z*)-propenylproline starting from 4-hydroxyproline

As already mentioned before, Negishi reaction looked to be a very attractive way to introduce the (*Z*)-propenyl fragment. To test this reaction for proline derivatives, the known iodide (2*S*)-**41** was obtained from Boc-Hyp-OMe^[46] by Mitsunobu reaction according to Dormoy.^[47] It was allowed to react with Zn dust (activated according to Knochel et al^[48]) and then a Pd catalyst and (*Z*)-propenyl bromide were added. The product of the elimination of the intermediate organozinc compound, the *N*-protected allylglycine ester (*S*)-**42**, was obtained as a sole product in 67% yield (Scheme 9).

Scheme 9

The reproduction of this reaction without redundant components starting from both pure diastereomers of iodide (*S*)-**41** or their mixture, gave the allylglycine derivative (*S*)-**42** in essentially same yield. The optical purity of (*S*)-**42** was not determined intentionally but the optical rotation value of this material was in good agreement with the literature value.^[49]

Another option would be to try constructing the double bond of the propenyl fragment using Wittig reaction. Towards this goal, the effective route for the diastereoselective introduction of

the formyl group into forth position of proline without use of either strong bases and acids or chromatographic purification as these reagents or SiO₂ might cause epimerization at C-4 in the target aldehyde. The reduction of nitriles with DIBAH^[50] is one of the mildest ways to obtain the aldehyde: this reagent is not basic, the reduction with this reagent often gives aldehydes which are pure enough to be directly used in the Wittig reaction without any chromatographic purification. The work-up procedure without use of strong bases or acids was reported.^[51] Moreover, the preparation of several *N,O*-protected 4-cyanoprolines was already described.^[52]

The *N*-Boc protected 4-hydroxyproline (2*S*,4*R*)-**43**^[53] was esterified with large excess of *tert*-butyl bromide and K₂CO₃ in the presence of 1 equivalent of TEBACl in DMA according to procedure Martinez et al.^[54] to give the corresponding *tert*-butyl ester (2*S*,4*R*)-**44**^[55] in 65% yield which was in turn treated with mesyl chloride and triethylamine in CH₂Cl₂ to give mesylate (2*S*,4*R*)-**45**^[56] in 86% yield. The latter was transformed into the fully protected derivative of 4-cyanoproline (2*S*,4*S*)-**46** by reaction with tetrabutylammonium cyanide in DMF at 60 °C in 57% yield (Scheme 10).

Scheme 10

Although the selective reduction of the nitrile group with DIBAH in the presence of a *tert*-butyl ester was described,^[56] attempts of the reduction of (2*S*,4*S*)-**46** even with submolar quantities of

the reducing agent at $-78\text{ }^{\circ}\text{C}$ always gave the appropriate prolinol as a side product. To exclude this side reaction and risk of epimerization at C-2 during the nitrile insertion or Wittig reaction (when strong base should be used) the reduction of the carboxylic group of hydroxyproline may be used. The mesylate (2*S*,4*R*)-**47**^[57] gave on the treatment with tetrabutylammonium cyanide mainly the cyclization product (2*S*)-**49** and the nitrile (2*S*,4*S*)-**48** was obtained only as a minor product (Scheme 11). Therefore, the compound was first *O*-MOM protected with MOMBr and DIEA in CH_2Cl_2 to give the corresponding MOM-ether (2*S*,4*R*)-**50** in 92% crude yield. The latter was further transformed into the nitrile (2*S*,4*S*)-**51** (41%) (Scheme 12).

Scheme 11

Compound (2*S*,4*S*)-**51** was smoothly reduced into the aldehyde (2*S*,4*S*)-**52** with DIBAH in CH_2Cl_2 at $-30\text{ }^{\circ}\text{C}$ in 90% crude yield. The Wittig reaction between this substance and non-stabilized ylide, prepared from ethyltriphenylphosponium bromide according to Bestmann et al.^[58] using NaHMDS as a base to increase the *Z*-selectivity of this reaction, gave the desired fully protected 4-(*Z*)-propenylprolinol (2*S*,4*RS*)-**53** (38%) with excellent (*Z*)-selectivity as a 3:1 mixture of (2*S*,4*R*)/(2*S*,4*S*)-isomers.

Scheme 12

On the next step MOM-group has to be selectively cleaved in the presence of *N*-Boc group with *p*TsOH·H₂O at the ambient temperature according to Katoh et al.^[59] Unexpectedly, MOM-ester was entirely stable under these conditions. In fact, *N*-Boc group in this case was even more acid-sensitive than the *O*-MOM ether, and its partial cleavage was observed. To obtain the alcohol $(2S,4RS)\text{-54}$ both protecting groups were cleaved by TFA, and the crude amino alcohol was immediately acylated with Boc₂O to give the required somewhat impure product $(2S,4R)\text{-54}$ in 67% yield as a mixture of diastereomers which could be separated by conventional column chromatography. At this point it was clear that I should concentrate my efforts on further optimization of this route: improve diastereoselectivity of the formation of the double bond and

try to achieve a higher yield of the alcohol **54** on the deprotection step.

Scheme 13

As an alternative to the conventional Wittig reaction Corey-Fuchs sequence^[60] was tested (Scheme 13). The aldehyde $(2S,4S)\text{-}\mathbf{52}$ was treated with excess of ylide, preliminary obtained from CBr_4 and PPh_3 in the presence of Zn dust, in CH_2Cl_2 to give the vicinal dibromalkene $(2S,4RS)\text{-}\mathbf{55}$ in 68% yield and with almost full epimerization (*cis/trans* ratio 1.4:1). This product was treated first with 2 equivalents of $n\text{BuLi}$ in THF and then with a large excess of methyl iodide to give the fully protected 4-propynylprolinol $(2S,4RS)\text{-}\mathbf{56}$ (44%) which was then hydrogenated on P-2 nickel catalyst^[61] in ethanol in the presence of diethylamine with excellent stereoselectivity and the target $(2S,4RS)\text{-}\mathbf{54}$ (80%) together with the overhydrogenation product $(2S,4RS)\text{-}\mathbf{57}$ (12%) was obtained. However an overall efficiency of this sequence was clearly insufficient, and no attempts to improve this scheme were made.

As the *O*-MOM group could not be selectively cleaved in the presence of the Boc group, it was substituted with the *tert*-butyldimethylsilyl group (Scheme 14). The *N*-Boc protected 4-hydroxyproline $(2S,4R)\text{-}\mathbf{43}$ was treated with ethyl chloroformate and triethylamine in CH_2Cl_2 at $-30\text{ }^\circ\text{C}$, and the resultant mixed anhydride was reduced with aqueous NaBH_4 in presence of

tetra-*n*-butylammonium bromide as a phase transfer catalyst to give the *N*-Boc protected 4-hydroxyprolinol (2*S*,4*R*)-**58** in 82% yield. A primary hydroxyl group of **58** was selectively TBDMS-protected, as it was described by Aoyagi and Williams, and (2*S*,4*R*)-**59** was prepared in 84% yield.^[62] The treatment of this substance with mesyl chloride and triethylamine in CH₂Cl₂ gave the mesylate (2*S*,4*R*)-**60** in almost quantitative yield, which was in turn transformed into the nitrile (2*S*,4*S*)-**61** on treatment with tetrabutylammonium cyanide in DMF at 65 °C for 6 h with reproducible yield in 30–50 g scale.^[63]

Scheme 14

The latter was reduced to aldehyde (2*S*,4*S*)-**62** with DIBALH in 97% crude yield, as described above. A slow addition of (2*S*,4*S*)-**62** to 3–4 equivalents of the ylide, obtained on treatment of ethyltriphenylphosponium bromide with THF solution of the freshly sublimed potassium *tert*-butoxide (application of this base also allows to improve the *Z*-selectivity of Wittig reaction),^[64]

in THF at $-78\text{ }^{\circ}\text{C}$ allowed to decrease the epimerization significantly. The fully protected 4-(*Z*)-propenylprolinol (2*S*,4*R*)-**63** with excellent *Z/E*-selectivity (*E/Z* ratio 1:14–30) and which contained only traces of the (2*S*,4*R*)-epimer was obtained. The TBDMS group was smoothly removed with tetrabutylammonium fluoride in THF to give the alcohol (2*S*,4*R*)-**54** in 42% yield over 3 steps. This substance was oxidized with 10 equivalent of Jones reagent in acetone at $4\text{ }^{\circ}\text{C}$ and the desired *N*-Boc protected 4-(*Z*)-propenylproline (2*S*,4*R*)-**16** was obtained in 65% yield (18% overall yield on 8 steps).^[65] The high diastereo- and enantiomeric purity of this product was confirmed by HPLC chromatography (*dr* > 98:1, *ee* 97%).^[66]

To investigate the biosynthesis of hormaomycin **3** as well as to confirm the assignment of the absolute configuration of (4-PE)Pro moiety in **3** once more, the deuterium labelled diastereo- and enantiomerically pure 4-(*Z*)-propenylproline should be synthesized. As the productive synthesis of the *N*-Boc protected 4-(*Z*)-propenylproline starting from 4-hydroxyproline had already been developed, synthesis of the deuterated H-(4-PE)Pro-OH labelled at C-2 starting from (2*S*,4*R*)-2-deutero-4-hydroxyproline D-(2*S*,4*R*)-**19** was first tried (Scheme 15).

Scheme 15

As the latter was prepared via bicyclic compound $(3R,6R,7S)\text{-64}$ according to Weber and Seebach^[67] only in 15% yield, two analogs of **64** were tested in attempt to prepare the desired deuterated product in higher yield. The trisilylated derivative $(2S,4R)\text{-65}$ (which was obtained on heating of a suspension of 4-hydroxyproline in HMDS/TMSCl mixture with catalytic quantity of H_2SO_4 in almost quantitative yield as it was described for proline^[68]) was allowed to react with 1 equivalent of pivalic aldehyde in pentane in the presence of TFA or NH_4Cl to give the bicyclic compound $(3R,6R,7S)\text{-66}$ (50%) with variable diastereomeric purity. The enolate obtained after the deprotonation of this substance with LDA in THF at -78°C was quenched with CH_3OD to give after decomposition of the reaction product with 6 M HCl at the ambient temperature the diastereomerically pure D-(2S,4R)-19 with improved degree of deuteration (87% against original 77%), but again only in 16% yield. The bicyclic derivative $(3R,6R,7S)\text{-67}$ with trichloromethyl substituent instead of *tert*-butyl group was prepared by the reaction between $(2S,4R)\text{-65}$ and

chloral in acetonitrile in the presence of TFA with *dr* 95:5 in 50% yield (the similar cyclization product from proline was successfully used by Wang and Germanas for the preparation of 2-allyl, benzyl and methyl substituted prolines with good diastereoselectivity^[69]). Possibly, because of the smaller sterical volume of trichloromethyl group compared with *tert*-butyl group, the deprotonation of (2*R*,5*S*,7*R*)-**67** with LDA followed by quenching of the intermediate enolate with CH₃OD gave, after decomposition of the reaction product with boiling 6 M HCl, the mixture of epimeric 2-deutero-4-hydroxiprolines D-(2*S*,4*RS*)-**19** in (2*S*,4*R*)/(2*S*,4*S*) 4:1 ratio, and again in unacceptably low 18% yield.

As yields were always unacceptably low, other positions for the introduction of the deuterium label were chosen. It was decided to prepare 4,1'-dideutero-4-(*Z*)-propenylproline. The deuteration positions were chosen to establish the possible biotransformation of the deuterated amino acid in its isomer: (*R*)-*cis* or (*S*)-*trans*. If epimerization of chiral centers of this amino acid in course of the biosynthesis of hormaomycin took place, it should cause the loss of the deuterium label at C-4, and the depsipeptide labelled only at C-1' of (4-PE)Pro fragment would have been obtained after feedings experiments. Additionally, the introduction of the second deuterium label at C-1' allowed to obtain a visible change of ¹H NMR spectrum of **3** in its relative empty region (between 5.3 and 6.0 ppm), as well as to avoid the possible scattering of the deuterium label during the construction of the double bond between C-4 and C-1' positions. The nitrile (2*S*,4*S*)-**61** was treated with a small excess of LDA in THF at -78 °C, and the intermediate enolate was quenched at the same temperature with the saturated solution of Na₂SO₄ in D₂O (to obtain more of the desired *cis*-isomer according to Takano et al)^[70] and thus the separable mixture of epimers at C-4, D-(2*S*,4*S*)-**61** and D-(2*S*,4*R*)-**61** in 2:1 ratio was obtained (Scheme 16).

Scheme 16

If D₂O was used for the quenching of the enolate, the reverse selectivity was observed. The mixture of epimers was separated, and the *trans*-isomer D-(2*S*,4*R*)-**61** was deprotonated again and the mixture of epimers in the same ratio as before was obtained after the quenching of the enolate. The required isomer was separated and combined with the first portion to give the deuterated product D-(2*S*,4*S*)-**61** (90% deuterium content according to ¹H NMR spectrum) in 67% overall yield.^[71] The aldehyde D-(2*S*,4*S*)-**62**, prepared as it was described for the nondeuterated analog, was allowed to react with ylide, obtained from (1,1-dideutero)ethyltriphenylphosphonium bromide^[72] (1.9 deuterium atoms pro molecule; the nondeuterated compound was unobservable in ¹H NMR spectrum) and potassium *tert*-butoxide, and the dideuterated fully protected prolinol D₂-(2*S*,4*R*)-**62** was then TBDMS-protected with tetrabutylammonium fluoride to give the *N*-Boc protected prolinol D₂-(2*S*,4*R*)-**54** (D₂ and D contents 65% and 34% respectively, as determined by MS) in 45% overall yield over 3 steps. The latter was oxidized with Jones reagent in acetone to give the dideuterated acid D₂-(2*S*,4*R*)-**16** in 61%. The cleavage of Boc group with TFA gave the necessary product only in 36% yield. Therefore, several another conditions for the Boc-deprotection were tried (Scheme 17).^[73] To avoid this problem, the *p*-nitrobenzyl ester D₂-(2*S*,4*R*)-**68**, which was prepared by esterification of D₂-(2*S*,4*R*)-**16**^[74] with *p*-nitrobenzyl bromide and K₂CO₃ in MeCN in 88% yield, was *N*-Boc deprotected with 5 M HCl in Et₂O to give the corresponding amino ester as a hydrochloride in 88% yield. The latter was hydrolyzed with equimolar quantity of 0.1 M NaOH to give, after

carefull acidification, the dideuterated amino acid D₂-(2*S*,4*R*)-**17** in almost quantitative yield as a mixture with ca. equimolar quantity of NaCl. This mixture was directly used for the feeding experiment.

Scheme 17

No one possible biotransformation occurred and the doubly deuterated **3a** was successfully obtained. These experiments also cleanly demonstrated that the biosynthesis of H-(4-PE)Pro-OH takes place before the assembly of the peptide chain of **3**.

2. Synthesis of (*R*)-*allo*-threonine and (2*S*,3*R*)- β -Methylphenylalanine

(*R*)-*allo*-threonine was prepared according to the previously described procedure (Scheme 18).^[75] (*R*)-Threonine (*R*)-**69** was first transformed in its methyl ester and then acylated with benzoyl chloride to give the fully protected amino acid (*R*)-**70** in 74% yield, which was further underwent cyclization in oxazoline (2*R*,3*R*)-**71** with inversion of the configuration at C-3 (90%). The latter was hydrolyzed with 6 N HCl to give the desired amino acid (*R*)-*a*-**72** in 52% yield.

Scheme 18

Among numerous published enantio- and diastereoselective syntheses of β -methylphenylalanines^[76] only approach described by Hruby et al,^[77] based on the method of Evans et al.,^[78] was feasible for preparation of multigram quantities of this amino acid. As the initial procedure required a notorious separation of the enantiomers of γ -phenylbutyric acid, the modification of this route developed by the same group, was chosen (Scheme 19).^[79] The mixed anhydride of γ -phenylbutyric acid **73** formed on treatment with triethylamine and pivalic acid in THF was coupled with lithium enolate of (4*R*)-phenyloxazolidinone-2 (*R*)-**74**^[80] to yield the epimeric mixture of (4*R*)-4-phenyl-3-(3'-phenylbutyryl)oxazolidinones-2 (*4R*)-**75**. The mixture was separated by crystallization to give the desired (4*R*,3'*S*)-isomer in 44% yield. The same

compound was obtained as described for its enantiomer in another publication of Hruby et al.⁸¹ by conjugated addition of phenylmagnesium cuprate to the acyloxazolidinone (*Z*)-(*R*)-**76** [prepared by analogy to (*4R*)-**75**] in 80% yield. (*4R,3'S*)-**75** was converted to a boron enolate by use of dibutylborontriflate in CH₂Cl₂.

Scheme 19

Selective bromination was accomplished using NBS, and S_N2 displacement of the resulting

crude bromide gave the azide (*4R, 2'S,3'R*)-**77** with high diastereoselectivity in 76% yield in two steps. Finally reduction of the azido acid obtained after removal of the chiral auxiliary gave (*2S,3R*)- β -methylphenylalanine (*2S,3R*)-**78** in 46% overall yield over 6 steps.

3. Elucidation of the absolute configuration of *trans*-(2'-nitrocyclopropyl)alanine residue in hormaomycin. Synthesis of (2*R*,1'*R*,2'*R*)-(2'-nitrocyclopropyl)alanine and (2*S*,1'*RS*,2'*RS*)-2-deutero-(2'-nitrocyclopropyl)alanine

Further progress in preparation of *trans*-(2'-nitrocyclopropyl)alanine was achieved, when this work was already under way. The enantioselective alkylation of the Oppolzer sultam derived glycine equivalent **79**^[82] with *trans*-(2'-nitrocyclopropyl)methyl iodide **80**, which had earlier routinely been applied as a key step in *trans*-(2'-nitrocyclopropyl)alanines syntheses, often gave the desired alkylation product only in low yield and was not suitable for the preparation of this amino acid in multigram quantities.

Already first experiment with the Belokon complex **81**^[83] instead of this chiral glycine equivalent, allowed to significantly improve yield on this crucially important step. As a further development of this methodology the procedure for productive preparation of all four possible isomers of *trans*-(2'-nitrocyclopropyl)alanine exploited a notable difference in solubility between the two diastereomeric products of the enantioselective alkylation **82** of the complex **81** with the racemic iodide **80**, which allowed their separation from the same reaction mixture was published by Larionov, de Meijere, et al. [Scheme 20; alkylation only (*S*)-**81** showed].^[84]

This approach allows to obtain *trans*-(2'-nitrocyclopropyl)alanines with high diastereo- and enantiomeric purity. Moreover whole (2*S*,1'*R*,2'*R*)-(2'-nitrocyclopropyl)alanine, used in course of

this work, which was generously presented by Oleg V. Larionov, was prepared in this way.

Scheme 20

Nevertheless applying this route only 50% maximal yield of the desired isomer can be achieved.

It may be not taking into account if it's necessary to prepare any of (2S)-isomers of this amino acid [(S)-proline, which must be used for preparation of the chiral auxiliary **81** in this case, is cheap] but it can be a problem if it is necessary to prepare a large quantity of any of (2R)-isomer.

This disadvantage was overcome when the enantiomerically pure iodide **80** was applied.

The mixture of the diastereomeric acyloxazolidinones **9** was prepared (Scheme 1). It was

separated by crystallization to give individual diastereomers, (4*R*,1'*R*,2'*R*)-**9** and (4*R*,1'*S*,2'*S*)-**9**, after final purification by column chromatography in 70% and 45% respectively. As described procedure of their reductive cleavage^[14] gave on scaling-up the desired alcohols of reproducibly low quality and low yield, an alternative two step approach was developed (Scheme 21).

Scheme 21

The chiral auxiliary was removed by hydrolysis with LiOH in presence of hydrogen peroxide at low temperature and the carboxylic acids, (1*R*,2*R*)-**83** and (1*S*,2*S*)-**83**, were obtained in 78% and 67% yield respectively. They were further reduced with BH₃·THF in THF to give the alcohols, (1'*R*,2'*R*)-**10** and (1'*S*,2'*S*)-**10**, in 94% and 90% yield respectively. On treatment of (1'*R*,2'*R*)-**10** with iodine, triphenylphosphine and imidazol in CH₂Cl₂ according to protocol of Gordon and Gottardo^[85] iodide (1'*R*,2'*R*)-**80** was obtained in 95% yield.

It was employed in the alkylation of the chiral glycine equivalent (*R*)-**81**^[86] and the complex (2*R*,2'*R*,1''*R*,2''*R*)-**82** was obtained in 74% yield. The latter was decomposed by treatment with 6 M HCl to give amino acid (2*R*,1'*R*,2'*R*)-**83** in 55% yield together with the recovered amide (*R*)-**84** (84%), which could be used for the preparation of the new portion of the Ni-complex (*R*)-**81** (Scheme 22).

Scheme 22

In order to begin with the assembling of the peptide chain of hormaomycin, the configuration of both (3-Ncp)Ala fragments of the depsipeptide at C-2 should be assigned. The previous unsuccessful attempt to determine it via feeding experiment with the doubly deuterated at C-3 *trans*-(2'-nitrocyclopropyl)alanine 3-D₂-(2*S*,1'*RS*,2'*RS*)-**83** (see introduction) showed that the new feeding experiment with the enantiomerically pure H-(3-Ncp)Ala-OH, labelled at C-2, should be carried out.

(2*S*)-D₂-**81**, which was prepared from unlabeled (2*S*)-**81**^[86] by isotope exchange in excellent yield and with excellent deuteration degree (Scheme 23) according to protocol of Ragnarsson and Elmes,^[87] was alkylated with the racemic iodide **80** in CD₃CN/DMF mixture^[88] using NaH as a base to give the alkylated *Ni*-complex (2*S*,2'*S*,1'*RS*,2'*RS*)-D-**82** in 70% yield, which was in turn cleaved with 6 M HCl to give after ion-exchange chromatography the desired deuterated amino acid 2-D-(2*S*,1'*RS*,2'*RS*)-**83** in 68 % yield (Scheme 24).

Scheme 23

The MS- and NMR-spectra of this substance showed that the partial enrichment with deuterium at 2' position of the cyclopropyl ring occurred.

Scheme 24

The feeding experiment with D-(2*S*,1'*RS*,2'*RS*)-**83** was successful and gave deuterated hormaomycin **3b**, which was enriched with deuterium at three positions according to NMR spectra. The deuterium labels were introduced at 2' position of (3-Ncp)Ala moiety in the ring part of **3** as well as at C-2 and C-2' of the (3-Ncp)Ala-II residue in the side chain of **3** (figure

2).^[89] These observations showed that hormaomycin contains (2*R*)-(3-Ncp)Ala moiety in the ring part and (2*S*)-(3-Ncp)Ala in the side chain.

Figure 2. Comparison of ^1H NMR (600 MHz, CDCl_3) spectra of hormaomycin **3** (upper trace) and deuterated hormaomycins after feeding experiments with deuterated 4-(*Z*)-propenylproline **3a** (middle trace) and with deuterated 3-(2'-nitrocyclopropyl)alanine (lower trace) hormaomycin **3b**.

Also noteworthy, that the deuteration degree at C-2 of (3-Ncp)Ala residue was significantly lower than expected (decrease of the absorption of H-2 of (3-Ncp)Ala moiety in ^1H NMR spectrum of **3b** was only 40% comparing with the corresponding signal in the spectrum of the nondeuterated **3**). It indicates that in this case epimerization at the second carbon in H-(3-Ncp)Ala-OH (at least partially) takes place *before* the assembling of the peptide backbone of hormaomycin and it goes faster than the latter.

Observation, that the partial enrichment with deuterium of at C-2'' of the alkylated *Ni*-complex (2*S*,2'*S*,1'*RS*,2'*RS*)-D-**82** (and therefore deprotonation of the nitrocyclopropyl ring) took place, was in remarkable contradiction with the earlier results of D. Seebach et al..^[90] Attempted to

deprotonate nitrocyclopropane or 2-methylnitrocyclopropane **84** with different bases and then catch the resulting carbocations with a variety of electrophiles including D₂O, only products of their dimerization **85** or/and oxidative dimerization **86** were obtained. This discrepancy prompted us to briefly reinvestigate the deprotonation of the nitrocyclopropyl ring (Scheme 25).

The *Ni*-complex (2*S*,2'*S*,1''*R*,2''*R*)-**82** was treated in CD₃CN/DMF mixture with NaH to give after quenching with dilute D₂SO₄ in D₂O *Ni*-complex of the same absolute configuration in 71% yield, which contained 55% and 65% deuterium at C-2 and C-2' of (3-Ncp)Ala moiety respectively.^[91] Under the same conditions the *O*-Tr protected (1'*S*,2'*S*)-(2'-nitrocyclopropyl)methanol (1'*S*,2'*S*)-**87**, which was obtained on treatment of the nondeuterated alcohol **10** with trityl chloride in pyridine using DMAP as a catalyst in 78% yield, and nitrocyclopropane **88**^[92] gave deuterated analogs in 72% and 25% yield respectively. The deuteration degree for these compounds was > 95%. In case of (1'*S*,2'*S*)-**87** ¹H NMR spectrum of the crude product showed that the partial (about 5–7%) epimerization took place.

Scheme 25

Once the deuterium labeling of the nitrocyclopropyl ring was achieved, the possibility of its alkylation should be investigated. Two examples of the successful addition of benzaldehyde to

the *gem*-disubstituted 2,2-dimethyl-1-nitrocyclopropane **89**^[93] and to the highly substituted *trans*-1,2-dinitrospiro[3.3]heptane **90**^[94] have only been described up-to-date.

The latter was also iodinated by treatment first with sodium methylate in methanol and followed with iodine. All attempts to obtain another substitution products even in case of these sterically hindered substrates were unsuccessful (the sterical hindrance should have prevented formation of the dimerization and oxidative dimerisation products similar to those that were described before). The tritylated alcohol (1'*S*,2'*S*)-**88** was chosen as a starting material for this set of experiments since enantiomerically pure product of its alkylation (epimerization at C-2' seemed to be hardly possible) could serve for the preparation of amino- and nitrocyclopropyl containing products (i. e., β - and γ -amino acids) in an enantioselective manner. The allylation and benzylation reactions of this substrate using a large excess of NaH and the corresponding halide in dimethoxymethane gave expected products in moderate yields (Scheme 26).

Scheme 26

According to ¹H NMR spectra of reaction mixtures, the partial epimerization at C-1' took place. The appropriate pure *trans*-isomers were obtained after column chromatography and/or preparative TLC. The methylation reaction, which was carried out in diglyme, gave larger

epimerization extent compared with experiments in DME (*cis/trans* ratio for methylation 1:2 against 1:7 for allylation and 1:4 for benzylation).

4. Synthesis of *O*-MOM protected 5-chloro-1-hydroxypyrrol-2-carboxylic acid

The most straightforward way for the preparation of the protected 5-chloro-1-hydroxypyrrol-2-carboxylic acid seemed to be the simultaneous dehydration/chlorination of the fully protected *N*-hydroxypyroglutamic acid^[95] with POCl₃, PCl₅ or related reagents as it was described for several derivatives of pyroglutamic acid.^[96] Nevertheless the obvious instability of such kind of compounds under harsh conditions, which should be applied, sharply diminished the attractiveness of this way. In attempt to prepare *O*-protected 2,5-dichloropyrrol **95**, which could be further monometallated and then quenched with CO₂ to give the desired acid, **94**^[97] was heated with trichlorobenzo-1,2-dioxaphosphole (Scheme 27). This attempt gave complex mixture, which didn't contain any heteroaromatic compounds.

Scheme 27

N-Hydroxy or *N*-alkoxypyrroles have not attracted much attention of chemists and among relatively poor data about this compound only the publication of Abramovitch et al.,^[98] who described the preparation of several 2-cyano-1-hydroxypyrroles by the thermal decomposition of the corresponding 3-unsubstituted 2-azidopyridine *N*-oxides in benzene at 85–95 °C, contained some helpful information. According to the postulated mechanism, this reaction proceeds via elimination of nitrogen with ring opening to give unsaturated nitrile, which in turn undergoes electrocyclic ring closure to give 2*H*-pyrrole-*N*-oxide. Finally prototropic shift leads to the aromatic 2-cyano-1-hydroxypyrroles.

2-Azido-6-chloropyridine-*N*-oxide **102** should be first synthesized to prepare 5-chloro-2-cyano-1-hydroxypyrrol **103** according to the abovementioned procedure. This became feasible via diazotization of 2-amino-6-chloropyridine-*N*-oxide **101** followed by addition of sodium azide in 76% yield. As an attempt of the direct oxidation of 2-amino-6-chloropyridine^[99] with *m*-chloroperbenzoic acid (MCPBA) according to Pentimalli's procedure^[100] unexpectedly did not give the desired *N*-oxide, two another approaches were applied to prepare the compound **102** (Scheme 28).

Scheme 28

According to the first one, 2-acetamido-6-chloropyridine **96**^[101] was oxidized with MCPBA in dichloromethane to give the *N*-oxide **97** in 93%, which in turn was *N*-acylated with Boc₂O to give the diacylated derivative **98** in 69% yield. This compound was further deacetylated with *N,N,N',N'*-tetramethylguanidine (TMG) in methanol to give the *N*-Boc protected amine **99** in 60% yield. The deprotection of the latter with 3.9 M HCl in isopropanol gave the desired amine

101 as a hydrochloride in 97% yield. The treatment of 2,6-dichloropyridine-*N*-oxide **100**^[102] with 25% NH₃ in methanol in a sealed tube at 100 °C for 24 h gave this compound as a free base in a single step and in 55% yield.

The thermal decomposition of the azide **102** in degassed benzene gave 5-chloro-2-cyano-1-hydroxypyrrol **103** in 49% yield and the product of its deoxygenation **104** in 11% yield (Scheme 29). As several attempts to hydrolyze **103** failed, this compound was MOM-protected on the hydroxy group. Hydrolysis of the nitrile group in the intermediate **105** according to protocol Cacchi et al.^[103] led to the amide **106** (all attempts to hydrolyze **105** or **106** were unsuccessful) which, in turn, was doubly acylated with Boc₂O to give the desired triacylated intermediate **107** together with the side product **108**. The alkaline hydrolysis of **107**^[104] gave the *O*-MOM-protected Chpca-OH **109** (43% overall yield over 4 steps from **103**).

Scheme 29

To test the cleavage of MOM group from the hydroxyl group of Chpca fragment, attached to some peptide (or *O*-protected amino acid), and applicability of the Chpca-acylated amino acid with free hydroxyl group for peptide synthesis the acyltripeptide **119** was prepared (Scheme 30).

Scheme 30

Phenylalanine 9-fluorenylmethyl ester **111** obtained after Boc-group removal from *N*-termini of *N*-Boc protected amino ester **110**^[105] with 5 M HCl in Et₂O was coupled with the acid **109** using the combination of *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBT) to give the acyl amino ester **112** in 93% yield, which after

removal of the methoxymethyl group with $\text{MgBr}_2 \cdot \text{Et}_2\text{O}$ and ethyl mercaptan in diethyl ether^[106] gave the ester **113** (88%). The latter was deprotected using 20% diethylamine in CH_2Cl_2 to give the unstable **114** in 100% crude yield, which was immediately coupled with the dipeptide **118** obtained after deprotection of the peptide **117** with 5 M HCl in Et_2O , which was previously prepared from *N*-Boc protected phenylalanine **115** and valine methyl ester **116** using dicyclohexylcarbodiimide (DCC) and HOBt in 82% yield, using the *O*-(7-azabenzotriazole-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU)^[107] to give the desired acyltripeptide **119** in 23% yield.

One of the possibilities to study the structure-activity relationship for hormaomycin is connected with varying of the halogen substituent at C-5 of Chpca moiety. While the bromine-des-chlorine analog of **3** was successfully obtained by varying of the ionic content of the culture media (see introduction), attempts to introduce fluorine or iodine constituent instead of chlorine atom using this methodology failed.

To synthesize 5-fluor-2-cyano-1-hydroxypyrrol 2-amino-6-fluoropyridine-*N*-oxide should be first prepared (Scheme 31). Surprisingly, an attempt to oxidize 2,6-difluoropyridine **120** with 30% aq. H_2O_2 in TFA failed, and only starting material was recovered. The oxidation of **120** with anhydrous trifluoroacetic acid in CH_2Cl_2 at 20 °C for 4 days gave only traces of the desired product **121**, which quickly decomposed at 20 °C.

According to the second approach, successfully used for preparation of 2-amino-6-chloropyridine-*N*-oxide **101**, 2-acetamido-6-fluoropyridine **123** had to be synthesized. This compound was successfully prepared by diazotization of the 2-acetamido-6-aminopyridine **122**^[108] in pyridinium hydrofluoride in 79% yield.^[109]

Scheme 31

The amide **123** was converted to the corresponding *N*-oxide **124** using MCPBA in CH_2Cl_2 in 38% yield. The latter was *N*-Boc acylated with Boc_2O in presence of DMAP in MeCN and the crude diacetylated intermediate was directly treated with 1 eq. of TMG in methanol to give *N*-Boc protected 2-amino-6-methoxypyridine-*N*-oxide **125** as the sole isolable product in 38% yield. A possibility to introduce fluorine atom into the *N*-hydroxypyrrol ring via fluorination of the *O*-protected 2-cyano-1-hydroxypyrrole or the corresponding derivative of 2-cyano-1-hydroxypyrrolecarboxylic acid with a suitable fluorination agent has not been tested yet.

5. Final assembling of hormaomycin

5.1. General remarks

Once the absolute configuration of hormaomycin **3** had been established and necessary quantities of (2*S*,1'*S*,2'*R*)- and (2*R*,1'*S*,2'*R*)-3-(*trans*-2-nitrocyclopropyl)alanine, *N*-Boc-protected (2*S*,4*R*)-4-(*Z*)-propenylproline, as well as the *O*-MOM protected 5-chloro-1-hydroxypyrrole-2-carboxylic acid and other building blocks were prepared, the assembly of hormaomycin could be initiated.

At first look the peptide synthesis of hormaomycin seems to be not a very complicated problem. The modern “state of art” of peptide coupling^[110] allows to prepare peptides, that do not contain extremely hindered fragments such as α,α -dialkyl amino acids, *N*-alkyl amino acids or even more challenging *N*-aryl amino acids, provided with a proper choice of the coupling reagent, solvent and other experimental conditions, in high yield and with high optical purity. As almost all amino acids, which comprise hormaomycin, are β -branched (the only exception is 3-(2'-nitrocyclopropyl)alanine) HATU, as well as the combination of EDC and 7-aza-1-hydroxybenzotriazole (HOAt)^[111] or HOBt were used on each condensation step to ensure the high-yielding coupling. The most unusual in hormaomycin is the ester bond between the secondary (4-PE)Pro moiety and the hydroxy group of *α*-Thr. Among several methods described in literature the dialkylaminopyridine-promoted carbodiimide-mediated esterification was chosen for its formation.^[112]

On the other hand the reactivities of a double bond in 4-(*Z*)-propenylproline and nitro-group in nitrocyclopropylalanine moieties as well as, what is not so obvious, the ester bond between propenylproline and (*R*)-*allo*-threonine residues make a proper choice of the protecting groups and also the conditions for their deprotection to be a real challenge. Thus, the presence of the

double bond hampers the application of catalytic hydrogenolysis and HBr/AcOH reagent for the deprotection of peptides containing the propenylproline residue. The aliphatic nitro-group in (3-Ncp)Ala is not compatible with the reductive cleavage conditions, and the threonine ester bond is sensitive to alkaline and basic conditions.^[113] Because of this base sensitivity all attempts to remove the Fmoc-group from the model depsipeptides **126** and **127** gave only complex mixtures (Scheme 32). Furthermore, attempted removal of the ONb group from the C-termini of the depsipeptide **128** with TBAF caused the fission of threonine ester bond and instead of the desired product **129** only dipeptide **130** was obtained in 90% yield.

Scheme 32

The key step in the synthesis of hormaomycin is the formation of the macrocycle. The greater facility with which amide bonds can be formed, a consequence of the superior nucleophilicity of the amine over the hydroxyl group, makes macrolactamization the preferred mode for ring closure. The amide bond between (βMe)Phe and Ile seems to be least suitable for cyclization because of the possibility of *cyclo*-[Ile-(4-PE)Pro] diketopiperazine formation (β-position to the ester bond) as well as significant epimerization and low yield, which are connected with the bulk

side chains of these amino acids. That between α -Thr and (β Me)Phe is more preferable because of the suppression of racemization by urethane protection and that between (β Me)Phe and (*R*)-(3-Ncp)Ala since the cyclization tends to proceed faster between residues of the opposite than of the same α -center configuration.^[114] The ring closure at the amide bond between Ile and (4-PE)Pro should give a greater epimerization extent because of the higher basicity of proline relative to primary amino acids and that between (*R*)-(3-Ncp)Ala and (β Me)Phe is also less preferable since the large side chain of latter shields its amino group.

To complete synthesis the cyclic depsipeptide should be deprotected leaving intact its sensitive elements and then side chain of hormaomycin should be added.

5.2. *Syntheses of the selected model peptides*

Keeping in mind the ring closure between α -Thr and (β Me)Phe the applicability of the acid hypersensitive 2-nitrophenylsulfenyl (Nps) group^[115] as a temporary protecting group for amino function together with Boc group as a permanent protecting group for amino function and methylthiomethyl (MTM) group, which can be removed in the presence of the Boc-group with $\text{MgBr}_2 \cdot \text{Et}_2\text{O}$ in Et_2O or CH_2Cl_2 , as a protecting group for carboxylic function was tested (Scheme 33).

The *N*-Boc protected MTM ester **132**, obtained via reaction between MTMI and **131** in MeCN in 77% yield, was acylated with *N*-Nps protected proline **133**^[116] to give the ester **134** (85%), which after removal of Nps group with 2 eq. 0.1 M HCl in Et_2O and EtSH, was coupled with *N*-Nps protected isoleucine **135**^[116] to give the depsitriptide **136** in 80% yield. Treatment of **134** with $\text{MgBr}_2 \cdot \text{Et}_2\text{O}$ in CH_2Cl_2 instead of the selective removal of MTM group unexpectedly caused also the concurrent cleavage of the Nps group and gave only traces of the target **137**. An additional

In order to study the possibility of construction of the linear precursor for the cyclization at the amide bond between (β Me)Phe and (3-Ncp)Ala via condensation of already assembled tetra- and dipeptide fragments, depsipeptide **142** was prepared (Scheme 34). The dipeptide **138**,^[117] after removal of the Boc group from the *N*-termini, was coupled with *N*-Boc protected β -methylphenylalanine **139**^[76b] to give the tripeptide **140** in 83% yield, methyl ester function of which was hydrolyzed with triethylbenzylammonium hydroxide (Triton X) to give the acid **141** (77%). The latter was coupled with the alcohol **132** to give the desired product **142** in a satisfactory 45% yield.

Scheme 34

The synthesis of hexadepsipeptide **150** was further embarked on (Scheme 35). After removal of the Boc group, the tripeptide **140** was coupled with *N*-Boc protected (2*S*,1'*RS*,2'*RS*)-3-(2'-nitrocyclopropyl)alanine (2*S*,1'*RS*,2'*RS*)-**143** to give tetrapeptide ester **144** (67%), which was hydrolyzed with tetrabutylammonium hydroxide^[118] to give the crude acid **145** in a quantitative yield. *N*-Fmoc protected (βMe)Phe-OH **146**^[119] was esterified with 3,4-dimethoxybenzyl alcohol to give the Dmob ester **147** in 57% yield.

This ester, after removal of the Fmoc group, was coupled with *N*-Alloc protected α-Thr-OH **148**, which was obtained from (*R*)-*allo*-threonine **72** and pentafluorophenyl allyl carbonate (AllocOPfp), to give the dipeptide **149** in 90% yield. Several attempts to esterify the acid **145** with the alcohol **149** were unsuccessful and gave in the best case **150** as a component of the mixture inseparable by conventional methods.

Scheme 35

In order to evaluate, if the Alloc group can be successfully removed from the amino group of the depsipeptides containing *O*-acylated α -Thr moiety as their *N*-terminal amino acid, the ester **156** was prepared (Scheme 36).

Scheme 36

The α -Thr derivative **151** (prepared as described for (2*S*,3*R*)-isomer),^[120] after removal of the Boc group, was again *N*-protected with *N*-hydroxybenzotriazolyl allyl carbonate (AllocOBt) to give **152** (71%), which was further *O*-acylated with *N*-Boc protected Pro-OH **153** to give the ester **154** in 93% yield. The latter, after removal of the Boc group, was coupled with *N*-Boc protected Ile-OH **155** to give the desired product **156** in 89% yield.

Scheme 37

All attempts to remove the Alloc-group from the *N*-termini of α -Thr residue of **156** failed to give a reasonable yield of the required product since the initially formed amino ester rearranged via *O*

→ *N* migration. Thus, transprotection and transacylation of this depsipeptide according to protocol of Hiemstra et al.^[121] were tested (Scheme 37). The palladium-catalyzed deprotection by means of tributyltin hydride (TBTH) as a nucleophile in the presence of Boc₂O or HOBt ester, formed in situ from (2*S*,1'*RS*,2'*RS*)-**141**, as active acylating agents gave the expected products, **157** and **158**, in excellent yields (*N*-methyl morpholinium *p*-toluene sulfinate was also used as a nucleophile but gave the transprotected product of somewhat lower quality).

5.3. Synthesis of *N*-Alloc protected cyclodepsipeptide **171a**

Once reasonably effective route for removal of the Alloc group from the *N*-termini of *O*-acetylated *α*-Thr moiety had been established, the synthesis of the linear precursor with the bond linking Ile and (4-PE)Pro as a point for macrocyclization could be commenced. The protection strategy for this synthesis based on use of the Boc-, Z-, Fmoc- and Alloc-groups as an amino protection and 2-trimethylsilylethyl (TMSE)^[122] or (2-trimethylsilyl)ethoxymethyl (SEM)^[123] groups for the protection of carboxylic functions.

The SEM ester **159**, which was applied for this synthesis, was obtained in 58% yield by treatment of *N*-Alloc protected *α*-Thr-OH **148** with SEM-Cl and Li₂CO₃ in DMF. *N*-Fmoc protected (2*R*,1'*R*,2'*R*)-(3-Ncp)Ala-OH **160** was obtained by acylation of the amino acid (2*R*,1'*R*,2'*R*)-**83** with FmocOSu (93%), and amino ester **161** was obtained by esterification of **155** with 2-trimethylsilylethanol, was followed by the selective removal of Boc group and decomposition of the intermediate tosylate (54% crude yield over 3 steps) (Scheme 38).

Scheme 38

The prepared amino ester was coupled with *N*-Boc protected (βMe)Phe-OH **139** to yield dipeptide **162** (86%), which, after removal of the Boc group, by treatment with 3.9 M HCl in dioxane (78%), in turn was condensed with the *N*-protected amino acid **160** to give tripeptide **164** in 73% yield (Scheme 38). This peptide, after removal of the Fmoc group (100%) was coupled with *N*-Fmoc protected (βMe)Phe-OH **146** to give tetrapeptide **167** (89%) (schema 39).

Alcohol **159** was esterified with the *N*-Boc protected (4-PE)Pro-OH (*2S,3R*)-**16** using EDC in the presence of DMAP to give **166** (64%). This, after removal of the SEM group by treatment with MgBr₂·Et₂O in CH₂Cl₂ (65%),^[124] was directly coupled with tetrapeptide **169** using HATU in the presence of HOAt to give hexadepsipeptide **170** in 80% yield.

Scheme 39. Reagents and conditions: a) EDC, HOAt, DIEA, 2,4,6-trimethylpyridine, CH_2Cl_2 , $0 \rightarrow 20^\circ\text{C}$, 14 h. b) 3 M HCl in dioxane, 20°C , 40 min. c) 50% $\text{Et}_2\text{NH}/\text{MeCN}$, 20°C , 40 h. d) EDC, DMAP, CH_2Cl_2 , $0 \rightarrow 20^\circ\text{C}$, 24 h. e) EDC, HOAt, 2,4,6-trimethylpyridine, DMF, $0 \rightarrow 20^\circ\text{C}$, 14 h. f) $\text{MgBr}_2 \cdot \text{Et}_2\text{O}$, CH_2Cl_2 , $-30 \rightarrow 0^\circ\text{C}$, 2 h. g) HATU, HOAt, DIEA, 2,4,6-trimethylpyridine, DMF, $0 \rightarrow 20^\circ\text{C}$, 24 h.

The acidolytic removal of the Boc and TMSE groups from the latter occurred quantitatively and was followed by macrocyclization, using HATU in the presence of HOAt under high dilution conditions,^[125] which gave macrocycle **171a** in 26% yield after HPLC purification (Scheme 40). Unfortunately, an attempted transprotection with Boc_2O and $\text{Pd}(\text{PPh}_3)_4$ in the presence of TBTH gave an inseparable mixture of unreacted and transprotected peptide, **171a** and **171b**, in a low yield, presumably, due to steric hindrance.

Scheme 40

5.4. Comparative study of acidolytic deprotection Boc, Z and several Z-derived amino protecting groups

As shown by model experiments, peptides with the *O*-acylated threonine moiety on the *N*-termini and free α -amino function are unstable in basic, neutral and even weakly acidic conditions supposedly probably due to a fast O $\rightarrow$ N acyl shift. Nevertheless this side reaction is completely suppressed under relatively strong acidic conditions. As a means of preserving the Boc group as an amino protecting group on the (4-PE)Pro moiety, first the Z group as a protecting group for the α -amino group of *O*-acetylated threonine was tested (Scheme 41).

Scheme 41

Unfortunately, the rate of the “push-pull”-based cleavage of the Z-protecting group^[126] [more acidic (liquid HF, MSA or TFMSA in TFA, TMSOTf) reagents in this case cause simultaneous cleavage of the ester bond in the threonine moiety] was unacceptably low even in case of the model ester Z-Thr(FmocPro)-OMe. In order to overcome this difficulty the easiness of the acidolytic removal of the Z-group should be finely attenuated.

	<i>p</i> -MeO	<i>p</i> -NO ₂	<i>p</i> -Me	<i>m</i> -Me
σ^+	-0.78	0.78	-0.31	-0.10

deprotection conditions: 1) 2 M HCl in EtOAc, 2) 3.9 M HCl in dioxane
3) TFA (neat), 4) TFA (scavengers)

Alloc-*a*-Thr[HCl·H-(4-PE)Pro]-(βMe)Phe-(3-Ncp)Ala-(βMe)Phe-Ile-OTMSE

174

Scheme 42

The stability of urethane-type protecting groups under acidic conditions depends on the stability of carbocations formed after their cleavage. This stability in case of the *m*- and *p*- substituted benzyl cations correlates with the corresponding σ^+ Hammett constants.^[127] Whereas the *p*-NO₂ substituted Z group [Z(NO₂)], $\sigma_{\text{NO}_2}^+ = 0.78$, is noticeably more stable to TFA and HBr in AcOH, than the original Z group, the *p*-methoxy substituted Z group (MeZ), $\sigma_{\text{OMe}}^+ = -0.78$, is cleaved with TFA at 0 °C within several minutes. Cleavage of *p*-methyl Z (MeZ), *m*-methyl Z (*m*-MeZ) and *m,m*-dimethyl Z groups (DmZ) in the model dipeptides **173** was briefly evaluated (Scheme 42). The MeZ and DmZ groups were stable to 2 M HCl in EtOAc for at least 2 h and the *m*-MeZ group was stable to this reagent, while the Boc group cleaved from hexadepsipeptide **170** within 2 min. Furthermore, the MeZ and DmZ groups were quantitatively cleaved with 10% anisole in TFA within 1 h and *m*-MeZ with 20% thioanisole in TFA for 3.5 h. For a further application the MeZ group was chosen.

5.5. Synthesis of *N*-MeZ protected cyclodepsipeptide **171c**

The dicyclopropylmethyl (DCPM)^[128] and methoxymethyl (MOM) groups were used in the course of this synthesis for the protection of the carboxylic group. The alkylation of the *N*-MeZ protected amino acid **175**, which was obtained by reaction between of H-*α*-Thr-OH and MeOSu (90%), with allyl bromide in the presence of K₂CO₃ in MeCN gave **176** in 84% yield (Scheme 43).

Scheme 43

N-Z Protected (βMe)Phe-OH **177** was obtained by acylation of the corresponding amino acid with ZOSu in 85% yield. The alkylation of *N*-Z protected Ile-OH **178** with MOMI (generated in situ) gave MOM-ester **179** in 76% yield, which was, immediately after removal of the Z-group (100%), used in the coupling step. The DCPM ester **180b** was obtained just before the next step by removal of the Fmoc group from the *N*-termini of **182** (100%), which was in turn prepared via esterification reaction of the intermediate acyl chloride Fmoc-Ile-Cl with dicyclopropylmethanol in the presence of pyridine in 57% yield.

Having in mind to ring-close an acyclic precursor already containing the ester bond between the (4-PE)Pro and the α -Thr residues by forming the peptide bond between the Ile and (4-PE)Pro moieties, the methoxymethyl or dicyclopropylmethyl ester of Ile, **180a** or **180b**, were condensed with *N*-Z-protected (β Me)Phe-OH **177** (Scheme 44). After removal of the Z group from the *N*-

termini of the resulting dipeptides, **183a** (66%) and **183b** (84%), by catalytic hydrogenation to give **184a** (54%) and **184b** (100%), the latter were coupled with *N*-Fmoc-protected-(2*R*,1'*S*,2'*R*)-(3-*Ncp*)Ala-OH **160** to yield tripeptides **185a** (80%) and **185b** (79%). The relatively low yield of the amino ester **184a** must be attributed to the noticeable susceptibility of the MOM ester to nucleophilic attack by the free amino group, especially if a formation of a diketopiperazine ring is possible.

Scheme 44. Reagents and conditions: a) EDC, HOAt, DIEA, 2,4,6-collidine, CH₂Cl₂, 0 → 20 °C, 14 h. b) H₂, Pd/C, EtOAc, 20 °C, 40 min. c) 50% Et₂NH/MeCN, 20 °C, 1 h. d) EDC, 4-pyrrolidinopyridine, CH₂Cl₂, 0 → 20 °C, 24 h. e) EDC, HOAt, 2,4,6-collidine, DMF, 0 → 20 °C, 14 h. f) Pd(PPh₃)₄, *N*-Methylaniline, DME, 20 °C, 1 h. g) HATU, HOAt, DIEA, 2,4,6-collidine, CH₂Cl₂, 0 → 20 °C, 24 h.

Tripeptides **185a** and **185b**, after removal of the Fmoc-group (100% yield), were coupled with *N*-Fmoc-protected (βMe)Phe-OH **10** to give tetrapeptides **188a** (78%) and **188b** (84%). The latter was used for the successful preparation of hormaomycin analogs (see Chapter 6).

The 4-pyrrolidinopyridine-catalyzed condensation of the *N*-Boc-protected (4-PE)Pro-OH **16** and *N,C*-protected α -Thr **176** gave the ester **187** (83%), which, after palladium-promoted removal of the allyl group (90%), was coupled with the tetrapeptide **190a** using the HATU reagent in the presence of HOAt to give the hexadepsipeptide **191a** in 79% yield.

The MOM and Boc groups were removed from the termini of the latter (the ESI-MS spectrum showed that the MeZ group stayed intact), and the cyclizing peptide condensation succeeded under high dilution conditions, using the HATU reagent. The cyclodepsipeptide **171c** was obtained after HPLC purification in 53% yield over 2 steps (Scheme 45).

Scheme 45

5.6. Final steps of hormaomycin synthesis

In order to complete the assembling of hormaomycin, the side chain should be attached. *N*-Boc protected (3-Ncp)Ala-OH (2*S*,1'*R*,2'*R*)-**143** was esterified with 9-fluorenylmethanol (FmOH) using EDC in the presence of 4-pyrrolidinopyridine giving **192** (74%) (Scheme 46). The latter, after removal of the Boc-group, was coupled with *O*-MOM protected Chpca-OH **109** to give the acyl amino ester **193** in 80% yield. After removal of the MOM group, by treatment with

MgBr₂·Et₂O, the resulted **194** (78%) was further transformed into acid **195**, which was too unstable (complete polymerization in THF or CDCl₃ solution within 30 min) to be used on the last coupling step. In order to overcome the difficulty, **193**, after removal of the Fm group, was coupled with macrocycle **171c**, the MeZ group of which was in turn removed with 10% anisole in TFA, using the (7-azabenzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate reagent (PyAOP)^[107] in the presence of HOAt.

Scheme 46

This coupling step was unexpectedly accompanied with significant racemization at C-2 of (S)-(3-Ncp)Ala moiety and separation of the obtained mixture of epimers by preparative TLC gave *O*-MOM protected hormaomycin *O*-MOM-**3** (26%) and diastereomeric *epi-O*-MOM-**3** (16%) (Scheme 47).

Scheme 47

To complete the assembly of **3** in a stepwise manner, the *N*-MeZ protected cyclic intermediate **171c** was deprotected and first coupled with *N*-Teoc-protected (2*S*,1'*R*,2'*R*)-(3-Ncp)Ala-OH **197** (Scheme 48). After removal of the Teoc-group, the intermediate in turn was coupled with the *O*-MOM-protected 5-chloro-1-hydroxypyrrole-2-carboxylic acid **109**.

Finally, the MOM group was removed by treatment with $\text{MgBr}_2 \cdot \text{Et}_2\text{O}$ and EtSH in dichloromethane to give a substance, which was expected to be identical with the native hormaomycin **3** (51% on 5 steps).

Scheme 48

It indeed disclose the expected $[M - H^+ + 2Na^+]$, $[M + Na^+]$ and $[M - H^+]$ peaks in its positive- and negative-mode ESI/MS spectra, had the same optical rotation, almost identical CD trace, at least in the wavelength region above 210 nm where no solvent interference is expected (these spectra were measured in methanol), identical UV spectrum, a very similar ^{13}C NMR spectrum and identical HPLC retention time as an authentic sample of the natural material. The synthetic product also showed the same capability to inhibit the growth of coryneform bacteria as native hormaomycin **3**. However, there were some differences in their 1H NMR spectra, measured in $CDCl_3$ and CD_3OH . These discrepancies were initially interpreted to indicate that a diastereomer of the natural product had been synthesized. Yet, this possibility was excluded by the results of LC/MS experiments according to the advanced Marfey method,^[38] which rigorously proved that the MePhe, Ile, Ncpa and α -Thr residues were not epimerized in any of the peptide coupling and deprotection steps. Moreover prof. W. A. König (Hamburg) kindly repeated some GC separations^[9] to determine the absolute configuration of the 3-phenylmethylalanine residue in hormaomycin **3**, using pure (2*S*,3*R*)-3-methylphenylalanine, provided by us, as one of the reference compounds (previously^[9] a mixture of all stereoisomers of 3-methylphenylalanine had been used as a reference). These latest results were in accordance with those obtained before. Furthermore the comparative 1H - 1H COSY experiments in CD_3OH , which were performed by Prof. C. Griesinger and Dr. U. Reinscheid (Göttingen), showed the same correlations for both samples between the four amide protons in 7.87–8.37 ppm region and four α -protons in 3.80–4.50 ppm (the two left correlations were undistinguished for each probe). Consequently, the small differences in the 1H NMR spectra of natural and synthetic hormaomycin must be attributed to slightly different conformational dynamics due to traces of different impurities in the two samples.

6. Synthesis of hormaomycin analogs. Evaluation of biological activity

6.1. Attempted synthesis of [(*S*)-(3-Acp)Ala⁷]-hormaomycin

Once the synthesis of hormaomycin **3** was successfully completed, the synthesis of its analogs was initiated. As a first target for this synthetic investigation analog, contained in the side chain (*2S,1'R,2'R*)-3-(2'-aminocyclopropyl)alanine [(*S*)-(3-Acp)Ala] instead of (*S*)-(3-Ncp)Ala moiety was chosen. Noteworthy, that the corresponding feeding experiment with H-(3-Acp)Ala-OH failed and no products contained this amino acid were isolated.^[12]

Schema 49.

As initial attempts of the reduction of the already *N*-Teoc protected (3-Ncp)Ala-OH (*S*)-**197** and its methyl ester (*S*)-**198** failed (Scheme 49), the free amino acid (*2S,1'R,2'R*)-**83** was hydrogenated on 10% Pd/C in methanol according to procedure of Larionov and de Meijere^[129] to give the desired diamino acid, which without any purification was further transformed via acylation of the corresponding copper complex with ZOSu in *N*_ω-Z protected H-(3-Acp)Ala(Z)-OH **199** in 52% yield over two steps (Scheme 50).

Scheme 50

The latter was further N_α -protected with Teoc-OSu to give the protected diamino acid **200** (66%), which, after hydrogenolytic removal of the Z-group, was in turn allowed to react with 3-nitro-2-pyridinesulfonyl chloride (NpysCl) to give the orthogonally protected derivative **201** in 44% yield.

The Npys group, which was proposed for the amino protection by Matsueda et al.,^[130] is stable to trifluoroacetic acid, but easily removed with very dilute (0.1–0.2 M) HCl in organic solvents. It is reported that it can be also cleaved under nearly neutral conditions with triphenylphosphine and a proton source as well as in essentially under neutral conditions with thiols such as 2-pyridinethiol *N*-oxide or 2-mercaptobenzothiazole. To evaluate the deprotection conditions for this group the N_α -Z, N_ω -Npys protected ornithine amide **204** was prepared and several deprotection conditions were examined (Scheme 51).

On the contrary to the related Nps group, Npys group was only partially cleaved with $\text{MgBr}_2 \cdot \text{Et}_2\text{O}$ and EtSH in CH_2Cl_2 for 4.5 h. It was rather insensitive to 2-pyridinethiol *N*-oxide and gave, by treatment with this reagent for 5 h, only traces of the deprotected material. Gratifyingly it was completely removed with 3 eq. 0.1 M triphenylphosphine and 3 eq. of 0.1 M

pyridinium chloride in CH_2Cl_2 for 2 min, although the yield of the deprotected amino amide was moderate.

Schema 51.

The cyclic intermediate **171c**, after removal of the MeZ group, was acylated with the orthogonally protected diamino acid **201** (Scheme 52). After removal of the Teoc group, the resultant intermediate product was in turn coupled with the *O*-MOM protected Chpca **109** to give the *N*-protected branched acyl depsipeptide [(3-Acp)Ala(Npys)⁷]-MOM-**3** in 64% yield over four steps. The latter was treated first with $\text{MgBr}_2\cdot\text{Et}_2\text{O}$ and EtSH in CH_2Cl_2 , and then with PPh_3 and $\text{Py}\cdot\text{HCl}$. Although the resulted mixture contained two Erlich reagent active compounds according to TLC, attempt of its separation by exclusion chromatography did not give any isolable compounds, containing pyrrole fragment.

Scheme 52

6.2. *Synthesis of [(S)-(NO₂)Nva⁷]-hormaomycin. Isolation of [(S)-(NO₂)Nva³]-horamomycin and [(S)-(NO₂)Nva³,(S)-(NO₂)Nva⁷]-hormaomycin*

From previous experiments it was already known that the presence of the *N*-hydroxy group at Chpca moiety is crucially important for both kinds of biological activity of hormaomycin. The fact, that analogs with transformed or substituted (3-Ncp)Ala moieties, obtained before, do not possess the hormaomycin-like biological activity, point out the significance of this structural elements of **3**. Nevertheless the importance of *each* (3-Ncp)Ala residue was absolute unclear and the question, could any analogs without one or two nitrocyclopropyl fragments retain some biological activity yet, stayed still open. To clarify situation the synthesis of hormaomycin analog with 5-nitronorvaline [(NO₂)Nva] moiety (methylen group instead of cyclopropyl ring) in place of (3-Ncp)Ala residue in the side chain was initiated. For the preparation of [(*R*)-(NO₂)Nva³,(*S*)-(NO₂)Nva⁷]- and [(*R*)-(NO₂)Nva³]-analogs feeding experiment with H-Nva-OH was used.

The N_{α} -Boc, N_{ω} -Z protected ornithine **205** was esterified to give the corresponding *tert*-butyl ester **206**^[131] in 94% yield, which, after removal of the Z-group, was transformed in **207** (58%) via oxidation of the amino group with MCPBA in refluxing 1,2-dichloroethane according to protocol of Gilbert and Borden.^[132]

Scheme 53

The simultaneous removal of the both protecting group gave amino acid as a hydrochloride **208** (56%), which was further treated with TeocOSu to give the *N*-protected (NO₂)Nva-OH **209** in 93% yield (scheme 53). This derivative was also prepared from N_{α} -Z, N_{ω} -Boc protected ornithine **202** but in noticeably lower yield.

To evaluate applicability of 5-nitronorvaline for peptide synthesis the acylated tripeptide **211** was prepared (Scheme 54). Dipeptide **117**, after removal of the Boc, was coupled with **209** to give tripeptide **210** in 59% yield. Sequential deprotection of its *N*-termini was followed by acylation with Chpca(MOM)-OH **109** and gave **211** (79%).

Scheme 54

After that, the assembling of analog [(S)-(NO₂)Nva⁷]-**3** could be initiated. The cyclic intermediate **171c**, after removal of the MeZ group, was coupled with the *N*-Teoc protected amino acid **209**. After removal of the Teoc group the resulted intermediate was acylated with Chpca(MOM)-OH **109**. Finally the desired analog was obtained after removal of the MOM group in overall 60% yield over 5 steps (schema 55).

Scheme 55

Feeding experiment with amino acid **208** (0.42 g, ca. 2 mmol) was performed by Ms. M. Klingibieli and Mr. H.-P. Kroll in prof. A. Zeeck's group (Goettingen) and gave 7 mg of mixture,

which, according to ESI-MS spectra, contained besides **3** ($m/z = 1151$ [$M + Na^+$]; $m/z = 1127$ [$M - H^+$]) analogs with one (3-Ncp)Ala residue substituted on $(NO_2)Nva$ moiety ($m/z = 1139$ [$M + Na^+$]; $m/z = 1115$ [$M - H^+$]) and analog with two $(NO_2)Nva$ residues instead of two (3-Ncp)Ala moieties ($m/z = 1127$ [$M + Na^+$]; $m/z = 1103$ [$M - H^+$]). HPLC analysis of this mixture showed four main peaks with hormaomycin-like UV spectra in ratio ca. 2:1:1:0.5. The preparative HPLC separation of mixture allowed to isolate chromatographically pure [(*S*)- $(NO_2)Nva^3$]-**3** and [(*S*)- $(NO_2)Nva^3$,(*S*)- $(NO_2)Nva^7$]-**3**. The careful evaluation of spectral and biological properties of these substances is continued.

6.3. Synthesis of [*α*-Dab¹]-hormaomycin and [*α*-Dab¹,*α*-Ile⁵]-hormaomycin

Hormaomycin analog with the amide bond instead of the ester bond in the ring part became next task of this investigation. This insignificant change could show how strong affects the conformation of the ring part of hormaomycin on its biological properties as the presence of the odd amide bond could have the significant influence on the intramolecular hydrogen bonds. Furthermore amide bonds are usually much more stable to enzymatic cleavage and it could cause a prolonged action of the aza-analog comparing with native hormaomycin **3**.

Scheme 56

To prepare this analog the orthogonally protected (2*R*,3*R*)-diaminobutyric acid (*a*-Dab) should be first synthesized (Scheme 56). The *tert*-butyl ester **213**, which was prepared according to published procedure^[133] with Sharpless asymmetric aminohydroxylation as a key step followed by stereospecific azidation, was transformed in fully protected *a*-Dab derivative **214** as it was described by Orgel et al..^[134] The latter, after simultaneous removal of the *N*-Boc and *O*-*tert*-butyl groups with trifluoroacetic acid, was esterified with methanol and the resulted *N*_β-protected diamino methyl ester was acetylated with MeZOSu to give **215** in 69% yield over three steps.

After removal of the Fmoc group diamino ester **215** was coupled with the *N*-Boc protected (4-PE)Pro-OH **16** to give methyl ester **216** in 83% yield, which was in turn saponified using tetrabutylammonium hydroxide (Scheme 57).

Scheme 57

Intermediate **217** (85%) was coupled with tetrapeptide **188b**, after removal of the Fmoc group from the *N*-termini of the latter, to yield the branched hexapeptide **218** (80%) (Scheme 58).

The acidolytic removal of the Boc and DCPM groups from **218** occurred quantitatively and was followed by macrocyclization, using HATU in the presence of HOAt under high dilution conditions, which gave the epimeric macrocycles, **219a** and **219b**, in 28% and 19% yield respectively after HPLC separation (Scheme 59).

Scheme 58

Investigation of the amino acid content of these products according to the advanced Marfey method^[38] showed that **219b** contained instead of Ile residue (*R*)-*a*-Ile [*a*-Ile] moiety.

Scheme 59

The cyclic branched peptide **219a**, after removal of the MeZ group, was acylated with the *N*-Teoc protected (2*S*,1'*R*,2'*R*)-(3-Ncp)Ala-OH **197** (Scheme 60). The resultant intermediate, after removal of the Teoc group, was coupled with acid **109**. MgBr₂·Et₂O/EtSH-mediated removal of the MOM group completed this synthesis and gave the desired [*a*-Dab¹]-**3** in 68% yield on five steps. The likeness of the ¹H-spectra of this substance and those of native hormaomycin **3** indicated that the expected remarkable conformational changes did not take place.

Scheme 60

Similarly [a-Dab¹,*α*-Ile⁶]-**3** was prepared from intermediate **219b** in 39% yield over five steps after HPLC purification. Its ¹H-spectrum differed sharply from ¹H-spectrum of **3** and showed (at least in CDCl₃) on the presence of several conformers.

6.4. Syntheses to [a-N_αMeDab¹]-hormaomycin

For a further synthetic investigation of **3** analog, contained (2*R*,3*R*)-*N*_β-methyl diaminobutyric acid [a-N_βMeDab] moiety instead of *α*-Thr residue, was chosen.

The fully protected amino acid **222** was synthesized starting from the known **220**^[135] (Scheme 61). After removal of the TBDMS group followed by removal of the *tert*-butyl ester, the *N*-Z protected isothreonine **220** was esterified with diazomethane to give intermediate, which was further converted into the corresponding mesylate.

Scheme 61

This transformation was followed by displacement of the mesylate with NaN_3 in DMF to give azide **221**, which was further transformed in the N_α -Boc, N_β -Z protected α - N_β MeDab methyl ester, by treatment first with triphenylphosphine and water, and then with Boc_2O . Further removal of the Z group by hydrogenolysis was followed by introduction of the Fmoc group to give the intermediate N_α -Boc, N_β -Fmoc protected α - N_β MeDab methyl ester, which, after removal of the Boc group, was finally acylated with MeZOSu to give **222** in 13% yield over ten steps.

This diamino acid, after removal of the Fmoc group, was coupled with the N -Boc protected (4-PE)Pro-OH **16** giving dipeptide **223** (79%) (Scheme 62). Noteworthy, that NMR-spectra of **223** showed on the presence of at least four conformers (instead of usual two) and the complicated temperature dependence (on heating till $100\text{ }^\circ\text{C}$ the coalescence point was almost reached but spectra, measured at $125\text{ }^\circ\text{C}$, showed again on the presence of several conformers; as at this

temperature substance already noticeably decomposed, no attempts to measure spectra at higher temperature has been made).

Scheme 62

6.5. *Evaluation of the biological activity of building blocks and hormaomycin analogs as well as selected model compounds*

All biological tests were performed by Ms. M. Klingebiel and Mr. H.-P. Kroll in prof. A. Zeeck's group (Göttingen).

To evaluate the possible antibiotic activity the plate diffusion tests on bacteriostatic activity against test strains of *E. coli*, *B. subtilis*, *S. aureus*, *C. albicans*, as well as against highly sensitive to hormaomycin *Arthrobacter oxydans* with two isomers of 3-(2'-nitrocyclopropyl)alanine, (2*S*,1'*R*,2'*R*)-**83** and (2*R*,1'*R*,2'*R*)-**83**, 4-(*Z*)-propenylproline (2*S*,4*R*)-**16**, 1-hydroxy-2-chloro-5-cyanopyrrole **103**, and with the model acyl tripeptide **119** were carried out. In these experiments all tested compounds did not show any noticeable activity with the only exception for **103**, which displayed significant activity against *S. aureus*.

As the evaluation of the morphogen activity was impossible due to the technical reasons only the antibiotic activity against *Arthrobacter* species was tested (table 1).

Compound	Relative activity(%)
119	0
193	0
194	19
<i>O</i> -MOM- 3	19
<i>epi-O</i> -MOM- 3	13
Synthetic 3	113
211	39
219a	0
[(NO ₂)Nva ⁷]- 3	90
[<i>a</i> -Dab ¹]- 3	103
[<i>a</i> -Dab ¹ , <i>a</i> -Ile ⁵]- 3	6

Table 1. Relative bacteriocidal activity of several compounds in plate diffusion tests against *Arthrobacter crystallopoites* (strain 20119): plates diameter 9 mm, thickness 0.5 mm; 0.05 mg substance pro plate. Relative activity = ($\varnothing$ of inhibition zone – 9/ $\varnothing$ of inhibition zone of **3** – 9) $\times$ 100 (%).

For the most potent compound [(NO₂)Nva⁷]-**3**, [*a*-Dab¹]-**3** and **211** the dilution row plate diffusion tests were also performed (table 2).

Compound mg pro plate	5×10^{-2}	5×10^{-3}	5×10^{-4}	5×10^{-5}
3	100	94	71	39
[(NO ₂)Nva ⁷]- 3	90	84	58	26
[<i>a</i> -Dab ¹]- 3	103	90	68	35
211	39	23	2	0

Table 2. Relative bacteriocidal activity of several compounds in dilution row plate diffusion tests against *Arthrobacter crystallopoites* (strain 20119) (estimated relative to activity of hormaomycin at 5×10^{-2} mg pro plate)

Even these preliminary tests allowed making some conclusions about SAR for the antibiotic activity of hormaomycin **3**:

- 1) side chain of hormaomycin causes its antibiotic activity;
- 2) lack of the aliphatic nitro-group in the side chain of amino acid, acetylated with Chpca, causes complete loss of the bactericidal activity (**109** vs. **211**);
- 3) substitution of the cyclopropyl ring in the side chain of amino acid, acetylated with Chpca, at least with the methylene group, causes only insignificant loss activity (**3** vs. [(NO₂)Nva⁷]-**3**);
- 4) presence of the free *N*-hydroxy group on Chpca moiety is very important; its alkylation causes significant decrease of activity (**3** vs. *O*-MOM-**3**, **193** vs. **194**);
- 5) cyclic part of hormaomycin **3** does not have any intrinsic antibiotic activity (**219**), nevertheless the significant changes of its conformations can give inactive compounds ([*α*-Dab¹,*α*-Ile⁵]-**3**);
- 6) generally, group or substituent, connected with the fragment, which is responsible for antibiotic action, can noticeably effect the antibiotic activity (**194** vs. **211**).

Further, it seems to be very possible, that the cyclic part of **3** diminishes its antibiotic activity (*O*-MOM-**3** vs. **211**). Nevertheless additional test with **211**, after removal of the MOM group, should be made. Consequently, if the resultant compound has similar or higher activity than hormaomycin **3**, it will mean that the postulated mechanism of the antibiotic action of hormaomycin **3** (perturbation of the cell membrane) is wrong.

C. Experimental Part

1. General methods

¹H NMR spectra: Bruker AM 250 (250 MHz), AMX 300 (300 MHz) or Varian Unity 300 (300 MHz), Inova 500 (500 MHz), Inova 600 (600 MHz). Proton chemical shifts are reported in ppm relative to residual peak of deuterated solvent or tetramethylsilane: δ (ppm) = 0 for TMS, 2.49 for [D₅]DMSO, 4.65 for HOD in D₂O, 7.26 for CHCl₃, 1.73 and 3.55 for [D₇]THF, 3.35 for CHD₂OD, 2.04 for [D₅]acetone. Higher order NMR spectra were approximately interpreted as first-order spectra, if possible. For the characterization of the observed signal multiplicities the following abbreviations were applied: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, as well as br = broad, Ar-H = aryl-H. ¹³C NMR spectra [additional DEPT (Distortionless Enhancement by Polarization transfer) or APT (Attached Proton Test)]: Bruker AM 250 (62.9 MHz), AMX 300 (75.5 MHz) or Varian Unity 300 (75.5 MHz), Inova 500 (125.7 MHz), Inova 600 (125.7 MHz) instruments. ¹³C chemical shifts are reported relative to peak of solvent or tetramethylsilane: 0 for TMS, 39.5 for [D₅]DMSO, 77.0 for CDCl₃, 25.5 and 3.55 for [D₇]THF, 3.35 for CHD₂OD, 29.8 for [D₅]acetone or to dioxane in D₂O (δ = 67.2 ppm). The following abbreviations were applied: DEPT: + = primary or tertiary (positive signal in DEPT), – = secondary (negative signal in DEPT), C_{quat} = quaternary (null signal in DEPT); APT: + = primary or tertiary (positive signal in DEPT), – = secondary or quaternary (negative signal in APT); if it was necessary and possible HMBS (Heteronuclear Multiple Bond Connectivity) and/or HMQS (Heteronuclear Multiple Quantum Coherence) were also measured. IR-spectra: Bruker IFS 66 (FT-IR) spectrometer as KBr pellets or oils between KBr plates. MS: EI-MS: Finnigan MAT 95, 70 eV, high resolution EI-MS spectra with perfluorkerosene as reference substance; DCI-MS: Finnigan MAT 95, 200 eV, reactant gas NH₃; ESI-MS: Finnigan LCQ. HPLC-MS: pump: Flux Instruments Rheos 4000; degasser: Flux Instruments ERC 3415 α ; detector: Linear UVIS-205; data system: Flux Instruments Janeiro; ESI: Finnigan LCQ, positive

and negative mode; data system: Finnigan LCQ Xcalibur; column: Crom Superspher 100 RP-18 endcapped (4 μm , 2 x 100 mm); HPLC conditions: eluent A: H_2O (0.05% HCOOH), eluent B: 90% MeCN (0.05% HCOOH), 30 $\rightarrow$ 70% B for 30 min, flow rate: 300 $\mu\text{L}/\text{min}$. HPLC: pump: Kontron 322 system, detector: Kontron DAD 440, mixer: Kontron HPLC 360, data system: Kontron Kromasystem 200, columns: Knauer Nucleosil-100 C18 (analytical, 5 μm , 3 mm x 250 mm), Knauer Nucleosil-100 C18 (preparative, 5 μm , 8 mm x 250 mm). Optical rotations: Perkin-Elmer 241 digital polarimeter, 1-dm cell; optical rotation values are given in $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$; concentrations (c) are given in g/100 mL. Circular dichroism: Jasco J 500A. Molar ellipticities (Θ) are given in $\text{grad cm}^2 10^{-1} \text{ mol}^{-1}$. M.p.: Büchi 510 capillary melting point apparatus, uncorrected values. TLC: Macherey-Nagel precoated sheets, 0.25 mm Sil G/UV₂₅₄. The chromatograms were viewed under UV light and/or by treatment with phosphomolibdic acid (10% in ethanol), or ninhydrin (0.2% in ethanol), or Ehrlich's reagent (freshly prepared solution of 1 g of 4-dimethylaminobenzaldehyde in 25 mL 36% HCl and 75 mL methanol). Column chromatography: Merck silica gel, grade 60, 230–400 mesh and Baker silica gel, 40–140 mesh. Preparative TLC: Macherey-Nagel, silica gel SIL G/UV₂₅₄, layer thickness 0.25 mm (100 x 200 mm or 200 x 200 mm). Elemental analyses: Mikroanalytisches Laboratorium des Instituts für Organische Chemie der Universität Göttingen. Starting materials: Anhydrous solvents were prepared according to standard methods by distillation over drying agents and were stored under argon. All other solvents were distilled before use. All reactions were carried out with magnetic stirring and, if air or moisture sensitive, in flame-dried glassware under argon or nitrogen.

The following compounds were prepared according to literature procedures:

(2*S*,4*R*)-4-Tosyloxi-*N*-Boc-proline,^[22] *tert*-butyl (*R*)-*N*-(*tert*-butyloxycarbonyl)pyroglutamate [as it was described for (*S*)-isomer],^[34] *tert*-butyl (2*R*,4*R*)-(N-*tert*-butyloxycarbonyl)-4-allyl-pyroglutamate [as it was described for (2*S*,4*S*)-isomer],^[35] methyl (2*S*,4*R*)-(N-*tert*-butyloxycarbonyl)-4-hydroxyproline,^[46] methyl (2*S*,4*RS*)-(N-*tert*-butyloxycarbonyl)-4-iodoproline,^[47] (2*S*,4*R*)-(N-*tert*-butyloxycarbonyl)-4-hydroxyproline,^[53] (2*S*,4*R*)-(N-*tert*-butyloxy-carbonyl)-4-mesyloxyprolinol,^[57] (2*S*,4*R*)-(N-*tert*-butyloxycarbonyl)-4-hydroxyprolinol *tert*-butyldimethylsilyl ester,^[62] adduct of (2*S*,4*R*)-4-hydroxyproline and pivalic aldehyde,^[64] *rac*-(*trans*-2'-nitrocyclopropyl)methanol,^[84] (1,1-dideutero)ethyltriphenylphosphonium bromide,^[72] (*R*)-*allo*-threonine,^[75] (4*R*)-phenyloxazolidinone-2,^[80] (4*R*)-4-phenyl-3-(3'-phenylbutyryl)oxazolidinone-2,^[79] (4*R*,3'*S*)-4-phenyl-3-(3'-phenylbutyryl)oxazolidinone-2 [as it was described for (4*S*,3'*R*)-isomer],^[81] 1,1,3,3-tetramethyl guanidinium azide,^[136] *rac*-β-methylphenylalanine,^[137] (2*S*,3*R*)-β-methylphenylalanine,^[79] (*S*) and (*R*) Belokon's chiral glycine equivalents,^[86] (4*R*)-4-isopropyl-1,3-*trans*-(2'-nitrocyclopropyl)carbonyloxazolidinones-2,^[14] *trans*-(2'-nitrocyclopropyl)methyl iodide,^[80] 1-methoxysuccinimide,^[97] 2-amino-6-chloropyridine,^[99] 2-acetamido-6-chloropyridine,^[101] 2,6-dichloropyridine-*N*-oxide,^[102] 9-fluorenylmethyl (*S*)-(N-*tert*-butyloxycarbonyl)phenylalaninate,^[105] 2-acetamido-6-aminopyridine,^[107] (*S*)-(2-nitrophenyl)sulfenylproline,^[116] (*S*)-(2-nitrophenyl)sulfenylisoleucine,^[116] methyl (N-*tert*-butyloxycarbonyl)-isoleucyl-proline,^[117] *p*-nitrobenzyl (*R*)-*allo*-(N-*tert*-butyloxycarbonyl)threoninate [as it was described for (*S*)-*threo*-isomer],^[120] (2*S*,3*R*)-(N-9-fluorenylmethyloxycarbonyl)-β-methylphenylalanine,^[119] *tert*-butyl (2*R*,3*R*)-2-azido-3-(benzyloxycarbonylamino)-butanoate,^[133] *tert*-butyl (2*R*,3*R*)-2-(*tert*-butyloxycarbonylamino)-3-(9-fluorenylmethyloxycarbonylamino)butanoate,^[134] *tert*-butyl (2*R*,3*R*)-3-(benzyloxycarbonylmethylamino)-2-*tert*-butyldimethylsilyloxybutanoate,^[135] tetrabutylammonium cyanide,^[138] 9-fluorenylmethyl *N*-hydroxysuccinyl carbonate,^[139] nitrophenylsulfenyl chloride,^[139] 3-nitropyridine-2-sulfenyl chloride,^[140] 1-hydroxy-7-aza-benzotriazol,^[141] (7-azabenzotriazol-1-yloxy)tris(pyrrolidino)-

phosphonium hexafluorophosphate,^[141] 2-(trimethylsilyl)ethyl *N*-succinyl carbonate.^[142] (2*S*,1'*R*,2'*R*)-3-(2'-Nitrocyclopropyl)alanine and nitrocyclopropane were kindly provided by Oleg V. Larionov (Göttingen).

2. Preparation of substances

2.1. *Determination of the relative configuration of 4-(Z)-propenylproline moiety in hormaomycin. Attempts to prepare pure diastereomers of 4-(Z)-propenylproline via alkylation with (Z)-propenyllithium cuprate*

(2*S*,4*R*)-4-Tosyloxi-*N*-Boc-proline (2*S*,4*R*)-(15):^[22] M.p. 138–139 °C; *R*_f = 0.19 (hexanes/EtOAc

1:2 (1.5% AcOH); $[\alpha]_{20}^D - 62.7$ (*c* = 0.33, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 1.39, 1.44 [2 x s, 9 H; C(CH₃)₃], 2.14–2.67 (m, 2 H; 3-H_A, 5-H_A), 2.46 (s, 3 H; CH₃), 3.45–3.81 (m, 2 H, 3-H_B, 5-H_B),

4.39, 4.42 (2 x t, *J* = 8 Hz, 1 H; 2-H), 5.02 (m, 1 H, 4-H), 7.37 (d, *J* = 8.25 Hz, 2 H, 3'-H), 7.79 (d, *J* = 8.25 Hz, 2 H, 2'-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 21.6 (+, CH₃), 28.0, 28.2 [+ , C(CH₃)₃], 35.3, 37.0 (–, C-3), 51.8, 52.3 (–, C-5), 51.2 (+, C-2), 78.2, 78.6 [C_{quat}, C(CH₃)₃], 81.2, 81.8 (+, C-4), 127.7 (+, C-3'), 130.1 (+, C-2'), 133.1, 133.24 (C_{quat}, C-4'), 145.3 (C_{quat}, C-1'), 153.2, 155.0 (C_{quat}, NCO₂), 175.2, 177.5 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 3062 \text{ cm}^{-1}$, 2985, 2956, 1754, 1635, 1433, 1171; elemental analysis calcd (%) for C₁₇H₂₃NO₇S (385.4): C 52.98, H 6.01, N 3.63; found C 52.67, H 6.19, N 3.55.

trans- and *cis*-*N*-Boc-4-(*Z*)-Propenylprolines *cis*-(16) and *trans*-(16): To a Li span (0.087g, 12.53 mmol) in anhydrous diethyl ester (7 mL) under stirring was added a solution of *cis*-1-

bromopropene (0.3 mL, 5.01 mmol) in diethyl ether (3 mL) at $-10\text{ }^{\circ}\text{C}$ over 5 min under argon (Ar). After this, the reaction mixture was stirred at the same temperature for additional 1 h and added to a suspension of $\text{CuBr}\cdot\text{Me}_2\text{S}$ (0.529 g, 2.26 mmol) in diethyl ester (15 mL) at $-45\text{--}40\text{ }^{\circ}\text{C}$ (internal temperature) for 10 min. After 1 h, a solution of (2*S*,4*R*)-**15** (0.29 g, 0.75 mmol) in THF (10.5 mL) was added to obtained almost clear green-yellowish solution of lithium dipropenylcuprate at the same temperature over 15 min and the reaction mixture was allowed to warm to $20\text{ }^{\circ}\text{C}$ over a period of 16 h. The dark-colored reaction mixture was cooled in water-ice bath and treated with saturated NH_4Cl (5–10 mL). Sodium hydroxide (10%) was added to bring the pH to 10 and the organic layer separated. The aqueous phase was washed with ether (3×10 mL), acidificated with 4 N H_2SO_4 to pH 2–3 and then extracted with ethyl acetate (2×20 mL). The combined organic layers were washed with water (2×10 mL), then with brine (2×5 mL), dried and concentrated under reduced pressure. The residue was filtered through pad of silica gel (6 cm) in $\text{MeOH}/\text{CHCl}_3$ 1:10 to give after evaporation of solvent the propenylproline (0.155 g, 81 %) as an inseparable mixture of diastereomers in ratio 2.2:1 (according to ^{13}C spectrum). The portion (25 mg) of this mixture was separated by HPLC (conditions A, flow rate 3 mL/min) to give *trans*-isomer *trans*-**16** (13.8 mg, t_{R} = 6.84 min), *cis*-isomer *cis*-**16** (5.4 mg, t_{R} = 6.35 min) in pure form.

trans-**16**: M.p. $41\text{--}45\text{ }^{\circ}\text{C}$; ^1H NMR (500 MHz, CDCl_3): δ = 1.42, 1.48 [$2 \times \text{s}$, 9 H, $\text{C}(\text{CH}_3)_3$], 1.66

(d, J = 6.0 Hz, 3 H; 3'-H), 1.82 (ddd, J = 11.9, 11.9, 9.1 Hz, 0.7 H, 3-H_a), 2.01 (ddd, J = 11.9, 11.9, 10.3 Hz, 0.3 H, 3-H_a), 2.11–2.24 (m, 0.3 H; 3-H_b), 2.41 (dd, J = 12.6, 6.2 Hz, 0.7 H; 3-H_b), 2.97 (dd, J =

10.0 Hz, 0.7 H, 5-H_a), 3.05 (dd, J = 9.5 Hz, 0.3 H, 5-H_a), 3.18–3.33 (m, 1 H, 4-H), 3.58 (dd, J = 8.5, 8.5 Hz, 0.7 H, 5-H_b), 3.76 (dd, J = 9.3, 9.3 Hz, 0.3 H, 5-H_b), 4.31 (d, J = 9.5 Hz, 0.3 H, 2-H), 4.40 (d, J = 8.5 Hz, 0.7 H, 2-H), 5.25 (dd, J = 9.8, 9.8 Hz, 1 H, 1'-H), 5.52 (dq, J = 9.8, 7.0 Hz, 1 H, 2'-H), 6.40–7.50 (br, 1 H, CO_2H); ^{13}C NMR (125.7 MHz, CDCl_3): δ = 13.2 (+, CH_3), 28.2, 28.3 [+ , $\text{C}(\text{CH}_3)_3$], 34.2, 35.2 (+, C-4), 35.1, 37.0 (–, C-3), 51.4, 51.9 (–, C-5), 58.9,

59.2 (+, C-2), 80.3, 81.3 [C_{quat} , $C(\text{CH}_3)_3$], 126.7, 126.9 (+, C-2'), 129.2, 129.6 (+, C-1'), 153.8, 156.0 (C_{quat} , NCO_2), 175.4, 178.4 (C_{quat} , C-1). IR (KBr): $\nu(\text{tilde}) = 3023 \text{ cm}^{-1}$, 2981, 2880, 1722, 1650, 1404, 1227, 1159; MS (EI, 70 eV), m/z (%): 255 (1) [M^+], 210 (8) [$M^+ - \text{CHO}_2$], 182 (2) [$M^+ - \text{C}_4\text{H}_9\text{O}$], 154 (100) [$M^+ - \text{C}_5\text{H}_9\text{O}_2$], 110 (70) [$\text{C}_7\text{H}_{12}\text{N}^+$], 87 (8), 57 (80) [C_4H_9^+]; HRMS (EI): calcd for $\text{C}_{13}\text{H}_{21}\text{NO}_4$: 255.1471; found 255.1471.

cis-**16**: M.p. 65–68 °C; ^1H NMR (500 MHz, CDCl_3): $\delta = 1.41$, 1.45 [$2 \times s$, 9 H, $\text{C}(\text{CH}_3)_3$], 1.65

(d, $J = 6.5 \text{ Hz}$, 3 H; 3'-H), 1.75 (ddd, $J = 12.5$, 10.3, 10.3 Hz, 0.5 H, 3-H_a), 1.88 (ddd, $J = 12.0$, 10, 10 Hz, 0.5 H, 3-H_a), 2.38 (dddd, $J = 13.0$, 7.0, 7.0, 7.0 Hz, 0.5 H; 3-H_b), 2.44 (dddd, $J = 12.5$, 6.5, 6.5, 6.5 Hz,

0.5 H; 3-H_b), 2.99–3.16 (m, 2 H, 4-H, 5-H_a), 3.67 (dd, $J = 9.3$, 7.3 Hz, 0.5 H, 5-H_b), 3.69–3.78 (m, 0.5 H, 5-H_b), 4.22 (dd, $J = 8.3$, 8.3 Hz, 0.5 H, 2-H), 4.28 (dd, $J = 8.3$, 8.3 Hz, 0.5 H, 2-H), 5.23 (ddq, $J = 10.8$, 10.8, 1.8 Hz, 1 H, 1'-H), 5.52 (dq, $J = 10.8$, 6.5 Hz, 1 H, 2'-H), 6.82–7.57 (br, 1 H, CO_2H); ^{13}C NMR (125.7 MHz, CDCl_3): $\delta = 13.2$ (+, CH_3), 28.2, 28.4 [+ , $\text{C}(\text{CH}_3)_3$], 35.8, 36.0 (+, C-4), 35.9, 37.4 (–, C-3), 51.5, 52.1 (–, C-5), 59.3, 59.6 (+, C-2), 80.4, 81.0 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 126.6, 126.9 (+, C-2'), 129.2, 129.3 (+, C-1'), 153.6, 155.6 (C_{quat} , NCO_2), 176.3, 178.6 (C_{quat} , C-1); IR (KBr): $\nu(\text{tilde}) = 3020 \text{ cm}^{-1}$, 2975, 2943, 1736, 1633, 1441, 1252, 1168; MS (EI, 70 eV), m/z (%): 255 (1) [M^+], 210 (8) [$M^+ - \text{CHO}_2$], 182 (2) [$M^+ - \text{C}_4\text{H}_9\text{O}$], 154 (100) [$M^+ - \text{C}_5\text{H}_9\text{O}_2$], 110 (70) [$\text{C}_7\text{H}_{12}\text{N}^+$], 87 (8), 57 (80) [C_4H_9^+]; HRMS (EI): calcd for $\text{C}_{13}\text{H}_{21}\text{NO}_4$: 255.1471; found 255.1471.

trans-4-(*Z*)-propenylproline trifluoroacetate *TFA-trans*-(**17**): Compound *trans*-**17** (13.8 mg,

0.054 mmol) was deprotected with TFA (1 mL) at 20 °C for 20 min.

The resulting solution was concentrated under reduced pressure to give trifluoroacetate *trans*-**17** (13.9 mg, 100%) as a hygroscopic faint

rose solid. ^1H NMR (250 MHz, CD_3OD): $\delta = 1.72$ (dd, $J = 7.0$, 1.8 Hz, 3 H, 3'-H), 2.11–2.29 (m, 1 H, 3-H_a), 2.35–2.53 (m, 1 H, 4-H), 3.02 (dd $J = 9.5$, 11 Hz, 1 H, 3-H_b), 3.60 (dd, $J = 7.5$,

11.1 Hz, 5-H_b), 4.55 (dd, $J = 4.7, 9.6$ Hz, 2-H), 5.35 (ddq, $J = 9.8, 9.8, 1.8$ Hz, 1 H, 1'-H), 5.74 (ddq, $J = 9.8, 1.0, 7.0$ Hz, 1 H, 2'-H); the residual peak of CD₂HOD masked absorption for 5-H_a; ¹³C NMR (62.9 MHz, CD₃OD): $\delta = 13.5$ (+, C-3'), 36.2 (–, C-3), 36.2 (+, C-4), 51.9 (–, C-5), 60.7 (+, C-2), 117.9 (q, $J = 289$ Hz, CF₃), 128.9 (+, C-2'), 129.7 (+, C-1'), 162.1 (q, $J = 37.4$ Hz, CF₃CO), 171.9 (C_{quat}, C-1); IR (film): $\nu(\text{tilde}) = 3250\text{--}1950\text{ cm}^{-1}$, 1679, 1631, 1410, 1203, 1137; MS (EI, 70 eV), m/z (%): 155 (3) [M⁺], 110 (100) [M⁺ – CHO₂], 87 (16), 69 (43) [CF₃⁺], 51 (16), 45 (45) [CHO₂⁺], 41 (15) [C₃H₅⁺]; HRMS (EI): calcd for C₈H₁₃NO₂·C₂HF₃O₂: 155.0946·113.9928; found 155.0946.

cis-4-(*Z*)-propenylproline hydrochloride HCl-*cis*-(**17**): Compound *cis*-**16** (10.2 mg, 0.040 mmol)

was deprotected with TFA (1 mL) at 20 °C for 20 min. The resulting

solution was concentrated under reduced pressure. The residue was

taken up with 2 M HCl/EtOAc (3 × 2 mL) and then concentrated under

reduced pressure. The residue was recrystallized four times from CH₂Cl₂/Et₂O to give after prolonged drying at 0.01 Torr hydrochloride *cis*-**17** (2.0 mg, 26%) as a hygroscopic brownish semisolid.

¹H NMR (250 MHz, D₂O): $\delta = 1.47$ (dd, $J = 6.9, 1.9$ Hz, 3 H, 3'-H), 1.61–1.81 (m, 1 H, 3-H_a), 2.45 (ddd, $J = 13.0, 7.1, 7.1$ Hz, 1 H, 3-H_b), 2.90 (dd $J = 10.1, 10.1$ Hz, 1 H, 5-H_a), 3.24 (dddd, $J = 9.0, 9.0, 9.0, 9.0, 9.0$ Hz, 4-H), 3.31–3.42 (m, 5-H_b), 4.25 (dd $J = 8.9$ Hz, 2-H), 5.11 (ddq, $J = 8.8, 8.8, 1.9$ Hz, 1 H, 1'-H), 5.44–5.58 (m, 2'-H); ¹³C NMR (62.9 MHz, D₂O): $\delta = 13.1$ (+, C-3'), 35.6 (–, C-3), 36.5 (+, C-4), 50.8 (–, C-5), 60.5 (+, C-2), 127.7 (+, C-2'), 129.6 (+, C-1'), 172.7 (C_{quat}, C-1); MS (ESI), positive m/z (%): 178 (6) [M + Na⁺], 156 (100) [M + H⁺].

tert-butyl (2)-3-oxo-2-oxa-5-azabicyclo[2.2.1]heptane-5-carboxylate (2*S*)-(22): A suspension of

dried Na₂CO₃ (0.72 g, 6.79 mmol) in a solution of (2*S*,3*R*)-**15** (1.28 g, 3.32 mmol) in butanone-2 (200 mL) was stirred under reflux for 24 h and MgSO₄

(one spoon) was then added. After 5 min the mixture was cooled to 20 °C and filtered. The filtrate was concentrated under reduced pressure to give yellow solid, which was recrystallized from EtOAc/hexanes 1:1 to give (2*S*)-**22** (0.645 g, 91%) as a colorless cubic crystals. M.p. 106.5–107 °C (lit.:^[30] m.p. 109–111 °C); $[\alpha]_{20}^D$ –48.3 (c = 1.04, CHCl₃) [lit.:^[30] $[\alpha]_{24}^D$ –49.6 (c = 1.40, CHCl₃)].

N-Carbophenoxy-(2*S*,4*R*)-4-hydroxyproline (2*S*,4*R*)-(**20**): Phenyl chloroformate (11.5 mL, 91.37

mmol) was added to a vigorously stirred ice-cold solution of 4-hydroxyproline (2*S*,4*R*)-**19** (10 g, 76.26 mmol) and NaHCO₃ (19.22 g, 228.8 mmol) in water (75 mL) for 5 min. The mixture was allowed to warm to 20 °C and stirring continued for a further 15 h. The mixture was filtered, the pH was adjusted to 0–1 with concentrated HCl and the resultant suspension or emulsion was extracted with THF (3 × 60 mL) under salting-out conditions. The organic layer was dried, filtered and concentrated under reduced pressure. The resulting solid was extracted with a big volume of boiling EtOAc. The resultant solution was concentrated to ca. 30 mL and the precipitate formed was filtered off, recrystallized from THF/Et₂O/hexanes and dried to give (2*S*,4*R*)-**20** (15.30 g, 80%) as a colorless pulver. $[\alpha]_{20}^D$ –45.8 (c = 0.86, THF); ¹H NMR (250 MHz, [D₆]DMSO): δ = 1.91–2.11 (m, 1 H, 3-H_a), 2.16–2.34 (m, 1 H, 3-H_b), 3.34–3.68 (m, 2 H, 5-H), 4.20–4.35 (m, 1 H, 4-H), 4.25, 4.44 (2 × dd, J = 8.0 Hz, 1 H, 2-H), 5.00–5.40 (br, 1 H, OH), 7.01–7.42 (m, 5 H, Ar-H); ¹³C NMR (62.9 MHz, [D₆]DMSO): δ = 38.3, 39.1 (–, C-3), 55.3, 55.4 (–, C-5), 55.3, 55.4 (+, C-2), 68.1, 68.9 (+, C-4), 121.9, 122.0 (+, Ar-C), 125.56, 126.00 (+, Ar-C), 129.6 (+, Ar-C), 151.2, 151.3 (C_{quat}, Ar-C), 152.6, 152.9 (C_{quat}, NCO₂), 173.6, 174.2 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde})$ = 3250–2750 cm^{–1}, 2990, 2975, 2953, 2894, 1744, 1677, 1414, 1200, 1074; MS (EI, 70 eV), m/z (%) = 251 (0.3) [M⁺], 130 (8), 94 (100) [C₆H₆O⁺], 77 (11) [C₆H₅⁺], 68 (28) [C₄H₆N⁺], 41 (13).

N-Carbophenoxy-(2*S*,4*R*)-4-hydroxyprolinol (2*S*,4*R*)-(21): A 1 M solution of BH₃ in THF (84

mL) was added dropwise to a stirred ice-cold solution of the acid (2*S*,4*R*)-20 (4.00 g, 15.92 mmol) in THF (80 mL) for 2 h, the mixture was allowed to warm to 20 °C and stirred at this temperature for a further 2 h, and finally refluxed for 30 min. The reaction flask was then cooled in

a water/ice bath and 1 M HCl in MeOH (90 mL) was carefully added for 1 h. All volatiles were then removed, the residue was redissolved in EtOAc (20 mL), cooled, filtered and the resultant clear solution was concentrated again. The oily residue was purified by column chromatography (MeOH/CHCl₃ 1:7, *R*_f = 0.44) to give (2*S*,4*R*)-21 (2.80 g, max. 74%) as a viscous colorless oil which contained ca. 4% of diphenyl carbonate according to ¹H NMR spectrum and was used without additional purification for the next step. ¹H NMR (250 MHz, CDCl₃): δ = 1.67–1.89 (m, 1 H, 3-H_a), 2.10 (dddd, *J* = 13.5, 7.0, 2.0, 2.0, 1 H, 3-H_b), 2.15–2.42 (br, 1 H, OH), 3.59–3.72 (m, 2 H, 5-H_a, 1-H_a), 3.72–3.86 (m, 2 H, 5-H_b, 1-H_b), 4.15–4.35 (m, 1 H, 4-H), 4.38–4.54 (br, 1 H, OH), 7.09–7.29 (m, 4 H, Ar-H), 7.30–7.43 (m, 1 H, Ar-H); The peak of 2-H was masked by absorption of OH; ¹³C NMR (62.9 MHz, CDCl₃): δ = 36.7, 37.0 (–, C-3), 55.5, 55.7 (–, C-5), 57.9, 59.2 (+, C-2), 63.2, 65.0 (–, C-1), 68.8 (+, C-4), 121.6 (+, Ar-C), 125.5 (+, Ar-C), 129.2, 129.3 (+, Ar-C), 150.7 (C_{quat}, Ar-C), 155.3 (C_{quat}, NCO₂).

(6*R*,7*aS*)-6-Hydroxyperhydro-1*H*-pyrrolo[1,2-*c*][1,3]oxazolone-3 (2*S*,4*R*)-(22): A 1.6 M *t*BuOK

in (10.5 mL, 16.80 mmol) was added dropwise to a stirred ice-cold solution of alcohol (2*S*,4*R*)-21 (4.00 g, 16.86 mmol) in THF (50 mL) for 15 min and stirring continued at the same temperature for 1 h. The pH was then carefully adjusted to 5–6 with 1 M HCl in MeOH and the mixture was filtered and

concentrated under reduced pressure. The residue was purified by column chromatography (CHCl₃/MeOH 15:1, *R*_f = 0.21) and then crystallized from EtOAc/hexanes to give (2*S*,4*R*)-21 (2.00 g, 83%) as a colorless solid. M.p. 66–69 °C; [α]₂₀^D –41.0 (*c* = 1.03, CHCl₃); ¹H NMR (500

MHz, CDCl₃): δ = 1.54 (ddd, J = 13.0, 10.7, 5.4 Hz, 1 H, 3-H_a), 2.03 (dd, J = 13.0, 5.5 Hz, 1 H, 3-H_b), 3.05 (ddd, J = 12.5, 1.5, 0.5 Hz, 1 H, 5-H_a), 3.21–3.25 (br, 1 H, OH), 3.79 (dd, J = 12.5, 5.5 Hz, 1 H, 5-H_b), 4.13 (dd, J = 8.9, 3.4 Hz, 1 H, 1-H_a), 4.17–4.24 (m, 1 H, 2-H), 4.51 (dd, J = 7.7, 8.9 Hz, 1 H, 1-H_b), 4.57 (ddd, J = 5.4, 1.0, 1.0 Hz, 1 H, 4-H); ¹³C NMR (125.7 MHz, CDCl₃): δ = 40.3 (–, C–3), 55.4 (–, C–5), 57.8 (+, C–2), 67.5 (–, C–1), 72.1 (+, C–4), 161.8 (C_{quat}, NCO₂); IR (KBr): $\nu(\text{tilde})$ = 3441 cm^{–1}, 2989, 2878, 2862, 1720, 1476, 1464, 1407, 1253, 1175, 1011; MS (EI, 70 eV), m/z (%) = 143 (40) [M⁺], 115 (20) [M⁺ – CO], 99 (100) [M⁺ – CO₂], 55 (24) [CHO₂⁺]; HRMS (EI): calcd for C₆H₉NO₃: 143.0582; found 143.0582; elemental analysis (%) for C₆H₉NO₃ (143.1): calcd. C 50.35, H 6.34, N 9.79; found C 50.66, H 6.28, N 9.67.

(6*R*,7*aS*)-6-Tosyloxyperhydro-1*H*-pyrrolo[1,2-*c*][1,3]oxazolone-3 (2*S*,4*R*)-(23): Pyridine (7.2

mL) was added to a stirring mixture of (2*S*,4*R*)-22 (1.03 g, 7.20 mmol) and tosyl chloride (2.74 g, 14.37 mmol) at the 4 °C for 10 min. The reaction flask was left at the same temperature for 4 days and the mixture was then stirred at 20 °C for 4 h. After this, the mixture was diluted with EtOAc (100

mL) and washed with ice-cold 1 M H₂SO₄ (5 × 40 mL), water (2 × 20 mL), brine (2 × 20 mL), dried, filtered and concentrated under reduced pressure. The residue was triturated with hexanes to give the crude product which was recrystallized from EtOAc/hexanes to give (2*S*,4*R*)-22 (1.78 g, 82%) as a colorless crystalline solid. R_f = 0.07 EtOAc/hexanes 1:2.5; $[\alpha]_{20}^D$ –53.8 (c = 1.56, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 1.43–1.73 (m, 1 H, 3-H_a), 2.32 (dd, J = 14.0, 5.0 Hz, 1 H, 3-H_b), 2.47 (s, 3 H, Me), 3.17 (dd, J = 13.8, 1.8 Hz, 1 H, 5-H_a), 3.91 (dd, J = 13.8, 6.0 Hz, 1 H, 5-H_b), 4.07–4.15 (m, 1 H, 2-H), 4.16 (dd, J = 9.4, 2.5 Hz, 1 H, 1-H_a), 4.53 (dd, J = 9.4, 8.9 Hz, 1 H, 1-H_b), 5.11–5.17 (m, 1 H, 4-H), 7.38 (d, J = 8.3 Hz, 2 H, Ar-H), 7.79 (d, J = 8.3 Hz, 2 H, Ar-H); ¹³C NMR (125.7 MHz, CDCl₃): δ = 21.4 (+, Me), 37.6 (–, C–3), 52.7 (–, C–5), 57.4 (+, C–2), 66.5 (–, C–1), 81.3 (+, C–4), 127.5, 130.0, 132.8 (+, Ar-C), 145.3 (C_{quat}, Ar-H), 160.9

(C_{quat}, NCO₂); IR (KBr): $\nu(\text{tilde}) = 3157\text{ cm}^{-1}$, 3068, 3058, 3024, 3003, 2980, 2960, 2918, 2895, 2803, 1759, 1597, 1405, 1328, 1320, 1262, 1185, 1175, 1092, 1060.

Attempt of the preparation of (6R,7aS)-6-(Z)-propenylperhydro-1H-pyrrolo[1,2-

c][1,3]oxazolone-3 (2S,4R)-(24): A solution of (2S,4R)-**23** (0.25 g, 0.84 mmol) in THF (5 mL) was added dropwise to a solution of (Z)-propenyllithium cuprate in Et₂O (10 mL) prepared as described for the preparation of *N*-Boc protected 4-(Z)-propenylprolines from

propenyllithium which was in turn prepared from lithium (90 mg, 11.76 mmol) and 1-(Z)-propenyl bromide (0.50 mL, 0.59 mmol) in Et₂O (5 mL) and suspension of CuBr₂·Me₂S (0.544 g, 2.65 mmol) in Et₂O (5 mL) at –40 °C for 30 min and stirring continued for a further 3 h. The reaction mixture was then allowed to warm to 0 °C and stirring continued for an additional 16 h. The reaction was then quenched with saturated aqueous NH₄Cl (4 mL) and the mixture was diluted with Et₂O (40 mL), the organic layer was separated, washed with 1 M H₂SO₄ (3 × 10 mL), water (3 × 10 mL), saturated NaHCO₃ (3 × 10 mL), water (3 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The residue (120 mg) was separated by column chromatography (EtOAc/hexanes 1:2.5, *R*_f = 0.22) to give (2S,4R)-**24** (11 mg, 5%) as a colorless oil and starting material (90 mg). According to NMR spectra *Z/E* ratio of this product was ca. 4:1. [α]₂₀^D –66.8 (*c* = 0.38, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 1.20–1.43 (m, 1 H, 3-H_a), 1.65 (dd, *J* = 7.0, 2.0 Hz, 3 H, 3'-H), 2.11 (ddd, *J* = 12.0, 5.5 Hz, 5.5 Hz, 1 H, 3-H_b), 3.14–3.46 (m, 3 H, 4-H, 5-H), 3.90–4.05 (m, 1 H, 2-H), 4.20 (dd, *J* = 9.0, 3.5 Hz, 1 H, 1-H_a), 4.49 (dd, *J* = 9.0, 8.3 Hz, 1 H, 2-H), 5.23–5.40 (m, 1 H, 1'-H), 5.53 (dq, *J* = 10.5, 7.5 Hz, 1 H, 2'-H); ¹³C NMR (62.9 MHz, CDCl₃): only spectrum for (*Z*)-isomer is described δ = 13.0 (+, C-3'), 38.4 (–, C-3), 38.5 (+, C-4), 51.3 (–, C-5), 59.2 (+, C-2), 67.3 (–, C-1), 126.1 (+, C-2'), 130.5 (+, C-1'), 158.3 (C_{quat}, NCO₂); IR (KBr): $\nu(\text{tilde}) = 3013\text{ cm}^{-1}$, 2974, 2935, 2917, 2887, 2859, 1753, 1393, 1328, 1252, 1230, 1206, 1077, 1061; MS (EI, 70 eV), *m/z* (%) = 167 (30) [*M*⁺], 99 (34)

[C₅H₉NO⁺], 82 (18) [C₅H₈N⁺], 67 (23), 55 (100) [CHO₂⁺], 41 (23) [C₃H₅⁺]; HRMS (EI): calcd for C₆H₉NO₃: 167.0946; found 167.0946.

2.2. Elucidation of the absolute configuration of 4-(Z)-propenylproline moiety in hormaomycin

tert-Butyl (2*R*,4*R*)-*N*-Boc-4-propylpyroglutamate (2*R*,4*R*)-**(27)**: (2*R*,4*R*)-**26** (0.22 g, 0.676 mmol)

was hydrogenated on 10 % Pd/C (90 mg) in EtOAc (10 mL) at 20 °C for 15 h. The reaction mixture was filtered, solvent was removed under reduced pressure and the product was purified by column chromatography (hexanes/EtOAc 4:1, *R_f* = 0.46) to give (2*R*,4*R*)-**27** as

a colorless oil (0.198 g, 90%). ¹H NMR (250 MHz, CDCl₃): δ = 0.89 (t, *J* = 7.1 Hz, 3 H, 3'-H), 1.28–1.50 (m, 3 H, 1'-H_a, 2'-H), 1.46 [s, 9 H, C(CH₃)₃], 1.48 [s, 9 H, C(CH₃)₃], 1.52–1.67 (m, 1 H, 3-H_a), 1.72–1.91 (m, 1 H, 1'-H_b), 2.42–2.61 (m, 2 H, 3-H_a, 4-H), 4.36 (dd, *J* = 8.8, 6.3 Hz, 1 H, 2-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 13.6 (+, C-3'), 20.1 (–, C-2'), 27.7 (–, C-1'), 27.7 [+ , 2 × C(CH₃)₃], 33.0(–, C-3), 42.2 (+, C-4), 57.9 (+, C-2), 81.8 [C_{quat}, C(CH₃)₃], 83.0 [C_{quat}, C(CH₃)₃], 149.3 (C_{quat}, NCO₂), 170.4 (C_{quat}, C-1), 175.4 (C_{quat}, C-5); IR (film): ν(tilde) = 2978 cm^{–1}, 2934, 2874, 1792, 1744, 1719, 1316, 1158; MS (CI), *m/z* (%): 362 (8) [M + 2 NH₄⁺ – H⁺], 345 (88) [M + NH₄⁺], 328 (6) [M + H⁺].

tert-Butyl (2*R*,4*R*)-*N*-Boc-4-propylprolinate (2*R*,4*R*)-**(28)**: A 1 M solution of lithium

triethylborohydride in THF (0.8 mL) was added to a solution of (2*R*,4*R*)-**(27)** (0.198 g, 0.605 mmol) in THF (5 mL) at –78 °C under nitrogen atmosphere. After 1 h the reaction mixture was quenched with saturated aqueous NaHCO₃ (2 mL) and warmed to 0 °C. 30%

H₂O₂ (3 drops) was added and the mixture was stirred at 0 °C. After 20 min the organic solvent

was removed under reduced pressure, and the aqueous layer was extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried, filtered and concentrated. The residue was dried at 0.01 Torr for 3 h, then dissolved in anhydrous CH_2Cl_2 (5 mL) containing triethylsilane (0.1 mL, 0.622 mmol) and was cooled to -78°C under nitrogen atmosphere. Boron trifluoride etherate (0.1 g, 0.705 mmol) was then added to the reaction mixture for 10 min. After 30 min, triethylsilane (0.1 mL, 0.622 mmol) and boron trifluoride etherate (0.1 g, 0.705 mmol) were added and the reaction mixture was stirred for 2 h at the same temperature. Saturated aqueous NaHCO_3 (2 mL) was added and the reaction mixture was extracted with CH_2Cl_2 (3×10 mL), dried, concentrated under reduced pressure and purified by column chromatography (EtOAc/hexanes 1:1, $R_f = 0.56$) to give (2*R*,4*R*)-**28** (0.150 g, 79%) as a colorless oil. $[\alpha]_{24}^D$ 75.4 ($c = 0.98$, CHCl_3) (lit. for (*S*)-isomer: ^[33b] $[\alpha]_{24}^D$ -52 ($c = 1.1$, CHCl_3)); ^1H NMR (250 MHz, CDCl_3): $\delta = 0.89$ (t, $J = 6.9$ Hz, 3 H, 3'-H), 1.22–1.38 (m, 5 H, 3-H_a, 1'-H, 2'-H), 1.42 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.42, 1.43 [$2 \times$ s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.97–2.21 (m, 1 H, 4-H), 2.37 (ddd, $J = 8.1, 8.1, 8.1$ Hz, 0.5 H, 3-H_b), 2.42 (ddd, $J = 7.8, 7.8, 7.8$ Hz, 0.5 H, 3-H_b), 2.94 (dd, $J = 10.1, 10.1$ Hz, 1 H, 5-H_a), 3.62 (dd, $J = 10.1, 7.3$ Hz, 0.3 H, 5-H_b), 3.74 (dd, $J = 10.1, 7.3$ Hz, 0.7 H, 5-H_b), 4.06 (dd, $J = 8.1, 8.1$ Hz, 0.7 H, 2-H), 4.11 (dd, $J = 8.1, 8.1$ Hz, 0.3 H, 2-H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 14.1$ (+, C-3'), 21.3 (–, C-2'), 27.9, 28.0 [+ , $\text{C}(\text{CH}_3)_3$], 28.3, 28.4 [+ , $\text{C}(\text{CH}_3)_3$], 35.0, 36.2 (–, C-1'), 37.3 (–, C-3), 37.6, 38.3 (+, C-4), 52.1, 52.4 (–, C-5), 59.8 (+, C-2), 79.4, 79.6 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 80.7 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 153.8 (C_{quat} , NCO_2), 172.4 (C_{quat} , C-1); IR (film): $\nu(\text{tilde}) = 2980\text{ cm}^{-1}$, 2931, 2871, 1717, 1703, 1398, 1367, 1174, 1152; MS (CI), m/z (%): 332 (12) $[\text{M} + \text{NH}_4^+]$, 314 (100) $[\text{M} + \text{H}^+]$.

(2*S*,4*S*)-Propylproline hydrochloride HCl·(2*S*,4*S*)-(29): (2*S*,4*R*)-16 (20 mg, 0.078 mmol) was

hydrogenated on 10 % Pd/C (5 mg) in EtOAc (2 mL) at 20 °C under ambient pressure of hydrogen for 15 h. The reaction mixture was

filtered, solvent was removed under reduced pressure and the residue

was treated with 2 N HCl in EtOAc at 20 °C for 1 h. Solvent was removed and (2*S*,4*S*)-29 was obtained as a an extremely hygroscopic colorless solid (12 mg, .79%). $[\alpha]_{20}^D -7.8$ ($c = 1.0$, CHCl₃).

(2*R*,4*R*)-4-propylproline hydrochloride (2*R*,4*R*)-(29): Compound (2*R*,4*R*)-9 (0.07 g, 0.223

mmol) was heated with 6 M HCl (2 mL) at 85 °C for 2 h. The reaction mixture was concentrated under reduced pressure. The traces of water was removed by codistillation with toluene (2 × 10

mL) and the residue was recrystallized thrice from CH₂Cl₂/Et₂O to give after prolonged drying at

0.01 Torr (2*R*,4*R*)-(6) (0.034 g, 79%) as an extremely hygroscopic white solid. $[\alpha]_{24}^D 7.7$ ($c =$

0.88, CHCl₃); ¹H NMR (250 MHz, D₂O): $\delta = 0.83$ (m, 3 H, 3'-H), 1.19–1.54 (m, 4 H, 1'-H, 2'-

H), 1.70 (dddd, $J = 12.8, 12.8, 10.0, 10.0$ Hz, 1 H; 4-H), 2.26–2.49 (m, 1 H, 3-H_a), 2.50–2.68 (m,

1 H, 3-H_b), 2.95 (dd, $J = 10.9, 10.9$ Hz, 1 H, 5-H_a), 3.50 (dd, $J = 10.9, 8.3$ Hz, 1 H, 5-H_b), 4.35

(dd, $J = 8.9, 8.9$ Hz, 1 H, 2-H); ¹H NMR (250 MHz, CD₃OD): $\delta = 0.99$ (t, $J = 7$ Hz, 3 H, 3'-H),

1.30–1.62 (m, 4 H, 1'-H, 2'-H), 1.74 (dddd, $J = 11.7, 11.7, 10.2, 10.2$ Hz, 1 H; 4-H), 2.33–2.68

(m, 1 H, 3-H_a), 2.65 (ddd, $J = 12.2, 6.7, 6.7$ Hz, 1 H, 3-H_b), 2.98 (dd, $J = 10.2, 10.2$ Hz, 1 H, 5-

H_a), 3.53 (dd, $J = 11.7, 7.8$ Hz, 1 H, 5-H_b), 4.37 (dd, $J = 8.6, 8.6$ Hz, 1 H, 2-H); ¹³C NMR (62.9

MHz, D₂O): $\delta = 14.1$ (+, C-3'), 21.5 (–, C-2'), 34.6 (–, C-1'), 35.3 (–, C-3), 38.8 (+, C-4), 51.6 (–,

C-5), 60.6 (+, C-2), 173.0 (C_{quat}, C-1); IR (film): $\nu(\text{tilde}) = 3432 \text{ cm}^{-1}$, 2960, 2931, 2873,

1736, 1389, 1252; MS (EI, 70 eV), m/z (%): 157 (1) [M⁺], 112 (100) [M⁺ – HCO₂], 87 (4), 70 (5)

[C₄H₈N⁺], 69 (11), 43 (15) [C₃H₇⁺], 41 (18) [C₃H₅⁺]; HRMS (EI): calcd for C₈H₁₅NO₂:

157.1103; found 157.1103.

Total hydrolysis of peptides (GP 1): Peptides were hydrolyzed with 6 M HCl/AcOH (1:1, 0.5 mL per 1 mg peptide) in sealed tube at 105 °C over 24 h. The solvent was then removed under reduced pressure and the residue was used for further derivatization.

Derivatisation with (S)-FDVA (GP 2): 0.4 mg probe was dissolved in 0.5 mL H₂O, pH of the solution was adjusted to 9 with 1 M NaHCO₃ and 1% acetone solution of (S)-FDVA (0.1 mL) was added. The reaction mixture was stirred at 40 °C for 1 h and then pH was adjusted to 6–7 with 0.1 M HCl. After this, the reaction mixture was diluted with 1 mL of MeCN and was directly used for HPLC/MS experiments.

Determination of the absolute configuration of (4-PE)Pro residue in hormaomycin 3: The hydrogenated hormaomycin (ca. 0.5 mg) was hydrolyzed and, after derivatisation with (S)-FDVA, was used for HPLC/MS experiments. Detection: UV: 340 nm, ESI-MS (positive) m/z: 437.7 – 438.7, ESI-MS (negative) m/z: 435.7 – 436.7; (S)-FDVA-(2*S*,4*S*)-**29**: *t*_R = 14.77 min, mix probe – 1 peak; (S)-FDVA-(2*R*,4*R*)-**29**: *t*_R = 17.98 min, mix probe – 2 peaks.

2.3. Attempts to prepare 4-(*Z*)-propenylproline starting from pyroglutamic acid

*N-Boc tert-Butyl (2*S*)-4-(1'-hydroxypropyl)pyroglutamate (S)-(38):* A solution of LiHMDS in THF was obtained by addition of a 2.47 M BuLi solution in hexanes (2.33 mL, 5.75 mmol) to a solution of HMDS (1.30 mL, 6.16 mmol) in THF (18 mL) at –78 °C followed by stirring of the resultant mixture at 4 °C for 1 h. It was then added to a solution of (S)-**25** (1.43 g, 5.01 mmol) in THF (40 mL) at –78 °C and the resultant mixture was stirred at the same temperature for a further 1 h. A solution of propionaldehyde (0.42 mL, 5.79 mmol) and BF₃·Et₂O (0.73 mL, 5.76 mmol) in THF (18 mL) was then added and stirring continued at the same

temperature for 75 min. The reaction was then quenched with saturated NH_4Cl (30 mL) and the mixture was diluted with Et_2O (250 mL). The organic layer was separated, washed with water (3×40 mL), 0.5 M H_2SO_4 (3×40 mL), water (3×40 mL), brine (2×30 mL), dried, filtered and concentrated under reduced pressure. The residue was separated by column chromatography (EtOAc/hexanes 1:3) to give one pure possible diastereomer (0.785 g, 46%) as a colorless oil and the inseparable mixture of two another diastereomers in ratio 1:3 [0.55 g, 78% overall yield of (*S*)-**38**]. No attempts to elucidate the configuration of these products were made. The pure diastereomer: $R_f = 0.26$ EtOAc/hexanes 1:3; ^1H NMR (250 MHz, CDCl_3): $\delta = 0.98$ (t, $J = 7.3$ Hz, 3 H, 3'-H), 1.48 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.50 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.84–2.12 (m, 2 H, 3-H), 2.63 (dt, $J = 12.2, 8.7$ Hz, 1 H, 4-H), 3.59–3.70 (m, 1 H, 1'-H), 4.24 (s, OH), 4.44 (d, $J = 8.0$ Hz, 1 H, 2-H); The signal of 2'-H was masked by absorptions of $\text{C}(\text{CH}_3)_3$; ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 8.8$ (+, C-3'), 24.9 (–, C-2'), 26.7 (–, C-3), 27.4 [+ , $\text{C}(\text{CH}_3)_3$], 27.5 [+ , $\text{C}(\text{CH}_3)_3$], 45.5 (+, C-4), 57.4 (+, C-2), 72.7 (+, C-1'), 82.1 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 83.2 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 148.5 (C_{quat} , NCO_2), 169.6 (C_{quat} , C-1), 176.1 (C_{quat} , C-5). The mixture of diastereomers: $R_f = 0.26$ EtOAc/hexanes 1:3; ^1H NMR (250 MHz, CDCl_3): $\delta = 0.99$ (t, $J = 7.3$ Hz, 3 H, 3'-H), 1.15–1.30 (m, 0.4 H, 2'-H_a), 1.48 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.50 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.85–2.10 (m, 0.6 H, 3-H), 2.31–2.80 (m, 2 H, 3-H, 4-H), 3.49–3.70 (m, 1 H, 1'-H), 4.12 (s, OH), 4.35 (dd, $J = 8.0, 8.0$ Hz, 0.7 H, 2-H), 4.43 (dd; $J = 1.2, 10.4$ Hz, 0.3 H, 2-H); The signal of 2'-H was partially masked by absorptions of $\text{C}(\text{CH}_3)_3$; ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 8.5, 9.0$ (+, C-3'), 18.5, 21.6 (–, C-2'), 24.6 (–, C-3), 27.0, 27.1 [+ , $\text{C}(\text{CH}_3)_3$], 27.7, 27.8 [+ , $\text{C}(\text{CH}_3)_3$], 46.7, 47.2 (+, C-4), 57.8, 57.9 (+, C-2), 70.0, 72.9 (+, C-1'), 82.1, 82.3 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 83.2, 83.8 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 148.9, 149.0 (C_{quat} , NCO_2), 169.8, 170.5 (C_{quat} , C-1), 174.6, 175.9 (C_{quat} , C-5).

tert-Butyl *N*-Boc 2-(*S*)-4-(*E*)-propylidenepyroglutamate (*S*)-(**39**): Et₃N (4.00 mL, 28.56 mmol)

followed by mesyl chloride (0.23 mL, 2.97 mmol) were added

dropwise to a stirred ice-cold solution of the mixture of diastereomers of (*S*)-**38** (0.55 g, 1.60 mmol) in CH₂Cl₂ (20 mL) and the mixture was

then allowed to warm to 20 °C. Stirring continued for a further 48 h and the reaction was then quenched with water (3 mL). EtOAc (80 mL)

was added and the organic layer was washed with water (4 × 20 mL) and brine (2 × 20 mL), dried, filtered and concentrated under reduced pressure. The resultant oily residue was identical with the one obtained by reaction of the pure diastereomer of (*S*)-**38** (0.785 g, 2.29 mmol) with Et₃N (5.8 mL, 41.44 mmol) and mesyl chloride (0.32 mL, 4.13 mmol) in CH₂Cl₂ (20 mL). Both portions were combined and separated by column chromatography (twice: first EtOAc/hexanes 3:7, then EtOAc/hexanes 1:3) to give (*E*)-**2** (0.76 g, 60%) as a faint tan solid and (*Z*)-isomer of (*S*)-**39** (55 mg, 4%). The configuration of the main product was confirmed with NOESY experiment.

(*S*)-**39**: *R*_f = 0.55 EtOAc/hexanes 3:7; ¹H NMR (250 MHz, CDCl₃): δ = 1.01 (t, *J* = 7.5 Hz, 3 H, 3'-H), 1.42 [s, 9 H, C(CH₃)₃], 1.45 [s, 9 H, C(CH₃)₃], 2.11 (dddd, *J* = 7.5, 7.5, 7.5, 7.5, 7.5 Hz, 2 H, 2'-H), 2.42–2.54 (m, 1 H, 3-H_a), 2.89–3.09 (m, 1 H, 3-H_b), 4.45 (dd, *J* = 7.8, 3.5 Hz, 1 H, 2-H), 6.57–6.70 (m, 1 H, 1'-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 12.6 (+, C-3'), 22.6 (–, C-2'), 25.5 (–, C-3), 27.7 [+ , C(CH₃)₃], 27.8 [+ , C(CH₃)₃], 56.4 (+, C-2), 82.0 [C_{quat}, C(CH₃)₃], 83.0 [C_{quat}, C(CH₃)₃], 127.9 (C_{quat}, C-4), 140.2 (+, C-1'), 149.8 (C_{quat}, NCO₂), 166.2 (C_{quat}, C-1), 170.2 (C_{quat}, C-5).

(*Z*)-isomer: *R*_f = 0.75 EtOAc/hexanes 3:7; δ = 1.01 (t, *J* = 7.5 Hz, 3 H, 3'-H), 1.45 [s, 9 H, C(CH₃)₃], 1.51 [s, 9 H, C(CH₃)₃], 2.43–2.58 (m, 1 H, 3-H_a), 2.75 (dddd, *J* = 7.5, 7.5, 7.5, 7.5, 7.5 Hz, 2 H, 2'-H), 2.89–3.09 (m, 1 H, 3-H_b), 4.42 (dd, *J* = 7.8, 3.5 Hz, 1 H, 2-H), 5.92–6.06 (m, 1 H, 1'-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 13.7 (+, C-3'), 20.6 (–, C-2'), 27.8 [+ , C(CH₃)₃],

27.9 [$+$, C(CH₃)₃], 29.1 ($-$, C-3), 56.5 ($+$, C-2), 82.0 [C_{quat}, C(CH₃)₃], 83.0 [C_{quat}, C(CH₃)₃], 126.0 (C_{quat}, C-4), 144.8 ($+$, C-1'), 150.1 (C_{quat}, NCO₂), 165.9 (C_{quat}, C-1), 170.2 (C_{quat}, C-5).

N-Boc *tert*-Butyl (2*S*,4*S*)-4-(*E*)-(1'-propenyl)pyroglutamate (2*S*,4*S*)-(40) and *N*-Boc *tert*-Butyl (2*S*,4*R*)-4-(*E*)-(1'-propenyl)pyroglutamate (2*S*,4*R*)-(40): A solution of LiHMDS in THF/HMPT was obtained by addition of a 2.47 M BuLi solution in hexanes (0.54 mL, 1.33 mmol) to a solution of HMDS (0.33 mL, 1.56 mmol) and HMPT (0.356 g, 1.99 mmol) in THF (5 mL) at $-78\text{ }^{\circ}\text{C}$ followed by stirring of the resultant mixture at $4\text{ }^{\circ}\text{C}$ for 15 min. It was then added dropwise to a solution of (*E*)-2 (0.375 g, 1.15 mmol) in THF (5 mL) at $-78\text{ }^{\circ}\text{C}$ within 20 min and the mixture was stirred at the same temperature for a further 1 h. A solution of 2,6-di-*tert*-butylphenol (0.713 g, 3.46 mmol) in THF (1 mL) was then added for 10 min. After that, a saturated aqueous NH₄Cl (ca. 8 mL) was added and the mixture was allowed to warm to $20\text{ }^{\circ}\text{C}$ and diluted with Et₂O (50 mL). The organic layer was separated, washed with water (3×40 mL), 0.5 M H₂SO₄ (3×40 mL), water (3×40 mL), brine (2×30 mL), dried, filtered and concentrated under reduced pressure. The residue containing the mixture of diastereomers and 2,6-di-*tert*-butylphenol was separated by column chromatography (twice, EtOAc/hexanes 1:3) to give (2*S*,4*S*)-40 (0.140 g, 37%) as a faint yellow oil and (2*S*,4*R*)-40 (70 mg, 19%) as a colorless solid. (2*S*,4*S*)-40 contained a little impurity of diastereomer (according to TLC; it was unobservable in ¹H NMR spectrum) and attempt to purify it by additional column chromatography was made. As the (2*S*,4*S*)-isomer isomerized to another diastereomer only material with essentially the same quality (70 mg) together with (2*S*,4*R*)-40 (40 mg) and the mixed fraction (25 mg) were obtained.

(2*S*,4*R*)-**40**: $[\alpha]_{24}^D$ 7.1 ($c = 0.31$, CHCl_3); $R_f = 0.42$ EtOAc/hexanes 1:3; ^1H NMR (250 MHz,

CDCl_3): $\delta = 1.48$ [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.50 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.71 (dd, $J = 6.3, 1.3$ Hz, 3 H, 3'-H), 2.04–2.31 (m, 2 H, 3-H), 3.19–3.24 (m, 1 H, 4-H), 4.44 (dd, $J = 9.0, 1.8$ Hz, 1 H, 2-H), 5.47 (dd, $J = 9.0, 1.3$ Hz, 1 H, 1'-H), 5.63 (dq, $J = 1.3, 6.3$ Hz, 1 H, 2'-H); ^{13}C NMR (62.9

MHz, CDCl_3): $\delta = 17.9$ (+, C-3'), 27.77 [+ , $\text{C}(\text{CH}_3)_3$], 27.79 [+ , $\text{C}(\text{CH}_3)_3$], 28.7 (–, C-3), 44.7 (+, C-4), 57.5 (+, C-2), 82.2 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 83.1 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 126.0 (+, C-2'), 129.7 (+, C-1'), 149.4 (C_{quat} , NCO_2), 170.1 (C_{quat} , C-1), 173.6 (C_{quat} , C-5); IR (KBr): $\nu(\text{tilde}) = 2979 \text{ cm}^{-1}$, 2934, 1781, 1743, 1708, 1457, 1367, 1296, 1221, 1155; MS (ESI), positive m/z (%) = 348 (75) [$\text{M} + \text{Na}^+$].

(2*S*,4*S*)-**40**: $R_f = 0.38$ EtOAc/hexanes 1:3; ^1H NMR (250 MHz, CDCl_3): $\delta = 1.47$ [s, 9 H,

$\text{C}(\text{CH}_3)_3$], 1.50 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.66 (dd, $J = 5.0, 1.3$ Hz, 3 H, 3'-H), 1.86 (dd, $J = 12.5, 6.5$ Hz, 1 H, 3-H_a), 2.53 (ddd, $J = 13.3, 6.5$ Hz, 1 H, 3-H_b), 3.09–3.23 (m, 1 H, 4-H), 4.38 (dd, $J = 9.0, 5.8$ Hz, 1 H, 2-H), 5.30–5.52 (m, 1 H, 1'-H), 5.52–5.72 (m, 1 H, 2'-H); ^{13}C NMR (62.9 MHz,

CDCl_3): $\delta = 17.8$ (+, C-3'), 27.7 [+ , $2 \times \text{C}(\text{CH}_3)_3$], 28.6 (–, C-3), 45.7 (+, C-4), 58.0 (+, C-2), 82.0 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 83.1 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 126.3 (+, C-2'), 129.3 (+, C-1'), 149.4 (C_{quat} , NCO_2), 170.1 (C_{quat} , C-1), 173.6 (C_{quat} , C-5).

2.4. Synthesis of *N*-Boc protected 4-(*Z*)-propenylproline and dideutero-4-(*Z*)-propenylproline starting from 4-hydroxyproline

N-Boc Methyl (*S*)-allylglicinate (2*S*)-**42**: A vigorously stirred suspension of zinc dust (0.926 g, 14.16 mmol) in anhydrous THF (2 mL) contained C₂H₄Br₂ (0.09 mL, 1.04 mmol) was heated with heat gun till boiling started and then cooled to 20 °C. After this, TMSCl (0.1 mL) was added and the suspension was stirred for a further 10 min. A solution of *N*-Boc protected 4-(2*S*,4*RS*)-iodoproline (2*S*)-**41** (1.30 g, 3.66 mmol) in THF (2.5 mL) was then added to the mixture, the temperature was increased to 30–35 °C and stirring continued at this temperature for 16 h. After this, the mixture was filtered, concentrated under reduced pressure and distributed between 0.5 M H₂SO₄ (20 mL) and Et₂O (40 mL). The organic layer was separated, washed with water (3 × 10 mL), brine (2 × 10 mL), dried, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/hexanes 1:4, *R*_f = 0.30) to give the title compound (0.56 g, 67%) as a colorless liquid. The spectral data were in accordance with literature. $[\alpha]_{20}^D$ 19.1 (*c* = 0.98, CHCl₃) [lit.:^[49] $[\alpha]_{20}^D$ 18.8 (*c* = 1.0, CHCl₃)].

N-Boc tert-Bityl (2*S*,3*R*)-4-hydroxyproline (2*S*,4*R*)-(**44**): Triethylbenzylammonium chloride (7.78 g, 34.16 mmol) was added to a vigorously stirred suspension of dried K₂CO₃ (123.0 g, 0.888 mol) in a solution of *N*-Boc protected hydroxyproline (2*S*,4*R*)-**43** (7.9 g, 34.16 mmol) and *tert*-butyl bromide (185 mL, 1.639 mol) in DMA (256 mL) and the mixture was stirred at 55–60 °C for 48 h. It was then poured in water (2 L) and the resultant suspension was extracted with Et₂O (500 mL). The organic layer was washed with water (8 × 100 mL), brine (2 × 50 mL), dried, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/hexanes 1:2, *R*_f = 0.32) and finally by two recrystallizations from hexanes to give

(2*S*,4*R*)-**44** (6.40 g, 65%) as a colorless solid. M.p. 64–67 °C (lit.:^[55] m.p. 62–64 °C); $[\alpha]_{20}^D = -58.0$ ($c = 0.84$, CHCl₃) [lit.:^[55] $[\alpha]_{20}^D = -64.8$ ($c = 1.3$, CHCl₃)]. The spectral data were in accordance with literature.

N-Boc *tert*-Bityl (2*S*,3*R*)-4-mesyloxyprolinate (2*S*,3*R*)-**(45)**: Mesyl chloride (1.97 mL, 25.40 mmol) was added to a solution of (2*S*,3*R*)-**44** (5.60 g, 19.49 mmol) and TEA (4.08 mL, 29.15 mmol) in CH₂Cl₂ (40 mL) at –20 °C for 5 min and the mixture was allowed to warm to 4 °C for 2 h. After this, the reaction was quenched with saturated NaHCO₃ (ca. 20 mL) and the mixture was diluted with Et₂O (200 mL). The organic layer was separated and washed with saturated NaHCO₃ (4 × 40 mL), water (2 × 40 mL), brine (2 × 40 mL), dried, filtered and concentrated under reduced pressure. The residue was recrystallized from Et₂O/pentane to give (2*S*,3*R*)-**45** (6.09 g, 86%) as a colorless solid. $R_f = 0.53$ EtOAc/hexanes 1:2. M.p. 67–69 °C (lit.:^[56] m.p. 78–79 °C); $[\alpha]_{20}^D -48.3$ ($c = 1.04$, CHCl₃) [lit.:^[56] $[\alpha]_{20}^D -49.6$ ($c = 1.40$, MeOH)]; ¹H NMR (250 MHz, CDCl₃): $\delta = 1.43$ [s, 9 H, C(CH₃)₃], 1.44 [s, 9 H, C(CH₃)₃], 2.19 (ddd, $J = 14.3, 5.8, 5.8$ Hz, 1 H, 3-H_a), 2.43–2.71 (m, 1 H, 3-H_b), 3.03 (s, 3 H, Me), 3.62–3.91 (m, 2 H, 5-H), 4.18–4.38 (m, 1 H, 2-H), 5.15–5.23 (m, 1 H, 4-H); ¹³C NMR (62.9 MHz, CDCl₃): $\delta = 27.9$ [+, C(CH₃)₃], 28.2 [+, C(CH₃)₃], 36.2, 37.5 (–, C–3), 38.7 (+, SO₂Me), 52.1, 52.4 (–, C–5), 57.9 (+, C–2), 77.9, 78.2 (+, C–4), 80.5, 80.6 [C_{quat}, C(CH₃)₃], 81.7 [C_{quat}, C(CH₃)₃], 153.5 (C_{quat}, NCO₂), 171.2 (C_{quat}, C–1).

N-Boc *tert*-Bityl (2*S*,3*S*)-4-cyanoprolinate (2*S*,4*S*)-**(46)**: A solution of the mesylate (2*S*,4*R*)-**45** (5.50 g, 15.05 mmol) and tetrabutylammonium cyanide (13.30 g, 49.55 mmol) in DMF (25 mL) was heated at 60–65 °C for 12 h. The mixture was then diluted with Et₂O (150 mL) and washed with water (7 × 40 mL),

brine (2×10 mL), dried and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/hexanes 1:2.5, $R_f = 0.65$) to give oil which was triturated with pentane to give (2*S*,4*S*)-**46** (2.52 g, 57%) as a colorless solid. M.p. 62–64 °C; ^1H NMR (250 MHz, CDCl_3): $\delta = 1.42, 1.45$ [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.47, 1.49 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 2.19 (ddd, $J = 13.8, 5.8, 5.8$ Hz, 1 H, 3- H_a), 2.53–2.75 (m, 1 H, 3- H_b), 3.08 (ddd, $J = 7.5, 7.5, 7.5$ Hz, 1 H, 5- H_a), 3.55–3.70 (m, 1 H, 5- H_b), 3.87–4.01 (m, 1 H, 4-H), 4.19, 4.22 ($2 \times$ dd, $J = 11.0, 5.8$ Hz, 1 H, 2-H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 14.7, 15.7$ (+, C-4), 27.7 [+ , $\text{C}(\text{CH}_3)_3$], 28.4 [+ , $\text{C}(\text{CH}_3)_3$], 33.2, 34.3 (–, C-3), 49.0, 49.2 (–, C-5), 58.67, 58.75 (+, C-2), 80.65, 80.72 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 81.2 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 119.3 (C_{quat} , CN), 153.0, 153.2 (C_{quat} , NCO_2), 170.1, 170.3 (C_{quat} , C-1); MS (CI), m/z (%): 314 (25) [$\text{M} + \text{NH}_4^+$], 297 (6) [$\text{M} + \text{H}^+$].

N-Boc (2*S*,3*R*)-4-mesyloxiprolinol methoxymethyl ether (2*S*,4*R*)-(**50**): Methoxymethyl bromide

(4.10 mL, 50.22 mmol) was added dropwise to a stirred solution of *N*-Boc protected 4-mesyloxiprolinol (2*S*,4*R*)-**47** (5.3 g, 17.94 mmol) and DIEA (9.20 mL, 51.50 mmol) in CH_2Cl_2 (30 mL) at -20 °C for

10 min. The temperature was allowed to reach 20 °C and stirring continued for a further 3 h. The reaction was then diluted with water (150 mL) and Et_2O (150 mL), the organic layer was separated and washed with water (3×30 mL), 1 M H_2SO_4 (3×30 mL), water (3×30 mL), brine (2×15 mL), dried and concentrated under reduced pressure to give (2*S*,4*R*)-**50** (5.60 g, 92% crude) as a faint turbid oil which was used for the next step without additional purification. ^1H NMR (250 MHz, CDCl_3): $\delta = 1.46$ [s, 9 H, $\text{C}(\text{CH}_3)_3$], 2.23–2.47 (m, 2 H, 3-H), 3.03, 3.04 ($2 \times$ s, 3 H, Me), 3.32, 3.33 ($2 \times$ s, 3 H, OMe), 3.43–3.65 (m, 2 H, 1- H_a , 5- H_a), 3.65–3.91 (m, 2 H, 1- H_b , 5- H_b), 3.99–4.23 (m, 1 H, 2-H), 4.59 (s, 2 H, OCH_2O), 5.14–5.19 (m, 1 H, 4-H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 28.3$ [+ , $\text{C}(\text{CH}_3)_3$], 34.9, 35.0 (–, C-3), 38.6 (+, Me), 52.2, 52.7 (–, C-5), 55.1, 55.3 (+, OMe), 67.6, 68.1 (–, C-1), 78.7 (+, C-4), 80.1 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 96.4 (–, OCH_2), 153.9 (C_{quat} , NCO_2); The absorptions of OMe masked the signals of C-2.

N-Boc (2*S*,3*S*)-4-cyanoprolinol methoxymethyl ether (2*S*,4*S*)-(**51**): A solution of the mesylate

(2*S*,4*S*)-**50** (1.63 g, 4.80 mmol) and tetrabutylammonium cyanide (2.60

g, 9.68 mmol) in DMF (15 mL) was heated at 60–65 °C for 10 h. The

mixture was then diluted with Et₂O (150 mL) and washed with water

(20 × 40 mL), brine (2 × 10 mL), dried and concentrated under reduced pressure. The residue

was purified by column chromatography (alumina, EtOAc/petrol ether 1:3, *R_f* = 0.26) to give

(2*S*,4*S*)-**51** (0.53 g, 41%) as an yellow oil. *R_f* = 0.43 EtOAc/hexanes 1:1.1 (1% TEA); ¹H NMR

(250 MHz, CDCl₃): δ = 1.46 [s, 9 H, C(CH₃)₃], 2.23–2.55 (m, 2 H, 3-H), 3.02 (dddd, *J* = 8.0, 8.0,

8.0, 8.0 Hz, 1 H, 4-H), 3.36 (s, OMe), 3.46 (dd, *J* = 11.0, 7.5 Hz, 1 H, 1-H_a), 3.57–3.83 (m, 2 H,

1-H_b, 5-H_a), 3.85–4.18 (m, 2 H, 2-H, 5-H_b), 4.63 (s, 2 H, OCH₂O); ¹³C NMR (62.9 MHz,

CDCl₃): δ = 26.1, 26.8 (+, C-4), 28.2, 28.3 [+ , C(CH₃)₃], 31.8, 32.7 (–, C-3), 49.5 (–, C-5), 55.2,

56.1 (+, OMe), 67.2, 67.8 (–, C-1), 80.3 [C_{quat}, C(CH₃)₃], 96.3 (–, OCH₂O), 120.1 (C_{quat}, CN),

153.4 (C_{quat}, NCO₂); The absorptions of OMe masked the signals of C-2; MS (CI), *m/z* (%): 288

(100) [M + NH₄⁺], 271 (55) [M + H⁺]; MS (EI, 70 eV): *m/z* (%) = 270 (4) [M⁺], 224 (3) [M⁺ –

C₂H₅O], 214 (6) [M⁺ – C₄H₈], 208 (23) [M⁺ – C₂H₆O₂], 195 (41) [M⁺ – C₃H₇O₂], 183 [M⁺ –

C₄H₇O₂], 167 (18), 139 (27) [C₇H₁₁N₂O⁺], 110 (10) [C₇H₁₂N⁺], 95 (91) [C₅H₇N₂⁺], 57 (100)

[C₄H₉⁺], 45 (25) [C₂H₅O⁺], 41 (10); HRMS: calcd for C₁₃H₂₂N₂O₄: 270.1580; found: 270.1580.

N-Boc (2*S*,3*S*)-4-formylprolinol methoxymethyl ether (2*S*,4*S*)-(**52**): A 1 M DIBAH in hexanes

(2.6 mL, 2.60 mmol) was added dropwise at –78 °C over 10 min to a

stirred solution of the cyanide (2*S*,4*S*)-**51** (0.53 g, 1.96 mmol) in

CH₂Cl₂ (12 mL). The reaction mixture was stirred at –25 – –20 °C for

2 h, whereafter methanol (1 mL) was added dropwise at 0 °C for 3 min and stirring was

continued at the same temperature for 20 min. A saturated aqueous NH₄Cl (10 mL) was then

added and the mixture was allowed to warm to 20 °C. After 20 min the reaction mixture was

diluted with Et₂O (100 mL) and saturated aqueous potassium sodium tartrate (20 mL) and

vigorous stirring continued for an additional hour. The phases were separated and the organic fraction was washed with 2% citric acid (3×25 mL), water (7×30 mL), brine (2×20 mL), the aqueous layer was back extracted with Et₂O (3×40 mL). The combined organic layers were dried, filtered and concentrated under reduced pressure to give aldehyde (2*S*,4*S*)-**52** (0.48 g, 90% crude yield) as an opaque oil, which was used for the next step without further purification. R_f = 0.54, EtOAc/hexanes 1:1.1 (0.1% TEA); ¹H NMR (250 MHz, CDCl₃): δ = 1.44 [s, 9 H, C(CH₃)₃], 2.15–2.42 (m, 2 H, 3-H), 2.77–2.91 (m, 1 H, 4-H), 3.27, 3.32 (2 $\times$ s, OMe), 3.41–3.83 (m, 4 H, 1-H, 5-H), 3.81–4.13 (m, 1 H, 2-H), 4.33–4.64 (m, 2 H, OCH₂O), 9.63 (s, 1 H, CHO); ¹³C NMR (62.9 MHz, CDCl₃): δ = 28.2 [+ , C(CH₃)₃], 28.6, 29.4 (–, C-3), 45.9 (–, C-5), 48.4, 49.0 (+, C-4), 55.0, 56.0 (+, OMe), 56.1, 56.5 (+, C-2), 68.3 (–, C-1), 79.6 [C_{quat}, C(CH₃)₃], 95.9, 96.3 (–, OCH₂O), 153.7 (C_{quat}, NCO₂), 200.4, 201.5 (+, CHO).

N-Boc (2*S*,4*RS*)-4-(*Z*)-propenylprolinol methoxymethyl ether (2*S*,4*RS*)-(**53**): A suspension of

NaHMDS (0.176 g, 0.96 mmol) and ethyltriphenylphosphonium bromide (0.356 g, 0.96 mmol) in hexanes was vigorously stirred under reflux for 3.5 h. The mixture was then cooled to –20 °C,

filtered in dry conditions through dried glass wool and the resultant turbid solution was concentrated under reduced pressure in dry conditions. The residue was then taken up with anhydrous THF (10 mL), cooled to –78 °C, and a solution of (2*S*,4*S*)-**52** (0.24 g, 0.89 mmol) in THF (5 mL) was added dropwise within 10 min. Stirring continued at the same temperature for a further 16 h, then the mixture was allowed to warm to 20 °C and stirred at this temperature for an additional 2 h. The reaction mixture was concentrated under reduced pressure, the residue was taken up with EtOAc/hexanes 1:5 (20 mL), filtered through Celite pad (1 cm), concentrated, dissolved in Et₂O (20 mL), concentrated, dissolved in Et₂O/hexanes 1:1 (20 mL), filtered, concentrated under reduced pressure. The residue was purified by column chromatography (alumina, EtOAc/hexanes 1:6) to give (2*S*,4*RS*)-**53** (95 mg, 38%) as a colorless oil, gradually

solidified in a colorless solid. The ratio of (2*S*,4*R*)/(2*S*,4*S*) was ca. 3/1 according to ¹H NMR spectrum. The signals of (*E*)-isomer couldn't be observed due to low content of it. *R*_f = 0.43 EtOAc/hexanes 1:5 (0.1% TEA); ¹H NMR (250 MHz, CDCl₃): δ = 1.43 [s, 9 H, C(CH₃)₃], 1.63 (d, *J* = 6.8 Hz, 3 H, 3'-H), 1.63–1.90 (m, 1 H, 3-H_a), 1.93–2.09 (m, 0.25 H, 3-H_b, *trans*-isomer), 2.01–2.35 (m, 0.75 H, 3-H_b, *cis*-isomer), 2.86 (ddd, *J* = 10.0, 10.0, 10.0 Hz, 1 H, 4-H), 2.87–3.05 (m, 1 H, 5-H_b), 3.33, 3.35 (2 × s, OMe), 3.37–4.07 (m, 4 H, 1-H, 2-H, 5-H_b), 4.53, 4.54 (2 × s, 2 H, OCH₂O), 5.24 (ddq, *J* = 10.8, 10.8, 1.8 Hz, 1 H, 1'-H), 5.46 (ddq, *J* = 1.0, 10.8, 6.8 Hz, 1 H, 1'-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 13.1 (+, C-3'), 28.4 [+ , C(CH₃)₃], 35.5 (+, C-4), 35.6, 36.6 (–, C-3), 51.9, 52.4 (–, C-5), 55.1 (+, OMe), 56.7 (+, C-2), 68.4, 69.3 (–, C-1), 79.3 [C_{quat}, C(CH₃)₃], 96.5 (–, OCH₂O), 125.8 (+, C-1'), 130.5, 130.8 (+, C-2'), 154.2 (C_{quat}, NCO₂); MS (CI), *m/z* (%): 303 (16) [M + NH₄⁺], 286 (100) [M + H⁺]; MS (EI, 70 eV): *m/z* (%) = 285 (3) [M⁺], 223 (3) [M⁺ – C₂H₆O], 210 (53) [M⁺ – C₃H₇O₂], 208 (23) [M⁺ – C₂H₆O₂], 154 (100) [C₉H₁₆NO⁺], 110 (45) [C₇H₁₂N⁺], 57 (30) [C₄H₉⁺], 45 (8) [C₂H₅O⁺], 41 (4) [C₃H₅⁺]; HRMS: calcd for C₁₅H₂₇NO₄: 285.1940; found: 285.1940.

N-Boc (2*S*,4*RS*)-4-(*Z*)-propenylprolinol (2*S*,4*RS*)-(**54**): The protected alcohol (2*S*,4*RS*)-**53** (54

mg, 0.19 mmol) was deprotected with TFA (0.5 mL) for 20 min. All volatiles was then removed under reduced pressure to leave the deprotected amino alcohol as a brown semisolid which was taken up

with CH₂Cl₂ (4 mL) and water (4 mL). NaHCO₃ (88 mg, 1.05 mmol) and then Boc₂O (100 mg, 0.46 mmol) were added to this vigorously stirred biphasic system and vigorous stirring continued for a further 3 h. *N,N*-dimethylaminopropylamine (30 mg, 0.29 mmol) was then added and after 10 min the mixture was taken up with Et₂O (50 mL). The organic layer was washed with 1 M H₂SO₄ (3 × 10 mL), water (3 × 10 mL), brine (2 × 10 mL), dried, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/hexanes 1:1, *R*_f = 0.74) to give somewhat impure mixture of diastereomers (2*S*,4*RS*)-**54** (31 mg, 67%) as a faint

yellow oil. This mixture could be separated by column chromatography (for details of this separation and spectral data of individual diastereomers see deprotection of the *N*-Boc, *O*-TBDMS protected 4-(*Z*)-propenylprolinol).

N-Boc (2*S*,4*RS*)-4-(2',2'-dibromovinyl)prolinol methoxymethyl ether (2*S*,4*RS*)-(**55**): To a stirred

ice-cold mixture of PPh₃ (2.016 g, 7.69 mmol), CBr₄ (1.70 g, 5.13 mmol) and activated Zn dust (0.34 g, 5.20 mmol) was added CH₂Cl₂ (5 mL), the temperature was allowed to reach 20 °C and the mixture was stirred for 24 h. The reaction flask was then

immersed with a water/ice bath and a solution of the aldehyde (2*S*,4*S*)-**52** (0.60 g, 2.20 mmol) in CH₂Cl₂ (5 mL) was added dropwise to the resultant beige suspension for 10 min. The mixture was stirred at the same temperature for 2 h, the temperature was then allowed to increase to 20 °C and stirring continued for a further 4 h. The mixture was then filtered through alumina/silica gel pad (0.5/1 cm), concentrated under reduced pressure, taken up with CH₂Cl₂/EtOAc/hexanes 1:0.7:0.3 (15 mL), filtered, concentrated under reduced pressure, taken up with CH₂Cl₂/EtOAc/hexanes 1:0.5:0.5 (15 mL), filtered and concentrated under reduced pressure, dissolved in EtOAc/hexanes 1:1 (15 mL), filtered and concentrated under reduced pressure, dissolved in EtOAc/hexanes 1:2.5, filtered and concentrated under reduced pressure again. The residue was purified by column chromatography (EtOAc/hexanes 1:2.5, *R_f* = 0.34) to give (2*S*,4*RS*)-**55** (6.36 g, 68%) as a colorless viscous oil. The ratio of (2*S*,4*R*)/(2*S*,4*S*)-isomers was 1/1.6 according to ¹H NMR spectrum. ¹H NMR (250 MHz, CDCl₃): δ = 1.45 [s, 9 H, C(CH₃)₃], 1.71–1.93 (m, 1 H, 3-H_a), 2.09–2.21 (m, 0.4 H, 3-H_b, *trans*-isomer), 2.32 (ddd, *J* = 11.0, 6.6, 6.6 Hz, 0.6 H, 3-H_b, *cis*-isomer), 2.91–3.22 (m, 2 H, 4-H, 5-H_a), 3.34, 3.35 (2 × s, OMe), 3.50–4.11 (m, 4 H, 1-H, 2-H, 5-H_b), 4.60, 4.61 (2 × s, 2 H, OCH₂O), 6.29 (d, *J* = 8.5 Hz, 0.4 H, 1'-H, *trans*-isomer), 6.37 (d, *J* = 8.5 Hz, 0.4 H, 1'-H, *cis*-isomer); ¹³C NMR (62.9 MHz, CDCl₃): δ = 28.2 [+ , C(CH₃)₃], 33.4, 34.0, 34.3 (–, C-3), 39.9, 40.8, 41.2 (+, C-4), 50.0, 50.1,

50.5 (–, C-5), 54.9 (+, OMe), 56.2 (+, C-2), 67.7, 68.0, 68.5 (–, C-1), 79.4 [C_{quat} , $C(\text{CH}_3)_3$], 90.1, 90.2 (C_{quat} , C-2'), 96.2 (–, OCH_2O), 138.7 (+, C-1'), 153.7 (C_{quat} , NCO_2); MS (CI), m/z (%): 447 (20) [$\text{M} + \text{NH}_4^+$], 430 (100) [$\text{M} + \text{H}^+$].

N-Boc (2*S*,4*RS*)-4-propynylprolinol methoxymethyl ether (2*S*,4*RS*)-**(56)**: A 2.34 M solution of

*n*BuLi in hexanes (1.61 mL, 3.77 mmol) was added to a solution of

(2*S*,4*RS*)-**(55)** (0.734 g, 1.71 mmol) in anhydrous THF (12 mL) at –

78 °C for 7 min and stirring continued at the same temperature for

a further 2 h. The temperature was then allowed to increase to

20 °C and the mixture was stirred at this temperature for an additional 1 h. The reaction flask

was immersed with dry ice/acetone bath and methyl iodide (0.47 mL, 7.55 mmol) was then

added for 20 min. The mixture was allowed to warm to 20 °C for 20 h and then stirred at this

temperature for an additional 1 h. After this, all volatiles were removed under reduced pressure

and the residue was purified by column chromatography (EtOAc/hexanes 1:4, R_f = 0.37) to give

(2*S*,4*RS*)-**(55)** (0.215 g, 44%) as a colorless oil which completely polymerized at the ambient

temperature in the presence of oxygen in several weeks. During the chromatographic purification

on this step the undesirable (2*S*, 4*S*)-isomer was partially separated. The ratio of (2*S*, 4*S*)/(2*S*,

4*R*)-isomers was 1:6 according to ^1H NMR spectrum. The signals of only main diastereomer

were observed in ^{13}C NMR spectrum. ^1H NMR (250 MHz, CDCl_3): δ = 1.45 [s, 9 H, $\text{C}(\text{CH}_3)_3$],

1.87–2.09 (m, 1 H, 3- H_a), 2.07–2.18 (m, 0.15 H, 3- H_b , *trans*-isomer), 2.30 (ddd, J = 11.0, 8.3,

8.3 Hz, 0.85 H, 3- H_b , *cis*-isomer), 2.72–2.89 (m, 1 H, 4-H), 3.12 (dd, J = 10.6, 8.6 Hz, 1 H, 5-

H_a), 3.33, 3.35 (2 $\times$ s, OMe), 3.49–4.03 (m, 4 H, 1-H, 2-H, 5- H_b); ^{13}C NMR (62.9 MHz, CDCl_3):

δ = 3.2 (+, C-4), 28.1 [+ , $\text{C}(\text{CH}_3)_3$], 35.2, 36.1 (–, C-3), 52.1, 52.6 (–, C-5), 54.8 (+, OMe), 56.1,

56.3 (+, C-2), 67.7, 68.5 (–, C-1), 77.2 (C_{quat} , C-2'), 79.0 (C_{quat} , C-1'), 79.4 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 96.2

(–, OCH_2O), 154.0 (C_{quat} , NCO_2); IR (film): $\nu(\text{tilde})$ = 2978 cm^{-1} , 2924, 2882, 1699, 1479,

1454, 1394, 1365, 1169, 1151, 1112, 1039; MS (EI, 70 eV): m/z (%) = 283 (0.04) [M^+], 251 (8)

$[M^+ - CH_4O]$, 227 (6) $[M^+ - C_4H_8]$, 208 (59) $[M^+ - C_3H_7O_2]$, 180 (6), 152 (100) $[C_9H_{14}NO^+]$, 108 (93) $[C_7H_{10}N^+]$, 57 (52) $[C_4H_9^+]$, 45 (15) $[C_2H_5O^+]$, 41 (6).

Attempt of the partial reduction of the triple bond on nickel catalyst in (2S,4RS)-56: To a vigorously stirred suspension of $Ni(OAc)_2 \cdot 5 H_2O$ (25 mg, 0.094 mmol) in EtOH (1 mL) in hydrogen atmosphere was added $NaBH_4$ (7 mg, 0.18 mmol). Ethylendiamine (3 drops) and then a solution of substance (2S,4RS)-53 (0.204 g, 0.72 mmol) in EtOH (2 mL) was added to the resultant thick black suspension and stirring continued for a further 1 h. The mixture diluted with EtOAc/hexanes 1:1 (3 mL) and filtered through silica gel pad (1 cm). The solvents were removed under reduced pressure and the residue was taken up with EtOAc/hexanes 1:2.5 (10 mL), filtered to remove the last traces of the catalyst and concentrated under reduced pressure to give colorless oil (0.190 g) of (2S,4RS)-53, which contained (according to 1H NMR spectrum) ca. 12–13% of *N*-Boc, *O*-MOM protected 4-propylprolinol.

(2S,4R)-*N*-Boc-4-Hydroxyprolinol (2S,4R)-(58): To a solution of (2S,4R)-43 (57.5 g, 248 mmol)

and triethylamine (38.4 mL, 273 mmol) in CH_2Cl_2 (1000 mL) at $-30\text{ }^\circ C$, ethyl chloroformate (25 mL, 261 mmol) was added and the mixture was stirred for 40 min. To this mixture tetra-*n*-butylammonium bromide (8.5 g, 26.4 mmol) and then suspension of $NaBH_4$ (40 g, 1057 mmol) in ice-cold water (50 mL) (carefully, by small portions) were added and the reaction mixture allowed to warm to $-10\text{ }^\circ C$ and stirred for 1 h. The temperature of the mixture was further increased to $0\text{ }^\circ C$ and stirring continued at this temperature for 1 h. pH of the aqueous layer was then carefully adjusted to 5 – 6 with 50% acetic acid and the mixture was filtered through Celite. The organic layer was separated and aqueous layer was extracted with CH_2Cl_2 ($3 \times 100\text{ mL}$). The aqueous layer was discarded and the combined organic fraction was dried, concentrated under reduced pressure and the residue was purified by column chromatography (EtOAc/hexanes 7:3, $R_f = 0.24$) to give

(2*S*,4*R*)-**58** (44.23 g, 82%) as a white solid. $[\alpha]_{24}^D - 58.8$ ($c = 1.05$, EtOH) (lit.:^[143] $[\alpha]_{24}^D - 58.87$, $c = 1.009$, EtOH); ^1H NMR (250 MHz, CDCl_3): $\delta = 1.47$ [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.57–1.80 (m, 1 H, 3- H_a), 1.89–1.98 (br, 1 H, OH), 1.98–2.11 (m, 1 H, 3- H_b), 3.35–3.63 (m, 3 H, 2-H, 5-H), 3.70 (dd, $J = 9.4$, 9.4 Hz, 1 H, 1- H_a), 4.04–4.25 (m, 1 H, 1- H_b), 4.29–4.45 (m, 1 H, 4-H), 4.95–5.09 (br, 1 H, OH); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 28.3$ [+ , $\text{C}(\text{CH}_3)_3$], 37.3 (–, C-3), 54.9, 55.6 (–, C-5), 57.7, 58.6 (+, C-2), 63.8, 66.4 (–, C-1), 68.8 (+, C-4), 80.4 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 155.0, 156.9 (C_{quat} , NCO_2); IR (film): $\nu(\text{tilde}) = 3381\text{ cm}^{-1}$, 2981, 2942, 2899, 1653, 1412, 1160, 1041.

(2*S*,4*R*)-*N*-Boc-*O*-TBDMS-4-Mesyloxyprolinol (2*S*,4*R*)-(**60**): To a solution of substance (2*S*,4*R*)-

59 (48.14 g, 145 mmol) and triethylamine (30.4 mL, 218 mmol) in CH_2Cl_2 (120 mL) at $-40\text{ }^\circ\text{C}$, mesyl chloride (15.2 mL, 196 mmol) was added for 10 min. The mixture was allowed to warm to $0\text{ }^\circ\text{C}$

for 3 h and saturated NaHCO_3 (100 mL) was added. The reaction mixture was taken up with Et_2O (500 mL), the organic layer was washed with H_2O ($3 \times 100\text{ mL}$), 1 M NaHSO_4 ($3 \times 100\text{ mL}$), H_2O ($2 \times 100\text{ mL}$), brine ($2 \times 100\text{ mL}$) and dried. Concentration under reduced pressure gave (2*S*,4*R*)-**60** (58.2 g, 98%) as a light yellow oil. $[\alpha]_{24}^D - 38.5$ ($c = 0.55$, CHCl_3); $R_f = 0.53$ ($\text{EtOAc/hexanes } 1:2.3$); ^1H NMR (250 MHz, CDCl_3): $\delta = 0.01$ [s, 6 H, $\text{Si}(\text{CH}_3)_2$], 0.85 [s, 9 H, $\text{SiC}(\text{CH}_3)_3$], 1.44 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 2.30–2.49 (m, 2 H, 3-H), 3.02 (s, 3 H, CH_3), 3.44–3.63 (m, 2 H, 5-H), 3.63–4.15 (m, 3 H, 1-H, 2-H), 5.25–5.33 (m, 1 H, 4-H); ^{13}C NMR (75.5 MHz, $\text{C}_2\text{D}_2\text{Cl}_4$, 353 K): $\delta = -5.7$ [+ , $\text{Si}(\text{CH}_3)_2$], 17.8 (C_{quat} , SiC), 25.6 [+ , $\text{SiC}(\text{CH}_3)_3$], 28.3 [+ , $\text{C}(\text{CH}_3)_3$], 34.8 (–, C-3), 38.4 (+, SO_2Me), 52.4 (–, C-5), 57.0 (+, C-2), 63.4 (–, C-1), 78.9 (+, C-4), 79.6 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 153.6 (C_{quat} , NCO_2); IR (film): $\nu(\text{tilde}) = 3381\text{ cm}^{-1}$, 2930, 2858, 1698, 1473, 1404, 1366, 1257, 1174, 1120; MS (ESI), (positive) m/z (%): 432 (91) [$\text{M} + \text{Na}^+$].

(2*S*,4*S*)-*N*-Boc-*O*-TBDMS-4-Cyanoprolinol (2*S*,4*S*)-**(61)**: A sealed flask containing a solution of

(2*S*,4*S*)-**60** (52.8 g, 129 mmol) and tetra-*n*-butylammonium cyanide

(72.0 g, 268 mmol) in anhydrous MeCN (50 mL) was placed into the

heated to 65–68 °C oil-bath. After stirring for 6 h, the mixture was

taken up with EtOAc/hexanes 1:4 (500 mL), washed with water (8 × 100 mL) and brine (2 × 50

mL), dried and filtered through a pad of silica gel (5 cm). The solvents were removed under

reduced pressure and the residue was purified by column chromatography (EtOAc/hexanes 1:3,

$R_f = 0.50$) to give (2*S*,4*S*)-**61** (26.50 g, 60%) as a yellowish oil solidified by seeding into a white

solid. M.p. 55 – 58 °C; $[\alpha]_{24}^D - 25.9$ ($c = 0.9$, CHCl₃); ¹H NMR (300 MHz, C₂D₂Cl₄, 358 K): $\delta =$

0.10 [s, 6 H, Si(CH₃)₂], 0.94 [s, 9 H, SiC(CH₃)₃], 1.48 [s, 9 H, C(CH₃)₃], 2.29–2.46 (m, 2 H, 3-

H), 2.96 (dddd, $J = 8.1, 8.1, 8.1, 8.1$ Hz, 1 H, 4-H), 3.42 (dd, $J = 8.4, 10.5$ Hz, 1 H, 5-H_a), 3.74

(dd, $J = 3.0, 9.8$ Hz, 1 H, 5-H_b), 3.79–3.95 (m, 2 H, 1-H_a, 2-H), 3.96 (dd, $J = 7.8, 10.8$ Hz, 1 H,

1-H_b); ¹³C NMR (75.5 MHz, C₂D₂Cl₄, 358 K): $\delta = -5.6$ [+, Si(CH₃)₂], 17.9 (C_{quat}, SiC), 25.7 [+,

SiC(CH₃)₃], 26.3 (+, C-4), 28.2 [+, C(CH₃)₃], 31.9 (–, C-3), 49.8 (–, C-5), 57.8 (+, C-2), 62.9 (–,

C-1), 80.0 [C_{quat}, C(CH₃)₃], 119.8 (C_{quat}, CN), 153.2 (C_{quat}, NCO₂); IR (film): $\nu(\text{tilde}) = 3381$

cm^{–1}, 2952, 2895, 2855, 2240, 1696, 1471, 1400, 1259, 1172, 1099; MS (CI), m/z (%): 358 (8)

[M + NH₄⁺], 341 (100) [M + H⁺]; elemental analysis calcd (%) for C₁₇H₃₂N₂O₃Si (340.22): C

59.96, H 9.47, N 8.23; found C 60.29, H 9.55, N 8.04.

(2*S*,4*S*)- and (2*S*,4*R*)-*N*-Boc-*O*-TBDMS-4-deutero-4-cyanoprolinols (2*S*,4*S*)-D-**(61)** and (2*S*,4*R*)-

D-**(61)**: To a solution of nitrile (2*S*,4*S*)-**61** (9.5 g, 27.90 mmol) in THF (50 mL) at –78 °C was

added dropwise a solution of ice-cold LDA, prepared from *n*BuLi (15 mL of 2.44 M solution in

hexanes, 36.27 mmol) and diisopropylamine (5.9 mL, 41.85 mmol) in THF (30 mL), for 40 min.

Stirring was continued for an additional 90 min and then the reaction was quenched with

saturated solution of Na₂SO₄ in D₂O (10 mL; under vigorous stirring) at the same temperature.

The mixture was allowed to warm to 20 °C and it was then concentrated under reduced pressure,

taken up with Et₂O (70 mL), filtered through Celite, concentrated again and separated by column chromatography (EtOAc/hexanes 1:3.7, *R_f* = 0.38, *trans*-isomer *R_f* = 0.54) to give (2*S*,4*S*)-D-**61** (4.21 g) and a mixed fraction enriched with (2*S*,4*R*)-D-**61** (3.62 g, 10.63 mmol), which was dissolved in THF (20 mL), again treated with solution of LDA, prepared from *n*BuLi (5.7 mL, 13.91 mmol) and diisopropylamine (2.23 mL, 15.91 mmol) in THF (15 mL) and quenched with saturated solution of Na₂SO₄ in D₂O (3 mL) as it was described above. Separation of the mixture by column chromatography gave second crop of *cis*-nitrile (2*S*,4*S*)-D-**61** (2.40 g). It was combined with the first fraction and finally purified by column chromatography as it was described above to give (2*S*,4*S*)-D-**61** (6.39 g, 67%, 90% D according to ¹H NMR spectrum) as a colorless oil, gradually solidified in a white solid.

(2*S*,4*S*)-D-**61**: m.p. 53 – 55 °C; [α]₂₄^D – 25.9 (*c* = 1.1, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ =

H, 2-H, 5-H_b); ¹³C NMR (62.9 MHz, CDCl₃): δ = –5.6 [+ , Si(CH₃)₂], 18.0 (C_{quat}, SiC), 25.7 [+ , SiC(CH₃)₃], 26.2 (t, *J* = 21.7 Hz, C–4), 28.2 [+ , C(CH₃)₃], 30.9, 32.1 (–, C–3), 49.5, 49.9 (–, C–5), 57.7 (+, C–2), 62.1, 63.3 (–, C–1), 80.0 [C_{quat}, C(CH₃)₃], 119.9 (C_{quat}, CN), 153.3 (C_{quat}, NCO₂); IR (film): ν (tilde) = 2952 cm^{–1}, 2895, 2858, 2241, 1700, 1471, 1405, 1258, 1177, 1101; MS (CI), *m/z* (%): 359 (1) [M + NH₄⁺], 342 (100) [M + H⁺]; elemental analysis (%) for C₁₇H₃₁DN₂O₃Si (341.23): C 59.78, H 9.74, N 8.20; found C 59.54, H 9.60, N 8.03.

The mixed fractions enriched with *trans*-epimer were purified by column chromatography as it

Si(CH₃)₂], 0.85 [s, 9 H, SiC(CH₃)₃], 1.44 [s, 9 H, C(CH₃)₃], 2.15–2.46 (m, 2 H, 3-H), 3.24–3.47 (m, 0.2 H, 4-H), 3.41–3.80 (m, 3.5 H, 0.5 1-H, 2-H, 5-H), 3.87–4.07 (m, 1.5 H, 1.5 1-H); ¹³C

NMR (62.9 MHz, CDCl₃): δ = -5.7 [+, Si(CH₃)₂], 18.0 (C_{quat}, SiC), 25.7 [+, SiC(CH₃)₃], 26.5 (t, J = 15.7 Hz, C-4), 28.2 [+, C(CH₃)₃], 32.4, 33.2 (-, C-3), 49.4, 49.5 (-, C-5), 57.5 (+, C-2), 63.5, 64.0 (-, C-1), 80.0 [C_{quat}, C(CH₃)₃], 120.0 (C_{quat}, CN), 153.2 (C_{quat}, NCO₂); IR (film): ν (tilde) = 2955 cm⁻¹, 2930, 2885, 2858, 2245, 1702, 1473, 1393, 1257, 1177, 1121; MS (ESI), (positive) m/z (%): 364 (48) [M + Na⁺].

(2*S*,4*S*)-*N*-Boc-*O*-TBDMS-4-Formylprolinol (2*S*,4*R*)-(62): A 1 M DIBAH in hexanes (36.1 mL,

for 2 h, whereafter methanol (2.1 mL) was added dropwise at 0 °C for 3 min and stirring was continued at the same temperature for 15 min. A saturated aqueous NH₄Cl (8.4 mL) was then added and the mixture was allowed to warm to 20 °C. After 45 min the reaction mixture was diluted with Et₂O (80 mL) and saturated aqueous potassium sodium tartrate (14 mL) and vigorous stirring continued for an additional hour. The phases were separated and the organic fraction was washed twice with solution of citric acid (5.14 g, 26.72 mmol) in water (120 mL), with water (5 × 50 mL), brine (2 × 20 mL), dried, filtered and concentrated under reduced pressure. The residue was taken up with hexanes (30 mL) filtered through Celite pad and concentrated under reduced pressure to give the aldehyde (2*S*,4*S*)-62 (9.0 g, 98% crude) as a colorless oil, which was used for the next step without further purification. R_f = 0.37, EtOAc/hexanes 1:4; ¹H NMR (250 MHz, CDCl₃): δ = 0.03 [s, 6 H, Si(CH₃)₂], 0.86 [s, 9 H, SiC(CH₃)₃], 1.43 [s, 9 H, C(CH₃)₃], 1.78–2.20 (m, 1 H, 3-H_a), 2.22–2.41 (m, 1 H, 3-H_b), 2.78–3.12 (m, 1 H, 4-H), 3.45–4.02 (m, 5 H, 1-H, 2-H, 5-H), 9.63 (s, 1 H, CHO).

(2*S*,4*S*)-*N*-Boc-*O*-TBDMS-4-Deutero-4-formylprolinol (2*S*,4*S*)-D-(62): A 1 M DIBAH in hexanes (22.6 mL, 22.6 mmol) was added dropwise at - 30 °C over 10 min to a stirred solution

of the cyanide (2*S*,4*S*)-D-**61** (5.72 g, 16.74 mmol) in CH₂Cl₂ (50 mL). The reaction mixture was stirred at –30 – –20 °C for 2 h, whereafter CH₃OD (1 mL) was added dropwise at 0 °C for 3 min and stirring was continued at the same temperature for 15 min. A saturated aqueous NH₄Cl (5.5 mL) was then added and the mixture was allowed to warm to 20 °C. After 45 min the reaction mixture was diluted with Et₂O (80 mL) and saturated aqueous potassium sodium tartrate (14 mL) and vigorous stirring continued for an additional hour. The phases were separated and the organic fraction was washed twice with solution of citric acid (3.21 g, 16.74 mmol) in water (65 mL), with water (5 × 50 mL), brine (2 × 20 mL), dried and concentrated under reduced pressure. The residue was taken up with hexanes (30 mL) filtered through Celite pad and concentrated under reduced pressure to give aldehyde (2*S*,4*S*)-D-**62** (5.52 g, 97% crude) as a colorless oil, which was used for next step without further purification. *R*_f = 0.37, EtOAc/hexanes 1:4; ¹H NMR (250 MHz, CDCl₃): δ = 0.02 [s, 6 H, Si(CH₃)₂], 0.87 [s, 9 H, SiC(CH₃)₃], 1.45 [s, 9 H, C(CH₃)₃], 2.18–2.31 (m, 2 H, 3-H), 3.43–4.03 (m, 5 H, 1-H, 2-H, 5-H), 9.65 (s, 1 H, CHO); ¹³C NMR (62.9 MHz, CDCl₃): δ = –5.5 [+ , Si(CH₃)₂], 18.3 (C_{quat}, SiC), 25.9 [+ , SiC(CH₃)₃], 28.4 [+ , C(CH₃)₃], 46.2, 46.5 (–, C–3), 48.7 (t, *J* = 19.7 Hz, C–4), 57.8 (+, C–2), 63.5, 64.2 (–, C–1), 79.4 [C_{quat}, C(CH₃)₃], 153.9 (C_{quat}, NCO₂), 201.7 (+, CHO).

(2*S*,4*R*)-*N*-Boc-4-(*Z*)-Propenylprolinol (2*S*,4*R*)-(**54**): A freshly prepared 0.85 M solution of *t*BuOK in THF (108 mL, 91.68 mmol) was added to a suspension of ethyltriphenylphosponium bromide (44.00 g, 118.52 mmol) in THF (50 mL) at 0 °C. The cooling bath was removed and stirring continued for an additional 2 h. The mixture was then cooled to –78 °C and a solution of (2*S*,4*S*)-**62** (9.00 g, 26.20 mmol) in THF (30 mL) was added dropwise within 2 h. Stirring continued at the same temperature for a further 24 h, and then the mixture was allowed to warm to 20 °C for 24 h. After 48 h, the reaction flask was immersed into an ice/water bath and a saturated aqueous Na₂SO₄ (50 mL) was added. The reaction mixture was concentrated under reduced pressure, the residue was taken up with CH₂Cl₂/Et₂O 1:4 (200 mL), filtered through a

silica gel pad (10 cm), concentrated, dissolved in Et₂O (20 mL), filtered, concentrated, dissolved in Et₂O/hexanes 1:1 (20 mL), filtered, concentrated, dissolved in hexanes (20 mL), filtered, concentrated, dissolved in pentane (20 mL), filtered, concentrated and finally purified by column chromatography (EtOAc/hexanes 1:8, R_f = 0.51) to give the impure (2*S*,4*R*)-*N*-Boc-*O*-TBDMS-4-(*Z*)-propenylprolinol (2*S*,4*S*)-**63** (5.3 g) which was used for the next step without further purification. TBAF·3H₂O (8.86 g, 28.12 mmol) was added to a stirring solution of crude alkene (5.0 g, max. 14.06 mmol) in THF (15 mL) at 20 °C. After 1.5 h the mixture was taken up with Et₂O (100 mL), washed with water (5 × 20 mL), brine (2 × 20 mL), dried, concentrated under reduced pressure and purified by column chromatography (EtOAc/hexanes 1:2.5, R_f = 0.32) to give (2*S*,4*R*)-**54** [2.69 g, 42% over 3 steps from (2*S*,4*S*)-**61**] as a colorless oil solidified by seeding into a white solid and (2*S*,4*S*)-epimer (2*S*,4*S*)-**54** (0.12 g, 2%). (2*S*,4*S*)-**54**: m.p. 41–43 °C; $[\alpha]_{24}^D$ – 47.9 (c = 0.97, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 1.23 (ddd, J = 11, 11, 11 Hz, 1 H, 3-H_a), 1.46 [s, 9 H, C(CH₃)₃], 1.65 (dd, J = 6.9, 0.8 Hz, 3 H, 3'-H), 2.13 (ddd, J = 6.7, 6.1, 6.1 Hz, 1 H, 3-H_b), 2.90 (dddd, J = 10.4, 10.4, 10.4, 10.4 Hz, 1 H, 5-H_a), 2.85–3.10 (m, 1 H, 4-H), 3.52–3.77 (m, 3 H, 5-H_b, 2-H, 1-H_a), 3.96 (dd, J = 7.6, 14.9 Hz, 1 H, 1-H_b), 5.18 (t, J = 9.8 Hz, 1 H, 1'-H), 5.30 (dd, J = 8.9, 1.8 Hz, 1 H, OH), 5.52 (dq, J = 9.8, 6.9 Hz, 1 H, 2'-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 13.2 (+, CH₃), 28.4 [+ , C(CH₃)₃], 35.2 (+, C-4), 35.8 (–, C-3), 52.7 (–, C-5), 61.1 (+, C-2), 67.6 (–, C-1), 80.4 [C_{quat}, C(CH₃)₃], 126.3 (+, C-2'), 129.8 (+, C-1'), 156.8 (C_{quat}, NCO₂); IR (film): ν (tilde) = 3398 cm^{–1}, 2976, 2932, 2871, 1696, 1402, 1164; MS (EI): m/z (%) = 241 (1) [M⁺], 210 (35) [M⁺–CH₃O], 168 (9), 154 (100) [M⁺–C₅H₁₁O], 110 (96) [C₇H₁₂N⁺], 67 (5), 57 (89) [C₄H₉⁺], 41 (15) [C₃H₅⁺]; HRMS: C₁₃H₂₃NO₃: 241.1678; elemental analysis (%) for C₁₃H₂₃NO₃ (241.3): calcd. C 64.70, H 9.61, N 5.80; found C 64.83, H 9.74, N 5.64.

(2*S*,4*R*)-**54**: R_f = 0.28 EtOAc/hexanes 1:2.5; ¹H NMR (250 MHz, CDCl₃): δ = 1.44 [s, 9 H, C(CH₃)₃], 1.60 (dd, J = 6.8, 1.8 Hz, 3 H, 3'-H), 1.68–1.85 (m, 2 H, 3-H), 3.01 (ddd, J = 9.5, 9.5, 9.5 Hz, 1 H, 5-H_a), 3.13 (dddd, J = 8.3, 8.3, 8.3, 8.3 Hz, 1 H, 4-H), 3.48 (dd, J = 6.9,

10.1 Hz, 1 H, 5-H_b), 3.54–3.59 (m, 2 H, 1-H_a, 2-H), 3.77–4.12 (m, 1 H, 1-H_b), 4.40 (br, 1 H, OH), 5.21 (dd, $J = 10.9, 10.9$ Hz, 1 H, 1'-H), 5.48 (dq, $J = 10.9, 6.8$ Hz, 1 H, 2'-H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 13.2$ (+, CH_3), 28.4 [+ , $\text{C}(\text{CH}_3)_3$], 34.8 (+, C-4), 35.1 (–, C-3), 52.7 (–, C-5), 59.6 (+, C-2), 67.7 (–, C-1), 80.2 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 125.9 (+, C-2'), 130.4 (+, C-1'), 158.3 (C_{quat} , NCO_2).

(2*S*,4*R*)-*N*-Boc-4,1'-Dideutero-4-(*Z*)-propenylprolinol (2*S*,4*R*)-D₂-(**54**): A freshly prepared 1.08 M solution of *t*BuOK in THF (45.0 mL, 41.67 mmol) was added to a suspension of (1,1-dideuteroethyl)triphenylphosponium bromide (18.5 g, 49.56 mmol) in THF (45 mL) at 0 °C, the cooling bath was removed and stirring continued for an additional 2 h. The mixture was then cooled to –78 °C and a solution of (2*S*,4*S*)-D-**62** (5.52 g, 16.02 mmol) in THF (30 mL) was added dropwise for 2 h. Stirring continued at the same temperature for further 24 h, and then the mixture was allowed to warm to 20 °C for 24 h. After 48 h, the reaction flask was immersed into an ice/water bath and a saturated aqueous Na_2SO_4 (50 mL) was added. The reaction mixture was concentrated under reduced pressure, taken up with $\text{CH}_2\text{Cl}_2/\text{Et}_2\text{O}$ (120 mL), filtered through silica gel pad (10 cm), concentrated, dissolved in Et_2O /hexanes 1:1 (50 mL), filtered, concentrated, dissolved in hexanes (50 mL), filtered, dissolved in pentane (50 mL), filtered, concentrated and finally purified by column chromatography (EtOAc /hexanes 1:10, $R_f = 0.39$) to give impure (2*S*,4*R*)-D₂-**63** (3.87 g) which was used for next step without further purification. TBAF·3H₂O (10.25 g, 32.48 mmol) was added to a stirring solution of crude alkene (3.87 g, max. 10.82 mmol) in THF (40 mL) at 20 °C. After 1.5 h the mixture was taken up with Et_2O (250 mL), washed with water (5 × 50 mL), brine (2 × 30 mL), dried, concentrated under reduced pressure and purified by column chromatography (EtOAc /hexanes 3:7, $R_f = 0.34$) to give (2*S*,4*R*)-D₂-**54** (1.84 g, 45% on 3 steps from **6**, D₂ and D contents 65% and 34% respectively, as determined by MS) as a colorless oil solidified by seeding into a white solid. M.p. 35–37 °C; $[\alpha]_{24}^{\text{D}} - 46.9$ ($c = 1.0$, CHCl_3); ^1H NMR (300 MHz, $\text{C}_2\text{D}_2\text{Cl}_4$, 373 K): the mixture of

isotopomers: δ = 1.36 (dd, J = 12 Hz, 12 Hz, 1 H, 3-H_a), 1.51 [s, 9 H, C(CH₃)₃], 1.68 (dd, J = 0.8 Hz, 3 H, 3'-H), 2.20 (dd, J = 7.1, 12.8 Hz, 1 H, 3-H_b), 2.93 (d, J = 11 Hz, 1 H, 5-H_a), 2.95–3.05 (m, 0.1 H, 4-H), 3.58–3.78 (m, 3 H, 2-H, 1-H_a, 5-H_b), 3.84–3.99 (m, 1 H, 1-H_b), 5.19–5.26 (m, 1 H, 1'-H), 5.33 (d, J = 7.8 Hz, OH), 5.58 (dq, J = 10.5, 6.0 Hz, 0.2 H, 2'-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 13.0, 13.1 (+, CH₃), 28.4 [+ , C(CH₃)₃], 34.7 (t, J = 18.6 Hz, C-4), 35.7 (–, C-3), 52.5 (–, C-5), 61.0 (+, C-2), 67.5 (–, C-1), 80.3 [C_{quat}, C(CH₃)₃], 125.3 (t, J = 23.2 Hz, C-2'), 126.2 (+, C-2'), 129.5, 129.7 (+, C-1'), 156.8 (C_{quat}, NCO₂); IR (film): ν (tilde) = 3416 cm^{–1}, 2976, 2932, 2870, 1696, 1411, 1174; MS (EI, 70 eV), m/z (%) = 243 (1) [M⁺], 212 (50) [M⁺ – CH₃O], 170 (9), 156 (100) [M⁺ – C₅H₁₁O], 112 (80) [C₇H₁₀D₂N⁺], 83 (6), 69 (5), 57 (92) [C₄H₉⁺]; HRMS (EI): calcd for C₁₃H₂₁D₂NO₃: 243.1803; found 243.1803; elemental analysis (%) for C₁₃H₂₂DNO₃ (242.3): calcd. C 64.43, H 9.98, N 5.78; found C 64.79, H 9.64, N 5.60.

(2*S*,4*R*)-*N*-Boc-4-(*Z*)-Propenylproline (2*S*,4*R*)-(16): A 2.67 M Jones reagent (36.5 mL, 97.46 mmol) was added to a solution of alcohol (2*S*,4*R*)-54 (2.35 g, 9.74 mmol) in acetone (800 mL) at 4 °C for 1 h and the mixture was stirred at the same temperature for an additional 2 h. Isopropanol (5 mL) was then added dropwise within 10 min and the mixture was allowed to warm to 20 °C. The reaction mixture was concentrated to 200 mL under reduced pressure at the bath temperature not higher than 30 °C, taken up with Et₂O (500 mL) and washed with water (3 × 100 mL). The aqueous fraction was back extracted with ether (3 × 50 mL) and the organic layers were combined, washed with brine (2 × 50 mL), dried, filtered, concentrated to 100 mL under reduced pressure and extracted with saturated aqueous NaHCO₃ (5 × 40 mL). The combined aqueous fractions were washed with Et₂O (2 × 50 mL), pH of the aqueous fractions was carefully adjusted to 2.5–3 with solid NaHSO₄, a formed emulsion was extracted with Et₂O (2 × 100 mL) and the organic fraction was washed with 1 M NaHSO₄ (3 × 50 mL), water (3 × 50 mL), brine (2 × 20 mL), dried, filtered and concentrated under reduced pressure. The residue was

recrystallized twice from hexanes and finally purified by column chromatography (EtOAc/hexanes 1:3 (2% AcOH), R_f = 0.27) to give (2*S*,4*R*)-**16** (1.63 g, 65%) as a white solid. HPLC: detection: 200 nm, t_R = 18.58 min, gradient: 20 → 50% MeCN in H₂O (0.1% TFA) for 30 min, purity > 99%; m.p. 84 – 85 °C; $[\alpha]_{24}^D$ –84.4 (c = 0.86, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 1.42, 1.48 [2 s, 9 H, C(CH₃)₃], 1.66 (d, J = 6.8 Hz, 3 H, 3'-H), 1.72–1.84, 1.93–2.12 (m, 1 H, 3-H_a), 2.27–2.54 (m, 1 H, 3-H_b), 3.06 (dddd, J = 9.9, 9.9, 9.9, 9.9, 9.9 Hz, 1 H, 4-H), 2.98–3.20 (m, 1 H, 5-H_a), 3.64–3.86 (m, 1 H, 5-H_b), 4.25, 4.35 (2 dd, J = 8.3, 8.3 Hz, 1 H, 2-H), 5.26 (ddq, J = 8.5, 8.5, 1.8 Hz, 1 H, 1'-H), 5.52 (dq, J = 8.5, 6.8 Hz, 1 H, 2'-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 13.1 (+, CH₃), 28.1, 28.3 [+ , C(CH₃)₃], 35.7, 36.1 (+, C-4), 36.1, 37.3 (–, C-3), 51.4, 51.9 (–, C-4), 58.9, 59.1 (+, C-2), 80.6 [C_{quat}, C(CH₃)₃], 126.6, 126.9 (+, C-2'), 129.0, 129.2 (+, C-1'), 153.6, 154.8 (C_{quat}, NCO₂), 177.3, 178.4 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde})$ = 3020, 2975, 2943, 2880, 2625, 1736, 1633, 1441, 1369, 1252, 1168; MS (ESI), positive m/z (%) = 300 (35) [M – H + 2Na⁺], 278 (16) [M + Na⁺]; negative m/z = 254 (100) [M – H[–]]; HRMS (EI): calcd for C₁₃H₂₁NO₄: 255.1471; found 255.1471; elemental analysis (%) for C₁₃H₂₁NO₄ (255.1): calcd. C 61.16, H 8.29, N 5.49; found C 61.16, H 8.23, N 5.31.

(2*S*,4*R*)-*N*-Boc-4,1'-Dideutero-4-(*Z*)-propenylproline (2*S*,4*R*)-D₂-(**16**): A 2.67 M Jones reagent (28.4 mL, 75.83 mmol) was added to a solution of alcohol (2*S*,4*R*)-

D₂-**54** (1.844 g, 7.58 mmol) in acetone (600 mL) at 4 °C within 1 h and the mixture was stirred at the same temperature for an additional 2h. Isopropanol (4 mL) was then added dropwise within 10 min and

the mixture was allowed to warm to 20 °C. The reaction mixture was concentrated to 150 mL under reduced pressure at the bath temperature not higher than 30 °C and was taken up with Et₂O (350 mL) and washed with water (3 × 100 mL). The aqueous fraction was back extracted with ether (3 × 50 mL) and the organic layers were combined, washed with brine (2 × 50 mL), dried,

filtered, concentrated to 75 mL under reduced pressure and extracted with saturated aqueous NaHCO_3 (5×40 mL). The combined aqueous fractions were washed with Et_2O (2×50 mL), pH of the aqueous fractions was carefully adjusted to 2.5–3 with solid NaHSO_4 and a formed emulsion was extracted with Et_2O (2×75 mL). The organic fraction was washed with 1 M NaHSO_4 (3×30 mL), water (3×30 mL), brine (2×10 mL), dried, filtered and concentrated under reduced pressure. The residue was recrystallized twice from hexanes and finally purified by column chromatography (EtOAc/hexanes 1:3 (2% AcOH), $R_f = 0.27$) to give (2*S*,4*R*)-**D₂-16** (1.18 g, 61%, D_2 and D contents 67% and 33% respectively, as determined by ESI-MS) as a white solid. HPLC: detection: 200 nm, $t_R = 18.54$ min, gradient: 20 $\rightarrow$ 50% MeCN in H_2O (0.1% TFA) for 30 min, purity > 99%; $[\alpha]_{24}^D - 68.6$ ($c = 1.05$, CHCl_3); ^1H NMR (250 MHz, CDCl_3): mixture of isotopo- and rotamers: $\delta = 1.41, 1.46$ [2 s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.64 (s, 3 H, 3'-H), 1.72–1.93 (m, 1 H, 3- H_a), 2.38, 2.46 (2 dd, $J = 7.5, 7.5$ Hz, 1 H, 3- H_b), 3.03 (d, $J = 11.5$ Hz, 0.5 H, 5- H_a), 3.08 (d, $J = 10.0$ Hz, 0.5 H, 5- H_a), 3.68, 3.76 (2 d, $J = 10.5$ Hz, 1 H, 5- H_b), 4.23, 4.33 (2 dd, $J = 8.4, 8.4$ Hz, 1 H, 2-H), 5.25 (m, 1'-H), 5.52 (dq, $J = 8.5, 6.8$ Hz, 0.15 H, 2'-H), 9.00–10.50 (br, 1 H, CO_2H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 12.8, 12.9$ (+, CH_3), 28.0, 28.1 [+ , $\text{C}(\text{CH}_3)_3$], 35.5 (t, C-4), 35.5 (+, C-4), 36.0, 37.0 (–, C-3), 51.2, 51.7 (–, C-5), 58.8, 59.0 (+, C-2), 80.4 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 126.4 (t, C-2'), 126.4, 126.7 (+, C-2'), 128.7, 128.9 (+, C-1'), 153.5, 154.6 (C_{quat} , NCO_2), 177.0, 177.9 (C_{quat} , C-1); IR (KBr): $\nu(\text{tilde}) = 3020, 2975, 2943, 2880, 2623, 1736, 1632, 1440, 1369, 1253, 1163$; MS (ESI), positive m/z (%) = 302 (14) [$\text{M} - \text{H} + 2\text{Na}^+$], 280 (100) [$\text{M} + \text{Na}^+$]; negative $m/z = 256$ (100) [$\text{M} - \text{H}^-$]; HRMS (ESI): calcd for $[\text{C}_{13}\text{H}_{19}\text{D}_2\text{NO}_4\text{Na}^+]$: 280.14883; found 280.14911.

(2*S*,4*R*)-4,1'-Dideutero-4-(*Z*)-propenylproline (2*S*,4*R*)-D₂-(**17**): The *N*-protected acid D₂-(2*S*,4*R*)-

D₂-**16** (0.155 g, 0.602 mmol) was dissolved in trifluoroacetic acid (2

mL) at 20 °C. After 20 min, all volatiles were removed under reduced

pressure to leave yellow oil, which was taken up with bidistilled water

(5 mL). The solution was then concentrated under reduced pressure (this operation was repeated

15 times). The residue was recrystallized thrice from MeOH/Et₂O to give (2*S*,4*R*)-D₂-**17** (34 mg,

36%) as a white solid. $[\alpha]_{24}^D - 4.2$ ($c = 0.31$, MeOH); ¹H NMR (250 MHz, D₂O): mixture of

isotopomers: $\delta = 1.63$ (s, 3 H, 3'-H), 1.75 (dd, $J = 11.3$ Hz, 1 H, 3-H_a), 2.54 (dd, $J = 8.3, 12.6$ Hz,

1 H, 3-H_b), 3.03 (d, $J = 11.5$ Hz, 1 H, 5-H_a), 3.36 (dddd, $J = 9.3, 9.3, 9.3, 9.3, 9.3$ Hz, 0.15 H, 4-

H), 3.48 (d, $J = 11.5$ Hz, 1 H, 5-H_b), 4.16 (dd, $J = 8.3$ Hz, 1 H, 2-H), 5.27 (m, 1'-H), 5.52 (m,

0.15 H, 2'-H); ¹³C NMR (62.9 MHz, D₂O): $\delta = 12.3, 12.4$ (+, CH₃), 35.5 (–, C-3), 35.8, (t, C-4),

36.1 (+, C-4), 50.0 (–, C-5), 61.2 (+, C-2), 127.3, 127.4 (+, C-1'), 127.8 (t, $J = 23.0$ Hz, C-2'),

128.5 (+, C-2'), 174.2 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 3101, 3008, 2969, 2915, 2856, 2361,$

1626, 1388, 1315; MS (EI, 70 eV), m/z (%): 157 (1) [M⁺], 112 (100) [M⁺ – CHO₂], 87 (16), 69

(43) [CF₃⁺], 54 (7), 41 (36) [C₃H₅⁺]; MS (ESI), positive m/z (%) = 202 (16) [M – H + 2Na⁺], 180

(6) [M + Na⁺]; negative $m/z = 156$ (100) [M – H[–]]; HRMS (EI): calcd for [C₈H₁₁D₂NO₂]:

157.1072; found 157.1072.

p-Nitrobenzyl (2*S*,4*R*)-*N*-Boc-4-(*Z*)-propenylprolinate (2*S*,4*R*)-(**68**): A suspension of K₂CO₃ (54

mg, 0.391 mmol) in a solution of (2*S*,4*R*)-**16** (0.100 g, 0.392 mmol) and *p*-nitrobenzylbromide

(89 mg, 0.412 mmol) in MeCN (1.5 mL) was stirred in a sealed tube at 70 °C for 3 h. The

mixture was then cooled, diluted with Et₂O (10 mL), filtered, concentrated under reduced

pressure and purified by column chromatography (EtOAc/hexanes 1:3, $R_f = 0.43$) to give

(2*S*,4*R*)-**68** (0.143 g, 88%) as a colorless glass. ¹H NMR (250 MHz, CDCl₃): $\delta = 1.34, 1.44$ [2 s,

9 H, C(CH₃)₃], 1.64 (d, $J = 6.8$ Hz, 3 H, 3'-H), 1.63–1.80 (m, 1 H, 3-H_a), 2.37–2.54 (m, 1 H, 3-

H_b), 2.98–3.26 (m, 2 H, 4-H, 5-H_a), 3.61–3.89 (m, 1 H, 5-H_b), 4.30, 4.37 (2 dd, $J = 8.3, 8.3$ Hz,

1 H, 2-H), 5.19 (dd, $J = 13.5$ Hz, 1 H, Bzl-H_a) 5.16–5.30 (m, 1 H, 1'-H), 5.35 (d, $J = 13.5$ Hz, 1 H, Bzl-H_b), 5.43–5.61 (m, 1 H, 2'-H), 7.52 (d, $J = 8.3$ Hz, 2 H, Ar-H), 8.21 (dd, $J = 7.5$, 7.5 Hz, 2 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): $\delta = 13.1$ (+, CH₃), 28.1, 28.3 [+ , C(CH₃)₃], 35.6, 36.3 (+, C-4), 36.5, 37.4 (–, C-3), 51.4, 51.9 (–, C-5), 59.0, 59.1 (+, C-2), 65.0 (–, C-Bzl), 80.0 [C_{quat}, C(CH₃)₃], 123.5, 123.7 (+, C-Ar), 126.7, 126.9 (+, C-2'), 128.1, 128.4 (+, C-Ar), 129.0, 129.1 (+, C-1'), 142.7, 143.1 (C_{quat}, C-Ar), 147.4, 147.6 (C_{quat}, C-Ar), 153.3, 154.1 (C_{quat}, NCO₂), 172.4, 172.5 (C_{quat}, C-1); IR (film): $\nu(\text{tilde}) = 3082$ cm^{–1}, 2976, 2932, 2869, 2623, 2241, 1754, 1699, 1524, 1400, 1347, 1258, 1174; MS (EI, 70 eV), m/z (%) = 390 (1) [M⁺], 289 (12) [M⁺ – C₅H₉O₂], 245 (4), 210 (56) [M⁺ – C₈H₆NO₄], 154 (100) [C₈H₁₂NO₂⁺], 110 (90) [C₇H₁₂N⁺], 57 (92) [C₄H₉⁺]; HRMS (EI): calcd for C₂₀H₂₆N₂O₆: 390.1791; found 390.1791; elemental analysis (%) for C₂₀H₂₆N₂O₆ (390.4): calcd. C 61.53, H 6.71, N 7.17; found C 61.20, H 6.61, N 6.99.

p-Nitrobenzyl (2*S*,4*R*)-*N*-Boc-4,1'-dideutero-4-(*Z*)-propenylprolinate (2*S*,4*R*)-D₂-(**68**): A

suspension of K₂CO₃ (0.467 g, 3.379 mmol) in a solution of (2*S*,4*R*)-D₂-**16** (0.87 g, 3.381 mmol) and *p*-nitrobenzylbromide (0.767 g, 3.550 mmol) in MeCN (5 mL) was stirred in a sealed

tube at 70 °C for 3 h. The mixture was then cooled, diluted with Et₂O (10 mL), filtered, concentrated under reduced pressure and purified by column chromatography (EtOAc/hexanes 1:3, $R_f = 0.43$) to give (2*S*,4*R*)-D₂-**68** (1.23 g, 88%) as a colorless glass. $[\alpha]_{24}^D -37.0$ ($c = 0.86$, CHCl₃); ¹H NMR (250 MHz, CDCl₃): mixture of isotopomers: $\delta = 1.34$, 1.44 [2 s, 9 H, C(CH₃)₃], 1.63 (s, 3 H, 3'-H), 1.63–1.80 (m, 1 H, 3-H_a), 2.37–2.51 (m, 1 H, 3-H_b), 3.06 (d, $J = 10.5$ Hz, 1 H, 5-H_a), 3.67, 3.78 (2 d, $J = 10.5$ Hz, 1 H, 5-H_b), 4.30, 4.37 (2 dd, $J = 8.8$, 8.8 Hz, 1 H, 2-H), 5.19 (dd, $J = 13.8$ Hz, 1 H, Bzl-H_a) 5.20–5.26 (m, 1 H, 1'-H), 5.35 (d, $J = 13.8$ Hz, 1 H, Bzl-H_b), 5.49–5.59 (m, 0.2 H, 2'-H), 7.52 (d, $J = 8.5$ Hz, 2 H, Ar-H), 8.20 (dd, $J = 7.8$, 7.8 Hz, 2 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): $\delta = 12.9$, 13.0 (+, CH₃), 28.0, 28.2 [+ ,

C(CH₃)₃], 35.1 (t, $J = 23.3$ Hz, C-4), 36.3 (+, C-4), 36.4, 37.3 (–, C-3), 51.3, 51.7 (–, C-5), 58.9, 59.0 (+, C-2), 64.9 (–, C-Bzl), 79.9 [C_{quat}, C(CH₃)₃], 123.5, 123.6 (+, C-Ar), 126.7, 126.8 (+, C-2'), 128.0, 128.3 (+, C-Ar), 128.9, 129.0 (+, C-1'), 142.7, 143.1 (C_{quat}, C-Ar), 147.4, 147.6 (C_{quat}, C-Ar), 153.2, 154.0 (C_{quat}, NCO₂), 172.3, 172.5 (C_{quat}, C-1); MS (EI, 70 eV), m/z (%) = 392 (1) [M⁺], 291 (12) [M⁺ – C₅H₉O₂], 247 (4), 212 (50) [M⁺ – C₈H₆NO₄], 156 (100) [C₈H₁₂NO₂⁺], 112 (80) [C₇H₁₂N⁺], 57 (92) [C₄H₉⁺]; HRMS (EI): calcd for C₂₀H₂₄D₂N₂O₆: 392.1916; found 392.1916; elemental analysis (%) for C₂₀H₂₅DN₂O₆ (391.8): calcd. C 61.37, H 6.95, N 7.16; found C 61.41, H 6.66, N 7.08.

(2*S*,4*R*)-4,1'-Dideutero-4-(*Z*)-propenylproline (2*S*,4*R*)-D₂-(**17**): To a solution of compound (2*S*,4*R*)-D₂-**68** (1.108 g, 2.679 mmol) in Et₂O (5 mL) a 5 M HCl solution in Et₂O (20 mL) was added and stirring continued in dark for 2 h. The mixture was then filtered and a fresh 5 M HCl solution in Et₂O (10 mL) was added. After 1 h, the mixture was diluted with hexanes (10 mL) and a formed precipitate was separated, washed with Et₂O and dried to give p-nitrobenzyl (2*S*,4*R*)-4,1'-dideutero-4-(*Z*)-propenylproline hydrochloride (0.775 g, 88%). MS (ESI), positive m/z (%): 293 (100) [M + H⁺]. A 1 M NaOH (4.89 mL) was added dropwise to an ice-cold solution of p-nitrobenzyl (2*S*,4*R*)-4,1'-dideutero-4-(*Z*)-propenylproline hydrochloride (0.775 g, 2.357 mmol) in MeOH (5 mL) for 10 min. Stirring continued at the same temperature for an additional 30 min, after that mixture was diluted with water (20 mL), methanol was removed under reduced pressure and the reaction mixture was extracted with Et₂O (10 × 10 mL). pH of the water fraction was carefully adjusted to 6.5–7.5 with 1 M HCl (ca. 2.40 mL) and water was removed under reduced pressure to give after prolonged drying at 0.01 Torr a mixture of (2*S*,4*R*)-D₂-**68** and NaCl (0.655 g, 0.292 g of NaCl, 99%) as a white solid which had the same spectral characteristics as described above and which was directly used for feeding experiments.

Trimethylsilyl (2*S*,4*R*)-1-trimethylsilyl-4-trimethylsilyloxyproline (2*S*,4*R*)-(65): To a

suspension of hydroxyproline (2*S*,4*R*)-**19** (13.1 g, 100 mmol) in HMDS (140 mL) was added H₂SO₄ (3 drops) and the mixture was refluxed till almost clear solution was obtained and then for a further 2 h. TMSCl (32 mL, 252 mmol) was then added and the mixture was refluxed for an additional 16 h. All volatiles were removed under reduced pressure (ca. 4 Torr, bath temperature not higher than 100 °C) to give the residue which was purified by bulb-to-bulb distillation to give (2*S*,4*R*)-**65** (34.4 g, 99%) as a turbid very sensitive to moisture liquid. ¹H NMR (250 MHz, CDCl₃): δ = 0.03 [s, 9 H, Si(CH₃)₃], 0.09 [s, 9 H, Si(CH₃)₃], 0.25 [s, 9 H, Si(CH₃)₃], 2.00 (dd, *J* = 8.6, 8.6 Hz, 2 H, 3-H), 2.84 (dd, *J* = 9.5, 3.8 Hz, 1 H, 5-H_a), 3.21 (dd, *J* = 9.5, 5.0 Hz, 1 H, 5-H_b), 3.90 (dd, *J* = 6.5, 6.5 Hz, 1 H, 2-H), 4.36 (dddd, *J* = 4.0, 4.0, 4.0, 4.0 Hz, 1 H, 4-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = -0.98 [+ , Si(CH₃)₃], -0.36 [+ , Si(CH₃)₃], -0.04 [+ , Si(CH₃)₃], 40.4 (-, C-3), 55.1 (-, C-5), 60.5 (+, C-2), 72.2 (+, C-4), 177.0 (C_{quat}, C-1).

(3*R*,6*R*,7*aS*)-3-*tert*-Butyl-6-[(trimethylsilyl)oxy]perhydro-1*H*-pyrrolo[1,2-*c*][1,3]oxazolone-1

(3*R*,6*R*,7*aS*)-(66): To a solution of (2*S*,4*R*)-**65** (13.16 g, 37.85 mmol) in pentane (15 mL) pivalic aldehyde (4.11 mL, 37.84 mmol) was added dropwise within 10 min and then NH₄Cl (ca. 0.05 g) was added (if exothermic reaction didn't occur, more NH₄Cl was added; in some runs TFA (several drops) were used instead of NH₄Cl). After 2 h, the mixture was diluted with pentane (25 mL), cooled to -5 °C and filtered while cold under anhydrous conditions. The resultant almost clear solution was concentrated under reduced pressure to give (3*R*,6*R*,7*aS*)-**66** (5.18 g, 50%) as a faint turbid very sensitive to moisture oil. The diastereomeric purity of this product varied strongly from run to run and was 80–99% according to ¹H NMR spectra. ¹H NMR (250 MHz, CDCl₃): δ = 0.11 [s, 9 H, Si(CH₃)₃], 0.92 [s, 9 H, C(CH₃)₃], 2.04–2.27(m, 2 H, 3-H), 2.89 (dd, *J* = 11.0, 8.0 Hz, 1 H, 5-H_a), 3.13 (dd, *J* = 11.0, 5.0 Hz, 1 H, 5-H_b), 4.27 (dd, *J* =

11.3, 5.5 Hz, 1 H, 2-H), 4.26 (dddd, $J = 5.5, 5.5, 5.5, 5.5$ Hz, 1 H, 4-H), 4.46 (s, 1 H, 1'-H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = -0.08$ [+ , Si(CH₃)₃], 24.0 [+ , C(CH₃)₃], 37.4 [C_{quat}, C(CH₃)₃], 38.0 (–, C-3), 61.3 (–, C-5), 64.9 (+, C-2), 70.7 (+, C-4), 108.2 (+, C-1'), 177.3 (C_{quat}, C-1).

(2*S*,4*R*)-2-deuteroxyproline (2*S*,4*R*)-D-(**19**): To a solution of diisopropylamine (2.94 mL, 20.98 mmol) in THF (50 mL) was added a 2.34 M *n*BuLi in hexanes (8.9 mL, 20.83 mmol) at –78 °C for 5 min. The temperature was then allowed to reach 4 °C and stirring continued at this temperature for a further 25 min. The resultant LDA solution was transferred through Teflon tube in a dropping funnel immersed with an ice/water bath and added dropwise to a solution of (3*R*,6*R*,7*aS*)-**66** (5.18 g, 19.08 mmol, *dr* > 95:5) in THF (80 mL) at –78 °C for 1 h. After this, stirring continued at the same temperature for a further 30 min. A solution of 2.34 M BuLi in hexanes (8.9 mL, 20.83 mmol) in THF (50 mL) was then added for 30 min and the mixture was stirred for an additional 1 h. The reaction was quenched with CH₃OD (4 mL) and the temperature was allowed to reach 20 °C. 6 M HCl (50 mL) was then added and the mixture was stirred for 2 h. The mixture was then concentrated under reduced pressure and the residue was dissolved in water and passed through column with Amberlite IR-120 in H⁺-form (ca. 250 mL). The column was washed with water till neutral pH of the eluat and then with 10% aqueous NH₃ till the eluat didn't contain ninhydrine active compounds according to TLC. These washings were combined and concentrated to ca. 200 mL. The resultant brown solution was extracted with *n*-butanol saturated with water (3 × 50 mL). The aqueous fraction was then concentrated under reduced pressure and the residue was taken up with EtOH (15 mL). The precipitate formed was filtered off, dried and precipitated from water with acetone to give (2*S*,4*R*)-D-**19** (0.40 g, 16%) as an off-white solid. The spectral data of this compound were in accordance with literature. The deuteration degree was ca. 87% according to ^1H NMR spectrum. $[\alpha]_{20}^D -73.4$ ($c = 0.95$, CHCl_3) [lit.:^[67] $[\alpha]_{20}^D -72.1$ ($c = 1.0$, CHCl_3)].

(3*R*,6*R*,7*aS*)-3-(Trichloromethyl)-6-[(trimethylsilyl)oxy]perhydro-1*H*-pyrrolo[1,2-*c*][1,3]oxazolone-1 (3*R*,6*R*,7*aS*)-(67): A mixture of (2*S*,4*R*)-65 (34.01 g, 97.8 mmol) and chloral (10 mL,

102.6 mmol) was stirred at 20 °C for 16 h. As according to the ¹H NMR spectrum of the reaction mixture the conversion was very low (< 10%), anhydrous MeCN (20 mL), chloral (10 mL, 102.6 mmol) and TFA (3 drops) were added. The temperature of the mixture started to increase

and the reaction flask was then immersed with a water/ice bath. The mixture was allowed to warm to 20 °C for 2 h and stirring continued at this temperature for an additional 1 h. All volatiles were removed under reduced pressure (first 4 Torr, then 0.02 Torr) and the black solid residue was extracted with anhydrous Et₂O (3 × 75 mL). The ethereal solution was concentrated under reduced pressure and the residue (ca. 28.5 g) was triturated with anhydrous hexanes (ca. 150 mL), the resultant solution was decanted and concentrated under reduced pressure. The residue was taken up with pentane (200 mL), the solution was filtered and cooled to –78 °C. The mother liquor was then decanted, the residual solid was taken up again with pentane (200 mL) and cooled to –78 °C. The mother liquor was decanted and the residual solid was washed with cold pentane (–78 °C) and dried to give (3*R*,6*R*,7*aS*)-67 (16.23 g, 50%) as a colorless reasonably stable to the atmospheric moisture solid. According to ¹H NMR spectrum, *dr* was > 94:6. $[\alpha]_{20}^D - 5.8$ (*c* = 1.32, THF); ¹H NMR (250 MHz, CDCl₃): δ = 0.13 [s, 9 H, Si(CH₃)₃], 2.17 (dd, *J* = 6.3, 6.3 Hz, 2 H, 3-H), 3.18 (dd, *J* = 11.3, 5.5 Hz, 1 H, 5-H_a), 3.33 (dd, *J* = 11.3, 5.3 Hz, 1 H, 5-H_b), 4.25 (dd, *J* = 7.3, 7.3 Hz, 1 H, 2-H), 4.35 (dddd, *J* = 5.5, 5.5, 5.5, 5.5 Hz, 1 H, 4-H), 5.11 (s, 1 H, 1'-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = – 0.05 [+ , Si(CH₃)₃], 38.1 (–, C-3), 61.0 (+, C-2), 64.1 (–, C-5), 70.7 (+, C-4), 100.6 (C_{quat}, CCl₃), 103.6 (+, C-1'), 175.1 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 2960 \text{ cm}^{-1}$, 2912, 2884, 1783, 1389, 1263, 1259, 1189, 1126, 997; MS (CI), *m/z* (%): 355/353/351 (6/18/18) [M + NH₄⁺], 336/334/332 (31/96/100) [M + H⁺].

2.5. *Elucidation of the absolute configuration of trans-(2'-nitrocyclopropyl)alanine residue in hormaomycin. Synthesis of (2R,1'R,2'R)-(2'-nitrocyclopropyl)alanine and (2S,1'RS,2'RS)-2-deutero-(2'-nitrocyclopropyl)alanine*

(1R,2R)-2-Nitrocyclopropanecarboxylic acid (1R,2R)-**(83)**: A 30% aqueous H₂O₂ (8.5 mL) and

then a 0.8 M aqueous LiOH (137 mL) (the latter dropwise for 50 min) were added to an effectively stirred solution of (2R,1'R,2'R)-**(83)** (16.55 g, 68.61 mmol) in undistilled THF (400 mL) at $-2 - 0^{\circ}\text{C}$ (internal temperature) and stirring continued at the same temperature for additional 40 min. A solution of NaHSO₃ (34.59 g, 274.44 mmol) in water (70 mL) was carefully added by portions at the same temperature. THF was removed under reduced pressure at the bath temperature $0-10^{\circ}\text{C}$ and the resultant suspension was diluted with ice-cold water (500 mL) and extracted with cold CH₂Cl₂ (5×100 mL). The organic layers were then pooled, washed with water (3×100 mL), brine (2×50 mL), dried, filtered and concentrated under reduced pressure to give (R)-4-isopropylloxazolidin-2-one (6.15 g, 69%). The pH of the aqueous layer was carefully adjusted to ca. 1 with solid NaHSO₄ and the resulting emulsion was extracted with EtOAc (5×200 mL) under salting-out conditions. The organic fractions were pooled, washed with brine (3×200 mL), dried, filtered and concentrated under reduced pressure to ca. 40 mL. Hexanes (ca. 100 mL) were then added, the mixture was left at 4°C for 16 h and then the precipitate was filtered off. This crude product was finally purified from CH₂Cl₂/hexanes to give the acid (1R,2R)-**(83)** (7.03 g, 78%) as a colorless solid. The spectral data of this compound were in accordance with those for the racemic acid. $[\alpha]_{20}^D -223.9$ ($c = 0.81$, THF).

(1S,2S)-2-Nitrocyclopropanecarboxylic acid (1S,2S)-**(83)**: A 30% aqueous H₂O₂ (1.95 mL) and then a 0.8 M aqueous LiOH (31.5 mL) (the latter dropwise for 50 min) were added to an

effectively stirred solution of (2*R*,1'*S*,2'*S*)-**9** (3.80 g, 15.75 mmol) in undistilled THF (92 mL) at $-3 - 0\text{ }^{\circ}\text{C}$ (internal temperature) and stirring continued at the same temperature for additional 50 min. A solution of NaHSO₃ (13.97 g, 110.84 mmol) in water (50 mL) was then carefully added by portions at the same temperature. THF was removed under reduced pressure at the bath temperature 0–10 °C and the resultant suspension was diluted with ice-cold water (250 mL) and extracted with cold CH₂Cl₂ (4 × 50 mL). The organic layers were then pooled, washed with water (3 × 50 mL), brine (2 × 30 mL), dried, filtered and concentrated under reduced pressure to give (*R*)-4-isopropylloxazolidin-2-one (1.36 g, 67%). The pH of the aqueous layer was carefully adjusted to ca. 1 with solid NaHSO₄ and the resulting emulsion was extracted with THF (3 × 100 mL) under salting-out conditions. The organic fractions were pooled, washed with brine (3 × 50 mL), dried, filtered and concentrated under reduced pressure. The residue was recrystallized from CH₂Cl₂/hexanes to give the acid (1*S*,2*S*)-**83** (1.27 g, 62%) as a colorless solid. The spectral data of this compound were in accordance with those for the racemic acid. $[\alpha]_{20}^D$ 204.3 ($c = 0.79$, THF).

(1'*S*,2'*S*)-(2'-Nitrocyclopropyl)methanol (1'*S*,2'*S*)-**(10)** and (1'*R*,2'*R*)-(2'-Nitrocyclopropyl)methanol (1'*R*,2'*R*)-**(10)**: A 1 M solution of BH₃·THF in THF (12.4 mL) was added to a solution of (1*S*,2*S*)-**83** (1.25 g, 9.54 mmol) in anhydrous THF (12 mL) at 4 °C for 50 min. The cooling bath was then removed and stirring continued for a further 48 h. Methanol (15 mL) was then added dropwise for 30 min and the mixture was concentrated under reduced pressure. The residue was taken up with MeOH (20 mL) and the resultant solution was refluxed for 4 h. All volatiles were then removed under reduced pressure and the residue was purified by column chromatography (EtOAc/hexanes 2:3, $R_f = 0.27$) and then by bulb-to-bulb distillation at the bath temperature 100–120 °C at 0.002 Torr to give (1'*S*,2'*S*)-**10** (1.00 g, 90%) as a colorless oil. The spectral data of this compound were in accordance with literature. $[\alpha]_{20}^D$ 100.9 ($c = 1.03$, CHCl₃) [lit.:^[13] $[\alpha]_{20}^D$

97.8 ($c = 1.60$, CHCl_3]. (1' R ,2' R)-Isomer was also prepared in the same manner in 94% yield. The spectral data of this compound were in accordance with literature. $[\alpha]_{20}^D -92.6$ ($c = 1.01$, CHCl_3) [lit.:^[13] $[\alpha]_{20}^D -95.1$ ($c = 1.10$, CHCl_3)].

Alkylation of (R)-Nickel complex (2R)-81 with (1' R ,2' R)-1-(iodomethyl)-2-nitrocyclopropane (1' R ,2' R)-80. Preparation of (2R,2' R ,1'' R ,2'' R)-82: A suspension of (2R)-81 (5.50 g, 11.00 mmol) in a mixture of DMF (5.5 mL) and MeCN (11 mL) was degassed with three freeze-pump-thaw cycles at -70°C . NaH (0.616 g, 60% in oil, 15.40 mmol) and iodide (1' R ,2' R)-80 (2.585 g, 11.39 mmol) were then added. The cooling bath was removed, and the reaction mixture was vigorously stirred for 40 min. The reaction flask was then immersed with ice/water bath and 60% AcOH (1.2 mL) was carefully added. The precipitated solid was filtered off, washed with cold (-35°C) MeCN and Et_2O . After this, it was dissolved in CHCl_3 and filtered from the tar. The solution was then concentrated under reduced pressure to ca. 10 mL and Et_2O (40 mL) was added. The precipitated solid was filtered off and dried to give (2R,2' R ,1'' R ,2'' R)-82 (4.85 g, 74%) as a red solid. The spectral data of this compound were in accordance with literature. The isotopic pattern [MS (EI, 70 eV)] was in accordance with the calculated one. $[\alpha]_{20}^D -2335$ ($c = 0.055$, MeOH) [lit.:^[84] $[\alpha]_{20}^D -2406$ ($c = 0.1$, CHCl_3)]; HRMS (EI): calcd for $\text{C}_{31}\text{H}_{30}\text{N}_4\text{NiO}_5$: 596.1570; found 596.1570.

(2R,1' R ,2' R)-3-(2'-nitrocyclopropyl)alanine (2R,1' R ,2' R)-83): 6 M HCl (40 mL) was added to

the suspension of finely grounded (2R,2' R ,1'' R ,2'' R)-82 (4.85 g, 8.12 mmol) in refluxing methanol (20 mL). The mixture was gently refluxed for 10 min. The resulting green syrup was then concentrated, was taken up with ice-cold water (50 mL), the precipitate was filtered off and washed with cold water (20 mL) to give after drying (R)-BPB-HCl HCl-(2R)-84 (2.50 g, 74% recovery) as a white solid. The filtrate was combined with washings, pH was carefully adjusted to 6 with aqueous ammonia and extracted

with CHCl_3 (3×50 mL). The organic layer was dried, filtered and concentrated under reduced pressure to give another portion of (*R*)-BPB (0.32 g, 84% overall recovery). Preswallowed Amberlite IRA-120 in H^+ form (110 mL) was added to the water fraction and the mixture was stirred for 15 h. The ion-exchange resin was filtered, washed with water to pH 5 and treated with 10% aqueous NH_3 (2×250 mL) for 2 h. The combined filtrates were concentrated under reduced pressure to give white solid, which was dissolved in hot water (7 mL). The solution was filtered and diluted with EtOH (15 mL). The formed precipitate was filtered off and dried. This material (0.88 g) was additionally purified by recrystallization from water (4 mL), the precipitated crystals were filtered off, washed with cold EtOH:H₂O 2:1 and dried at 50 °C and 0.002 Torr for 16 h to give (*2R,1'R,2'R*)-**83** (0.77 g, 55%) as a colorless solid. The spectral data of this compound were in agreement with literature. $[\alpha]_{20}^D -81.0$ ($c = 0.33$, H₂O) [lit.:^[84] $[\alpha]_{20}^D -72.2$ ($c = 0.32$, H₂O)]; MS (ESI), positive m/z (%) = 219 (40) $[\text{M} - \text{H}^+ + 2\text{Na}^+]$, 175 (14) $[\text{M} + \text{H}^+]$; negative m/z (%) = 369 (92) $[2\text{M} - 2\text{H}^+ + \text{Na}^+]$, 347 (62) $[2\text{M} - \text{H}^+]$, 173 (18) $[\text{M} - \text{H}^+]$.

2-Dideuterated Nickel complex (2S)-D₂-81: Unlabelled (*2S*)-**81** (6 g, 12.043 mmol) was

dissolved in a mixture of CH_3OD (10 mL), D_2O (7 mL) and CDCl_3 (5 mL) and Na_2CO_3 (0.2 g, 2.410 mmol) was added. The mixture was stirred at 50 °C for 24 h in sealed flask, concentrated under reduced pressure, CH_3OD (10 mL), D_2O (7 mL) and CDCl_3 (5 mL) was added again and the resultant biphasic reaction mixture was stirred at 50 °C for an additional

24 h. The reaction mixture was then cooled, diluted with CHCl_3 (30 mL), organic phase was separated, washed with H_2O (2×30 mL), dried and concentrated to give after recrystallization from $\text{CHCl}_3/\text{Et}_2\text{O}/\text{hexanes}$ (*2S*)-D₂-**81** (5.66 g, 94%; D₂ and D contents > 97% and < 3% respectively, as determined by MS) as a dark red solid. M.p. 210–213 °C; ¹H NMR (250 MHz, CDCl_3): $\delta = 1.97\text{--}2.19$ (m, 2 H, 4-H), 2.28–2.46 (m, 1 H, 3-H_a), 2.46–2.63 (m, 1 H, 3-H_b), 3.21–3.41 (m, 1 H, 5-H_a), 3.45 (dd, $J = 10.9, 5.6$ Hz, 1 H, 5-H_b), 3.59–3.72 (m, 1 H, 2-H), 3.68 (d, $J =$

25.0 Hz, 1 H, Bzl-H_a), 4.46 (d, $J = 25$ Hz, 1 H, Bzl-H_b), 6.63–6.72 (m, 1 H, Ar-H), 6.74–6.81 (m, 1 H, Ar-H), 6.88–7.01 (m, 1 H, Ar-H), 7.03–7.12 (m, 1 H, Ar-H), 7.14–7.24 (m, 1 H, Ar-H), 7.24–7.33 (m, 1 H, Ar-H), 7.35–7.46 (m, 2 H, Ar-H), 7.44–7.58 (m, 3 H, Ar-H), 8.04 (d, $J = 8$ Hz, 2 H, Ar-H), 8.26 (d, $J = 8.5$ Hz, 1 H, Ar-H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 23.4$ (–, C-4), 30.3 (–, C-3), 57.2 (–, C-5), 62.8 (–, C-Bzl), 69.5 (+, C-2), 120.4, 123.9, 125.3, 125.9, 128.5, 128.7, 129.0, 129.2, 129.4, 131.4, 131.8 and 132.8 (+, Ar-C), 124.8, 133.0, 134.2, 142.2 (C_{quat} , Ar-C), 171.3 (C_{quat} , C=N), 176.9 (C_{quat} , C=O), 181.0 (C_{quat} , CO–N); the signals of CD_2 carbon could not be detected because of their low intensity; IR (KBr): $\nu(\text{tilde}) = 2973\text{ cm}^{-1}$, 2869, 1673, 1642, 1589, 1442, 1336, 1264, 1176; MS (EI, 70 eV), m/z (%): 499 (40) [M^+], 455 (100) [$\text{M}^+ - \text{CO}_2$], 363 (8), 267 (10), 265 (17), 217 (41), 160 (41), 91 (78) [C_7H_7^+]; HRMS (EI): calcd for $\text{C}_{27}\text{H}_{23}\text{D}_2\text{N}_3\text{NiO}_3$: 499.1375; found 499.1375; elemental analysis calcd (%) for $\text{C}_{27}\text{H}_{23}\text{D}_2\text{N}_3\text{NiO}_3$ (500.2): C 64.83, H 5.44, N 8.40; found C 65.03, H 5.77, N 8.32.

Alkylation of 2-Dideuterated Nickel complex (2S)-D₂-81 with rac-trans-1-(iodomethyl)-2-nitrocyclopropane 80. Preparation of (2S,2'S,1''RS,2''RS)-D-82: A suspension of (2S)-D₂-81 (4.6

g, 9.196 mmol) in a mixture of DMF (4.0 mL) and CD_3CN (8 mL) was degassed with three freeze-pump-thaw cycles at -70°C . NaH (0.504 g, 60% in oil, 12.595 mmol) and iodide **80** (2.12 g, 9.334 mmol) were then added. The cooling bath was removed, and the reaction mixture was vigorously stirred

for 40 min. The reaction flask was then immersed with ice/water bath and D_2O (2 mL) and followed by D_2SO_4 (1.26 g, 12.6 mmol) in D_2O (5 mL) were carefully added. After this, water (70 mL) was added and the separated dark red oil was triturated with ether to give red-brown solid (5.7 g) which was further purified by column chromatography (CHCl_3 /acetone 6 : 1, $R_f = 0.37$) and then by recrystallization from CHCl_3 /MeOH to give (2S,2'S,1''RS,2''RS)-D-82 (3.87 g, 70%) as a dark red solid. According to ^1H NMR spectrum deuterium content was 85% at C-2'

and 60% at C-2" respectively. M.p. > 260 °C, $[\alpha]_{20}^D$ 1708 ($c = 0.05$, MeOH); ^1H NMR (250 MHz, CDCl_3): mixture of diastereo- and isotopomers: $\delta = 0.46\text{--}0.58$ (m, 0.5 H, $3''\text{-H}_a$), $0.85\text{--}0.96$ (m, 0.5 H, $3''\text{-H}_a$), $1.05\text{--}1.28$ (m, 0.5 H, $3'\text{-H}_a$), 1.45 (dd, $J = 8.4, 14.4$ Hz, 0.5 H, $3'\text{-H}_a$), $1.73\text{--}1.84$ (m, 0.5 H, $3''\text{-H}_b$), $1.84\text{--}1.96$ (m, 0.5 H, $3''\text{-H}_b$), $1.97\text{--}2.32$ (m, 2.5 H, 4-H, 0.5 $3'\text{-H}_b$), $2.38\text{--}2.58$ (m, 2 H, 3- H_a , $1''\text{-H}$), $2.58\text{--}2.83$ (m, 1.5 H, 3- H_a , 0.5 $3'\text{-H}_b$), $3.33\text{--}3.41$ (m, 0.15 H, $2'\text{-H}$), $3.40\text{--}3.73$ (m, 3 H, 2-H, 5-H), 3.56 (dd, $J = 2.3, 12.8$ Hz, 1 H, Bzl- H_a), 3.92 (ddd, $J = 3.4, 3.4, 7.0$ Hz, 0.30 H, $2''\text{-H}$, $2'\text{-H}$), 4.00 (dd, $J = 14.6, 3.9$ Hz, 0.13 H, $2''\text{-H}$), 4.44 (d, $J = 12.8$ Hz, 1 H, Bzl- H_b), $6.56\text{--}6.73$ (m, 2 H, Ar-H), 6.88 (t, $J = 8$ Hz, 1 H, Ar-H), $7.08\text{--}7.24$ (m, 2 H, Ar-H), $7.29\text{--}7.59$ (m, 5 H, Ar-H), $7.59\text{--}7.65$ (m, 1 H, Ar-H), 8.07 (dt, $J = 7.8, 1.5$ Hz, 2 H, Ar-H), 8.13 (dd, $J = 3.3, 8.5$ Hz, 1 H, Ar-H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 17.7, 17.8, 18.2, 18.4$ (–, C- $3''$), $21.5, 21.6, 21.6, 21.7$ (+, C- $1''$), 23.8 (–, C-4), 30.6 (–, C-3), $36.1, 36.4$ (–, C- $3'$), 57.2 (–, C-5), 58.5 (+, C-2), $63.1, 63.2$ (–, C-Bzl), 68.3 (t, $J = 19.3$ Hz, C- $2'$), $70.0, 70.0$ (+, C- $2''$), $120.6, 123.5, 123.6, 127.1, 127.1, 127.2, 127.2, 128.7, 128.7, 128.8, 129.0, 129.3, 129.8, 130.0, 131.3, 132.3$ and 133.0 (+, Ar-C), $125.9, 133.2, 133.4, 133.4, 142.3$ (C_{quat} , Ar-C), $170.9, 171.0$ (C_{quat} , C=N), $178.1, 178.2$ (C_{quat} , C=O), 180.4 (C_{quat} , CO-N); IR (KBr): $\nu(\text{tilde}) = 3071\text{ cm}^{-1}, 2972, 2922, 2866, 1667, 1624, 1589, 1537, 1436, 1365, 1339, 1257, 1166$; MS (EI, 70 eV), m/z (%): 598 (7) [M^+], 553 (15) [$\text{M}^+ - \text{CO}_2$], 439 (9), 348 (7), 217 (22), 160 (100), 91 (23) [C_7H_7^+]; HRMS (EI): calcd for $\text{C}_{31}\text{H}_{29}\text{DN}_4\text{NiO}_5$: 597.1632; found 597.1632; elemental analysis (%) for $\text{C}_{31}\text{H}_{29}\text{DN}_4\text{NiO}_5$ (598.3): C 62.23, H 5.22, N 9.36; found C 61.94, H 5.12, N 9.22.

(2*S*,1'*RS*,2'*RS*)-2-Deutero-3-(2'-nitrocyclopropyl)alanine (2*S*,1'*RS*,2'*RS*)-D-(**83**): 6 M HCl (32

mL) was added to the suspension of finely grounded (2*S*,2'*S*,1''*RS*,2''*RS*)-D-**82** (3.87 g, 6.472 mmol) in refluxing methanol (16 mL). The mixture was gently refluxed for 10 min.

The resulting green syrup was then concentrated, taken up with ice-cold water (50 mL), the precipitate was filtered off and washed with cold water (20 mL). The filtrate was combined with

washings, pH was carefully adjusted to 6 with aqueous ammonia and the resultant emulsion was extracted with CHCl_3 (3×50 mL). Preswallowed Amberlite IRA-120 in H^+ form (90 mL) was added to the water fraction and the mixture was stirred for 15 h. The ion-exchange resin was filtered off, washed with water to pH 5 and treated with 10% aqueous NH_3 (2×150 mL) for 2 h. The combined basic filtrates were concentrated under reduced pressure to give white solid, which was dissolved in hot water (10 mL). The solution was filtered and diluted with EtOH (20 mL). The formed precipitate was filtered off to give (2*S*,1'*RS*,2'*RS*)-D-**83** (0.77 g, 68%; 60% of (2*S*,1'*S*,2'*R*)-isomer according to ^{13}C NMR spectrum) as a white solid. ^1H spectrum showed 88% content of deuterium at C-2 and 58% at C-2' respectively. According to MS-ESI spectrum D_2 and D contents were 52% and 42% respectively. M.p. 193–195 °C (decomp.); $[\alpha]_{20}^{\text{D}}$ 3.3 ($c = 0.3$, H_2O); ^1H NMR (250 MHz, D_2O): mixture of diastereo- and isotopomers: $\delta = 1.24\text{--}1.39$ (m, 1 H, 3'-H), 1.73–2.25 (m, 4 H, 3-H, 1'-H, 3'-H), 3.8 (dd, $J = 5.9, 5.9$ Hz, 1 H, 2-H), 4.18–4.26 (m, 1 H, 2'-H); ^{13}C NMR (62.9 MHz, D_2O): $\delta = 20.4, 20.5, 20.7, 20.9$ (–, C-3'), 24.3, 24.4, 24.6, 24.7 (+, C-1'), 33.7, 33.8 (–, C-3), 55.9 (t, $J = 23.4$ Hz, C-2), 61.6, 61.7 (+, C-2'), 176.0, 176.1 (C_{quat} , C-1); IR (film): $\nu(\text{tilde}) = 3418, 3034, 2102, 1598, 1530, 1440, 1394, 1365$; MS (ESI), positive m/z (%): 220/221 (30/40) [$\text{M} + 2 \text{Na}^+ - \text{H}^+$]; negative m/z (%): 175/176 (3/3) [$\text{M} - \text{H}^-$]; elemental analysis calcd (%) for $\text{C}_6\text{H}_9\text{DN}_2\text{O}_4$ (175.2): C 41.14, H 6.33, N 15.99; found C 40.98, H 6.29, N 15.65.

(1'*S*,2'*S*)-Triphenylmethoxy-(2'-Nitrocyclopropyl)methan (1'*S*,2'*S*)-**(87)**: Trityl chloride (1.166 g, 4.18 mmol) was added to an ice-cold solution of the alcohol (1'*S*,2'*S*)-**10** (0.50 g, 4.27 mmol) and DMAP (ca. 10 mg) in anhydrous pyridine (8 mL). The mixture was allowed to warm to 20 °C and stirring continued for a further 4 days. Methanol (3 mL) was then added and the mixture was stirred for an additional 3 h. All volatiles were then removed and the residue was partitioned between Et_2O and water (40 mL of each). The organic layer was washed with water (2×10 mL), 1 M NaHSO_4 (3×10 mL), water (3×10 mL), brine (2×10 mL), dried, filtered and concentrated

under reduced pressure. The resultant crude product was recrystallized from hexanes to give (1'S,2'S)-**87** (1.20 g, 78%) as a colorless solid. The spectral data of this compound were in accordance with literature.^[13]

Deuterium exchange experiments (GP 3): To a stirred solution or suspension of the corresponding nitro-compound (0.3 mmol) in anhydrous CD₃CN/DMF 2:1 (1.5 mL), NaH (0.6 mmol, 60% suspension in oil) was added at –25 °C. The mixture was allowed to warm to 20 °C and stirring continued for an additional 50 min. The reaction quenched with 0.5 M D₂SO₄ in D₂O (1.2 mL) and diluted then with water (15 mL). The crude products were separated by filtration or extraction with Et₂O and purified by recrystallization, column chromatography or distillation.

*Deuteration of (2S,2'S,1''R,2''R)-**82**:* Ni-complex (2S,2'S,1''R,2''R)-**82** (0.25 g, 0.42 mmol) was treated with NaH (0.038 g, 60% suspension in oil, 0.95 mmol) in CD₃CN/DMF 2:1 (1.5 mL) according to GP 3 and the crude product was recrystallized from CHCl₃/Et₂O to give deuterated product (0.181 g, 71%; 55% and 65% inclusion of the deuterium label at the 2 and 2' positions of the (3-Ncp)Ala fragment respectively according to ¹H NMR spectrum).

*Deuteration of (1'S,2'S)-**87**:* Compound (1'S,2'S)-**87** (0.30 g, 0.835 mmol) was treated with NaH

(0.040 g, 60% suspension in oil, 1.00 mmol) in CD₃CN/DMF 2:1 (1.5 mL) according to GP 3 and the crude product was purified by column chromatography (EtOAc/hexanes 1:14, *R_f* = 0.41) to give deuterated product (1'S,2'S)-D-**87** (0.215 g, 72%; D content > 99%, as determined by MS) as a white solid. [α]₂₀^D 63.4 (*c* = 0.61, CHCl₃) [lit. for **17**:^[13] [α]₂₀^D 66.2 (*c* = 1.00, CHCl₃)]; ¹H NMR (250 MHz, CDCl₃): δ = 1.31 (dd, *J* = 6.0, 7.5 Hz, 1 H, 3'-H_a), 1.84 (dd, *J* = 5.8, 10.5 Hz, 1 H, 3'-H_b), 2.17–2.31 (m, 1 H, 1'-H), 3.08 (dd, *J* = 5.6, 10.1 Hz, 1 H, 1-H), 3.30 (dd, *J* = 4.5, 10.3 Hz, 1-H), 7.22–7.43 (m, 15 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 15.3 (–, C-3'), 25.2 (+, C-1'), 57.3 (t, *J* = 27.7 Hz, C-2'), 61.6 (–, C-1), 86.7 (C_{quat}, CPh₃), 127.1, 127.9, 128.4 (+, Ar-C), 143.4 (C_{quat}, Ar-C); IR (KBr):

$\nu(\text{tilde}) = 3111 \text{ cm}^{-1}$, 3089, 3062, 3029, 2969, 2942, 2893, 2871, 1524, 1355, 1218, 1115, 1033;
MS (EI, 70 eV), m/z (%): 360 (26) $[\text{M}^+]$, 283 (12) $[\text{M}^+ - \text{C}_6\text{H}_5]$, 243 (100) $[\text{CPh}_3^+]$, 165 (44) $[\text{CPh}_2^+]$; HRMS (EI): calcd for $\text{C}_{23}\text{H}_{20}\text{DNO}_3$: 360.1584; found 360.1584; elemental analysis calcd (%) for $\text{C}_{23}\text{H}_{20}\text{DNO}_3$ (360.4): C 76.65, H 6.15, N 3.89; found C 76.47, H 6.06, N 3.72.

Deuteration of nitrocyclopropane 88: Nitrocyclopropane **88** (0.255 g, 2.928 mmol) was treated with NaH (0.152 g, 60% suspension in oil, 3.800 mmol) in $\text{CD}_3\text{CN}/\text{DMF}$ 2:1 (2.4 mL) according to GP 3. The reaction was quenched with 5% acetic acid in D_2O (3 mL) and the mixture was extracted with Et_2O (3×10 mL). The combined organic layers were washed with water (3×10 mL), brine (2×10 mL), dried, filtrated through short (5 cm) column with alumina and concentrated under atmospheric pressure at bath temperature $< 45^\circ\text{C}$. The residue was subjected to bulb-to-bulb distillation under reduced pressure (125 Torr) and the fraction passed at the bath temperature $> 50^\circ\text{C}$ was collected to give D-**88** (72 mg, 90% purity, 25%; D content $> 97\%$, as determined by ^1H NMR) as a colorless liquid. ^1H NMR (250 MHz, CDCl_3): $\delta = 1.12\text{--}1.22$ (m, 2 H), 1.62 (dd, $J = 5.3, 5.3$ Hz, 2 H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 11.4$ (–), 53.8 (t, $J = 28.3$ Hz); MS (EI, 70 eV), m/z (%): 46 (38) $[\text{NO}_2^+]$, 42 (100) $[\text{C}_3\text{H}_4\text{D}^+]$.

(1'S,2'S)-(2-allyl-2-nitrocyclopropyl)methyl trityl ether (1'S,2'S)-(91): NaH (0.156 g, 60%

suspension in mineral oil, 3.90 mmol) was added to a degassed solution of (1'S,2'S)-**87** (0.200 g, 0.56 mmol) and allyl bromide (0.67 g, 55.38 mmol) in anhydrous DME (7 mL) and stirring continued for a further 36 h. The mixture was then heated to $55\text{--}60^\circ\text{C}$ and stirred for an additional 3 h. After this, the reaction flask was cooled in ice/water bath and water (3 mL) was carefully added. The mixture was diluted with Et_2O (40 mL) washed with water (2×10 mL), 1 M NaHSO_4 (3×10 mL), again water (3×10 mL), brine (2×10 mL), dried, filtered and concentrated under reduced pressure. The residue was purified by column chromatography ($\text{EtOAc}/\text{hexanes}$ 1:30, $R_f = 0.11$) to give the crude product (0.104 g)

which was according to ^1H NMR spectrum impure mixture of *cis*- and *trans*-isomers in 1:7. This material was purified by preparative TLC (3 plates 200×200 mm, EtOAc/hexanes 1:30, 3 runs) to give (1'*S*,2'*S*)-**91** (72 mg, 49%) as a turbid oil which solidified in a colorless solid during prolonged drying at 65 °C and at 0.002 Torr. M.p. 79–80 °C; $[\alpha]_{20}^D -33.0$ ($c = 0.33$, CHCl_3); ^1H NMR (250 MHz, CDCl_3): $\delta = 1.31$ (dd, $J = 9.0, 6.0$ Hz, 1 H, 3'-H_a), 1.84 (dddd, $J = 5.5, 9.0, 9.0, 9.0, 9.0$ Hz, 1 H, 3'-H_b), 1.85 (dddd, $J = 9.0, 7.5, 6.0, 1.2$ Hz, 1 H, 1'-H), 2.44 (dddd, $J = 13.0, 6.5, 1.2, 1.2$ Hz, 1 H, 1''-H_a), 3.09–3.23 (m, 2 H, 1-H_a, 1''-H_b), 3.51 (dd, $J = 10.5, 5.5$ Hz, 1 H, 1-H_b), 5.11–5.18 (m, 1 H, *cis*-2''-H), 5.18 (dddd, $J = 17.4, 1.4, 1.4, 1.4$ Hz, *trans*-2''-H), 5.74–5.92 (m, 1 H, 3''-H), 7.17–7.43 (m, 15 H, Ar-H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 18.5$ (–, C-3'), 29.1 (+, C-1'), 38.2 (–, C-1''), 61.0 (–, C-1), 68.2 (C_{quat}, C-2'), 86.6 (C_{quat}, CPh₃), 118.7 (–, C-3''), 127.0, 127.8, 128.5 (+, Ar-C), 132.1 (+, C-2''), 143.8 (C_{quat}, Ar-C); IR (film): $\nu(\text{tilde}) = 3088\text{ cm}^{-1}$, 3059, 3028, 2983, 2926, 2882, 1960, 1644, 1598, 1535, 1492, 1448, 1361, 1344, 1222, 1070; MS (EI, 70 eV), m/z (%): 399 (9) [M^+], 322 (4) [$\text{M}^+ - \text{C}_6\text{H}_5$], 243 (100) [CPh_3^+], 165 (15) [CPh_2^+], 140 (37) [$\text{C}_7\text{H}_{10}\text{NO}_2^+$], 105 (33); HRMS (EI): calcd for $\text{C}_{26}\text{H}_{25}\text{NO}_3$: 399.1834; found 399.1834; elemental analysis calcd (%) for $\text{C}_{26}\text{H}_{25}\text{NO}_3$ (399.5): C 78.17, H 6.31, N 3.51; found C 77.87, H 6.31, N 3.29.

(1'*S*,2'*R*)-(2-benzyl-2-nitrocyclopropyl)methyl trityl ether (1'*S*,2'*R*)-(**92**): NaH (0.111 g, 60%

suspension in mineral oil, 2.78 mmol) was added to a degassed solution of (1'*S*,2'*S*)-**87** (0.100 g, 0.28 mmol) and benzyl bromide (0.33 mL, 2.78 mmol) in anhydrous DME (5 mL) and stirring continued for a further 5 days. After this, the reaction flask was cooled in ice/water bath and

water (3 mL) was carefully added. The mixture was diluted with Et_2O (40 mL) washed with water (2×10 mL), 1 M NaHSO_4 (3×10 mL), water (3×10 mL), brine (2×10 mL), dried, filtered and concentrated under reduced pressure. All volatiles were then removed at 180 °C (bath temperature) and at 0.002 Torr by bulb-to-bulb distillation. The residual oil was triturated

with pentane and then purified by preparative TLC (200 × 200 mm, EtOAc/hexanes 1:30, 3 runs) to give (1'S,2'R)-**92** (50 mg, 40%) as a turbid oil. The fraction contained mainly somehow more polar (1'S,2'S)-isomer (15 mg, 12%) also was separated. ¹H NMR (250 MHz, CDCl₃): δ = 1.24 (dd, *J* = 9.5, 6.5 Hz, 1 H, 3'-H_a), 1.40–1.58 (m, 1 H, 3'-H_b), 1.91 (dddd, *J* = 8.0, 6.5, 6.5, 1.3 Hz, 1 H, 1'-H), 3.08 (d, *J* = 15.8 Hz, 1 H, H_a, Bzl-H), 3.18 (dd, *J* = 11.0, 8.5 Hz, 1 H, 1-H_a), 3.52 (dd, *J* = 11.0, 5.5 Hz, 1 H, 1-H_b), 3.84 (d, *J* = 15.8 Hz, 1 H, H_b, Bzl-H), 7.17–7.44 (m, 20 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 18.4 (–, C-3'), 29.1 (+, C-1'), 39.5 (–, C-1''), 61.1 (–, C-1), 69.2 (C_{quat}, C-2'), 86.7 (C_{quat}, CPh₃), 127.0, 127.1, 127.8, 127.9, 128.5, 129.1 (+, Ar-C), 136.1, 143.8 (C_{quat}, Ar-C); IR (film): ν(tilde) = 3088 cm^{–1}, 3060, 3028, 2930, 2880, 1955, 1600, 1533, 1493, 1448, 1362, 1323, 1221, 1071; MS (EI, 70 eV), *m/z* (%): 449 (9) [M⁺], 372 (4) [M⁺ – C₆H₅], 259 (36), 243 (100) [CPh₃⁺], 190 (16) [C₁₁H₁₂NO₂⁺], 165 (15) [CPh₂⁺], 105 (33); HRMS (EI): calcd for C₃₀H₂₇NO₃: 449.1991; found 449.1991.

(1'S,2'S)-**92**: ¹H NMR (250 MHz, CDCl₃): δ = 1.06 (dd, *J* = 7.8, 6.0 Hz, 1 H, 3'-H_a), 2.12 (dddd, *J* = 10.0, 6.0, 4.5, 1.5 Hz, 1 H, 3'-H_b), 2.32–2.47 (m, 1 H, 1'-H), 2.65 (d, *J* = 16.5 Hz, 1 H, H_a, Bzl-H), 2.90 (dd, *J* = 10.8, 8.5 Hz, 1 H, 1-H_a), 3.56 (dd, *J* = 10.8, 5.0 Hz, 1 H, 1-H_b), 3.85 (d, *J* = 16.5 Hz, 1 H, H_b, Bzl-H), 7.05–7.35 (m, 15 H, Ar-H), 7.37–7.46 (m, 5 H, Ar-H).

(1'S,2'S)-(2-methyl-2-nitrocyclopropyl)methyl trityl ether (1'S,2'S)-(**93**) and (1'S,2'R)-(2-methyl-2-nitrocyclopropyl)methyl trityl ether (1'S,2'R)-(**93**): NaH (0.111 g, 60% suspension in mineral oil, 2.78 mmol) was added to a degassed solution of (1'S,2'S)-**87** (0.100 g, 0.28 mmol) and methyl iodide (0.39 g, 2.75 mmol) in anhydrous diglym (5 mL) and stirring continued for 10 h. The second portions of NaH (0.111 g, 60% suspension in mineral oil, 2.78 mmol) and methyl iodide (0.39 g, 2.75 mmol) were added and the mixture was stirred for a further 12 h. The third portions of NaH (0.111 g, 60% suspension in mineral oil, 2.78 mmol) and methyl iodide (0.39 g, 2.75 mmol) were added and stirring continued for an additional 16 h. After this, the reaction flask was cooled in ice/water bath and water (3 mL) was carefully added. The mixture was

diluted with Et₂O (40 mL) washed with water (2 × 10 mL), 1 M NaHSO₄ (3 × 10 mL), water (3 × 10 mL), brine (2 × 10 mL), dried, filtered and concentrated under reduced pressure. The residue was separated by column chromatography (EtOAc/hexanes 1:30) to give the *trans*-isomer (1'*S*,2'*S*)-**93** (27 mg, 26%) as a viscous colorless oil and the *cis*-isomer (1'*S*,2'*R*)-**93** (15 mg, 15%) as an yellow solid. Also their mixture (5 mg) was obtained.

(1'*S*,2'*S*)-**93**: *R*_f = 0.13 EtOAc/hexanes 1:30; ¹H NMR (250 MHz, CDCl₃): δ = 1.07 (dd, *J* = 9.3,

6.3 Hz, 1 H, 3'-H_a), 1.40–1.57 (m, 1 H, 3'-H_b), 1.74 (s, 3 H, Me), 1.82 (dd, *J* = 8.8, 6.3 Hz, 1 H, 1'-H), 3.13 (dd, *J* = 11.0, 8.8 Hz, 1 H, 1-H_a),

3.48 (dd, *J* = 11.0, 5.8 Hz, 1 H, 1-H_b), 7.10–7.41 (m, 15 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 20.7 (–, C-3'), 21.1 (+, Me), 30.9 (+, C-1'), 61.1 (–, C-1), 64.5 (C_{quat}, C-2'), 86.6 (C_{quat}, CPh₃), 127.0, 127.8, 128.5 (+, Ar-C), 143.8 (C_{quat}, Ar-C); IR (film): ν(tilde) = 3088 cm^{–1}, 3060, 3027, 2983, 2939, 2882, 1960, 1598, 1539, 1492, 1445, 1388, 1364, 1340, 1221, 1070, 1031; MS (EI, 70 eV), *m/z* (%): 373 (11) [M⁺], 296 (5) [M⁺ – C₆H₅], 259 (27), 243 (100) [CPh₃⁺], 165 (53) [CPh₂⁺], 114 (38) [C₅H₈NO₂⁺], 105 (43), 77 (12) [C₆H₅⁺]; HRMS (EI): calcd for C₂₄H₂₃NO₃: 373.1678; found 373.1678.

(1'*S*,2'*R*)-**93**: *R*_f = 0.16 EtOAc/hexanes 1:30; ¹H NMR (250 MHz, CDCl₃): δ = 0.83 (dd, *J* = 7.5, 5.5 Hz, 1 H, 3'-H_a), 1.58 (s, 3 H, Me), 1.94 (dd, *J* = 10.5, 5.5 Hz, 1 H, 3'-H_b), 2.25–2.43 (m, 1 H, 1'-H), 2.76 (dd, *J* = 10.8, 8.8 Hz, 1 H, 1-H_a), 3.48 (dd, *J* = 10.8, 5.3 Hz, 1 H, 1-H_b), 7.15–7.45 (m, 15 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 14.7 (+, Me), 22.4 (–, C-3'), 29.7 (+, C-1'), 62.4 (–, C-1), 64.0 (C_{quat}, C-2'), 86.8 (C_{quat}, CPh₃), 127.1, 127.9, 128.5 (+, Ar-C), 143.7 (C_{quat}, Ar-C); IR (KBr): ν(tilde) = 3086 cm^{–1}, 3056, 3025, 2926, 2887, 1817, 1597, 1530, 1489, 1446, 1390, 1364, 1340, 1226, 1182, 1070, 1030.

2.6. Synthesis of *O*-MOM protected 5-chloro-1-hydroxypyrrol-2-carboxylic acid

2-Acetamido-6-chloro-pyridine-*N*-oxide (**97**): m-Chloroperbenzoic acid (11.97 g, 90% purity,

62.44 mmol) was added to a solution of 2-acetamido-6-chloro-pyridine **96** (8.24 g, 48.30 mmol) in CH₂Cl₂ (100 mL) and stirring continued for an additional 60 h. K₂CO₃ (6.5 g) was then added to the mixture and

after stirring for 1 h the mixture was filtered, concentrated under reduced pressure and the residue was recrystallized from CH₂Cl₂/hexanes to give **97** (8.35 g, 93%) as a white solid. ¹H NMR (250 MHz, CDCl₃): δ = 2.29 (s, 3 H, CH₃), 7.15–7.31 (m, 2 H, 4-H, 5-H), 8.35 (dd, *J* = 2.5, 10.0 Hz, 1 H, 3-H), 9.80–10.20 (br, 1 H, NH); ¹³C NMR (62.9 MHz, CDCl₃): δ = 25.0 (+, CH₃), 112.1 (+, C-3), 119.4 (+, C-5), 127.2 (+, C-4), 140.0 (C_{quat}, C-6), 145.4 (C_{quat}, C-2), 169.0 (C_{quat}, CH₃CO); MS (EI, 70 eV): *m/z* (%) = 188/186 (30/100) [M⁺], 146/144 (30/100) [M⁺ – C₂H₂O], 130/128 (3/8), 43 (78) [C₂H₃O⁺]; HRMS (EI): calcd for C₇H₇ClN₂O₂: 186.0196; found 186.0196.

tert-Butyl acetyl(6-chloropyridin-2-yl)carbamate-*N*-oxide (**98**): DMAP (0.54 g, 4.42 mmol) was

added to a solution of **97** (8.35 g, 44.75 mmol) and Boc₂O (11.09 g, 50.81 mmol) in acetonitrile (60 mL) and stirring continued for an additional 12 h. The reaction mixture diluted with EtOAc (250 mL)

and washed with 1 M NaHSO₄ (3 × 40 mL), saturated aqueous NaHCO₃ (3 × 40 mL), H₂O (3 × 40 mL), brine (2 × 20 mL), dried, filtered and concentrated under reduced pressure. The crude product was recrystallized from EtOAc/hexanes to give **98** (8.31 g, 69%) as a white solid. *R*_f = 0.59, EtOAc/hexanes 1:1; ¹H NMR (250 MHz, CDCl₃): δ = 1.35 [s, 9 H, C(CH₃)₃], 2.58 (s, 3 H, CH₃), 7.08–7.22 (m, 1 H, 3-H, 5-H), 7.41–7.49 (m, 1 H, 4-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 25.5 (+, CH₃), 27.5 [+ , C(CH₃)₃], 84.5 [C_{quat}, C(CH₃)₃], 124.0 (+, C-3), 124.5 (+, C-5), 125.8 (+, C-4), 141.9 (C_{quat}, C-6), 145.8 (C_{quat}, C-2), 150.0 (C_{quat}, NCO₂), 171.8 (C_{quat}, CH₃CO); MS (EI,

70 eV): m/z (%) = 288/286 (5/14) [M^+], 172/170 (30/100) [$M^+ - C_6H_{12}O_2$], 146/144 (30/90) [$M^+ - C_6H_{12}O_3$], 57 (93) [$C_4H_9^+$], 43 (32) [$C_2H_3O^+$]; HRMS (EI): calcd for $C_7H_7ClN_2O_2$: 286.0720; found 286.0720.

2-tert-butyloxycarbonylamino-6-chloro-pyridine-N-oxide (**99**): To a solution of substance **98**

(8.31 g, 28.98 mmol) in methanol (70 mL) 1,1,3,3-tetramethylguanidine

(4.53 mL, 36.10 mmol) was added and stirring continued for an

additional 40 min. The mixture was concentrated under reduced

pressure to ca. 30 mL, was diluted with Et_2O (100 mL), washed with 1 M $NaHCO_3$ (2×20 mL),

brine (2×20 mL), dried, filtered and concentrated under reduced pressure to leave a solid

residue which was recrystallized from octane to give **99** (4.25 g, 60%) as a white solid. 1H NMR

(250 MHz, $CDCl_3$): δ = 1.50 [s, 9 H, $C(CH_3)_3$], 7.09 (dd, J = 2.0, 8.3 Hz, 1 H, 3-H), 7.19 (dd, J =

8.3, 8.3 Hz, 1 H, 4-H), 8.06 (dd, J = 2.0, 8.3 Hz, 1 H, 5-H), 9.13–9.31 (br, 1 H, NH); ^{13}C NMR

(62.9 MHz, $CDCl_3$): δ = 28.0 [$^+$, $C(CH_3)_3$], 82.5 [C_{quat} , $C(CH_3)_3$], 110.6 ($^+$, C-3), 118.2 ($^+$, C-5),

126.9 ($^+$, C-4), 140.0 (C_{quat} , C-6), 146.0 (C_{quat} , C-2), 151.2 (C_{quat} , NCO_2); IR (KBr): ν (tilde) =

3297 cm^{-1} , 3122, 2980, 1728, 1608, 1560, 1500, 1377, 1275, 1257, 1182, 1159; MS (EI, 70 eV):

m/z (%) = 246/244 (6/20) [M^+], 190/188 (3/8) [$M^+ - C_4H_8O$], 173/171 (5/17) [$M^+ - C_4H_9O_2$],

146/144 (11/36) [$C_7H_7ClN_2O_2^+$], 57 (100) [$C_4H_9^+$], 41 (8); HRMS (EI): calcd for $C_{10}H_{13}ClN_2O_3$:

244.0615; found 244.0615.

2-Amino-6-chloro-pyridine-N-oxide hydrochloride $HCl \cdot$ (**101**): Amide **99** (4.22 g, 17.30 mmol)

was deprotected with 3.9 M HCl in dioxane (100 mL) for 24 h. All volatiles were then removed

and the residue was triturated with ether to give $HCl \cdot$ **101** (3.03 g, 97%) as a colorless solid.

2-Amino-6-chloropyridine-*N*-oxide (101): 2,6-Dichloropyridine *N*-oxide **100** (6.30 g, 38.4

mmol) was heated with 25% NH₃ in MeOH (100 mL) in a sealed tube at

105 °C for 26 h (TLC control). The tube was cooled in a water-ice bath,

then opened and the reaction mixture was filtered and concentrated under

reduced pressure. The residue was dissolved in MeOH/CHCl₃ 1:4, the solution was filtered again

and concentrated to give a dark-red oil, which was purified by column chromatography (silica

gel, MeOH/CHCl₃ 1:4, *R_f* = 0.50) and then by recrystallization (CHCl₃/hexanes) to give the title

compound (3.04 g, 55%) as a colorless solid. *R_f* = 0.23 EtOAc/hexanes 1:1; m.p. 133–135 °C; ¹H

NMR (250 MHz, CDCl₃): δ = 5.03 (br, 2 H, NH₂), 6.31 (dd, *J* = 0.5, 8.3 Hz, 1 H, 3-H), 6.57 (dd,

J = 0.5, 7.5 Hz, 1 H, 5-H), 7.29 (dd, *J* = 7.8 Hz, 1 H, 4-H); ¹³C NMR (62.9 MHz, CDCl₃): δ =

106.4 (+, C-3), 112.5 (+, C-5), 139.9 (+, C-4), 149.1 (C_{quat}, C-6), 158.7 (C_{quat}, C-2); IR (KBr):

ν(tilde) = 3400–2500 cm^{−1}, 3192, 3147, 1653, 1628, 1550, 1502, 1412, 1216, 1188; MS (EI, 70

eV): *m/z* (%) = 146/144 (30/100) [M⁺], 128/126 (10/30) [M⁺ − H₂O], 119/117 (5/16) [M⁺ −

HCN], 109 (2) [M⁺ − Cl], 100 (7), 99 (10), 92 (17), 93/91 (5/16), 81 (7); HRMS: C₅H₅ClN₂O:

calcd for C₅H₅ClN₂O₂: 144.0090; found: 144.0090; elemental analysis calcd (%) for C₅H₅ClN₂O

(144.6): C 41.54, H 3.49, N 19.38; found C 41.41, H 3.54, N 19.46.

2-Azido-6-chloropyridine-*N*-oxide (102): A 2.5 M aqueous NaNO₂ (11.3 ml) was added

dropwise to an ice-cold solution of 2-Amino-6-chloropyridine-*N*-oxide **101**

(3.80 g, 26.3 mmol) in 85 ml 10% HCl so that the internal temperature was

not higher than 5 °C. The reaction mixture was stirred for 10 min, then a

2.5 M NaN₃ (11.3 ml) was added dropwise so that the internal temperature was not higher than 5

°C and stirring continued at the same temperature for an additional 1 h. The reaction mixture was

extracted with CH₂Cl₂ (8 × 50 ml), dried and filtered through short column with silica gel using

first CH₂Cl₂ (1 l) and then CH₂Cl₂/Et₂O 1:1 (1 l) as an eluent. The combined fractions were

concentrated to ca. 15 ml under reduced pressure and hexanes (150 ml) were added. The

precipitate was separated by filtration and washed with CH₂Cl₂/hexanes 1:10 to give after drying the title compound (3.4 g, 76%) as a faint yellow solid. ¹H NMR (250 MHz, CDCl₃): 6.84 (dd, *J* = 2.0, 8.3 Hz, 1 H, 3-H), 7.11 (dd, *J* = 8.3, 8.3 Hz, 1 H, 4-H), 7.24 (dd, *J* = 2.0, 8.6 Hz, 1 H, 5-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 114.5 (+, C-3), 121.5 (+, C-5), 126.2 (+, C-4), 145.4 (C_{quat}, C-6), 156.8 (C_{quat}, C-2); IR (KBr): ν(tilde) = 3126 cm⁻¹, 3077, 2130, 1597, 1470, 1390, 1212; MS (EI, 70 eV): *m/z* (%) = 172/170 (14/44) [M⁺], 144/142 (5/16) [M⁺ – N₂], 114/112 (18/56), 87/85 (6/18), 76 (100); HRMS (EI): calcd for C₅H₃ClN₄O: 169.9995; found: 169.9995; elemental analysis (%) for C₅H₃ClN₄O (144.6): C 35.21, H 1.77, N 32.85; found C 35.35, H 2.01, N 32.66.

5-Chloro-1-hydroxypyrrole-2-carbonitrile (103) and 5-chloro-2-carbonitrile (104): A solution of 2-Azido-6-chloropyridine-*N*-oxide **102** (1.27 g, 7.45 mmol) in anhydrous benzene (32 mL) under argon was sealed in a thick-walled tube. The tube was heated first at 90 °C, then the temperature was allowed to increase to 105 °C, and the reaction mixture was left at this temperature for 15 min. Then the temperature was decreased to 85 °C, and stirring continued for an additional 4 h. After this, the reaction mixture was allowed to cool to 20 °C and was concentrated under reduced pressure at a temperature not higher than 35 °C to give a red solid, which was purified by column chromatography (silica gel, EtOAc/hexanes 1:4) to give **103** (0.517g, 49%, *R_f* = 0.37) as a light tan solid and **109** (0.12 g, 11%, *R_f* = 0.49) as a white solid.

103: *R_f* = 0.52, EtOAc/Hexanes = 1 : 3; m.p. 101 – 102 °C; ¹H NMR (250 MHz, CDCl₃): δ = 6.03 (d, *J* = 5.0 Hz, 1 H, 4-H), 6.67 (d, *J* = 5.0 Hz, 1 H, 3-H), 8.96 (br, 1 H, OH); ¹³C NMR (62.9 MHz, CDCl₃): δ = 100.5 (C_{quat}, C-2), 104.9 (+, C-4), 111.0 (C_{quat}, CN), 116.0 (+, C-3), 131.9 (C_{quat}, C-5); IR (KBr): ν(tilde) = 3139 cm⁻¹, 3127, 3007, 2900, 2241, 1516, 1425, 1403, 1378; MS (EI, 70 eV): *m/z* (%) = 144/142 (30/100) [M⁺], 127/125 (17/57) [M⁺ – OH], 107 (2) [M⁺ – Cl], 89 (3), 80 (49), 73 (9), 64 (26), 52

(9); HRMS (EI): calcd for $C_5H_3ClN_2O$: 141.9934, found: 141.9934; elemental analysis (%) for $C_5H_3ClN_2O$ (142.5): C 42.13, H 2.12, N 19.65; found C 42.03, H 2.35, N 19.44.

104: 1H NMR (250 MHz, $CDCl_3$): δ = 6.03 (d, J = 7.5 Hz, 1 H, 4-H), 6.67 (d, J = 7.5 Hz, 1 H, 3-H), 8.96 (bs, 1 H, NH); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 100.0 (C_{quat} , C-

2), 108.6 (+, C-4), 113.6 (C_{quat} , CN), 120.8 (C_{quat} , C-5), 121.5 (+, C-3); MS (EI, 70 eV): m/z (%) = 128/126 (30/100) [M^+], 101/99 (1/3) [$M^+ - HCN$],

91 (4) [$M^+ - Cl$], 77/75 (1/4), 64 (16); HRMS (EI): calcd for $C_5H_3ClN_2$: 125.9985, found: 125.9985.

5-Chloro-1-methoxymethoxypyrrole-2-carbonitrile (105): To a vigorously stirred emulsion of a

24% aqueous NaOH (0.89 g, 5.34 mmol) in a solution of 5-Chloro-1-hydroxypyrrole-2-carbonitrile **103** (0.25 g, 1.75 mmol) and TBACl· H_2O (0.05 g, 0.18 mmol) in CH_2Cl_2 was added MOMCl (1.07 ml, 14.09 mmol)

and vigorous stirring continued for an additional 30 min. The organic layer was then separated and the aqueous fraction was extracted with CH_2Cl_2 (3 $\times$ 10 ml). Combined organic layers were then concentrated under reduced pressure and the crude product was purified first by column chromatography (EtOAc/hexanes 1:7) and finally by bulb-to-bulb distillation at the bath temperature 60–100 °C and at 0.02 Torr to give the title compound (0.310 g, 94%) as a colorless liquid which solidified in a freezer in a colorless solid. R_f = 0.55, EtOAc/hexanes = 1 : 4; 1H NMR (250 MHz, $CDCl_3$): δ = 3.73 (s, 3 H, CH_3), 5.18 (s, 2 H, CH_2), 6.05 (d, J = 4.8 Hz, 1 H, 4-H), 6.65 (d, J = 4.8 Hz, 1 H, 3-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 58.9 (+, CH_3), 102.1 (C_{quat} , C-2), 104.2 (–, CH_2), 105.1 (+, C-4), 111.6 (C_{quat} , CN), 115.5 (+, C-3), 118.6 (C_{quat} , C-5); IR (film): ν (tilde) = 3136 cm^{-1} , 2945, 2839, 2227, 1430, 1218, 1167, 1091; MS (EI, 70 eV): m/z (%) = 188/186 (3/8) [M^+], 127/125 (2/5) [$M^+ - C_2H_5O_2$], 64 (3), 45 (100) [$C_2H_5O^+$]; HRMS (EI): calcd for $C_7H_7ClN_2O_2$: 186.0196, found: 186.0196; elemental analysis calcd (%) for $C_7H_7ClN_2O_2$ (186.6): C 45.06, H 3.78, N 15.01; found C 44.87, H 3.79, N 14.81.

5-Chloro-1-methoxymethoxypyrrole-2-carboxamide (106): To a vigorously stirred solution of 5-

Chloro-1-methoxymethoxypyrrole-2-carbonitrile **105** (0.63 g, 2.30 mmol) and tetra-*n*-butylammonium hydrogen sulfate (0.16 g, 0.47 mmol) in CH₂Cl₂ (4.3 ml) a 20% aqueous NaOH (0.86 g, 4.30 mmol)

and 30% H₂O₂ (1.64 ml, 16.22 mmol) were added and vigorous stirring continued for an additional 90 min. The layers were separated and the water layer was additionally extracted with CH₂Cl₂ (4 × 5 ml) under salt-out conditions. The combined organic layers were concentrated under reduced pressure and the residue was purified by column chromatography (EtOAc/hexanes 1:1, *R_f* = 0.29) to give the title compound (0.445 g, 94%) as a colorless oil. ¹H NMR (250 MHz, CDCl₃): δ = 3.55 (s, 3 H, CH₃), 5.25 (s, 2 H, CH₂), 5.50–6.50 (br, 2 H, NH₂) 6.05 (d, *J* = 4.8 Hz, 1 H, 4-H), 6.73 (d, *J* = 4.8 Hz, 1 H, 3-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 59.5 (+, CH₃), 104.2 (+, C-4), 104.8 (–, CH₂), 111.4 (+, C-3), 118.6 (C_{quat}, C-2), 122.1 (C_{quat}, C-5), 160.4 (C_{quat}, CONH₂); IR (film): ν(tilde) = 3466 cm^{–1}, 3339, 3192, 2943, 2838, 1658, 1606, 1440, 1166, 1085; MS (EI, 70 eV): *m/z* (%) = 206/204 (11/32) [M⁺], 174/172 (23/70) [M⁺ – CH₄O], 145/143 (4/13) [M⁺ – C₂H₅O₂], 90/88 (2/6), 45 (100) [C₂H₅O⁺]; HRMS (EI): calcd for C₇H₉ClN₂O₂: 204.0302, found: 204.0302; elemental analysis calcd (%) for C₇H₉ClN₂O₂ (204.6): C 41.09, H 4.43, N 13.69; found C 41.32, H 4.21, N 13.47.

Di-*tert*-butyl [5-chloro-1-(methoxymethoxy)-pyrrol-2-yl]carbonylimidodicarbomate (107)

and *tert*-butyl bis-[5-chloro-1-(methoxymethoxy)-pyrrol-2-yl]carbonylcarbamate (108): To a solution of 5-Chloro-1-methoxymethoxypyrrole-2-carboxamide (0.41 g, 2.00 mmol) and Boc₂O (2.11 g, 9.67 mmol) in anhydrous MeCN (10 ml) 4-pyrrolidinopyridine (50 mg, 0.34 mmol) was added and stirring continued for an additional 2.5 h. The mixture was then carefully heated with a heat-gun to ca. 50 °C, cooled, concentrated under reduced pressure and the crude product was purified by column chromatography (EtOAc/hexanes 1:8) to give the desired doubly N-Boc-

acylated product **107** (0.665 g, 82%, $R_f = 0.13$) as a colorless solid and the by-product **108** (50 mg, 5%, $R_f = 0.07$) as a viscous opaque oil.

107: M.p. 67–68 °C; ^1H NMR (250 MHz, CDCl_3): $\delta = 1.42$ [s, 18 H, $2 \times \text{C}(\text{CH}_3)_3$], 3.68 (s, 3 H,

CH₂), 104.9 (+, C-4), 116.0 (+, C-3), 122.3 (C_{quat} , C-2), 124.2 (C_{quat} , C-5), 149.3 (C_{quat} , NCO₂), 157.0 (C_{quat} , CON); IR (KBr): $\nu(\text{tilde}) = 3123$ cm^{-1} , 3012, 2971, 2946, 1793, 1696, 1431, 1391, 1371, 1275, 1255, 1156, 1102; MS (EI, 70 eV): m/z (%) = 406/404 (2/6) [M^+], 350/348 (2/5) [$\text{M}^+ - \text{C}_4\text{H}_8$], 294/292 (2/6) [$\text{M}^+ - \text{C}_8\text{H}_{16}$], 250/248 (11/35) [$\text{M}^+ - \text{C}_9\text{H}_{17}\text{O}_2$], 190/188 (11/35), 174/172 (30/100) [$\text{C}_7\text{H}_7\text{NO}_2\text{Cl}^+$] 143 (31), 57 [C_4H_9^+] (100), 45 (58) [$\text{C}_2\text{H}_5\text{O}^+$]; HRMS (EI): calcd for $\text{C}_{17}\text{H}_{25}\text{ClN}_2\text{O}_7$: 404.1350, found: 404.1350; elemental analysis calcd (%) for $\text{C}_{17}\text{H}_{25}\text{ClN}_2\text{O}_7$ (404.9): C 50.44, H 6.22, N 6.92; found C 50.26, H 5.99, N 6.85.

108: ^1H NMR (250 MHz, CDCl_3): $\delta = 1.39$ [s, 9 H, $\text{C}(\text{CH}_3)_3$], 3.64 (s, 3 H, CH₃), 5.24 (s, 2 H,

104.8 (–, $2 \times$ CH₂), 104.8 (+, $2 \times$ C-4), 115.2 (+, $2 \times$ C-3), 122.5 (C_{quat} , $2 \times$ C-2), 123.6 (C_{quat} , $2 \times$ C-5), 151.1 (C_{quat} , NCO₂), 158.9 (C_{quat} , $2 \times$ CON); IR (film): $\nu(\text{tilde}) = 3131$ cm^{-1} , 2979, 2940, 2946, 2840, 1751, 1700, 1429, 1395, 1370, 1256, 1149, 1087; MS (CI), m/z (%): 511/509 (33/52) [$\text{M} + \text{NH}_4^+$], 494/492 (9/14) [$\text{M} + \text{H}^+$].

5-Chloro-1-methoxymethoxypyrrole-2-carboxylic acid (109): To a solution of the doubly *N*-Boc-

acylated derivative **107** (0.630 g, 1.55 mmol) in dioxane (8 mL) was

added 1 N NaOH (2.02 mL) and the mixture was stirred at 55 °C for 2

h. Then, all volatiles were removed under reduced pressure, the residue

was taken up with H₂O (20 mL) and washed with CH₂Cl₂ (5 × 5 mL). The pH of the water layer

was adjusted with 1 N NaHSO₄ to 3, and the product was extracted with CH₂Cl₂ (4 × 10 mL)

under salting-out conditions. The organic phase was dried and concentrated to give after

recrystallization from CH₂Cl₂/hexanes **109** (193 mg, 60%) as a colorless solid. *R*_f = 0.36,

EtOAc/hexanes = 1:2.5 (5% AcOH); m.p. 120–122 °C (decomp.); ¹H NMR (250 MHz, CDCl₃):

δ = 3.71 (s, 3 H, CH₃), 5.25 (s, 2 H, CH₂), 6.06 (d, *J* = 4.9 Hz, 1 H, 4-H), 6.95 (d, *J* = 4.9 Hz,

1 H, 3-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 58.9 (+, CH₃), 104.6 (–, CH₂), 104.7 (+, C-4),

116.0 (+, C-3), 117.7 (C_{quat}, C-2), 122.8 (C_{quat}, C-5), 163.6 (C_{quat}, CO); IR (KBr): ν(tilde) =

3451 cm^{–1}, 3135, 3006, 2964, 2944, 2618, 2562, 1671, 1543, 1533, 1447, 1437, 1323, 1263,

1159, 1119; MS (EI, 70 eV): *m/z* (%) = 207/205 (13/43) [M⁺], 177/175 (2/5) [M⁺ – CH₂O],

145/143 (2/7) [M⁺ – C₂H₆O₂], 129 (7), 91/89 (2/6), 75/73 (1/3), 45 (100) [C₂H₅O⁺]; HRMS (EI):

calcd for C₇H₈ClN₄O: 205.0142; found 205.0142; elemental analysis calcd (%) for C₇H₈ClNO₄

(205.6): C 40.89, H 3.92, N 6.81; found C 40.93, H 3.94, N 6.68.

Chpca(MOM)-Phe-OFm (112): Boc-Phe-OFm **110** (0.260 g, 0.59 mmol) was deprotected with 5

M HCl in Et₂O (25 mL) for 1.5 h. All volatiles were then removed and the resultant white solid

residue was triturated with Et₂O and filtered off to give HCl·H-Phe-OFm **111** which was directly

coupled with acid **109** (0.100 g, 0.48 mmol) using EDC (0.100 g, 0.52 mmol), HOBt (80 mg,

hydrate with 13% H₂O, 0.515 mmol) and DIEA (0.138 g, 1.07 mmol) in CH₂Cl₂ (7 mL)

according to GP 5 for 4 h. After usual aqueous work-up the crude product was purified by

column chromatography (EtOAc/hexanes 1:3, *R*_f = 0.30) to give **112** (0.24 g, 93%) as a turbid

glass. ¹H NMR (250 MHz, CDCl₃): δ = 3.12 (ddt, *J* = 5.8, 14.0, 14.0 Hz, 2 H, 3-H), 3.49 (s, 3 H,

OMe), 4.17 (t, $J = 6.6$ Hz, 1 H, 1'-H, *Fm*), 4.38–4.59 (m, 2 H, 2'-H, *Fm*), 5.00 (s, 2 H, OCH₂O), 5.03 (dt, $J = 5.8, 5.8$ Hz, 1 H, 2-H), 5.99 (d, $J = 4.8$ Hz, 1 H, 4-H, *Chpca*), 6.63 (d, $J = 4.8$ Hz, 1 H, 3-H, *Chpca*), 6.84 (d, $J = 7.5$ Hz, 1 H, NH), 7.03–7.11 (m, 2 H, Ar-H), 7.18–7.33 (m, 5 H, Ar-H), 7.33–7.46 (m, 2 H, Ar-H), 7.52 (d, $J = 7.5$ Hz, 2 H, Ar-H), 7.74 (d, $J = 7.5$ Hz, 2 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): $\delta = 37.6$ (–, C-3), 46.6 (+, C-1', *Fm*), 53.0 (+, C-2), 59.1 (+, OMe), 67.0 (–, C-2', *Fm*), 104.0 (+, C-4, *Chpca*), 104.7 (–, OCH₂O), 111.0 (+, C-3, *Chpca*), 118.5 (C_{quat}, C-2, *Chpca*), 119.9 (+, Ar-C), 121.9 (C_{quat}, C-5, *Chpca*), 124.8 ($\times 2$), 127.1, 127.8, 128.6, 129.2 (+, Ar-C), 135.7 (C_{quat}, Ar-C), 141.2 (C_{quat}, Ar-C), 143.2, 143.3 (C_{quat}, Ar-C), 158.0 (C_{quat}, C-1, *Chpca*), 171.4 (C_{quat}, C-1); MS (EI, 70 eV), m/z (%) = 532/530 (1/2) [M^+], 354/352 (2/7) [$M^+ - C_{14}H_{10}$], 178 (100) [$C_{14}H_{10}^+$], 128 (8), 91 (8) [$C_7H_7^+$], 45 (12) [$C_2H_5O^+$]; HRMS (EI): calcd for C₃₀H₂₇ClN₂O₅: 530.1608; found 530.1608.

Chpca-Phe-OFm (**113**): Compound **112** (0.235 g, 0.44 mmol) was deprotected using MgBr₂·Et₂O (2.50 g, 96.81 mmol) and EtSH (0.12 mL, 1.62 mmol) in Et₂O (20 mL) according to GP 10 for 4 h. 1 M NaHSO₄ (10 mL) and Et₂O (40 mL) were then added, the organic layer was separated washed with 1 M NaHSO₄ (3 $\times$ 10 mL), water (5 $\times$ 10 mL), brine (2 $\times$ 10 mL), dried, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (twice, EtOAc/hexanes 1:3, $R_f = 0.35$) to give **113** (0.19 g, 88%) as a white foam. ¹H NMR (250 MHz, CDCl₃): $\delta = 3.11$ (dd, $J = 6.3, 6.3$ Hz, 2 H, 3-H), 4.18 (t, $J = 6.3$ Hz, 1 H, 1'-H, *Fm*), 4.47–4.59 (m, 2 H, 2'-H, *Fm*), 5.00 (s, 2 H, OCH₂O), 5.03 (dt, $J = 7.5, 6.3$ Hz, 1 H, 2-H), 5.93 (d, $J = 5.0$ Hz, 1 H, 4-H, *Chpca*), 6.19 (d, $J = 7.5$ Hz, 1 H, NH), 6.35 (d, $J = 5.0$ Hz, 1 H, 3-H, *Chpca*), 7.00–7.09 (m, 2 H, Ar-H), 7.21–7.29 (m, 5 H, Ar-H), 7.36–7.47 (m, 2 H, Ar-H), 7.52 (dd, $J = 7.5, 3.0$ Hz, 2 H, Ar-H), 7.76 (dd, $J = 7.5, 4.3$ Hz, 2 H, Ar-H), 13.2–13.5 (br, 1 H, OH); ¹³C NMR (62.9 MHz, CDCl₃): $\delta = 37.5$ (–, C-3), 46.4 (+, C-1', *Fm*), 52.8 (+, C-2), 67.1 (–, C-2', *Fm*), 102.5 (+, C-4, *Chpca*), 106.0 (+, C-3, *Chpca*), 114.8 (C_{quat}, C-2, *Chpca*), 115.6 (C_{quat}, C-5, *Chpca*), 119.9 (+, Ar-C), 124.6, 127.0, 127.2, 127.7, 127.8, 128.5,

128.9 (+, Ar-C), 135.1 (C_{quat}, Ar-C), 141.1 (C_{quat}, Ar-C), 142.9, 143.0 (C_{quat}, Ar-C), 161.9 (C_{quat}, C-1, *Chpca*), 171.3 (C_{quat}, C-1); MS (EI, 70 eV), m/z (%) = 488/486 (6/18) [M⁺], 310/308 (2/8) [M⁺ – C₁₄H₁₀], 178 (100) [C₁₄H₁₀⁺], 146/144 (5/16) [C₅H₃ClNO₂⁺], 91 (8) [C₇H₇⁺]; HRMS (EI): calcd for C₂₈H₂₃ClN₂O₄: 486.1346; found 486.1346.

Chpca-Phe-OH (**114**): Compound **113** (70 mg, 0.013 mmol) was deprotected using 20% Et₂NH in CH₂Cl₂ (2 mL) for 40 min. All volatiles were removed, the residue was taken up with EtOAc (40 mL) and washed with 1 M NaHSO₄ (3 × 10 mL), water (3 × 10 mL), brine (2 × 10 mL), dried, filtered and concentrated under reduced pressure to give after drying at 0.02 Torr crude **114** (44 mg, 100%) as a rose oil which was immediately used for the next step.

Chpca-Phe-Phe-Val-OMe (**119**): Boc-Phe-Val-OMe **117** (60 mg, 0.16 mmol) was deprotected with 5 M HCl in Et₂O (5 mL) for 2 h. The mixture was concentrated under reduced pressure and the residual hydrochloride of H-Phe-Val-OMe HCl·**118** was directly coupled with *N*-acyl amino acid **114** (44 mg, 0.13 mmol) using HATU (97 mg, 0.25 mmol) and DIEA (0.070 mL, 0.41 mmol) in CH₂Cl₂ (4 mL) according to GP 7 for 1.5 h. The mixture was then taken up with Et₂O (40 mL) and the crude product obtained after usual aqueous work-up (GP 5) was purified by column chromatography (EtOAc/hexanes 1:1, R_f = 0.27) and finally recrystallized from EtOAc/hexanes 1:1 to give **119** (17 mg, 23%) as an off-white solid. $[\alpha]_{20}^D$ –32.0 (c = 1.10, CHCl₃); ¹H NMR (300 MHz, CD₃OD): δ = 0.92 (d, J = 7.0 Hz, 6 H, 4-H, *Val*), 2.08 (octet, J = 7.0 Hz, 1 H, 3-H, *Val*), 2.93 (dt, J = 13.7, 8.0 Hz, 2 H, 3-H, *Phe*), 3.11 (dt, J = 13.7, 6.5 Hz, 2 H, 3-H, *Phe*), 3.67 (s, 3 H, OMe), 4.25–4.33 (m, 1 H, 2-H, *Val*), 4.70 (dd, J = 6.2, 8.0 Hz, 1 H, 2-H, *Phe*), 4.77 (dd, J = 5.9, 8.0 Hz, 1 H, 2-H, *Phe*), 5.98 (d, J = 4.8 Hz, 1 H, 4-H, *Chpca*), 6.63 (d, J = 4.8 Hz, 1 H, 3-H, *Chpca*), 7.09–7.28 (m, 10 H, Ar-H), 8.25 (d, J = 8.5 Hz, 1 H, NH); ¹³C NMR (75.5 MHz, CD₃OD): δ = 18.7, 19.5 (+, C-4, *Val*), 31.9 (+, C-3, *Val*), 38.9 (–, C-3, *Phe*), 39.0 (–, C-3, *Phe*), 52.5 (+, C-2), 55.5 (+, C-2), 55.8 (+, C-2), 59.4 (+, OMe), 103.9 (+, C-4, *Chpca*),

110.9 (+, C-3, *Chpca*), 119.3 (C_{quat}, C-2, *Chpca*), 122.0 (C_{quat}, C-5, *Chpca*), 119.9 (+, Ar-C), 127.7, 127.9, 129.4, 129.5, 130.4, 130.4 (+, Ar-C), 137.8, 138.0 (C_{quat}, Ar-C), 161.2 (C_{quat}, C-1, *Chpca*), 173.1, 173.1, 173.3 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 3063 \text{ cm}^{-1}$, 3029, 2964, 2933, 1746, 1644, 1630, 1551, 1412, 1259, 1144; MS (EI, 70 eV), m/z (%) = 570/568 (9/32) [M⁺], 439/437 (9/31) [M⁺ – C₆H₁₃NO₂], 290 (32), 279 (40), 262 (12), 146/144 (100) [C₅H₃ClNO₂⁺], 91 (12) [C₇H₇⁺]; HRMS (EI): calcd for C₂₉H₃₃ClN₄O₆: 568.2089; found 568.2089.

2-Acetamido-6-fluoropyridine (123): 2-Acetamido-6-aminopyridine **122** (2.6 g, 17.20 mmol)

was dissolved in pyridinium fluoride (11 mL), cooled to – 10 °C (internal temperature) and then NaNO₂ (1.305 g, 18.92 mmol) was added at the same temperature in small portions and stirring continued for an additional 10 min. The mixture was allowed to warm to 20 °C, poured into ice-cold water (50 mL) and the water fraction was extracted with CH₂Cl₂ (10 × 20 mL). The combined organic layers were stirred with K₂CO₃ (4 g) and water (1 mL) for 1 h, magnesium sulfate (10 g) was then added and stirring continued for an additional 20 min. Salts were filtered off, volatiles were removed under reduced pressure and the residue was recrystallized from CH₂Cl₂/hexanes to give **123** (2.09 g, 79%) as an off-white solid. ¹H NMR (250 MHz, CDCl₃): δ = 2.21 (s, 3 H, CH₃), 6.65 (dd, J = 2.5, 8 Hz, 1 H, 5-H), 7.79 (ddd, J = 8.0, 8.0, 8.0 Hz, 1 H, 4-H), 7.95–8.10 (br, NH), 8.00–8.07 (m, 1 H, 3-H).

2-Acetamido-6-fluoropyridine-N-oxide (124): A solution of **123** (8.89 g, 57.67 mmol) and m-

chloroperbenzoic acid (33.26 g, 90% purity, 173.53 mmol) in CH₂Cl₂ (70 mL) was stirred at 20 °C for 9 days. The mixture was then stirred with K₂CO₃ (10 g) for 1 h, filtered, intensely shaken with saturated aqueous K₂CO₃ (60 mL) for 15 min, filtered through a pad of magnesium sulfate (5 cm) and concentrated under reduced pressure to ca. 20 mL. Hexanes (200 mL) were then added and the

precipitate formed was filtered off to give **124** (3.78 g, 38%) as a white solid. $R_f = 0.10$, EtOAc/hexanes = 1:1; m.p. 155–157 °C (decomp.); ^1H NMR (250 MHz, CDCl_3): δ = 2.33 (s, 3 H, CH_3), 6.86–6.95 (m, 1 H, 5-H), 7.24–7.38 (m, 1 H, 4-H), 8.28 (d, J = 7.5 Hz, 1 H, 3-H), 9.80–10.20 (br, 1 H, NH); ^{13}C NMR (62.9 MHz, D_2O): δ = 24.4 (+, CH_3), 106.0 (+, d, J = 19.6 Hz, C-5), 112.2 (+, d, J = 4.3 Hz, C-3), 134.1 (+, d, J = 8.3 Hz, C-4), 144.7 (C_{quat} , d, J = 7.0 Hz, C-2), 157.0 (C_{quat} , d, J = 259.9 Hz, C-6), 173.3 (C_{quat} , CO); IR (KBr): $\nu(\text{tilde})$ = 3244 cm^{-1} , 3139, 3108, 3059, 2993, 2904, 1703, 1583, 1529, 1502, 1398, 1286, 1264, 1215; MS (EI, 70 eV): m/z (%) = 170 (34) [M^+], 128 (100) [$\text{M}^+ - \text{C}_2\text{H}_2\text{O}$], 112 (10), 101 (8), 83 (8), 70 (8), 43 (72) [$\text{C}_2\text{H}_3\text{O}^+$]; HRMS (EI): calcd for $\text{C}_7\text{H}_7\text{FN}_2\text{O}_2$: 170.0492; found 170.0492; elemental analysis calcd (%) for $\text{C}_7\text{H}_7\text{FN}_2\text{O}_2$ (170.1): C 49.42, H 4.15, N 16.46; found C 49.36, H 4.28, N 16.27.

2.7. Final assembling of hormaomycin

Preparation and HPLC behavior of (S)-FDVA-derivatives of the amino acids used for synthesis of hormaomycin: The mixture of H-(R)-(3-Ncp)Ala-OH, H-(S)-(3-Ncp)Ala-OH, H-(2S,3R)-(β Me)Phe-OH, (S)-Ile, H-(2S,4R)-(PE)Pro-OH, H-(2R)- α -Thr (ca. 0.4 mg each) was divided in two equal portions, one of each was directly transformed into mixture of the (S)-FDVA derivatives (GP 2) and the second one was dissolved in water (0.2 mL) and treated with Et_3N (0.08 mL) and Ac_2O (0.08 mL). The mixture was then heated at 60 °C for 1 h, concentrated under reduced pressure to give the residue which was finally hydrolyzed according to GP 1 within 12 h to give the mixture of pairs of corresponding epimers at C-2 which was further also transformed into the mixture of the appropriate (S)-FDVA derivatives (GP 2). Also [(S)-FDVA] $_2$ -(2R)- α -Dab-OH was prepared. Detection: UV: 340 nm; ESI-MS, positive, m/z : 454.7–455.7 [(3-Ncp)Ala], 434.7–435.7 [(4-PE)Pro], 459.7–460.7 [(β Me)Phe], 411.7–412.7 (Ile), 399.7–400.7 (α -Thr), 678.7–679.7 (α -Dab); ESI-MS, negative, m/z : 452.6–453.6 [(3-Ncp)Ala],

433.6–434.6 [(4-PE)Pro], 457.6–458.6 [(βMe)Phe], 409.6–410.6 (Ile), 397.6–398.6 (α-Thr), 676.7–677.7 (α-Dab); retention times: (3-Ncpa)Ala: (2*S*,1'*R*,2'*R*)-isomer – t_R = 8.96 min, (2*S*,1'*R*,2'*R*)-isomer – t_R = 11.44 min; (4-PE)Pro: (2*S*,4*R*)-isomer – t_R = 13.08 min; the concentration of this amino acid after hydrolysis (GP 1) and derivatization was usually lower than detection limit; (βMe)Phe: (2*S*,3*R*)-isomer – t_R = 13.75 min; (2*R*,3*R*)-isomer – t_R = 18.53 min; Ile: (*S*)-isomer – t_R = 11.69 min; (*R*)-α-isomer – t_R = 17.25 min; α-Thr: (*R*)-isomer – t_R = 5.56 min; this amino acid did not epimerize under conditions applied; α-Dab: (*R*)-isomer – 20.21 min.

Deprotection of N-Fmoc-protected peptides (GP 4): The protected peptides (1 mmol) were taken up with acetonitrile or THF (2 mL), diethylamine (2 mL) was added, and the resulting mixture left at ambient temperature for 40 min. All volatiles were evaporated under reduced pressure, the residue was taken up with toluene (2 × 5 mL), which was evaporated under reduced pressure to remove the last traces of diethylamine. Obtained crude *N*-deprotected peptides were directly used in the next condensation step.

Peptide condensation step for the preparation of peptides using EDC/HOAt mediated coupling (GP 5): EDC (1.03 mmol) and HOAt (1.05 mmol) were added to a cooled (4 °C) solution of the respective *N*-protected amino acid (1 mmol) in anhydrous CH₂Cl₂ (3 mL). After 20 min, the solution of the appropriate crude *N*-deprotected peptide (0.97 mmol) and TMP (3 mmol) in anhydrous CH₂Cl₂ (1 mL) was added at the same temperature. The temperature was allowed to reach 20 °C and stirring was continued for 15 h. Then the reaction mixture was diluted with Et₂O or EtOAc (30 mL) and washed with water (2 × 5 mL), 1 M KHSO₄ (3 × 5 mL), water (2 × 5 mL), 3% aqueous solution of NaHCO₃ (3 × 5 mL), water (3 × 5 mL), brine (2 × 5 mL), dried and concentrated under reduced pressure. The residue was purified by column chromatography or recrystallization.

Preparation of esters using EDC/4-dialkylaminopyridine mediated coupling (GP 6): EDC (1.1–2.2 mmol) was added to a cooled (4 °C) solution of the respective *N*-protected amino acid (1.03 mmol), alcohol or *N,C*-protected threonine (1 mmol) and DMAP or 4-pyrrolidinopyridine (0.1–1 mmol) in anhydrous CH₂Cl₂ (3 mL). The temperature was allowed to reach 20 °C and stirring was continued for 15 h. Then the reaction mixture was diluted with Et₂O or EtOAc (30 mL) and washed with 1 M KHSO₄ (3 × 5 mL), water (2 × 5 mL), 3% aqueous solution of NaHCO₃ (3 × 5 mL), water (3 × 5 mL), brine (2 × 5 mL), dried and concentrated under reduced pressure. The residue was purified by column chromatography.

Preparation of depsipeptides using HATU/HOAt mediated coupling (GP 7): Deprotected according to GP 4 tpeptide (0.1 mmol) was dissolved anhydrous MeCN (3 mL), depsidipeptide (0.11 mmol), HATU (0.107 mmol) and HOAt (0.11 mmol) were added and the reaction mixture was cooled to 4 °C. DIEA (0.11 mmol) and TMP (0.3–0.5 mmol) were then added, the mixture was allowed to warm to 20 °C and stirring continued for an additional 15 h. The mixture was then taken up with Et₂O or EtOAc (40 mL) and after usual aqueous work-up (GP 5) the organic layer was concentrated to leave crude depsihexapeptide, which was purified by recrystallization and/or column chromatography.

Preparation of cyclohexadepsipeptides (GP 8): Hexadepsipeptide (0.105 mmol) was deprotected and then dissolved with CH₂Cl₂ (1.0 l). The solution was cooled to 4 °C (internal temperature), HATU (0.105 mmol) and HOAt (0.105 mmol) were added and then the solution of DIEA (0.315 mmol) in CH₂Cl₂ (50 mL) was added over 30 min. The cooling bath was removed and stirring continued for an additional 2 h at the ambient temperature. Then the reaction mixture was cooled again to 4 °C and the second portions of HATU (0.105 mmol) and HOAt (0.105 mmol) were added and then the solution of DIEA (0.315 mmol) in CH₂Cl₂ (50 mL) was added over 30 min.

The temperature was allowed to reach 20 °C and stirring was continued for 15 h. After this, the solvent was removed under reduced pressure, the residue was taken up with Et₂O, and after usual water work-up (see GP 5) and concentration under reduced pressure, was purified first by column chromatography and then by recrystallization (Et₂O/pentane) to give crude product, which was finally purified by preparative HPLC.

Preparation of OSu-esters from alcohols (GP 9): To a vigorously stirred suspension of dried K₂CO₃ (1.2 g) in a solution of alcohol (10 mmol) in anhydrous CH₂Cl₂ (15 mL) a 20% phosgene solution in toluene (7.5 mL) was added at –10 °C for 15 min and stirring continued at the same temperature for 1 h. The cooling bath was then removed and the mixture stirred for a further 1 h. It was then concentrated under reduced pressure at 20 °C, taken up with Et₂O (20 mL), filtered through a pad of MgSO₄ and concentrated under reduced pressure to give the crude chloroformate which was further used without any purification. To a vigorously stirred ice-cold solution of the crude chloroformate (10 mmol) and HOSu (9.5 mmol) in THF (70 mL) Et₃N (10.1 mmol) was added for 5 min. The reaction mixture was then allowed to warm to 20 °C and stirred for an additional 15 h. The reaction mixture was filtered, concentrated under reduced pressure, taken up with Et₂O or EtOAc, filtered again and concentrated. The residue was crystallized from EtOAc/hexanes to give appropriate OSu ester.

Cleavage of MOM-group using MgBr₂·Et₂O and EtSH (GP 10): MgBr₂·Et₂O (1 mmol) and EtSH (0.5 mmol) were added to a vigorously stirred solution of the O-MOM protected derivative (0.1 mmol) in CH₂Cl₂ or Et₂O (15 mL) and stirring continued for a further 3.5 h (the TLC control was impossible as starting material and product in all cases showed exactly the same *R_f* in all chromatographic systems). The mixture was then taken up with EtOAc (40 mL) and washed with 1 M NaHSO₄ (3 × 10 mL), water (5 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated

under reduced pressure. The residue was purified by column chromatography or preparative TLC, or/and crystallization.

Deprotection of N-MeZ protected cyclohexadepsipeptides (GP 11): The NMe-Z protected cyclodepsipeptide (10 μ mol) was treated with 10% anisole in TFA (1 mL) in dark for 2 h. All volatiles were then removed under reduced pressure at 20 °C. The solid residue was taken up with toluene (2 $\times$ 10 mL) which was distilled off under reduced pressure and then washed with pentane (2 mL $\times$ 3) and hexanes (2 mL $\times$ 3). The resultant crude deprotected depsipeptide was directly used for the appropriate coupling reaction.

Boc-Thr-OMTM (132): MTMCl (0.918 g, 9.51 mmol) was added to a vigorously stirred suspension of dry NaI (1.80 g, 12.01 mmol) in anhydrous MeCN (10 mL). After 15 min, this mixture was added to a solution of Boc-Thr-OH **131** (2.79 g, 10.00 mmol) in MeCN (20 mL) and the reaction mixture was heated at 80 °C for 3 h. The mixture was then cooled and concentrated under reduced pressure. The residue was taken up with Et₂O (50 mL) and subjected usual aqueous work-up. The organic layer was then dried, filtered and concentrated under reduced pressure. The resultant crude product was purified by column chromatography (EtOAc/hexanes 1:3, R_f = 0.15) to give **132** (2.05 g, 77%) as a colorless oil. ¹H NMR (250 MHz, CDCl₃): δ = 1.27 (d, J = 6.3 Hz, 3 H, 4-H), 1.45 [s, 9 H, C(CH₃)₃], 2.04 (d, J = 5.0 Hz, 1 H, OH), 2.25 (s, 3 H, SMe), 4.20–4.42 (m, 2 H, 2-H, 3-H), 5.17 (d, J = 11.8 Hz, 1 H, H_a, OCH₂SMe), 5.21–5.39 (br, 1 H, NH), 5.30 (d, J = 11.8 Hz, 1 H, H_b, OCH₂SMe).

Boc-Thr-(Nps-Pro)-OMTM (134): Nps-Pro-OH **133** (0.763 g, 1.70 mmol) was coupled with alcohol **132** (0.439 g, 1.64 mmol) using EDC (0.326 g, 1.18 mmol) and DMAP (42 mg, 0.34 mmol) in CH₂Cl₂ (10 mL) according to GP 6 for 15 h. The crude product obtained after usual

aqueous work-up was finally purified by column chromatography (EtOAc/hexanes 1:4, R_f = 0.13) to give the ester **134** (0.717 g, 85%) as a bright yellow oil. ^1H NMR (250 MHz, CDCl_3): δ = 1.11–1.40 (m, 3 H, 4-H, *Thr*), 1.46 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.85–2.51 (m, 4 H, 3-H, 4-H, *Pro*), 2.04 (s, 3 H, SMe), 3.01–3.26, 3.27–3.63 ($2 \times$ m, 2 H, 5-H, *Pro*), 3.97 (d, J = 8.0 Hz, 1 H, 2-H, *Thr*), 4.22–4.35, 4.36–4.60 ($2 \times$ m, 1 H, 2-H, *Pro*), 4.82–5.34 (m, 3 H, OCH_2SMe , C-3, *Thr*), 5.34–5.62 (br, 1 H, NH), 7.26 (dd, J = 8.3, 8.3 Hz, 1 H, 4-H, *Nps*), 7.46–7.79 (m, 2 H, 5-H, 6-H, *Nps*), 8.28 (d, J = 8.3 Hz, 1 H, 3-H, *Nps*); ^{13}C NMR (62.9 MHz, CDCl_3): δ = 15.0 (+, SMe), 16.2 (+, C-4, *Thr*), 22.7, 24.2 (–, C-3, *Pro*), 27.8 [+ , $\text{C}(\text{CH}_3)_3$], 31.1 (–, C-4, *Pro*), 55.2, 58.9 (–, C-5, *Pro*), 56.6 (+, C-2, *Thr*), 66.1, 68.6 (+, C-2, *Pro*), 69.4 (–, OCH_2SCH_3), 70.9 (+, C-3, *Thr*), 79.8 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 123.2 (+, C-3, *Nps*), 124.4 (+, C-6, *Nps*), 125.1, 125.4 (+, C-4, *Nps*), 133.5 (+, C-5, *Nps*), 141.6 (C_{quat} , C-1, *Nps*), 144.6 (C_{quat} , C-2, *Nps*), 155.3 (C_{quat} , NCO_2), 169.2, 171.9 (C_{quat} , C-1); MS (ESI), positive m/z (%) = 552 (75) [$\text{M} + \text{Na}^+$]; negative m/z (%) = 528 (100) [$\text{M} - \text{H}^+$].

Boc-Thr(Nps-Ile-Pro)-MTM (136): The ester **134** (0.109 g, 0.21 mmol) was deprotected with 0.2 M HCl in Et_2O (2.1 mL, 0.42 mmol) and EtSH (0.075 mL, 1.0 mmol) for 1 min. The mixture was then concentrated under reduced pressure and the solid residue was washed with boiled hexanes to give the crude deprotected amino ester which was immediately coupled with *Nps-N*-protected isoleucine **135** (59 mg, 0.21 mmol) using EDC (41 mg, 0.21 mmol), HOAt (29 mg, 0.21 mmol) and TMP (0.125 g, 1.03 mmol) in CH_2Cl_2 (4 mL) according to GP 5 for 16 h. The crude product obtained after usual aqueous work-up (GP 5) was finally purified by column chromatography (EtOAc/hexanes 1:2.5, R_f = 0.23) to give **136** (0.107 g, 80%) as a bright yellow oil. ^1H NMR (250 MHz, CDCl_3): δ = 0.97 (t, J = 7.0 Hz, 3 H, 5-H, *Ile*), 1.04 (d, J = 6.8 Hz, 3 H, 1'-H, *Ile*), 1.16–1.39 (m, 1 H, 4-H_a, *Ile*), 1.30 (d, J = 6.3 Hz, 4-H, *Thr*), 1.46 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.71–2.05 (m, 5 H, 4-H_b, *Ile*, 3-H, 4-H, *Pro*), 2.05–2.20 (m, 1 H, 3-H, *Ile*), 2.23 (s, 3 H, SMe), 3.27–3.43 (m, 2 H, 5-H_a, *Pro*, NH, *Ile*), 3.43–3.59 (m, 2 H, 2-H, *Ile*, 5-H_b, *Pro*), 4.44–4.56 (m, 2 H, 2-H), 5.21 (d, 2 H, OCH_2SMe), 5.28 (d, J = 9.5 Hz, 1 H, NH), 5.48 (dq, J = 3.0, 6.3 Hz, 3-

H, *Thr*), 7.25 (dd, $J = 8.0, 8.0$ Hz, 1 H, 4-H, *Nps*), 7.62 (dd, $J = 8.0$ Hz, 1 H, 6-H, *Nps*), 8.08 (d, $J = 8.0$ Hz, 1 H, 5-H, *Nps*), 8.25 (d, $J = 8.0$ Hz, 1 H, 3-H, *Nps*); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 11.1$ (+, C-5, *Ile*), 15.2 (+, SMe), 15.5 (+, C-1', *Ile*), 16.7 (+, C-4, *Thr*), 24.3 (–, C-4, *Pro*), 24.8 (+, C-4, *Ile*), 28.1 [+ , $\text{C}(\text{CH}_3)_3$], 29.5 (–, C-3, *Pro*), 38.6 (+, C-3, *Ile*), 47.1 (–, C-5, *Pro*), 57.0 (+, C-2, *Thr*), 58.9 (+, C-2, *Pro*), 68.4 (+, C-2, *Ile*), 69.9 (–, OCH_2SCH_3), 71.3 (+, C-3, *Thr*), 80.1 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 124.6 (+, C-3, *Nps*), 124.7 (+, C-6, *Nps*), 125.5 (+, C-4, *Nps*), 133.4 (+, C-5, *Nps*), 142.3 (C_{quat} , C-1, *Nps*), 145.6 (C_{quat} , C-2, *Nps*), 155.7 (C_{quat} , NCO_2), 169.6, 170.7, 172.4 (C_{quat} , C-1); MS (EI, 70 eV), m/z (%) = 642 (5) [M^+], 586 (6) [$\text{M}^+ - \text{C}_4\text{H}_8$], 521 (8), 509 (17), 437 (17), 239 (100), 154 (75) [$\text{C}_6\text{H}_4\text{NO}_2\text{S}^+$], 138 (36) [$\text{C}_6\text{H}_4\text{NOS}^+$], 86 (52), 61 (38) [$\text{C}_2\text{H}_5\text{S}^+$], 57 (22) [C_4H_9^+]; HRMS (EI): calcd for $\text{C}_{28}\text{H}_{42}\text{N}_4\text{O}_9\text{S}_2$: 642.2393; found 642.2393.

Attempt of the selective cleavage of MTM-group from 134: $\text{MgBr}_2 \cdot \text{Et}_2\text{O}$ (0.45 g, 1.74 mmol) was added to a solution of ester **134** (0.119 g, 0.23 mmol) in CH_2Cl_2 (15 mL) and stirring continued for 45 min. TLC analysis of reaction mixture showed full consumption of the starting material and unexpectedly polar, ninhydrin active main product. After usual aqueous work-up (GP 5, excluding washings with an aqueous solution of NaHCO_3) only several mg of the mixture containing desired acid (according to ^1H NMR) was obtained. An additional experiment with *Nps-Ile-OH* showed that in this case *Nps* group completely cleaved within 20 min.

N-Boc-3-(2S,3R)Methylphenylalanine (139): A solution of Boc_2O (0.607 g, 2.78 mmol) in acetone (5 mL) was added to a solution 3-(2*S*,3*R*)-methylphenylalanine hydrochloride (2*S*,3*R*)-**78** (0.5 g, 2.32 mmol) in 1 M NaOH (4.7 mL) and the resulting mixture was stirred for 15 h. The mixture was then concentrated to ca. 5 mL under reduced pressure, diluted with water (20 mL) and extracted with Et_2O (3×5 mL). pH of the aqueous layer was adjusted to 2 with solid NaHSO_4 and the emulsion obtained was extracted with Et_2O (40 mL). The organic layer was washed with 1 M aqueous NaHSO_4 (3×10 mL), water (3×10 mL), brine (2×5 mL), dried, filtered and concentrated

under reduced pressure to give crude product which was recrystallized from hexanes to give **2** (0.53 g, 82%) as a white solid. $[\alpha]_{20}^D$ 18.6 ($c = 1.73$, CHCl_3) [lit.:^[76b] $[\alpha]_{20}^D$ 17.0 ($c = 1.70$, CHCl_3); ^1H NMR (250 MHz, CDCl_3): $\delta = 1.19$ (s, 3 H, CH_3), 1.39 (s, 9 H, $\text{C}(\text{CH}_3)_3$), 3.33 (dddd, $J = 6.3$ Hz, 1 H, 3-H), 4.36–4.50 (m, 0.3 H, 2-H), 4.45 (dd, $J = 5.0, 9.8$ Hz, 1 H, 2-H), 5.04 (d, $J = 8.3$ Hz, 0.6 H, NH), 6.42 (d, $J = 8.3$ Hz, 0.3 H, NH), 7.18–7.39 (m, 5 H, Ph-H), 11.10–12.00 (br, 1 H, CO_2H).

Boc-(βMe)Phe-Ile-Pro-OMe (140): Boc-Ile-Pro-OMe **138** (0.155 g, 0.45 mmol) was deprotected with 2 M HCl in MeOH (5 mL) for 2 h. The mixture was then concentrated under reduced pressure and the residue was triturated with Et_2O /hexanes 1:1. The resultant crude deprotection product was directly coupled with *N*-Boc protected β-methylphenylalanine **139** (0.135 g, 0.48 mmol) using EDC (89 mg, 0.46 mmol), HOAt (63 mg, 0.47 mmol) and TMP (0.442 g, 3.65 mmol) in CH_2Cl_2 (5 mL) according to GP 5 for 7 h. The solid residue obtained after usual aqueous work-up (GP 5) was taken up with boiling *tert*-BuOMe (20 mL), the resultant suspension was cooled and hexanes were then added to complete precipitation. The precipitate was filtered off and dried to give **140** (0.190 g, 83%) as a colorless solid. $R_f = 0.30$ EtOAc/hexanes 1:1; $[\alpha]_{20}^D$ –56.9 ($c = 0.36$, CHCl_3); ^1H NMR (250 MHz, CDCl_3): $\delta = 0.84$ (t, $J = 7.3$ Hz, 3 H, 5-H, *Ile*), 0.89 (d, $J = 7.3$ Hz, 3 H, 1'-H, *Ile*), 0.95–1.17 (m, 1 H, 4-H_a, *Ile*), 1.31 [d, $J = 7.0$ Hz, 3 H, 4-H, (*βMe*)*Phe*], 1.39 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.40–1.58 (m, 1 H, 4-H_b, *Ile*), 1.59–1.82 (m, 1 H, 3-H, *Ile*), 1.82–2.10 (m, 3 H, 3-H_a, 4-H, *Pro*), 2.10–2.31 (m, 1 H, 3-H_b, *Pro*), 3.17 [dq, $J = 7.0, 7.0$ Hz, 1 H, 3-H, (*βMe*)*Phe*], 3.42–3.63 (m, 2 H, 5-H, *Pro*), 3.69 (s, 3 H, OMe), 4.22 (dd, $J = 6.5, 6.5$ Hz, 1 H, 2-H, *Ile*), 4.29–4.44 [m, 2 H, 2-H, (*βMe*)*Phe*, 2-H, *Pro*], 5.05 (d, $J = 6.8$ Hz, 1 H, NH), 6.16 (d, $J = 8.8$ Hz, 1 H, NH), 7.08–7.30 (m, 5 H, Ar-H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 10.9$ (+, C-5, *Ile*), 14.7 (+, C-1', *Ile*), 17.0 [+ , C-4, (*βMe*)*Phe*], 24.2 (–, C-4, *Pro*), 24.6 (–, C-4, *Ile*), 28.1 [+ , $\text{C}(\text{CH}_3)_3$], 28.9 (–, C-3, *Pro*), 37.7 (+, C-3, *Ile*), 42.4 [+ , C-3,

(β Me)Phe], 47.0 (–, C-5, *Pro*), 51.9 (+, OMe), 54.4 (+, C-2), 58.4 (+, C-2), 60.1 (+, C-2), 79.6 [C_{quat} , C(CH₃)₃], 126.4, 127.5, 128.1 (+, Ar-C), 142.4 (C_{quat} , Ar-C), 155.4 (C_{quat} , NCO₂), 169.7, 170.5, 172.1 (C_{quat} , C-1); IR (KBr): $\nu(\text{tilde}) = 3320\text{ cm}^{-1}$, 2970, 2878, 1754, 1706, 1642, 1516, 1451, 1367, 1174; MS (EI, 70 eV), m/z (%) = 503 (18) [M^+], 398 (32), 298 (18), 269 (13), 206 (20), 178 (33), 134 (38), 130 (86), 86 (100), 57 (62) [$C_4H_9^+$]; HRMS (EI): calcd for C₂₇H₄₁N₃O₆: 503.2995; found 503.2995.

Boc-(β Me)Phe-Ile-Pro-OH (141): A 40% methanolic solution of Triton X (0.464 g, 1.11 mmol) was added to a stirred solution of **140** (0.190 g, 0.36 mmol) in THF/H₂O 5:1 (4 mL) at 4 °C and stirring continued at the same temperature for a further 4 h. A 1 M H₂SO₄ (2 mL) EtOAc (40 mL) were then added. Organic layer was separated, washed with 1 M NaHSO₄ (3 × 10 mL), water (3 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (MeOH/CHCl₃ 1:10, R_f = 0.29) to give **141** (0.142 g, 77%) as a colorless solid. ¹H NMR (250 MHz, CDCl₃): δ = 0.70–0.95 (m, 3 H, 5-H, *Ile*), 0.89 (d, J = 5.5 Hz, 3 H, 1'-H, *Ile*), 0.95–1.18 (m, 2 H, 4-H, *Ile*), 1.20–1.33 [m, 3 H, 4-H, (β Me)Phe], 1.40 [s, 9 H, C(CH₃)₃], 1.62–1.84 (m, 1 H, 3-H, *Ile*), 1.86–2.32 (m, 4 H, 3-H, 4-H, *Pro*), 3.00–3.23 [m, 1 H, 3-H, (β Me)Phe], 3.51–3.71 (m, 2 H, 5-H, *Pro*), 4.19–4.53 (m, 3 H, 2-H), 5.09–5.41 (m, 1 H, NH), 6.48–6.97 (m, 1 H, NH), 7.00–7.29 (m, 5 H, Ar-H); MS (EI, 70 eV), m/z (%) = 489 (21) [M^+], 384 (50), 347 (17), 291 (13), 284 (30), 206 (22), 178 (57), 134 (63), 116 (80), 86 (100), 57 (76) [$C_4H_9^+$]; HRMS (EI): calcd for C₂₆H₃₉N₃O₆: 489.2839; found 489.2839.

Boc-Thr[Boc-(β Me)Phe-Ile-Pro-OMe]-OMTM (142): The acid **141** (0.142 g, 0.28 mmol) was coupled with **132** (77 mg, 0.28 mmol) using EDC (55 mg, 0.29 mmol) and DMAP (5 mg, 0.04 mmol) in CH₂Cl₂ (5 mL) according to GP 6 for 16 h. The crude product obtained after usual aqueous work-up was recrystallized from Et₂O to give **142** (95 mg, 45%) as a colorless solid. R_f

= 0.30 EtOAc/hexanes 1:1; $[\alpha]_{20}^D$ -56.9 (c = 0.36, CHCl_3); ^1H NMR (250 MHz, CDCl_3): δ = 0.85 (t, J = 7.0 Hz, 3 H, 5-H, *Ile*), 0.91 (d, J = 6.8 Hz, 3 H, 1'-H, *Ile*), 0.94–1.17 (m, 1 H, 4-H_a, *Ile*), 1.26 [d, J = 6.5 Hz, 3 H, 4-H, (βMe)*Phe*], 1.31 (d, J = 7.0 Hz, 3 H, 4-H, *Thr*), 1.40 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.43 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.62–1.79 (m, 1 H, 3-H, *Ile*), 1.82–2.05 (m, 3 H, 3-H_a, 4-H, *Pro*), 2.06–2.31 (m, 1 H, 3-H_b, *Pro*), 2.21 (s, 3 H, SMe), 3.06–3.27 [m, 1 H, 3-H, (βMe)*Phe*], 3.39–3.62 (m, 2 H, 5-H, *Pro*), 4.06–4.30 (m, 2 H, 2-H, *Ile*, 2-H, *Pro*), 4.32 [dd, J = 8.8, 8.8 Hz, 1 H, 2-H, (βMe)*Phe*], 4.47 (dd, J = 10.0, 2.0 Hz, 1 H, 2-H, *Thr*), 5.03 (d, J = 9.3 Hz, 1 H, NH), 5.18 (d, J = 2.0 Hz, 2 H, OCH_2S), 5.43 (dq, J = 7.0, 2.0 Hz, 1 H, 3-H, *Thr*), 6.10 (d, J = 8.5 Hz, 1 H, NH), 7.05–7.31 (m, 5 H, Ar-H); The absorption of 4-H_b of *Ile* was masked by the signals of $\text{C}(\text{CH}_3)_3$ and of C-4 of *Thr*, the signal of one amide proton was masked by the peak of OCH_2S ; ^{13}C NMR (62.9 MHz, CDCl_3): δ = 10.8 (+, C-5, *Ile*), 15.0 (+, SMe), 15.2 (+, C-1', *Ile*), 16.6 (+, C-4, *Thr*), 17.2 [+ , C-4, (βMe)*Phe*], 24.3 (–, C-4, *Pro*), 24.6 (–, C-4, *Ile*), 28.1 (+, $2 \times \text{C}(\text{CH}_3)_3$), 29.0 (–, C-3, *Pro*), 37.6 (+, C-3, *Ile*), 42.3 [+ , C-3, (βMe)*Phe*], 47.0 (–, C-5, *Pro*), 54.2 (+, C-2), 56.9 (+, C-2), 58.7 (+, OMe), 60.3 (+, C-2), 69.8 (–, OCH_2S), 71.3 (+, C-3, *Thr*), 79.5, 80.1 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 126.5, 127.5, 128.2 (+, Ar-C), 142.3 (C_{quat} , Ar-C), 155.4, 155.5 (C_{quat} , NCO_2), 169.5 ($\times 2$), 170.5 ($\times 2$) (C_{quat} , C-1); MS (EI, 70 eV), m/z (%) = 750 (34) [M^+], 677 (15) [$\text{M}^+ - \text{C}_4\text{H}_9\text{O}$], 645 (44), 589 (10), 545 (15), 445 (12), 428 (18), 375 (18), 315 (21), 262 (20), 206 (36), 134 (26), 86 (100), 57 (72) [C_4H_9^+]; HRMS (EI): calcd for $\text{C}_{37}\text{H}_{58}\text{N}_4\text{O}_{10}\text{S}$: 750.3874; found 750.3874.

N-Boc-3-(2*S*,1'*S*,2'*R*)-(trans-2'-Nitrocyclopropyl)alanine (2*S*,1'*S*,2'*R*)-(143): A solution of Boc_2O

(0.500 g, 2.29 mmol) in acetone (2 mL) was added to a vigorously stirred solution of 3- (2*S*,1'*R*,2'*R*)-(trans-2'-nitrocyclopropyl)alanine (2*S*,1'*R*,2'*R*)-**83** (0.266 g, 1.53 mmol) in

1 M NaOH (1.53 mL) with some amount of NaHCO_3 (if precipitate formed acetone and/or water were added to obtain homogeneous solution) and stirring continued for a further 15 h. *N,N*-

dimethylaminopropylamine (0.11 mL, 0.88 mmol) was then added. After an additional 10 min acetone was removed under reduced pressure and pH of the residual water solution was adjusted to 2–3 with 1 M NaHSO₄. The resulting emulsion was extracted with Et₂O (50 mL) and the ethereal layer was washed with 1 M NaHSO₄ (2 × 10 mL), water (3 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The residue was taken up with hexanes, filtered through Celite pad and concentrated under reduced pressure to give (2*S*,1'*S*,2'*R*)-**143** (0.353 g, 84%) as an extremely viscous colorless oil. R_f = 0.06 EtOAc/hexanes 1:3 (2% AcOH); $[\alpha]_{20}^D$ 20.6 (c = 0.81, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 1.14 (ddd, J = 6.5, 6.5, 6.5 Hz, 1 H, 3'-H_a), 1.45 [s, 9 H, C(CH₃)₃], 1.59–1.82 (m, 1 H, 3'-H_b), 1.82–1.94 (m, 1 H, 1'-H), 1.94–2.21 (m, 2 H, 3-H), 4.10–4.23 (m, 1 H, 2'-H), 4.25–4.38, 4.42–4.53 (2 × m, 1 H, 2-H), 5.22–5.40, 6.97–7.05 (m, 1 H, NH), 6.10–7.30 (br, 1 H, CO₂H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 17.5 (–, C-3'), 22.0 (+, C-1'), 28.1 [+ , C(CH₃)₃], 33.4, 33.6 (–, C-3), 52.5, 53.7 (+, C-2), 59.2 (+, C-2'), 80.6, 82.5 [C_{quat}, C(CH₃)₃], 155.4, 156.9 (C_{quat}, NCO₂), 175.1, 175.4 (C_{quat}, C-1); IR (film): $\nu(\text{tilde})$ = 3700–2250 cm^{–1}, 3101, 2979, 2935, 1715, 1545, 1437, 1394, 1369, 1253, 1163; MS (ESI): positive mode, m/z (%) = 319 (32) [M – H⁺ + 2Na⁺], 297 (86) [M + Na⁺]; negative mode, m/z (%) = 273 (30) [M – H⁺].

Boc-(2*S*,1'*SR*,2'*SR*)-(3-*Ncp*)Ala-(β Me)Phe-Ile-Pro-OMe (**144**): Tripeptide **140** (0.300 g, 0.60 mmol) was deprotected with 3 M HCl in MeOH (7 mL) for 2 h. The mixture was then concentrated under reduced pressure, the residue was triturated with Et₂O and the resultant precipitate was filtered off and dried to give hydrochloride of deprotected tripeptide which was coupled with *Boc*-(2*S*,1'*SR*,2'*SR*)-(3-*Ncp*)Ala-OH (2*S*,1'*SR*,2'*SR*)-**143** (0.163 g, 0.59 mmol) (obtained as it was described for (2*S*,1'*R*,2'*R*)-isomer) using EDC (0.118 g, 0.62 mmol), HOAt (83 mg, 0.61 mmol), DIEA (77 mg, 0.60 mmol) and TMP (0.217 g, 1.79 mmol) in CH₂Cl₂ (15 mL) according to GP 5 for 15 h. The crude product obtained after usual aqueous work-up (GP 5) was finally purified by column chromatography (MeOH/CHCl₃ 1:18, R_f = 0.42) to give **144**

(0.264 g, 67%) as a colorless solid. $[\alpha]_{20}^D -87.7$ ($c = 0.43$, CHCl_3); ^1H NMR (250 MHz, CDCl_3): $\delta = 0.78$ (dt, $J = 2.8, 7.3$ Hz, 3 H, 5-H, *Ile*), 0.85 (d, $J = 6.8$ Hz, 3 H, 1'-H, *Ile*), 0.91–1.13 (m, 1 H, 4-H_a, *Ile*), 1.27 [ddd, $J = 7.0, 7.0, 7.0$ Hz, 2'-H_a, (*3-Ncp*)*Ala*], 1.36 [d, $J = 7.0$ Hz, 3 H, 4-H, (βMe)*Phe*], 1.52 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.67–2.25 [m, 9 H, 3-H, *Ile*, 3-H, 1'-H, 2'-H_b, (*3-Ncp*)*Ala*, 3-H, 4-H, *Pro*], 2.99–3.31 (m, 2 H, 5-H, *Pro*), 3.49–3.79 [m, 1 H, 3-H, (βMe)*Phe*], 3.71 (s, 3 H, OMe), 3.92–4.03 [m, 0.5 H, 2'-H, (*3-Ncp*)*Ala*], 4.24–4.59 [m, 3.5 H, 2-H, 2'-H, (*3-Ncp*)*Ala*], 5.19 [ddd, $J = 9.6, 9.6, 9.6$ Hz, 1 H, 2-H, (*3-Ncp*)*Ala*], 6.01–6.19, 6.29–6.43 ($2 \times$ br, 1 H, NH), 6.85–6.79 (br, 0.5 H, NH), 7.05–7.17 (m, 1.5 H, Ar-H, NH), 7.18–7.41 (m, 4-H, Ar-H), 7.41–7.51, 7.54–7.71 ($2 \times$ m, 1 H, NH); The absorption of $\text{C}(\text{CH}_3)_3$ masked the signal of 4-H_b of *Ile*; ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 11.3$ (+, C-5, *Ile*), 14.2, 14.3 (+, C-1', *Ile*), 16.2, 18.1 [–, C-3', (*3-Ncp*)*Ala*], 19.47, 19.55 [+ , C-4, (βMe)*Phe*], 22.5, 22.9 [+ , C-1', (*3-Ncp*)*Ala*], 24.4 (–, C-4, *Pro*), 24.7 (–, C-4, *Ile*), 28.2, 28.3 [+ , $\text{C}(\text{CH}_3)_3$], 28.9 (–, C-3, *Pro*), 33.5 [–, 3-H, (*3-Ncp*)*Ala*], 38.4 (+, C-3, *Ile*), 43.7 [+ , C-3, (βMe)*Phe*], 47.0 (–, C-5, *Pro*), 51.9 (+, OMe), 53.9 (+, C-2), 57.9 (+, C-2), 58.2 (+, C-2), 58.4 (+, C-2), 58.8 [+ , C-2', (*3-Ncp*)*Ala*], 79.2 [C_{quat} , $\text{C}(\text{CH}_3)_3$], 126.1, 128.1 ($\times 2$) (+, Ar-C), 142.3 (C_{quat} , Ar-C), 155.5, 155.7 (C_{quat} , NCO_2), 169.75, 169.78 (C_{quat} , C-1), 170.7, 170.8 (C_{quat} , C-1), 172.0, 172.1 (C_{quat} , C-1), 172.7 (C_{quat} , C-1); IR (KBr): $\nu(\text{tilde}) = 3085\text{ cm}^{-1}$, 2971, 2933, 2878, 1750, 1711, 1634, 1545, 1453, 1367, 1174; MS (EI, 70 eV), m/z (%) = 659 (4) [M^+], 603 (7), 531 (10), 503 (27), 418 (29), 362 (38), 334 (58), 290 (18), 269 (24), 225 (12), 182 (12), 130 (100), 86 (28), 57 (6) [C_4H_9^+]; HRMS (EI): calcd for $\text{C}_{33}\text{H}_{49}\text{N}_5\text{O}_9$: 659.3530; found 659.3530.

Boc-(2S,1'SR,2'SR)-(3-Ncp)Ala-(βMe)Phe-Ile-Pro-OH (145): A 40% aqueous solution of tetrabutylammonium hydroxide (0.416 g, 0.64 mmol) was added to a vigorously stirred solution of tetrapeptide **144** (0.13 g, 0.20 mmol) in THF (2.0 mL) at 4 °C and stirring continued at the same temperature for 3.5 h. The reaction was then quenched with 1 M H_2SO_4 (3 mL) and diluted

with EtOAc (40 mL), the organic layer was washed with 1 M NaHSO₄ (3 × 10 mL), water (3 × 10 mL), brine (2 × 10 mL), dried, filtered and concentrated under reduced pressure to give the crude tetrapeptide with free carboxylic group **145** (0.125 g, 100% crude, *R*_f = 0.21 MeOH/CHCl₃ 1:18) as a white solid which was used for the next step without additional purification.

Fmoc-(*β*Me)*Phe*-OD*mob* (**147**): The *N*-Fmoc protected *β*-methylphenylalanine **146** (0.19 g, 0.47 mmol) was esterified with 2,4-dimethoxybenzyl alcohol (78 mg, 0.46 mmol) using EDC (93 mg, 0.49 mmol) and DMAP (12 mg, 0.10 mmol) in CH₂Cl₂ (5 mL) according to GP 7 for 16 h. The crude product obtained after usual aqueous work-up (GP 7) was finally purified by column chromatography (EtOAc/hexanes 1:3, *R*_f = 0.33) to give **147** (0.133 g, 57%) as a turbid glass. $[\alpha]_{20}^D$ -2.6 (*c* = 0.27, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 1.35 (d, *J* = 7.0 Hz, 3 H, 4-H), 3.25 (dq, *J* = 7.0, 7.0 Hz, 1 H, 3-H), 3.80 (s, 3 H, OMe), 3.82 (s, 3 H, OMe), 4.18 (t, *J* = 7.1 Hz, 1 H, 1'-H, *Fmoc*), 4.23–4.45 (m, 2 H, 2'-H, *Fmoc*), 4.60 (dd, *J* = 9.0, 6.0 Hz, 1 H, 2-H), 5.32 (s, 2 H, Bzl-H), 6.41–6.49 (m, 2 H, Ar-H), 7.04–7.18 (m, 3 H, Ar-H), 7.19–7.47 (m, 7 H, Ar-H), 7.57 (d, *J* = 7.3 Hz, 2 H, Ar-H), 7.77 (d, *J* = 7.3 Hz, 2 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 16.3 (+, C-4), 42.6 (+, C-3), 47.0 (+, C-1', *Fmoc*), 55.1 (+, OMe), 55.2 (+, OMe), 59.2 (+, C-2), 62.6 (–, Bzl-C), 66.7 (–, C-2', *Fmoc*), 98.3 (+, C-3, *Dmob*), 103.8 (+, C-5, *Dmob*), 115.7 (C_{quat}, C-1, *Dmob*), 119.8, 124.9, 126.9, 127.5, 127.6 (× 2), 128.2, 131.7 (+, Ar-C), 141.0, 141.1 (C_{quat}, Ar-C, *Fmoc*), 141.1 (C_{quat}, Ar-C, (*β*Me)*Phe*), 143.7, 143.8 (C_{quat}, Ar-C, *Fmoc*), 155.5 (C_{quat}, NCO₂), 158.8 (C_{quat}, C-2, *Dmob*), 161.3 (C_{quat}, C-4, *Dmob*), 171.1 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde})$ = 3063 cm⁻¹, 3028, 2963, 2835, 1724, 1616, 1510, 1451, 1209, 1159, 1040; MS (EI, 70 eV), *m/z* (%) = 551 (1) [M⁺], 288 (10), 178 (100) [C₁₄H₁₀⁺], 151 (32) [C₉H₁₁O₂⁺], 105 (27) [C₈H₉⁺]; HRMS (EI): calcd for C₃₄H₃₃NO₆: 551.2308; found 551.2308.

Alloc-a-Thr-OH (**148**): A solution of AllocOPfp (0.295 g, 1.10 mmol) in acetone (7 mL) was

added to a vigorously stirred solution of (*R*)-*a*-threonine (*R*)-**72** (0.203 g, 1.00 mmol) and NaHCO₃ (0.185 g, 2.20 mmol) in water (5 mL) (if emulsion formed acetone or water was added to obtain homogeneous solution) and stirring continued for an additional 1 h. The mixture was then concentrated under reduced pressure, diluted with methanol (10 mL), treated with 3.9 M HCl in dioxane (0.64 mL) and concentrated again. The residue was purified by column chromatography (MeOH/CHCl₃ 1:8 (2.5% AcOH), *R*_f = 0.39) to give **148** (0.154 g, 74%) as a colorless oil polymerized easily in the presence of the traces of acid. ¹H NMR (250 MHz, CD₃OD): δ = 1.26 (d, *J* = 6.5 Hz, 3 H, 4-H), 4.13 (dq, *J* = 6.1, 6.1 Hz, 1 H, 3-H), 4.25 (d, *J* = 5.0 Hz, 1 H, 2-H), 4.60 (d, *J* = 7.8 Hz, 2 H, 1-H, *Alloc*), 5.16–5.28 (m, 1 H, *trans*-3-H, *Alloc*), 5.30–5.43 (m, 1 H, *cis*-3-H, *Alloc*), 5.87–6.08 (m, 1 H, 2-H, *Alloc*); ¹³C NMR (62.9 MHz, CD₃OD): δ = 19.5 (+, C-4), 61.3 (+, C-2), 67.0 (–, C-1, *Alloc*), 69.0 (+, C-3), 118.0 (–, C-3, *Alloc*), 134.3 (+, C-2, *Alloc*), 158.7 (C_{quat}, NCO₂), 174.0 (C_{quat}, C-1); IR (film): ν(tilde) = 3750–2500 cm^{–1}, 2981, 2934, 1708, 1535, 1411, 1260, 1100, 1042; MS (CI), *m/z* (%): 221 (100) [M + NH₄⁺], 204 (12) [M + H⁺].

Alloc-a-Thr-(βMe)Phe-ODmob (**149**): Compound **147** (0.128 g, 0.23 mmol) was deprotected according to GP 4 and the resultant amino ester was coupled with *N*-Alloc protected *allo*-(*R*)-threonine **148** (52 mg, 0.26 mmol) using EDC (50 mg, 0.26 mmol), HOAt (35 mg, 0.26 mmol) and TMP (56 mg, 0.46 mmol) in CH₂Cl₂/DMF 1:1 (5 mL) according to GP 5. The crude product obtained after usual aqueous work-up (GP 5) was triturated with hot hexanes, filtered off, washed with cold Et₂O and dried to give **149** (0.129 g, 90%) as a colorless solid. *R*_f = 0.29 EtOAc/hexanes 1:1; [α]₂₀^D 29.6 (*c* = 0.27, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 1.15 (d, *J* = 6.5 Hz, 3 H, 4-H, *a*-Thr), 1.32 [d, *J* = 7.3 Hz, 3 H, 4-H, (βMe)Phe], 3.25 [dq, *J* = 1.0, 7.3 Hz, 1 H, 3-H, (βMe)Phe], 3.30–3.42 (br, 1 H, OH), 3.81 (s, 3 H, OMe), 3.82 (s, 3 H, OMe), 3.87 (dd, *J* = 6.5, 5.5 Hz, 1 H, 3-H, *a*-Thr), 4.01–4.13 [m, 1 H, 2-H, (βMe)Phe], 4.56 (d, *J* = 5.5 Hz, 2 H,

Alloc), 4.82 (dd, $J = 9.0, 5.5$ Hz, 1 H, 2-H, *a-Thr*), 5.08 (dd, $J = 11.8, 11.8$ Hz, 2 H, 1-H, *Alloc*), 5.17–5.26 (m, 1 H, *trans*-3-H, *Alloc*), 5.26–5.37 (m, 1 H, *cis*-3-H, *Alloc*), 5.61 (d, $J = 7.3$ Hz, 1 H, NH), 5.78–5.99 (m, 1 H, 2-H, *Alloc*), 6.40–6.49 (m, 2 H, Ar-H), 6.82 (d, $J = 9.0$ Hz, 1 H, NH), 7.07–7.19 (m, 3 H, Ar-H), 7.20–7.30 (m, 3 H, Ar-H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 16.0$ [+ , C-4, (βMe)*Phe*], 41.9 [+ , C-3, (βMe)*Phe*], 55.29 (+ , OMe), 55.34 (+ , OMe), 57.4 [+ , C-2, (βMe)*Phe*], 59.1 (+ , C-2, *a-Thr*), 62.8 (– , Bzl-C), 66.1 (– , C-1, *Alloc*), 69.0 (+ , C-3, *a-Thr*), 98.4 (+ , C-3, *Dmob*), 103.9 (+ , C-5, *Dmob*), 115.8 (C_{quat} , C-1, *Dmob*), 117.9 (– , C-3, *Alloc*), 127.0, 127.6, 128.3, 131.7 (+ , Ar-C), 132.4 (+ , C-2, *Alloc*), 141.0 (C_{quat} , Ar-C), 156.6 (C_{quat} , NCO_2), 158.9 (C_{quat} , C-2, *Dmob*), 161.4 (C_{quat} , C-4, *Dmob*), 170.2, 170.9 (C_{quat} , C-1); IR (KBr): $\nu(\text{tilde}) = 3299\text{ cm}^{-1}$, 3065, 2962, 2934, 2838, 1743, 1694, 1539, 1510, 1259, 1239, 1045; MS (EI, 70 eV), m/z (%) = 514 (6) [M^+], 151 (100) [$\text{C}_9\text{H}_{11}\text{O}_2^+$], 134 (19), 41 (3) [C_3H_5^+]; HRMS (EI): calcd for $\text{C}_{27}\text{H}_{34}\text{N}_2\text{O}_8$: 514.2315; found 514.2315.

Attempts of the preparation of Alloc-a-Thr[Boc-(2S,1'SR,2'SR)-(3-Ncp)Ala-(βMe)Phe-Ile-Pro]-(βMe)Phe-ODmob (150): Acid **145** (0.117 g, 0.18 mmol) was treated with the alcohol **149** (89 mg, 0.17 mmol) using DCC (78 mg, 0.38 mmol) and DMAP (22 mg, 0.18 mmol) in THF (5 mL) at 20 °C according to GP 6 (the carboxylic component was absolute insoluble in CH_2Cl_2 or toluene). Solvent was then blown out with the strong flow of nitrogen, dioxane (10 mL) was then added and stirring continued for a further 24 h. A dilute aqueous acetic acid (1 mL) was then added and after 10 min the mixture was filtered and diluted with Et_2O (50 mL). The residue (78 mg) obtained after usual aqueous work-up (GP 6) contained according to ^1H NMR only the impure alcohol **149**.

To a solution of the carboxylic component (10 mg, 15.16 μmol), alcohol **149** (7.0 mg, 13.64 μmol) and HOAt (2.0 mg, 15.19 μmol) in THF (0.5 mL) was added EDC (8.7 mg, 45.47 μmol) and stirring continued for 0.5 h. Solvent was then blown out with the strong flow of nitrogen, CH_2Cl_2 (0.5 mL) was added and stirring continued for a further 48 h. The separation of the

mixture obtained after usual aqueous work-up by column chromatography didn't give the desired product.

To a solution of DMAP (50 mg, 0.36 mmol) in CH_2Cl_2 (ca. 3 mL) was added TFA (20 mg, 0.18 mmol) and the resultant suspension was added to a solution of the carboxylic component **145** (0.120 g, 0.18 mmol) and alcohol **149** (0.112 g, 0.22 mmol) in THF (1.5 mL) followed by EDC (0.105 g, 0.55 mmol) and stirring continued for 3 h. All volatiles were then removed under reduced pressure under dry conditions, CH_2Cl_2 (5 mL) was added to the residue and stirring continued for a further 48 h. $\text{C}_2\text{H}_4\text{Cl}_2$ (10 mL) was added and the mixture was heated and stirred at 60 °C for another 16 h. The crude product obtained after the usual aqueous work-up (GP 6) was then purified by column chromatography (EtOAc/hexanes 1:1, $R_f = 0.15$, elongated spot) to give an amorphous solid (0.22 g), which was triturated with Et_2O to give the residue (54 mg), which was the mixture contained besides the desired depsipeptide **150** at least one side product (ratio 2:1 according to ^{13}C NMR) inseparable by conventional methods.

Alloc-a-(R)-Thr-ONb (**152**): Boc-*a*-(R)-Thr-ONb **151** (0.61 g, 1.72 mmol) was deprotected with

4 M HCl in EtOAc (prepared in situ by a careful addition of 1 equivalent of AcCl to a solution of 1.2 equivalent of MeOH in EtOAc at 4 °C) for 3 h. All volatiles were removed under reduced pressure and

the residue was triturated with Et_2O , filtered off and dried to give hydrochloride of the amino ester (0.48 g, 96%) as a colorless solid which was directly used for the next step. To a solution of this material and DIEA (0.224 g, 1.73 mmol) in CH_2Cl_2 (15 mL) was added AllocOBt (0.38 g, 1.73 mmol) and the mixture was stirred for 2 h. *N,N*-dimethylaminopropylamine (0.1 g, 0.98 mmol) was then added and after 10 min the mixture was diluted with Et_2O (60 mL). The crude product obtained after usual aqueous work-up (GP 6) was finally purified by column chromatography (EtOAc/hexanes 1:1, $R_f = 0.33$) to give **152** (0.44 g, 76% yield on 2 steps) as a colorless solid. $[\alpha]_{20}^D -12.3$ ($c = 0.44$, CHCl_3); ^1H NMR (250 MHz, CDCl_3): $\delta = 1.22$ (d, $J =$

6.8 Hz, 3 H, 4-H), 2.85 (d, $J = 5.8$ Hz, 1 H, OH), 4.16 (dq, $J = 3.5, 6.8$ Hz, 1 H, 3-H), 4.46 (dd, $J = 8.0, 3.5$ Hz, 1 H, 2-H), 4.59 (dt, $J = 5.5, 1.3$ Hz, 2 H, 1-H, *Alloc*), 5.17–5.24 (m, 1 H, *trans*-3-H, *Alloc*), 5.24–5.36 (m, 1 H, *cis*-3-H, *Alloc*), 5.29 (s, 2 H, Bzl-H), 5.74 (d, $J = 8.0$ Hz, 1 H, NH), 5.89 (ddt, $J = 10.5, 10.5, 5.5$ Hz, 1 H, 2-H, *Alloc*), 7.52 (d, $J = 8.8$ Hz, 2 H, Ar-H), 8.22 (d, $J = 8.8$ Hz, 2 H, Ar-H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 19.1$ (+, C-4), 59.4 (+, C-2), 65.5 (–, Bzl-C), 66.0 (–, C-1, *Alloc*), 68.4 (+, C-3), 117.9 (–, C-3, *Alloc*), 123.6, 128.3 (+, Ar-C), 132.1 (+, C-2, *Alloc*), 142.3, 147.5 (C_{quat} , Ar-C), 156.2 (C_{quat} , NCO_2), 170.0 (C_{quat} , C-1); IR (KBr): $\nu(\text{tilde}) = 3315\text{ cm}^{-1}$, 2974, 2910, 1739, 1690, 1549, 1531, 1348, 1334, 1196, 1041; MS (CI), m/z (%): 356 (100) $[\text{M} + \text{NH}_4^+]$, 339 (12) $[\text{M} + \text{H}^+]$.

Alloc-a-Thr(Boc-Pro)-ONb (**154**): Substance **152** (0.43 g, 1.27 mmol) was coupled with Boc-Pro-OH **153** (0.287 g, 1.33 mmol) using EDC (0.261 g, 1.36 mmol) and DMAP (31 mg, 0.25 mmol) in CH_2Cl_2 (10 mL) according to GP 6 for 15 h. The crude product obtained after usual aqueous work-up (GP 6) was purified by column chromatography (EtOAc/hexanes 1:1, $R_f = 0.68$) to give **154** (0.63 g, 93%) as a colorless oil. ^1H NMR (250 MHz, CDCl_3): $\delta = 1.33, 1.37$ ($2 \times \text{d}$, $J = 6.8$ Hz, 3 H, 4-H, *a-Thr*), 1.40, 1.45 [$2 \times \text{s}$, 9 H, $\text{C}(\text{CH}_3)_3$], 1.76–2.26 (m, 4 H, 3-H, 4-H, *Pro*), 3.32–3.55 (m, 2 H, 5-H, *Pro*), 4.08, 4.22 (dd, $J = 9.5, 3.5$ Hz, 1 H, 2-H, *Pro*), 4.54, 4.56 ($2 \times \text{d}$, $J = 5.8$ Hz, 2 H, 1-H, *Alloc*), 4.62, 4.64 ($2 \times \text{dd}$, $J = 3.5, 6.8$ Hz, 1 H, 2-H, *Thr*), 5.12–5.42 (m, 5 H, 3-H, *Alloc*, 3-H, *a-Thr*, Bzl-H), 5.50, 6.55 ($2 \times \text{d}$, $J = 9.5$ Hz, 1 H, NH), 5.89 (ddt, $J = 11.3, 11.3, 5.8$ Hz, 1 H, 2-H, *Alloc*), 7.54, 7.58 ($2 \times \text{d}$, $J = 8.3$ Hz, 2 H, Ar-H), 8.21, 8.25 (d, $J = 8.3$ Hz, 2 H, Ar-H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 16.0, 16.5$ (+, C-4, *a-Thr*), 23.1, 24.3 (–, C-4, *Pro*), 28.0 [+ , $\text{C}(\text{CH}_3)_3$], 29.3, 30.4 (–, C-3, *Pro*), 46.0, 46.3 (–, C-5, *Pro*), 57.2, 57.6 (+, C-2, *a-Thr*), 58.8, 58.9 (+, C-2, *Pro*), 65.2, 65.5 (–, Bzl-C), 65.67, 65.72 (–, C-1, *Alloc*), 70.79, 70.85 (+, C-3, *a-Thr*), 117.3, 117.6 (–, C-3, *Alloc*), 123.4, 123.5 (+, Ar-C), 128.0, 128.3 (+, Ar-C), 132.1, 132.4 (+, C-2, *Alloc*), 141.9, 142.4 (C_{quat} , Ar-C), 147.3, 147.5 (C_{quat} , Ar-C), 153.2, 154.0 (C_{quat} , NCO_2), 155.4, 155.8 (C_{quat} , NCO_2), 168.4 (C_{quat} , C-1), 171.9, 172.4 (C_{quat} , C-1); IR

(film): $\nu(\text{tilde}) = 3317 \text{ cm}^{-1}$, 3082, 2979, 2882, 1700, 1521, 1399, 1348, 1162; MS (EI, 70 eV), m/z (%) = 535 (1) $[\text{M}^+]$, 479 (3) $[\text{M}^+ - \text{C}_4\text{H}_8]$, 434 (22) $[\text{M}^+ - \text{C}_5\text{H}_9\text{O}_2]$, 321 (14), 281 (8), 170 (36), 114 (100), 70 (94) $[\text{C}_4\text{H}_8\text{N}^+]$, 57 (39) $[\text{C}_4\text{H}_9^+]$, 41 (21) $[\text{C}_3\text{H}_5^+]$; HRMS (EI): calcd for $\text{C}_{25}\text{H}_{33}\text{N}_3\text{O}_{10}$: 535.2166; found 535.2166.

Alloc-a-(R)-Thr(Boc-Ile-Pro)-ONb (156): Ester **154** (0.315 g, 0.59 mmol) was deprotected with TFA (5 mL) for 40 min. All volatiles were removed, the residue was taken up with toluene (2 $\times$ 20 mL) which was distilled off to remove the last traces of TFA. The resultant amino ester was coupled with Boc-Ile-OH **155** (0.15 g, 0.65 mmol) using EDC (0.127 g, 0.66 mmol), HOAt (89 mg, 0.66 mmol), DIEA (0.80 g, 0.62 mmol) and TMP (0.214 g, 1.77 mmol) in CH_2Cl_2 (5 mL) according to GP 5 for 15 h. The crude product, after the usual aqueous work-up (GP 5), was finally purified by column chromatography (EtOAc/hexanes 1:2, $R_f = 0.29$) to give **156** (0.34 g, 89%) as white foam. $[\alpha]_{20}^D -15.7$ ($c = 0.42$, CHCl_3); ^1H NMR (250 MHz, CDCl_3): $\delta = 0.88$ (t, $J = 7.3$ Hz, 3 H, 5-H, *Ile*), 0.94 (d, $J = 6.8$ Hz, 3 H, 1'-H, *Ile*), 0.99–1.21 (m, 1 H, 4-H_a, *Ile*), 1.35 (d, $J = 6.8$ Hz, 3 H, 4-H, *a-Thr*), 1.42 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.64–1.82 (m, 1 H, 4-H_b, *Ile*), 1.84–2.27 (m, 5 H, 3-H, *Ile*, 3-H, 4-H, *Pro*), 3.54–3.72 (m, 1 H, 5-H_a, *Pro*), 3.78–3.92 (m, 1 H, 5-H_b, *Pro*), 4.18–4.33 (m, 2 H, 2-H, *Pro*, 2-H, *Ile*), 4.56 (d, $J = 5.3$ Hz, 2 H, 1-H, *Alloc*), 4.62 (dd, $J = 3.0$, 7.0 Hz, 1 H, 2-H, *a-Thr*), 5.07–5.45 (m, 6 H, 3-H, *Alloc*, 3-H, *a-Thr*, Bzl-H, NH), 5.90 (ddt, $J = 11.3$, 11.3, 5.3 Hz, 1 H, 2-H, *Alloc*), 6.58 (d, $J = 9.0$ Hz, 1 H, NH), 7.57 (d, $J = 9.0$ Hz, 2 H, Ar-H), 8.21 (d, $J = 9.0$ Hz, 2 H, Ar-H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 11.0$ (+, C-5, *Ile*), 15.1 (+, C-1', *Ile*), 16.7 (+, C-4, *a-Thr*), 24.1 (–, C-4, *Pro*), 25.2 (–, C-4, *Ile*), 28.2 [+ , $\text{C}(\text{CH}_3)_3$], 28.8 (–, C-3, *Pro*), 37.7 (+, C-3, *Ile*), 47.4 (–, C-5, *Pro*), 56.0 (+, C-2), 57.6 (+, C-2), 59.3 (+, C-2), 65.5 (–, Bzl-C), 65.7 (–, C-1, *Alloc*), 71.2 (+, C-3), 117.5 (–, C-3, *Alloc*), 123.6, 128.4 (+, Ar-C), 132.6 (+, C-2, *Alloc*), 142.6, 147.6 (C_{quat} , Ar-C), 155.5, 156.0 (C_{quat} , NCO_2), 168.6, 171.4, 171.6 (C_{quat} , C-1); IR (KBr): $\nu(\text{tilde}) = 3396 \text{ cm}^{-1}$, 3323, 3083, 2974, 2936, 2878, 1749, 1715, 1638,

1525, 1444, 1348, 1248, 1171; MS (ESI), positive m/z (%) = 671 (30) $[M + Na^+]$, 1319 (100) $[2M + Na^+]$; negative m/z (%) = 647 (15) $[M - H^+]$.

Boc-a-Thr(Boc-Ile-Pro)-ONb (**157**): $Pd(PPh_3)_4$ (1.5 mg, 1.30 μ mol) and then immediately Bu_3SnH (66 mg, 0.23 mmol) were added to a vigorously stirred solution of the depsipeptide **156** (65 mg, 0.10 mmol) and Boc_2O (87 mg, 0.40 mmol) in anhydrous degassed DMA (2 mL) and stirring continued for a further 80 min. All volatiles were removed under reduced pressure and the residue was purified by column chromatography (EtOAc/hexanes 1:2, R_f = 0.19) to give **157** (60 mg, 90%) as a faint yellow viscous oil. $[\alpha]_{20}^D$ -14.2 (c = 0.60, $CHCl_3$); 1H NMR (250 MHz, $CDCl_3$): δ = 0.89 (t, J = 7.3 Hz, 3 H, 5-H, *Ile*), 0.97 (d, J = 6.8 Hz, 3 H, 1'-H, *Ile*), 0.99–1.21 (m, 1 H, 4-H_a, *Ile*), 1.32 (d, J = 6.5 Hz, 3 H, 4-H, *a-Thr*), 1.42 [s, 9 H, $C(CH_3)_3$], 1.44 [s, 9 H, $C(CH_3)_3$], 1.49–1.63 (m, 1 H, 4-H_b, *Ile*), 1.62–1.85 (m, 1 H, 3-H, *Ile*), 1.87–2.31 (m, 4 H, 3-H, 4-H, *Pro*), 3.51–3.70 (m, 1 H, 5-H_a, *Pro*), 3.75–3.88 (m, 1 H, 5-H_b, *Pro*), 4.21–4.37 (m, 2 H, 2-H, *Pro*, 2-H, *Ile*), 4.53 (dd, J = 3.0, 7.0 Hz, 1 H, 2-H, *a-Thr*), 5.16 (d, J = 9.3 Hz, 1 H, NH), 5.32 (d, J = 3.5 Hz, 2 H, Bzl-H), 6.17 (d, J = 7.0 Hz, 1 H, NH), 7.56 (d, J = 8.8 Hz, 2 H, Ar-H), 8.21 (d, J = 8.8 Hz, 2 H, Ar-H); The peak of Bzl-H masked absorption for 3-H of *a-Thr*; ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 11.1 (+, C-5, *Ile*), 15.3 (+, C-1', *Ile*), 16.8 (+, C-4, *a-Thr*), 24.0 (–, C-4, *Pro*), 25.2 (–, C-4, *Ile*), 28.2 [+ , $2 \times C(CH_3)_3$], 28.8 (–, C-3, *Pro*), 37.8 (+, C-3, *Ile*), 47.3 (–, C-5, *Pro*), 56.0 (+, C-2), 57.2 (+, C-2), 59.2 (+, C-2), 65.5 (–, Bzl-C), 71.2 (+, C-3), 79.5 [C_{quat} , $C(CH_3)_3$], 79.9 [C_{quat} , $C(CH_3)_3$], 123.7, 128.4 (+, Ar-C), 142.6, 147.6 (C_{quat} , Ar-C), 155.6 (C_{quat} , $2 \times NCO_2$), 169.0, 171.3, 171.6 (C_{quat} , C-1); IR (KBr): $\nu(\tilde{)} = 2979\text{ cm}^{-1}$, 2932, 1751, 1717, 1646, 1526, 1456, 1368, 1348, 1251, 1165; MS (EI, 70 eV), m/z (%) = 664 (1) $[M^+]$, 608 (3) $[M^+ - C_4H_8]$, 491 (8), 186 (45), 130 (100), 86 (69), 57 (45) $[C_4H_9^+]$; HRMS (EI): calcd for $C_{32}H_{48}N_4O_{11}$: 664.3320; found 664.3320.

Boc-(3-Ncp)Ala-a-Thr(Boc-Ile-Pro)-ONb (**158**): DCC (29 mg, 0.14 mmol) was added to an ice-cold solution of *N*-Boc protected (3-nitrocyclopropyl)alanine (2*S*)-**143** (39 mg, 0.14 mmol) (the mixture of diastereomers partially enriched with (1'*S*,2'*R*)-isomer was used for this synthesis) depsipeptide **156** (30 mg, 46.24 μ mol) and HOBt (19 mg, hydrate, 87% purity, 0.14 mmol) in CH₂Cl₂ (5 mL). Cooling bath was then removed and stirring continued for a further 90 min. The mixture was then degassed with three freeze-pump-thaw cycles at -70°C . Pd(PPh₃)₄ (1.1 mg, 1.0 μ mol) and Bu₃SnH (28 mg, 96.20 μ mol) was then added and the mixture was stirred for a further 3 h. AcOH (1 drop) and then 5 min later *N,N*-dimethylaminopropylamine (1 drop) were added, and after an additional 10 min, the mixture was diluted with Et₂O (50 mL) and filtered. The residue obtained after usual aqueous work-up (GP 5) was dissolved in Et₂O (4 mL), the resultant solution was filtered and concentrated again under reduced pressure. The crude product was purified by column chromatography (twice, EtOAc/hexanes 1:2, R_f = 0.11) and finally by preparative TLC (EtOAc/hexanes 1:2.5, 3 runs) to give **158** (36 mg, 95%) as an extremely viscous colorless oil or white foam. $[\alpha]_{20}^D$ -4.3 (c = 0.40, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ = 0.87 (t, J = 7.0 Hz, 3 H, 5-H, *Ile*), 0.94 (d, J = 6.8 Hz, 3 H, 1'-H, *Ile*), 0.99 [ddd, J = 6.5, 6.5, 6.5 Hz, 1 H, 3'-H_a, (3-*Ncp*)*Ala*], 1.14–1.24 [m, 2 H, 4-H_a, *Ile*, 3'-H_b, (3-*Ncp*)*Ala*], 1.37 (d, J = 6.8 Hz, 3 H, 4-H, *a-Thr*), 1.40 [s, 9 H, C(CH₃)₃], 1.41 [s, 9 H, C(CH₃)₃], 1.56–1.67 [m, 2 H, 4-H_b, *Ile*, 1'-H, (3-*Ncp*)*Ala*], 1.70–1.80 (m, 1 H, 3-H, *Ile*), 1.85–2.01 [m, 4 H, 4-H, *Pro*, 3-H, (3-*Ncp*)*Ala*], 2.06–2.15 (m, 1 H, 3-H_a, *Pro*), 2.17–2.25 (m, 1 H, 3-H_b, *Pro*) 3.64 (ddd, J = 8.0, 8.0, 8.0 Hz, 1 H, 5-H_a, *Pro*), 3.94–4.02 (m, 1 H, 5-H_b, *Pro*), 4.04–4.16 (m, 2 H, 2-H), 4.27 (dd, J = 9.5, 9.5 Hz, 1 H, 2-H), 4.63–4.72 [m, 1.7 H, 2-H, 0.7 2'-H, (3-*Ncp*)*Ala*], 5.27 (d, J = 14.5 Hz, 1 H, H_a, Bzl-H), 5.34 (d, J = 14.5 Hz, 2 H, H_b, Bzl-H), 5.38–5.45 (m, 1 H, *a-Thr*), 5.49 (d, J = 8.5 Hz, 1 H, NH), 5.66 (d, J = 9.0 Hz, 1 H, NH), 7.52 (d, J = 8.5 Hz, 2 H, Ar-H), 8.12–8.20 (br, 1 H, NH), 8.20 (d, J = 8.5 Hz, 2 H, Ar-H); The peak of H_b of Bzl-H masked absorption for 0.7 H, 2'-H of (3-*Ncp*)*Ala*; ¹³C NMR (125.7 MHz, CDCl₃): δ = 10.6 (+, C-5, *Ile*), 14.8 (+, C-1', *Ile*), 17.1 (+, C-4, *a-Thr*), 17.26 [–, C-3', (3-*Ncp*)*Ala*], 21.9 [+ , C-1', (3-*Ncp*)*Ala*], 24.7 (–, C-4, *Pro*),

25.3 (–, C-4, *Ile*), 28.2 [+ , C(CH₃)₃], 28.3 [+ , C(CH₃)₃], 29.0 (–, C-3, *Pro*), 34.4 [–, C-3, (*3-Ncp*)*Ala*], 37.7 (+, C-3, *Ile*), 47.8 (–, C-5, *Pro*), 52.9 (+, C-2), 56.1 (+, C-2), 59.4 (+, C-2), 59.6 [+ , C-2 and C-2', (*3-Ncp*)*Ala*], 65.4 (–, Bzl-C), 70.6 (+, C-3), 79.6 [C_{quat}, C(CH₃)₃], 80.0 [C_{quat}, C(CH₃)₃], 123.7, 128.3 (+, Ar-C), 142.5, 147.6 (C_{quat}, Ar-C), 154.9, 155.8 (C_{quat}, 2 × NCO₂), 167.9, 171.3, 171.5, 172.2 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 2977 \text{ cm}^{-1}$, 2935, 2879, 1751, 1700, 1630, 1543, 1525, 1448, 1368, 1348, 1252, 1170; MS (ESI), positive m/z (%) = 843 (100) [M + Na⁺]; negative m/z (%) = 820 (100) [M – H⁺].

Alloc-a-Thr-OSEM (**159**): To a vigorously stirred suspension of Li₂CO₃ (0.122 g, 1.65 mmol) in

a solution of **148** (0.305 g, 1.50 mmol) in DMF (6 mL), SEMCl (0.2 g, 1.2 mmol) was added for 5 min and stirring continued for 23 h. The second portion of SEMCl (0.1 g, 0.6 mmol) was then added and after stirring for an additional 3 h the mixture was diluted with Et₂O (50 mL), washed with water (8 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/hexanes 1:3, R_f = 0.22) to give **159** (0.288 g, 58%) as a colorless oil. $[\alpha]_{20}^D -7.3$ (c = 0.33, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 0.02 [s, 9 H, Si(CH₃)₃], 0.96 (t, J = 8.4 Hz, 2 H, 2'-H, *SEM*), 1.23 (d, J = 6.5 Hz, 3 H, 4-H), 2.73–2.82 (br, 1 H, OH), 3.72 (t, J = 8.4 Hz, 2 H, 1'-H, *SEM*), 4.11–4.28 (m, 1 H, 3-H), 4.47 (dd, J = 7.8, 4.0 Hz, 1 H, 2-H), 4.59 (d, J = 5.5 Hz, 2 H, 1-H, *Alloc*), 5.22–5.29 (m, 1 H, *trans*-3-H, *Alloc*), 5.30–5.39 (m, 1 H, *cis*-3-H, *Alloc*), 5.33 (d, J = 6.0 Hz, 1 H, 1-H, *SEM*), 5.66 (d, J = 7.8 Hz, 1 H, NH), 5.89 (ddt, J = 10.4, 10.4, 5.6 Hz, 1 H, 2-H, *Alloc*); ¹³C NMR (62.9 MHz, CDCl₃): δ = –1.7 [+ , Si(CH₃)₃], 17.7 (–, C-2', *SEM*), 18.6 (+, C-4), 59.3 (+, C-2), 65.8 (–, C-1', *SEM*), 67.9 (–, C-1, *Alloc*), 68.2 (+, C-3), 89.6 (–, C-1, *SEM*), 117.7 (–, C-3, *Alloc*), 132.2 (+, C-2, *Alloc*), 156.2 (C_{quat}, NCO₂), 169.9 (C_{quat}, C-1); IR (film): $\nu(\text{tilde}) = 3390 \text{ cm}^{-1}$, 3086, 2954, 2898, 1728, 1521, 1457, 1412, 1382, 1250, 1115; MS (ESI), positive m/z (%) = 356 (100) [M + Na⁺].

N-Fmoc-3-(2*R*,1*R*,2*R*)-(trans-2'-Nitrocyclopropyl)alanine (2*R*,1'*R*,2'*R*)-(160): A solution of

FmocOSu (0.416 g, 1.36 mmol) in acetone (7 mL) was added

to a vigorously stirred solution of 3-(2*R*,1'*R*,2'*R*)-(trans-2'-nitrocyclopropyl)alanine (2*R*,1'*R*,2'*R*)-**83** (0.2 g, 1.15 mmol) and NaHCO₃ (0.202 g, 2.40 mmol) in water (5 mL) (if precipitate formed acetone and/or water were added to obtain homogeneous solution) and stirring continued for an additional 3 h. Acetone was then removed under reduced pressure and pH of the residual water solution was adjusted to 1 with 3 M HCl. The resulting emulsion was extracted with Et₂O (30 mL) and the ethereal layer was then back extracted with 3% aqueous NaHCO₃ (5 × 10 mL, TLC control of the completeness of extraction was necessary). The aqueous fractions were pooled, washed with Et₂O (2 × 10 mL), acidified to pH 2 with 3 M HCl and the resulting emulsion was extracted with Et₂O (40 mL). The organic phase was washed with 3 M HCl (2 × 10 mL), water (3 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The residue was triturated with cold pentane and filtered. The resulting semisolid was dried at 0.02 Torr for prolonged time to give (2*R*,1'*R*,2'*R*)-**160** (0.423 g, 93%) as a white foam. *R*_f = 0.08 EtOAc/hexanes 1:1; m.p. (softening) 50–57 °C; [α]₂₀^D –56.7 (*c* = 0.36, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 0.71–0.82 (m, 0.4 H, 3'-H_a), 1.11 (ddd *J* = 6.2, 6.2, 6.2 Hz, 0.6 H, 3'-H_a), 1.17–1.51 (m, 1 H, 3'-H_b), 1.75–2.13 (m, 2 H, 3-H), 3.61–3.76, 3.76–3.89 (2 × m, 1 H, 1'-H), 3.99–4.12 (m, 0.5 H, 2-H), 4.12–4.27 (m, 1.5 H, 2''-H, 2-H), 4.27–4.56 (m, 2 H, 1''-H), 4.56–4.69, 4.71–4.87 (2 × m, 1 H, 2'-H), 5.48 (d, *J* = 7.0 Hz, 0.6 H, NH), 7.01–7.13 (m, 0.4 H, NH), 7.23–7.42 (m, 5 H, Ar-H), 7.42–7.61 (m, 2 H, Ar-H), 7.75 (d, *J* = 7.5 Hz, 2 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 17.3, 17.6 (–, C-3'), 21.4, 22.0 (+, C-1'), 32.8, 33.2 (–, C-3), 46.8 (+, C-2''), 53.0 (+, C-2), 59.0 (+, C-2'), 66.7, 67.0 (–, C-1''), 119.8 (+, Ar-C), 124.2, 124.4 (+, Ar-C), 124.8 (+, Ar-C), 126.9, 127.6 (+, Ar-C), 141.1 (C_{quat}, Ar-C), 143.0, 143.3 (C_{quat}, Ar-C), 143.3, 143.5 (C_{quat}, Ar-C), 155.9, 156.8 (C_{quat}, NCO₂), 173.8, 174.6 (C_{quat}, C-1); IR (KBr): ν(tilde) = 3331 cm^{–1}, 2965, 2934, 1736, 1689, 1653, 1525,

1455, 1391, 1367, 1251, 1176; MS (EI, 70 eV), m/z (%) = 396 (1) $[M^+]$, 196 (36) $[C_{14}H_{12}O^+]$, 178 (51) $[C_{14}H_{10}^+]$, 165 (100), 139 (6); HRMS (EI): calcd for $C_{21}H_{20}N_2O_6$: 396.1321; found 396.1321; elemental analysis calcd (%) for $C_{21}H_{20}N_2O_6$ (396.4): C 63.63, H 5.09, N 7.07; found C 63.56, H 5.21, N 7.00.

Boc-(βMe)Phe-Ile-OTMSE (162): To a solution of Boc-Ile-OH **155** (0.5 g, 2.16 mmol), 2-trimethylsilylethanol (0.256 g, 2.16 mmol) and DMAP (0.053 g, 0.43 mmol) in CH_2Cl_2 (5 mL) EDC (0.427 g, 2.23 mmol) was added at $-10\text{ }^\circ\text{C}$ and the mixture was then allowed to warm to $20\text{ }^\circ\text{C}$ and stirred for 3h. The mixture was then diluted with Et_2O (50 mL) and washed with water ($2 \times 10\text{ mL}$), 1 M $NaHSO_4$ ($3 \times 10\text{ mL}$), water ($2 \times 10\text{ mL}$), brine ($2 \times 5\text{ mL}$), dried, filtered and concentrated to give after prolonged drying at 5 Torr crude Boc-Ile-OTMSE (0.47 g, 66% crude yield) as a colorless oil which was used without purification for the next step. This material (0.47 g, 1.42 mmol) and $pTsOH \cdot H_2O$ (0.297 g, 1.56 mmol) were dissolved in dry benzene (15 mL) and heated under reflux for 1.5 h. The mixture was concentrated under reduced pressure and the residue was recrystallized from EtOAc/hexanes to give $TsOH \cdot H_2N$ -Ile-OTMSE (0.505 g, 88% crude yield) as a white solid which was used without further purification for the next step. This tosylate (0.283 g, 0.7 mmol) was dissolved Et_2O (40 mL) and was washed with saturated aqueous $NaHCO_3$ ($3 \times 5\text{ mL}$), brine ($2 \times 5\text{ mL}$), dried, filtered and concentrated under reduced pressure to leave H_2N -Ile-OTMSE **161** (0.150 g, 93% crude) as a colorless oil which was directly used for the peptide condensation in CH_2Cl_2 (5 mL) according to GP 5 with *N*-Boc protected β-methylphenylalanine **139** (0.193 g, 0.65 mmol) using EDC (0.127 g, 0.66 mmol), HOAt (0.089 g, 0.66 mmol) and TMP (0.089 g, 0.73 mmol). The organic layer, after aqueous work-up, was concentrated under reduced pressure to leave pure **162** (0.275 g, 86%) as a colorless glass. R_f = 0.38 EtOAc/hexanes 1:4; $[\alpha]_{20}^D -3.3$ (c = 0.4, $CHCl_3$); 1H NMR (250 MHz, $CDCl_3$): δ = 0.04 [s, 9 H, $Si(CH_3)_3$], 0.79 (d, J = 6.8 Hz, 3 H, 1'-H, *Ile*), 0.86 (d, J = 7.4 Hz, 3 H, 5-H, *Ile*), 0.82–0.95 (m, 2 H, 2-H, *TMSE*), 0.95–1.15 (m, 1 H, 4- H_a , *Ile*), 1.31 [d, J = 7.3 Hz, 3 H,

4-H, (β Me)Phe], 1.40 [s, 9 H, C(CH₃)₃], 1.73–1.82 (m, 1 H, 3-H, *Ile*), 3.16 [quint, J = 6.9 Hz, 1 H, 3-H, (β Me)Phe], 3.95–4.19 (m, 2 H, 1-H, *TMSE*), 4.20–4.36 [m, 2 H, 2-H, *Ile* and 2-H, (β Me)Phe], 5.15 (d, J = 8.8 Hz, 1 H, NH), 6.17 (d, J = 8.3 Hz, 1 H, NH), 7.01–7.33 (m, 5 H, Ar-H); the peaks of C(CH₃)₃ and CH₃, (β Me)Phe masked absorption for 4-H_b, *Ile*; ¹³C NMR (62.9 MHz, CDCl₃): δ = –1.79 [+ , Si(CH₃)₃], 11.3 (+, C-5, *Ile*), 15.0 (+, C-1', *Ile*), 16.9 [+ , C-4, (β Me)Phe], 17.1 (–, C-2, *TMSE*), 24.9 (–, C-4, *Ile*), 28.0 [+ , C(CH₃)₃], 37.7 (+, C-3, *Ile*), 42.1 [+ , C-3, (β Me)Phe], 56.2 (+, C-2, *Ile*), 60.0 [+ , C-2, (β Me)Phe], 63.0 (–, C-1, *TMSE*), 79.4 [C_{quat}, C(CH₃)₃], 126.5, 127.4, 128.2 (+, Ar-C), 142.3 (C_{quat}, Ar-C), 155.5 (C_{quat}, NCO₂), 170.6 (C_{quat}, C-1, *Ile*), 170.8 [C_{quat}, C-1, (β Me)Phe]; IR (film): ν (tilde) = 3500–2500 cm^{–1}, 3066, 2953, 1720, 1542, 1478, 1369, 1234, 1106, 1078; MS (EI, 70 eV), m/z (%) = 492 (7) [M⁺], 436 (4) [M⁺ – C₄H₈], 375 (8) [M⁺ – C₅H₁₃OSi], 259 (38), 178 (52), 134 (58), 73 (40), 57 (100) [C₄H₉⁺]; HRMS (EI): calcd for C₂₆H₄₄N₂O₅Si: 492.3020; found 492.3020.

Fmoc-(2*S*)-(3-*Ncp*)Ala-(β Me)Phe-*Ile*-OTMSE (**164**): The dipeptide **162** (0.268 g, 0.544 mmol) was deprotected with 3.9 M HCl in dioxane (4 mL) for 1 h. The mixture was then concentrated under reduced pressure and the residue was taken up with Et₂O (40 mL) and washed with saturated aqueous NaHCO₃ (3 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure to give H-(β Me)Phe-*Ile*-OTMSE (0.167 g, 78%) as a colorless oil which was directly coupled with **160** (0.178 g, 0.449 mmol) in CH₂Cl₂ (4 mL) according to GP 5 employing EDC (89 mg, 0.463 mmol), HOAt (61 mg, 0.450 mmol) and TMP (54 mg, 0.449 mmol) to give after usual water work-up crude product which was crystallized twice from CH₂Cl₂/hexanes and finally purified by column chromatography (CHCl₃/EtOAc 7:1) to give **164** (0.240 g, 73%) as a white solid. R_f = 0.28 EtOAc/hexanes 1:2; [α]₂₀^D –7.5 (c = 0.12, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 0.04 [s, 9 H, Si(CH₃)₃], 0.79 (d, J = 7.0 Hz, 3 H, 1'-H, *Ile*), 0.86 (t, J = 7.6 Hz, 3 H, 5-H, *Ile*), 0.87–1.14 [m, 4 H, 2-H, *TMSE*, 2'-H_a, (3-*Ncp*)Ala, 4-H_a, *Ile*], 1.24–1.41 (m, 1 H, 4-H_b,

Ile), 1.31 [d, $J = 7.0$ Hz, 3 H, 4-H, (β Me)*Phe*], 1.49–1.67 [m, 1 H, 2'-H_b, (*3-Ncp*)*Ala*], 1.67–1.82 [m, 2 H, 3-H, *Ile*, 1'-H, (*3-Ncp*)*Ala*], 1.82–2.03 [m, 2 H, 3-H, (*3-Ncp*)*Ala*], 3.21 [quint, $J = 7.5$ Hz, 1 H, 3-H, (β Me)*Phe*], 3.97–4.15 (m, 3 H, 1-H, *TMSE*, 2-H), 4.16–4.54 (m, 5 H, 2 $\times$ 2-H and 1'-H, 2'-H, *Fmoc*), 4.62 [ddd, $J = 8.5$ Hz, 1 H, 2'-H, (*3-Ncp*)*Ala*], 5.49–5.83 (br, 1 H, NH), 6.00–6.34 (br, 1 H, NH), 7.08–7.46 (m, 9 H, Ar-H), 7.52–7.66 (m, 2 H, Ar-H), 7.77 (d, $J = 7.3$ Hz, 2 H, Ar-H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = -1.79$ [+], $\text{Si}(\text{CH}_3)_3$, 11.3 (+, C-5, *Ile*), 15.0 (+, C-1', *Ile*), 17.1 [+], C-4, (β Me)*Phe*, 17.2 (–, C-2, *TMSE*), 17.9 [–, C-3', (*3-Ncp*)*Ala*], 22.2 [+], C-1', (*3-Ncp*)*Ala*, 25.0 (–, C-4, *Ile*), 34.0 [–, C-3, (*3-Ncp*)*Ala*], 37.9 (+, C-3, *Ile*), 41.9 [+], C-3, (β Me)*Phe*, 46.9 (+, C-1', *Fmoc*), 54.0 [+], C-2, (*3-Ncp*)*Ala*, 56.3 (+, C-2, *Ile*), 58.7 [+], C-2, (β Me)*Phe*, 58.9 [+], C-2', (*3-Ncp*)*Ala*, 63.3 (–, C-1, *TMSE*), 67.0 (–, C-2', *Fmoc*), 112.3 (+, Ar-C), 124.9 (+, Ar-C), 126.8 (+, Ar-C), 126.9 (+, Ar-C), 127.0 (+, Ar-C), 127.6 (+, Ar-C), 128.3 (+, Ar-C), 141.1, 141.1 (C_{quat} , Ar-C), 141.7 (C_{quat} , Ar-C), 143.5, 143.7 (C_{quat} , Ar-C), 156.0 (C_{quat} , NCO_2), 170.2, 171.2, 171.4 (C_{quat} , C-1); IR (KBr): $\nu(\text{tilde}) = 3341\text{ cm}^{-1}$, 3261, 3068, 2964, 2877, 1732, 1712 1642, 1544, 1368, 1251, 1228; MS (ESI), positive m/z (%) = 794 (30) [$\text{M} + \text{Na}^+$], 1564 (100) [$2\text{M} + \text{Na}^+$]; negative m/z (%) = 805 (20) [$\text{M} + \text{Cl}^-$].

*Fmoc-(\beta*Me)*Phe*-(2*S*)-(3-*Ncp*)*Ala*-(\betaMe)*Phe*-*Ile*-OTMSE (**167**): The *N*-Fmoc protected amino acid **146** (0.132 g, 0.328 mmol) was coupled according to GP2 with the tripeptide obtained after deprotection of **164** (0.230 g, 0.298 mmol) according to GP 4 in $\text{CH}_2\text{Cl}_2/\text{DMF}$ 3:1 (3 mL) employing EDC (65 mg, 0.339 mmol), HOAt (46 mg, 0.340 mmol) and TMP (41 mg, 0.338 mmol). The mixture was then taken up with EtOAc (30 mL) and concentrated after usual aqueous work-up to give crude product which was triturated with Et_2O /hexanes 1:1 and then recrystallized from THF/hexanes to give **167** (0.248 g, 89%) as an off-white solid. $R_f = 0.21$, EtOAc/ CHCl_3 2:9; $[\alpha]_{20}^D -7.5$ ($c = 0.12$, CHCl_3); ^1H NMR (250 MHz, $[\text{D}_8]\text{THF}$): $\delta = 0.03$ [s, 9 H, $\text{Si}(\text{CH}_3)_3$], 0.78 (q, $J = 7.1$ Hz, 6 H, 5-H, 1'-H, *Ile*), 0.91 (dd, $J = 8.6, 8.6$ Hz, 2 H, 2-H,

TMSE), 0.98–1.17 [m, 2 H, 2'-H_a, (3-*Ncp*)*Ala*, 4-H_a, *Ile*], 1.20 [d, $J = 7$ Hz, 3 H, 4-H, (β Me)*Phe*], 1.30–1.51 [m, 4 H, 1'-H, 2'-H_b, (3-*Ncp*)*Ala* and 3-H, 4-H_b, *Ile*], 1.33 [d, $J = 7.0$ Hz, 4-H, CH₃, (β Me)*Phe*], 1.54–1.79 [m, 2 H, 3-H, (3-*Ncp*)*Ala*], 3.10 [m, 1 H, 3-H, (β Me)*Phe*], 3.24 [t, $J = 7.3$ Hz, 1 H, 3-H, (β Me)*Phe*], 3.91–4.08 (m, 3 H, 1-H, *TMSE*, 2-H), 4.19–4.48 [m, 6 H, 2 $\times$ 2-H and 1'-H, 2'-H, *Fmoc* and 2'-H, (3-*Ncp*)*Ala*], 4.55 (dd, $J = 8.5, 8.5$ Hz, 1 H, 2-H), 7.02–7.38 (m, 16 H, Ar-H), 7.52 (d, $J = 9.0$ Hz, 1 H, NH), 7.57 (d, $J = 8.3$ Hz, 1 H, NH), 7.64 (d, $J = 7.5$ Hz, 1 H, NH), 7.68 (d, $J = 7.3$ Hz, 1 H, NH), 7.77 (d, $J = 7.3$ Hz, 2 H, Ar-H); ¹³C NMR (62.9 MHz, [D₈]THF): $\delta = -1.3$ [+ , Si(CH₃)₃], 11.9 (+, C-5, *Ile*), 15.9 (+, C-1', *Ile*), 18.0 [+ , C-4, (β Me)*Phe*], 18.2 (–, C-2, *TMSE*), 18.7 [+ , C-4, (β Me)*Phe*], 18.7 [–, C-3', (3-*Ncp*)*Ala*], 23.1 [+ , C-1', (3-*Ncp*)*Ala*], 26.0 (–, C-4, *Ile*), 34.8 [–, C-3, (3-*Ncp*)*Ala*], 38.8 (+, C-3, *Ile*), 42.8 [+ , C-3, (β Me)*Phe*], 44.1 [+ , C-3, (β Me)*Phe*], 48.4 (+, C-1', *Fmoc*), 52.7 (+, C-2), 57.1 (+, C-2), 59.5 (+, C-2), 60.1 (+, C-2), 62.0 [+ , C-2', (3-*Ncp*)*Ala*], 63.5 (–, C-1, *TMSE*), 67.8 (–, C-2', *Fmoc*), 120.7 (+, Ar-C), 126.3 (+, Ar-C), 127.4 (+, Ar-C), 127.6 (+, Ar-C), 128.0 (+, Ar-C), 128.5 (+, Ar-C), 129.0 (+, Ar-C), 129.1 (+, Ar-C), 129.2 (+, Ar-C), 129.3 (+, Ar-C), 142.4 (2 $\times$ C_{quat}, Ar-C), 143.9, 144.3 (C_{quat}, Ar-C), 145.3, 145.5 (C_{quat}, Ar-C), 157.7 (C_{quat}, NCO₂), 171.2, 171.4, 172.0, 172.1 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 3276$ cm^{–1}, 3064, 2965, 1710, 1668, 1637, 1543, 1452, 1369, 1249; MS (ESI), positive m/z (%) = 955 (90) [M + Na⁺], 1886 (80) [2M + Na⁺]; negative m/z (%) = 966 (22) [M + Cl[–]].

Alloc-a-Thr[*Boc*-(4-*PE*)*Pro*]-*OSEM* (**166**): The ester **166** (0.203 g, 64%) was prepared from the protected threonine **159** (0.185 g, 0.555 mmol), *N*-Boc protected propenylproline **16** (0.146 g, 0.572 mmol), 4-pyrrolidinopyridine (0.01 g, 0.067 mmol) and EDC (0.113 g, 0.589 mmol) in CH₂Cl₂ (5 mL) according to GP 6 after column chromatography (EtOAc/hexanes 1:6, $R_f = 0.19$) as a turbid oil. $[\alpha]_{20}^D -21.0$ ($c = 0.40$, CHCl₃); ¹H NMR (250 MHz, CDCl₃): $\delta = 0.01$ [s, 9 H, Si(CH₃)₃], 0.96 (t, $J = 8.6$ Hz, 2 H, 2'-H, *SEM*), 1.32, 1.36 (2 $\times$ d, $J = 6.7$ Hz, 3 H, 4-H, *a-Thr*),

1.41, 1.43 [$2 \times s$, 9 H, C(CH₃)₃], 1.63, 1.64 [$2 \times d$, $J = 7.0$ Hz, 3 H, 3'-H, (4-*PE*)*Pro*], 1.67–1.86 [m, 1 H, 3-H_a, (4-*PE*)*Pro*], 2.17–2.49 [m, 1 H, 3-H_b, (4-*PE*)*Pro*], 2.94–3.19 [m, 2 H, 4-H, 5-H_a, (4-*PE*)*Pro*], 3.51–3.64 [m, 0.5 H, 5-H_b, (4-*PE*)*Pro*], 3.72 (t, $J = 8.3$ Hz, 2 H, 1'-H, *SEM*), 4.15 [dd, $J = 9.6, 6.9$ Hz, 0.5 H, 2-H, (4-*PE*)*Pro*], 4.22 [dd, $J = 8.2, 8.2$ Hz, 0.5 H, 2-H, (4-*PE*)*Pro*], 4.45–4.67 (m, 3 H, 1-H, *Alloc* and 2-H, *a*-*Thr*), 5.09–5.44 [m, 6 H, 1-H, *SEM* and 2-H, 3-H, *Alloc* and 3-H, *a*-*Thr* and 1'-H, (4-*PE*)*Pro*], 5.53 [dq, $J = 9.2, 7.0$ Hz, 1 H, 2'-H, (4-*PE*)*Pro*], 6.51 (d, $J = 9.2$ Hz, 1 H, NH); the peak of 1'-H, *SEM* masked absorption for 0.5 H, 5-H_b of (4-*PE*)*Pro*; ¹³C NMR (62.9 MHz, CDCl₃): $\delta = -1.6$ [+ , Si(CH₃)₃], 13.0 [+ , C-3', (4-*PE*)*Pro*], 15.8, 16.6 (+ , C-4, *a*-*Thr*), 17.8 (– , C-2', *SEM*), 28.2, 28.3 [+ , C(CH₃)₃], 35.5, 36.3 [+ , C-4, (4-*PE*)*Pro*], 36.1, 37.1 [– , C-3, (4-*PE*)*Pro*], 51.3, 51.6 [– , C-5, (4-*PE*)*Pro*], 57.1 57.6 [+ , C-2, (4-*PE*)*Pro*], 59.2, 59.3 (+ , C-2, *a*-*Thr*), 65.6, 65.9 (– , C-1', *SEM*), 68.0, 68.3 (– , C-1, *Alloc*), 70.9, 71.1 (+ , C-3, *a*-*Thr*), 80.0, 80.2 (C_{quat}, C(CH₃)₃), 89.7, 90.1 (– , C-1, *SEM*), 117.4, 117.9 (– , C-3, *Alloc*), 126.6, 126.7 [+ , C-2', (4-*PE*)*Pro*], 129.1, 129.2 [+ , C-1', (4-*PE*)*Pro*], 132.3, 132.7 (+ , C-2, *Alloc*), 153.2, 153.8 [C_{quat}, NCO₂, (4-*PE*)*Pro*], 155.4, 156.0 (C_{quat}, NCO₂, *a*-*Thr*), 168.7 (C_{quat}, C-1), 171.9, 172.3 (C_{quat}, C-1); IR (film): $\nu(\text{tilde}) = 3333$ cm^{–1}, 3082, 2977, 2953, 2930, 1746, 1703, 1367, 1250, 1113; MS (EI, 70 eV), m/z (%) = 570 (1) [M⁺], 469 (16) [M⁺ – C₅H₉O₂], 339 (15), 168 (9), 210 (17), 154 (100) [C₈H₁₂NO₂⁺], 110 (98) [C₇H₁₂N⁺], 57 (92) [C₄H₉⁺], 41 (12) [C₃H₅⁺]; HRMS (EI): calcd for C₂₇H₄₆N₂O₉Si: 570.2972; found 570.2972.

Alloc-*a*-*Thr*[*Boc*-(4-*PE*)*Pro*]-*OH* (**168**): MgBr₂·Et₂O (0.335 g, 1.297 mmol) was added to a solution of ester **166** (0.200 g, 0.350 mmol) in CH₂Cl₂ (15 mL) at – 30 °C, the mixture was allowed to warm to 4 °C and stirring continued for 2 h. The reaction mixture was then diluted with Et₂O (50 mL), washed with 1 M NaHSO₄ (3 × 10 mL), water (3 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure to give crude **168** (0.103 g, 65%) as a turbid oil which was used for next step without purification. $R_f = 0.17$ EtOAc/hexanes 1:3 (2% AcOH).

Alloc-a-Thr[Boc-(4-PE)Pro]-(βMe)Phe-(2S)-(3-Ncp)Ala-(βMe)Phe-Ile-OTMSE (170):

Tetrapeptide **167** (0.165 g, 0.174 mmol) was deprotected according to GP 4 and the condensation between the resultant *N*-deprotected peptide **169** and peptide acid **168** (88 mg, 0.193 mmol) was then carried out in MeCN (3 mL) employing HATU (82 mg, 0.216 mmol), HOAt (27 mg, 0.200 mmol), DIEA (25 mg, 0.193 mmol) and TMP (70 mg, 0.578 mmol) according to GP 7 to give after usual aqueous work-up and evaporation of solvents the red solid which was triturated with hexanes, filtered and finally purified by column chromatography (EtOAc/hexanes 1:1.5, R_f = 0.28) to give **170** (0.156 g, 80%) as white solid. $[\alpha]_{20}^D$ 16.9 (c = 0.16, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ = 0.07 [s, 9 H, Si(CH₃)₃], 0.72 (d J = 7.0 Hz, 3 H, 1'-H, *Ile*), 0.86 (t, J = 7.0 Hz, 3 H, 5-H, *Ile*), 0.97 [ddd, J = 6.5 Hz, 1 H, 2'-H_a, (3-*Ncp*)*Ala*], 1.02 (dd, J = 8.8 Hz, 2 H, 2-H, *TMSE*), 1.06–1.21 [m, 2 H, 1'-H, (3-*Ncp*)*Ala*, 4-H_a, *Ile*], 1.21–1.50 [m, 4 H, 2'-H_b, 3-H, (3-*Ncp*)*Ala*, 4-H_b, *Ile*], 1.25 [d, J = 6.5 Hz, 3 H, 4-H, (βMe)*Phe*], 1.35 [d, J = 7.0 Hz, 3 H, 4-H, (βMe)*Phe*], 1.42 (d, J = 6.5 Hz, 3 H, 4-H, *a-Thr*), 1.49 [s, 9 H, C(CH₃)₃], 1.69 [dd, J = 1.5, 6.5 Hz, 3 H, 3'-H, (4-*PE*)*Pro*], 1.75 [ddd, J = 11.8, 10.3, 12.0 Hz, 1 H, 3-H_a, (4-*PE*)*Pro*], 1.79–1.89 (m, 1 H, 3-H, *Ile*), 2.43 [ddd, J = 6.5, 6.5, 6.5 Hz, 1 H, 3-H_b, (4-*PE*)*Pro*], 3.17 [dd, J = 10.5, 10.5 Hz, 1 H, 5-H_a, (4-*PE*)*Pro*], 3.21–3.35 [m, 2 H, 4-H, (4-*PE*)*Pro*, 3-H, (βMe)*Phe*], 3.72 [dd, J = 7.8, 10.5 Hz, 5-H, (4-*PE*)*Pro*], 3.86 [dq, J = 3.0, 7.0 Hz, 1 H, 3-H, (βMe)*Phe*], 4.17–4.32 (m, 3 H, 1-H, *TMSE*, 2-H), 4.35 (dd, J = 4.5, 9 Hz, 1 H, 2-H), 4.40 (dd, J = 1.5, 8.5 Hz, 1 H, 2-H), 4.49 (dd, J = 7.0, 10.0 Hz, 1 H, 2-H), 4.51–4.62 [m, 4 H, 1-H, *Alloc* and 2'-H, (3-*Ncp*)*Ala* and 2-H], 4.63 (dd, J = 10.3, 10.3 Hz, 1 H, 2-H), 5.16–5.21 (m, 1 H, *trans*-3-H, *Alloc*), 5.24–5.33 [m, 2 H, 1'-H, (4-*PE*)*Pro*, *cis*-3-H, *Alloc*], 5.50 (dq, J = 1.5, 6.5 Hz, 1 H, 3-H, *a-Thr*), 5.58 [dq, J = 10.5, 6.5 Hz, 1 H, 2'-H, (4-*PE*)*Pro*], 5.86 (ddt, J = 17.0, 10.5, 6.0 Hz, 1 H, 2-H, *Alloc*), 6.59 (d, J = 8 Hz, NH), 6.85 (d, J = 9.5 Hz, NH), 7.05 (d, J = 10 Hz, 1 H, NH), 7.14–7.37 (m, 10 H, Ar-H), 7.50 (d, J = 9.5 Hz, 1 H, NH), 7.91 (d, J = 6.0 Hz, 1 H, NH); ¹³C NMR (125.7 MHz, CDCl₃): δ = –1.5 [+ , Si(CH₃)₃], 11.7 (+, C-5, *Ile*), 13.3, [+ , C-3', (4-*PE*)*Pro*], 15.9 (+, C-1', *Ile*), 17.3 [–, C-3',

(*3-Ncp*)Ala], 17.9 (+, C-4, *a*-Thr), 18.3 (–, C-2, *TMSE*), 19.0 [+ , C-4, (*β*Me)*Phe*], 19.5 [+ , C-4, (*β*Me)*Phe*], 21.9 [+ , C-1', (*3-Ncp*)Ala], 25.2 (–, C-4, *Ile*), 28.5 [+ , C(CH₃)₃], 31.6 [–, C-3, (*3-Ncp*)Ala], 36.4 [–, C-3, (*4-PE*)Pro], 36.4 [+ , C-4, (*4-PE*)Pro], 37.4 (+, C-3, *Ile*), 40.6 [+ , C-3, (*β*Me)*Phe*], 42.0 [+ , C-3, (*β*Me)*Phe*], 50.9 (+, C-2), 52.1 [–, C-5, (*4-PE*)Pro], 56.2 (+, C-2), 59.4 (+, C-2), 59.5 (+, C-2), 61.1 (+, C-2), 61.6 (+, C-2), 62.0 [+ , C-2', (*3-Ncp*)Ala], 63.2 (–, C-1, *TMSE*), 65.6 (–, C-1, *Alloc*), 70.7 (+, C-3, *a*-Thr), 118.1 (–, C-3, *Alloc*), 127.0, 127.2, 127.7, 127.8, 128.5 (x2), 128.7, 128.9 [+ , Ar-C and C-1', C-2', (*4-PE*)Pro], 132.7 (+, C-2, *Alloc*), 141.8, 142.0 (C_{quat}, Ar-C), 154.9, 155.6 (C_{quat}, NCO₂), 170.5 (x2), 170.8, 171.3, 173.8, 174.2 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 3390\text{ cm}^{-1}$, 3086, 2954, 2898, 1728, 1521, 1412, 1382, 1250, 1150; MS (ESI), positive *m/z* (%) = 1155 (100) [M + Na⁺]; negative *m/z* (%) = 1131 (95) [M – H⁺].

N-Alloc protected cyclodepsipeptide (**171a**): Depsipeptide **170** (0.136 g, 0.120 mmol) was deprotected with TFA (2 mL) at 20 °C for 2 h. The mixture was then concentrated under reduced pressure to give residue which was triturated with Et₂O, filtered and quickly washed with cold CH₂Cl₂ to give trifluoroacetate of deprotected depsipeptide (0.126 g, 100%) as a white solid which was used without further purification for the cyclisation step. *R*_f = 0.10 CH₂Cl₂/MeOH 10:1 (1% of 28% aqueous NH₃). It was dissolved in anhydrous CH₂Cl₂ (1.20 l) and cyclized according to GP 9 employing HATU (2 × 0.059 g, 0.155 mmol) and DIEA (2 × 0.062 g, 0.480 mmol) to give after aqueous work-up, column chromatography (EtOAc/hexanes 1:1.5, *R*_f = 0.24) and crystallization from Et₂O/pentane the crude product which was finally purified by preparative HPLC to give **171a** (29 mg, 26%) as a colorless solid. Preparative HPLC: isocratic, 70% MeCN in H₂O (0.1% TFA); analytical HPLC 1: column A, isocratic 70% MeCN in H₂O (0.1% TFA), flow rate 1 mL/min, *t*_R = 12.96 min, purity > 98%; analytical HPLC 2: column B, gradient 0 → 99 % MeCN in H₂O (0.1% TFA) for 25 min, then isocratic 99% MeCN (0.1% TFA), flow rate = 0.5 mL/min, *t*_R = 28.61 min, purity > 98%; [α]₂₀^D – 8.5 (*c* = 0.13, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ = 0.61 [ddd, *J* = 14.3, 7.5, 7.5 Hz, 1 H, 3-H_a, (*3-Ncp*)Ala], 0.76

[ddd, $J = 6.5, 6.5, 6.5$ Hz, 1 H, 2'-H_a, (3-*Ncp*)Ala], 0.88 (d $J = 6.5$ Hz, 3 H, 1'-H, *Ile*), 0.91 (t, $J = 7.5$ Hz, 3 H, 5-H, *Ile*), 1.10–1.20 (m, 1 H, 4-H_a, *Ile*), 1.27 [d, $J = 7.0$ Hz, 3 H, 4-H, (β Me)Phe], 1.28–1.34 [m, 1 H, 1'-H, (3-*Ncp*)Ala], 1.38 [d, $J = 7.0$ Hz, 4-H, (β Me)Phe], 1.42–1.53 [m, 1 H, 3-H_b, (3-*Ncp*)Ala], 1.53–1.61 [m, 2 H, 2'-H_b, (3-*Ncp*)Ala, 4-H_b, *Ile*], 1.59 (d, $J = 6.5$ Hz, 3 H, 4-H, *a*-Thr), 1.65 [dd, $J = 1.5, 6.8$ Hz, 3 H, 3'-H, (4-*PE*)Pro], 1.74 [ddd, $J = 11.5, 11.5, 11.5$ Hz, 1 H, 3-H_a, (4-*PE*)Pro], 1.77–1.87 (m, 1 H, 3-H, *Ile*), 2.23 [ddd, $J = 5.5, 5.5, 12.5$ Hz, 1 H, 3-H_b, (4-*PE*)Pro], 3.07 [dq, $J = 7.0, 7.0$ Hz, 1 H, 3-H, (β Me)Phe], 3.15–3.30 [m, 1 H, 4-H, (4-*PE*)Pro], 3.25 [dd, $J = 11.0, 11.0$ Hz, 1 H, 5-H_a, (4-*PE*)Pro], 3.55 [dq, $J = 5.0, 7.0$ Hz, 1 H, 3-H, (β Me)Phe], 3.71 (dd, $J = 6.5, 6.5$ Hz, 1 H, 2-H), 3.78 (ddd, $J = 3.0, 3.0, 7.0$ Hz, 1 H, 2-H), 3.98 [dd, $J = 6.3, 11.0$ Hz, 5-H, (4-*PE*)Pro], 4.07 [dd, $J = 7.3, 7.3$ Hz, 1 H, 2-H, (4-*PE*)Pro], 4.49 (dd, $J = 1.5, 9.5$ Hz, 2-H), 4.53 [dd, $J = 4.5, 7.0$ Hz, 1 H, 2-H, (β Me)Phe], 4.54–4.60 [m, 2 H, 2'-H, (3-*Ncp*)Ala, 1-H_a, Alloc], 4.60–4.64 (m, 1 H, 1-H, Alloc), 4.66 [dd, $J = 8.0, 8.0$ Hz, 1 H, 2-H, (β Me)Phe], 5.19–5.26 [m, 1 H, 1'-H, (4-*PE*)Pro], 5.22 (ddt, $J = 1.5, 1.5, 10.3$ Hz, 1 H, *trans*-3-H, Alloc), 5.32 (ddt, $J = 1.5, 1.5, 17.3$ Hz, 1 H, *cis*-3-H, Alloc), 5.42 (dq, $J = 10.5, 7.0$ Hz, 1 H, 3-H, *a*-Thr), 5.58 [dq, $J = 10.5, 6.8$ Hz, 1 H, 2'-H, (4-*PE*)Pro], 5.91 (ddt, $J = 17.3, 10.5, 5.5$ Hz, 1 H, 2-H, Alloc), 6.12–6.21 (br, 1 H, NH), 6.36 (d, $J = 7.0$ Hz, 1 H, NH), 6.46 (d, $J = 9.5$ Hz, NH), 6.54 (d, $J = 9.5$ Hz, NH), 7.11–7.34 (m, 10 H, Ar-H), 7.45 (d, $J = 8.0$ Hz, 1 H, NH); ^{13}C NMR (125.7 MHz, CDCl₃): $\delta = 10.5$ (+, C-5, *Ile*), 13.3, [+ , C-3', (4-*PE*)Pro], 14.6 [+ , C-4, (β Me)Phe], 14.8 (+, C-1', *Ile*), 17.3 [–, C-3', (3-*Ncp*)Ala], 17.8 [+ , C-4, (β Me)Phe], 18.4 (+, C-4, *a*-Thr), 21.4 [+ , C-1', (3-*Ncp*)Ala], 24.8 (–, C-4, *Ile*), 32.0 [–, C-3, (3-*Ncp*)Ala], 35.4 [–, C-3, (4-*PE*)Pro], 36.6 [+ , C-4, (4-*PE*)Pro], 37.0 (+, C-3, *Ile*), 39.6 [+ , C-3, (β Me)Phe], 44.7 [+ , C-3, (β Me)Phe], 52.5 [–, C-5, (4-*PE*)Pro], 53.3 [+ , C-2, (3-*Ncp*)Ala], 54.6 (+, C-2, *Ile*), 58.5 [+ , C-2, (β Me)Phe], 59.1 (+, C-2, *a*-Thr), 59.2 [+ , C-2, (β Me)Phe], 59.9 [+ , C-2, (4-*PE*)Pro], 60.6 [+ , C-2', (3-*Ncp*)Ala], 65.9 (–, C-1, Alloc), 72.6 (+, C-3, *a*-Thr), 117.7 (–, C-3, Alloc), 126.9, 127.2, 127.4, 127.7, 127.8, 127.9, 128.5, 128.7 [+ , Ar-C and C-1', C-2', (4-*PE*)Pro], 132.7 (+, C-2,

Alloc), 141.1, 142.6 (C_{quat} , Ar-C), 156.0 (C_{quat} , NCO_2), 168.7, 170.0, 170.5, 171.2, 171.3, 173.0 (C_{quat} , C-1); MS (ESI), positive m/z (%) = 937 (100) [$\text{M} + \text{Na}^+$]; negative m/z (%) = 913 (100) [$\text{M} - \text{H}^+$].

Attempt of the transprotection of cyclodepsipeptide 171a: To a vigorously stirred suspension of $\text{Pd}(\text{P}(\text{Ph})_3)_4$ (1 mg, 0.87 μmol) in a solution of the *N*-Alloc protected cyclohexadepsipeptide **171a** (25 mg, 27.38 μmol) and Boc_2O (2 mg, 91.64 μmol) in DME (4 mL) Bu_3SnH (24 mg, 82.45 μmol) was added and stirring continued for 2.5 h. All volatiles were removed under reduced pressure and the residue was purified by preparative TLC (20×20 , EtOAc/hexanes 1:2, $R_f = 0.18$, 3 runs) to give 10 mg of the mixture of unreacted and transprotected peptide, **171a** and **171b**, in ratio 40:60 (according to ESI-MS spectra).

Study of the acidolytic cleavage of Z, m-MeZ, MeZ and DmZ: To test a cleavage of Z-group by “push-pool” mechanism ester Z-Thr(FmocPro)-OMe **172** (prepared according to GP 6) (0.005 g) was treated with mixture of TFA (1.5 mL), dimethylsulfide (0.2 mL) and thioanisole (0.4 mL) at 20 °C for 7.5 h and then at 4 °C for 12 h. According to TLC the deprotection was incomplete. Dipeptide Boc-Phe-Val-OMe **117** was deprotected by TFA for 20 min. The aliquots of the resultant trifluoroacetate of dipeptide (3×0.25 mmol) were dissolved in CH_2Cl_2 (5 mL), DIEA (0.510 mmol) and then appropriate OSu-ester: MeZOSu or *m*-MeZOSu, or DmZ-OSu (0.275 mmol) (prepared according to GP 9) was added to each portion of the deprotected dipeptide. After 3 h, the reaction mixtures were diluted with Et_2O (50 mL) and subjected usual aqueous work-up (GP 5). The ethereal solutions were dried, filtered and concentrated under reduced pressure. The residues were purified by crystallization from EtOAc/hexanes (MeZ-Phe-Val-OMe **173a**) or by column chromatography (*m*-MeZ-Phe-Val-OMe **173b** and DmZ-Phe-Val-OMe **173c**) to give the desired dipeptides in 75–90% yield. These dipeptides and hexadepsipeptide **170** (6 mg each) were placed in four 10-mL flasks and then freshly prepared 2 M HCl in dry

EtOAc (1 mL to each sample) was added. TLC control (EtOAc/hexanes 1:1, MeOH/CH₂Cl₂ 1:10 (1% of 28% aqueous NH₃); visualization with phosphomolibdic acid or with ninhydrin) showed full *N*-deprotection of **170** for 2 min, MeZ-group and 3.5-DeZ-groups were stable to these conditions at least for 2 h and showed only traces of the deprotected products after 5 h. *m*-MeZ-group was stable to these conditions. TFA or 20% *N*-methylindole, or 10% anisole, or 20% thioanisole in TFA (1 mL) were added to aliquots of *N*-protected dipeptides (4 × 5 mg each). TLC control (see above) showed smooth cleavage (full consumption of the starting material and one ninhydrin active spot on chromatogram) of MeZ and DmZ groups with 10% anisole and 20% thioanisole in TFA for 1 h and *m*-MeZ with 20% thioanisole in TFA for 3.5 h.

MeZ- α -Thr-OH (**175**): NaHCO₃ (0.180 g, 2.14 mmol) and then a solution of MeZOSu (0.608 g, 2.31 mmol) in dioxane (7 mL) were added to a vigorously stirred solution of *allo*-D-threonine (*R*)- α -**72** (0.25 g, 2.10 mmol) in water (7 mL) and stirring continued for 3 h (if precipitate formed dioxane and/or water were added to obtain homogeneous solution). The mixture was then concentrated under reduced pressure, diluted with water (40 mL) and washed with CH₂Cl₂ (4 × 10 mL). pH of the water fraction was adjusted to ca. 1–2 with solid NaHSO₄ and the resulting emulsion was extracted with EtOAc (2 × 40 mL). The organic layer was washed with water (4 × 20 mL), brine 2 × 10 mL), dried and concentrated under reduced pressure. The residue was recrystallized from Et₂O/hexanes and then from CH₂Cl₂/hexanes to give **175** (0.175 g, 39%) as a white solid. The mother liquor from the second crystallization was concentrated and recrystallized again from Et₂O/hexanes to give a second crop of **175** (0.23 g, 90% overall yield). R_f = 0.13 EtOAc/hexanes (2% AcOH), 3 runs; m.p. 78 – 80 °C; $[\alpha]_{20}^D$ – 24.6 (c = 0.32, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 1.27 (d, J = 6.5 Hz, 3 H, 4-H), 2.34 (s, 3 H, 1'-H), 3.70–4.40 (br, 1 H, CO₂H), 4.05–4.27 (m, 1 H, 3-H), 4.33–4.44 (m, 1 H, 2-H), 5.07 (s, 2 H, Bzl-H), 5.77 (d, J = 7.5 Hz, 1 H, NH), 7.15 (d, J = 8.0 Hz, 2 H, Ar-H), 7.24 (d, J = 8.0 Hz, 2 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 18.7 (+, C-4), 21.1 (+, C-1'), 59.3 (+, C-2), 67.4 (–, Bzl-C), 69.0 (+, C-

3), 128.3, 129.2 (+, Ar-C), 132.7, 138.1 (C_{quat}, Ar-C), 156.9 (C_{quat}, NCO₂), 173.3 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 3400 \text{ cm}^{-1}$, 3213, 2988, 2931, 2889, 1723, 1678, 1537, 1422, 1275, 1225, 1210; MS (EI, 70 eV), m/z (%) = 267 (3) [M⁺], 223 (5) [M⁺ – CO₂], 162 (8) [M⁺ – C₈H₉], 105 (100) [C₈H₉⁺], 77 (6) [C₆H₅⁺], 45 (5) [CHO₂⁺]; HRMS (EI): calcd for C₁₃H₁₇NO₅: 267.1107; found 267.1107; elemental analysis calcd (%) for C₁₃H₁₇NO₅ (267.3): calcd. C 58.42, H 6.41, N 5.24; found C 58.59, H 6.13, N 5.08.

MeZ-*a*-Thr-OAll (176): A suspension of dried K₂CO₃ (0.034 g, 0.247 mmol) in a solution of the *N*-protected acid **175** (0.12 g, 0.449 mmol) and allyl bromide (0.08 mL, 0.946 mmol) in anhydrous MeCN (4 mL) was vigorously stirred in a sealed tube at 85 °C for 2 h. The mixture was then allowed to cool to 60 °C and stirring continued for an additional 16 h. The reaction mixture was cooled to 20 °C, and Et₂O (50 mL) and water (20 mL) were then added. The organic layer was washed with water (4 × 10 mL), saturated aqueous NaHCO₃ (2 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The residual oil was triturated with Et₂O/pentane 1:2 to give a white solid. Then more pentane was added to complete precipitation and **176** was filtered off and dried under reduced pressure (0.116 g, 84%). $R_f = 0.16$ EtOAc/hexanes 1:3; m.p. 47–48 °C; $[\alpha]_{20}^D - 20.4$ ($c = 0.30$, CHCl₃); ¹H NMR (250 MHz, CDCl₃): $\delta = 1.20$ (d, $J = 6.5$ Hz, 3 H, 4-H), 2.35 (s, 3 H, 1'-H), 2.74 (d, $J = 6.3$ Hz, 1 H, OH), 4.09–4.26 (m, 1 H, 3-H), 4.47 (dd, $J = 7.5, 3.8$ Hz, 1 H, 2-H), 4.67 (d, $J = 5.3$ Hz, 2 H, 1-H, *All*), 5.08 (s, 2 H, Bzl-H), 5.27 (ddt, $J = 10.3, 1.0, 1.0$ Hz, 1 H, *trans*-3-H, *All*), 5.34 (ddt, $J = 17.3, 1.0, 1.0$ Hz, 1 H, *cis*-3-H, *All*), 5.66 (d, $J = 7.3$ Hz, 1 H, NH), 5.90 (ddt, $J = 17.3, 10.3, 5.8$ Hz, 1 H, 2-H, *All*), 7.17 (d, $J = 8.0$ Hz, 2 H, Ar-H), 7.26 (d, $J = 8.0$ Hz, 2 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): $\delta = 18.7$ (+, C-4), 20.9 (+, C-1'), 59.3 (+, C-2), 65.9 (–, C-1, *All*), 67.4 (–, Bzl-C), 68.4 (+, C-3), 118.7 (–, C-3, *All*), 128.0, 128.9 (+, Ar-C), 132.8 (+, C-2, *All*), 132.8, 137.7 (C_{quat}, Ar-C), 156.4 (C_{quat}, NCO₂), 169.9 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 3409 \text{ cm}^{-1}$, 3327, 3056,

2972, 2941, 2889, 1744, 1690, 1536, 1463, 1382, 1338, 1284, 1233, 1183; MS (EI, 70 eV), m/z (%) = 307 (2) [M^+], 263 (4) [$M^+ - C_2H_4O$], 202 (8) [$M^+ - C_8H_9$], 105 (100) [$C_8H_9^+$], 77 (5) [$C_6H_5^+$], 41 (5) [$C_3H_5^+$]; HRMS (EI): calcd for $C_{16}H_{21}NO_5$: 307.1420; found 307.1420; elemental analysis calcd (%) for $C_{16}H_{21}NO_5$ (307.5): C 62.53, H 6.89, N 4.56; found C 62.80, H 6.84, N 4.41.

N-Z-3-(2S,3R)- β -Methylphenylalanine (**177**): A solution of ZOSu (0.396 g, 1.59 mmol) in acetone (5 mL) was added to a vigorously stirred solution of 3-(2S,3R)-methylphenylalanine hydrochloride HCl-**78** (0.35 g, 1.62 mmol) and $NaHCO_3$ (0.409 g, 4.87 mmol) in water (5 mL) and stirring continued for 2 h (if emulsion formed acetone and/or water were added to obtain homogeneous solution). Acetone was then removed under reduced pressure, the residual fraction was diluted with water (25 mL) and washed with Et_2O (3×10 mL). The organic fraction was back extracted with 5% aqueous $NaHCO_3$ (3×10 mL), pH of the combined water fractions were adjusted to 1 with 1 M HCl and the resulting emulsion was extracted with Et_2O (2×50 mL). The organic layer was washed with 1 M HCl (2×10 mL), water (10×10 mL), brine (2×10 mL), dried, filtered and concentrated under reduced pressure. The residual oil was dissolved in Et_2O (3 mL) and dicyclohexylamine (0.277 g, 1.53 mmol) was added followed by hexanes (20 mL) and the resulting precipitate was filtered and crystallized twice from EtOAc/hexanes to give dicyclohexylammomium salt of **177** (0.67 g, 85%) as a white solid. To obtain an analytical sample of **177** a small quantity of the dicyclohexylammionium salt dissolved in EtOAc and washed twice with 1 M HCl, thrice with water, twice with brine to give after prolonged drying at 0.02 Torr and at 60 °C **177** as a colorless solid. M.p. 76.5–79 °C; $[\alpha]_{20}^D$ 17.3 ($c = 0.76$, $CHCl_3$) 1H NMR (250 MHz, $CDCl_3$): $\delta = 1.31$, 1.36 ($2 \times d$, $J = 7.0$ Hz, 3 H, 4-H), 3.33 (dq, $J = 7.0$, 7.0 Hz, 1 H, 3-H), 4.43–4.55 (m, 0.25 H, 2-H), 4.60 (dd, $J = 5.5$ Hz, 9 Hz, 0.75 H, 3-H), 4.78 (d, $J = 12.5$ Hz, 0.25 H, Bzl-H), 4.96–5.20 (m, 1.75 H, Bzl-H), 5.30 (d, $J = 9$ Hz, 0.75 H, NH), 6.25

(d, $J = 8.8$ Hz, 0.20 H, NH), 7.03–7.52 (m, 10 H, 1''-H), 4.56–4.69, 4.71–4.87 ($2 \times$ m, 1 H, 2'-H), 5.48 (d, $J = 7.0$ Hz, 0.6 H, NH), 7.01–7.13 (m, 10 H, Ar-H), 7.30–7.90 (br, 1 H, CO₂H); ¹³C NMR (62.9 MHz, CDCl₃): $\delta = 14.3$, 15.8 (+, C-4), 41.5, 41.8 (+, C-3), 59.1, 59.8 (+, C-2), 67.0, 67.4 (–, Bzl-C), 127.0 (+, Ar-C), 127.5 (+, Ar-C), 127.6 (+, Ar-C), 127.9 (+, Ar-C), 128.0 (+, Ar-C), 128.3, 128.3 (+, Ar-C), 135.2, 135.9 (C_{quat}, Ar-C), 140.9, 141.4 (C_{quat}, Ar-C), 156.0, 157.1 (C_{quat}, NCO₂), 175.1, 175.4 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 3750\text{--}2250\text{ cm}^{-1}$, 1718, 1518, 1497, 1454, 1416, 1347, 1217; MS (EI, 70 eV), m/z (%) = 313 (1) [M⁺], 209 (4) [M⁺ – C₈H₈], 162 (10), 105 (100) [C₈H₉⁺], 91 (48) [C₇H₇⁺], 65 (7) [C₅H₅⁺]; HRMS (EI): calcd for C₁₈H₁₉NO₄: 313.1314; found 313.1314; elemental analysis calcd (%) for C₁₈H₁₉NO₄ (313.4): C 69.00, H 6.11, N 4.47; found C 69.16, H 6.00, N 4.45.

N-Z Isoleucine methoxymethyl ester (179): To an ice-cold solution of MOM-Cl (0.43 mL, 5.65 mmol) in acetonitrile (7 mL) LiI (0.783 g, 5.65 mmol) was added. The mixture was stirred at the same temperature for 20 min and a solution of Z-Ile-OH **178** (0.75 g, 2.83 mmol), DIEA (0.731 g, 5.65 mmol) in acetonitrile (5 mL) was then added dropwise for 5 min. The reaction mixture was allowed to warm to 20 °C, stirred for an additional 2 h and then diluted with Et₂O (50 mL). After usual aqueous work-up (GP 5), the organic layer was dried, and concentrated under reduced pressure to give the oily residue which was purified by column chromatography (twice, EtOAc/hexanes 1:6, $R_f = 0.21$) to give **179** (0.665 g, 76%) as a colorless oil. $[\alpha]_{20}^D$ 3.9 ($c = 1.90$, CHCl₃); ¹H NMR (250 MHz, CDCl₃): $\delta = 0.93$ (t, $J = 6.9$ Hz, 3 H, 5-H), 0.97 (d, $J = 6.8$ Hz, 3 H, 1'-H), 1.06–1.32 (m, 1 H, 4-H_a), 1.35–1.54 (m, 1 H, 4-H_b), 1.81–2.03 (m, 1 H, 3-H), 3.47 (s, 3 H, OMe), 4.38 (dd, $J = 9, 4.5$ Hz, 1 H, 2-H), 5.11 (s, 2 H, Bzl-H), 5.22 (d, $J = 5.8$ Hz, 1 H, H_a, OCH₂O), 5.28 (d, $J = 6.0$ Hz, 1 H, NH), 5.35 (d, $J = 5.8$ Hz, 1 H, H_b, OCH₂O), 7.28–7.42 (m, 5 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): $\delta = 11.2$ (+, C-5), 15.1 (+, C-1'), 24.5 (–, C-4), 57.3 (+, OMe), 37.4 (+, C-3), 58.1 (+, C-2), 66.5 (–, Bzl-C), 90.6 (–, OCH₂O), 127.7, 127.7, 128.1 (+,

Ar-C), 136.0 (C_{quat}, Ar-C), 155.9 (C_{quat}, NCO₂), 171.4 (C_{quat}, C-1); IR (film): $\nu(\text{tilde}) = 3390 \text{ cm}^{-1}$, 3066, 3034, 2965, 2937, 2878, 2832, 1724, 1525, 1455, 1412, 1337, 1220, 1087, 1041.

N-Fmoc Isoleucine dicyclopropylmethyl ester (**182**): To a stirred ice-cold solution of *N*-Fmoc protected isoleucine **181** (1 g, 28.29 mmol) in anhydrous CH₂Cl₂ (10 mL) oxalyl chloride (0.898 g, 7.07 mmol) and then DMF (5 drops) were added and stirring continued at the same temperature for 2 h. The mixture was then allowed to warm to 20 °C and stirred for an additional 1 h. The solvent was then flow out with nitrogen stream and the crude chloranhydride was dried at 0.01 Torr for 2 h and used further without purification. The chloranhydride was dissolved in anhydrous CH₂Cl₂ (10 mL) and the mixture of pyridine/dicyclopropylmethanol 1:1 (1.5 mL) was then added. After 40 min DMAP (0.02 g) was added to the mixture and stirring continued for an additional 3 h. The reaction mixture was then diluted with Et₂O (50 mL), washed (according to GP 5), dried, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/hexanes 1:10 (0.5% Et₃N), *R*_f = 0.24). The appropriate fractions were pooled, concentrated under reduced pressure, taken up with Et₂O/hexanes 1:1 (100 mL), washed with water (3 × 20 mL), 3% aqueous NaHCO₃ (3 × 20 mL), water (3 × 20 mL), brine (2 × 10 mL), dried, filtered and concentrated under reduced pressure to give **182** (0.72 g, 57%) as a turbid oil. $[\alpha]_{20}^D - 3.8$ (*c* = 0.26, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 0.16–0.38 (m, 4 H, 2'-H_a, DCPM), 0.38–0.51 (m, 2 H, 2'-H_b, DCPM), 0.51–0.64 (m, 2 H, 2'-H_c, DCPM), 0.94 (t, *J* = 7.5 Hz, 3 H, 5-H, *Ile*), 0.96 (d, *J* = 7.5 Hz, 3 H, 1'-H, *Ile*), 1.02–1.16 (m, 2 H, 1'-H, DCPM), 1.17–1.34 (m, 1 H, 4-H_a, *Ile*), 1.39–1.47 (m, 1 H, 4-H_b, *Ile*), 1.86–2.09 (m, 1 H, 3-H, *Ile*), 3.90 (t, *J* = 8.8 Hz, 1 H, 1-H, DCPM), 4.23 (dd, *J* = 7.3, 7.3 Hz, 1 H, 2-H, *Ile*), 4.34–4.44 (m, 3 H, 1'-H, 2'-H, *Fmoc*), 5.36 (d, *J* = 9.8 Hz, 1 H, NH), 5.16 (d, *J* = 6.0 Hz, 1 H, NH), 7.23–7.46 (m, 4 H, Ar-H), 7.6 (d, *J* = 7.5 Hz, 2 H, Ar-H), 7.76 (d, *J* = 8.3 Hz, 2 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 2.4, 2.6, 2.9 (–, C-2', DCPM), 11.6 (+, C-5, *Ile*), 14.5, 15.3 (+, C-1', DCPM), 14.6

(+, C-1', *Ile*), 24.9 (–, C-4, *Ile*), 38.1 (+, C-3, *Ile*), 47.1 (+, C-2', *Fmoc*), 58.3 (+, C-2, *Ile*), 66.8 (–, C-1', *Fmoc*), 83.4 (+, C-1, *DCPM*), 119.8, 125.0, 126.9, 127.5 (+, Ar-C), 141.1 (C_{quat}, Ar-C), 143.7, 143.8 (C_{quat}, Ar-C), 156.0 (C_{quat}, NCO₂), 171.5, (C_{quat}, C-1, *Ile*); IR (film): $\nu(\text{tilde}) = 3400\text{ cm}^{-1}$, 3068, 3009, 2964, 2935, 2876, 2832, 1805, 1717, 1508, 1451, 1331, 1209, 1220, 1091, 1030.

Z-(β Me)*Phe-Ile-OMOM* (**183a**): The compound **179** (0.415 g, 1.34 mmol) was hydrogenated on 10% Pd on charcoal (0.150 g) under ambient pressure of hydrogen in EtOAc (10 mL) with AcOH (0.080 g, 1.34 mmol) for 1 h. The mixture was then filtered, concentrated under reduced pressure, taken up with Et₂O (40 mL), washed with saturated aqueous NaHCO₃ (3 $\times$ 10 mL), brine (2 $\times$ 10 mL), dried, filtered and concentrated under reduced pressure to give deprotected ester of isoleucine **180a** as a free base which was directly used for the condensation with *N-Z* protected β -methylphenylalanine **177** (0.400 g, 1.28 mmol) employing EDC (0.257 g, 1.34 mmol), HOAt (0.173 g, 1.28 mmol) and TMP (0.500 mL, 3.78 mmol) in CH₂Cl₂ (5 mL) according to GP 5. After 2 h, the reaction mixture was subjected usual aqueous work-up and the resulting crude product was purified by crystallization from EtOAc/hexanes to give dipeptide **183a** (0.400 g, 66%) as a white solid. $R_f = 0.17$, EtOAc/hexanes 1:6; $R_f = 0.35$, EtOAc/hexanes 1:4; m.p. 111–112 °C; $[\alpha]_{20}^D - 8.5$ ($c = 0.30$, CHCl₃); ¹H NMR (250 MHz, CDCl₃): $\delta = 0.81$ (d, $J = 6.8$ Hz, 3 H, 1'-H, *Ile*), 0.87 (t, $J = 6.8$ Hz, 3 H, 5-H, *Ile*), 0.94–1.17 (m, 1 H, 4-H_a, *Ile*), 1.24–1.45 (m, 1 H, 4-H_b, *Ile*), 1.35 [d, $J = 7.3$ Hz, 3 H, 4-H, (β Me)*Phe*], 1.68–1.87 (m, 1 H, 3-H, *Ile*), 3.17 [dq, $J = 7.3$ Hz, 1 H, 3-H, (β Me)*Phe*], 3.42 (s, 3 H, OMe), 4.27–4.41 (m, 2 H, 2 $\times$ 2-H), 5.09 (s, 2 H, Bzl-H), 5.13 (d, $J = 6.0$ Hz, 1 H, H_a, OCH₂O), 5.21 (d, $J = 5.8$ Hz, 1 H, H_b, OCH₂O), 5.43 (d, $J = 9$ Hz, 1 H, NH), 5.98 (d, $J = 7.8$ Hz, NH), 7.15–7.30 (m, 6 H, Ar-H), 7.30–7.42 (m, 4 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): $\delta = 11.2$ (+, C-5, *Ile*), 14.9 (+, C-1', *Ile*), 16.9 [+ , C-4, (β Me)*Phe*], 24.8 (–, C-4, *Ile*), 37.5 (+, C-3, *Ile*), 42.2 [+ , C-3, (β Me)*Phe*], 56.1 (+, C-2,

Ile), 57.5 (+, OMe), 60.2 [+ , C-2, (β Me)*Phe*], 66.5 (–, Bzl-C), 90.6 (–, OCH₂O), 126.6, 127.5, 127.5, 127.7, 128.2 ($\times 2$) (+, Ar-C), 136.2, 141.9 (C_{quat}, Ar-C), 156.2, 156.8 (C_{quat}, NCO₂), 170.4, 170.6 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 3330\text{ cm}^{-1}$, 3275, 3065, 3033, 2965, 2928, 2874, 2832, 1741, 1689, 1654, 1536, 1293, 1275, 1253, 1242, 1155, 1071, 1012; MS (EI, 70 eV), m/z (%) = 470 (4) [M^+], 366 (11) [$M^+ - C_8H_8$], 319 (18) [$M^+ - C_8H_8NO_2$], 289 (15), 268 (8), 224 (20), 199 (6), 105 (10) [$C_8H_9^+$], 91 (100) [$C_7H_7^+$], 86 (10), 45 (12) [CHO_2^+]; HRMS (EI): calcd for C₂₆H₃₄N₂O₆: 470.2417; found 470.2417; elemental analysis calcd (%) for C₂₆H₃₄N₂O₆ (470.6): C 66.36, H 7.28, N 5.95; found C 66.58, H 7.19, N 6.10.

Z-(β Me)*Phe-Ile-ODCPM* (**183b**): The substance **182** (0.540 g, 1.21 mmol) was *N*-deprotected according to GP 4 and the resultant amino ester was coupled with *N-Z* protected (β -methylphenylalanine) **177** (0.36 g, 1.15 mmol) employing EDC (0.277 g, 1.18 mmol), HOAt (0.160 g, 1.18 mmol) and TMP (0.418 g, 3.45 mmol) in CH₂Cl₂ (5 mL) according to GP 5. After 6 h, the reaction mixture was subjected usual aqueous work-up and the resulting crude product was triturated with pentane and then purified by crystallization from hexanes to give dipeptide **183b** (0.500 g, 84%) as a white solid. $R_f = 0.17$, EtOAc/hexanes 1:6 (0.5% Et₃N); m.p 105–106 °C; $[\alpha]_{20}^D$ 9.0 ($c = 0.31$, CHCl₃); ¹H NMR (250 MHz, CDCl₃): $\delta = 0.23\text{--}0.41$ (m, 4 H, 2'-H_a, *DCPM*), 0.41–0.53 (m, 2 H, 2'-H_b, *DCPM*), 0.53–0.64 (m, 2 H, 2'-H_c, *DCPM*), 0.81 (d, $J = 6.8$ Hz, 3 H, 1'-H, *Ile*), 0.89 (t, $J = 7.3$ Hz, 3 H, 5-H, *Ile*), 0.95–1.21 (m, 3 H, 4-H_a, *Ile*, 1'-H, *DCPM*), 1.35 [d, $J = 7.3$ Hz, 3 H, (β Me)*Phe*], 1.37–1.48 (m, 1 H, 4-H_b, *Ile*), 1.71–1.90 (m, 1 H, 3-H, *Ile*), 3.19 [dq, $J = 7.3$ Hz, 1 H, (β Me)*Phe*], 3.90 (t, $J = 8.5$ Hz, 1 H, 1-H, *DCPM*), 4.26–4.42 (m, 2 H, 2 $\times$ 2-H), 5.09 (s, 2 H, Bzl-H), 5.42 (d, $J = 9.0$ Hz, 1 H, NH), 6.06 (d, $J = 7.5$ Hz, 1 H, NH), 7.15–7.30 (m, 6 H, Ar-H), 7.30–7.40 (m, 4 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): $\delta = 2.4$, 2.7 (–, C-2', *DCPM*), 11.4 (+, C-5, *Ile*), 14.3, 14.5 (+, C-1', *DCPM*), 14.8 (+, C-1', *Ile*), 16.7 [+ , C-4, (β Me)*Phe*], 24.9 (–, C-4, *Ile*), 37.9 (+, C-3, *Ile*), 42.2 [+ , C-3, (β Me)*Phe*], 56.1 (+, C-2,

Ile), 60.1 [+, C-2, (β Me)*Phe*], 66.5 (–, Bzl-C), 82.9 (+, C-1, *DCPM*), 126.5, 127.4, 127.5, 127.6, 128.1 ($\times$ 2) (+, Ar-C), 136.2, 141.9 (C_{quat} , Ar-C), 156.1 (C_{quat} , NCO_2), 170.2, 170.5 (C_{quat} , C-1, *Ile*); IR (KBr): $\nu(\text{tilde}) = 3300 \text{ cm}^{-1}$, 3270, 3010, 2965, 2875, 1732, 1690, 1655, 1535, 1454, 1379, 1334, 1292, 1275, 1255, 1240, 1196, 1137, 1013; MS (EI, 70 eV), m/z (%) = 520 (8) [M^+], 416 (7) [$\text{M}^+ - \text{C}_8\text{H}_8$], 381 (8) [$\text{M}^+ - \text{C}_7\text{H}_9\text{NO}_2$], 296 (20) [$\text{C}_{18}\text{H}_{18}\text{NO}_2$], 268 (11), 224 (20), 176 (6), 105 (7) [C_8H_9^+], 95 (100), 86 (17), 67 (10); HRMS (EI): calcd for $\text{C}_{31}\text{H}_{40}\text{N}_2\text{O}_5$: 520.2937; found 520.2937; elemental analysis calcd (%) for $\text{C}_{26}\text{H}_{34}\text{N}_2\text{O}_6$ (470.6): C 71.51, H 7.74, N 5.38; found C 71.51, H 7.42, N 5.40.

Fmoc-(2*S*)-(3-*Ncp*)*Ala*-(β Me)*Phe*-*Ile*-OMOM (**185a**): The compound **183a** (0.370 g, 0.786 mmol) was hydrogenated on 10% Pd on charcoal (0.100 g) under ambient pressure of hydrogen in EtOAc (10 mL) with AcOH (0.048 g, 0.800 mmol) for 1 h. The mixture was then filtered, concentrated under reduced pressure, taken up with Et₂O (40 mL), washed with saturated aqueous NaHCO₃ (3 $\times$ 10 mL), brine (2 $\times$ 10 mL), dried, filtered and concentrated under reduced pressure to give deprotected dipeptide **184a** as a free base together with *cyclo*-[(β Me)*Phe*-*Ile*]. This mixture was directly used for the condensation with *N*-Fmoc protected amino acid **160** (0.320 g, 0.807 mmol) employing EDC (0.160 g, 0.835 mmol), HOAt (0.111 g, 0.821 mmol) and TMP (0.430 mL, 3.254 mmol) in CH₂Cl₂ (5 mL) according to GP 5. After 15 h, the reaction mixture was subjected usual aqueous work-up and the resulting crude product was first crystallized from CH₂Cl₂/hexanes and then purified by column chromatography (CHCl₃/MeOH = 60:1 (1% EtOH), R_f = 0.28) to give tripeptide **185a** (0.220 g, 39%) as a white solid. $[\alpha]_{20}^D$ – 15.3 (c = 0.40, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 0.82 (q, J = 7.3 Hz, 6 H, 5-H, 1'-H, *Ile*), 0.93–1.17 [m, 2 H, 4-H_a, *Ile*, 3'-H_a, (3-*Ncp*)*Ala*], 1.26–1.43 (m, 1 H, 4-H_b, *Ile*), 1.32 [d, J = 6.5 Hz, 3 H, 4-H, (β Me)*Phe*], 1.48–1.70 [m, 1 H, 3'-H_b, (3-*Ncp*)*Ala*], 1.70–1.84 [m, 2 H, 3-H, *Ile*, 1'-H, (3-*Ncp*)*Ala*], 1.84–1.99 [m, 2 H, 3-H, (3-*Ncp*)*Ala*], 3.22 [dq, J = 7.4 Hz, 1 H, 3-H,

(β Me)Phe], 3.40 (s, 3 H, OMe), 4.00–4.10 (m, 1 H, 2-H), 4.20 (dd, J = 6.5, 6.5 Hz, C-2), 4.26–4.44 [m, 3 H, 1'-H, 2'-H_a, Fmoc, 2'-H, (3-Ncp)Ala], 4.49 (dd, J = 10.6, 6.9 Hz, 1 H, 2'-H, Fmoc), 4.62 (dd, J = 8.5, 8.5 Hz, 1 H, C-2), 5.09 (d, J = 5.9 Hz, 1 H, H_a, OCH₂O), 5.20 (d, J = 5.9 Hz, 1 H, H_b, OCH₂O), 5.63 (d, J = 8.0 Hz, 1 H, NH), 6.17 (d, J = 8.0 Hz, NH), 6.95 (d, J = 8.5 Hz, 1 H, NH), 7.14–7.31 (m, 7 H, Ar-H), 7.40 (dd, J = 7.5, 7.5 Hz, 2 H, Ar-H), 7.58 (dd, J = 7.3, 5.0 Hz, 2 H, Ar-H), 7.77 (d, J = 7 Hz, 2 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 11.4 (+, C-5, Ile), 15.0 (+, C-1', Ile), 17.1 [+ , C-4, (β Me)Phe], 17.9 [–, C-3', (3-Ncp)Ala], 22.1 [+ , C-1', (3-Ncp)Ala], 24.9 (–, C-4, Ile), 34.0 [–, C-3, (3-Ncp)Ala], 37.7 (+, C-3, Ile), 41.9 [+ , C-3, (β Me)Phe], 46.9 (+, C-1', Fmoc), 54.0 [+ , C-2, (β Me)Phe], 56.3 [+ , C-2, Ile], 57.7 (+, OMe), 58.7 [+ , C-2, (3-Ncp)Ala], 59.0 [+ , C-2', (3-Ncp)Ala], 67.1 (+, C-2', Fmoc), 90.9 (–, OCH₂O), 119.9, 124.9, 126.9, 127.0, 127.6, 127.7, 128.4 (+, Ar-C), 141.1, 141.2 (C_{quat}, Ar-C, Fmoc), 141.6 [C_{quat}, Ar-C, (β Me)Phe], 143.5, 143.7 (C_{quat}, Ar-C, Fmoc), 156.0 (C_{quat}, NCO₂), 170.2, 170.6, 171.2 (C_{quat}, C-1); IR (KBr): ν (tilde) = 3330 cm^{–1}, 3275, 3067, 2966, 1718, 1643, 1542, 1451, 1368, 1227, 1163, 1139, 1091; MS (EI, 70 eV), m/z (%) = 715 (0.04) [M⁺], 518 (3), 429 (13), 321 (5), 196 (20), 178 (100), 165 (54), 199 (6), 134 (16), 86 (23), 45 (20) [CHO₂⁺]; MS (ESI): positive mode, m/z (%) = 737 (30) [M + Na⁺]; negative mode, m/z (%) = 749 (40) [M + Cl[–]].

Fmoc-(3-Ncp)Ala-(β Me)Phe-Ile-ODCPM (**185b**): Dipeptide **183b** (0.35 g, 0.67 mmol) was taken up with EtOAc (10 mL) and hydrogenated over 10% Pd/C (0.15 g) under ambient pressure of hydrogen for 2 h. The reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure to give deprotected dipeptide **184b**, which was directly used for the coupling with **160** (274 mg, 0.69 mmol), using EDC (137 mg, 0.72 mmol), HOAt (96 mg, 0.71 mmol) and TMP (0.25 mL, 2.02 mmol) according to GP 5 to give compound **185b** (405 mg, 79%) as a colorless solid after 2 recrystallizations from THF/hexanes 1:1. R_f = 0.52,

EtOAc/hexanes 2:3; m.p 151–155 °C, $[\alpha]_{20}^D - 3.8$ ($c = 0.26$, CHCl_3); ^1H NMR (250 MHz, CDCl_3): $\delta = 0.20\text{--}0.38$ (m, 4 H, 2'-H, *DCPM*), 0.37–0.49 (m, 2 H, 2'-H, *DCPM*), 0.49–0.62 (m, 2 H, 2'-H, *DCPM*), 0.80 (d, $J = 7.0$ Hz, 3 H, 1'-H, *Ile*), 0.86 (t, $J = 7.5$ Hz, 3 H, 5-H, *Ile*), 0.94–1.20 [m, 4 H, 4-H_a, *Ile*, 3'-H_a, 3-(*Ncp*)*Ala*, 1'-H, *DCPM*], 1.33–1.49 [m, 1 H, 3'-H_b, 3-(*Ncp*)*Ala*], 1.34 [d, $J = 7.0$ Hz, 3 H, 4-H, (β -*Me*)*Phe*], 1.50–1.68 (m, 1 H, 4-H_b, *Ile*), 1.71–1.85 [m, 2 H, 3-H, *Ile*, 1'-H, 3-(*Ncp*)*Ala*], 1.85–2.05 [m, 2 H, 3-H, 3-(*Ncp*)*Ala*], 3.22 [dq, $J = 7.0$ Hz, 1 H, 3-H, (β -*Me*)*Phe*], 3.79 (t, $J = 8.6$ Hz, 1 H, 1-H, *DCPM*), 4.01–4.1 (m, 1 H, 2-H), 4.21 (dd, $J = 6.5$ Hz, 1 H, 2-H), 4.26–4.43 [m, 3 H, 2'-H, 3-(*Ncp*)*Ala*, 1'-H, 2'-H_a, *Fmoc*], 4.49 (dd, $J = 10.3, 7.0$ Hz, 1 H, 2'-H_b, *Fmoc*), 4.61 (dd, $J = 5.5$ Hz, 1 H, 2-H), 5.58 (d, $J = 8.3$ Hz, 1 H, NH), 6.14 (d, $J = 7.8$ Hz, 1 H, NH), 6.84 (d, $J = 8.0$ Hz, 1 H, NH), 7.12–7.31 (m, 5 H, Ar-H), 7.33 (d, $J = 7.8$ Hz, 2 H, Ar-H), 7.40 (dd, $J = 7.3, 7.3$ Hz, 2 H, Ar-H), 7.58 (d, $J = 7.0$ Hz, 2 H, Ar-H), 7.77 (d, $J = 7.3$ Hz, 2 H, Ar-H); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 2.4, 2.7$ (–, C-2', *DCPM*), 11.4 (+, C-5, *Ile*), 14.1, 14.4 (+, C-1', *DCPM*), 14.8 (+, C-1', *Ile*), 16.7 [+ , C-4, (β -*Me*)*Phe*], 17.7 [–, C-3', 3-(*Ncp*)*Ala*], 21.97 [+ , C-1', 3-(*Ncp*)*Ala*], 24.9 (–, C-4, *Ile*), 33.9 [–, C-3, 3-(*Ncp*)*Ala*], 38.1 (+, C-3, *Ile*), 41.7 [+ , C-3, (β -*Me*)*Phe*], 46.8 (+, C-1', *Fmoc*), 53.8 (+, C-2), 56.2 (+, C-2), 58.5 (+, C-2), 58.8 [+ , C-2', 3-(*Ncp*)*Ala*], 67.0 (–, C-2', *Fmoc*), 83.1 (+, C-1, *DCPM*), 119.7, 124.8, 126.6, 127.4 ($\times 2$), 128.2 ($\times 2$), 140.96, 141.49 (C_{quat}, Ar-C, *Fmoc*), 141.5 [C_{quat}, Ar-C, (β -*Me*)*Phe*], 143.5, 143.6 (C_{quat}, Ar-C, *Fmoc*), 155.9 (C_{quat}, NCO₂), 169.8, 170.5, 171.0 (C_{quat}, C-1); IR (KBr): $\nu = 2966\text{ cm}^{-1}$, 1717, 1645, 1543, 1451, 1368, 1147; MS (ESI): positive mode, m/z (%) = 787 (70) [$\text{M} + \text{Na}^+$]; negative mode, m/z (%) = 799 (38) [$\text{M} + \text{Cl}^-$]; elemental analysis calcd (%) for $\text{C}_{44}\text{H}_{52}\text{N}_4\text{O}_8$ (764.9): C 69.09, H 6.85, N 7.32; found C 69.06, H 6.79, N 7.19.

*Fmoc-(β Me)*Phe*-(3-*Ncp*)*Ala*-(β Me)*Phe*-*Ile*-OMOM (188a)*: Tripeptide **185a** (126 mg, 0.29 mmol) was deprotected according to GP 4 and then directly coupled with **146** (126 mg, 0.31 mmol) according to GP 5 using EDC (6 mg, 0.31 mmol), HOAt (42 mg, 0.31 mmol) and TMP

(106 mg, 0.88 mmol) in CH₂Cl₂/MeCN 2:1 (3 mL) to give **188a** (195 mg, 78%) as a colorless solid after recrystallization from CHCl₃/hexanes. *R*_f = 0.27, CHCl₃/MeOH 70:1 (1% EtOH); m.p. 209–212 °C (decomp.), $[\alpha]_{20}^D - 40.2$ (*c* = 0.3, THF); ¹H NMR (250 MHz, [D₈]THF): δ = 0.78 (d, *J* = 7.0 Hz, 3 H, 1'-H, *Ile*), 0.80 (t, *J* = 7.0 Hz, 3 H, 5-H, *Ile*), 0.94–1.17 [m, 2 H, 4-H_a, *Ile*, 3'-H_a, (3-*Ncp*)*Ala*], 1.20 [d, *J* = 6.8 Hz, 3 H, 4-H, (β-*Me*)*Phe*], 1.33 [d, *J* = 7.0 Hz, 3 H, 4-H, (β-*Me*)*Phe*], 1.34–1.54 [m, 4 H, 3-H, 4-H_a, *Ile*, 1'-H, 3'-H_b, (3-*Ncp*)*Ala*], 1.59–1.87 [m, 2 H, 3-H, (3-*Ncp*)*Ala*], 3.10 [dq, *J* = 10.3, 7.1 Hz, 1 H, 3-H, (β-*Me*)*Phe*], 3.21 [dq, *J* = 7.5, 7.5 Hz, 1 H, 3-H, (β-*Me*)*Phe*], 3.31 (s, 3 H, OMe), 3.95 [ddd, *J* = 6.8, 3.3, 3.3 Hz, 1 H, 2'-H, (3-*Ncp*)*Ala*], 4.13–4.40 (m, 5 H, 2 × 2-H, 1'-H, 2'-H, *Fmoc*), 4.46 [ddd, *J* = 3.3, 3.3, 3.3 Hz, 1 H, 2-H, (3-*Ncp*)*Ala*], 4.55 (dd, *J* = 8.6, 8.6 Hz, 1 H, 2-H), 5.03 (d, *J* = 5.6 Hz, 1 H, H_a, OCH₂O), 5.11 (d, *J* = 5.6 Hz, 1 H, H_b, OCH₂O), 6.97–7.46 (m, 16 H, 4 × NH, 12 × Ar-H), 7.54 (dd, *J* = 10.9, 8.9 Hz, 2 H, Ar-H), 7.66 (dd, *J* = 9.9, 7.6 Hz, 2 H, Ar-H), 7.77 (d, *J* = 7.5 Hz, 2 H, Ar-H); ¹³C NMR (62.9 MHz, [D₈]THF): δ = 11.9 (+, C-5, *Ile*), 15.9 (+, C-1', *Ile*), 17.9 [+ , C-4, (β-*Me*)*Phe*], 18.6 [+ , C-4, (β-*Me*)*Phe*], 18.8 [–, C-3', (3-*Ncp*)*Ala*], 23.1 [+ , C-1', (3-*Ncp*)*Ala*], 26.0 (–, C-4, *Ile*), 34.7 [–, C-3, (3-*Ncp*)*Ala*], 38.6 (+, C-3, *Ile*), 42.7 [+ , C-3, (β-*Me*)*Phe*], 44.0 [+ , C-3, (β-*Me*)*Phe*], 48.4 (+, C-1', *Fmoc*), 52.7 (+, C-2), 57.3 (+, C-2), 57.9 (+, OMe), 59.6 (+, C-2), 60.1 [+ , C-2', (3-*Ncp*)*Ala*], 62.1 (+, C-2), 67.6 (–, C-2', *Fmoc*), 91.4 (–, OCH₂O), 126.3, 126.3, 127.4, 127.7, 128.0, 128.5, 128.9, 129.1, 129.2, 129.3 (+, Ar-C), 142.4, 142.4 (C_{quat}, Ar-C, *Fmoc*), 143.9 [C_{quat}, Ar-C, (β-*Me*)*Phe*], 144.2 [C_{quat}, Ar-C, (β-*Me*)*Phe*], 145.3, 145.5 (C_{quat}, Ar-C, *Fmoc*), 157.7 (C_{quat}, NCO₂), 171.3, 171.4, 171.6, 172.1 (C_{quat}, C-1); IR (KBr): ν = 3300 cm^{–1}, 2967, 1708, 1668, 1637, 1543, 1370, 1247; MS (ESI): positive mode, *m/z* (%) = 898 (100) [M + Na⁺]; elemental analysis calcd (%) for C₄₉H₅₇N₅O₁₀ (876.0): C 67.18, H 6.56, N 7.99; found C 67.15, H 6.46, N 8.06.

Fmoc-(β*Me*)*Phe*-(2*S*)-(3-*Ncp*)*Ala*-(β*Me*)*Phe*-*Ile*-ODCPM (**188b**): The tripeptide **185b** (0.420 g, 0.549 mmol) was deprotected according to GP 4 and the resultant product **186b** was then directly

coupled with **146** (0.242 g, 0.603 mmol) according to GP 5 using EDC (0.114 g, 0.595 mmol), HOAt (0.080 g, 0.592 mmol) and TMP (0.200 g, 1.650 mmol) in CH₂Cl₂ (3 mL). After 1 h, a precipitate fell down from the reaction mixture and anhydrous DMF (2 mL) was added to obtain homogeneous solution. After 15 h the reaction mixture was concentrated under reduced pressure. The resulting solid was washed with water (100 mL), 3% aqueous NaHCO₃ (100 mL), water (100 mL), Et₂O (100 mL), pentane (50 mL), dried at 0.5 Torr and then crystallized twice from THF/hexanes to give **188b** (0.430 g, 85%) as an off-white solid. $R_f = 0.29$, CHCl₃/MeOH 70:1; m.p 210–215 °C (decomp.), $[\alpha]_{20}^D - 26.3$ ($c = 0.32$, THF); ¹H NMR (250 MHz, [D₈]THF): $\delta = 0.22$ – 0.57 (m, 7 H, 2'-H, *DCPM*), 0.67 – 0.90 (m, 2 H, 1'-H, 2'-H, *DCPM*), 0.78 – 0.86 (m, 1 H, 1'-H, *DCPM*), 0.81 (d, $J = 6.5$ Hz, 3 H, 1'-H, *Ile*), 0.83 (t, $J = 7.3$ Hz, 3 H, 5-H, *Ile*), 0.92 – 1.19 [m, 4 H, 4-H, *Ile*, 3'-H, (3-*Ncp*)*Ala*], 1.20 [d, $J = 7.3$ Hz, 3 H, 4-H, (β *Me*)*Phe*], 1.32 [d, $J = 7.0$ Hz, 3 H, 4-H, (β *Me*)*Phe*], 1.30 – 1.50 [m, 4 H, 3-H, *Ile*, 1'-H, 3-H, (3-*Ncp*)*Ala*], 3.11 [dq, $J = 9.6$, 7.2 Hz, 1 H, 3-H, (β *Me*)*Phe*], 3.24 [dq, $J = 7.4$, 7.4 Hz, 1 H, 3-H, (β *Me*)*Phe*], 3.81 (t, $J = 8.5$ Hz, 1 H, 1-H, *DCPM*), 3.94 [ddd, $J = 6.8$, 3.3 , 3.3 Hz, 1 H, 2'-H, (3-*Ncp*)*Ala*], 4.13 – 4.41 (m, 5 H, 2 × 2-H, 1'-H, 2'-H, *Fmoc*), 4.41 – 4.51 (m, 1 H, 2-H), 4.61 (dd, $J = 8.5$, 7.8 Hz, 1 H, 2-H), 6.97 – 7.40 (m, 16 H, 2 × NH, 14 × Ar-H), 7.48 (d, $J = 8.5$ Hz, 1 H, NH), 7.58 (d, $J = 8.3$ Hz, 1 H, NH), 7.66 (dd, $J = 8.1$ Hz, 2 H, Ar-H), 7.77 (d, $J = 7.8$ Hz, 2 H, Ar-H); ¹³C NMR (62.9 MHz, [D₈]THF): $\delta = 3.3$, 3.5 , 3.6 , 3.7 (–, C-2', *DCPM*), 12.2 (+, C-5, *Ile*), 15.6 , 15.9 (+, C-1', *DCPM*), 16.1 (+, C-1', *Ile*), 17.7 [+ , C-4, (β *Me*)*Phe*], 18.7 [+ , C-4, (β *Me*)*Phe*], 18.9 [–, C-3', (3-*Ncp*)*Ala*], 23.2 [+ , C-1', (3-*Ncp*)*Ala*], 26.2 (–, C-4, *Ile*), 35.0 [–, C-3, (3-*Ncp*)*Ala*], 39.1 (+, C-3, *Ile*), 42.8 [+ , C-3, (β *Me*)*Phe*], 44.1 [+ , C-3, (β *Me*)*Phe*], 48.5 (+, C-1', *Fmoc*), 52.8 (+, C-2), 57.4 (+, C-2), 59.3 (+, C-2), 60.2 [+ , C-2', (3-*Ncp*)*Ala*], 62.1 (+, C-2), 67.8 (–, C-2', *Fmoc*), 83.4 (+, C-1, *DCPM*), 126.4 (× 2), 127.5 , 127.7 , 128.1 , 128.6 , 129.1 , 129.1 , 129.3 , 129.4 (+, Ar-C), 142.5 (C_{quat}, Ar-C, *Fmoc*), 144.1 [C_{quat}, Ar-C, (β *Me*)*Phe*], 144.4 [C_{quat}, Ar-C, (β *Me*)*Phe*], 145.4 , 145.6 (C_{quat}, Ar-C, *Fmoc*), 157.8 (C_{quat}, NCO₂), 171.1 , 171.5 , 171.9 , 172.1 (C_{quat}, C-1); IR (KBr): $\nu = 3250$ cm^{–1},

3065, 2967, 2933, 1709, 1665, 1636, 1542, 1451, 1369, 1246; MS (ESI): positive mode, m/z (%) = 949 (100) $[M + Na^+]$; elemental analysis calcd (%) for $C_{54}H_{63}N_5O_9$ (926.1): C 70.03, H 6.86, N 7.56; found C 69.94, H 6.68, N 7.32.

MeZ-a-Thr[(4-PE)Pro]-OAll (**187**): Ester **187** (0.588 g, 83%) was prepared from protected threonine **176** (0.400 g, 1.30 mmol) and *N*-Boc protected 4-(*Z*)-propenylproline **16** (0.340 g, 1.33 mmol), 4-pyrrolidinopyridine (0.250 g, 1.69 mmol) and EDC (0.324 g, 1.69 mmol) in CH_2Cl_2 (7 mL) according to GP 6 after column chromatography (EtOAc/hexanes 1:6) as a turbid oil. R_f = 0.43 EtOAc/hexanes 1:3; $[\alpha]_{20}^D$ – 35.4 (c = 0.28, $CHCl_3$); 1H NMR (250 MHz, $CDCl_3$): δ = 1.32, 1.34 (2 $\times$ d, J = 6.5 Hz, 3 H, 4-H, *a*-Thr), 1.36, 1.39 [2 $\times$ s, 9 H, $C(CH_3)_3$], 1.64, 1.64 [2 $\times$ dd, J = 1.5, 7.0 Hz, 3 H, 3'-H, (4-PE)Pro], 1.63–1.81 [m, 1 H, 3-H_a, (4-PE)Pro], 2.21–2.46 [m, 1 H, 3-H_b, (4-PE)Pro], 2.33, 2.35 (2 $\times$ s, 3 H, 1'-H, *MeZ*), 2.93–3.19 [m, 2 H, 4-H, 5-H_a, (4-PE)Pro], 3.51–3.67, 3.69–3.83 [2 $\times$ m, 1 H, 5-H_b, (4-PE)Pro], 4.10–4.27 [m, 1 H, 2-H, (4-PE)Pro], 4.48–4.65 (m, 1 H, 2-H, *a*-Thr), 4.68 (d, J = 5.2 Hz, 2 H, 1-H, *All*), 4.97–5.13 (m, 2 H, Bzl-H, *MeZ*), 5.17–5.43 [m, 4 H, 3-H, *All*, 3-H, *a*-Thr, 1'-H, (4-PE)Pro], 5.54 [dq, J = 10.2, 7.0 Hz, 1 H, 2'-H, (4-PE)Pro], 5.80–6.01 (m, 1 H, 2-H, *All*), 6.42 (d, J = 9.2 Hz, 1 H, NH), 7.13, 7.17 (2 $\times$ d, J = 7.9 Hz, 2 H, Ar-H), 7.23, 7.25 (d, J = 7.9 Hz, 2 H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 12.8 [+ , C-3', (4-PE)Pro], 15.8, 16.3 (+ , C-4, *a*-Thr), 20.8 (+, C-1', *MeZ*), 27.9 [+ , $C(CH_3)_3$], 35.3, 36.1 [+ , C-4, (4-PE)Pro], 35.8, 36.9 [– , C-3, (4-PE)Pro], 51.1, 51.3 [– , C-5, (4-PE)Pro], 57.0, 57.4 [+ , C-2, (4-PE)Pro], 59.0, 59.1 (+ , C-2, *a*-Thr), 65.5, 66.0 (– , C-1, *All*), 66.4, 67.0 (– , Bzl-C), 70.8, 70.8 (+ , C-3), 79.6, 79.7 [C_{quat} , $C(CH_3)_3$] 118.2, 118.9 (– , C-3, *All*), 126.3, 126.3 (+ , Ar-C), 127.9 (+ , Ar-C), 128.7, 128.8 [+ , C-2', (4-PE)Pro], 129.0, 129.1 [+ , C-1', (4-PE)Pro], 130.9, 131.2 (+ , C-2, *All*), 132.7, 133.1 (C_{quat} , Ar-C), 137.2, 137.6 (C_{quat} , Ar-C), 153.0, 153.5 (C_{quat} , NCO_2 , *Boc*), 155.4, 155.9 (C_{quat} , NCO_2 , *MeZ*), 168.4, 168.4 (C_{quat} , C-1, *a*-Thr), 171.6, 171.9 [C_{quat} , C-1, (4-PE)Pro]; IR (KBr): ν (tilde) = 3009 cm^{-1} , 2977, 2932, 2871, 1747, 1703, 1520, 1478, 1453, 1399, 1367, 1253, 1161; MS (EI, 70 eV), m/z (%) = 544 (2) $[M^+]$, 488 (6) $[M^+$

– C₄H₈], 444 (5) [M⁺ – C₅H₈O₂], 339 (26), 210 (11), 154 (100) [C₈H₁₂NO₂⁺], 110 (83) [C₇H₁₂N⁺], 105 (100) [C₈H₉⁺], 57 (49) [C₄H₉⁺], 41 (15) [C₃H₅⁺]; HRMS (EI): calcd for C₂₉H₄₀N₂O₈: 544.2785; found 544.2785; elemental analysis calcd (%) for C₂₉H₄₀N₂O₈ (544.7): C 63.95, H 7.40, N 5.14; found C 63.99, H 7.32, N 4.99.

MeZ-a-Thr[(4-*PE*)*Pro*]-*OH* (**189**): Pd(P(Ph)₃)₄ (0.034 g, 2.94 μmol) was added to a vigorously stirred solution of ester **187** (0.108 g, 0.198 mmol) and *N*-methyl aniline (0.08 mL, 0.738 mmol) in DME (3.5 mL) and the resulting suspension was carefully heated with heat-gun to obtain a homogeneous solution. The mixture was left to stir at 20 °C for 45 min, was then again carefully heated with heat-gun till catalyst dissolved and stirred for a further 15 min. The reaction mixture was then diluted with Et₂O (40 mL), washed with 1 M NaHSO₄ (3 × 10 mL), water (10 × 10 mL), brine (2 × 10 mL), dried, filtered and concentrated under reduced pressure. The residue was taken up with Et₂O/hexanes 1:2, filtered, concentrated and purified by column chromatography (EtOAc/hexanes 1:2 (1.5% AcOH), *R*_f = 0.34) to give **189** (0.121 g, 90%) as an yellow oil. $[\alpha]_{20}^D$ – 71.7 (*c* = 0.32, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 1.35, 1.40 [2 × s, 9 H, C(CH₃)₃], 1.41, 1.43 (2 × d, *J* = 5.7 Hz, 3 H, 4-H, *a*-*Thr*), 1.63, 1.65 [2 × dd, *J* = 1.5, 6.7 Hz, 3 H, 3'-H, (4-*PE*)*Pro*], 1.67–1.86 [m, 1 H, 3-H_a, (4-*PE*)*Pro*], 2.24–2.49 [m, 1 H, 3-H_b, (4-*PE*)*Pro*], 2.33, 2.34 (2 × s, 3 H, 1'-H, *MeZ*), 2.91–3.20 [m, 2 H, 4-H, 5-H_a, (4-*PE*)*Pro*], 3.40–4.30 (br, 1 H, CO₂H), 3.61, 3.73 [2 × ddd, *J* = 2.7 Hz, 1 H, 5-H_b, (4-*PE*)*Pro*], 4.11–4.29 [m, 1 H, 2-H, (4-*PE*)*Pro*], 4.51 (dd, *J* = 8.6, 3.5 Hz, 1 H, 2-H, *a*-*Thr*), 5.05 (s, 2 H, Bzl-H, *MeZ*), 5.15–5.31 [m, 1 H, 1'-H, (4-*PE*)*Pro*], 5.31–5.44 (m, 1 H, 3-H, *a*-*Thr*), 5.45–5.60 [m, 1 H, 2'-H, (4-*PE*)*Pro*], 5.63 (d, *J* = 7.6 Hz, 0.4 H, NH), 6.46 (d, *J* = 9.2 Hz, 0.6 H, NH), 7.13, 7.17 (2 × d, *J* = 7.9 Hz, 2 H, Ar-H), 7.21, 7.22 (d, *J* = 7.9 Hz, 2 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 13.0 [+ , C-3', (4-*PE*)*Pro*], 15.8, 16.3 (+, C-4, *a*-*Thr*), 21.0 (+, C-1', *MeZ*), 28.1 [+ , C(CH₃)₃], 35.5, 36.2 [+ , C-4, (4-*PE*)*Pro*], 36.0, 37.0 [–, C-3, (4-*PE*)*Pro*], 51.3, 51.6 [–, C-5, (4-*PE*)*Pro*], 57.0, 57.5 [+ , C-2,

(*4-PE*)*Pro*], 59.1, 59.3 (+, C-2, *a-Thr*), 66.8, 67.0 (–, Bzl-C), 71.2, 71.4 (+, C-3), 80.5, 80.6 [C_{quat} , C(CH₃)₃] 126.5, 126.5 (+, Ar-C), 128.1 (+, Ar-C), 128.1 [+ , C-2', (*4-PE*)*Pro*], 128.9, 129.0 [+ , C-1', (*4-PE*)*Pro*], 132.8, 133.0 (C_{quat} , Ar-C), 137.6, 137.9 (C_{quat} , Ar-C), 153.7, 154.2 (C_{quat} , NCO₂, *Boc*), 155.8, 156.4 (C_{quat} , NCO₂, *MeZ*), 171.2, 171.7 (C_{quat} , C-1), 172.0, 172.2 (C_{quat} , C-1); IR (KBr): $\nu(\text{tilde}) = 3700\text{--}2750\text{ cm}^{-1}$, 2979, 1729, 1520, 1404, 1369, 1257, 1162, 1061; MS (EI, 70 eV), m/z (%) = 504 (2) [M^+], 448 (7) [$M^+ - C_4H_8$], 404 (27) [$M^+ - C_5H_8O_2$], 299 (21), 210 (14), 154 (100) [$C_8H_{12}NO_2^+$], 110 (92) [$C_7H_{12}N^+$], 105 (100) [$C_8H_9^+$], 57 (50) [$C_4H_9^+$], 41 (16) [$C_3H_5^+$]; HRMS (EI): calcd for C₂₆H₃₆N₂O₈: 504.2472; found 504.2472.

MeZ-a-Thr[*Boc*-(*4-PE*)*Pro*]-(β *Me*)*Phe*-(3-*Ncp*)*Ala*-(β *Me*)*Phe*-*Ile*-OMOM (**191a**): Tetrapeptide **188a** (180 mg, 0.21 mmol) was deprotected according to GP 4 in MeCN (2 mL), taken up with anhydrous CH₂Cl₂ (5 mL), dipeptide acid **189** (0.114 g, 0.23 mmol), HATU (96 mg, 0.25 mmol) and HOAt (31 mg, 0.23 mmol) were added and the reaction mixture was cooled to 4 °C. After this, a solution of DIEA (29 mg, 0.22 mmol) and TMP (75 mg, 0.62 mmol) in CH₂Cl₂ (2 mL) were added at the same temperature within 5 min. The temperature was allowed to reach 20 °C, and stirring continued for an additional 15 h. After aqueous work-up according to GP 5 and two recrystallizations from EtOAc/hexanes 1:2, depsipeptide **191a** (185 mg, 79%) was obtained as a colorless powder. $R_f = 0.46$, EtOAc/hexanes 1:1; m.p. 125–127 °C, $[\alpha]_{20}^D 0$ ($c = 0.21$, CHCl₃), $[\alpha]_{20}^D -29.0$ ($c = 0.2$, THF); ¹H NMR (250 MHz, CDCl₃): $\delta = 0.76$ (d, $J = 6.8$ Hz, 3 H, 1'-H, *Ile*), 0.89 (t, $J = 7.3$ Hz, 3 H, 5-H, *Ile*), 0.95–1.11 [m, 2 H, 4-H_a, *Ile*, 3'-H_a, (3-*Ncp*)*Ala*], 1.11–1.60 [m, 5 H, 3-H, 4-H_b, *Ile*, 3-H_a, 1'-H, 3'-H_a, (3-*Ncp*)*Ala*], 1.24 [d, $J = 7.5$ Hz, 3 H, (β -*Me*)*Phe*], 1.27 [d, $J = 7.5$ Hz, 3 H, 4-H, (β -*Me*)*Phe*], 1.36 (s, 9 H, C(CH₃)₃), 1.43 (d, $J = 6.5$ Hz, 3 H, 4-H, *a-Thr*), 1.68 [dd, $J = 6.6, 1.6$ Hz, 3 H, 3'-H, (*4-PE*)*Pro*], 1.76 [dd, $J = 11.6, 11.6$ Hz, 1 H, 3-H_a, (*4-PE*)*Pro*], 1.87–2.02 [m, 1 H, 3-H_b, (3-*Ncp*)*Ala*], 2.32 (s, 3 H, 1'-H, *MeZ*), 2.43 [ddd, $J = 12.3, 6.5, 6.5$ Hz, 1 H, 3-H_b, (*4-PE*)*Pro*], 2.99–3.20 [m, 1 H, 4-H, (*4-PE*)*Pro*], 3.11 [dd, $J = 9.8,$

9.8 Hz, 1 H, 5-H_a, (4-PE)Pro], 3.21–3.38 [m, 2 H, 3-H, (β-Me)Phe, 5-H_b, (4-PE)Pro], 3.49 (s, 3 H, OMe), 3.70 (dd, *J* = 9.8, 7.0 Hz, 1 H, 2-H), 3.86 [dq, *J* = 3.9, 6.5 Hz, 1 H, 3-H, (β-Me)Phe], 4.14 (dd, *J* = 11.1, 6.3 Hz, 1 H, 2-H), 4.34–4.55 (m, 3 H, 2-H, 2'-H, (3-Ncp)Ala), 4.59 (dd, *J* = 9.9, 5.1 Hz, 1 H, 2-H), 4.67 (dd, *J* = 10.4, 10.4 Hz, 1 H, 2-H), 5.03 (dd, *J* = 2.9, 2.9 Hz, 2 H, Bzl-H), 5.27 [ddq, *J* = 10.5, 10.5, 1.6 Hz, 1 H, 1'-H, (4-PE)Pro], 5.46–5.60 [m, 2 H, 3-H, *a*-Thr, 2'-H, (4-PE)Pro], 5.61 (d, *J* = 6.3 Hz, 1 H, H_b, OCH₂O), 6.57 (d, *J* = 8.3 Hz, 1 H, NH), 6.99 (d, *J* = 6.0 Hz, 1 H, NH), 7.02 (d, *J* = 6.0 Hz, 1 H, NH), 7.07–7.38 (m, 14 H, Ar-H), 7.57 (d, *J* = 10.0 Hz, 1 H, NH), 7.95 (d, *J* = 5.8 Hz, 1 H, NH); The peak of Bzl-H masked absorption for H_a of OCH₂O; ¹³C NMR (62.9 MHz, CDCl₃): δ = 11.5 (+, C-5, *Ile*), 13.0 [+ , C-3', (4-PE)Pro], 15.6 (+, C-1', *Ile*), 17.5 [+ , C-4, (β-Me)Phe], 18.2 [–, C-3', (3-Ncp)Ala], 18.4 [+ , C-4, (β-Me)Phe], 19.2 (+, C-4, *a*-Thr), 20.9 (+, C-1', *MeZ*), 21.7 [+ , C-1', (3-Ncp)Ala], 24.9 (–, C-4, *Ile*), 28.1 [+ , C(CH₃)₃], 31.5 [–, C-3, (3-Ncp)Ala], 36.1 [–, C-3, (4-PE)Pro], 36.1 [+ , C-4, (4-PE)Pro], 36.9 (+, C-3, *Ile*), 40.4 [+ , C-3, (β-Me)Phe], 41.8 [+ , C-3, (β-Me)Phe], 50.8 (+, C-2), 51.9 [–, C-5, (4-PE)Pro], 56.1 (+, C-2), 57.5 (+, OMe), 59.1 (+, C-2), 59.3 (+, C-2), 60.7 (+, C-2), 61.2 (+, C-2), 61.6 [+ , C-2', (3-Ncp)Ala], 66.7 (–, Bzl-C), 70.4 (+, C-3, *a*-Thr), 80.7 [C_{quat}, C(CH₃)₃], 90.9 (–, OCH₂O), 126.3, 126.7, 127.5, 127.7, 128.2, 128.3, 128.4, 128.5, 128.7, 128.8 [+ , Ar-C and C-1', C-2', (4-PE)Pro], 133.0, 137.6, 141.6, 141.8 (C_{quat}, Ar-C), 154.5, 154.6 (C_{quat}, NCO₂), 170.31, 170.33, 170.8, 171.1, 173.0, 173.8 (C_{quat}, C-1); IR (KBr): ν = 3062 cm^{–1}, 2972, 2934, 1729, 1648, 1543, 1454, 1368, 1254, 1162, 1091; MS (ESI): positive mode, *m/z* (%) = 1163 (100) [M + Na⁺]; negative mode, *m/z* (%) = 1138 (50) [M – H⁺], 1175 (50) [M + Cl[–]]; elemental analysis calcd (%) for C₆₀H₈₁N₇O₁₅ (1140.4): C 63.20, H 7.16, N 8.60; found C 63.09, H 7.27, N 8.46.

N-MeZ protected cyclohexadepsipeptide (**171c**): To the hexadepsipeptide **191a** (0.188 g, 0.165 mmol) 2 M HCl in EtOAc (2 mL) was added, the reaction mixture was stirred for 45 min at 20 °C in the dark place and then was concentrated under reduced pressure at 20 °C. The residue was triturated with dry Et₂O to give deprotected material as a white solid (0.160 g; MS (ESI):

positive mode, m/z (%) = 997 (100) $[M + H^+]$, 1019 (5) $[M + Na^+]$; negative mode, m/z (%) = 995 (50) $[M - H^+]$, which was taken up with anhydrous CH_2Cl_2 (1.5 l) and cyclized employing HATU (2×0.073 g, 2×0.192 mmol) and HOAt (2×0.022 g, 2×0.163 mmol) and solution of DIEA (2×0.062 g, 2×0.480 mmol) in CH_2Cl_2 (2×50 mL) according to GP 8 for 16 h. After this, the solvent was removed under reduced pressure, the residue was taken up with Et_2O (50 mL), and after usual aqueous work-up (GP 5), drying and filtration, the organic layer was concentrated under reduced pressure. The residue was purified first by column chromatography (acetone/hexanes 1:3, R_f = 0.17) and then by recrystallization (Et_2O /pentane) to give crude product (0.12 g), which was finally purified by preparative HPLC to give cyclodepsipeptide **171c** (86 mg, 53% on 2 steps) as a white solid. Preparative HPLC: isocratic, 75% MeCN in H_2O (0.1% TFA); analytical HPLC 1: column B, isocratic, 50% MeCN in H_2O (0.1% TFA) for 20 min, then gradient 50% $\rightarrow$ 99% MeCN (0.1% TFA) for 15 min, then isocratic, 99% MeCN in H_2O (0.1% TFA) for 10 min, flow rate = 0.5 mL/min, t_R = 34.93 min, purity > 98%; analytical HPLC 2: column B, gradient 40% $\rightarrow$ 70% MeCN (0.1% TFA) for 20 min, then gradient 70% $\rightarrow$ 99% MeCN (0.1% TFA) for 15 min, flow rate = 0.5 mL/min, t_R = 29.00 min, purity > 98%; $[\alpha]_{20}^D$ – 15.5 (c = 0.20, $CHCl_3$); 1H NMR (600 MHz, $CDCl_3$): δ = 0.64 [ddd, J = 14.4, 7.2, 7.2 Hz, 1 H, 3- H_a , (3-*Ncp*)*Ala*], 0.72 (d, J = 6.6 Hz, 3 H, 1'-H, *Ile*), 0.74 [ddd, J = 6.6, 6.6, 6.6 Hz, 1 H, 3'- H_a , (3-*Ncp*)*Ala*], 0.79 (t, J = 7.2 Hz, 3 H, 5-H, *Ile*), 1.04–1.12 (m, 1 H, 4- H_a , *Ile*), 1.23 [d, J = 6.6 Hz, 3 H, 4-H, (β -*Me*)*Phe*], 1.27–1.34 [m, 1 H, 1'-H, (3-*Ncp*)*Ala*], 1.37 [d, J = 6.6 Hz, 3 H, 4-H, (β -*Me*)*Phe*], 1.37–1.43 [m, 1 H, 3- H_b , (3-*Ncp*)*Ala*], 1.45–1.54 (m, 1 H, 4- H_b , *Ile*), 1.54–1.57 [m, 1 H, 3'- H_b , (3-*Ncp*)*Ala*], 1.57 (d, J = 6.6 Hz, 3 H, 4-H, *a*-*Thr*), 1.65 [dd, J = 6.6, 1.5 Hz, 3 H, 3'-H, (4-*PE*)-*Pro*], 1.66–1.76 [m, 2 H, 3-H, *Ile*, 3- H_a , (4-*PE*)*Pro*], 2.23 [ddd, J = 12.0, 5.4, 5.4 Hz, 1 H, 3- H_b , (4-*PE*)-*Pro*], 2.35 (s, 3 H, 1'-H, *MeZ*), 3.05 [dq, J = 6.6, 6.6 Hz, 1 H, 3-H, (β -*Me*)*Phe*], 3.15–3.28 [m, 2 H, 4-H, 5- H_a , (4-*PE*)-*Pro*], 3.54 [dq, J = 7.2, 6.6 Hz, 1 H, 3-H, (β -*Me*)*Phe*], 3.71 (dd, J = 6.0, 5.4 Hz, 1 H, 2-H), 3.76 [ddd, J = 6.6, 3.3, 3.3 Hz, 1 H, 2'-H,

(*3-Ncp*)*Ala*], 3.98 (dd, $J = 10.5, 6.3$ Hz, 1 H, 2-H), 4.01–4.08 [m, 1 H, 5-H_b, (*4-PE*)-*Pro*], 4.46–4.54 (m, 2 H, 2-H), 4.54 (dd, $J = 9.6, 9.6$ Hz, 1 H, 2-H), 4.69 (dd, $J = 8.4$ Hz, 1 H, 2-H), 5.03 (d, $J = 12.0$ Hz, 1 H, Bzl-H_a), 5.15 (d, $J = 12.0$ Hz, 1 H, Bzl-H_b), 5.19–5.25 [m, 1 H, 1'-H, (*4-PE*)-*Pro*], 5.39 (dq, $J = 1.8, 6.6$ Hz, 1 H, 3-H, *a-Thr*), 5.56 [dq, $J = 10.8, 6.6$ Hz, 1 H, 2'-H, (*4-PE*)-*Pro*], 6.21–6.37 (br, 2 H, NH), 6.44 (d, $J = 8.4$ Hz, 1 H, NH), 6.48 (d, $J = 9.6$ Hz, 1 H, NH), 7.06–7.18 (m, 4 H, Ar-H), 7.19–7.27 (m, 9 H, Ar-H, NH), 7.26–7.34 (m, 2 H, Ar-H), 7.48 (d, $J = 8.4$ Hz, 1 H, NH); ^{13}C NMR (150.8 MHz, CDCl_3): $\delta = 10.3$ (+, C-5, *Ile*), 13.3 [+ , C-3', (*4-PE*)-*Pro*], 14.6 [+ , C-1', *Ile*, C-4, (β -*Me*)*Phe*], 17.3 [–, C-3', (*3-Ncp*)*Ala*], 17.7 [+ , C-4, (β -*Me*)*Phe*], 18.4 (+, C-4, *a-Thr*), 21.2 (+, C-1', *MeZ*), 21.3 [+ , C-1', (*3-Ncp*)*Ala*], 24.7 (–, C-4, *Ile*), 32.0 [–, C-3, (*3-Ncp*)*Ala*], 35.4 [–, C-3, (*4-PE*)-*Pro*], 36.6 [+ , C-3, *Ile*, C-4, (*4-PE*)-*Pro*], 39.4 [+ , C-3, (β -*Me*)*Phe*], 44.5 [+ , C-3, (β -*Me*)*Phe*], 52.5 [–, C-5, (*4-PE*)-*Pro*], 53.3 (+, C-2), 54.6 (+, C-2), 58.6 (+, C-2), 59.0 [+ , C-2', (*3-Ncp*)*Ala*], 59.4 (+, C-2), 60.1 (+, C-2), 60.7 (+, C-2), 67.2 (–, Bzl-C), 72.6 (+, C-3, *a-Thr*), 127.1, 127.2, 127.5, 127.6, 128.3, 128.6, 128.8, 129.2 (+, Ar-C), 127.8 [+ , C-1', (*4-PE*)-*Pro*], 128.0 [+ , C-2', (*4-PE*)-*Pro*], 133.2, 137.9, 140.9, 142.6 (C_{quat} , Ar-C), 156.3 (C_{quat} , NCO_2), 169.0, 170.3, 170.6, 171.1, 171.4, 173.1 (C_{quat} , C-1); IR (KBr): $\nu = 2969$ cm^{-1} , 2936, 2878, 1730, 1637, 1541, 1515, 1452, 1370, 1179; MS (ESI): positive mode, m/z (%) = 1001 (100) [$\text{M} + \text{Na}^+$]; negative mode, m/z (%) = 977 (100) [$\text{M} - \text{H}^+$]; HRMS (ESI): calcd for [$\text{C}_{53}\text{H}_{67}\text{N}_7\text{O}_{11}\text{Na}^+$]: 1000.4792; found 1000.4792.

Boc-(S)-(3-Ncp)Ala-OFm (192): The ester **192** was obtained from *N*-protected aminoacid (2*S*,1'*R*,2'*R*)-**143** (0.213 g, 0.777 mmol) employing EDC (0.223 g, 1.163 mmol), 4-pyrrolidinopyridine (0.036 g, 0.242 mmol) and 9-fluorenylmethanol (0.152 g, 0.775 mmol) in CH_2Cl_2 (6 mL) according to GP 6 for 5 h. The mixture was then diluted with Et_2O (50 mL) and subjected to usual aqueous work-up. The organic layer was dried, filtered and concentrated under reduced pressure. The residue was crystallized from hexanes, taken up with Et_2O (4 mL) and filtered through a silica gel pad (1.5 cm) to give after concentration of the filtrate under reduced

pressure the desired product **192** (0.26 g, 74%) as a colorless solid. $R_f = 0.30$ EtOAc/hexanes 1:4; m.p 79–81 °C; $[\alpha]_{20}^D - 27.0$ ($c = 0.3$, CHCl₃); ¹H NMR (250 MHz, CDCl₃): $\delta = 1.14$ (ddd, $J = 6.8, 6.8, 5.3$ Hz, 1 H, 3'-H_a), 1.43 [s, 9 H, C(CH₃)₃], 1.43–1.60 (m, 1 H, 3'-H_b), 1.58–1.71 (m, 1 H, 1'-H), 1.72–1.92 (m, 2 H, 3-H), 3.90 (ddd, $J = 6.9, 3.5, 3.5$ Hz, 1 H, 2-H), 4.23 (t, $J = 6.0$ Hz, 1 H, 1''-H), 4.35 (ddd, $J = 6.8, 6.8, 6.8$ Hz, 1 H, 1'-H), 4.55 (dd, $J = 10.8, 6.0$ Hz, 1 H, 2''-H_a), 4.66 (dd, $J = 10.8, 6.0$ Hz, 1 H, 2''-H_b), 5.13 (d, $J = 8$ Hz, 1 H, NH), 7.26–7.48 (m, 4 H, Ar-H), 7.57 (d, $J = 7.4$ Hz, 2 H, Ar-H), 7.77 (d, $J = 7.5$ Hz, 2 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): $\delta = 17.5$ (–, C-3'), 21.8 (+, C-1'), 28.1 [+ , C(CH₃)₃], 33.6 (–, C-3), 46.6 (+, C-1''), 52.6 (+, C-2), 59.0 (+, C-2'), 66.9 (–, C-2''), 80.2 [C_{quat}, C(CH₃)₃], 119.9, 119.9 (+, Ar-C), 124.6, 124.7 (+, Ar-C), 127.1 (+, Ar-C), 127.9 (+, Ar-C), 141.2, 141.3 (C_{quat}, Ar-C), 143.0, 143.2 (C_{quat}, Ar-C), 155.0 (C_{quat}, NCO₂), 175.4 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 3420$ cm^{–1}, 3067, 2980, 2934, 1738, 1715, 1684, 1545, 1521, 1451, 1369, 1208, 1163; MS (EI, 70 eV), m/z (%) = 452 (3) [M⁺], 178 (100) [C₁₄H₁₀⁺], 129 (2) [C₅H₉N₂O₂⁺], 93 (3) [C₇H₇⁺], 57 (16) [C₄H₉⁺], 41 (5) [C₃H₅⁺]; HRMS (EI): calcd for C₂₅H₂₈N₂O₆: 452.1947; found 452.1947; elemental analysis calcd (%) for C₂₅H₂₈N₂O₆ (452.5): C 66.36, H 6.24, N 6.19; found C 66.11, H 5.99, N 6.02.

Chpca(MOM)-(2S)-(3-Ncp)Ala-OFm (**193**): Ester **192** (0.223 g, 0.493 mmol) was deprotected with 4 M HCl in EtOAc (5 mL) for 90 min to give HCl·H-(3-Ncp)Ala-OFm (0.182 g, 95%) as a white solid, which was coupled with acid *Chpca(MOM)-OH* **109** (96 mg, 0.467 mmol) using EDC (93 mg, 0.485 mmol), HOAt (64 mg, 0.474 mmol), DIEA (61 mg, 0.472 mmol) and TMP (0.114 g, 0.941 mmol) in CH₂Cl₂ (5 mL) according to GP 5. After 3 h, the reaction mixture was diluted with Et₂O (50 mL) and subjected to usual aqueous work-up (GP 5). The organic layer was dried, filtered and concentrated under reduced pressure. The residue was crystallized from Et₂O/pentane to give **193** (0.202 g, 80% on two steps) as a white solid. $R_f = 0.20$ EtOAc/hexanes 1:3; m.p 94–95 °C; $[\alpha]_{20}^D - 33.3$ ($c = 0.3$, CHCl₃); ¹H NMR (250 MHz, CDCl₃): $\delta = 1.14$ [ddd, J

= 6.8, 6.8, 5.5 Hz, 1 H, 3'-H_a, (3-*Ncp*)*Ala*], 1.47–1.60 [m, 1 H, 3'-H_b, (3-*Ncp*)*Ala*], 1.69–1.90 [m, 3 H, 1'-H, 3-H, (3-*Ncp*)*Ala*], 3.57 (s, 3 H, OMe), 3.89 [ddd, $J = 7.0, 3.3, 3.3$ Hz, 1 H, 2-H, (3-*Ncp*)*Ala*], 4.25 (t, $J = 5.8$ Hz, 1 H, 1'-H, *Fm*), 4.63 (dd, $J = 10.6, 5.8$ Hz, 1 H, 2'-H_a, *Fm*), 4.73 (dd, $J = 10.6, 5.8$ Hz, 1 H, 2'-H_b, *Fm*), 5.17 (d, $J = 6.8$ Hz, 1 H, H_a, OCH₂O), 5.25 (d, $J = 6.8$ Hz, 1 H, H_b, OCH₂O), 6.04 (d, $J = 4.7$ Hz, 1 H, 4-H, *Chpca*), 6.68 (d, $J = 4.7$ Hz, 1 H, 3-H, *Chpca*), 7.17 (d, $J = 7.0$ Hz, 1 H, NH), 7.26–7.37 (m, 2 H, Ar-H, *Fm*), 7.42 (dd, $J = 7.0, 7.0$ Hz, 2 H, Ar-H, *Fm*), 7.57 (d, $J = 7.3$ Hz, 2 H, Ar-H, *Fm*), 7.77 (dd, $J = 7.5, 3.6$ Hz, 2 H, Ar-H, *Fm*); The adsorption of 2'-H_b, *Fm* masked signal of 1'-H, (3-*Ncp*)*Ala*; ¹³C NMR (62.9 MHz, CDCl₃): δ = 17.5 [–, C-3', (3-*Ncp*)*Ala*], 21.7 [+C-1', (3-*Ncp*)*Ala*], 33.3 [–, C-3, (3-*Ncp*)*Ala*], 46.6 (+, C-1', *Fm*), 51.3 [+C-2, (3-*Ncp*)*Ala*], 58.9 [+C-2', (3-*Ncp*)*Ala*], 59.4 (+, OMe), 66.9 (–, C-2', *Fm*), 104.2 (+, C-4, *Chpca*), 104.8 (–, OCH₂O), 111.1 (+, C-3, *Chpca*), 118.7 (C_{quat}, C-2, *Chpca*), 119.9, 120.0 (+, Ar-C, *Fm*), 121.7 (C_{quat}, C-5, *Chpca*), 124.5, 124.6 (+, Ar-C, *Fm*), 127.1, 127.2 (+, Ar-C, *Fm*), 127.9 (+, Ar-C, *Fm*), 141.2, 141.3 (C_{quat}, Ar-C, *Fm*), 143.0, 143.1 (C_{quat}, Ar-C, *Fm*), 158.0 (C_{quat}, C-1, *Chpca*), 171.1 [C_{quat}, C-1, (3-*Ncp*)*Ala*]; IR (KBr): $\nu(\text{tilde}) = 3370\text{ cm}^{-1}$, 3038, 2962, 2935, 2836, 1729, 1638, 1538, 1428, 1374, 1339, 1239, 1164; MS (EI, 70 eV), m/z (%) = 542/540 (1/2) [M⁺], 510/508 (1/3) [M⁺ – CH₂O], 191 (10), 178 (100) [C₁₄H₁₀⁺], 165 (12), 130/128 (3/10) [C₅H₃ClNO⁺], 45 (36) [C₂H₅O⁺]; HRMS (EI): calcd for C₂₇H₂₆ClN₃O₇: 539.1459; found 539.1459; elemental analysis (%) for C₂₇H₂₆ClN₃O₇ (540): calcd. C 60.06, H 4.85, N 7.78; found C 60.10, H 5.00, N 7.71.

Chpca-(2*S*)-(3-*Ncp*)*Ala*-OFm (**194**): The acyl amino ester **193** (50 mg, 92.60 μmol) was deprotected with MgBr₂·Et₂O (0.239 g, 0.925 mmol) and EtSH (0.07 mL, 0.945 mmol) in CH₂Cl₂ (15 mL) according to GP 10 for 3 h. The usual aqueous work-up (GP 10) followed by preparative TLC (200 × 200 mm, EtOAc/hexanes 1:4, 2 runs) gave **194** (0.036 g, 78%) as an extremely viscous oil which was unlimitedly stable by storage under argon at –28 °C. $R_f = 0.20$ EtOAc/hexanes 1:3; ¹H NMR (250 MHz, CDCl₃): δ = 0.83–0.96 [m, 1 H, 3'-H_a, (3-*Ncp*)*Ala*],

1.42–1.56 [m, 1 H, 3'-H_b, (3-*Ncp*)Ala], 1.67–1.82 [m, 3 H, 1'-H, 3-H, (3-*Ncp*)Ala], 3.89 [ddd, $J = 7.0, 3.1, 3.1$ Hz, 1 H, 2-H, (3-*Ncp*)Ala], 4.25 (t, $J = 5.1$ Hz, 1 H, 1'-H, *Fm*), 4.67 (dd, $J = 10.7, 5.1$ Hz, 1 H, 2'-H_a, *Fm*), 4.82 (dd, $J = 10.7, 5.1$ Hz, 1 H, 2'-H_b, *Fm*), 5.96 (d, $J = 5.0$ Hz, 1 H, 4-H, *Chpca*), 6.37 (d, $J = 7.0$ Hz, 1 H, NH), 6.40 (d, $J = 5.0$ Hz, 1 H, 3-H, *Chpca*), 7.26–7.37 (m, 2 H, Ar-H, *Fm*), 7.42 (dd, $J = 7.3, 7.3$ Hz, 2 H, Ar-H, *Fm*), 7.56 (d, $J = 7.3$ Hz, 2 H, Ar-H, *Fm*), 7.76 (dd, $J = 7.1, 5.3$ Hz, 2 H, Ar-H, *Fm*), 13.0–13.3 (br, 1 H, OH); The adsorption of 2'-H_a, *Fm* masked signal of 1'-H, (3-*Ncp*)Ala; ¹³C NMR (62.9 MHz, CDCl₃): $\delta = 17.4$ [–, C-3', (3-*Ncp*)Ala], 21.6 [+C-1', (3-*Ncp*)Ala], 33.4 [–, C-3, (3-*Ncp*)Ala], 46.7 (+, C-1', *Fm*), 51.2 [+C-2, (3-*Ncp*)Ala], 58.9 [+C-2', (3-*Ncp*)Ala], 66.9 (–, C-2', *Fm*), 102.8 (+, C-4, *Chpca*), 106.1 (+, C-3, *Chpca*), 114.4 (C_{quat}, C-2, *Chpca*), 116.0 (C_{quat}, C-5, *Chpca*), 120.0, 120.1 (+, Ar-C, *Fm*), 124.4, 124.5 (+, Ar-C, *Fm*), 127.2, 127.3 (+, Ar-C, *Fm*), 128.0 (+, Ar-C, *Fm*), 141.3, 141.4 (C_{quat}, Ar-C, *Fm*), 142.9, 143.0 (C_{quat}, Ar-C, *Fm*), 162.2 (C_{quat}, C-1, *Chpca*), 170.9 [C_{quat}, C-1, (3-*Ncp*)Ala]; IR (KBr): $\nu(\text{tilde}) = 3750\text{--}1800\text{ cm}^{-1}$, 3067, 1740, 1542, 1451, 1426, 1368, 1198; MS (EI, 70 eV), m/z (%) = 497/495 (2/7) [M⁺], 319/317 (1/4) [M⁺ – C₁₄H₁₀], 178 (100) [C₁₄H₁₀⁺], 146/144 (3/10) [C₅H₂ClNO₂⁺]; HRMS (EI): calcd for C₂₅H₂₂ClN₃O₆: 495.1197; found 495.1197.

Chpca-(2*S*)-(3-*Ncp*)Ala-OH (**195**): Ester **194** (35 mg, 70.58 μmol) was deprotected with 10% tris(2-aminoethyl)amine (TAEA) in CH₂Cl₂ (1 mL) for 20 min and the mixture was then taken up with EtOAc (30 mL). The organic layer briefly washed 1 M NaHSO₄ (3 $\times$ 10 mL), water (3 $\times$ 5 mL), brine (2 $\times$ 5 mL), dried, filtered and concentrated to give **195** as a turbid oil which completely polymerized into white insoluble solid at 20 °C for ca. 2 h. In solution **195** was even less stable. ¹H NMR (250 MHz, [D₆]-acetone): $\delta = 1.30$ [ddd, $J = 7.0, 7.0, 7.0$ Hz, 1 H, 3'-H_a, (3-*Ncp*)Ala], 1.71–1.87 [m, 1 H, 3'-H_b, (3-*Ncp*)Ala], 1.90–2.21 [m, 3 H, 1'-H, 3-H, (3-*Ncp*)Ala], 4.42 [ddd, $J = 6.3, 3.5, 3.5$ Hz, 1 H, 2-H, (3-*Ncp*)Ala], 4.71–4.84 [m, 2'-H, (3-*Ncp*)Ala], 6.03 (d, $J = 5.0$ Hz, 1 H, 4-H, *Chpca*), 6.82 (d, $J = 5.0$ Hz, 1 H, *Chpca*), 7.0–8.0 (br, 2 H, OH, CO₂H),

8.17 (d, $J = 7.5$ Hz, 1 H, NH); It was impossible to measure ^{13}C NMR because of complete decomposition of **195** during measurement; MS (EI, 70 eV), m/z (%) = 319/317 (12/40) [M^+], 283 (6), 144 (100), 130/128 (15/46) [$\text{C}_5\text{H}_2\text{ClNO}^+$], 112 (6), 91/89 (4/12), 80 (18) [$\text{C}_5\text{H}_6\text{N}^+$], 64 (12), 45 (10) [CO_2H^+]; HRMS (EI): calcd for $\text{C}_{11}\text{H}_{12}\text{ClN}_3\text{O}_6$: 317.0414; found 317.0414.

O-MOM protected hormaomycin *O*-MOM-**3** and its epimer *epi-O*-MOM-**3**: The ester **193** (5.4 mg, 10.00 μmol) was deprotected with 20% Et_2N in MeCN (1 mL) for 40 min. The mixture was then concentrated under reduced pressure, the residue was washed with hexanes, taken up with Et_2O (30 mL), washed with 1 M NaHSO_4 (3×5 mL), water (3×5 mL), brine (2×5 mL). The organic layer was dried, filtered and concentrated under reduced pressure. The resultant crude *N*-acyl amino acid **196** ($R_f = 0.42$ EtOAc/hexanes 1:2 (2% AcOH)) was dried at 0.01 Torr for 1 h and then introduced in the coupling reaction with the deprotected depsipeptide obtained after treatment of the *N*MeZ protected cyclodepsipeptide **171c** (8.0 mg, 8.179 μmol) with 10% anisole in TFA (1 mL) according to GP 11 using PyAOP (8.4 mg, 16.11 μmol), HOAt (2.2 mg, 16.28 μmol), DIEA (1.3 mg, 10.06 μmol) and TMP (3.8 mg, 31.36 μmol) in CH_2Cl_2 (1 mL) according to GP 7 for 16 h. The mixture was then diluted with Et_2O and subjected to usual aqueous work-up (GP 5). TLC analysis showed the presence of the two main reaction products which were separated by preparative TLC (100 $\times$ 200 mm, acetone/hexanes 1:3, 3 runs). Each fraction was additionally purified by preparative TLC (40 $\times$ 80 mm, acetone/hexanes 1:3, 3 runs) to give *O*-MOM-**3** (2.5 mg, 26%) as a colorless glass and *epi-O*-MOM-**3** (1.5 mg, 16%) as colorless glass. *O*-MOM-**3**: $R_f = 0.11$ acetone/hexanes 1:3; ^1H NMR (600 MHz, CDCl_3): $\delta = -0.26$ [ddd, $J = 6.0, 6.0, 6.0$ Hz, 1 H, 3'- H_a , (3-*Ncp*)*Ala*], 0.07–0.17 [m, 1 H, 3- H_a , (3-*Ncp*)*Ala*], 0.52–0.61 [m, 1 H, 1'-H, (3-*Ncp*)*Ala*], 0.68–0.79 [m, 1 H, 3- H_b , (3-*Ncp*)*Ala*], 0.88 (t, $J = 7.2$ Hz, 3 H, 5-H, *Ile*), 1.02 (d, $J = 6.6$ Hz, 3 H, 1'-H, *Ile*), 1.05 [ddd, $J = 6.0, 6.0, 6.0$ Hz, 1 H, 3'- H_b , (3-*Ncp*)*Ala*], 1.10–1.14 [m, 1 H, 3'- H_a , (3-*Ncp*)*Ala*], 1.19–1.27 [m, 2 H, 4- H_a , *Ile*, 3'- H_b , (3-*Ncp*)*Ala*], 1.29 [d, $J = 6.6$ Hz, 3 H, 4-H, (βMe)*Phe*], 1.39 [d, $J = 6.6$ Hz, 3 H, 4-H, (βMe)*Phe*], 1.50–1.56 (m, 1 H, 4- H_b ,

Ile), 1.59 (d, $J = 7.2$ Hz, 3 H, 4-H, *a-Thr*), 1.64–1.70 [m, 1 H, 3-H_a, (*3-Ncp*)*Ala*], 1.69 [dd, $J = 7.2$, 1.8 Hz, 3 H, 3'-H, (*4-PE*)-*Pro*], 1.75–1.85 [m, 2 H, 3-H, *Ile*, 3-H_a, (*4-PE*)*Pro*], 1.90–1.95 [m, 1 H, 3-H_b, (*3-Ncp*)*Ala*], 1.95–2.00 [m, 1 H, 1'-H, (*3-Ncp*)*Ala*], 2.40 [ddd, $J = 12.0$, 6.0, 6.0 Hz, 1 H, 3-H_b, (*4-PE*)-*Pro*], 3.05 [dq, $J = 10.8$, 6.6 Hz, 1 H, 3-H, (β Me)*Phe*], 3.11–3.17 [m, 1 H, 2'-H, (*3-Ncp*)*Ala*], 3.24–3.32 [m, 2 H, 4-H, 5-H_a, (*4-PE*)-*Pro*], 3.71 [dq, $J = 4.2$, 7.2 Hz, 1 H, 3-H, (β Me)*Phe*], 3.75 (s, 3 H, OMe), 3.90 (dd, $J = 6.6$, 5.4 Hz, 1 H, 2-H), 3.93–3.98 [m, 1 H, 5-H_b, (*4-PE*)-*Pro*], 4.09 [ddd, $J = 6.6$, 3.0, 3.0 Hz, 1 H, 2'-H, (*3-Ncp*)*Ala*], 4.26 (dd, $J = 13.2$, 13.2 Hz, 1 H, 2-H), 4.30 (dd, $J = 11.4$, 6.0 Hz, 1 H, 2-H), 4.61 (dd, $J = 8.4$, 4.8 Hz, 1 H, 2-H), 4.64–4.70 (m, 2 H, 2-H), 5.10–5.17 [m, 1 H, 2-H, (*3-Ncp*)*Ala*], 5.24–5.29 [m, 1 H, 1'-H, (*4-PE*)-*Pro*], 5.39 (dq, $J = 1.8$, 7.2 Hz, 1 H, 3-H, *a-Thr*), 5.47 (d, $J = 6.0$ Hz, 1 H, H_a, OCH₂O), 5.50 (d, $J = 6.0$ Hz, 1 H, H_b, OCH₂O), 5.64 [dq, $J = 10.5$, 7.2 Hz, 1 H, 2'-H, (*4-PE*)-*Pro*], 5.75–5.81 (br, 2 H, NH), 6.17 (d, $J = 4.5$ Hz, 1 H, 4-H, *Chpca*), 6.70 (d, $J = 9.0$ Hz, 1 H, NH), 6.84 (d, $J = 4.5$ Hz, 1 H, 3-H, *Chpca*), 7.06 (dd, $J = 7.5$, 7.5 Hz, 1 H, Ar-H), 7.09 (d, $J = 7.2$ Hz, 2 H, Ar-H), 7.15 (dd, $J = 7.5$, 7.5 Hz, 3 H, Ar-H), 7.16 (d, $J = 7.2$ Hz, 1 H, NH), 7.22–7.31 (m, 5 H, Ar-H, NH), 7.38 (d, $J = 9.6$ Hz, 1 H, NH), 7.75 (d, $J = 9.0$ Hz, 1 H, NH), 8.79 (d, $J = 9.0$ Hz, 1 H, NH); ¹³C NMR (150.8 MHz, CDCl₃): $\delta = 10.8$ (+, C-5, *Ile*), 13.2 [+ , C-4, (β Me)*Phe*], 13.3 [+ , C-3', (*4-PE*)-*Pro*], 15.5 (+, C-1', *Ile*), 17.2 [–, C-3', (*3-Ncp*)*Ala*], 17.3 [–, C-3', (*3-Ncp*)*Ala*], 17.5 (+, C-4, *a-Thr*), 18.3 [+ , C-4, (β Me)*Phe*], 20.7 [+ , C-1', (*3-Ncp*)*Ala*], 21.3 [+ , C-1', (*3-Ncp*)*Ala*], 24.9 (–, C-4, *Ile*), 29.7 [–, C-3, (*3-Ncp*)*Ala*], 34.0 [–, C-3, (*3-Ncp*)*Ala*], 35.4 [–, C-3, (*4-PE*)-*Pro*], 36.7 [+ , C-4, (*4-PE*)-*Pro*], 38.6 (+, C-3, *Ile*), 39.4 [+ , C-3, (β Me)*Phe*], 42.7 [+ , C-3, (β Me)*Phe*], 50.2 [+ , C-2', (*3-Ncp*)*Ala*], 51.5 (+, C-2), 52.9 [–, C-5, (*4-PE*)-*Pro*], 54.7 (+, C-2), 54.9 (+, C-2), 58.5 [+ , C-2', (*3-Ncp*)*Ala*], 59.4 (+, OMe, C-2), 59.6 (+, C-2), 60.2 (+, C-2), 61.5 (+, C-2), 69.9 (+, C-3, *a-Thr*), 104.5 (–, OCH₂O), 106.1 (+, C-4, *Chpca*), 111.5 (+, C-3, *Chpca*), 119.8 (C_{quat}, C-2, *Chpca*), 121.5 (C_{quat}, C-2, *Chpca*), 126.9, 127.1, 127.5, 127.6, 128.5, 128.6 (+, Ar-C), 127.4 [+ , C-1', (*4-PE*)-*Pro*], 128.5 [+ , C-2', (*4-PE*)-*Pro*], 142.3 (2 × C_{quat}, Ar-C), 158.6 (C_{quat}, C-1,

Chpca), 168.8, 169.9, 170.1, 170.2, 171.1, 171.3, 171.4 (C_{quat} , C-1); UV (MeOH): neutral: $\lambda_{\text{max}}(\epsilon) = 268 (1.8 \times 10^4)$, 201 (7.5×10^5) nm; basic: 341 (1.4×10^3), 268 (1.5×10^4), 205 (9.1×10^4); acidic: 341 (2.7×10^3), 268 (1.4×10^4) nm; CD (MeOH): $\lambda_{\text{max}}[\Theta] = 268.2 (2.71 \times 10^4)$, 259.3 (2.40×10^4), 222.1 (-41.7×10^4) nm ($c = 8.18 \times 10^{-6}$ M); MS (ESI): positive mode, m/z (%) = 1196 (100) $[M + Na^+]$; negative mode, m/z (%) = 1171 (100) $[M - H^+]$.

epi-O-MOM-3: $R_f = 0.09$ acetone/hexanes 1:3; ^1H NMR (600 MHz, CDCl_3): $\delta = 0.68$ [ddd, $J = 6.6, 6.6, 6.6$ Hz, 1 H, 3'-H_a, (3-*Ncp*)Ala], 0.79–0.92 [m, 2 H, 1'-H, 3-H_a, (3-*Ncp*)Ala], 0.98 (t, $J = 7.2$ Hz, 3 H, 5-H, *Ile*), 1.01 (d, $J = 6.6$ Hz, 3 H, 1'-H, *Ile*), 1.15 [ddd, $J = 6.6, 6.6, 6.6$ Hz, 1 H, 3'-H_b, (3-*Ncp*)Ala], 1.19–1.32 [m, 6 H, 3'-H, (3-*Ncp*)Ala, 4-H, (β Me)Phe, 4-H_a, *Ile*], 1.34 [d, $J = 7.2$ Hz, 3 H, 4-H, (β Me)Phe], 1.46–1.62 [m, 3 H, 4-H_b, *Ile*, 3-H, (3-*Ncp*)Ala], 1.64–1.70 [m, 1 H, 3-H_a, (3-*Ncp*)Ala], 1.69 [dd, $J = 7.2, 1.8$ Hz, 3 H, 3'-H, (4-*PE*)-Pro], 1.76 [ddd, $J = 11.4, 11.4, 11.4$ Hz, 3-H_a, (4-*PE*)Pro], 1.90–1.99 (m, 1 H, 3-H, *Ile*), 2.05–2.13 [m, 1 H, 1'-H, (3-*Ncp*)Ala], 2.40 [ddd, $J = 11.4, 6.0, 6.0$ Hz, 1 H, 3-H_b, (4-*PE*)-Pro], 3.13–3.23 [m, 1 H, 3-H, (β Me)Phe], 3.23–3.32 [m, 2 H, 4-H, 5-H_a, (4-*PE*)-Pro], 3.62 (s, 3 H, OMe), 3.67–3.75 [m, 1 H, 3-H, (β Me)Phe], 3.85 [ddd, $J = 6.6, 3.0, 3.0$ Hz, 2'-H, (3-*Ncp*)Ala], 3.96–4.01 (m, 1 H, 2-H), 4.05–4.09 [m, 1 H, 5-H_b, (4-*PE*)-Pro], 4.13 [ddd, $J = 6.6, 3.0, 3.0$ Hz, 1 H, 2'-H, (3-*Ncp*)Ala], 4.13–4.21 (m, 1 H, 2-H), 4.56–4.72 (m, 3 H, 2-H), 4.61 (dd, $J = 9.0, 4.5$ Hz, 1 H, 2-H), 4.84 (dd, $J = 9.0, 2.4$ Hz, 1 H, 2-H), 5.20–5.35 [m, 2 H, 1'-H, (4-*PE*)Pro, 3-H, *a*-Thr], 5.22 (d, $J = 6.0$ Hz, 1 H, H_a, OCH₂O), 5.28 (d, $J = 6.0$ Hz, 1 H, H_b, OCH₂O), 5.61 [dq, $J = 10.5, 7.2$ Hz, 1 H, 2'-H, (4-*PE*)-Pro], 6.03 (d, $J = 4.8$ Hz, 1 H, 4-H, *Chpca*), 6.33–6.46 (br, 1 H, NH), 6.57–6.69 (br, 1 H, NH), 6.70 (d, $J = 4.8$ Hz, 1 H, 3-H, *Chpca*), 7.13–7.34 (m, 14 H, Ar-H, NH), 7.57–7.71 (br, 1 H, NH); Absorption of water masked signal of 4-H, *a*-Thr ; ^{13}C NMR (150.8 MHz, CDCl_3): $\delta = 10.2$ (+, C-5, *Ile*), 13.4 [+ , C-4, (β Me)Phe, C-3', (4-*PE*)-Pro], 14.7 (+, C-1', *Ile*), 17.4 [–, C-3', (3-*Ncp*)Ala], 17.9 [–, C-3', (3-*Ncp*)Ala], 18.6 (+, C-4, *a*-Thr), 21.5 [+ , C-4, (β Me)Phe], 24.4 [+ , C-1', (3-*Ncp*)Ala], 24.9 (–, C-4, *Ile*), 29.3 [+ , C-1', (3-*Ncp*)Ala], 29.7 [–, C-3, (3-*Ncp*)Ala], 30.9

[−, C-3, (3-*Ncp*)*Ala*], 35.6 [−, C-3, (4-*PE*)-*Pro*], 36.6 [+ , C-4, (4-*PE*)-*Pro*], 37.5 (+, C-3, *Ile*), 39.0 [+ , C-3, (β*Me*)*Phe*], 40.2 [+ , C-3, (β*Me*)*Phe*], 52.3 (+, C-2), 52.9 [−, C-5, (4-*PE*)-*Pro*], 53.2 (+, C-2), 54.4 (+, C-2), 55.8 (+, C-2), 59.0 [+ , C-2′, (3-*Ncp*)*Ala*], 59.3 [+ , C-2′, (3-*Ncp*)*Ala*], 59.5 (+, OMe, C-2), 59.5 (+, C-2), 60.7 (+, C-2), 72.2 (+, C-3, *α*-*Thr*), 104.2 (−, OCH₂O), 104.9 (+, C-4, *Chpca*), 110.9 (+, C-3, *Chpca*), 118.7 (C_{quat}, C-2, *Chpca*), 122.1 (C_{quat}, C-2, *Chpca*), 126.8, 127.1, 127.3, 128.6, 128.7, 128.8 (+, Ar-C), 127.7 [+ , C-1′, (4-*PE*)-*Pro*], 128.1 [+ , C-2′, (4-*PE*)-*Pro*], 141.6 (2 × C_{quat}, Ar-C), 157.9 (C_{quat}, C-1, *Chpca*), 169.8, 170.0, 170.2, 170.9 (× 2), 171.2, 171.3 (C_{quat}, C-1); UV (MeOH): neutral: λ_{max}(ε) = 261 (1.7 × 10⁴), 201 (6.6 × 10⁵) nm; basic: 341 (1.4 × 10³), 267 (1.4 × 10⁴), 226 (2.1 × 10⁴), 204 (7.6 × 10⁴); acidic: 341 (2.7 × 10³), 269 (2.0 × 10⁴) nm; CD (MeOH): λ_{max}[Θ] = 274 (2.08 × 10⁴), 221.6 (−4.99 × 10⁴) nm (*c* = 6.48 × 10^{−6} M); MS (ESI): positive mode, *m/z* (%) = 1196 (100) [M + Na⁺]; negative mode, *m/z* (%) = 1207 (40) [M + Cl[−]], 1171 (100) [M − H⁺].

N-Teoc-3-(2*S*,1*S*,2*R*)-(trans-2′-Nitrocyclopropyl)alanine (*S*)-(197): A solution of TeocOSu

(0.358 g, 1.38 mmol) in acetone (5 mL) was added to a vigorously stirred solution of 3-(2*S*,1′*R*,2′*R*)-(trans-2′-nitrocyclopropyl)alanine (2*S*,1′*R*,2′*R*)-**83** (0.200 g, 1.15 mmol) and NaHCO₃ (0.202 g, 2.40 mmol) in water (7 mL) (if emulsion formed acetone and/or water were added to obtain homogeneous solution) and stirring continued for a further 2 h. *N,N*-dimethylaminopropylamine (0.055 mL, 0.44 mmol) was then added. After an additional 10 min acetone was removed under reduced pressure and pH of the residual water solution was adjusted to 2–3 with 1 M NaHSO₄. The resulting emulsion was extracted with Et₂O (50 mL) and the ethereal layer was washed with 1 M NaHSO₄ (2 × 10 mL), water (10 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The residual oil (0.300 g) was dissolved in Et₂O (5 mL) and cyclohexylamine (0.094 g, 0.95 mmol) was added. The mixture was concentrated under reduced pressure and treated with boiling hexanes. The resultant precipitate was filtered off and washed

with Et₂O/pentane 1:4 to give cyclohexylammonium salt of (*S*)-**197** (0.386 g, 81%) as a white solid. $R_f = 0.24$ EtOAc/hexanes 1:3 (2% AcOH); $[\alpha]_{20}^D$ 22.80 ($c = 0.46$, CHCl₃) for CHA salt; ¹H NMR (250 MHz, CDCl₃): $\delta = 0.04$ [s, 9 H, Si(CH₃)₃], 1.00 (dd, $J = 9.5, 7.3$ Hz, 2 H, 2-H, *Teoc*), 1.14 (ddd, $J = 6.0, 6.0, 6.0$ Hz, 1 H, 3'-H_a), 1.60–1.95 (m, 1 H, 1'-H, 3'-H_b), 1.98–2.19 (m, 2 H, 3-H), 4.18 (dd, $J = 8.4, 8.4$ Hz, 2 H, 1-H, *Teoc*), 4.33–4.46, 4.46–4.59 (2 $\times$ m, 1 H, 2-H), 5.33–5.46 (m, 1 H, NH), 7.08–7.25 (br, 1 H, CO₂H); ¹³C NMR (62.9 MHz, CDCl₃): $\delta = -1.9$ [+ , Si(CH₃)₃], 17.3 (–, C-3' and C-2, *Teoc*), 22.0 (+, C-1'), 33.1, 33.3 (–, C-3), 52.7, 53.2 (+, C-2), 59.0 (+, C-2'), 63.7, 64.8 (–, C-1, *Teoc*), 156.4, 157.4 (C_{quat}, NCO₂), 174.5, 174.8 (C_{quat}, C-1); IR (KBr; for CHA salt): $\nu(\text{tilde}) = 3419$ cm^{–1}, 3250–2600, 2952, 2863, 1722, 1632, 1541, 1492, 1406, 1368, 1248, 1206, 1163, 1053; MS (ESI): positive, $m/z = 363$ (28) [M – H⁺ + 2Na⁺], 341 (26) [M + Na⁺]; negative, $m/z = 657$ (100) [2M – 2H⁺ + Na⁺], 317 (28) [M – H⁺].

Hormaomycin (**3**): An ethereal solution (50 mL) of the CHA salt of *N*-Teoc protected (2*S*,1'*R*,2'*R*)-(3-nitrocyclopropyl)alanine (8.1 mg, 19.41 μ mol) was washed with 1 M H₂SO₄ (3 $\times$ 5 mL), 1 M KHSO₄ (2 $\times$ 5 mL), water (3 $\times$ 5 mL), brine (2 $\times$ 5 mL), dried, filtered and concentrated under reduced pressure. The resultant *N*-protected amino acid (*S*)-**197** was dried at 0.02 Torr for 2 h and then coupled with the depsipeptide [obtained after deprotection of *N*-MeZ protected cyclodepsipeptide **171c** (9.5 mg, 9.71 μ mol) with 10% anisole in TFA (1.1 mL) for 2 h according to GP 11] using HATU (7.4 mg, 19.46 μ mol), HOAt (2.6 mg, 19.24 μ mol), DIEA (1.25 mg, 9.67 μ mol) and TMP (7.04 mg, 58.10 μ mol) in CH₂Cl₂ (0.7 mL) according to GP 7 for 15 h. The mixture was then diluted with EtOAc/Et₂O 1:1 (20 mL) and the crude product obtained after usual aqueous work-up (GP 2) was purified by preparative TLC (100 $\times$ 200 mm, acetone/hexanes 1:3, 3 runs) to give Teoc-(*S*)-(3-Ncp)Ala-*cyclo*-hexadepsipeptide (8.0 mg, 73%, $R_f = 0.43$ acetone/hexanes 1:2) as a colorless glass which was used for the next step without any characterization. Teoc group was cleaved from this substance (8.0 mg, 7.08 μ mol) with TFA (0.6

mL) for 1 h. The mixture was concentrated under reduced pressure at 20 °C and then taken up with toluene (3 × 15 mL) which was distilled off to remove the last traces of TFA. The resultant deprotected depsipeptide was coupled with **109** (2.9 mg, 14.10 μmol) using HATU (5.4 mg, 14.20 μmol), DIEA (0.92 mg, 7.12 μmol) and TMP (5.14 mg, 42.42 μmol) in CH₂Cl₂ (1 mL) according to GP 7 for 2.5 h. The mixture was then taken up with Et₂O (20 mL) and the crude product obtained after usual aqueous work-up (GP 7) was purified by preparative TLC (200 × 200 mm, acetone/hexanes 1:2.5) to give *O*-MOM-**3** (8.0 mg, 96%, *R*_f = 0.36 acetone/hexanes 1:2) as a colorless glass which was used for the next step without any characterization. MOM-**3** (8.0 mg, 6.82 μmol) was deprotected using MgBr₂·Et₂O (52 mg, 201.36 μmol) and EtSH (0.10 mL, 1.918 mmol) in CH₂Cl₂ (10 mL) according to 10 for 3 h. The mixture was taken up with EtOAc and the crude product obtained after usual aqueous work-up was purified by preparative TLC (200 × 200 mm, acetone/hexanes 1:3, 2 runs) and finally by trituration with pentane to give **3** (5.5 mg, 72%, 50% on 5 steps from **171c**) as a white solid. *R*_f = 0.24 acetone/hexanes 3:7; analytical HPLC: column B, isocratic, 65% MeCN in H₂O for 15 min, then gradient 65 → 99% MeCN in H₂O for 5 min, then isocratic, 99% MeCN, flow rate = 0.5 mL/min, *t*_R = 14.54 min, purity > 92%; [α]₂₀^D 20.0 (*c* = 0.1, MeOH); ¹H NMR (600 MHz, CDCl₃): δ = −0.65 – −0.36 [m, 1 H, 3'-H_a, (3-*Ncp*)Ala], −0.11–0.16 [m, 1 H, 3-H_a, (3-*Ncp*)Ala], 0.27–0.36 [m, 1 H, 1'-H, (3-*Ncp*)Ala], 0.38–0.51 [m, 1 H, 3-H_b, (3-*Ncp*)Ala], 0.90 (t, *J* = 7.2 Hz, 3 H, 5-H, *Ile*), 0.98–1.07 [m, 1 H, 3'-H_b, (3-*Ncp*)Ala], 1.04 (d, *J* = 6.6 Hz, 3 H, 1'-H, *Ile*), 1.08 [(ddd, *J* = 7.2, 7.2, 7.2 Hz, 1 H, 3'-H_a, (3-*Ncp*)Ala], 1.27–1.35 (m, 1 H, 4-H_a, *Ile*), 1.30 [d, *J* = 7.2 Hz, 3 H, 4-H, (β-*Me*)Phe], 1.42 [d, *J* = 7.2 Hz, 3 H, 4-H, (β-*Me*)Phe], 1.53–1.59 (m, 1 H, 4-H_b, *Ile*), 1.54 (d, *J* = 6.6 Hz, 3 H, *α*-*Thr*), 1.59–1.67 [m, 1 H, 3-H_a, (3-*Ncp*)Ala], 1.70 [dd, *J* = 6.6, 1.8 Hz, 3 H, 3'-H, (4-*PE*)Pro], 1.73–1.99 [m, 4 H, 3-H_b, 1'-H, 3'-H_b, (3-*Ncp*)Ala, 3-H, *Ile*], 1.82 [ddd, *J* = 11.4, 11.4, 11.4 Hz, 1 H, 3-H_a, (4-*PE*)Pro], 2.40 [ddd, *J* = 11.4, 6.0, 5.4 Hz, 1 H, 4-H_b, (4-*PE*)Pro], 2.91–2.98 [m, 1 H, 2'-H, (3-*Ncp*)Ala], 3.02 [dq, *J* = 11.1, 7.2 Hz, 1 H, 3-H, (β-*Me*)Phe], 3.24–3.34 [m,

2 H, 4-H, 5-H_a, (*4-PE*)*Pro*], 3.53–3.63 [m, 1 H, 2-H, (*3-Ncp*)*Ala*], 3.68 [dq, $J = 4.5, 7.2$ Hz, 1 H, 3-H, (β -*Me*)*Phe*], 3.94–4.00 [m, 1 H, 5-H_b, (*4-PE*)*Pro*], 4.05 [ddd, $J = 6.6, 3.6, 3.6$ Hz, 1 H, 2'-H, (*3-Ncp*)*Ala*], 4.30 [dd, $J = 12.0, 6.6$ Hz, 1 H, 2-H, (*4-PE*)*Pro*], 4.33 [dd, $J = 10.8, 10.8$ Hz, 1 H, 2-H, (β -*Me*)*Phe*], 4.50–4.58 [m, 1 H, 2-H, (β -*Me*)*Phe*], 4.54 (dd, $J = 9.0, 1.8$ Hz, 1 H, 2-H, *a-Thr*), 4.69 (dd, $J = 9.0, 9.0$ Hz, 1 H, 2-H, *Ile*), 5.19 [ddd, $J = 7.8, 7.8, 7.8$ Hz, 1 H, 2-H, (*3-Ncp*)*Ala*], 5.24–5.31 [m, 1 H, 1'-H, (*4-PE*)*Pro*], 5.43 (dq, $J = 6.6, 2.4$ Hz, 1 H, 3-H, *a-Thr*), 5.65 [dq, $J = 10.2, 6.6$ Hz, 1 H, 2'-H, (*4-PE*)*Pro*], 6.13 (d, $J = 4.8$ Hz, 1 H, 4-H, *Chpca*), 6.26–6.36 (br, 1 H, NH), 6.83 (d, $J = 4.8$ Hz, 1 H, 3-H, *Chpca*), 6.84 (d, $J = 9.6$ Hz, 1 H, NH), 7.02–7.07 (m, 1 H, Ar-H), 7.08–7.13 (m, 2 H, Ar-H), 7.14–7.19 (m, 3 H, Ar-H), 7.22–7.28 (m, 7 H, Ar-H, NH), 7.31 (d, $J = 8.4$ Hz, 1 H, NH), 9.03 (d, $J = 7.8$ Hz, 1 H, NH); ^{13}C NMR (150.8 MHz, CDCl_3): $\delta = 10.6$ (+, C-5, *Ile*), 13.3 [+ , C-3', (*4-PE*)*Pro*], 13.3 [+ , C-4, (β -*Me*)*Phe*], 15.0 (+, C-1', *Ile*), 17.0 (+, C-4, *a-Thr*), 17.4 [–, $2 \times$ C-3', (*3-Ncp*)*Ala*], 18.0 [+ , C-4, (β -*Me*)*Phe*], 20.5 [+ , C-1', (*3-Ncp*)*Ala*], 21.8 [+ , C-1', (*3-Ncp*)*Ala*], 24.9 (–, C-4, *Ile*), 33.4 [–, C-3, (*3-Ncp*)*Ala*], 35.3 [–, C-3, (*3-Ncp*)*Ala*], 35.6 [–, C-3, (*4-PE*)*Pro*], 36.7 [+ , C-4, (*4-PE*)*Pro*], 38.1 (+, C-3, *Ile*), 39.3 [+ , C-3, (β -*Me*)*Phe*], 41.9 [+ , C-3, (β -*Me*)*Phe*], 50.7 [+ , C-2, (*3-Ncp*)*Ala*], 52.0 [+ , C-2, (*3-Ncp*)*Ala*], 52.8 [–, C-5, (*4-PE*)*Pro*], 54.7 (+, C-2, *Ile*), 55.2 (+, C-2, *a-Thr*), 58.2 [+ , C-2', (*3-Ncp*)*Ala*], 59.3 [+ , C-2', (*3-Ncp*)*Ala*], 59.9 [+ , C-2, (β -*Me*)*Phe*], 60.2 [+ , C-2, (β -*Me*)*Phe*], 61.4 [+ , C-2, (*4-PE*)*Pro*], 69.2 (+, C-3, *a-Thr*), 103.2 (+, C-4, *Chpca*), 109.3 (+, C-3, *Chpca*), 119.5 (C_{quat}, C-2, *Chpca*), 121.4 (C_{quat}, C-5, *Chpca*), 126.9, 127.2, 127.5, 127.6, 128.5, 128.7 (+, Ar-C), 127.5 [+ , C-1', (*4-PE*)*Pro*], 128.4 [+ , C-2', (*4-PE*)*Pro*], 141.6, 142.2 (C_{quat}, Ar-C), 159.6 (C_{quat}, C-1, *Chpca*), 168.7, 168.9, 169.8, 170.9, 171.3, 171.5, 172.1 (C_{quat}, C-1); UV (MeOH): λ_{max} (ϵ) = 204 (5×10^4), 275 (9.6×10^3) nm; CD (MeOH): λ_{max} [Θ] = 279 (6.01×10^4); 224.2 (-6.500×10^3) nm ($c = 1.754 \times 10^{-4}$ M); MS (ESI): positive, $m/z = 1174$ (100) [$\text{M} - \text{H}^+ + 2\text{Na}^+$], 1151 (80) [$\text{M} + \text{Na}^+$]; negative, $m/z = 1127$ (100) [$\text{M} - \text{H}^+$].

Comparison of amino acidic content and their configuration of synthetic and natural hormaomycins 3 by HPLC/MS experiments: The natural and synthetic hormaomycins **3** (0.6 mg each) were hydrolyzed according to GP 1 and concentrated under reduced pressure. A half portion of each probe was directly treated with (S)-FDVA and other half was first epimerized and then treated with (S)-FDVA according GP 2. The HPLC/MS experiments with this material using the mixtures of (S)-FDVA derivatives of synthetic amino acids as standards failed to find any difference between synthetic and natural products.

2.8. *Synthesis of hormaomycin analogs. Evaluation of biological activity*

N_ω-Z protected (2S,1'S,2'R)-3-(2'-Aminocyclopropyl)alanine (S)-(199): (2S,1'S,2'R)-3-(2'-Amino-

cyclopropyl)alanine (2S,1'S,2'R)-**83** (0.150 g, 0.863 mmol) was

hydrogenated on 10% Pd on charcoal (0.150 g) in anhydrous methanol (20 mL) under ambient pressure of hydrogen for 15 h. The mixture was then filtered through Celite pad and concentrated under reduced pressure to give a crude diamino acid as oil which was directly used without purification for the next step. This material was dissolved in water (4 mL) and CuSO₄ (72 mg, 0.451 mmol) was added. NaHCO₃ (0.145 g, 1.726 mmol) and then a solution of ZOSu (0.28 g, 1.123 mmol) in acetone (2 mL) were added to the resultant deep violet solution after 1 h and stirring continued for a further 1.5 h. The precipitate formed was then filtered off and washed with water (100 mL), Et₂O (100 mL) and dried. The resulting copper complex (0.265 g) was dissolved in boiling water (5 mL) and Trilon B (0.509 g, 1.367 mmol) was then added. The mixture was vigorously stirred for 10 min and then cooled in ice bath for 30 min. The precipitate formed was filtered off, washed with ice-cold water (50 mL), Et₂O (100 mL) and dried to give the first crop of (S)-**199** (0.112 g) as a white solid. The mother liquor was concentrated under reduced pressure to ca. 5 mL and cooled in ice bath. The precipitate formed was filtered off and crystallized from methanol to give the second crop of (S)-**199** (12 mg, 52%

overall yield over 2 steps). $R_f = 0.19$ MeCN/H₂O/AcOH 1:1:1; m.p 212 – 216 °C; $[\alpha]_{20}^D -14.47$ ($c = 0.32$, 0.1 N HCl); ¹H NMR (250 MHz, CD₃OD): $\delta = 0.56$ – 0.68 (m, 1 H, 3'-H_a), 0.78 – 0.98 (m, 2 H, 3'-H_b, 3-H_a), 1.06 (ddd, $J = 10.5, 10.5, 10.5$ Hz, 1 H, 3-H_b), 2.34 (ddd, $J = 7.0, 3.5, 3.5$ Hz, 1 H, 1'-H), 2.60 (d, $J = 14.3$ Hz, 1 H, 2'-H), 3.74 (d, $J = 10.8$ Hz, 1 H, 2-H), 5.20 (s, 2 H, Bzl-H), 7.28 – 7.44 (m, 5 H, Ar-H); ¹³C NMR (62.9 MHz, DCl in D₂O): $\delta = 11.5$ (–, C-3'), 17.3 (+, C-1'), 29.1 (+, C-2'), 32.4 (–, C-3), 54.2 (+, C-2), 68.4 (–, Bzl-C), 128.3 , 129.1 , 129.4 (+, Ar-C), 136.8 (C_{quat}, Ar-C), 160.6 (C_{quat}, NCO₂), 172.2 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 3730$ – 2700 cm^{–1}, 3341 , 3035 , 2949 , 1696 , 1599 , 1529 , 1276 ; MS (ESI): positive, $m/z = 323$ (8) [M – H⁺ + 2Na⁺], 301 (80) [M + Na⁺], 279 (3) [M + H⁺]; negative, $m/z = 277$ (100) [M – H⁺]; elemental analysis calcd (%) for C₁₄H₁₈N₂O₄ (278.3): calcd. C 60.42, H 6.52, N 10.07; found C 60.26, H 6.32, N 9.89.

Teoc-(2*S*)-(3-*Acp*)*Ala*(*Z*)-*OH* (**200**): The *N*_ω-monoprotected diamino acid **199** (0.112 g, 0.402

mmol) and NaHCO₃ (75 mg, 0.893 mmol) were dissolved in boiling water (7 mL) and cooled in ice/water bath. A solution of

TeocOSu (0.131 g, 0.505 mmol) in acetone (7 mL) was added, the cooling bath was then removed and stirring continued for an additional 15 h. Acetone was then removed from the mixture under reduced pressure and the resulting water fraction was filtered through Celite pad and treated with 3-*N,N*-dimethylaminopropylamine (3 drops). pH was adjusted to 2 with 1 M KHSO₄ and the resulting emulsion was extracted with Et₂O (50 mL). The organic layer was washed with 1 M KHSO₄ (3 × 10 mL), water (10 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure to give *N,N'*-diprotected amino acid **200** (0.112 g, 66%) as an extremely viscous colorless oil. $R_f = 0.20$ EtOAc/hexane 1:3 (2% AcOH); $[\alpha]_{20}^D 37.1$ ($c = 1.1$, CHCl₃); ¹H NMR (250 MHz, CDCl₃): $\delta = 0.03$ [s, 9 H, Si(CH₃)₃], 0.56 (ddd, $J = 6.0, 6.0, 6.0$ Hz, 1 H, 3'-H_a), 0.71 – 0.84 (m, 1 H, 3'-H_b), 0.98 (t, $J = 8.5$ Hz, 2 H, 2-H, *Teoc*), 1.22 – 1.43

(m, 1 H, 3-H_b), 2.02–2.22, 2.25–2.36 (2 × m, 1 H, 1'-H), 2.36–2.51 (m, 1 H, 2'-H), 4.14 (t, J = 8.5 Hz, 2 H, 1-H, *Teoc*), 4.39–4.65 (m, 1 H, 2-H), 5.10 (s, 2 H, Bzl-H), 5.22–5.28, 5.60–5.71 (2 × br, 1 H, NH), 5.76–5.88, 6.81–6.90 (m, 1 H, NH), 7.27–7.39 (m, 5 H, Ar-H), 10.50–11.40 (br, 1 H, CO₂H); The peak of 2-H, *Teoc* masked absorption of 3-H_a; ¹³C NMR (62.9 MHz, CDCl₃): δ = -1.7 [+ , Si(CH₃)₃], 13.6, 13.8 (–, C-3'), 15.4, 17.0 (+, C-1'), 17.6 (–, C-2, *Teoc*), 28.9, 29.4 (+, C-2'), 34.7, 35.1 (–, C-3), 53.0, 53.7 (+, C-2), 63.4, 64.3 (–, C-1, *Teoc*), 67.1 (–, Bzl-C), 127.5, 128.2, 128.5 (+, Ar-C), 135.9 (C_{quat}, Ar-C), 155.9, 156.3 (C_{quat}, NCO₂), 157.8, 159.2 (C_{quat}, NCO₂), 174.8, 175.4 (C_{quat}, C-1); IR (KBr): ν (tilde) = 3730–2500 cm⁻¹, 3035, 2954, 2898, 1724, 1690, 1531, 1349, 1269, 1250, 1065; MS (ESI): positive, m/z = 867 (100) [2M + Na⁺], 445 (26) [M + Na⁺]; negative, m/z = 421 (78) [M – H⁺].

Teoc-(2*S*)-(3-*Acp*)Ala(*Npys*)-OH (**201**): The *N,N'*-Diprotected diamino acid **200** (60 mg, 0.142

mmol) was hydrogenated on 10% Pd on charcoal (30 mg) in methanol (7 mL) under ambient pressure of hydrogen for 2.5 h. The mixture was then filtered and

concentrated under reduced pressure to give *Teoc*-(3-*Acp*)Ala-OH as a white solid which was directly used for the next step. This material was taken up with anhydrous DMF (1 mL), triethylamine (60 mg, 0.593 mmol) was added, and the mixture was heated to 80 °C with heat-gun and cooled then in water/ice bath. A solution of *Npys*Cl (25 mg, 0.131 mmol) in DMF (0.4 mL) was then added dropwise for 5 min and the mixture was stirred at 4 °C for 1 h. After this, triethylamine (14 mg, 0.138 mmol), then (dropwise) solution of *Npys*Cl (25 mg, 0.131 mmol) in DMF (0.4 mL) and finally a third portion of triethylamine (28 mg, 0.277 mmol) were added and the mixture was allowed to reach 20 °C. Stirring continued for a further 15 h, the reaction mixture was then diluted with water (30 mL), filtered and extracted with Et₂O (3 × 10 mL). pH of the water fraction was adjusted to 2 with solid KHSO₄ and the resulting emulsion was extracted with Et₂O (50 mL). The organic layer was washed with 1 M KHSO₄ (2 × 10 mL),

water (10×10 mL), brine (2×5 mL), dried, filtered and concentrated under reduced pressure. The resultant bright orange oil (0.049 g) was purified by preparative TLC (200×200 mm, EtOAc/hexane 1:2 (1.5% AcOH), 1 run) to give **201** (0.028 g, 44%) as an orange foam. To prevent decomposition this acid was transformed in its *tert*-butyl ammonium salt. $R_f = 0.26$ EtOAc/hexane 1:2 (1.5% AcOH); $[\alpha]_{20}^D = 32.0$ ($c = 0.8$, CHCl_3) (for *tert*-butyl ammonium salt); ^1H NMR (250 MHz, CDCl_3): $\delta = 0.03$ [s, 9 H, $\text{Si}(\text{CH}_3)_3$], 0.44 (ddd, $J = 6.0, 6.0, 6.0$ Hz, 1 H, 3'- H_a), 0.80–0.94 (m, 1 H, 3'- H_b), 0.94–1.15 (m, 1 H, 1'-H), 0.99 (t, $J = 8.5$ Hz, 2 H, 2-H, *Teoc*), 1.54–1.71 (m, 1 H, 3- H_a), 1.71–1.86 (m, 1 H, 3- H_b), 2.62 (ddd, $J = 6.8, 3.5, 3.5$ Hz, 1 H, 2'-H), 4.16 (t, $J = 8.5$ Hz, 2 H, 1-H, *Teoc*), 4.40 (ddd, $J = 6.4, 6.4, 6.4$ Hz, 1 H, 2-H), 4.60–6.30 (br, 2 H, CO_2H , NH), 5.34 (d, $J = 7.8$ Hz, 1 H, CONH), 8.52 (dd, $J = 8.3, 1.3$ Hz, 1 H, 4-H, *Npys*), 8.83 (dd, $J = 4.5, 1.3$ Hz, 1 H, 6-H, *Npys*); Absorption of 5-H, *Npys* was masked by residual signal of CDCl_3 ; ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = -1.5$ [+ , $\text{Si}(\text{CH}_3)_3$], 14.5 (–, C-3'), 15.2, 18.0 (+, C-1'), 17.7 (–, C-2, *Teoc*), 34.5 (–, C-3), 38.5 (+, C-2'), 53.4, 53.8 (+, C-2), 63.7, 65.8 (–, C-1, *Teoc*), 119.3 (+, C-5, *Npys*), 133.6 (+, C-4, *Npys*), 140.1 (C_{quat} , C-3, *Npys*), 153.6 (+, C-6, *Npys*), 156.4 (C_{quat} , NCO_2), 164.0 (C_{quat} , C-2, *Npys*), 176.7 (C_{quat} , C-1); IR (KBr): $\nu(\text{tilde}) = 3730$ – 2500 cm^{-1} , 3077, 2954, 2896, 1718, 1587, 1557, 1511, 1397, 1336, 1254, 1230, 1069; MS (ESI): positive, $m/z = 487$ (50) [$\text{M} - \text{H}^+ + 2\text{Na}^+$], 466 (100) [$\text{M} + \text{Na}^+$]; negative, $m/z = 441$ (100) [$\text{M} - \text{H}^+$].

N $_{\alpha}$ -Z,*N* $_{\delta}$ -*Npys*-(*S*)-Ornithine 1-(*S*)-phenethylamide (**204**): *N* $_{\alpha}$ -Z,*N* $_{\delta}$ -Boc-(*S*)-Ornithine 1-(*S*)-

phenethylamide **203** (0.145 g, 76%) was obtained from *Z*-Orn(Boc)-OH **202** (0.150 g, 0.409 mmol) and 1-(*S*)-phenethylamine (99 mg, 0.919 mmol) employing EDC (81 mg, 0.423 mmol) and HOBt (65 mg, crystallohydrate with 13%

H_2O , 0.422 mmol) in CH_2Cl_2 (5 mL) according to GP 5 as a white solid. This amide was treated with TFA (2 mL) within 20 min. All volatiles were then removed under reduced pressure and the

residue was taken up with toluene (2×15 mL) which was then distilled off to remove the last traces of TFA. A solution of NpysCl (77 mg, 0.404 mmol) was added dropwise for 5 min to an ice-cold solution of the amino amide trifluoroacetate and TEA (90 mg, 0.889 mmol) in THF (5 mL), the mixture was allowed to warm to 20 °C and stirring continued for a further 15 h. The reaction mixture was then taken up with Et₂O (50 mL) and subjected to usual aqueous work-up (GP 5). The organic layer was dried, filtered and concentrated under reduced pressure. The residue was purified by two preparative TLC (200 × 200 mm, EtOAc/hexane 1:2, 2 runs and then 200 × 200 mm, CHCl₃/MeOH 110:1, 3 runs) and finally by crystallization from Et₂O/hexane to give **204** (95 mg, 59%) as a bright yellow solid. $R_f = 0.24$ CHCl₃/MeOH 110:1, 2 runs; $[\alpha]_{20}^D = -27.3$ ($c = 0.34$, CHCl₃); ¹H NMR (250 MHz, CDCl₃): $\delta = 1.47$ (d, $J = 7.0$ Hz, 3 H, 2'-H, *Phet*), 1.50–1.65 (m, 2 H, 4-H), 1.65–1.82 (m, 1 H, 3-H_a), 1.83–2.00 (m, 1 H, 3-H_b), 1.54–1.71 (m, 1 H, 3-H_a), 1.71–1.86 (m, 1 H, 3-H_b), 2.82–2.98 (m, 2 H, 5-H), 3.53–3.64 (m, 1 H, 1'-H, *Phet*), 4.20 (ddd, $J = 8.0, 6.8, 6.8$ Hz, 1 H, 2-H), 4.99–5.14 (br, NH), 5.10 (s, 2 H, Bzl-H), 5.42 (d, $J = 8.0$ Hz, 1 H, CONH), 6.20–6.43 (br, 1 H, CONH), 7.14–7.38 (m, 10 H, Ar-H), 7.22 (dd, $J = 8.4, 4.5$ Hz, 1 H, 5-H, *Npys*), 8.49 (dd, $J = 8.4, 1.5$ Hz, 1 H, 4-H, *Npys*), 8.72 (dd, $J = 4.5, 1.5$ Hz, 1 H, 6-H, *Npys*); ¹³C NMR (62.9 MHz, CDCl₃): $\delta = 21.7$ (+, C-2', *Phet*), 25.8 (–, C-4), 30.2 (–, C-3), 45.4 (+, C-1', *Phet*), 48.7 (–, C-5), 54.5 (+, C-2), 66.8 (+, Bzl-C), 119.3 (+, C-5, *Npys*), 125.9, 127.1, 127.7, 128.0, 128.4, 128.5 (+, Ar-C), 133.6 (+, C-4, *Npys*), 136.0, 140.0 (C_{quat}, Ar-C), 143.0 (C_{quat}, C-5, *Npys*), 153.4 (+, C-6, *Npys*), 156.2 (C_{quat}, NCO₂), 163.9 (C_{quat}, C-2, *Npys*), 170.8 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 3066$ cm^{–1}, 3030, 2954, 2868, 1710, 1650, 1586, 1556, 1510, 1451, 1394, 1334, 1233, 1068; MS (ESI): positive, $m/z = 1069$ (100) [2M + Na⁺], 546 (16) [M + Na⁺].

Attempts of the cleavage of Npys-group from 204: An aliquot of the amide **204** (10 mg, 19.10 μmol) was dissolved in CH₂Cl₂ (15 mL) and treated with MgBr₂·Et₂O (99 mg, 0.383 mmol) and EtSH (12 mg, 0.193 mmol) according to GP 10. After 4.5 h only the partial deprotection took

place (ca. 30% according to TLC). Another aliquot of **204** (10 mg, 19.10 μmol) was dissolved in CH_2Cl_2 (4 mL) and 2-mercaptopyridine-N-oxide (0.150 g, 1.175 mmol) was added. After 5 h, the mixture contained (according to TLC) only traces of the deprotected material. The third aliquot of **34** (10 mg, 19.10 μmol) was dissolved in CH_2Cl_2 (0.5 mL) and treated with 0.1 M PPh_3 in CH_2Cl_2 (0.2 mL) and 0.1 M $\text{Py}\cdot\text{HCl}$ in CH_2Cl_2 (0.2 mL). The starting material was completely consumed in 2 min (according to TLC). The mixture was concentrated under reduced pressure and the residue was triturated with EtOAc and filtered to give the mixture of the product of deprotection and $\text{Py}\cdot\text{HCl}$ (4 mg, 2:1 molar ratio according to ^1H NMR).

[(*S*)-(3-Acp)Ala(Npys)⁷]-MOM-**3**: An ethereal solution (50 mL) of the *tert*-butylammonium salt of **201** (10.0 mg, 19.39 μmol) was washed with 1 M KHSO_4 (3×5 mL), water (3×5 mL), brine (2×5 mL), dried, filtered and concentrated under reduced pressure. The resultant *N*-protected amino acid was dried at 0.02 Torr for 2 h and then coupled with the depsipeptide, obtained after deprotection of **171c** (10.0 mg, 10.22 μmol) with 10% anisole in TFA (1.1 mL) according to GP 11 for 2 h, using HATU (7.4 mg, 19.46 μmol), HOAt (2.6 mg, 19.24 μmol), DIEA (1.25 mg, 9.67 μmol) and TMP (7.04 mg, 58.10 μmol) in CH_2Cl_2 (0.7 mL) according to GP 7 for 15 h. The mixture was then taken up with Et_2O (20 mL) and the crude product obtained after usual aqueous work-up (GP 5) was purified by preparative TLC (100 $\times$ 200 mm, acetone/hexane 1:3, 2 runs) to give Teoc-(*S*)-(3-Acp)Ala(Npys)-*cyclo*-hexadepsipeptide (10.9 mg, 83%, R_f = 0.22 acetone/hexane 1:2) as a yellow foam which was used for the next step without any characterization. This substance (10.9 mg, 8.50 μmol) was deprotected with TFA (1 mL) for 1 h. The mixture was concentrated under reduced pressure at 20 $^\circ\text{C}$ and then taken up with toluene (3×15 mL) which was distilled off to remove the last traces of TFA. The resultant deprotected depsipeptide was coupled with Chpca(MOM)-OH **109** (3.5 mg, 17.02 μmol) using HATU (6.5 mg, 17.09 μmol), DIEA (1.10 mg, 8.51 μmol) and TMP (6.2 mg, 51.16 μmol) in CH_2Cl_2 (1 mL)

according to GP 7 for 3.5 h. The mixture was then taken up with Et₂O (20 mL) and the crude product obtained after usual aqueous work-up (GP 5) was purified by preparative TLC (200 × 200 mm, acetone/hexane 3:8, 2 runs) to give [(*S*)-(3-*Acp*)Ala(*Npys*)⁷]-MOM-**3** (7.1 mg, 64%) as an yellow foam. $R_f = 0.09$ acetone/hexane 3:8; $[\alpha]_{20}^D 103.0$ ($c = 0.60$, CHCl₃); ¹H NMR (600 MHz, CDCl₃): $\delta = -0.33 - -0.19$ [m, 1 H, 3'-H_a, (3-*Ncp*)Ala], 0.02–0.15 [m, 1 H, 3-H_a, (3-*Ncp*)Ala], 0.38 [ddd, $J = 6.0, 6.0, 6.0$ Hz, 1 H, 3'-H_a, (3-*Acp*)Ala], 0.53–0.62 [m, 1 H, 1'-H, (3-*Ncp*)Ala], 0.68–0.76 [m, 1 H, 3-H_b, (3-*Ncp*)Ala], 0.86–0.90 [m, 1 H, 3'-H_b, (3-*Acp*)Ala], 0.92 (t, $J = 7.2$ Hz, 3 H, 5-H, *Ile*), 0.95–1.01 [m, 1 H, 1'-H, (3-*Acp*)Ala], 1.07–1.15 [m, 1 H, 3'-H_b, (3-*Ncp*)Ala], 1.09 (d, $J = 6.6$ Hz, 3 H, 1'-H, *Ile*), 1.27–1.32 (m, 1 H, 4-H_a, *Ile*), 1.28 [d, $J = 7.2$ Hz, 3 H, 4-H, (β Me)*Phe*], 1.35–1.46 [m, 1 H, 3-H_a, (3-*Acp*)Ala] 1.41 [d, $J = 7.8$ Hz, 3 H, 4-H, (β Me)*Phe*], 1.56 (d, $J = 6.6$ Hz, 3 H, 4-H, *a*-Thr), 1.56–1.62 (m, 1 H, 4-H_b, *Ile*), 1.62–1.68 [m, 1 H, 3-H_b, (3-*Acp*)Ala] 1.69 [dd, $J = 6.6, 1.8$ Hz, 3 H, 3'-H, (4-*PE*)-*Pro*], 1.81 [ddd, $J = 12.0, 12.0, 12.0$ Hz, 1 H, 3-H_a, (4-*PE*)*Pro*], 1.82–1.87 (m, 1 H, 3-H, *Ile*), 2.38 [ddd, $J = 12.0, 6.0, 6.0$ Hz, 1 H, 3-H_b, (4-*PE*)-*Pro*], 2.61 [ddd, $J = 6.0, 2.5, 2.5$ Hz, 1 H, 1'-H, (3-*Acp*)Ala], 2.82–2.95 [m, 1 H, 3-H, (β Me)*Phe*], 3.09–3.18 [m, 1 H, 2'-H, (3-*Ncp*)Ala], 3.21–3.33 [m, 2 H, 4-H, 5-H_a, (4-*PE*)-*Pro*], 3.64–3.74 [m, 1 H, 3-H, (β Me)*Phe*], 3.74 (s, 3 H, OMe), 3.85–3.94 (m, 1 H, 2-H), 3.94–4.00 [m, 1 H, 5-H_b, (4-*PE*)-*Pro*], 4.10–4.17 (br, 1 H, NH, *Npys*), 4.25 (dd, $J = 9.6, 9.6$ Hz, 1 H, 2-H), 4.28 (dd, $J = 10.8, 6.0$ Hz, 1 H, 2-H), 4.57 (dd, $J = 9.6, 1.8$ Hz, 1 H, 2-H), 4.60–4.69 (m, 1 H, 2-H), 4.67 [ddd, $J = 8.4, 8.4, 8.4$ Hz, 1 H, 2-H, (3-*Acp*)Ala], 5.01–5.12 (m, 1 H, 2-H), 5.24–5.29 [m, 1 H, 1'-H, (4-*PE*)-*Pro*], 5.38 (q, $J = 7.2$ Hz, 1 H, 3-H, *a*-Thr), 5.46 (d, $J = 6.0$ Hz, 1 H, H_a, OCH₂O), 5.51 (d, $J = 6.0$ Hz, 1 H, H_b, OCH₂O), 5.63 [dq, $J = 10.8, 6.6$ Hz, 1 H, 2'-H, (4-*PE*)-*Pro*], 5.74 (d, $J = 7.2$ Hz, 1 H, NH), 6.12 (d, $J = 4.8$ Hz, 1 H, 4-H, Chpca), 6.79 (d, $J = 4.5$ Hz, 1 H, 3-H, Chpca), 6.81 (d, $J = 9.6$ Hz, 1 H, NH), 7.03–7.11 (m, 4 H, Ar-H), 7.12–7.18 (m, 4 H, Ar-H, 5-H, *Npys*), 7.21–7.30 (m, 4 H, Ar-H, NH), 7.37 (d, $J = 9.0$ Hz, 1 H, NH), 7.50–7.61 (br, 1 H, NH), 7.52 (d, $J = 10.2$ Hz, 1 H, NH), 8.48 (dd, $J = 8.4, 1.8$ Hz, 1 H, 4-H, *Npys*),

8.75 (d, $J = 3.6$ Hz, 1 H, 6-H, *Npys*), 8.78–8.88 (br, 1 H, NH); ^{13}C NMR (150.8 MHz, CDCl_3): δ = 10.8 (+, C-5, *Ile*), 13.3 [+ , C-4, (βMe)*Phe*, C-3', (*4-PE*)-*Pro*], 14.5 [–, C-3', (*3-Acp*)*Ala*], 15.2 (+, C-1', *Ile*), 17.4 (+, C-4, *a-Thr*), 17.6 [–, C-3', (*3-Ncp*)*Ala*], 18.2 [+ , C-1', (*3-Acp*)*Ala*], 18.3 [+ , C-4, (βMe)*Phe*], 20.8 [+ , C-1', (*3-Ncp*)*Ala*], 25.0 (–, C-4, *Ile*), 34.1 [–, C-3, (*3-Ncp*)*Ala*], 35.5 [–, C-3, (*4-PE*)-*Pro*], 36.7 [+ , C-4, (*4-PE*)-*Pro*], 38.5 [–, C-3, (*3-Acp*)*Ala*], 38.7 [+ , C-2', (*3-Acp*)*Ala*], 38.8 (+, C-3, *Ile*), 39.6 [+ , C-3, (βMe)*Phe*], 42.7 [+ , C-3, (βMe)*Phe*], 51.3 (+, C-2), 51.5 (+, C-2), 52.8 [–, C-5, (*4-PE*)-*Pro*], 54.7 [+ , C-2, (*3-Acp*)*Ala*], 54.8 (+, C-2), 58.5 [+ , C-2', (*3-Ncp*)*Ala*], 59.3 (+, OMe, C-2), 59.5 (+, C-2), 60.1 (+, C-2), 61.4 (+, C-2), 69.8 (+, C-3, *a-Thr*), 104.3 (+, C-4, *Chpca*), 106.1 (–, OCH_2O), 111.0 (+, C-3, *Chpca*), 119.1 (+, C-5, *Npys*), 119.3 (C_{quat} , C-2, *Chpca*), 122.0 (C_{quat} , C-2, *Chpca*), 126.8, 127.0, 127.5, 127.6, 128.5, 128.6 (+, Ar-C), 128.3 [+ , C-2', (*4-PE*)-*Pro*], 133.5 (+, C-4, *Npys*), 140.0 (C_{quat} , C-3, *Npys*), 142.3, 142.4 (C_{quat} , Ar-C), 153.4 (+, C-6, *Npys*), 158.4 (C_{quat} , C-1, *Chpca*), 164.5 (C_{quat} , C-2, *Npys*), 169.1, 170.0, 170.1 ($\times 2$), 171.3 ($\times 2$), 172.4 (C_{quat} , C-1); Peak 127.6 (+, Ar-C) masked absorption for [+ , C-1', (*4-PE*)-*Pro*]; MS (ESI): positive mode, m/z (%) = 1320 (100) [$\text{M} + \text{Na}^+$]; negative mode, m/z (%) = 1296 (100) [$\text{M} - \text{H}^+$].

*Attempt of the deprotection of [(3-Acp)Ala(*Npys*)⁷]-MOM-3:* A solution of the protected

depsipeptide (6.0 mg, 4.62 μmol) in CH_2Cl_2 (4 mL) was treated with

$\text{MgBr}_2 \cdot \text{Et}_2\text{O}$ (48 mg, 18.59 μmol) and EtSH (14 μL , 18.90 μmol)

according to GP 10 for 4 h. The crude product after usual aqueous work-

up (GP 10) was dried, dissolved in CH_2Cl_2 (0.5 mL) and treated with 0.1

M PPh_3 in CH_2Cl_2 (0.4 mL) and 0.1 M $\text{Py} \cdot \text{HCl}$ in CH_2Cl_2 (0.4 mL) for 10 min. The mixture, containing (according to TLC) two Erlich reagent active compounds, was then placed on the top

of the column with Sephadex LH-20. Elution with CH_2Cl_2 , then $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 10:1, then

$\text{CH}_2\text{Cl}_2/\text{MeOH}$ 3:1, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 1:1 and finally with pure MeOH didn't give the desired

product. *Boc-(S)-Orn(Z)-OtBu* (**206**): To a solution of *Boc-(S)-Orn(Z)-OH* **205** (1.50 g, 4.09

mmol) in DMA (31 mL) *tert*-butyl bromide (22.2 mL, 197 mmol), K₂CO₃ (14.71 g, 106.4 mmol) and benzyltriethylammonium chloride (0.932 g, 4.09 mmol) were added and the mixture was vigorously stirred at 55 – 60 °C for 48 h. The reaction mixture was then poured in water (0.7 l) and the resultant emulsion was extracted with Et₂O (2 × 200 mL). The organic layer was washed with water (14 × 50 mL), brine (2 × 50 mL), dried, filtered and concentrated to give **206** (1.63 g, 94%) as a colorless oil. *R*_f = 0.21 EtOAc/hexane 1:4.

Boc-(S)-(NO₂)Nva-OtBu (207): Ester **206** (1.63 g, 3.86 mmol) was hydrogenated on 10% Pd on charcoal (0.28 g) in EtOAc (15 mL) under ambient pressure of hydrogen for 3 h. The mixture was then filtered and concentrated under reduced pressure to give the crude monoprotected diamino ester (*R*_f = 0.20 CHCl₃/MeOH 10:1) which was immediately used without any purification for the next step. This material was dissolved in 1,2-dichloroethane (50 mL) and added to the vigorously stirred solution MCPBA (7.397 g, 90% purity, 42.86 mmol) in 1,2-dichloroethane (200 mL) under intensive reflux for 2 min. Stirring and refluxing continued for an additional 20 min and the reaction flask was then placed in a water/ice bath. After 15 min the reaction mixture was concentrated under reduced pressure to ca. 120 mL, diluted with Et₂O (400 mL), washed with aqueous saturated Na₂SO₃ (2 × 100 mL), saturated aqueous NaHCO₃ (3 × 100 mL), water (2 × 100 mL), brine (2 × 50 mL), dried, filtered and concentrated under reduced pressure. The residual brown oil was purified by column chromatography (EtOAc/hexane 1:6, *R*_f = 0.36) to give **207** (0.73 g, 58%) as a faint yellow oil. ¹H NMR (250 MHz, CDCl₃): δ = 1.44 [s, 9 H, C(CH₃)₃], 1.46 [s, 9 H, C(CH₃)₃], 1.63–1.80 (m, 1 H, 4-H_a), 1.80–1.95 (m, 1 H, 4-H_b), 1.95–2.05 (m, 2 H, 3-H), 4.10–4.28 (m, 1 H, 2-H), 4.43 (t, *J* = 6.6 Hz, 2 H, 5-H), 5.12 (d, *J* = 7.5 Hz, 1 H, NH); ¹³C NMR (62.9 MHz, CDCl₃): δ = 23.0 (–, C-4), 27.8 [+ , C(CH₃)₃], 28.1 [+ , C(CH₃)₃], 29.6 (–, C-3), 52.9 (+, C-2), 74.8 (–, C-5), 79.8 [C_{quat}, C(CH₃)₃], 82.4 [C_{quat}, C(CH₃)₃], 155.3 (C_{quat}, NCO₂), 170.9 (C_{quat}, C-1); IR (film): ν(tilde) = 3443 cm^{–1}, 3008, 2980, 2868, 1743, 1556, 1499, 1456, 1393, 1369, 1253, 1149, 1055; MS (ESI): positive mode, *m/z* = 341 (100) [M

+ Na⁺]; elemental analysis calcd (%) for C₁₄H₂₆N₂O₆ (318.4): C 52.82, H 8.23, N 8.80; found C 52.56, H 8.57, N 8.84.

Z-(*S*)-(NO₂)Nva-*O**t*Bu: This compound was obtained from *Z*-(*S*)-(NO₂)Nva-*O**t*Bu in a similar manner as compound **207** in 41% yield as a faint yellow oil. *R*_f = 0.21 EtOAc/hexane 1:4; [α]₂₀^D 10.7 (*c* = 0.60, CHCl₃); ¹H NMR (250 MHz, CDCl₃): δ = 1.47 [s, 9 H, C(CH₃)₃], 1.65–1.82 (m, 1 H, 4-H_a), 1.82–1.98 (m, 1 H, 4-H_b), 1.98–2.12 (m, 2 H, 3-H), 4.13–4.25 (m, 1 H, 2-H), 4.41 (t, *J* = 6.6 Hz, 2 H, 5-H), 5.11 (s, 2 H, Bzl-H), 5.32 (d, *J* = 7.5 Hz, 1 H, NH), 7.26–7.39 (m, 5 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 22.8 (–, C-4), 27.7 [+ , C(CH₃)₃], 29.3 (–, C-3), 53.3 (+, C-2), 66.7 (–, Bzl-C), 74.6 (–, C-5), 82.5 [C_{quat}, C(CH₃)₃], 127.9, 128.0, 128.3 (+, Ar-H), 136.0 (C_{quat}, Ar-C), 155.7 (C_{quat}, NCO₂), 170.5 (C_{quat}, C-1); IR (film): ν(tilde) = 3440 cm^{–1}, 3034, 2980, 2935, 1742, 1555, 1500, 1451, 1371, 1349, 1257, 1218, 1153, 1058; MS (CI), *m/z* (%): 370 (100) [M + NH₄⁺]; elemental analysis calcd (%) for C₁₇H₂₄N₂O₆ (352.4): C 57.94, H 6.86, N 7.95; found C 57.75, H 6.99, N 8.10.

(*S*)-5-Nitronorvaline hydrochloride (**208**): Compound **207** (1.20 g, 3.77 mmol) was deprotected

with TFA (5 mL) for 1 h. All volatiles were then removed under reduced pressure and the residue was taken up with toluene (2 × 20 mL) which was then distilled off to remove the last traces of TFA. To transform the resulting trifluoroacetate into hydrochloride it was taken up with 2 M

HCl in EtOAc (3 × 20 mL) which was then distilled off. The resulting oil was triturated with acetone to give light brown precipitate. Et₂O was then added to complete precipitation. The crude product was filtered off and washed with acetone/Et₂O 1:1 (200 mL) to give HCl·**208** (0.420 g, 56%) as an off-white solid. M.p 132–133 °C (decomp.); [α]₂₀^D 16.8 (*c* = 0.22, 0.1 M HCl) [lit. (for free base):^[144] [α]₂₀^D 22.8 (*c* = 0.56, dil. HCl)]; ¹H NMR (300 MHz, D₂O): δ =

1.89–2.24 (m, 4 H, 3-H, 4-H), 4.06 (t, $J = 5.0$ Hz, 1 H, 2-H), 4.56 (t, $J = 5.4$ Hz, 2 H, 5-H); ^{13}C NMR (50.3 MHz, D_2O): $\delta = 22.4$ (–, C-4), 26.8 (–, C-3), 52.5 (+, C-2), 74.5 (–, C-5), 171.7 (C_{quat} , C-1); IR (KBr): $\nu(\text{tilde}) = 3750\text{--}1800\text{ cm}^{-1}$, 2933, 1735, 1552, 1500, 1379, 1349, 1244; MS (ESI): positive mode, $m/z = 229$ (5) $[\text{M} - 2\text{H}^+ + 3\text{Na}^+]$, 207 (8) $[\text{M} - \text{H}^+ + 2\text{Na}^+]$, 185 (12) $[\text{M} + \text{Na}^+]$; negative mode, $m/z = 345$ (100) $[2\text{M} - 2\text{H}^+ + \text{Na}^+]$; elemental analysis calcd (%) for $\text{C}_5\text{H}_{11}\text{N}_2\text{O}_4\text{Cl}$ (198.6): C 30.24, H 5.58, N 14.10; found C 30.50, H 5.30, N 13.97.

Teoc-(*S*)-(NO₂)*Nva*-OH (**209**) from **207**: Compound **207** (0.220 g, 0.691 mmol) was deprotected

with TFA (1 mL) for 1 h. All volatiles were then removed under reduced pressure and the residue was taken up with toluene (2×20 mL) which was then distilled off to remove the last traces of TFA. The crude product was filtered off and washed with acetone/ Et_2O 1:1 (50 mL) to

give TFA-**208** (0.105 g) as a white solid. A solution of TeocOSu (0.180 g, 0.694 mmol) in acetone (5 mL) was added to a vigorously stirred solution of this material and NaHCO_3 (0.159 g, 1.893 mmol) in water (5 mL) (if emulsion formed acetone and/or water were added to obtain homogeneous solution) and stirring continued for a further 2 h. *N,N*-dimethylaminopropylamine (0.07 mL, 0.56 mmol) was then added. After an additional 10 min acetone was removed under reduced pressure and pH of the residual water solution was adjusted to 2–3 with 1 M NaHSO_4 . The resulting emulsion was extracted with Et_2O (50 mL) and the ethereal layer was washed with 1 M KHSO_4 (3×10 mL), water (10×10 mL), brine (2×5 mL), dried, filtered and concentrated under reduced pressure. The residual oil solidified on seeding and the crude product was crystallized from Et_2O /pentane to give **209** (0.108 g, 51% on 2 steps) as a white solid. $R_f = 0.24$ EtOAc/hexane 1:2 (1.5% AcOH) (staining with ninhydrin); M.p 70–72 °C (decomp.); $[\alpha]_{20}^D$ 23.7 ($c = 0.30$, CHCl_3); ^1H NMR (250 MHz, CDCl_3): $\delta = 0.04$ [s, 9 H, $\text{Si}(\text{CH}_3)_3$], 1.00 (t, $J = 8.5$ Hz, 2 H, 2-H, *Teoc*), 1.69–1.96 (m, 1 H, 4-H_a), 1.96–2.22 (m, 3 H, 4-H_b, 3-H), 4.18 (t, $J = 8.3$ Hz, 2 H, 1-H, *Teoc*), 4.20–4.45 (m, 1 H, 2-H), 4.44 (t, $J = 6.1$ Hz, 2 H, 5-H), 5.18–5.26, 6.95–7.07 (2

× br, 1 H, NH); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = -1.6$ [+ , $\text{Si}(\text{CH}_3)_3$], 17.6 (–, C-2, *Teoc*), 22.9, 23.2 (–, C-4), 29.2 (–, C-3), 52.8, 53.3 (+, C-2), 64.0, 65.0 (–, C-1, *Teoc*), 74.7 (–, C-5), 156.6, 157.6 (C_{quat} , NCO_2), 175.0, 175.7 (C_{quat} , C-1); IR (KBr): $\nu(\text{tilde}) = 3750\text{--}2800\text{ cm}^{-1}$, 2957, 2898, 1754, 1731, 1657, 1556, 1249, 1205, 1182, 1070; MS (ESI): positive mode, $m/z = 329$ (100) [$\text{M} + \text{Na}^+$]; negative mode, $m/z = 305$ (100) [$2\text{M} - \text{H}^+$]; elemental analysis calcd (%) for $\text{C}_{11}\text{H}_{22}\text{N}_2\text{O}_6\text{Si}$ (306.4): C 43.12, H 7.24, N 9.14; found C 43.07, H 7.55, N 9.03.

Teoc-(*S*)-(NO₂)*Nva*-OH (**38**) from *Z*-(*S*)-(NO₂)*Nva*-*OtBu*: *Z*-(*S*)-(NO₂)*Nva*-*OtBu* (0.300 g, 0.851 mmol) was deprotected with 3.4 M HBr in AcOH (1.5 mL) for 40 min. Ether (50 mL) was then added to the reaction mixture and the precipitating red oil was washed with Et₂O (3 × 50 mL), dried and used without further purification for the next step. The treatment with *Teoc*OSu (1.033 mmol) and NaHCO₃ (0.315 g, 3.750 mmol) in a water/acetone mixture gave after usual work-up (see above) the crude product as a red oil which was further purified by column chromatography (EtOAc/hexane 1:2 (1.5% AcOH), $R_f = 0.24$ (staining with ninhydrin)) and finally by trituration with hexane to give **38** (0.111 g, 43% on 2 steps) as a white solid.

Chpca(*MOM*)-(NO₂)*Nva*-*Phe*-*Val*-OMe (**211**): Dipeptide Boc-*Phe*-*Val*-OMe **117** (10 mg, 26.42 μmol) was deprotected with TFA (1 mL) for 20 min. All volatiles were then removed and the residue was taken up with toluene (2 × 15 mL) which was distilled off to remove the last traces of TFA to give corresponding trifluoroacetate as a white solid which was further coupled with *N*-*Teoc* protected nitronorvaline **209** (16 mg, 52.22 μmol) using HATU (20.1 mg, 52.86 μmol), HOAt (7.1 mg, 52.54 μmol), DIEA (3.4 mg, 26.31 μmol) and TMP (19.2 mg, 158.5 μmol) in CH₂Cl₂ (3 mL) according to GP 7 for 16 h. The crude product obtained after usual aqueous work-up (GP 5) was further purified by preparative TLC (200 × 200 mm, EtOAc/hexane 1:3, 2 runs, then EtOAc/hexane 3:7, 1 run) to give *Teoc*-(NO₂)*Nva*-*Phe*-*Val*-OMe **210** (8.9 mg, 59%) as a colorless glass. $R_f = 0.27$ EtOAc/hexane 1:1.5; ^1H NMR (250 MHz, CDCl_3): $\delta = 0.02$ [s,

9 H, Si(CH₃)₃], 0.81 (d, $J = 6.8$ Hz, 3 H, 4-H_a, *Val*), 0.85 (d, $J = 6.8$ Hz, 3 H, 4-H_b, *Val*), 0.97 (dd, $J = 8.8, 8.8$ Hz, 2 H, 2-H, *Teoc*), 1.53–2.02 [m, 4 H, 3-H, 4-H, (*NO*₂)*Nva*], 2.10 (octet, $J = 6.8$ Hz, 1 H, 3-H, *Val*), 3.06 (d, $J = 7.3$ Hz, 2 H, 3-H, *Phe*), 3.70 (s, 3 H, OMe), 4.01–4.40 (m, 3 H, 2-H, *Val*, 1-H, *Teoc*), 4.35 [t, $J = 6.6$ Hz, 2 H, 5-H, (*NO*₂)*Nva*], 4.43 [dd, $J = 8.5, 5.3$ Hz, 1 H, 2-H, (*NO*₂)*Nva*], 4.71 (dd, $J = 7.3, 7.3$ Hz, 1 H, 2-H, *Phe*), 5.28 (d, $J = 8.5$ Hz, 1 H, NH), 6.46 (d, $J = 8.8$ Hz, 1 H, NH), 6.79 (d, $J = 7.8$ Hz, 1 H, NH), 7.13–7.32 (m, 5 H, Ar-H). This compound (8.6 mg, 15.17 μ mol) was deprotected with TFA (1 mL) for 1 h. All volatiles were removed under reduced pressure, the residue was taken up with toluene (2 $\times$ 15 mL) which was then distilled off to remove the last traces of TFA to give trifluoroacetate of the deprotected tripeptide as a colorless solid which was coupled with acid **109** (4.1 mg, 19.94 μ mol) using HATU (7.2 mg, 18.93 μ mol), DIEA (2.0 mg, 15.47 μ mol) and TMP (7.3 mg, 60.24 μ mol) in CH₂Cl₂ (3 mL) according GP 7 for 2 h. The crude product obtained after usual aqueous work-up (GP 5) was finally purified by preparative TLC (200 $\times$ 200 mm, EtOAc/hexane 1:3, 3 runs) to give the acylated tripeptide **211** (7.3 mg, 79%). $R_f = 0.10$ EtOAc/hexane 1:3; $[\alpha]_{20}^D -32.3$ ($c = 0.50$, CHCl₃); ¹H NMR (600 MHz, CDCl₃): $\delta = 0.83$ (d, $J = 6.6$ Hz, 3 H, 4-H_a, *Val*), 0.86 (d, $J = 6.6$ Hz, 3 H, 4-H_b, *Val*), 1.68–1.75 [m, 1 H, 3-H_a, (*NO*₂)*Nva*], 1.86–1.94 [m, 1 H, 3-H_a, (*NO*₂)*Nva*], 2.09 (octet, $J = 6.6$ Hz, 1 H, 3-H, *Val*), 2.99 (dd, $J = 13.8, 7.8$ Hz, 1 H, 3-H_a, *Phe*), 3.09 (dd, $J = 13.8, 6.6$ Hz, 2 H, 3-H_b, *Phe*), 3.53 (s, 3 H, OMe, *MOM*), 3.69 (s, 3 H, OMe), 4.363 [dd, $J = 6.6, 6.6$ Hz, 2 H, 5-H, (*NO*₂)*Nva*], 4.35 (dd, $J = 8.4, 5.4$ Hz, 1 H, 2-H, *Val*), 4.70 (dd, $J = 13.8, 8.4$ Hz, 1 H, 2-H, *Phe*), 4.72 [dd, $J = 7.2, 7.2$ Hz, 1 H, 2-H, (*NO*₂)*Nva*], 5.19 (dd, $J = 12.6, 12.6$ Hz, 2 H, OCH₂O), 6.05 (d, $J = 4.8$ Hz, 1 H, 4-H, *Chpca*), 6.63 (d, $J = 8.4$ Hz, 1 H, NH), 6.74 (d, $J = 4.8$ Hz, 1 H, 3-H, *Chpca*), 7.12–7.20 (m, 5 H, Ar-H), 7.21 (d, $J = 7.8$ Hz, 1 H, NH); ¹³C NMR (150.8 MHz, CDCl₃): $\delta = 17.8, 18.8$ (+, C-4, *Val*), 23.2 [–, C-4, (*NO*₂)*Nva*], 29.1 [–, C-3, (*NO*₂)*Nva*], 31.1 (+, C-3, *Val*), 37.9 (–, C-3, *Phe*), 51.9 (+, C-2), 52.1 (+, C-2), 54.7 (+, C-2), 57.4 (+, OMe, *MOM*), 59.7 (+, OMe), 74.8 [–, C-5, (*NO*₂)*Nva*], 104.4 (+, C-4, *Chpca*), 104.8 (–, OCH₂O), 111.6 (+, C-3, *Chpca*), 118.8 (C_{quat}, C-2, *Chpca*), 121.9 (C_{quat}, C-5, *Chpca*), 126.9,

128.5, 129.2 130.4 (+, Ar-C), 136.3 (C_{quat},Ar-C), 158.6 (C_{quat}, C-1, *Chpca*), 170.6, 171.0, 171.9 (C_{quat}, C-1); MS (ESI): positive, $m/z = 632$ (48) [M + Na⁺]; negative, $m/z = 608$ (100) [M – H⁺].

*[(S)-(NO₂)Nva⁷]-Hormaomycin [(S)-(NO₂)Nva⁷]-**(3)***: Cyclodepsipeptide **171c** (19.0 mg, 19.42 μmol) was deprotected with 10% anisole in TFA (1.1 mL) according to GP 11 for 2 h and the resultant deprotected depsipeptide was coupled with *N*-Teoc protected amino acid **209** (16.9 mg, 55.16 μmol) using HATU (21.0 mg, 55.23 μmol), HOAt (7.5 mg, 55.50 μmol), DIEA (2.86 mg, 22.09 μmol) and TMP (24.12 mg, 199.04 μmol) in CH₂Cl₂ (2 mL) according to GP 7 for 15 h. The mixture was then diluted with Et₂O (20 mL) and the crude product obtained after usual aqueous work-up (GP 5) was purified by preparative TLC (200 × 200 mm, acetone/hexane 1:3, 3 runs) to give Teoc-(*S*)-(NO₂)Nva-*cyclo*-hexadepsipeptide (19.0 mg, 88%, $R_f = 0.06$ acetone/hexane 1:3, 3 runs) as a colorless glass which was used for the next step without any characterization. Teoc group was cleaved from this substance (19.0 mg, 16.99 μmol) with TFA (1.0 mL) for 1 h. The mixture was concentrated under reduced pressure at 20 °C and then taken up with toluene (3 × 15 mL) which was distilled off to remove the last traces of TFA. The resultant deprotected depsipeptide was coupled with *Chpca*(MOM)-OH **109** (7.0 mg, 34.04 μmol) using HATU (12.9 mg, 33.93 μmol), DIEA (2.85 mg, 22.08 μmol) and TMP (16.06 mg, 132.49 μmol) in CH₂Cl₂ (2 mL) according to GP 7 for 2.5 h. The mixture was then diluted with Et₂O (20 mL) and the crude product obtained after usual aqueous work-up (GP 5) was purified by preparative TLC (200 × 200 mm, acetone/hexane 1:2.7) to give *O*-MOM protected *[(S)-(NO₂)Nva⁷]-3* (15.0 mg, 76%, $R_f = 0.17$ acetone/hexane 1:2.5) as a colorless glass which was used for the next step without any characterization. This substance (15.0 mg, 12.91 μmol) was deprotected using MgBr₂·Et₂O (178 mg, 0.688 mmol) and EtSH (0.03 mL, 0.405 mmol) in CH₂Cl₂ (10 mL) according to GP 10 for 3 h. The mixture was taken up with Et₂O and the crude product obtained after usual aqueous work-up (GP 10) was crystallized from CH₂Cl₂/pentane. The mother liquor was concentrated under reduced and crystallized from Et₂O/pentane. This

second crop of the desired product was combined with first one to give [(*S*)-(NO₂)Nva⁷]-**3** (12.9 mg, 90%, 60% on 5 steps from **24**) as a white solid. R_f = 0.11 acetone/hexane 1:2.5; analytical HPLC: column B, gradient 25% → 85% MeCN in 0.15% ammonium acetate buffer (pH = 5.5) for 25 min, flow rate = 0.5 mL/min, t_R = 20.22 min, purity > 93%; $[\alpha]_{20}^D$ 13.0 (c = 0.1, CHCl₃); ¹H NMR (600 MHz, CDCl₃): δ = −0.72 – −0.59 [m, 1 H, 3'-H_a, (*3-Ncp*)Ala], −0.19 – −0.06 [m, 1 H, 3-H_a, (*3-Ncp*)Ala], 0.25–0.34 [m, 1 H, 1'-H, (*3-Ncp*)Ala], 0.55 [ddd, J = 14.4, 4.8, 4.8 Hz, 1 H, 3-H_b, (*3-Ncp*)Ala], 0.92 (t, J = 7.2 Hz, 3 H, 5-H, *Ile*), 0.97–1.04 [m, 1 H, 3'-H_b, (*3-Ncp*)Ala], 1.06 (d, J = 7.2 Hz, 3 H, 1'-H, *Ile*), 1.24–1.32 (m, 1 H, 4-H_a, *Ile*), 1.30 [d, J = 7.2 Hz, 3 H, 4-H, (*βMe*)Phe], 1.42 [d, J = 7.2 Hz, 3 H, 4-H, (*βMe*)Phe], 1.53 (d, J = 6.6 Hz, 3 H, *α-Thr*), 1.58–1.65 (m, 1 H, 4-H_b, *Ile*), 1.67–1.76 [m, 1 H, 3-H_a, (NO₂)Nva], 1.69 [dd, J = 7.2, 1.8 Hz, 3 H, 3'-H, (*4-PE*)Pro], 1.76–1.84 [m, 2 H, 3-H_b, (NO₂)Nva, 3-H_a, (*4-PE*)Pro], 1.85–1.92 (m, 1 H, 3-H, *Ile*), 1.92–2.09 [m, 2 H, 4-H, (NO₂)Nva], 2.38 [ddd, J = 12.0, 5.4, 5.4 Hz, 1 H, 4-H_b, (*4-PE*)Pro], 2.91 [ddd, J = 6.6, 3.0, 3.0 Hz, 1 H, 2'-H, (*3-Ncp*)Ala], 3.04 [dq, J = 11.1, 7.2 Hz, 1 H, 3-H, (*βMe*)Phe], 3.24–3.34 [m, 2 H, 4-H, 5-H_a, (*4-PE*)Pro], 3.45–3.52 [m, 1 H, 2-H, (*3-Ncp*)Ala], 3.69 [dq, J = 4.2, 7.2 Hz, 1 H, 3-H, (*βMe*)Phe], 3.95–4.03 [m, 1 H, 5-H_b, (*4-PE*)Pro], 4.26 [dd, J = 11.7, 6.0 Hz, 1 H, 2-H, (*4-PE*)Pro], 4.36 [t, J = 6.6 Hz, 2 H, 5-H, (NO₂)Nva], 4.37 [dd, J = 11.0, 11.0 Hz, 1 H, 2-H, (*βMe*)Phe], 4.49 [dd, J = 9.3, 4.2 Hz, 1 H, 2-H, (*βMe*)Phe], 4.56 (dd, J = 9.0, 2.4 Hz, 1 H, 2-H, *α-Thr*), 4.66 (dd, J = 9.0, 9.0 Hz, 1 H, 2-H, *Ile*), 5.04 [ddd, J = 9.0, 9.0, 7.2 Hz, 1 H, 2-H, (NO₂)Nva], 5.24–5.31 [m, 1 H, 1'-H, (*4-PE*)Pro], 5.38 (dq, J = 7.2, 2.4 Hz, 1 H, 3-H, *α-Thr*), 5.64 [dq, J = 10.8, 7.2 Hz, 1 H, 2'-H, (*4-PE*)Pro], 6.14 (d, J = 4.8 Hz, 1 H, 4-H, *Chpca*), 6.46 (d, J = 6.6 Hz, 1 H, NH), 6.75–6.84 (br, 1 H, NH), 6.82 (d, J = 4.8 Hz, 1 H, 3-H, *Chpca*), 6.88 (d, J = 9.6 Hz, 1 H, NH), 7.02–7.05 (m, 1 H, Ar-H), 7.10–7.20 (m, 5 H, Ar-H), 7.21–7.27 (m, 5 H, Ar-H, NH), 7.33 (d, J = 9.0 Hz, 1 H, NH), 7.94–8.09 (br, 1 H, NH), 9.08 (d, J = 9.0 Hz, 1 H, NH); ¹³C NMR (150.8 MHz, CDCl₃): δ = 10.5 (+, C-5, *Ile*), 13.3 [+ , C-3', (*4-PE*)Pro, C-4, (*βMe*)Phe], 14.8 (+, C-1', *Ile*), 17.1 (+, C-4, *α-Thr*), 17.1 [−, C-3', (*3-Ncp*)Ala],

17.7 [+ , C-4, (β Me)Phe], 20.1 [+ , C-1', (3-Ncp)Ala], 23.7 [- , C-4, (NO₂)Nva], 25.1 (- , C-3, Ile), 30.7 [- , C-3, (NO₂)Nva], 33.0 [- , C-3, (3-Ncp)Ala], 35.5 [- , C-3, (4-PE)Pro], 36.7 [+ , C-4, (4-PE)Pro], 38.1 (+ , C-3, Ile), 39.3 [+ , C-3, (β Me)Phe], 41.7 [+ , C-3, (β Me)Phe], 51.2 [+ , C-2, (NO₂)Nva], 51.8 [+ , C-2, (3-Ncp)Ala], 52.8 [- , C-5, (4-PE)Pro], 54.5 (+ , C-2, Ile), 54.9 (+ , C-2, α -Thr), 58.1 [+ , C-2', (3-Ncp)Ala], 59.9 [+ , C-2, (β Me)Phe], 59.9 [+ , C-2, (β Me)Phe], 61.4 [+ , C-5, (4-PE)Pro], 69.3 (+ , C-3, α -Thr), 75.0 [- , C-5, (NO₂)-Nva], 103.5 (+ , C-4, Chpca), 109.6 (+ , C-3, Chpca), 119.8 (C_{quat}, C-2, Chpca), 121.7 (C_{quat}, C-5, Chpca), 127.0, 127.2, 127.4, 127.7, 128.5, 128.6 (+ , Ar-C), 127.5 [+ , C-1', (4-PE)Pro], 128.3 [+ , C-2', (4-PE)Pro], 141.6, 142.1 (C_{quat}, Ar-C), 159.3 (C_{quat}, C-1, Chpca), 168.5, 168.7, 170.7, 171.3, 171.6, 172.3 (C_{quat}, C-1); UV (MeOH): neutral: $\lambda_{\max}(\epsilon) = 278 (1.2 \times 10^4) \text{ nm}$; basic: 282 (1.0×10^4), 205 (5.7×10^4) nm; acidic: 274 (1.1×10^4) nm;; CD (MeOH): $\lambda_{\max}[\Theta] = 272 (2.76 \times 10^4)$, 256 (1.94×10^4), 220 (-5.50×10^4) nm ($c = 6.63 \times 10^{-6} \text{ M}$); MS (ESI): positive, $m/z = 1162 (40) [\text{M} - \text{H}^+ + 2\text{Na}^+]$, 1140 (100) $[\text{M} + \text{Na}^+]$; negative, $m/z = 1115 (100) [\text{M} - \text{H}^+]$; HRMS (ESI): calcd for $[\text{C}_{54}\text{H}_{70}\text{N}_{10}\text{O}_{14}\text{Cl}^+]$: 1117.4756; found 1117.4752.

MeZ- α -Dab(Fmoc)-OMe (**215**): Boc-(*R*)- α -Dab(Fmoc)-OtBu **214** (0.39 g, 0.785 mmol) was

deprotected with TFA (5 mL) for 1 h. All volatiles were removed under reduced pressure at 20 °C. The solid residue was taken up with 1 M HCl (5 mL) and methanol (20 mL) and after 10 min the mixture was concentrated and the residue was dried at 0.02 Torr for 16 h and used for the next step without further purification. SOCl₂ (0.60 mL, 8.272 mmol) was added dropwise to a solution of the crude hydrochloride of amino acid in anhydrous methanol (35 mL) at -20 °C for 5 min and stirring continued at the same temperature for a further 15 min. The mixture was then allowed to warm to 20 °C and after stirring at this temperature for 1 h the reaction flask was sealed and the mixture was heated to 50 °C and stirred for an additional 20 h. The reaction mixture was then concentrated under reduced pressure and the residue was triturated with Et₂O

to give crude HCl·H-Dab(Fmoc)-OMe (0.29 g, 94%) as a white solid. $R_f = 0.30$ MeOH/CHCl₃ 1:100; ¹H NMR (250 MHz, CD₃OD): $\delta = 1.36$ (d, $J = 7$ Hz, 3 H, H-4), 3.97 (s, 3 H, OMe), 4.16–4.34 (m, 3 H, 2-H and 1'-H, 2'-H_a, *Fmoc*), 4.40–4.59 (m, 2 H, 3-H and 2'-H, *Fmoc*), 7.27–7.50 (m, 4 H, Ar-H), 7.54 (d, $J = 6.8$ Hz, 1 H, NH), 7.70 (d, $J = 7.3$ Hz, 2 H, Ar-H), 7.84 (d, $J = 7.3$ Hz, 1 H, Ar-H). A solution of MeZOSu (0.244 g, 0.927 mmol) in acetone (5 mL) was added to a solution of this material (0.29 g, 0.742 mmol) in water (5 mL) (if emulsion formed acetone and/or water were added to obtain homogeneous solution) and stirring continued for a further 1.5 h. Acetone was then distilled off under reduced pressure and the resultant suspension was filtered. The crude product was washed with Et₂O/pentane 1:1 (50 mL), water (100 mL), 3% NaHCO₃ (50 mL), water (20 mL), 1 M HCl, water (50 mL), pentane (50 mL) dried and finally crystallized from CH₂Cl₂/hexane to give **215** (0.272 g, 69% over 3 steps) as a colorless solid. M.p. 167–168 °C; $[\alpha]_{20}^D$ 8.5 ($c = 0.40$, THF); ¹H NMR (250 MHz, CDCl₃): $\delta = 1.47$ (d, $J = 6.8$ Hz, 3 H, 4-H), 2.34 (s, 3 H, 1'-H, *MeZ*), 3.77 (s, 3 H, OMe), 4.10–4.51 (m, 4 H, 3-H and 1'-H, 2'-H, *Fmoc*), 4.60 (dd, $J = 8.1, 3.1$ Hz, 1 H, 2-H), 5.08 (s, 2 H, Bzl-H), 5.32 (d, $J = 8.0$ Hz, 1 H, NH), 5.67 (d, $J = 6.8$ Hz, 1 H, NH), 7.16 (d, $J = 7.3$ Hz, 2 H, Ar-H), 7.21–7.46 (m, 6 H, Ar-H), 7.61 (d, $J = 6.8$ Hz, 2 H, Ar-H), 7.77 (d, $J = 7.3$ Hz, 2 H, Ar-H); ¹³C NMR (62.9 MHz, CDCl₃): $\delta = 16.3$ (+, C-4), 21.0 (+, C-1', *MeZ*), 47.0 (+, C-3), 48.9 (+, C-1', *Fmoc*), 52.5 (+, OMe), 57.8 (+, C-2), 66.8 (–, Bzl-H, *MeZ*), 67.1 (–, C-2', *Fmoc*), 119.8, 125.0, 126.9, 127.5, 128.3, 129.1 (+, Ar-C), 132.8 (C_{quat}, Ar-C), 137.9 (C_{quat}, Ar-C), 141.1 (C_{quat}, Ar-C), 143.7, 143.9 (C_{quat}, Ar-C), 155.8, 156.4 (C_{quat}, NCO₂), 170.7 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 3316$ cm^{–1}, 3067, 2948, 1748, 1691, 1542, 1450, 1338, 1316, 1282, 1231, 1169; MS (EI, 70 eV), m/z (%) = 502 (1) [M⁺], 266 (10) [C₁₄H₂₀NO₄⁺], 178 (100) [C₁₄H₁₀⁺], 165 (5), 105 (22) [C₈H₉⁺], 44 (11) [CO₂⁺]; HRMS (EI): calcd for C₂₉H₃₀N₂O₆: 502.2104; found 502.2104; elemental analysis calcd (%) for C₂₉H₃₀N₂O₆ (502.6): C 69.31, H 6.02, N 5.57; found C 69.08, H 5.88, N 5.38.

MeZ-a-Dab[Boc-(4-PE)Pro]-OMe (**216**): The compound **215** (0.191 g, 0.380 mmol) was deprotected according to GP 4 and the resultant crude N_α -protected diamino ester was coupled with *N*-Boc protected (4-propenyl)proline **16** (0.100 g, 0.392 mmol) using EDC (0.077 g, 0.402 mmol), HOAt (0.055 g, 0.407 mmol) and TMP (0.142 g, 1.172 mmol) in CH_2Cl_2 (4 mL) according to GP 5 for 6 h. The crude product obtained after usual aqueous work-up (GP 5) was finally purified by column chromatography (EtOAc/hexane 1:1.5, R_f = 0.35) to give **216** (0.163 g, 83%) as a turbid oil, which solidified during drying at 60 °C and 0.02 Torr in a white solid. M.p. 94–95 °C; $[\alpha]_{20}^D$ –41.6 (c = 0.32, CHCl_3); ^1H NMR (250 MHz, CDCl_3): δ = 1.04–1.19 (m, 3 H, 4-H, *a-Dab*), 1.41 [s, 9 H, $\text{C}(\text{CH}_3)_3$], 1.64 [dd, J = 7.0, 1.5 Hz, 3 H, 3'-H, (*4-PE*)*Pro*], 1.72–2.00 [m, 1 H, 3-H_a, (*4-PE*)*Pro*], 2.34 (s, 3 H, 1'-H, *MeZ*), 2.34–2.54 [m, 1 H, 3-H_b, (*4-PE*)*Pro*], 2.92–3.15 [m, 2 H, 4-H, 5-H_a, (*4-PE*)*Pro*], 3.76 (s, 3 H, OMe), 3.80–3.96 [m, 1 H, 5-H_b, (*4-PE*)*Pro*], 4.03–4.22 (m, 1 H, 3-H, *a-Dab*), 4.35–4.57 (m, 2 H, 2-H), 5.01 (d, J = 12.3 Hz, 1 H, Bzl-H_a), 5.09 (d, J = 12.3 Hz, 1 H, Bzl-H_b), 5.20–5.37 [m, 1 H, 1'-H, (*4-PE*)*Pro*], 5.54 [dq, J = 10.0, 7.0 Hz, 1 H, 2'-H, (*4-PE*)*Pro*], 5.59–5.77, 6.20–6.40 (2 × m, 1 H, NH), 6.81 (d, J = 8.8 Hz, 1 H, NH), 7.15 (d, J = 7.8 Hz, 2 H, Ar-H), 7.25 (d, J = 7.8 Hz, 2 H, Ar-H); ^{13}C NMR (62.9 MHz, CDCl_3): δ = 13.0 [+ , C-3', (*4-PE*)*Pro*], 15.3, 16.8 (+ , C-4, *a-Thr*), 21.0 (+ , C-1', *MeZ*), 28.1 [+ , $\text{C}(\text{CH}_3)_3$], 35.8 [+ , C-4, (*4-PE*)*Pro*], 37.8 [– , C-3, (*4-PE*)*Pro*], 46.6, 47.1 (+ , C-3, *a-Dab*), 52.0 [– , C-5, (*4-PE*)*Pro*], 52.4 (+ , OMe), 57.5 (+ , C-2, *a-Dab*), 60.9, 61.5 [+ , C-2, (*4-PE*)*Pro*], 66.8, 67.2 (– , Bzl-H, *MeZ*), 80.2 [C_{quat} , $\text{C}(\text{CH}_3)_3$] 126.4 [+ , C-2', (*4-PE*)*Pro*], 128.2, 129.0 (+ , Ar-C), 129.4 [+ , C-1', (*4-PE*)*Pro*], 132.7, 137.8 (C_{quat} , Ar-C), 154.2, 156.5 (C_{quat} , NCO_2), 170.2, 171.0 (C_{quat} , C-1), 172.0, 172.3 (C_{quat} , C-1); IR (KBr): $\nu(\text{tilde})$ = 3012 cm^{-1} , 2978, 2929, 2869, 1728, 1703, 1678, 1541, 1519, 1394, 1368, 1259, 1212, 1162; MS (EI, 70 eV), m/z (%) = 517 (1) [M^+], 444 (3) [$\text{M}^+ - \text{C}_4\text{H}_9\text{O}$], 416 (6) [$\text{M}^+ - \text{C}_5\text{H}_9\text{O}_2$], 281 (52) [$\text{C}_{15}\text{H}_{24}\text{N}_2\text{O}_3^+$], 238 (15) [$\text{C}_{13}\text{H}_{20}\text{N}_2\text{O}_3^+$], 225 (32) [$\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_3^+$], 182 (11), 154 (100) [$\text{C}_8\text{H}_{12}\text{NO}_2^+$], 110 (88) [$\text{C}_7\text{H}_{12}\text{N}^+$], 105 (70) [C_8H_9^+], 57 (49) [C_4H_9^+], 44 (68) [CO_2^+]; HRMS (EI): calcd for $\text{C}_{27}\text{H}_{39}\text{N}_3\text{O}_7$: 517.2788; found

517.2788; elemental analysis calcd (%) for $C_{27}H_{39}N_3O_7$ (517.6): C 62.65, H 7.59, N 8.12; found C 62.48, H 7.35, N 7.90.

MeZ-a-Dab[Boc-(4-PE)Pro]-OH (217): A 40% aqueous solution of tetra-*n*-butylammonium hydroxide (0.545 g, 0.840 mmol) was added dropwise to an ice-cold solution of ester **216** (0.145 g, 0.280 mmol) in THF (1.8 mL) and stirring continued at the same temperature for a further 45 min. A 1 M aqueous H_2SO_4 (2.1 mL) was then added and the mixture was diluted with Et_2O (50 mL) and washed with 1 M $KHSO_4$ (2×10 mL), water (5×10 mL), brine (2×5 mL), dried, filtered and concentrated under reduced pressure. The residue was recrystallized from Et_2O /hexane to give the first crop of the acid **217** (0.108 g) as a colorless solid. The mother liquor was concentrated under reduced pressure and the residue was recrystallized twice from Et_2O /hexane to give the second crop of the title compound (0.012 g, 85% overall yield). R_f = 0.06 $EtOAc$ /hexane 1:2 (1.5% $AcOH$); 1H NMR (250 MHz, $CDCl_3$): δ = 1.13–1.47 (m, 3 H, 4-H, *a-Dab*), 1.35, 1.39 [$2 \times s$, 9 H, $C(CH_3)_3$], 1.64 [d, J = 5.8 Hz, 3 H, 3'-H, (*4-PE*)*Pro*], 1.73–2.00 [m, 1 H, 3-H_a, (*4-PE*)*Pro*], 2.33 (s, 3 H, 1'-H, *MeZ*), 2.33–2.57 [m, 1 H, 3-H_b, (*4-PE*)*Pro*], 2.87–3.20 [m, 2 H, 4-H, 5-H_a, (*4-PE*)*Pro*], 3.48–3.73, 3.73–3.95 [$2 \times m$, 1 H, 5-H_b, (*4-PE*)*Pro*], 4.07–4.29 (m, 1 H, 3-H, *a-Dab*), 4.41 (d, J = 6.5 Hz, 1 H, 2-H), 4.43–4.69 (m, 1 H, 2-H), 5.03 (s, 2 H, Bzl-H), 5.18–5.33 [m, 1 H, 1'-H, (*4-PE*)*Pro*], 5.53 [dq, J = 10.8, 7.0 Hz, 1 H, 2'-H, (*4-PE*)*Pro*], 5.83–6.02, 6.31–6.48 ($2 \times m$, 1 H, NH), 6.81 (d, J = 8.8 Hz, 1 H, NH), 7.14 (d, J = 8.0 Hz, 2 H, Ar-H), 7.21–7.38 (br, 1 H, NH, CO_2H), 7.23 (d, J = 8.0 Hz, 2 H, Ar-H).

MeZ-a-Dab[Boc-(4-PE)Pro]-(βMe)Phe-(R)-(3-Ncp)Ala-(βMe)Phe-Ile-ODCPM (218):

Tetrapeptide **188b** (0.172 g, 0.186 mmol), after removal of the Fmoc-group according to GP 4, was coupled with ester acid **216** (0.104 g, 0.207 mmol) using HATU (79 mg, 0.208 mmol), HOAt (30 mg, 0.222 mmol) and TMP (75 mg, 0.619 mmol) in CH_2Cl_2 (5 mL) according to GP 7 for 15 h. The mixture was then diluted with Et_2O (50 mL), subjected to the usual aqueous work-

up (GP 5), dried, filtered and concentrated under reduced pressure. The resulting oil was recrystallized from hexane, then purified by column chromatography (EtOAc/hexane 4:3, R_f = 0.34) and finally recrystallized from hexane again to give the branched hexapeptide **218** (0.176 g, 80%) as a white solid. m.p. 101–103 °C (decomp.), $[\alpha]_{20}^D$ 52.8 (c = 0.29, THF); ^1H NMR (600 MHz, CDCl_3): δ = 0.34 (dddd, J = 4.8, 4.8, 4.8, 4.8 Hz, 1 H, 2'-H, *DCPM*), 0.40 (dddd, J = 4.8, 4.8, 4.8, 4.8 Hz, 1 H, 2'-H, *DCPM*), 0.43 (dddd, J = 4.8, 4.8, 4.8, 4.8 Hz, 1 H, 2'-H, *DCPM*), 0.45–0.54 (m, 2 H, 2'-H, *DCPM*), 0.54–0.59 (m, 2 H, 2'-H, *DCPM*), 0.66 (dddd, J = 4.2, 4.2, 4.2, 4.2 Hz, 1 H, 2'-H, *DCPM*), 0.79 (d, J = 6.6 Hz, 3 H, 1'-H, *Ile*), 0.84–0.92 [m, 1 H, 1'-H, (*3-Ncp*)*Ala*] 0.90 (t, J = 7.2 Hz, 3 H, 5-H, *Ile*), 1.04 [ddd, J = 6.0, 7.2, 7.2 Hz, 1 H, 3'-H_a, (*3-Ncp*)*Ala*], 1.06–1.14 (m, 1 H, 1'-H_a, *DCPM*), 1.14–1.23 (m, 1 H, 1'-H_b, *DCPM*), 1.23 [d, J = 6.6 Hz, 3 H, 4-H, (β *Me*)*Phe*], 1.25 [d, J = 6.6 Hz, 3 H, 4-H, (β *Me*)*Phe*], 1.28–1.39 [m, 3 H, 4-H_b, *Ile*, 3-H, (*3-Ncp*)*Ala*], 1.33 (d, J = 7.2 Hz, 3 H, 4-H, *a-Dab*), 1.34 [s, 9 H, C(CH₃)₃], 1.45–1.53 [m, 1 H, 3'-H_b, (*3-Ncp*)*Ala*], 1.69 [dd, J = 6.6, 1.2 Hz, 3'-H, (*4-PE*)*Pro*], 1.81 [ddd, J = 12.0, 12.0, 12.0 Hz, 1 H, 3-H_a, (*4-PE*)*Pro*], 1.85–1.93 (m, 1 H, 3-H, *Ile*), 2.32 (s, 3 H, 1'-H, *MeZ*), 2.36 [ddd, J = 12.0, 6.0, 6.0 Hz, 3-H_b, (*4-PE*)*Pro*], 3.12–3.23 [m, 2 H, 3-H, (β *Me*)*Phe*, 5-H_a, (*4-PE*)*Pro*], 3.21–3.30 [m, 2 H, 3-H, (β *Me*)*Phe*, 4-H, (*4-PE*)*Pro*], 3.67 [dd, J = 9.0, 7.8 Hz, 1 H, 5-H_b, (*4-PE*)*Pro*], 3.89 [ddd, J = 7.2, 3.0, 3.0 Hz, 1 H, 2'-H, (*3-Ncp*)*Ala*], 4.14 (t, J = 7.8 Hz, 1 H, 1-H, *DCPM*), 4.22 [dd, J = 4.8, 2.4 Hz, 1 H, 2-H, (*4-PE*)*Pro*], 4.24 (dd, J = 9.6, 6.6 Hz, 1 H, 2-H, *a-Dab*), 4.30 [dd, J = 10.8, 6.0 Hz, 1 H, 2-H, (β *Me*)*Phe*], 4.34 (dd, J = 9.3, 4.5 Hz, 1 H, 2-H, *Ile*), 4.60 [ddd, J = 10.5, 5.4, 5.4 Hz, 1 H, 2-H, (*3-Ncp*)*Ala*], 4.62–4.70 [m, 2 H, 2-H, (β *Me*)*Phe*, 3-H, *a-Dab*], 5.00 (d, J = 12.0 Hz, Bzl-H_a), 5.06 (d, J = 12.0 Hz, Bzl-H_b), 5.26–5.33 [m, 1 H, 1'-H, (*4-PE*)*Pro*], 5.56 [dq, J = 11.1, 6.6 Hz, 1 H, 2'-H, (*4-PE*)*Pro*], 6.61 (d, J = 6.6 Hz, 1 H, NH), 6.97 (d, J = 10.2 Hz, 1 H, NH), 7.01 (d, J = 9.0 Hz, 1 H, NH), 7.10 (d, J = 8.4 Hz, 2 H, Ar-H), 7.16–7.32 (m, 13 H, Ar-H, NH), 7.49 (d, J = 9.6 Hz, 1 H, NH), 7.60 (d, J = 9.6 Hz, 1 H, NH); The absorptions of 4-H, (β *Me*)*Phe* masked the peak of 4-H_a, *Ile*; ^{13}C NMR (150.8 MHz, CDCl_3):

δ = 2.5, 2.8, 2.8, 3.0 (–, C-2', *DCPM*), 11.6 (+, C-5, *Ile*), 13.2 [+ , C-3', (*4-PE*)*Pro*], 14.1, 14.6 (+, C-1', *DCPM*), 15.7 (+, C-1', *Ile*), 18.5 [+ , C-4, (β *Me*)*Phe*], 18.6 [–, C-3', (*3-Ncp*)*Ala*], 19.7 [+ , C-4, (β *Me*)*Phe*], 19.9 (+, C-4, *a-Dab*), 21.1 (+, C-1', *MeZ*), 21.6 [+ , C-1', (*3-Ncp*)*Ala*], 25.2 (–, C-4, *Ile*), 28.3 [+ , C(CH₃)₃], 30.8 [–, C-3, (*3-Ncp*)*Ala*], 36.2 [–, C-3, (*4-PE*)*Pro*], 36.5 (+, C-3, *Ile*), 37.1 [+ , C-4, (*4-PE*)*Pro*], 40.2 [+ , C-3, (β *Me*)*Phe*], 41.9 [+ , C-3, (β *Me*)*Phe*], 46.3 (+, C-3, *a-Dab*), 50.8 [+ , C-2, (*3-Ncp*)*Ala*], 52.5 [–, C-5, (*4-PE*)*Pro*], 56.6 (+, C-2, *Ile*), 59.5 [+ , C-2', (*3-Ncp*)*Ala*], 60.9 (+, C-2, *a-Dab*), 61.5 [+ , C-2, (β *Me*)*Phe*], 63.3 [+ , C-2, (*4-PE*)*Pro*], 63.4 [+ , C-2, (β *Me*)*Phe*], 66.7 (–, Bzl-C), 80.2 [C_{quat}, C(CH₃)₃], 83.1 (+, C-1, *DCPM*), 126.7, 127.4, 127.6, 127.7, 128.4, 128.4, 128.9, 128.9, (+, Ar-C), 127.0 [+ , C-2', (*4-PE*)*Pro*], 129.2 [+ , C-1', (*4-PE*)*Pro*], 133.5, 137.7, 141.6, 141.7 (C_{quat}, Ar-C), 154.4, 155.9 (C_{quat}, NCO₂), 169.7, 170.9, 173.6, 173.6, 174.1, 174.1 (C_{quat}, C-1); IR (KBr): ν = 3374 cm^{–1}, 3087, 3010, 2973, 2934, 2876, 1730, 1673, 1545, 1513, 1390, 1368, 1162; MS (ESI): positive mode, m/z (%) = 1212 (100) [M + Na⁺]; negative mode, m/z (%) = 1188 (100) [M – H⁺]; elemental analysis calcd (%) for C₆₅H₈₈N₈O₁₃ (1189.5): C 65.64, H 7.46, N 9.42; found C 65.63, H 7.22, N 9.26.

MeZ-Protected branched cyclohexapeptide (**219a**) and its epimer (**219b**): To the branched hexapeptide **218** (0.188 g, 0.165 mmol) was added freshly prepared 2 M HCl in EtOAc (3 mL). The reaction mixture was stirred for 1 h at 20 °C in the dark room and then was concentrated under reduced pressure at 20 °C. The residue was recrystallized from Et₂O/hexane to give the deprotected peptide as a white solid (0.145 g; MS (ESI): positive mode, m/z (%) = 996 (100) [M + H⁺]; negative mode, m/z (%) = 994 (100) [M – H⁺]), which was taken up with anhydrous CH₂Cl₂ (1.5 l) and cyclized employing HATU (2 × 61 mg, 2 × 0.160 mmol) and HOAt (2 × 18 mg, 2 × 0.133 mmol) and solution of DIEA (2 × 55 mg, 2 × 0.426 mmol) in CH₂Cl₂ (2 × 20 mL) according to GP 8 for 16 h. After this, the solvent was removed under reduced pressure, the residue was taken up with Et₂O (50 mL), and after usual aqueous work-up (GP 5), drying and filtration, the organic layer was concentrated under reduced pressure. The residue was purified

first by column chromatography (acetone/hexane 1:1.75, R_f = 0.29) and then by recrystallization (Et₂O/pentane) to give crude product (0.081 g), which contained two components according to analytical HPLC. The mixture was separated by preparative HPLC to give cyclodepsipeptide **219a** (41 mg, 28% on 2 steps) and its epimer **219b** (28 mg, 19% on 2 steps) as white solids. Preparative HPLC: isocratic, 75% MeCN in H₂O (0.1% TFA).

219a: analytical HPLC 1: column B, isocratic, 60% MeCN in H₂O (0.1% TFA), flow rate = 0.5 mL/min, t_R = 20.18 min, purity > 99%; analytical HPLC 2: column C, isocratic, 75% MeCN in H₂O (0.1% TFA), flow rate = 0.8 mL/min, t_R = 14.83 min, purity > 99%; $[\alpha]_{20}^D$ 8.6 (c = 0.21, CHCl₃); ¹H NMR (600 MHz, CDCl₃): δ = 0.15–0.31 [m, 1 H, 3'-H_a, (3-*Ncp*)Ala], 0.32–0.47 [m, 1 H, 3-H_a, (3-*Ncp*)Ala], 0.78 (t, J = 7.2 Hz, 3 H, 5-H, *Ile*), 0.84 (d, J = 6.6 Hz, 3 H, 1'-H, *Ile*), 0.88–0.97 [m, 1 H, 1'-H, (3-*Ncp*)Ala], 0.97–1.06 (m, 1 H, 4-H_a, *Ile*), 1.08–1.25 [m, 1 H, 3-H_b, (3-*Ncp*)Ala], 1.25 [d, J = 7.2 Hz, 3 H, 4-H, (β Me)*Phe*], 1.26–1.34 (m, 1 H, 4-H_b, *Ile*), 1.29 [d, J = 7.2 Hz, 3 H, 4-H, (β Me)*Phe*], 1.34–1.44 [m, 1 H, 3'-H_b, (3-*Ncp*)Ala], 1.40 (d, J = 7.2 Hz, 3 H, 4-H, *a-Dab*), 1.63–1.73 (m, 1 H, 3-H, *Ile*), 1.66 [d, J = 6.6 Hz, 3'-H, (4-*PE*)Pro], 1.87 [ddd, J = 11.4, 11.4, 11.4 Hz, 1 H, 3-H_a, (4-*PE*)Pro], 2.18 [ddd, J = 11.4, 6.0, 6.0 Hz, 1 H, 3-H_b, (4-*PE*)Pro], 2.38 (s, 3 H, 1'-H, *MeZ*), 3.01 [dq, J = 1.2, 7.2 Hz, 1 H, 3-H, (β Me)*Phe*], 3.07–3.19 [m, 1 H, 4-H, (4-*PE*)Pro], 3.23 [dd, J = 9.6, 9.6 Hz, 1 H, 5-H_a, (4-*PE*)Pro], 3.40–3.51 [m, 1 H, 2-H, (3-*Ncp*)Ala], 3.64–3.71 [m, 1 H, 3-H, (β Me)*Phe*], 3.71–3.79 (m, 1 H, 2-H), 3.89 [dd, J = 8.4, 8.4 Hz, 1 H, 5-H_b, (4-*PE*)Pro], 3.89–3.98 (m, 1 H, 2-H), 4.28–4.34 [m, 2 H, 2'-H, (3-*Ncp*)Ala, 3-H, *a-Dab*], 4.34–4.50 (m, 1 H, 2-H), 4.50–4.60 (m, 2 H, 2-H), 4.97 (d, J = 12.0 Hz, Bzl-H_a), 5.19 (d, J = 12.0 Hz, Bzl-H_b), 5.25 [dd, J = 9.6, 9.6 Hz, 1 H, 1'-H, (4-*PE*)Pro], 5.58 [dq, J = 9.6, 7.2 Hz, 1 H, 2'-H, (4-*PE*)Pro], 5.63–5.80 (br, 1 H, NH), 6.04–6.37 (br, 1 H, NH), 6.51–6.67 (br, 1 H, NH), 6.86–7.02 (br, 1 H, NH), 7.02–7.12 (m, 1 H, NH), 7.14–7.31 (m, 15 H, Ar-H, NH); ¹³C NMR (150.8 MHz, CDCl₃): δ = 10.5 (+, C-5, *Ile*), 13.2 [+ , C-3', (4-*PE*)Pro], 13.5 [+ , C-4, (β Me)*Phe*], 14.9 (+, C-1', *Ile*), 17.8 [–, C-3', (3-*Ncp*)Ala], 18.0 [+ , C-4, (β Me)*Phe*], 18.7 (+, C-4,

a-Dab), 21.0 [+ , C-1', (*3-Ncp*)*Ala*], 21.1 (+, C-1', *MeZ*), 24.3 (–, C-4, *Ile*), 32.7 [–, C-3, (*3-Ncp*)*Ala*], 35.2 [–, C-3, (*4-PE*)*Pro*], 36.6 [+ , C-4, (*4-PE*)*Pro*], 37.3 (+, C-3, *Ile*), 38.8 [+ , C-3, (*βMe*)*Phe*], 43.6 [+ , C-3, (*βMe*)*Phe*], 47.5 (+, C-3, *a-Dab*), 52.5 [+ , C-2, (*3-Ncp*)*Ala*], 52.7 [–, C-5, (*4-PE*)*Pro*], 54.5 (+, C-2), 58.9 [+ , C-2 and C-2', (*3-Ncp*)*Ala*], 59.3 (+, C-2), 59.5 (+, C-2), 62.3 [+ , C-2, (*4-PE*)*Pro*], 67.0 (–, Bzl-C), 126.8, 127.1, 127.2, 127.5, 127.7, 128.0, 128.4, 128.5, 128.6, 129.1 [+ , Ar-C, C-1', C-2', (*4-PE*)*Pro*], 133.4, 137.8, 141.4, 142.3 (C_{quat}, Ar-C), 157.3 (C_{quat}, NCO₂), 169.1, 170.5 (× 2), 170.9 (× 2), 172.0 (C_{quat}, C-1); IR (KBr): ν = 3406 cm^{–1}, 3060, 3029, 2969, 2936, 2877, 1670, 1634, 1542, 1517, 1452, 1369, 1205; MS (ESI): positive mode, m/z (%) = 1000 (100) [M + Na⁺]; negative mode, m/z (%) = 976 (100) [M – H⁺]; HRMS (ESI): calcd for [C₅₃H₆₈N₈O₁₀Na⁺]: 999.4951; found 999.4959.

219b: analytical HPLC 1: column B, isocratic, 60% MeCN in H₂O (0.1% TFA), flow rate = 0.5 mL/ min, t_R = 17.25 min, purity > 97%; analytical HPLC 2: column C, isocratic, 75% MeCN in H₂O (0.1% TFA), flow rate = 0.8 mL/ min, t_R = 10.12 min, purity > 97%; [α]₂₀^D – 42.68.6 (c = 0.27, CHCl₃); ¹H NMR (600 MHz, CDCl₃): δ = 0.65–0.73 (m, 3 H, 1'-H, *a-Ile*), 0.84 (t, J = 7.2 Hz, 3 H, 5-H, *a-Ile*), 0.89 [ddd, J = 5.4, 5.4, 5.4 Hz, 1 H, 3'-H_a, (*3-Ncp*)*Ala*], 1.10 [d, J = 5.4 Hz, 1 H, 4-H, (*βMe*)*Phe*], 1.10–1.15 [m, 1 H, 3-H_a^{*}, (*3-Ncp*)*Ala*], 1.17–1.26 [m, 4 H, 4-H, (*βMe*)*Phe*, 4-H_a^{*}, *a-Ile*], 1.40–1.48 [m, 1 H, 3-H_b^{*}, (*3-Ncp*)*Ala*], 1.44 (d, J = 7.8 Hz, 3 H, 4-H, *a-Dab*), 1.50–1.59 [m, 1 H, 1'-H, (*3-Ncp*)*Ala*], 1.59–1.63 [m, 2 H, 3'-H_b, (*3-Ncp*)*Ala*, 3-H, *a-Ile*], 1.63 [d, J = 6.6 Hz, 3'-H, (*4-PE*)*Pro*], 2.08–2.22 [m, 1 H, 3-H_a, (*4-PE*)*Pro*], 2.28 (s, 3 H, 1'-H, *MeZ*), 2.25–2.33 [m, 1 H, 3-H_b, (*4-PE*)*Pro*], 3.06 [dddddd, J = 7.8, 7.8, 7.8, 7.8, 7.8 Hz, 1 H, 4-H, (*4-PE*)*Pro*], 3.17–3.30 [m, 9.6 Hz, 2 H, 5-H_a, (*4-PE*)*Pro*, 3-H^{*}, (*βMe*)*Phe*], 3.31–3.44 [m, 2 H, 2'-H^{*}, (*3-Ncp*)*Ala*, 3-H, (*βMe*)*Phe*], 3.74 [dd, J = 7.8, 7.8 Hz, 1 H, 5-H_b, (*4-PE*)*Pro*] 3.74–3.84 (m, 1 H, 2-H), 4.08–4.20 (m, 1 H, 3-H, *a-Dab*), 4.31–4.84 (m, 2 H, 2-H), 4.48–4.62 (m, 2 H, 2-H), 4.73 (dd, J = 7.8, 7.8 Hz, 1 H, 2-H), 4.98 (d, J = 12.0 Hz, Bzl-H_a), 5.09 (d, J = 12.0 Hz, Bzl-H_b), 5.29 [dd, J = 9.0, 9.0 Hz, 1 H, 1'-H, (*4-PE*)*Pro*], 5.52 [dq, J = 10.8, 6.6 Hz, 1 H, 2'-H, (*4-*

PE)Pro], 5.92–6.04 (br, 1 H, NH), 6.74 (d, $J = 7.2$ Hz, 1 H, NH), 7.05–7.12 (br, 1 H, NH), 7.07 (d, $J = 7.8$ Hz, 2 H, Ar-H), 7.16–7.29 (m, 10 H, Ar-H, NH), 7.30–7.36 (m, 4 H, Ar-H, NH), 7.44–7.51 (br, 1 H, NH); The absorption of 4-H_b, *a-Ile* is masked by the signal of 3'-H, (*4-PE)Pro*; ^{13}C NMR (150.8 MHz, CDCl_3): $\delta = 11.7$ (+, C-5, *a-Ile*), 13.2 [+ , C-3', (*4-PE)Pro*], 14.0 (+, C-1', *a-Ile*), 17.3 [+ , C-4, ($\beta\text{Me})\text{Phe}$], 17.5 (+, C-4, *a-Dab*), 17.6 [–, C-3', (*3-Ncp)Ala*], 17.6 [+ , C-4, ($\beta\text{Me})\text{Phe}$], 21.1 (+, C-1', *MeZ*), 21.9 [+ , C-1', (*3-Ncp)Ala*], 26.3 (–, C-4, *a-Ile*), 31.8 [–, C-3, (*3-Ncp)Ala*], 34.2 [–, C-3, (*4-PE)Pro*], 36.1 [+ , C-4, (*4-PE)Pro*], 36.9 (+, C-3, *a-Ile*), 40.4 [+ , 2 $\times$ C-3, ($\beta\text{Me})\text{Phe}$], 49.3 (+, C-3, *a-Dab*), 51.0 (+, C-2), 52.9 [–, C-5, (*4-PE)Pro*], 54.1 (+, C-2), 58.8 [+ , C-2', (*3-Ncp)Ala*], 59.3 (+, C-2*), 59.4 (+, C-2*), 60.3 (+, 2 $\times$ C-2), 67.2 (–, Bzl-C), 126.7, 127.0, 127.6, 127.7, 128.2, 128.3, 128.5, 128.7, 129.0, 129.1 [+ , Ar-C, C-1', C-2', (*4-PE)Pro*], 132.8, 138.0, 142.0, 142.6 (C_{quat} , Ar-C), 156.2 (C_{quat} , NCO_2), 170.3 ($\times 2$), 170.8 ($\times 2$), 170.9, 171.0 (C_{quat} , C-1); IR (KBr): $\nu = 3420\text{ cm}^{-1}$, 3061, 3030, 2969, 2934, 2877, 1654, 1540, 1453, 1369, 1270; MS (ESI): positive mode, m/z (%) = 1000 (100) [$\text{M} + \text{Na}^+$]; negative mode, m/z (%) = 976 (100) [$\text{M} - \text{H}^+$].

Comparison of amino acid composition of cyclopeptides **219a and **219b**:** Aliquots of **219a** and **219b** (0.5 mg each) were hydrolyzed according to GP 1 and the resultant hydrolyzates were after concentration under reduced pressure treated with (*S*)-FDVA according to GP 2 and analyzed by HPLC/MS using the mixtures of (*S*)-FDVA derivatives of synthetic amino acids as standards. These experiments showed the only difference between these two products: cyclopeptide **219a** contained (*R*)-*a*-Ile residue and cyclopeptide **219b** contained Ile moiety.

[*a-Dab*¹]-Hormaomycin [*a-Dab*¹]-(3**):** An ethereal solution (50 mL) of the CHA salt of **197** (26.6 mg, 63.75 μmol) was washed with 1 M H_2SO_4 (3 $\times$ 5 mL), 1 M KHSO_4 (2 $\times$ 5 mL), water (3 $\times$ 5 mL), brine (2 $\times$ 5 mL), dried, filtered and concentrated under reduced pressure. The resultant *N*-protected amino acid was dried at 0.02 Torr for 2 h and then coupled with the depsipeptide,

obtained after deprotection of **219a** (19.5 mg, 19.96 μmol) with 10% anisole in TFA (1.1 mL) according to GP 11 for 2 h, using HATU (22.8 mg, 59.96 μmol), HOAt (8.1 mg, 59.94 μmol), DIEA (2.57 mg, 19.88 μmol) and TMP (21.8 mg, 179.00 μmol) in CH_2Cl_2 (3 mL) according to GP 7 for 15 h. The mixture was then diluted with Et_2O (40 mL) and the crude product obtained after usual aqueous work-up (GP 5) was purified by preparative TLC (200 $\times$ 200 mm, acetone/hexane 1:2.7) to give Teoc-(*S*)-(3-Ncp)Ala-*cyclo*-hexapeptide (21.6 mg, 96%, R_f = 0.18 acetone/hexane 1:2.5) as a colorless glass which was used for the next step without any characterization. This substance (21.6 mg, 19.13 μmol) was deprotected with TFA (2.0 mL) for 1 h. The mixture was concentrated under reduced pressure at 20 °C and then taken up with toluene (3 $\times$ 15 mL) which was distilled off to remove the last traces of TFA. The resultant deprotected branched peptide was coupled with Chpca(MOM)-OH **109** (7.0 mg, 34.04 μmol) using HATU (12.9 mg, 33.93 μmol), DIEA (2.47 mg, 19.13 μmol) and TMP (12.37 mg, 102.08 μmol) in CH_2Cl_2 (3 mL) according to GP 7 for 2.5 h. The mixture was then diluted with Et_2O (40 mL) and the crude product obtained after usual aqueous work-up (GP 5) was purified by preparative TLC (200 $\times$ 200 mm, acetone/hexane 1:2.7, 2 runs) and finally by recrystallization from Et_2O /hexane to give MOM-[*a*-Dab¹]-**3** (20.2 mg, 90%, R_f = 0.09 acetone/hexane 1:3) as a colorless solid which was used for the next step without any characterization. MOM-[*a*-Dab¹]-**3** (19.1 mg, 16.92 μmol) was deprotected using $\text{MgBr}_2 \cdot \text{Et}_2\text{O}$ (164 mg, 633.89 μmol) and EtSH (0.018 mL, 243.07 μmol) in CH_2Cl_2 (10 mL) according to GP 10 for 3 h. The mixture was diluted Et_2O (50 mL) and the crude product obtained after usual aqueous work-up (GP 10) was recrystallized from Et_2O /pentane and then from CH_2Cl_2 /pentane to give [*a*-Dab¹]-**3** (15.4 mg, 84%, 68% on 5 steps from **219a**) as a colorless solid. R_f = 0.14 acetone/hexane 1:2.5; analytical HPLC: column B, gradient 25% $\rightarrow$ 85% MeCN in 0.15% ammonium acetate buffer (pH = 5.5) for 25 min, flow rate = 0.5 mL/min, t_R = 21.72 min, purity > 99%; $[\alpha]_{20}^D$ 23.0 (c = 0.1, CHCl_3); ^1H NMR (600 MHz, CDCl_3): δ = -0.69 [ddd, J = 6.6, 6.6, 6.6 Hz, 1 H, 3'-H_a, (3-Ncp)Ala], -

0.17 – –0.07 [m, 1 H, 3-H_a, (3-*Ncp*)*Ala*], 0.20–0.27 [m, 1 H, 1'-H, (3-*Ncp*)*Ala*], 0.54 [ddd, $J = 14.4, 4.8, 4.8$ Hz, 1 H, 3-H_b, (3-*Ncp*)*Ala*], 0.90 (t, $J = 7.2$ Hz, 3 H, 5-H, *Ile*), 0.98–1.05 [m, 1 H, 3'-H_b, (3-*Ncp*)*Ala*], 1.03 [ddd, $J = 7.2, 7.2, 7.2$ Hz, 1 H, 3'-H_a, (3-*Ncp*)*Ala*], 1.07 (d, $J = 7.2$ Hz, 3 H, 1'-H, *Ile*), 1.32 [d, $J = 7.2$ Hz, 3 H, 4-H, (β Me)*Phe*], 1.35 [d, $J = 7.2$ Hz, 3 H, 4-H, (β Me)*Phe*], 1.41 (d, $J = 7.8$ Hz, 3 H, *a*-Dab), 1.53–1.59 (m, 1 H, 4-H_b, *Ile*), 1.63–1.75 [m, 2 H, 3-H, (3-*Ncp*)*Ala*], 1.69 [dd, $J = 7.2, 1.8$ Hz, 3 H, 3'-H, (4-*PE*)*Pro*], 1.84–1.95 [m, 3 H, 3'-H_b, (3-*Ncp*)*Ala*, 3-H, *Ile*, 3-H_a, (4-*PE*)*Pro*], 1.95–2.01 [m, 1 H, 3-H_a, (4-*PE*)*Pro*], 2.27 [ddd, $J = 12.0, 6.0, 6.0$ Hz, 1 H, 4-H_b, (4-*PE*)*Pro*], 2.85 [ddd, $J = 6.6, 4.8, 4.8$ Hz, 1 H, 2'-H, (3-*Ncp*)*Ala*], 3.02 [dq, $J = 11.4, 7.2$ Hz, 1 H, 3-H, (β Me)*Phe*], 3.22–3.32 [m, 2 H, 4-H, 5-H_a, (4-*PE*)*Pro*], 3.46–3.52 [m, 1 H, 2-H, (3-*Ncp*)*Ala*], 3.70 [dq, $J = 3.6, 7.2$ Hz, 1 H, 3-H, (β Me)*Phe*], 3.92 [dd, $J = 11.4, 5.4$ Hz, 2-H, (4-*PE*)*Pro*], 3.95–3.99 [m, 1 H, 5-H_b, (4-*PE*)*Pro*], 4.03 [ddd, $J = 7.2, 3.6, 3.6$ Hz, 1 H, 2'-H, (3-*Ncp*)*Ala*], 4.35 (dd, $J = 10.8, 10.8$ Hz, 1 H, 2-H), 4.42–4.48 [m, 1 H, 3-H, *a*-Dab], 4.45 (dd, $J = 10.2, 4.2$ Hz, 1 H, 2-H), 4.50 (dd, $J = 9.0, 3.0$ Hz, 1 H, 2-H), 4.66 (dd, $J = 9.0, 9.0$ Hz, 1 H, 2-H), 5.11–5.16 [m, 1 H, 2-H, (3-*Ncp*)*Ala*], 5.26–5.31 [m, 1 H, 1'-H, (4-*PE*)*Pro*], 5.63 [dq, $J = 10.8, 6.6$ Hz, 1 H, 2'-H, (4-*PE*)*Pro*], 6.15 (d, $J = 4.2$ Hz, 1 H, 4-H, *Chpca*), 6.43 (d, $J = 7.8$ Hz, 1 H, NH), 6.77–6.86 (br, 1 H, NH), 6.85 (d, $J = 4.2$ Hz, 1 H, 3-H, *Chpca*), 7.02–7.06 (m, 2 H, Ar-H), 7.12–7.19 (m, 6 H, Ar-H, NH), 7.21–7.30 (m, 4 H, Ar-H, NH), 7.44 (d, $J = 10.8$ Hz, 1 H, NH), 8.87 (d, $J = 9.0$ Hz, 1 H, NH), 10.70–11.00 (br, 1 H, OH); The signal of 4-H_a, *Ile* was masked by absorptions of C-4, (β Me)*Phe*; ¹³C NMR (150.8 MHz, CDCl₃): $\delta = 10.4$ (+, C-5, *Ile*), 13.1 [+ , C-3', (4-*PE*)*Pro*], 13.3 [+ , C-4, *a*-Dab], 14.9 (+, C-1', *Ile*), 17.0 [–, C-3', (3-*Ncp*)*Ala*], 17.2 [–, C-3', (3-*Ncp*)*Ala*], 17.6 [+ , C-4, (β Me)*Phe*], 17.7 [+ , C-4, (β Me)*Phe*], 20.0 [+ , C-1', (3-*Ncp*)*Ala*], 21.7 [+ , C-1', (3-*Ncp*)*Ala*], 25.1 (–, C-3, *Ile*), 32.8 [–, C-3, (3-*Ncp*)*Ala*], 35.2 [–, C-3, (3-*Ncp*)*Ala*], 35.9 [–, C-3, (4-*PE*)*Pro*], 36.4 [+ , C-4, (4-*PE*)*Pro*], 38.0 (+, C-3, *Ile*), 39.2 [+ , C-3, (β Me)*Phe*], 41.2 [+ , C-3, (β Me)*Phe*], 45.3 (+, C-3, *a*-Dab), 51.0 [+ , C-2, (3-*Ncp*)*Ala*], 51.8 [+ , C-2, (3-*Ncp*)*Ala*], 53.1 [–, C-5, (4-*PE*)*Pro*], 54.6 (+, C-2), 55.2 (+, C-2), 58.1

[+, C-2', (3-Ncp)Ala], 59.3 [+, C-2', (3-Ncp)Ala], 59.8 (+, C-2), 60.0 (+, C-2), 63.8 [+, C-2, (4-PE)Pro], 103.6 (+, C-4, Chpca), 109.9 (+, C-3, Chpca), 119.9 (C_{quat}, C-2, Chpca), 121.7 (C_{quat}, C-5, Chpca), 127.0, 127.3, 127.4 ($\times 2$), 127.7, 127.9, 128.5 ($\times 2$), 128.7 [+, Ar-C, C-1', C-2', (4-PE)Pro], 141.3, 142.2 (C_{quat}, Ar-C), 159.3 (C_{quat}, C-1, Chpca), 168.3, 169.6, 170.0, 170.2, 171.6 ($\times 2$), 171.9 (C_{quat}, C-1); UV (MeOH): neutral: $\lambda_{\max}(\epsilon) = 277 (1.6 \times 10^4)$ nm; basic: 281 (1.5×10^4), 205 (7.0×10^4) nm; acidic: 272 (1.4×10^4) nm; CD (MeOH): $\lambda_{\max}[\Theta] = 280.2 (2.01 \times 10^4)$; 276.5 (2.05×10^4), 225.6 (-4.55×10^{-4}), 221.5 (-5.06×10^4) nm ($c = 1.45 \times 10^{-5}$ M); MS (ESI): positive, $m/z = 1151$ (100) $[M + Na^+]$; negative, $m/z = 1127$ (100) $[M - H^+]$; HRMS (ESI): calcd for $[C_{55}H_{70}N_{11}O_{13}ClNa^+]$: 1128.4916; found 1128.4921.

[*a*-Dab¹, *a*-Ile⁵]-Hormaomycin [*a*-Dab¹, *a*-Ile⁵]-**(3)**: An ethereal solution (50 mL) of the CHA salt of **197** (27.3 mg, 65.39 μ mol) was washed with 1 M H₂SO₄ (3 $\times$ 5 mL), 1 M KHSO₄ (2 $\times$ 5 mL), water (3 $\times$ 5 mL), brine (2 $\times$ 5 mL), dried, filtered and concentrated under reduced pressure. The resultant *N*-protected amino acid was dried at 0.02 Torr for 2 h and then coupled with the depsipeptide, obtained after deprotection of **219b** (20.0 mg, 20.47 μ mol) with 10% anisole in TFA (1.1 mL) according to GP 11 for 2 h, using HATU (23.4 mg, 61.54 μ mol), HOAt (8.3 mg, 61.42 μ mol), DIEA (2.64 mg, 20.39 μ mol) and TMP (22.36 mg, 183.6 μ mol) in CH₂Cl₂ (3 mL) according to GP 7 for 15 h. The mixture was then diluted with Et₂O (40 mL) and the crude product obtained after usual aqueous work-up (GP 5) was purified by two crystallizations from Et₂O/hexane to give Teoc-(*S*)-(3-Ncp)Ala-*epi*-cyclo-hexapeptide (17.0 mg, 74%, $R_f = 0.19$ acetone/hexane 1:2.5) as a colorless glass which was used for the next step without any characterization. This substance (17.0 mg, 14.50 μ mol) was deprotected with TFA (2.0 mL) for 1 h. The mixture was concentrated under reduced pressure at 20 °C and then taken up with toluene (3 $\times$ 15 mL) which was distilled off to remove the last traces of TFA. The resultant deprotected branched peptide was coupled with Chpca(MOM)-OH **109** (5.5 mg, 26.74 μ mol)

using HATU (10.15 mg, 26.69 μmol), DIEA (1.95 mg, 15.09 μmol) and TMP (12.37 mg, 102.08 μmol) in CH_2Cl_2 (3 mL) according to GP 7 for 2.5 h. The mixture was then diluted with Et_2O (40 mL) and the crude product obtained after usual aqueous work-up (GP 5) was crystallized twice from $\text{CH}_2\text{Cl}_2/\text{hexane}$ to give MOM-[*a*-Dab¹,*a*-Ile⁵]-**3** (14.2 mg, 80%, $R_f = 0.11$ acetone/hexane 1:3) as a colorless solid which was used for the next step without any characterization. MOM-[*a*-Dab¹,*a*-Ile⁵]-**3** (14.2 mg, 12.11 μmol) was deprotected using $\text{MgBr}_2 \cdot \text{Et}_2\text{O}$ (110 mg, 425.17 μmol) and EtSH (0.018 mL, 243.07 μmol) in CH_2Cl_2 (10 mL) according to GP 10 for 3 h. The mixture was taken up with Et_2O (50 mL) and the crude product obtained after usual aqueous work-up (GP 10) was recrystallized from $\text{Et}_2\text{O}/\text{pentane}$ and then from $\text{CH}_2\text{Cl}_2/\text{pentane}$ to give the crude product (13.1 mg), which was finally purified by preparative HPLC to give [*a*-Dab¹,*a*-Ile⁵]-**3** (9.0 mg, 39% on 5 steps from **219b**) as a colorless solid. $R_f = 0.14$ acetone/hexane 1:2.5; preparative HPLC: Nucleosil RP 18, JASCO, 250×8 mm, 62% MeCN in H_2O (0.07% TFA), flow rate = 2.5 mL/min; analytical HPLC: the same column, the same conditions, $t_R = 17.72$ min, purity > 99%; MS (ESI): positive, $m/z = 1151$ (100) [$\text{M} + \text{Na}^+$]; 1129 [$\text{M} + \text{H}^+$]; negative, $m/z = 1127$ (100) [$\text{M} - \text{H}^+$].

MeZ-N β Me-a-Dab(Fmoc)-OMe (**222**): *O*-TBDMS Derivate of *N*Me-Z protected *tert*-Butyl ester

of (*S*)-isothreonine **220** (0.73 g, 1.67 mmol) was dissolved in MeCN (38 mL) and treated with 5% aqueous HF (40 mL) at 4 °C for 10 min. The mixture was allowed to warm to 20 °C and stirring continued for a further 4 h. A saturated aqueous NaHCO_3 was then carefully added to adjust pH to ca. 8 and the mixture was extracted with Et_2O (2×50 mL). The organic fraction was washed with water (5×20 mL), brine (2×10 mL), dried, filtered and concentrated under reduced pressure. The resultant crude alcohol was dried under 0.02 Torr for 2 h and then deprotected with TFA (6 mL). After 1 h, all volatiles were removed under reduced pressure, the residue was dissolved in toluene (2×20 mL) which was distilled off

to remove the last traces of TFA. The residue was dried under 0.02 Torr for 2 h and then taken up with Et₂O (10 mL; some quantity of methanol was added to obtain homogeneous solution) and treated with excess of diazomethane till the steady yellow coloration of the mixture was obtained. The mixture was then concentrated under reduced pressure and the residue was purified by column chromatography to give *N*Me-Z protected (*S*)-isothreonine methyl ester (0.361 g, 71%, *R*_f = 0.22 EtOAc/hexane 1:3) as a turbid oil which was directly used for the next step. Mesyl chloride (0.14 mL, 1.81 mmol) as added dropwise to a solution of this substance (0.36 g, 1.28 mmol) and TEA (0.254 mL, 1.81 mmol) in CH₂Cl₂ (7 mL) at – 30 °C for 3 min and stirring continued at the same temperature for 1 h. The mixture was then allowed to warm to 4 °C and it was stirred at this temperature for a further 1 h. Finally, the cooling bath was removed and stirring continued at 20 °C for an additional 3 h. Saturated aqueous NaHCO₃ (3 mL) was then added and the mixture was taken up with Et₂O (50 mL). After usual aqueous work-up (GP 5) the organic layer was dried, filtered and concentrated under reduced pressure to give crude mesylate of *N*Me-Z protected (*S*)-isothreonine methyl ester (0.46 g, 100% crude yield, *R*_f = 0.11, EtOAc/hexane 1:6) as a colorless oil. NaN₃ (0.086 g, 1.32 mmol) was added to a solution of the mesylate (0.46 g, 1.28 mmol) in DMF (8 mL) and stirring continued at 70 °C for 14.5 h. The mixture was then cooled, concentrated under reduced pressure and the residue was taken up with Et₂O (50 mL). After usual aqueous work-up (GP 5) the organic layer was dried, filtered and concentrated under reduced pressure. The resultant crude product was purified by column chromatography (EtOAc/hexane 1:6, *R*_f = 0.19) to give methyl (2*R*,3*R*)-*N*Me-Z protected 2-azido-butanoate (0.191 g, 49%) as a mobile colorless oil. This substance (0.191 g, 0.62 mmol) was dissolved in THF/H₂O (20:1) (15.8 mL), Ph₃P (0.262 g, 1.00 mmol) was added and stirring continued for 24 h. Boc₂O (0.272 g, 1.25 mmol) was then added and stirring continued for a further 24 h. The mixture was then concentrated and the residue was purified by column chromatography (twice, hexane 1:4, *R*_f = 0.22) to give N_β-methylated Boc-*α*-Dab(Z)-OMe (0.135 g, 57%) as a viscous colorless oil. This material (0.135 g, 0.35 mmol) was

hydrogenated on 10% Pd on charcoal (0.07 g) under ambient pressure of hydrogen in EtOAc (7 mL) for 3 h. The mixture was then filtered and concentrated under reduced pressure to give crude Boc- α -N β MeDab-OMe. FmocOPfp (0.159 g, 0.39 mmol) was added to a solution of this material, TMP (43 mg, 0.35 mmol) and a little bit of HOAt in EtOAc (5 mL) and stirring continued for 15 h. The mixture was then taken up with Et₂O (50 mL) and subjected to usual aqueous work-up (GP 5). The organic layer was dried, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/hexane 1:4, R_f = 0.22) to give N β -methylated Boc- α -Dab(Fmoc)-OMe (0.135 g, 81%) as a colorless oil. This material (0.135 g, 0.29 mmol) was deprotected with 2 M HCl in EtOAc (4 mL) for 1 h. The mixture was then concentrated under reduced pressure and the residue was dissolved in MeCN (4 mL), TMP (45 mg, 0.37 mmol), DIEA (37 mg, 0.29 mmol) and finally MeZOSu (83 mg, 0.32 mmol) were added to this solution and stirring continued for a further 16 h. *N,N*-Dimethylaminopropylamine (20 mg, 0.20 mmol) was then added and after 10 min the mixture was concentrated under reduced pressure. The residue was taken up with Et₂O and subjected to usual aqueous work-up (GP 5). The organic layer was dried, filtered and concentrated under reduced pressure. The resultant crude product was purified by column chromatography (EtOAc/hexane 1:3, R_f = 0.30) to give **222** (0.122 g, 13% overall yield on 10 steps) as a turbid glass. $[\alpha]_{20}^D$ 9.2 (c = 0.25, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ = 1.16, 1.25 (2 $\times$ d, J = 6.9 Hz, 3 H, 4-H), 2.33 (s, 3 H, 1'-H, *MeZ*), 2.81 (s, 3 H, *NMe*), 3.66 (s, 3 H, OMe), 4.12–4.61 (m, 5 H, 2-H, 3-H and 1'-H, 2'-H, *Fmoc*), 5.06 (d, J = 5.1 Hz, 2 H, Bzl-H), 5.31, 5.60 (2 $\times$ d, J = 7.5 Hz, 1 H, NH), 7.15 (d, J = 7.8 Hz, 2 H, 2-H, *MeZ*), 7.24 (d, J = 7.8 Hz, 2 H, 3-H, *MeZ*), 7.31 (dd, J = 7.5, 7.5 Hz, 2 H, 3-H, *Fmoc*), 7.40 (dd, J = 7.5, 7.5 Hz, 2 H, 4-H, *Fmoc*), 7.58–7.64 (m, 2 H, 2-H, *Fmoc*), 7.76 (d, J = 7.2 Hz, 5-H, *Fmoc*); ¹³C NMR (50.3 MHz, CDCl₃): δ = 14.3 (+, C-4), 21.0 (+, C-1', *MeZ*), 28.9 (+, *NMe*), 47.1 (+, C-3), 52.4 (+, C-2), 52.9 (+, C-1', *Fmoc*), 56.6 (+, OMe), 67.0 (–, Bzl-H, *MeZ*), 67.5 (–, C-2', *Fmoc*), 119.8, 124.9, 126.9, 127.5, 128.2, 129.1 (+, Ar-C), 133.0 (C_{quat}, Ar-C), 137.9 (C_{quat}, Ar-C), 141.2 (C_{quat}, Ar-C), 143.8, 143.9 (C_{quat}, Ar-C), 155.9, 156.4 (C_{quat},

NCO₂), 170.9 (C_{quat}, C-1); IR (KBr): $\nu(\text{tilde}) = 2951 \text{ cm}^{-1}$, 1751, 1725, 1700, 1521, 1451, 1320, 1273, 1242, 1204, 1018; MS (ESI): positive, $m/z = 539$ (100) [M + Na⁺].

MeZ-N β Me-a-Dab[Boc-(4-PE)Pro]-OMe (**223**): Compound **222** (0.108 g, 0.21 mmol) was deprotected according to GP 4 and the resultant crude monodeprotected diamino ester was coupled with *N*-Boc protected 4-(*Z*)-propenylproline **16** (64 mg, 0.25 mmol) using EDC (48 mg, 0.25 mmol), HOAt (34 mg, 0.25 mmol) and TMP (76 mg, 0.63 mmol) in CH₂Cl₂ (3 mL) according to GP 5 for 6 h. The crude product, obtained after usual aqueous work-up (GP 5), was finally purified by column chromatography (EtOAc/hexane 1:1.5, $R_f = 0.35$) to give **223** (88 mg, 79%) as a turbid oil. Analytical HPLC: column B, gradient 20% $\rightarrow$ 90% MeCN in water (0.1% TFA) for 35 min, flow rate = 0.5 mL/min, $t_R = 26.01$ min, purity > 97%; $[\alpha]_{20}^D$ 12.0 ($c = 0.35$, CHCl₃); ¹H NMR (300 MHz, C₂D₂Cl₄, 373 K): $\delta = 1.31$ (d, $J = 7.2$ Hz, 3 H, 4-H, *NMe-a-Dab*), 1.42 [s, 9 H, C(CH₃)₃], 1.50–1.74 [m, 1 H, 3-H_a, (*4-PE*)Pro], 1.67 [dd, $J = 7.2, 1.8$ Hz, 3 H, 3'-H, (*4-PE*)Pro], 2.26–2.44 [m, 1 H, 3-H_b, (*4-PE*)Pro], 2.36 (s, 3 H, 1'-H, *MeZ*), 2.90 (s, 3 H, NMe), 3.01–3.21 [m, 2 H, 4-H, 5-H_a, (*4-PE*)Pro], 3.23–3.48 [m, 0.5 H, 5-H_b, (*4-PE*)Pro], 3.74 (s, 3 H, OMe), 4.47 (dd, $J = 7.8, 7.8$ Hz, 1 H, 3-H, *NMe-a-Dab*), 4.57 (dd, $J = 7.8, 7.8$ Hz, 1 H, 2-H, *NMe-a-Dab*), 4.50–4.95 [m, 1 H, C-2, (*4-PE*)Pro], 5.08 (s, 2 H, Bzl-H), 5.25–5.36 [m, 1 H, 1'-H, (*4-PE*)Pro], 5.50–5.85 (br, 1 H, NH), 5.54 [dq, $J = 11.4, 7.2$ Hz, 1 H, 2'-H, (*4-PE*)Pro], 7.15 (d, $J = 7.8$ Hz, 2 H, Ar-H), 7.23 (d, $J = 7.8$ Hz, 2 H, Ar-H); The peak of OMe masked absorption of 0.5 H, 5-H_b of (*4-PE*)Pro; ¹³C NMR (75.5 MHz, C₂D₂Cl₄, 373 K): $\delta = 12.7$ [+ , C-3', (*4-PE*)Pro], 14.5 (+, C-4, *NMe-a-Dab*), 20.7 (+, C-1', *MeZ*), 28.2 (+, C(CH₃)₃, NMe), 36.0 [+ , C-4, (*4-PE*)Pro], 51.9 [–, C-5, (*4-PE*)Pro], 52.1 (+, C-2, *NMe-a-Dab*), 57.2 (+, OMe), 66.7 (–, Bzl-H, *MeZ*), 79.3 [C_{quat}, C(CH₃)₃], 125.8 [+ , C-2', (*4-PE*)Pro], 127.7, 128.8 (+, Ar-C), 130.0 [+ , C-1', (*4-PE*)Pro], 133.3, 137.5 (C_{quat}, Ar-C), 153.5, 155.6 (C_{quat}, NCO₂), 170.6, 173.2 (C_{quat}, C-1); The signals of C-2, C-3 of (*4-PE*)Pro and C-3 of *NMe-a-Dab* were unobservable because of their low

intensity; IR (KBr): $\nu(\text{tilde}) = 2977 \text{ cm}^{-1}$, 1751 1728, 1700, 1521, 1402, 1281, 1163; MS (ESI): positive, $m/z = 554$ (100) $[\text{M} + \text{Na}^+]$.

D. Summary

This work has been focused on the synthesis of hormaomycin **3** and analogs, and prior to that, on the assignment of the absolute structure of unusual amino acids, which comprise hormaomycin, their stereoselective synthesis, and development of methodology of the fine “tuning” of protecting groups applicable towards the synthesis of complicated objects and particularly cyclic peptides with very rare amino acids.

N-Boc protected *cis*- and *trans*-4-(*Z*)-propenylprolines, *cis*-**16** and *trans*-**16b**, were obtained after HPLC separation of the mixture of diastereomers, prepared in high yield by reaction between tosylate **15** and lithium di-(*Z*)-propenylcuprate. After removal of the Boc groups and derivatization with *p*-dimethylaminoazobenzenesulfonyl chloride, they, together with derivatized total hydrolyzate of hormaomycin **3**, were used for HPLC experiments, which showed that the (4-PE)Pro moiety of natural **3** had *cis*-configuration.

The results of LC/MS experiments according to the advanced Marfey method with the total hydrolyzate of the hydrogenated hormaomycin allowed one to assign the (2*S*,4*R*) absolute configuration of the (4-PE)Pro fragment unambiguously. Synthetic 4-propylprolines, (2*S*,4*R*)-**29** and (2*R*,4*S*)-**29**, were used as standards in this set of experiments.

The developed stereoselective synthesis of the *N*-Boc-protected (2*S*,4*R*)-4-(*Z*)-propenylproline (2*S*,4*R*)-**16**, starting from (2*S*,4*R*)-Boc-Hyp-OH **43**, allowed to prepare this compound on a multigram scale in 16% yield over 8 steps.

Based on this protocol, labelled (2*S*,4*R*)-(4,1')-dideutero-4-(*Z*)-propenylproline (2*S*,4*R*)-D₂-**17** was synthesized. A feeding experiment with this amino acid was successful and confirmed the previously proposed absolute configuration of the (4-PE)Pro fragment of **3**.

A preparation of the enantiomerically pure (2*S*,1'*RS*,2'*RS*)-2-deutero-3-(2'-nitrocyclopropyl)alanine D-(2*S*,1'*RS*,2'*RS*)-**83** by alkylation of the Belokon' glycine chiral equivalent became feasible after the deuterium-labelled nickel complex (*S*)-D₂-**81** had been synthesized. Alkylation of the latter with racemic *trans*-(2'-nitrocyclopropyl)methyl iodide **80** was followed by acidolytic decomposition of the intermediate product and gave D-(2*S*,1'*RS*,2'*RS*)-**83** in 48% yield over 2 steps. It is noteworthy, that deuteration at C-2' of the nitrocyclopropyl ring also occurred. The corresponding feeding experiment allowed one to assign the configuration at C-2 of the (3-Ncp)Ala residue in the cyclic part of hormaomycin as *R* and that in the side chain of **3** as *S*.

The O-MOM protected 5-chloro-1-hydroxypyrrol-2-carboxylic acid **109** was prepared from known 2,6-dichloropyridine-*N*-oxide **100**, using thermolysis of the azide **102** to the nitrile **103** according to protocol of Abramovitch and Cue as a key step, in 9% yield over 7 steps.

The new 4-methylbenzyloxycarbonyl *N*-protecting group (MeZ) had to be applied in the course of assembling of the backbone of hormaomycin **3**, since all other convenient *N*-protective groups orthogonal to the Boc group turned out to be incompatible with the restrictions imposed on the deprotection conditions due to the nitro group on the (3-Ncp)Ala moiety, the double bond in the (4-PE)Pro residue as well as the base-sensitive ester bond between the (4-PE)Pro and α -Thr fragments.

The linear precursor **191a**, prepared by sequential elongation of the growing peptide chain, was cyclized under high dilution conditions to give the cyclic intermediate **171c** in moderate yield

(53% over 2 steps). Assembly of **3** was completed in a stepwise manner and final removal of the MOM group gave the artificial hormaomycin.

Starting from **173c**, the branched acyl depsipeptide [(3-Acp)-Ala(Npys)⁷]-MOM-**3** was prepared. However, an attempted removal of the protecting groups failed to give the target hormaomycin analog.

The analog [NO₂Nva⁷]-**3** was successfully synthesized, employing the same methodology. Feeding experiment, performed with (*S*)-5-nitronorvaline **208**, was successful and [NO₂Nva³]-**3** as well as [NO₂Nva⁷,NO₂Nva³]-**3** were isolated.

After removal of the Boc and DCPM groups, the branched peptide **218** was cyclized to give the mixture of epimeric **219a** and **219b**, which was separated by HPLC to give individual diastereomers. They were further used for a successful preparation of [Dab¹]-**3** and [Dab¹,*a*-Ile]-**3** analogs.

Plate diffusion tests with hormaomycin **3**, its analogs as well as with some intermediates and model compounds clearly showed that the side chain of **3** defines its specific antibiotic activity.

Among other results, some observations not directly connected with chemistry of hormaomycin, deserve to be mentioned: deconjugative isomerisation of (*S*)-(Z)-**39** in (2*S*)-(Z)-**40**, which proceeds with full retention of the configuration of the double bond, zinc mediated elimination of iodide (*S*)-**41** in the *N,O*-protected allylglycine (*S*)-**42** and the successful alkylation of the trityl-protected alcohol (1'*S*,2'*S*)-**87** at C-2' of the cyclopropyl ring, and deuteration of several nitrocyclopropyl-containing species at the same position.

MeZ-*a*-Dab[Boc-(4-PE)Pro]-(β Me)Phe-(*R*)-(3-Ncp)Ala-(β Me)Phe-Ile-ODCPM

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E. References

- [1] M. F. Vincente, A. Basilio, A. Cabello, F. Peláez, *Clinical Microbiology and Infection* **2003**, 9, 15–32.
- [2] K. Stephens, *CRC Crit. Rev. Microbiol.* **1986**, 13, 309–334.
- [3] J. Engebrecht, K. Nealson, M. Silverman, *Cell* **1983**, 32, 773–781. M. Perego, J. A. Hoch, *Proc. Natl. Acad. Sci. USA* **1996**, 93, 1549–1553. H. B. Kaplan, L. Plamam, *FEMS Microbiol. Lett.* **1996**, 139, 89–95.
- [4] a) A. S. Khokhlow in *Frontiers of bioorganic chemistry and molecular biology* (S. N. Ananchenko, ed.), Pergamon Press, Oxford **1980**, 201–210. b) K. Shigeru, K. Hiroshi, N. Takuya, Y. Yasuhiro, *J. Bacteriol.* **1999**, 181, 5081–5084.
- [5] A.L. Demain, *Internatl. Microbiol.* **1998**, 1, 259–264.
- [6] N. Andres, H. Wolf, H. Zähler, *Z. Naturforsch.* **1990**, 45, 851–855.
- [7] N. Andres, H. Wolf, H. Zähler, E. Rössner, A. Zeeck, W. A. König, V. Sinnwell, *Helv. Chim. Acta* **1989**, 426–437.
- [8] E. Rössner, *Dissertation*, Universität Göttingen, **1989**.
- [9] E. Rössner, A. Zeeck, W. A. König, *Angew. Chem.* **1990**, 102, 84–85; *Angew. Chem. Int. Ed. Engl.* **1990**, 29, 64–65.
- [10] a) J. Zindel, A. de Meijere, *J. Org. Chem.* **1995**, 60, 2968–2973. b) J. Zindel, A. Zeeck, W. A. König, A. de Meijere, *Tetrahedron Lett.* **1993**, 34, 1917–1920.
- [11] K. Loscha, *Diplomarbeit*, Universität Göttingen, **2001**.
- [12] P. Alvermann, *Dissertation*, Universität Göttingen, **2001**.
- [13] J. Zindel, *Dissertation*, Universität Göttingen, **1993**.
- [14] M. Brandl, S. I. Kozhushkov, K. Loscha, O. V. Kokoreva, D. S. Yufit, J. A. K. Howard, A. de Meijere, *Synlett* **2000**, 12, 1741–1744
- [15] K. Osugi *Yakugaki Zasshi* **1958**, 78, 1338–1342; C. A. 53, 8110e.

- [16] E. Melotto, *Dissertation*, Universität Göttingen, **1999**.
- [17] M. Ritzau, *Dissertation*, Universität Göttingen, **1992**.
- [18] M. Brandl, *Dissertation*, Universität Göttingen, **2000**.
- [19] K. Wagner-Gillen, *Diplomarbeit*, Universität Göttingen, **1998**.
- [20] K. Ohba, H. Nakayama, K. Furihata, A. Shimazu, T. Endo, U. Seto, N. Otake, *J. Antibiot.* **1987**, *40*, 709–713.
- [21] S. Hanessian, M. Bayrakdarian, X. Luo, *J. Am. Chem. Soc.* **2002**, *124*, 4716–4721.
- [22] J. Thottathil, J. Moniot, *Tetrahedron Lett.* **1986**, *27*, 151–154.
- [23] Although known, this compound was not characterized even a melting point.
- [24] An noticeable (three-fold) excess of the alkylating agent should be used because of its low reactivity and instability at the reaction temperature.
- [25] This and all other HPLC experiments, described in this work with several exceptions, were performed by H.-P. Kroll and P. Alvermann in Prof. A. Zeeck group (Göttingen).
- [26] H. Brückner, C. Keller-Hoehl, *Chromatographia* **1990**, *30*, 621–629.
- [27] P. Marfey, *Carlsberg Res. Communications* **1984**, *49*, 591–596.
- [28] R. Knecht, J.-Y. Chang, *Anal. Chem.*, **1986**, *58*, 2375–2379.
- [29] K. Hashimoto, Y. Shima, H. Shirahama, *Heterocycles* **1996**, *42*, 489–492.
- [30] M. M. Bowers-Nemia, M. M. Joullie, *Heterocycles* **1983**, *20*, 817–828.
- [31] P. S. Portogese, J. G. Turcotte, *Tetrahedron* **1971**, *27*, 961–967.
- [32] P. G. M. Wuts, L. E. Pruitt, *Synthesis* **1989**, 622–623.
- [33] a) A. Koskinen, H. Rapoport, *J. Org. Chem.* **1989**, *54*, 1859–1866. b) C. Moody; D. Young, *J. Chem. Soc. Perkin Trans. I* **1997**, 3519–3530.
- [34] This compound was prepared as it was described for its (*S*)-isomer: E. Morera, F. Pinnen, G. Lucente, *Org. Lett.* **2002**, *4*, 1139–1142.
- [35] M. Steger, D. Young, *Tetrahedron* **1999**, *55*, 7935–7956.

- [36] J. Ezquerra, A. Escribano, A. Rubio, M. J. Remuinan, J. J. Vaquero, *Tetrahedron Lett.* **1995**, *36*, 6149–6152.
- [37] A sample of this compound and was kindly provided by P. Alvermann, Prof. A. Zeeck group (Göttingen).
- [38] Cf. a) K. Fujii, Y. Ikai, H. Oka, M. Suzuki, K. Harada, *Anal. Chem.* **1997**, *69*, 3346–3352.
b) Y. Suzuki, M. Ojika, Y. Sakagami, K. Kaida, R. Fudou, T. Kameyama, *J. Antibiot.* **2001**, *54*, 22–28.
- [39] A. F. Coudert, R. Azerad, *Synthesis* **1997**, 863–865.
- [40] (2*S*,4*R*)-**33**, (2*S*,4*R*)-**34** and (2*S*,4*R*)-**35** were also prepared.
- [41] For a review see: Negishi, E.-i. In: *Metal-Catalyzed Cross-Coupling Reactions* (F. Diederich, P. J. Stang, Eds.) Wiley-VCH: New York, **1998**, Chapter 1.
- [42] P. Galatsis, J. J. Manwell, S. D. Millan, *Tetrahedron Lett.* **1996**, *37*, 5261–5264 and references cited therein.
- [43] J. Ezquerra, C. Pedregal, B. Yruretagoyena, A. Rubio, M. C. Carreno, *J. Org. Chem.* **1995**, *60*, 2925–2930.
- [44] J. E. Baldwin, T. Miranda, M. Moloney, T. Hokelek, *Tetrahedron* **1989**, *45*, 7459–7468.
- [45] J.-D. Charrier, J. E. S. Duffy, P. B. Hitchcock, D. W. Young, *Tetrahedron Lett.* **1998**, *39*, 2199–2204.
- [46] P. Merino, S. Anoro, S. Franco, F. L. Merchan, T. Tejero, V. Tunon, *J. Org. Chem.* **2000**, *65*, 1590–1596.
- [47] J.-R. Dormoy, *Synthesis* **1982**, 753–756.
- [48] P. Knochel, M. C. P. Yeh, S. C. Berk, J. Talbert, *J. Org. Chem.* **1988**, *53*, 2390–2392.
- [49] P. N. Collier, A. D. Campbell, I. Patel, R. J. K. Taylor, *Tetrahedron Lett.* **2000**, *41*, 7115–7120.
- [50] Review on the chemistry of DIBAH: E. Winterfeld, *Synthesis* **1975**, 617–630.

- [51] Cf. H. Ishiyama, T. Takemura, M. Tsuda, J. Kobayashi *Tetrahedron* **1999**, *55*, 4583–4594.
- [52] a) R. J. Bridges, M. S. Stanley, M. W. Anderson, C. W. Cotman, A. R. Chamberlin, *J. Med. Chem.* **1991**, *34*, 717–725. b) T. R. Webb, C. Eigenbrot, *J. Org. Chem.* **1991**, *56*, 3009–3016.
- [53] M. Shigeyasu, M. Kuwahara, M. Sisido, T. Ishikawa, *Chem. Lett.* **2001**, *7*, 634–635.
- [54] P. Chevallet, P. Garrouste, B. Malawska, J. Martinez, *Tetrahedron Lett.* **1993**, 7409–7412.
- [55] K.-i. Tanaka, H. Sawanishi, *Tetrahedron: Asymmetry* **1998**, *9*, 71–78.
- [56] S. Hagishita, K. Seno, *Chem. Pharm. Bull.* **1989**, *37*, 327–335.
- [57] Y. Iso, T. Irie, T. Iwaki, M. Kii, Y. Sendo, K. Motokawa, Y. Nishitani, *J. Antibiot.* **1996**, *49*, 478–484.
- [58] a) H. J. Bestmann, W. Stransky, O. Vostrowsky, *Chem. Ber.* **1976**, *109*, 1694–1700. b) C. A. Henrick, *Tetrahedron* **1977**, *33*, 1845–1889. c) A. Albeck, N. Friedman, M. Sheves, M. Ottolenghi, *J. Am. Chem. Soc.* **1986**, *108*, 4614–4618.
- [59] T. Katoh, Y. Nagata, Y. Kobayashi, K. Arai, J. Minami, S. Terashima, *Tetrahedron* **1994**, *50*, 6221–6238.
- [60] E. J. Corey, P. L. Fuchs, *Tetrahedron Lett.* **1972**, *36*, 3769–3772.
- [61] C. A. Brown, V. K. Ahuja, *J. Org. Chem.* **1973**, *38*, 2226–2230.
- [62] Y. Aoyagi, R. M. Williams, *Tetrahedron* **1998**, *54*, 13045–13058.
- [63] When the synthesis of the *N*-Boc protected 4-(*Z*)-propenylproline had already been completed, a very similar procedure for the preparation of allyl 4-cyano-2-([dimethyl(1,1,2-trimethylpropyl)silyl]oxy)methylpyrrolidine-1-carboxylate was published: M. Sunagawa, M. Itoh, K. Kubota, A. Sasaki, Y. Ueda, P. Angehrn, A. Bourson, E. Goetschi, P. Hebeisen, R. L. Then, *J. Antibiot.* **2002**, *55*, 722–757.

- [64] B. Zheng, H. Maeda, M. Mori, S. Kusaka, O. Yonemitsu, T. Matsushima, N. Nakajima, J. Uenishi *Chem. Pharm. Bull.* **1999**, *47*, 1288–1296.
- [65] a) Several other ways to oxidize the alcohol **54** to the acid **16** were tried, but all of them (PDC in DMF, first Swern or Dess-Martin oxidation and then the oxidation of the intermediate alcohol with sodium chlorite in *tert*-butanylic alcohol/phosphate buffer) gave the product in lower yield and purity than the oxidation with Jones reagent. b) A. Barco, S. Benetti, G. Spalluto, A. Casolari, G. P. Pollini, V. Zanirato, *J. Org. Chem.* **1992**, *57*, 6279–6286.
- [66] a) The peak of the epimer (2*S*,4*S*)-**16** was unobservable. b) Enantiomeric excess was determined according to method of Merfey for the corresponding (*S*)-FDVA derivative, obtained after removal of the Boc group from (2*S*,4*R*)-**16**.
- [67] T. Weber, D. Seebach, *Helv. Chim. Acta* **1985**, *68*, 155–161.
- [68] R. Annunziata, M. Ferrari, G. Papeo, M. Resmini, M. Sisti, *Synth. Commun.* **1997**, *27*, 23–38.
- [69] H. Wang, J. P. Germanas, *Synlett.* **1999**, 33–36.
- [70] S. Takano, W. Uchida, S. Hatakeyama, K. Ogasawara, *Chem. Lett.* **1982**, 733–736.
- [71] For runs on a small scale (0.5–1.5 mmol) yields were ca. 10% higher.
- [72] H. Heimgartner, H. Schmid, H. J. Hansen, *Helv. Chim. Acta* **1972**, *55*, 1385–1401.
- [73] T. W. Green, P. G. M. Wuts, *Protecting Groups in Organic Synthesis*, 3rd Ed., Wiley, New York, **1999**.
- [74] The nondeuterated (2*S*,4*R*)-**16** was also prepared. See experimental section for details.
- [75] a) D. F. Elliot *J. Chem. Soc.* **1950**, 62. b) P. G. Anderson, D. Guijaro, D. Tanner *J. Org. Chem.* **1997**, *62*, 7364–7375. c) For several examples of other syntheses of (*R*)-*allo*-threonine see: a) D. Pons, M. Savignac, J.-P. Genet, *Tetrahedron Lett.* **1990**, *31*, 5023–

5026. b) P. J. Maurer, H. Takahata, H. Rapoport, *J. Am. Chem. Soc.* **1984**, *106*, 1095–1098. c) H. Shao, M. Goodman, *J. Org. Chem.* **1996**, *61*, 2582–2583.
- [76] Selected examples see: a) R. Fitzi, D. Seebach, *Tetrahedron* **1988**, *17*, 5277–5292. b) M. Pasto, A. Moyano, M. A. Pericas, A. Riera, *J. Org. Chem.* **1997**, *62*, 8425–8431. c) F. A. Davis, H. Liu, P. Zhou, T. Fang, G. V. Reddy, Y. Zhang, *J. Org. Chem.* **1999**, *64*, 7559–7567.
- [77] V. J. Hruby, R. Dharanipragada, K. VanHulle, A. Bannister, S. Bear, L. Kennedy, *Tetrahedron* **1992**, *48*, 4733–4748.
- [78] D. A. Evans, T. C. Britton, J. A. Ellman, R. L. Dorrow, *J. Am. Chem. Soc.* **1990**, *112*, 4011–4030.
- [79] G. Li, D. Patel, V. J. Hruby, *J. Chem. Soc. Perkin Trans. I* **1994**, 3057–3059.
- [80] This compound was prepared from (*R*)-phenylglycine: (*R*)-phenylglycinol, which was obtained by reduction of (*R*)-phenylglycine with NaBH₄/H₂SO₄ in THF (*cf.*: A. Abiko, S. Masamune, *Tetrahedron Lett.* **1992**, *33*, 5517–5518), was cyclized by heating in dimethylcarbonate with K₂CO₃ (*cf.*: V. I. Tararov, T. F. Savel'eva, Yu. T. Struchkov, A. P. Pisarevskii, N. I. Raevskii, Yu. N. Belokon', *Russ. Chem. Bull.* **1996**, *45*, 600–609, *Izv. Akad. Nauk Ser. Khim.* **1996**, *3*, 640–648).
- [81] E. Nicolas, K. C. Russel, V. J. Hruby *J. Org. Chem.* **1993**, *58*, 766–770.
- [82] H. Josien, A. Martin, G. Chassaing, *Tetrahedron Lett.* **1991**, *32*, 6547–6550.
- [83] For a review on these chiral auxiliary see: Yu. N. Belokon', *Janssen Chim. Acta* **1992**, *10*, 4–12.
- [84] O. V. Larionov, T. F. Savelieva, K. A. Kochetkov, N. S. Ikonnikov, S. I. Kozhushkov, D. S. Yufit, J. A. K. Howard, V. N. Khrustalev, Yu. N. Belokon, A. de Meijere, *Eur. J. Org. Chem.* **2003**, 869–877.
- [85] L. L. Gordon, C. Gottardo, *Synth. Commun.* **1990**, *20*, 1473–1479.

- [86] Yu. N. Belokon', V. I. Taranov, V. I. Maleev, T. F. Savel'eva, M. G. Ryzhov, *Tetrahedron: Asymetry* **1998**, 9, 4249–4252.
- [87] a) Y. Elemes, U. Ragnarsson, *J. Chem. Soc. Perkin Trans.1* **1996**, 537–540. b) To the best of our knowledge no procedure for the preparation of deuterated **81** was published before.
- [88] If nondeuterated acetonitrile was used, ca. 50% loss of deuterium occurred.
- [89] For the full assignment of ^1H - and ^{13}C -NMR spectra of **3**, see: P. Henne, *Dissertation*, Universität Göttingen, **1994**.
- [90] Y. Kai, P. Knochel, S. Kwiatkowski, J. D. Dunitz, J. F. M. Oth, D. Seebach, H. O. Kalinowski, *Helv. Chim. Acta* **1982**, 65, 137–161.
- [91] A similar experiment with (2*S*,2'*S*,1"*R*,2"*S*)-**16** was unsuccessful because of solubility problems.
- [92] This compound was generously donated by Oleg V. Larionov, Göttingen.
- [93] P. E. O'Bannon, W. P. Dailey, *J. Amer. Chem. Soc.* **1989**, 111, 9244–9245.
- [94] P. A. Wade, P. A. Kondracki, P. A. Carroll, *J. Am. Chem. Soc.* **1991**, 113, 8807–8811.
- [95] A. Thomas, S. Rajappa, *Tetrahedron* **1995**, 51, 10571–10580.
- [96] a) F. Effenberger, W. Mueller, H. Isak, *Chem. Ber.* **1987**, 120, 45–54. b) P. Netchitailo, M. Othman, A. Daich, B. Decroix, *Tetrahedron Lett.* **1997**, 38, 3227–3230.
- [97] G. Zinner, R. Moll, *Chem. Ber.* **1966**, 99, 1292–1298.
- [98] R. A. Abramovitch, B. W. Cue, *J. Am. Chem. Soc.* **1976**, 98, 1478–1486.
- [99] T. Ikemoto, T. Kawamoto, H. Wada, T. Ishida, T. Ito, Y. Isogami, Y. Miyano, Y. Mizuno, K. Tomimatsu, K. Hamamura, M. Takatani, M. Wakimasu, *Tetrahedron* **2002**, 58, 489–494.
- [100] L. Pentimalli, *Gazz. Chim. Ital.* **1964**, 94, 458.

- [101] L. P. Ferrarini, G. Biagi, O. Livi, G. Primofiore, M. Carpena *J. Heterocycl. Chem.* **1981**, *18*, 1007–1010.
- [102] S. V. D'Andrea, D. Bonner, J. J. Bronson, J. Clark, K. Denbleyker, J. Fung-Tome, S. E. Höft, T. W. Hudyma, J. D. Matiskella, R. F. Miller, P. F. Misco, M. Pucci, R. Sterzycki, Y. Tsai, Y. Ueda, J. A. Wichtowski, J. Singh, T. P. Kissick, J. T. North, A. Pullockaran, M. Humora, B. Boyhan, T. Vu, A. Fritz, J. Heikes, R. Fox, J. D. Godfrey, R. Perrone, M. Kaplan, D. Kronenthal, R. H. Müller, *Tetrahedron* **2000**, *56*, 5687–5698.
- [103] S. Cacchi, D. Misiti, P. La Torre, *Synthesis* **1980**, 243–244.
- [104] S. K. Davidsen, P. D. May, J. B. Summers, *J. Org. Chem.* **1991**, *56*, 5482–5485.
- [105] S. A. M. Merette, A. P. Burd, J. J. Deadman, *Tetrahedron Lett.* **1999**, *40*, 753–754.
- [106] S. Kim, I. S. Kee, Y. H. Park, J. H. Park, *Synlett* **1991**, *3*, 183–184.
- [107] L. A. Carpino, *J. Am. Chem. Soc.* **1993**, *115*, 4397–4398.
- [108] J. Bernstein, B. Stearns, E. Shaw, W. A. Lott, *J. Am. Chem. Soc.* **1947**, *69*, 1151–1158.
- [109] This compound was also obtained according to P. L. Ferrarini, C. Mori, C. Manera, F. Mori, V. Calderone, E. Martinotti, *J. Heterocycl. Chem.* **1999**, *36*, 1123–1128 in 33% yield.
- [110] For a review on peptide coupling agents see: F. Albericio, R. Chinchilla, D. J. Dodsworth, C. Najera, *Org. Prep. Proc. Int.* **2001**, *33*, 203–303.
- [111] L. A. Carpino, A. El-Faham, *Tetrahedron* **1999**, *55*, 6813–6830.
- [112] W. R. Kobertz, J. M. Essigmann, *J. Am. Chem. Soc.* **1996**, *118*, 7101–7107.
- [113] W.-R. Li, W. R. Ewing, B. D. Harris, M. M. Joulie, *J. Am. Chem. Soc.* **1990**, *112*, 7659–7672.
- [114] S. F. Brady, S. L. Varga, R. M. Freidinger, D. A. Schwenk, M. Mendlowski, F. W. Holly, D. F. Veber, *J. Org. Chem.* **1979**, *44*, 3101–3105.
- [115] L. Zervas, D. Borovas, E. Gazis, *J. Am. Chem. Soc.* **1963**, *85*, 3660–3667.

- [116] R. E. Vegner, L. K. Polevaja, I. A. Vosekalna, *Izv. AN LatSSR* **1979**, 103–106 (in Russian).
- [117] R. Sharan, B. Kundu, K. B. Mathur, *Indian J. Chem. Sect. B* **1991**, 30, 728–731.
Dichloromethane instead of DMF was used as a solvent.
- [118] A. F. Abdel-Magid, J. H. Cohen, C. A. Maryanoff, R. D. Shah, F. J. Villiani, F. Zhang, *Tetrahedron Lett.* **1998**, 39, 3391–3394.
- [119] D. M. Birney, D. C. Cole, C. E. Crosson, B. F. Kahl, B. W. Neff, T. W. Reid, K. Ren, R. D. Walkup, *J. Med. Chem.* **1995**, 38, 2478–2482.
- [120] J. Robles, M. Beltran, V. Marchan, Y. Perez, I. Travesset, E. Pedroso, A. Grandas, *Tetrahedron* **1999**, 55, 13251–13264.
- [121] E. C. Roos, P. Bernabe, H. Hiemstra, W. N. Speckamp, B. Kaptein, W. H. J. Bösten, *J. Org. Chem.* **1995**, 60, 1733–1740.
- [122] P. Sieber, *Helv. Chim. Acta* **1977**, 60, 2711–2716.
- [123] B. H. Lipshutz, J. J. Pegram, *Tetrahedron Lett.* **1980**, 21, 3343–3346.
- [124] W.-C. Chen, M. D. Vera, M. M. Joullie, *Tetrahedron Lett.* **1997**, 38, 4025–4028.
- [125] A. Ehrlich, H. U. Heyne, R. Winter, M. Beyermann, H. Haber, L. A. Carpino, M. Bienert, *J. Org. Chem.* **1996**, 61, 8831–8838.
- [126] Y. Kiso, K. Ukawa, T. Akita, *J. Chem. Soc. Chem. Commun.* **1980**, 101–102.
- [127] a) M. B. Smith, *March's Advanced Organic Chemistry: Reactions, Mechanisms and Structure* (M. B. Smith, J. March, eds.), Wiley, New-York **2001**, 370. b) C. G. Swain, E. C. Lupton, *J. Am. Chem. Soc.* **1968**, 90, 4328–4339.
- [128] L. A. Carpino, H. G. Chao, S. Ghassemi, E. M. Mansour, C. Riemer, R. Warrass, D. Sadat-Aalae, G. A. Truran, H. Imazumi, A. El-Faham, D. Ionescu, M. Ismail, T. L. Kowaleski, C. H. Han, H. Wenschuh, M. Beyermann, M. Bienert, H. Shroff, F. Albericio, S. A. Triolo, N. A. Sole, S. A. Kates, *J. Org. Chem.* **1995**, 60, 7718–7719.

- [129] O. V. Larionov, S. I. Kozhushkov, M. Brandl, A. de Meijere, *Mendeleev Commun.*, 2003, in press.
- [130] R. Matsueda, R. Walter, *Int. J. Peptide Protein Res.* **1980**, *16*, 392–401.
- [131] F. Yokokawa, H. Sugiyama, T. Shioiri, N. Katagiri, O. Oda, H. Ogawa, *Tetrahedron* **2001**, *57*, 4759–4766.
- [132] a) K. E. Gilbert, W. T. Borden, *J. Org. Chem.* **1979**, *44*, 659–661. b) P. Peterli-Roth, M. P. Maguire, E. Leon, H. Rapoport, *J. Org. Chem.* **1994**, *59*, 4186–4193.
- [133] H. Han, J. Yoon, K. D. Janda, *J. Org. Chem.* **1998**, *63*, 2045–2048.
- [134] K. Wen, H. Han, T. Z. Hoffman, K. D. Janda, L. E. Orgel, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 689–692.
- [135] D. D. Hennings, R. M. Williams, *Synthesis* **2000**, 1310–1314.
- [136] K. Sakai, J.-P. Anselme, *J. Org. Chem.* **1971**, *36*, 2387–2388.
- [137] Y. Kataoka, Y. Seto, M. Yamamoto, T. Yamada, S. Kuwata, H. Watanabe, *Bull. Chem. Soc. Jpn.* **1976**, *49*, 1081–1084.
- [138] V. Fedij, E. A. Lenoir III, M. J. Suto, J. R. Zeller, J. Wemple, *Tetrahedron: Asymmetry* **1998**, *5*, 1131–1134.
- [139] A. A. Gerschkovich, V. K. Kibirev, *Himicheskij syntez peptidov* **1992**, Kiev, Naukova Dumka (in Russian).
- [140] M. Ueki, M. Honda, Y. Kazama, T. Katoh, *Synthesis* **1994**, 21–22.
- [141] L. A. Carpino, US Patent 5,580,981, **1996**.
- [142] R. E. Shute, D. H. Rich, *Synthesis* **1987**, 346–349.
- [143] I. Ojima, T. Kogure, N. Yoda, *J. Org. Chem.* **1980**, *45*, 4728–4739.
- [144] B. Maurer, W. Keller-Schierlein, *Helv. Chim. Acta* **1969**, *52*, 388–396.

F. Spectral Data**1. ^1H -NMR Spectra**

$(2S,4R)$ - N -(*tert*-Butyloxycarbonyl)-4-(*Z*)-propenylproline ($2S,4R$)-16

(2S,4R)-N-(tert-Butyloxycarbonyl)-4,1'-dideutero-4-(Z)-propenylproline (2S,4R)-D₂-16

(2S,4R)-4-(Z)-propenylproline hydrochloride (2S,4R)-17·HCl

(2*S*,4*R*)-4,1'-Dideutero-4-(*Z*)-propenylproline (2*S*,4*R*)-17

Ni-complex (2*S*,2'*S*,1''*RS*,2''*RS*)-D-82

(2S,1'RS,2'RS)-2-Deutero-3-(2'-nitrocyclopropyl)alanine (2S,1'RS,2'RS)-D-(83)

5-Chloro-1-hydroxypyrrole-2-carbonitrile (103)

5-Chloro-1-methoxymethoxypyrrole-2-carboxylic acid (109)

MeZ-*a*-Thr[Boc-(4-PE)Pro]-(βMe)Phe-(3-Ncp)Ala-(βMe)Phe-Ile-OMOM (191a)

N-MeZ protected cyclohexadepsipeptide (**171c**)

O-MOM protected hormaomycin *O*-MOM-(**3**)

Hormaomycin (**3**) N_{ω} -Z protected (2S,1'S,2'R)-3-(2'-Aminocyclopropyl)alanine (*S*)-(199)

151 cdcl3
1s/AdM

se Sequence: s2pul
20 2003

TRUMENT: INOVA-600
ERVE H1
equency 599.740 MHz
ectral width 10000.0 Hz
quisition time 3.200 sec
laxation delay 0.000 sec
lse 45.0 degrees
bient temperature
repetitions
hle precision acquisition
A PROCESSING
size 131072
al acquisition time 1 minute

$[(S)-(3\text{-Acp})\text{Ala}(\text{Npys})^7]\text{-MOM-3}$

$(S)\text{-5-Nitronorvaline hydrochloride (208)}$

Chpca(MOM)-(NO₂)Nva-Phe-Val-OMe (**211**)

[(S)-(NO₂)Nva⁷]-Hormaomycin [(S)-(NO₂)Nva⁷]-(**3**)

[(S)-(NO₂)Nva³,(S)-(NO₂)Nva⁷]-Hormaomycin [(S)-(NO₂)Nva³,(S)-(NO₂)Nva⁷]-**(3)**

MeZ- α -Dab[Boc-(4-PE)Pro]-(β Me)Phe-(R)-(3-Ncp)Ala-(β Me)Phe-Ile-ODCPM (**218**)

MeZ-Protected branched cyclohexapeptide (**219a**)

219b

bzd172_cdcl3
Boris/AdM

Pulse Sequence: s2pul
Jun 4 2003

INSTRUMENT: INOVA-600
OBSERVE H1
Frequency 599.742 MHz
Spectral width 13793.1 Hz
Acquisition time 3.200 sec
Relaxation delay 0.000 sec
Pulse 45.0 degrees
Temperature 22.0 deg. C / 295.1 K
64 repetitions
Double precision acquisition
DATA PROCESSING
FT size 131072
Total acquisition time 3 minutes

[*a*-Dab¹]-Hormaomycin [*a*-Dab¹]-3

AZ-186_cd10d
Boris / AdM / CS

Pulse Sequence: s2pul
Sep 16 2003

INSTRUMENT: INOVA-600
OBSERVE H1
Frequency 599.743 MHz
Spectral width 10000.0 Hz
Acquisition time 3.200 sec
Relaxation delay 0.000 sec
Pulse 45.0 degrees
Temperature 22.0 deg. C / 295.1 K
64 repetitions
Double precision acquisition
DATA PROCESSING
FT size 131072
Total acquisition time 3 minutes

AZ-186_cd10d
Boris / AdM / CS
Pulse Sequence: s2pul
DATE: Sep 16 2003 FILE:

[*a*-Dab¹,*a*-Ile⁵]-Hormaomycin [*a*-Dab¹,*a*-Ile⁵]-3

2. ^{13}C -NMR Data

(2*S*,4*R*)-*N*-(*tert*-Butyloxycarbonyl)-4-(*Z*)-propenylproline (2*S*,4*R*)-**16**

(2*S*,4*R*)-*N*-(*tert*-Butyloxycarbonyl)-4,1'-dideutero-4-(*Z*)-propenylproline (2*S*,4*R*)- D_2 -**16**

(2*S*,4*R*)-4-(*Z*)-propenylproline hydrochloride (2*S*,4*R*)-**17**·HCl

(2*S*,4*R*)-4,1'-Dideutero-4-(*Z*)-propenylproline (2*S*,4*R*)-**17** (66.5 ppm – dioxane)

Ni-complex (2*S*,2'*S*,1''*RS*,2''*RS*)-D-82

(2*S*,1'*RS*,2'*RS*)-2-Deutero-3-(2'-nitrocyclopropyl)alanine (2*S*,1'*RS*,2'*RS*)-D-(83)

276

5-Chloro-1-hydroxypyrrole-2-carbonitrile (**103**) 5-Chloro-1-methoxymethoxypyrrole-2-carboxylic acid (**109**)

MeZ-*a*-Thr[Boc-(4-PE)Pro]-(βMe)Phe-(3-Ncp)Ala-(βMe)Phe-Ile-OMOM (**191a**)

N-MeZ protected cyclohexadepsipeptide (**171c**)

Pulse Sequence: s2pul
Nov 11 2002
INSTRUMENT: INOVA-600
OBSERVE C13
Frequency 150.820 MHz
Spectral width 34995.6 Hz
Acquisition time 1.829 sec
Relaxation delay 0.000 sec
Pulse 35.0 degrees
Ambient temperature
23504 repetitions
DECOUPLE H1
Frequency 599.740 MHz
Power 44 dB
Decoupler continuously on
WALTZ-16 modulated
Double precision acquisition
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total acquisition time 11.9 hours

O-MOM protected hormaomycin O-MOM-(3)

Hormaomycin (3)

N_{ω} -Z protected (2*S*,1'*S*,2'*R*)-3-(2'-Aminocyclopropyl)alanine (*S*)-(199)

bzd151 cdc13
Boris/ADM
Pulse Sequence: s2pul
Feb 27 2003
INSTRUMENT: INOVA-600
OBSERVE C13
Frequency 150.821 MHz
Spectral width 40712.5 Hz
Acquisition time 1.829 sec
Relaxation delay 0.000 sec
Pulse 35.0 degrees
Ambient temperature
24000 repetitions
DECOUPLE H1
Frequency 599.740 MHz
Power 44 dB
Decoupler continuously on
MALTZ-16 modulated
Double precision acquisition
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total acquisition time 12.2 hours

$[(S)-(3\text{-Acp})\text{Ala}(\text{Npys})^7]\text{-MOM-3}$

DAVIDO 6400
Zlatopolski / AdM
Jun 27 2003

INSTRUMENT: UNITY-200
OBSERVE C13
Frequency 50.293 MHz
Spectral width 12001.2 Hz
Acquisition time 1.365 sec
Relaxation delay 0.000 sec
Pulse 35.0 degrees
Temperature 35.0 deg. C / 308.1 K
12800 repetitions
DECOUPLE H1
Frequency 199.992 MHz
Power 40 dB
Decoupler continuously on
MALTZ-16 modulated
Double precision acquisition
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total acquisition time 4.9 hours

(S)-5-Nitronorvaline hydrochloride (**208**) (methanol as a standard)

REPT 65536
Zlatopolski / AdM / CS
Pulse Sequence: s2pul
Jul 21 2003

INSTRUMENT: INOVA-600
OBSERVE C13
Frequency 150.821 MHz
Spectral width 40713.5 Hz
Acquisition time 1.829 sec
Relaxation delay 0.000 sec
Pulse 35.0 degrees
Ambient temperature
2987 repetitions
DECOUPLE H1
Frequency 599.740 MHz
Power 44 dB
Decoupler continuously on
MALTZ-16 modulated
Double precision acquisition
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total acquisition time 91 minutes

Chpca(MOM)-(NO₂)Nva-Phe-Val-OMe (**211**)

bsd171 cdc13
Boris/AdM
Pulse Sequence: s2pul
Jun 11 2003
INSTRUMENT: INOVA-600
OBSERVE C13
Frequency 150.820 MHz
Spectral width 34995.6 Hz
Acquisition time 1.852 sec
Relaxation delay 0.000 sec
Pulse 35.0 degrees
Ambient temperature
21000 repetitions
DECOUPLE H1
Frequency 599.740 MHz
Power 44 dB
Decoupler continuously on
WALTZ-16 modulated
Double precision acquisition
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total acquisition time 10.7 hours

[(S)-(NO₂)Nva⁷]-Hormaomycin [(S)-(NO₂)Nva⁷]-**(3)**

bsd161 cdc13
Boris/AdM
Pulse Sequence: s2pul
Mar 17 2003
INSTRUMENT: INOVA-600
OBSERVE C13
Frequency 150.820 MHz
Spectral width 34995.6 Hz
Acquisition time 1.829 sec
Relaxation delay 0.000 sec
Pulse 35.0 degrees
Ambient temperature
2496 repetitions
DECOUPLE H1
Frequency 599.740 MHz
Power 44 dB
Decoupler continuously on
WALTZ-16 modulated
Double precision acquisition
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total acquisition time 76 minutes

MeZ-*a*-Dab[Boc-(4-PE)Pro]-(βMe)Phe-(R)-(3-Ncp)Ala-(βMe)Phe-Ile-ODCPM (**218**)

```

b1h01: cdc13
Boris/Adm
Pulse Sequence: #2pul
Apr 16 2003

INSTRUMENT: INOVA-600
OBSERVE C13
Frequency 150.820 MHz
Spectral width 34995.6 Hz
Acquisition time 1.820 sec
Relaxation delay 0.000 sec
P1 35.0 degrees
Solvent temperature
3000 repetitions
COUPLE H1
Frequency 500.740 MHz
Power 44 dB
ecoupler continuously on
ALT-16 modulated
wide precision acquisition
TA PROGRESSING
line broadening 1.0 Hz
F1 size 131072
Total acquisition time 9.1 hours

```

MeZ-Protected branched cyclohexapeptide (**219a**)

```

:13
/ Adm
ice: #2pul

INOVA-600
150.820 MHz
Spectral width 34995.6 Hz
Acquisition time 1.820 sec
Relaxation delay 0.000 sec
P1 35.0 degrees
Solvent temperature
3000 repetitions
COUPLE H1
Frequency 500.740 MHz
Power 44 dB
ecoupler continuously on
ALT-16 modulated
wide precision acquisition
TA PROGRESSING
line broadening 1.0 Hz
F1 size 131072
Total acquisition time 10.0 hours

```

219a

brd172 cdc13
Boris/AdH
Pulse Sequence: mzgpg1
Jun 3 2003
INSTRUMENT: INOVA-600
OBSERVE C13
Frequency 150.820 MHz
Spectral width 30400.6 Hz
Acquisition time 1.629 sec
Relaxation delay 0.000 sec
Pulse 35.0 degrees
Ambient temperature
21000 repetitions
DECOUPLE H1
Frequency 599.740 MHz
Power 44.00
Decoupler continuously on
WALTZ-16 modulated
Double precision acquisition
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total acquisition time 2.7 hours

[α -Dab¹]-Hormaomycin [α -Dab¹]-**(3)**

Lebenslauf

Am 07.03.1970 wurde ich als Sohn des Ingenieurs Dmitrij Zlatopolski und der Konstrukteurin Galina Zlatopolskaja, geborene Avdeeva, in Leningrad (Russische Föderation) geboren.

Von September 1976 bis Juli 1986 besuchte ich die Grund- und Mittelschule N°234 in Leningrad, an der ich im Juli 1986 mein Abitur absolvierte.

Zum Herbstsemester 1986 begann ich das Studium der Chemie an der Leningrader (St.-Petersburger) Staatsuniversität. Nach dem Abschluss des 4. Semesters wurde ich in die Sowjetarmee einberufen, in der ich von Juni 1988 bis September 1989 meinen Grundwehrdienst leistete. Zum Herbstsemester 1989 nahm ich das Studium wieder auf, absolvierte den vollen Studiengang und fertigte unter der wissenschaftlichen Anleitung von Herrn Dr. S. V. Nikolaev meine Diplomarbeit zu dem Thema „*Synthese der in sechster Position modifizierten LHRH Analoga*“ an. Am 22. Juni 1992 bestand ich meine Diplomhauptprüfung vor der Staatlichen Prüfungskommission, wobei mir die Qualifizierung des Chemikers verliehen wurde.

Im Zeitraum von September 1992 bis Oktober 1995 war ich in der Forschungsabteilung der „SPARK GmbH“ angestellt.

Von Oktober 1995 bis Juni 2000 arbeitete ich im Arbeitskreis von Prof. Dr. A. G. Shavva zum Thema „Synthetische Untersuchung amphiphiler Peptidantibiotika“.

Im Dezember 1999 heiratete ich Elizaveta Ourousova.

Im August 2000 kam ich als Gastwissenschaftler nach Deutschland und arbeite seit Oktober 2000 im Arbeitskreis von Prof. Dr. A. de Meijere an meiner Dissertation „*Endgültige Strukturauklärung des Hormaomycins und Totalsynthese von Hormaomycin sowie Analoga*“.

Mit Erfolg absolvierte ich im Wintersemester 2002/2003 den Kurs zur Allgemeinen Pharmakologie und Toxikologie.

Im September 2003 wurde mir das an der St.-Petersburger Universität ausgegebene Diplom als Äquivalent zu einem Diplom in Chemie an einer deutschen Universität anerkannt.

Danksagungen

Für die Aufnahme in seinen Arbeitskreis und sein Bemühen, mir eine finanzielle Unterstützung zu sichern, möchte ich ganz herzlich Herrn Prof. Dr. A. de Meijere danken.

Mein allergrößter Dank gehört meiner Frau Elizaveta, die mir von Anfang an in jedem schwierigen Moment geholfen hat.

Herrn Prof. A. Zeeck danke ich für viele wertvolle Diskussionen und wichtige Tips rund um die Chemie und Biochemie des Hormaomycins.

Für die sehr gute Ausführung zahlreicher biologischer und HPLC-Experimente sowie für die Aufnahme der UV- und CD-Spektren gilt mein Dank Herrn H.-P. Kroll und Frau M. Klingebiel. Ohne ihre Zusammenarbeit hätte diese Arbeit nicht durchgeführt werden können.

Ein großes Dankeschön an Herrn Dr. S. V. Nikolaev (Sankt-Petersburg) für die schnelle und exzellente Durchführung sehr wichtiger HPLC-Experimente sowie Herrn Prof. Dr. W. A. König (Hamburg) für sehr wichtige GC-Experimente.

Für die finanzielle Unterstützung im Rahmen des Sonderforschungsbereiches SFB 416 danke ich der Deutschen Forschungsgemeinschaft.

Für die Aufnahme der NMR-Spektren danke ich den Mitarbeitern der NMR-Abteilung und besonders Herrn Dipl. Chem. R. Machinek, Herrn M. Weitemeyer und Frau C. Zolke für die stete Hilfsbereitschaft und die anregenden Diskussionen.

Herrn Hambloch danke ich für die Durchführung der Elementaranalysen, Frau G. Udvarnoki und Herrn Dr. H. Frauendorf für die aufgenommenen Massenspektren und durchgeführten HPLC/MS-Experimente, und Herrn Dipl. Chem. O. Senge für die Ausführung präparativer HPLC-Trennungen.

Frau G. Keil-Knepel verdient für ihre Unterstützung bei den verschiedensten Problemen Anerkennung und Dank.

Für stetige Hilfs- und Diskussionsbereitschaft sowie zahlreiche chemische Tips und Tricks bedanke ich mich bei den Herren Dr. S. I. Kozhushkov, V. N. Belov, V. V. Sokolov, den Herren Dipl. Chem. O. V. Larionov, V. Bagutski, der Frau Dr. L. A. Maslovskaja und ganz besonders den Herren Dr. A. I. Savchenko, A. Leonov und S. Nizamov.

Mein besonderer Dank gilt Frau Dipl. Chem. F. Brackmann und Herrn Dipl. Chem. H. Schill für ihre Hilfe in meinem schwierigen „Kampf“ mit der deutschen Orthographie und Grammatik.

Für das sorgfältige Korrekturlesen dieser Arbeit bedanke ich mich bei den Herren Dipl. Chem. I. Freifeld, O. Larionov, Herrn Dr. S. Nizamov und in besonderem Maße bei den Herrn Dipl. Chem. D. Ovchinnikov, Dr. V. N. Belov und Prof. Dr. S. L. Mironov.

Ich möchte allen Mitarbeitern und Gastwissenschaftlern des Arbeitskreises danken, die mir in diesen Jahren ihre Freundschaft erwiesen haben: Farina, Martina, Björn, Gerd, Steffi, Gabriel, Michael (× 2),

Heiko, Mario, Daniel, Karsten, Andrej ($\times 2$), Shamil, Oleg, Victor, Hans, Alessandra, Regina, Jelena, Markus und Stefan ($\times 2$). Auch den restlichen Mitarbeitern des Arbeitskreises danke ich für ihre stete Diskussions- und Hilfsbereitschaft.