

Synthesis of Jasplakinolide Analogues Containing a Novel ω -Amino Acid

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Dedicated to Prof. R. R. Schmidt for his 70th birthday

Abstract: The synthesis of the ω -amino acid **4** is described utilizing a two-dimensional synthesis strategy combined with an enzymatic differentiation of homotopic ester groups. The amino acid **4** features two non-bonded interactions that result in conformational constraints on a cyclic construct. This amino acid was incorporated into the four macrolactams **17**, **22**, **31**, and **37**. The ring in **17** and **22** is 18-membered, whereas **31** and **37** have a 19-membered

ring. The pairs with the same ring size differ in a N-methyl group. For the larger macrolactams (**31** and **37**) conformational analysis showed that the macrocyclic rings are somewhat more rigid than in the natural lead, the depsipeptide jasplakinolide.

Keywords: amino acids • conformation analysis • natural products • peptidomimetic • total synthesis

Nevertheless, their conformations are comparable to the natural product. There are no intramolecular hydrogen bonds, neither is the *cis*-rotamer populated in the N-methyl compound **37**. Due to the increased flexibility of the smaller macrolactams **17** and **22** and signal overlap, a distinct solution structure could not be obtained for these compounds. The amino acid **4** should be useful for restricting the conformation of other small peptides.

Introduction

Constraining the conformation of a peptide fragment by incorporating it in a macrocyclic structure represents an important strategy for enhancing both binding strength and selectivity. In addition, this maneuver can suppress unwanted proteolysis. Studying the solution conformation of such a macrocyclic construct can provide important information on the peptide surface structure and the area that is presented to a receptor. The application of this strategy is common in medicinal chemistry.^[1] In a class of natural products, such as the cyclodepsipeptides, the presence of the macrocyclic ring most likely also improves binding and stability. Besides a macrocyclic ring, cyclodepsipeptides are characterized by the presence of at least one ester bond because they contain

a hydroxy acid. Furthermore, they contain unusual amino acids, which may be extended, N-methylated, hydroxylated, or halogenated. Occasionally, they contain fragments from other biosynthetic pathways, for example polyketides. The substituents on the polyketide fragment might be used as conformational control elements. An illustrative example is the depsipeptide jasplakinolide A (**1**) (Figure 1). This natural product, which was isolated from the sponge *Jaspis* sp.,^[2,3] shows potent antifungal, insecticidal, and antitumor activity. Related to the latter activity is its use as a tool in cytoskeletal research, since it stabilizes F-actin.^[4] A related compound is the cyclodepsipeptide geodiamolide A,^[5,6] (**2**), which has somewhat different activity.^[7]

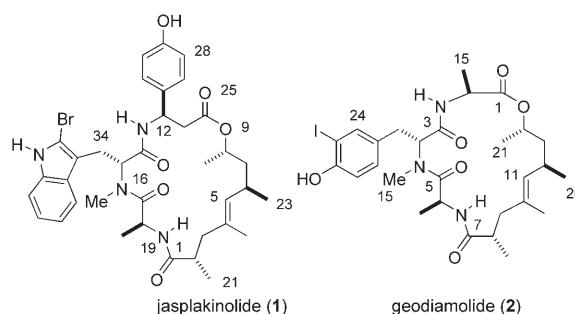


Figure 1. Structures of the cyclodepsipeptides jasplakinolide (**1**) and geodiamolide (**2**).

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Besides the tripeptide fragment, the ω -hydroxyacid **3** is part of these depsipeptides (Figure 2). This hydroxy acid contains four methyl groups in a 1,3-distance giving rise to two *syn*-pentane interactions and one 1,3-allylic interaction.^[8] As a result of this the carboxyl and hydroxyl groups at the end of the chain point in one direction, thereby allowing bridging with a peptide fragment. While the synthesis of the hydroxy acid **3** is feasible,^[9] the preparation of a larger amount is quite costly. We therefore turned to the design of simpler analogues of this hydroxy acid that also contains conformation controlling elements.

The design of the amino acid **4** followed from looking at the conformational control elements in the hydroxy acid **3** (Figure 2). Thus, the 1,3-allylic interaction around the central trisubstituted double bond should position the allylic C–H in an eclipsed orientation to the double bond.^[8] As a consequence, the methyl group at C-6 will point downwards and orient the 2-hydroxypropyl terminus to the other side, out of the plane of the double bond. The conformational situation at the carboxyl terminus seems to be less defined. Nevertheless, avoidance of *syn*-pentane interactions between C2–Me and C4–Me will cause the carboxyl group to point out of the plane of the central double bond as well. Our design plan then called for a rigidification of the vinylic C5–C6 bond. Accordingly, the allylic H was replaced with a two-carbon segment (see dashed lines in Figure 2), resulting in a *meta*-disubstituted aryl core. This simplification removes the stereocenter at C-6.

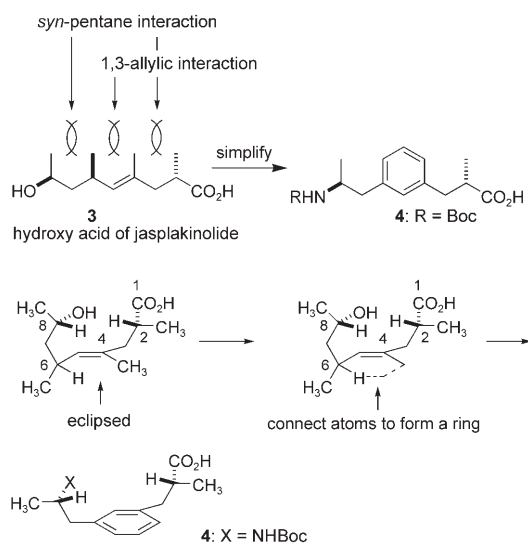


Figure 2. Design of the novel ω -amino acid **4**.

In order to probe the design process, conformational search runs (Macromodel 7.0, MM2* force field, 1000 starting structures) were carried out on hydroxy acid **3** and the amino acid **4** (without the N-Boc protecting group). The search for the hydroxy acid **3** found four conformers within 4.184 kJ mol^{−1} (1.0 kcal mol^{−1}) of the minimum (E = 36.14 kJ mol^{−1}). The lowest conformer **3a** has the allylic

C–H eclipsing the C4–Me, but only the carboxyl group is pointing out of the plane of the double bond (Figure 3). In the next lowest conformer **3b** (ΔE = 0.93 kJ mol^{−1}) 6-H, surprisingly, is *anti* to the C4–Me. Basically, C4–Me is bisecting

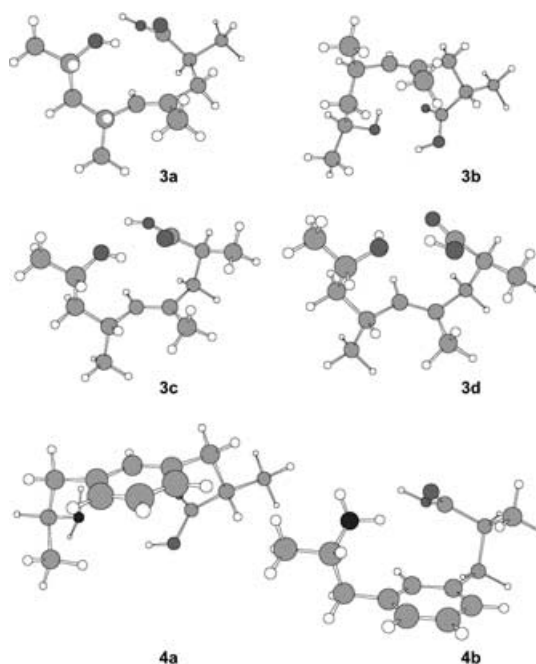


Figure 3. Calculated low energy conformations of the hydroxy acid **3** and the amino acid **4** (Chem3D representations).

the angle C6–Me/C-6/C-7. This orients both termini to the other side of the central π -system. Conformers **3c** (ΔE = 2.00 kJ mol^{−1}) and **3d** (ΔE = 4.10 kJ mol^{−1}) actually match the expected conformation. Thus, 6-H eclipses the vinylic methyl and the termini extend nicely to one side. For the amino acid **4** we found two conformers within 4.184 kJ mol^{−1} of the absolute minimum (E = 35.96 kJ mol^{−1}). In all cases, the termini are oriented more or less orthogonal to the plane of the aryl ring. In the second lowest conformer **4b** (ΔE = 2.45 kJ mol^{−1}) the termini point to the opposite side of the aryl ring. Conformer **4b** does have a striking similarity to conformer **3c** of the hydroxy acid. Most likely electrostatic interaction and hydrogen bonding cause a substantial gain in energy if both groups point to the same side. By looking at several of the calculated minima, it seems that the conformation of the aryl analogue is more ordered and less flexible.

An overlay of conformers **3c** and **4b** shows a decent overlap validating our original design (Figure 4). In another run, the configuration at the amino bearing carbon was inverted. In this case we found also several reasonable conformers, in which both termini extend from the same face of the aryl ring.

It should be mentioned that alternative strategies for constraining the conformation of peptides are known. Frequently, this involves the use of designed amino acids containing a

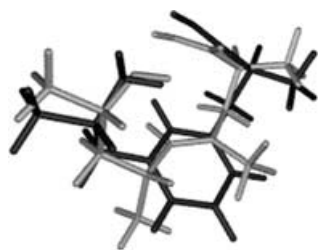
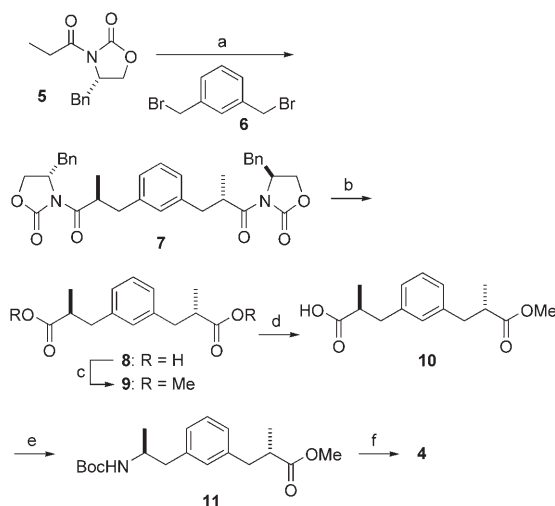


Figure 4. Overlay of the calculated conformers **3c** and **4b** (grey, hydroxy acid; black, aromatic amino acid).

cyclic backbone.^[10] In contrast to our work, a recent paper describes the synthesis of three jasplakinolide analogues in which the ω -hydroxy acid was replaced with ω -amino acids, for example 6-amino hexanoic acid, lacking conformational constraints.^[11]

The synthesis of the amino acid **4** began with the commercially available 1,3-bis(bromomethyl)benzene (**6**), which was subjected to a double alkylation^[12,13] with the propionyloxazolidinone^[14] **5** (Scheme 1). This way the C-2 symmetric alkylation product **7** was obtained in satisfactory yield (58 %). Subsequently, the chiral auxiliary was removed by hydrolysis

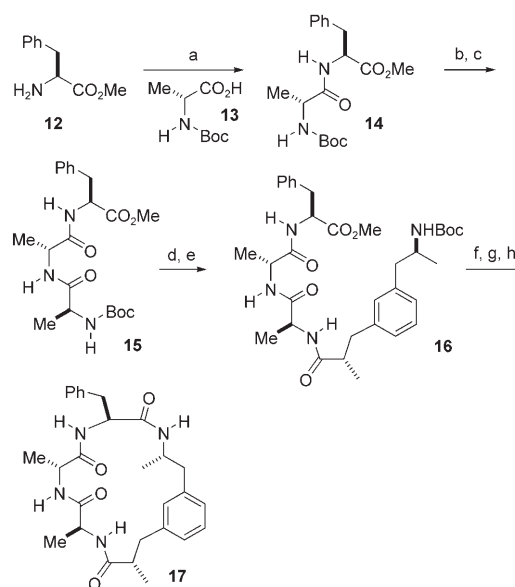


Scheme 1. Preparation of the N-protected ω -amino acid **4**. a) LDA (2.4 equiv), THF, -78°C , then add **6** (58 %); b) LiOH, H_2O_2 , 23°C , 5 h (95 %); c) MeOH, DCC, DMAP, CH_2Cl_2 , 23°C (71 %); d) PLE, H_2O , pH 7.5, 12 h, 23°C (64 %); e) $(\text{PhO})_2\text{P}(\text{O})\text{N}_3$, toluene, 23°C , 1 h, then reflux, 3 h, add *t*BuOH, reflux, 20 h (72 %); f) NaOH, THF/ H_2O , 23°C , 12 h (80 %); PLE=pig liver esterase.

to the diacid **8**, which was in turn converted to the dimethyl ester **9** via DCC-mediated esterification. This maneuver was necessary in order to allow for a monohydrolysis of the homotopic ester groups. This could be achieved by esterase-induced hydrolysis.^[15] Treatment of the monoacid **10** with diphenyl-diphosphoryl azide followed by heating of the reaction mixture in the presence of *tert*-butanol affected a Curtius rearrangement resulting in the N-Boc protected ω -amino acid ester **11**. Basic hydrolysis of the ester group led

to the desired acid **4**. This route secures this novel amino acid in gram quantities.

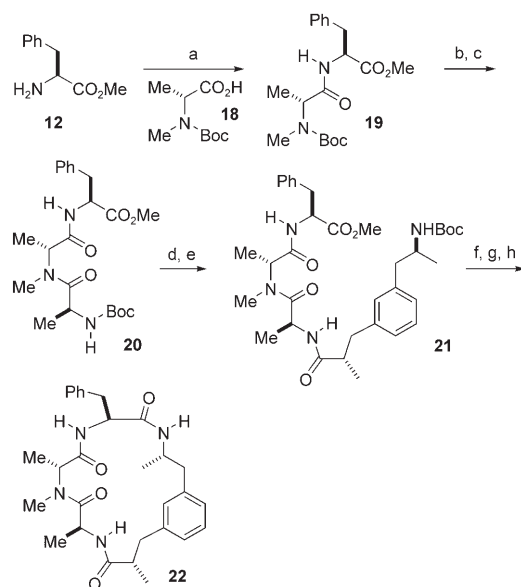
With the acid **4** in hand, the synthesis of various macrocycles was targeted, in which the two ends of acid **4** are bridged with some tripeptide fragments. Our goal was to prepare pairs of tripeptide fragments with one of them carrying a N-methyl group at the middle amino acid. The conformational analysis of the corresponding macrocycles should provide some hints on the mutual influence of the parts contained in the macrocycle. Thus, tripeptide **15** was assembled by a classical Boc strategy (Scheme 2). That is, DCC-mediated condensation of the phenylalanine derivative **12** with N-Boc-D-



Scheme 2. Preparation of the geodiamolide analogue **17**. a) DCC, HOBT, amino acid **13**, THF, $0 \rightarrow 23^{\circ}\text{C}$, 12 h (80 %); b) TFA, CH_2Cl_2 , 23°C ; c) EDCI, HOBT, Boc-L-Ala-OH, Et_3N , THF/ CH_2Cl_2 5:1, $0 \rightarrow 23^{\circ}\text{C}$, 16 h (75 %); d) TFA, CH_2Cl_2 , 23°C ; e) TBTU, HOBT, amino acid **4**, $i\text{Pr}_2\text{NEt}$, DMF, 23°C , 3 h (95 %, crude); f) NaOH, THF/ H_2O , 23°C , 3 h; g) TFA, CH_2Cl_2 , 23°C , 1 h; h) TBTU, HOBT, $i\text{Pr}_2\text{NEt}$, DMF, 23°C , 14 h (50 %, three steps).

alanine **13** gave the dipeptide **14**. After cleavage of the Boc protecting group, coupling of the free amine with N-Boc-L-alanine with the coupling reagent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) provided compound **15** in 75 % yield. Boc cleavage (TFA) and condensation of the resulting amine with the amino acid **4** led to the acyclic tetrapeptide **16**. Hydrolysis of the methyl ester, removal of the Boc group and macrolactam formation with 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) in DMF (0.001 M) gave rise to the jasplakinolide (geodiamolide) analogue **17** in 50 % yield.

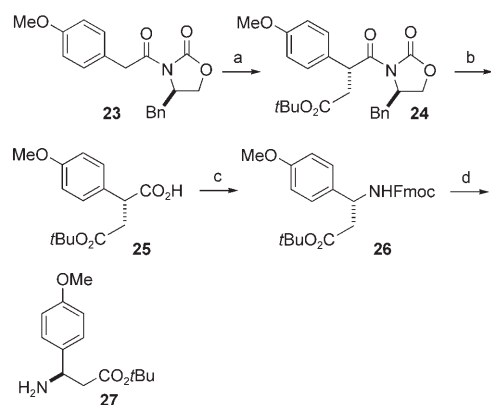
In a similar manner, the tripeptide **20** was assembled (Scheme 3). Here, N-Boc-N-methyl-D-alanine^[16,17] became the central amino acid of the tripeptide fragment. For the formation of the peptide bond to the N-methylated amine, the coupling reagent bromotrispyrrolidinophosphonium hexafluorophosphate (PyBroP) came to use. After liberation of



Scheme 3. Preparation of the geodiamolide analogue **22**. a) DCC, HOBT, amino acid **18**, THF, 0 $\rightarrow$ 23 $^{\circ}\text{C}$, 12 h (70%); b) TFA, CH_2Cl_2 , 23 $^{\circ}\text{C}$; c) PyBroP, $i\text{Pr}_2\text{NEt}$, N-Boc-L-Ala-OH (**13**), CH_2Cl_2 , 23 $^{\circ}\text{C}$, 1 h (61%); d) TFA, CH_2Cl_2 , 23 $^{\circ}\text{C}$; e) EDCI, HOBT, amino acid **4**, $\text{CH}_2\text{Cl}_2/\text{THF}$, 0 $\rightarrow$ 23 $^{\circ}\text{C}$, 7 h (52%); f) NaOH, THF/ H_2O , 23 $^{\circ}\text{C}$, 3 h (90%); g) TFA, CH_2Cl_2 , 23 $^{\circ}\text{C}$, 1 h; h) TBTU, HOBT, $i\text{Pr}_2\text{NEt}$, DMF, 23 $^{\circ}\text{C}$, 14 h (65%).

the terminal amine, EDCI-mediated condensation with the amino acid **4** provided the *seco*-compound **21**. Ester hydrolysis, cleavage of the Boc protecting group and macrolactam formation delivered the geodiamolide analogue **22** with a N-methyl amide group.

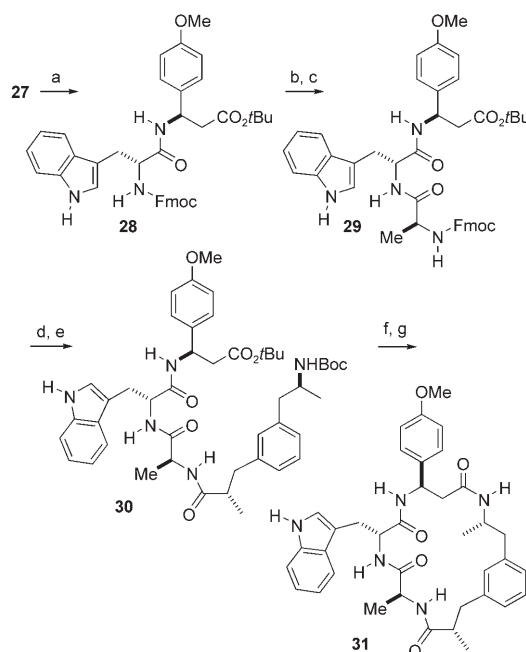
The difference between jasplakinolide and geodiamolide is the presence of a β -amino acid in the former one. While several methods for the synthesis of β -amino acids exist, the methyl ether derivative **27** was prepared via an asymmetric alkylation reaction of the oxazolidinone^[18] **23** by using *tert*-butyl-bromoacetate as electrophile (Scheme 4).^[19] The alkylation product **24** was hydrolyzed to the acid **25**, which in



Scheme 4. Synthesis of the β -amino acid **27** via asymmetric alkylation and Curtius rearrangement. a) $\text{NaN}(\text{SiMe}_3)_2$, THF, -78°C , 2.5 h, then add $\text{BrCH}_2\text{CO}_2t\text{Bu}$, -78°C , 3 h (71%); b) H_2O_2 , LiOH, THF, 0 $^{\circ}\text{C}$, 5 h (78%); c) $(\text{PhO})_2\text{P}(\text{O})\text{N}_3$, Et_3N , toluene, reflux, add fluorenyl-OH, reflux, 3 h (45%); d) Et_2NH , THF, 0 $\rightarrow$ 23 $^{\circ}\text{C}$, 12 h (60%).

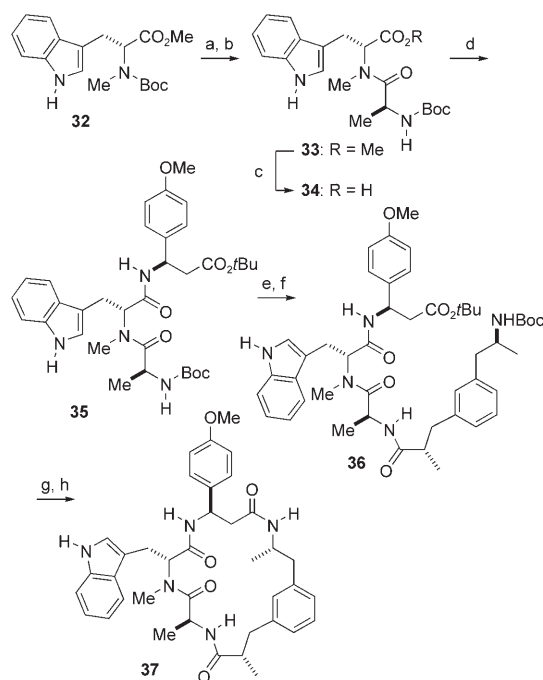
turn was subjected to a Curtius rearrangement. The resulting isocyanate was reacted with 9H-fluoren-9-ylmethanol to yield the protected β -amino acid **26**. Subsequent treatment of the urethane **26** gave free amine **27**.

The carboxyl-protected amino acid **27** was then condensed with N-Fmoc-D-tryptophan to yield compound **28** (Scheme 5). Deprotection and another condensation provided the tripeptide **29**. Thereafter, liberation of the terminal amine and condensation with the ω -amino acid **4** gave the protected *seco*-compound **30**. Both protecting groups could now be removed in one step by using trifluoroacetic acid. After concentration of the reaction mixture, macrolactam formation with TBTU in the presence of HOBT at room temperature led to compound **31** in very good yield.



Scheme 5. Synthesis of the tripeptide **29**, coupling with amino acid **4**, and formation of macrocycle **31**. a) Fmoc-D-Trp-OH, DCC, HOBT, THF, $-10 \rightarrow 0^{\circ}\text{C}$, 12 h (91%); b) Et_2NH , THF, 0 $\rightarrow$ 23 $^{\circ}\text{C}$, 80 min (78%); c) Fmoc-L-Ala-OH, DCC, HOBT, THF, 0 $^{\circ}\text{C}$, 12 h (97%); d) Et_2NH , THF, 0 $\rightarrow$ 23 $^{\circ}\text{C}$, 80 min (85%); e) DCC, HOBT, THF, amino acid **4**, $-20 \rightarrow 23^{\circ}\text{C}$, 8.5 h (92%); f) TFA, CH_2Cl_2 , 0 $\rightarrow$ 23 $^{\circ}\text{C}$, 1.5 h; g) TBTU, HOBT, $i\text{Pr}_2\text{NEt}$, DMF, 23 $^{\circ}\text{C}$, 14 h (92%, two steps).

In order to reach the N-methyl analogue **37** of macrocycle **31**, the assembly of the tripeptide part needed to be changed, since N-methylation was not compatible with the Fmoc protecting group. Accordingly, the known tryptophan derivative^[3d] **32** was deprotected at the nitrogen and elongated with N-Boc-L-alanine (Scheme 6). At this point, the methyl ester at the C terminus was cleaved, followed by amide formation of the resulting acid with the amino acid **27**. The selective cleavage of the N-Boc group could be achieved with TBDMSOTf and 2,6-lutidine.^[20] The remainder of the synthesis proceeded as before. Thus, DCC-mediated condensation of the resulting amine with the amino acid **4** gave the tetrapeptide **36**. Deprotection and macrolac-



Scheme 6. Synthesis of the macrocycle **37** with a N-methyl group at the tryptophan amino acid. a) TFA, CH₂Cl₂, 23 °C, 1 h (68 %); b) Boc-L-Ala-OH, DCC, HOBT, THF, 0 → 23 °C, 14 h (59 %); c) NaOH, THF, 23 °C, 2 h (74 %); d) amino acid **27**, DCC, HOBT, −20 → 0 °C, 12 h (96 %); e) TBDMSOTf, 2,6-lutidine, CH₂Cl₂, 0 °C, 1 h, then add H₂O (56 %); f) amino acid **4**, DCC, HOBT, THF, −20 → 23 °C, 18.5 h (60 %); g) TFA, CH₂Cl₂, 0 → 23 °C, 1.5 h; h) TBTU, HOBT, *i*Pr₂NEt, 23 °C, 14 h (45 %, two steps).

tam formation led to **37**. However, in this case the yield for the macrocyclization was lower.

Conformational Studies

Aside from variations in the side-chains, the major difference of the four analogues **17**, **22**, **31**, and **37** is the replacement of the polypropionate subunit with a *m*-xylyl containing amino acid **4**. The 19-membered systems **31** and **37** are structurally closely related to the natural compound jasplakinolide. Compound **31** possesses a secondary amide in position 16 (Figure 5). ¹H and ¹³C resonance assignments via DQFCOSY, ROESY/NOESY and HMQC spectra were performed in [D₆]DMSO and gave a single signal set for **31** and a doubled signal set for **37** with the *trans* isomer of the N-methylated amide populated for > 99 %.

One large and one small ³J_{HH} coupling constant (Table 1) for the methylene groups in positions 2, 6, and 10 of **31** and in positions 6 and 10 for **37** are typical for a well-defined *gauche-anti* orientation with preference for the major rotamer(s) as distinguished by characteristic ROE signals. This allowed the assignment of the *pro-R* and *pro-S* protons of the methylene groups. Due to signal overlay, a statement on the methylene protons in position 2 of **37** was not possible.

With ROESY and NOESY measurements, characteristic proton–proton interactions could be determined and trans-

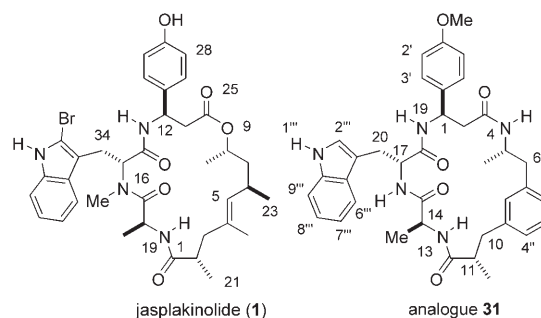


Figure 5. Structures for the natural compound jasplakinolide and the 19-membered ring analogue **31** with the numbering systems used.

Table 1. ³J_{HH} coupling constants in Hz of the methylene protons in **17**, **22**, **31** and **37**. Due to identical chemical shift for H10^b/H10ⁱ of **17** and H2^b/H2ⁱ of **37**, the coupling constants could not be determined.

	17	22	31	37
² J _{H1^b,H1ⁱ}	13.5	14.0	² J _{H2^b,H2ⁱ}	13.6
³ J _{H1^b,H2}	n.d.	12.7	³ J _{H2^b,H1}	4.3
³ J _{H1ⁱ,H2}	3.7	3.6	³ J _{H2ⁱ,H1}	10.4
² J _{H6^b,H6ⁱ}	13.1	13.1	² J _{H6^b,H6ⁱ}	13.0
³ J _{H6^b,H5}	6.1	~7 (from COSY)	³ J _{H6ⁱ,H5}	10.6
³ J _{H6ⁱ,H5}	4.1	~5 (from COSY)	³ J _{H6ⁱ,H5}	3.7
² J _{H10^b,H10ⁱ}	n.d.	12.6	² J _{H10^b,H10ⁱ}	11.0
³ J _{H10^b,H11}	n.d.	11.3 (via H11)	³ J _{H10^b,H11}	2.7
³ J _{H10ⁱ,H11}	n.d.	4.0	³ J _{H10ⁱ,H11}	13.7

ferred into proton–proton distances by integration. They are in very good agreement for compounds **31** and **37** corresponding to a minor influence of the N-methylation in position 16 on the overall structure. No intensive cross signals were found between H_α protons of adjacent amino acids, thus proving *trans* conformation for all amide bonds. The methylated amide in **37** leads to an energy barrier that gives rise to a separated signal set for the *cis*-amide. The signal set for the *cis* isomer is populated to less than 1 %. The significant preference for the *trans* rotamer, to such an extent unusual for a tertiary amide, was found for the natural compound jasplakinolide as well,^[21] thus emphasizing the good analogy of synthetic analogue and natural product.

The dynamical properties of the whole macrocycles **31** and **37** is consistent including the side-chains having the same dynamical behavior as the macrocyclic ring. The newly inserted *m*-xylyl unit performs an oscillating movement as ROESY data yield only mean proton–proton distances for several possible orientations of the phenyl ring in relation to the macrocyclic ring.

Via temperature measurements, which gave temperature coefficients for the amidic NH protons in the range of −3.7 to −6.0 ppb K^{−1}, transannular hydrogen bonds could be excluded, but nevertheless, as coupling constants and ROESY data show, the 19-membered analogues **31** and **37** are rigid macrocyclic structures.

Within a MD simulation, calculated structures of **31** and **37** are very well comparable with each other, the only difference being the orientation of the NH proton respective to

the N-methyl group in position 16 with the proton in **31** pointing into the middle of the macrocyclic ring and the methyl group in **37** oriented towards the lower side of the ring (Figure 6).

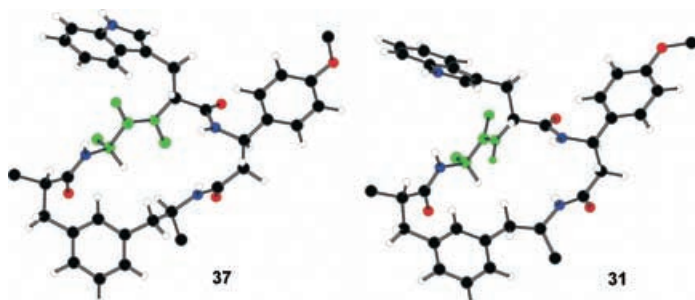


Figure 6. Energy-minimized structures for **31** and **37** after a 100 ps MD simulation at 300 K. In green, torsion with the largest difference for **31** and **37** with the N-methyl group in **37** oriented onto the lower side of the ring and the NH proton in position 16 of **31** pointing into the macrocyclic ring.

The results presented above are in accordance with NMR structural investigations of jasplakinolide.^[21] Jasplakinolide does not possess any hydrogen bonds, nor a higher fraction of *cis*-amide bonds either. The 3J coupling constants yield a more flexible structure for the natural analogue in comparison to the compounds **31** and **37**, however, the difference is small. The orientation of the two aromatic side-chains were not determined in detail in the study presented here. But a possible “tweezer” structure as stated in literature^[21] cannot be supported by NOEs between side-chain protons.

The 18-membered rings of compounds **17** and **22** show several structural differences when compared to jasplakinolide. They are rather analogues of geodiamolide with a difference in the position of the aromatic amino acid (Figure 7).^[5]

^1H and ^{13}C signal assignment was done by DQF-COSY, ROESY/NOESY, and HMQC spectra in $[\text{D}_6]\text{DMSO}$ with results comparable to the 19-membered macrocycles: a single signal set for **17**, the non-methylated amide and a doubled signal set for **22** with the *cis* isomer of the N-methylated amide populated to less than 1%.

Investigation of the NOESY/ROESY data results in mean values of proton–proton distances being significant for the fast exchange of several conformers. Thus the 18-membered rings **17** and **22** are more flexible than the systems described above and a preferred structure can not be calculated on the basis of experimental ROE data. ROESY data confirm a *trans* configuration for all amide bonds in **17** and **22**. Additionally the

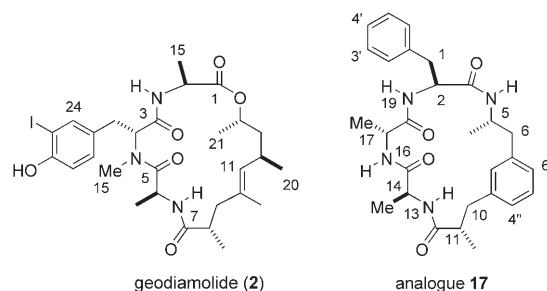


Figure 7. Structures for the natural compound geodiamolide and the 18-membered ring analogue **17** with the numbering systems used.

ROESY data again reveal an oscillatory movement for the *m*-xylyl unit like in **31** and **37**. In case of the geodiamolide analogues **17** and **22** there are no consistent dynamics for the whole molecule, but rather an independent dynamical behavior of the side-chains, that is, benzyl and methyl groups, as seen in ROESY spectra with cross signals of different sign.

The methylene groups in position 1 of **17** and in positions 1 and 10 of **22** exhibit one large and one small $^3J_{\text{HH}}$ coupling constant (Table 1) defining a preferred *gauche*–*anti* orientation, whereas in position 6 the coupling constants possess mediated values upon the presence of several rotamers.

Temperature measurements yield low temperature coefficients for NH4 and NH13 for both structures (NH4: -1.0 , -1.1 ppb K^{-1} , respectively; NH13: $+0.4$, -1.1 ppb K^{-1} , respectively) with a high probability for those NH protons to take part in an intramolecular hydrogen bond.

When calculating only a partial structure for **22** with NOESY/ROESY data a $\beta\text{II}'$ -turn-like structure is obtained for the macrocyclic part containing the phenylalanine unit. For this case NH4 is part of a transannular hydrogen bond with CO(15) as partner, whereas for NH13 there is no geometrically reasonable arrangement for the formation of a hydrogen bond (Figure 8). At least for this section of the macrocyclic ring the ROESY data obtained are in accordance with the distances typical for $\beta\text{II}'$ turns.

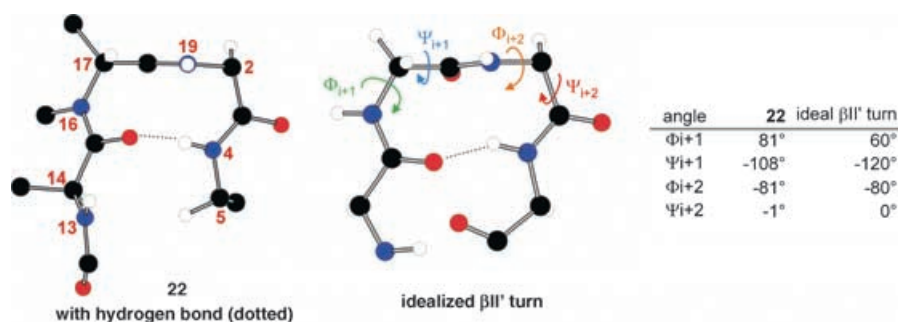


Figure 8. Comparison of the calculated partial structure of **22** with a $\beta\text{II}'$ -turn structure.

Nevertheless, the 18-membered rings **17** and **22** are more flexible compared with **31**, **37**, or jasplakinolide and an equilibrium of several fast-exchanging conformers is existent.

Biological Studies

The four analogues were tested for cytotoxicity against two cell lines.^[22] Compound **37** turned out to be the most active one. It inhibits the growth of L929 mouse fibroblasts with an IC_{50} of $25 \mu\text{g mL}^{-1}$. For the ovary cancer cell line SKOV-3 the IC_{50} amounts to $20 \mu\text{g mL}^{-1}$. Compound **31** did show weak activity against the L929 cells. The geodiamolide analogues **17** and **21** were devoid of any activity. While the activity of **37** is moderate it does show that the N-Me group makes a difference. Most remarkably, at a concentration of $40 \mu\text{g mL}^{-1}$, the assay with SKOV-3 cells shows many cells with two nuclei. This is a clear indication that the actin polymerization is disturbed. Based on the activity data it can be said that analogue **37** is the most jasplakinolide-like among the four compounds.

Summary

In this paper we describe the synthesis of a novel ω -amino acid **4** that incorporates conformational constraints due to non-bonded interactions (*syn*-pentane interactions). The design was guided by the polypropionate sector of the depsipeptide jasplakinolide. This novel amino acid was prepared by a double alkylation, enzyme-mediated hydrolysis of homotopic ester groups, and a Curtius rearrangement on the carboxylic acid. The amino acid was incorporated into the four macrolactams **17**, **22**, **31**, and **37**. The former two feature an 18-membered macrocycle, whereas the latter two have a 19-membered ring. For the two larger ones, conformational analysis showed that the macrocyclic rings are more rigid than the jasplakinolide ring, but all in all their conformations are very well comparable to the natural product. Like stated for jasplakinolide in the literature,^[21] the 19-membered analogues exhibit neither intramolecular hydrogen bonds, nor is the *cis*-rotamer populated in case of N-methylation (**37**). The conformational features of the 18-membered (geodiamolide) analogues are quite different. The macrocyclic ring of compounds **17** and **22** is more flexible than the other investigated systems. Due to the increased flexibility and signal overlap a distinct solution structure for **17** and **22** could not be gained. Whether the ring size or the additional aromatic side-chains in **31** and **37** cause a stabilizing effect on the macrocyclic system could not be determined. But both features are differing for **17** and **22** and might thus be an explanation for the higher flexibility of these smaller macrocycles. In addition, both N-methylated analogues (**22** and **37**) populate the *trans*-amide conformer to more than 99%.

The amino acid **4** could serve as a novel workbench for restricting the conformation of small peptides. Furthermore, the aryl group might serve as a handle for attachment of derived macrocycles to a solid surface. The incorporation of the *m*-xylene subunit into the amino acid indicates that small structural modifications can have subtle effects on a macrocyclic structure. Finally, it should be noted that the N-

methyl analogues **22** and **37** only populate the *trans*-amide conformer.

Experimental Section

General methods: ^1H and ^{13}C NMR: Bruker Avance 400, spectra were recorded at 295 K either in CDCl_3 , C_6D_6 , or $[\text{D}_6]\text{acetone}$; chemical shifts are calibrated to the residual proton and carbon resonance of the solvent: CDCl_3 (δ_{H} 7.25, δ_{C} 77.0 ppm), C_6D_6 (δ_{H} 7.16, δ_{C} 128.0 ppm), CD_3OD (δ_{H} 4.78, 3.21, δ_{C} 49.0 ppm), or $[\text{D}_6]\text{acetone}$ (δ_{H} 2.04, δ_{C} 29.8, 206.7 ppm). Conformational NMR analysis: Bruker Avance 600, recorded at 600.13 MHz proton resonance frequency at 300 K respectively 320 K (for **22**) in $[\text{D}_6]\text{DMSO}$. Chemical shift calibration to $[\text{D}_6]\text{DMSO}$ (δ_{H} 2.49 ppm, δ_{C} 39.5 ppm). Melting points: Büchi Melting Point B-540, uncorrected. IR: Jasco FT/IR-430. Optical rotation: Jasco polarimeter P-1020, reported in degree $[\alpha]_{\text{D}}$ (c [g per 100 mL], solvent). HRMS (FT-ICR): Bruker Daltonic APEX 2 with electron spray ionization (ESI). Flash chromatography: J. T. Baker silica gel 43–60 μm . Thin-layer chromatography Machery–Nagel Polygram Sil G/UV₂₅₄. Analytical HPLC–MS: HP 1100 Series connected with an ESI MS detector Agilent G1946C, positive mode with fragmentor voltage of 40 eV, column: Nucleosil 100–5, C-18 HD, 5 μm , 70 $\times$ 3 mm Machery Nagel, eluent: NaCl solution (5 mM)/acetonitrile, gradient: 0/10/15/17/20 min with 20/80/80/99/99% acetonitrile, flow: 0.6 mL min^{−1}. All solvents used in the reactions were purified before use. Dry diethyl ether, tetrahydrofuran, and toluene were distilled from sodium and benzophenone, whereas dry dichloromethane, dimethylformamide, pyridine, and triethylamine were distilled from CaH_2 . Petroleum ether with a boiling range of 40–60 °C was used. The pH 7 buffer was prepared by dissolving KH_2PO_4 (85.0 g, 0.625 mol) and NaOH (14.5 g, 0.3625 mol) in water (1 L). Reactions were generally run under an argon or nitrogen atmosphere. All commercially available compounds (Acros, Aldrich, Fluka, Merck) were used as received unless stated otherwise. L-Phenylalanine methyl ester and Boc-N-methyl-D-alanine (**18**) were prepared according to the literature reported.^[16,17]

(2S)-3-(3-((2S)-2-[(*tert*-Butoxycarbonyl)amino]propyl)phenyl)-2-methylpropanoic acid (4**):** NaOH (80 mg) in H_2O (5 mL) was added to a stirred solution of methyl ester **11** (0.55 g, 1.64 mmol) in THF (12 mL). The reaction mixture was stirred for 14 h at room temperature before being poured into water (25 mL) and extracted with diethyl ether (3 $\times$ 10 mL). The aqueous layer was acidified to pH 2–3 with 1 N HCl and extracted with ethyl acetate (3 $\times$ 15 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by flash chromatography (ethyl acetate/petroleum ether 1:3) resulting in acid **4** as a white solid (0.44 g, 85%). M.p. 104–106 °C; R_f = 0.45 (ethyl acetate/petroleum ether 1:3); $[\alpha]_{\text{D}}^{25}$ = +6.0 (c = 0.56, CH_2Cl_2); ^1H NMR (400 MHz, CD_3OD): δ = 0.94 (d, J = 6.1 Hz, 3H; CH_3NH), 0.99 (d, J = 6.6 Hz, 3H; $\text{CH}_3\text{CO}_2\text{H}$), 1.28 (s, 9H; $\text{C}(\text{CH}_3)_3$), 2.45–2.66 (m, 4H; benzylic H, CH), 2.86 (dd, J = 12.6, 6.1 Hz, 1H; benzylic H), 3.19 (s, 1H; NH), 3.61–3.66 (m, 1H; CHNH), 6.91–6.92 (m, 3H; aryl H), 7.06 ppm (t, J = 7.5 Hz, 1H; H_{m} , aryl H); ^{13}C NMR (100 MHz, CD_3OD): δ = 17.2 (CH_3CHNH), 20.5 ($\text{CH}_3\text{CHCO}_2\text{H}$), 28.8 ($\text{C}(\text{CH}_3)_3$), 40.7 ($\text{CH}_2\text{CHCO}_2\text{H}$), 42.7 (CHCO_2H), 43.8 (CH_2CHNH), 49.5 (CHNH), 79.8 (Boc C), 127.9, 128.3, 129.2, 131.2, 140.3, 140.7 (aryl), 157.7 (Boc C=O), 179.9 ppm (CO_2H); IR (film): $\tilde{\nu}$ = 3326, 2974, 2930, 1706, 1653, 1507, 1248, 1169 cm^{-1} ; HRMS (ESI): m/z : calcd for $\text{C}_{18}\text{H}_{27}\text{NO}_4\text{Na}$: 344.18323; found: 344.18313 [$M+\text{Na}$]⁺.

(4R)-4-Benzyl-3-[(2S)-3-(3-((2S)-3-[(4R)-4-benzyl-2-oxo-1,3-oxazolidin-3-yl]-2-methyl-3-oxopropyl)phenyl)-2-methylpropanoyl]-1,3-oxazolidin-2-one (7**):** *n*-Butyllithium (22.5 mL, 56.2 mmol, 2.5 M in hexane) was added at 0 °C to a solution of diisopropylamine (8.0 mL, 56.2 mmol) in THF (190 mL). The reaction mixture was stirred at 0 °C for 30 min, before it was cooled to −78 °C. At this point, propionyl oxazolidinone **5** (12.0 g, 51.5 mmol), dissolved in THF (210 mL), was added. After being stirred for 1.5 h at −78 °C, the solid 1,3-bis(bromomethyl)-benzene (**6**) (6.17 g, 23.4 mmol) was added in one portion. Stirring was continued for 24 h with simultaneous warming of the reaction mixture to room temperature.

The reaction was quenched with NH_4Cl (60 mL), and then most of the organic solvent was removed in vacuo. The residue was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4 , filtered, and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/EtOAc 7:3) to get **7** as a hygroscopic compound (7.72 g, 58%). $R_f=0.45$ (petroleum ether/EtOAc 7:3); $[\alpha]_D^{25}=-21.9$ ($c=1.503$, CH_2Cl_2); $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta=1.18$ (d, $J=6.6$ Hz, 6H; CH_3), 2.54–2.69 (m, 4H; benzylic H), 3.11–3.18 (m, 4H; PhCH_2), 4.10–4.20 (m, 6H; CHCH_3 , OCH_3), 4.64–4.70 (m, 2H; NCH), 7.09–7.32 ppm (m, 14H; aryl H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3): $\delta=16.2$ (CH_3), 37.3 (PhCH_2), 39.1 (CHCH_3), 39.9 (benzylic), 54.7 (NCH), 65.5 (OCH_2), 126.8, 127.9, 128.5, 129.0, 130.1, 134.9, 138.9 (aryl), 152.6 (NCO_2), 176.1 ppm (CO); IR (film): $\tilde{\nu}=3028, 2976, 2932, 1770, 1694, 1604, 1588, 1487, 1455, 1393, 1288, 1210, 1103, 1053\text{ cm}^{-1}$; HRMS (EI): m/z : calcd for $\text{C}_{34}\text{H}_{36}\text{N}_2\text{O}_6$: 568.257798; found: 568.257289 [M] $^+$.

(2S)-3-[3-[(2S)-2-Carboxypropyl]phenyl]-2-methylpropanoic acid (8): H_2O_2 (11.7 mL of a 30 wt% solution, 102.7 mmol) was added at 0°C through a syringe to a solution of the bisalkylated compound **7** (7.50 g, 13.2 mmol) in THF (250 mL), followed by the addition of $\text{LiOH}\cdot\text{H}_2\text{O}$ (2.20 g, 51.4 mmol), dissolved in water (120 mL). The solution was stirred at 0°C for 5 h. Subsequently, saturated Na_2SO_3 solution (100 mL) and saturated NaHCO_3 solution (100 mL) were added at 0°C. The whole mixture was partially concentrated in vacuo and diluted with water (100 mL). The aqueous layer was extracted with dichloromethane (3 × 75 mL) to recover the auxiliary. The aqueous layer was then acidified at 0°C to pH 1.5 by using 6 M HCl and then extracted with ethyl acetate (4 × 100 mL). The combined organic layers were dried (MgSO_4), filtered, and concentrated in vacuo yielding an oily residue (2.95 g, 90%). $[\alpha]_D^{25}=+35.5$ ($c=0.42$, CH_2Cl_2); $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta=1.20$ (d, $J=6.8$ Hz, 6H; CH_3), 2.62–2.72 (m, 2H; benzylic H), 2.75–2.83 (m, 2H; CH), 3.10 (dd, $J=13.1, 6.1$ Hz, 2H; benzylic H), 7.07 (d, $J=7.1$ Hz, aryl H), 7.10 (s, 1H; H_α , aryl H), 7.26 (t, $J=7.6$ Hz, 1H; H_m , aryl H), 11.79 ppm (brs, 2H; CO_2H); $^{13}\text{C NMR}$ (400 MHz, CDCl_3): $\delta=16.3$ (CH_3), 39.1 (benzylic), 41.3 (CH), 127.1, 128.4, 129.7, 139.0 (aryl), 182.7 ppm (CO_2H); IR (film): $\tilde{\nu}=3500\text{--}2500$ (broad), 1702, 1589, 1463, 1292, 1199, 1044 cm^{-1} ; HPLC-MS (ESI): m/z : 250.2, 204.2, 186.2, 177.2, 171.2, 131.2; HRMS (EI): m/z : calcd for $\text{C}_{14}\text{H}_{18}\text{O}_4$: 250.12049; found: 250.118291 [M] $^+$.

Methyl (2S)-3-[3-[(2S)-3-methoxy-2-methyl-3-oxopropyl]phenyl]-2-methylpropanoate (9): A solution of DCC (8.12 g, 39.4 mmol) in CH_2Cl_2 (26 mL) was added at 0°C to a solution of diacid **8** (3.20 g, 12.8 mmol), methanol (1.4 mL, 32.8 mmol) and DMAP (96 mg) in dry CH_2Cl_2 (37 mL). The solution was stirred at 0°C for 30 min and then at room temperature for 6 h. The white precipitate was filtered off, the solvent evaporated, and the residue redissolved in diethyl ether. The organic solution was washed successively with cold 1 N HCl, NaHCO_3 solution, and brine. The dried (Na_2SO_4) organic layer was filtered, and concentrated. The crude product was purified by flash chromatography (ethyl acetate/petroleum ether 1:9) to provide the diester **9** as an oily compound (2.52 g, 71%). $R_f=0.38$ (ethyl acetate/petroleum ether 1:9); $[\alpha]_D^{25}=+40.1$ ($c=0.61$, CH_2Cl_2); $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta=1.15$ (d, $J=6.5$ Hz, 6H; CH_3), 2.61–2.67 (m, 2H; benzylic H), 2.69–2.77 (m, 2H; CH), 3.00 (dd, $J=13.1, 6.7$ Hz, 2H; benzylic H), 3.64 (s, 6H; OCH_3), 6.97 (s, 1H; H_α , aryl H), 7.01 (d, $J=7.6$ Hz, 2H; aryl H), 7.20 ppm (t, $J=7.6$ Hz, 1H; H_m , aryl H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3): $\delta=16.6$ (CH_3), 39.6 (benzylic), 41.6 (CH), 51.5 (OCH_3), 126.9, 128.3, 129.6, 139.3 (aryl), 176.4 ppm (CO_2Me); IR (film): $\tilde{\nu}=2974, 2951, 1736, 1459, 1375, 1361, 1164\text{ cm}^{-1}$; HPLC-MS (ESI): m/z : 278.2, 218.2, 186.2, 171.2, 131.2, 105.2, 91.2; HRMS (EI): m/z : calcd for $\text{C}_{16}\text{H}_{22}\text{O}_4$: 278.15179; found: 278.152648 [M] $^+$.

(2S)-3-[3-[(2S)-3-Methoxy-2-methyl-3-oxopropyl]phenyl]-2-methylpropanoic acid (10): A solution of diester **9** (2.50 g, 9.0 mmol) in MeOH (5 mL) was emulsified under vigorous stirring in NaCl solution (0.1 M, 646.75 mL) to which pH 7 phosphate buffer (3.25 mL) was added, making the solution 3 mM in phosphate. Then a suspension of PLE (25 mg, 1000 units, Sigma Aldrich, E-3019) in 3.2 M $(\text{NH}_4)_2\text{SO}_4$ solution (1 mL) was added. During the hydrolysis the pH was kept between 7 and 7.5 by the controlled addition of NaOH solution (0.1 N). After observing

the formation of diacid in HPLC-MS (maximum 12 h), the reaction mixture was washed with CH_2Cl_2 (2 × 500 mL). The aqueous phase was acidified to pH 2.5 with 25% hydrochloric acid and extracted with ethyl acetate (3 × 500 mL). The combined organic layers (CH_2Cl_2 and EtOAc) were dried with Na_2SO_4 , filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (ethyl acetate/petroleum ether 1:4) to provide the monoacid monoester as an oily compound (1.52 g, 64%). $R_f=0.4$ (ethyl acetate/petroleum ether 1:4); $[\alpha]_D^{25}=+46.15$ ($c=1.07$, CH_2Cl_2); $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta=1.05$ (d, $J=6.82$ Hz, 3H; $\text{CH}_3\text{CHCO}_2\text{CH}_3$), 1.08 (d, $J=7.1$ Hz, 3H; $\text{CH}_3\text{CHCO}_2\text{H}$), 2.53–2.58 (m, 2H; benzylic H), 2.62–2.68 (m, 2H; CH), 2.94 (ddd, $J=19.4, 13.1, 6.4$ Hz, 2H; benzylic H), 3.55 (s, 3H; OCH_3), 6.90–6.96 (m, 3H; aryl H), 7.12 (t, $J=7.5$ Hz, 1H; H_m , aryl H), 10.49 ppm (broad, 1H; CO_2H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3): $\delta=16.4$ ($\text{CH}_3\text{CO}_2\text{CH}_3$), 16.6 ($\text{CH}_3\text{CO}_2\text{H}$), 39.1 ($\text{CH}_2\text{CHCO}_2\text{CH}_3$), 39.6 ($\text{CH}_2\text{CHCO}_2\text{H}$), 41.4 (CHCO_2H), 51.5 (OCH_3), 126.9, 127.0, 128.4, 129.8, 139.0 (aryl), 176.4 (CO_2Me), 182.3 ppm (CO_2H); IR (film): $\tilde{\nu}=3025, 2975, 1736, 1707\text{ cm}^{-1}$; HPLC-MS: m/z : 264.2, 218.2, 186.2, 171.2, 158.2, 131.2, 91.2; HRMS (EI): m/z : calcd for $\text{C}_{15}\text{H}_{20}\text{O}_4$: 264.133877; found: 264.136141 [M] $^+$.

Methyl (2S)-3-[3-[(2S)-2-[(*tert*-butoxycarbonyl)amino]propyl]phenyl]-2-methylpropanoate (11): A solution of the monoester **10** (0.80 g, 3.03 mmol) in toluene (23 mL) was treated with triethylamine (0.5 mL, 3.33 mmol) and DPPA (0.66 mL, 3.03 mmol). After stirring for 30 min, the mixture was heated under reflux for 3.5 h. The isocyanate formation was monitored by IR for the appearance of a strong signal in the 2300–2200 cm^{-1} region and disappearance of the carboxylic acid carbonyl peak. The reaction mixture was cooled to 50°C, *tert*-butanol (3 mL, 10 equiv) was added via syringe and the solution heated to reflux for 20 h. The reaction was cooled to room temperature and quenched with saturated NaHCO_3 solution (25 mL). The mixture was extracted with diethyl ether (3 × 25 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (ethyl acetate/petroleum ether 1:5), to provide compound **11** as an oil (0.73 g, 72%). $R_f=0.55$ (ethyl acetate/petroleum ether 1:5); $[\alpha]_D^{25}=+12.3$ ($c=0.21$, CH_2Cl_2); $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta=0.99$ (d, $J=6.6$ Hz, 3H; $\text{CH}_3\text{CHCO}_2\text{Me}$), 1.06 (d, $J=6.6$ Hz, 3H; CH_3CHNR), 1.36 (s, 9H; $\text{C}(\text{CH}_3)_3$), 2.51–2.58 (m, 2H; benzylic H), 2.63–2.68 (m, 1H; CHCO_2Me), 2.75 (dd, $J=13.1, 5.3$ Hz, 1H; benzylic H), 2.93 (dd, $J=13.1, 6.4$ Hz, 1H; benzylic H), 3.57 (s, 3H; OCH_3), 3.81 (broad, 1H; CHNHR), 4.31 (broad, 1H; NH), 6.90–7.32 ppm (m, 4H; aryl H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3): $\delta=16.3$ ($\text{CH}_3\text{CHCO}_2\text{Me}$), 19.7 (CH_3CHNHR), 28.0 ($\text{C}(\text{CH}_3)_3$), 39.2 ($\text{CH}_2\text{CHCO}_2\text{Me}$), 41.0 (CH_2CHNHR), 42.5 (CH_2CHNHR), 47.1 (CHNHR), 51.2 (OCH_3), 78.8 (Boc quaternary C), 126.6, 127.2, 127.9, 129.9, 137.9, 139.0 (aryl), 154.8 (Boc CO), 176.2 ppm (CO_2Me); IR (film): $\tilde{\nu}=3361, 2973, 2930, 2359, 1735, 1710, 15516, 1364, 1166\text{ cm}^{-1}$; HRMS (EI): m/z : calcd for $\text{C}_{19}\text{H}_{29}\text{NO}_4$: 335.209636; found: 335.207186 [M] $^+$.

Methyl *N*-(*tert*-butoxycarbonyl)-*D*-alanyl-*L*-phenylalaninate (14): A solution of DCC (1.5 g, 7.25 mmol) in THF (11 mL) at 0°C was added to a stirred solution of Boc-*D*-Ala-OH (**13**) (1.10 g, 5.6 mmol), *H*-*L*-Phe-*O*Me (**12**) (1.00 g, 5.6 mmol), hydroxybenzotriazole (0.75 g, 5.6 mmol) in dry THF (45 mL). Stirring was continued for 12 h at room temperature. The dicyclohexylurea was filtered off, washed with cold diethyl ether, and the filtrate was concentrated. Purification was done by flash chromatography (ethyl acetate/petroleum ether 1:3) yielding a colorless solid (1.55 g, 80%). M.p. 98–99°C; $R_f=0.35$ (ethyl acetate/petroleum ether 1:3); $[\alpha]_D^{25}=+59.8$ ($c=0.40$, CH_2Cl_2); $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta=1.21$ (d, $J=7.1$ Hz, 3H; CH_3), 1.36 (s, 9H; $\text{C}(\text{CH}_3)_3$), 3.00 (dd, $J=13.8, 6.2$ Hz, 1H; benzylic H), 3.05–3.10 (m, 1H; benzylic H), 4.04–4.18 (m, 1H; CHNH), 4.77–4.81 (m, 1H; CHCO_2Me), 4.95 (brs, 1H; NH(Boc)), 6.60 (brs, 1H; NHCHCO_2Me), 7.03–7.22 ppm (m, 5H; aryl H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3): $\delta=18.4$ (CH_3), 28.2 ($\text{C}(\text{CH}_3)_3$), 37.8 (benzylic), 49.9 (CHCO_2Me), 52.3 (OCH_3), 53.0 (CHNH(Boc)), 80.0 (Boc C), 127.1, 128.5, 129.2, 135.7 (aryl), 155.3 (Boc C=O), 171.7 (CO_2Me), 172.2 ppm (CONH); IR (KBr): $\tilde{\nu}=3304, 2987, 2930, 1732, 1664, 1517, 1317\text{ cm}^{-1}$; HRMS (EI): m/z : calcd for $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_5$: 294.119319; found: 294.121541 [$M-\text{C}(\text{CH}_3)_3$] $^+$.

Methyl *N*-(*tert*-Butoxycarbonyl)-L-alanyl-D-alanyl-L-phenylalaninate (15): A solution of peptide **14** (1.0 g, 2.8 mmol) in CH_2Cl_2 (22 mL) was treated with $\text{CF}_3\text{CO}_2\text{H}$ (2.2 mL) and the mixture stirred at room temperature for 1 h. The solvent was removed in vacuo, and the residue dried by azeotropic removal of H_2O with toluene. The crude material was subjected to the next reaction without further purification. To a cooled (0°C) solution of the crude amine salt (240 mg, 0.96 mmol) and Boc-L-Ala-OH (181 mg, 0.96 mmol) in THF (11 mL) and CH_2Cl_2 (2.5 mL) were added 1-hydroxybenzotriazole (130 mg, 0.96 mmol), Et_3N (0.32 mL, 2.3 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (239 mg, 1.25 mmol). The mixture was then stirred at room temperature for 16 h. The solvent was removed in vacuo, and the residue purified by flash chromatography (40% ethyl acetate in petroleum ether) to provide tripeptide **15** as a colorless solid (0.30 g, 75%). M.p. 149–151 $^\circ\text{C}$; R_f =0.33 (40% ethyl acetate in petroleum ether); $[\alpha]_{\text{D}}^{25}$ =+19.1 (c =0.48, CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3): δ =1.19 (d, J =7.1 Hz, 3H; $\text{CH}_3\text{CHNHBoc}$), 1.24 (d, J =7.0 Hz, 3H; CH_3CONH), 1.36 (s, 9H; C-(CH_3)₃), 2.96 (dd, J =13.9, 7.1 Hz, 1H; benzylic H), 3.07–3.12 (m, 1H; benzylic H), 3.62 (s, 3H; OCH_3), 4.02–4.17 (m, 1H; CHNHBoc), 4.40–4.47 (m, 1H; CHNHCO), 4.73–4.78 (m, 1H; CHCO_2Me), 5.11 (d, J =7.3 Hz, 1H; NHBOC), 6.84 (d, J =7.3 Hz, 1H; NHCO_2Me), 6.95 (d, J =6.6 Hz, 1H; NHCO), 7.05–7.22 ppm (m, 5H; aryl H); ^{13}C NMR (100 MHz, CDCl_3): 18.1 ($\text{CH}_3\text{CHNHBoc}$), 18.4 (CH_3CHCONH), 28.3 C-(CH_3)₃, 37.8 (benzylic), 48.5 (CHCONH), 50.2 (CHNHBoc), 53.2 (CHCO_2Me), 80.1 (Boc C), 127.0, 128.5, 129.2, 135.8 (aryl), 155.3 (Boc C=O), 171.7 (CO_2Me), 171.8 (NHCO), 172.6 ppm (CH_2NHCO); IR (KBr): $\tilde{\nu}$ =3277, 2979, 2748, 1753, 1707, 1645, 1519, 1456, 1370, 1166 cm^{-1} ; HRMS (EI): m/z : calcd for $\text{C}_{21}\text{H}_{31}\text{N}_3\text{O}_6$: 421.221247; found: 421.225247 [M] $^+$.

Methyl *N*-[(2*S*)-3-(3-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]propyl]phenyl)-2-methylpropanoyl]-L-alanyl-D-alanyl-L-phenylalaninate (16): A solution of peptide **15** (100 mg, 0.24 mmol) in CH_2Cl_2 (2 mL) was treated with $\text{CF}_3\text{CO}_2\text{H}$ (0.18 mL), and the mixture stirred at room temperature for 1 h. The solvent was removed in vacuo, and the residue was dried by azeotropic removal of H_2O with toluene. The crude material was subjected to the next reaction without further purification. To a solution of crude amine salt and Boc-protected ω -amino acid **4** (77 mg, 0.24 mmol) in DMF (2 mL) were added TBTU (77 mg, 0.24 mmol) HOBt (34 mg, 0.24 mmol), DIEA (0.1 mL, 0.576 mmol) and the mixture was stirred for 3 h at room temperature. The reaction mixture was diluted with water (3 mL) and extracted with ethyl acetate (3 $\times$ 4 mL). The combined organic layers were washed with water resulting in almost pure tetrapeptide **16** (133 mg, 90%) as judged by HPLC-MS. This material was used for the macrolactam formation without any further purification. *N*-Boc-*N*-methyl-D-alanine (**18**) was prepared according to the literature.^[23,24]

(3*S*,6*S*,9*R*,12*S*,15*S*)-6-Benzyl-3,9,12,15-tetramethyl-4,7,10,13-tetraazabicyclo[15.3.1]henicosa-1(21),17,19-triene-5,8,11,14-tetrone (17) (deprotection of the carboxylic and amino groups of compound **16** and macrocyclization): NaOH (7.7 mg), dissolved in H_2O (0.5 mL), was added to a stirred solution of tetrapeptide **16** (100 mg, 0.160 mmol) in THF (3 mL). The reaction mixture was stirred for 1 h at room temperature before being poured into water (5 mL) and extracted with diethyl ether (3 $\times$ 5 mL). The aqueous layer was acidified to pH 2–3 with 1 *N* HCl and extracted with ethyl acetate (3 $\times$ 5 mL). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated providing the carboxylic acid in almost quantitative yield. This compound was used directly in the next step. A solution of above Boc-protected tetrapeptide acid (90 mg, 0.145 mmol) in CH_2Cl_2 (1.2 mL) was treated with $\text{CF}_3\text{CO}_2\text{H}$ (0.11 mL, 1.45 mmol), and the mixture stirred at room temperature for 1 h. The solvent was removed in vacuo, and the residue dried by azeotropic removal of H_2O with toluene. The crude material was subjected to the macrolactamization without any further purification. Thus, the residue was dissolved in DMF (140 mL) and the stirred solution treated successively with TBTU (140 mg, 0.435 mmol), HOBt (59 mg, 0.435 mmol), and $i\text{Pr}_2\text{EtN}$ (0.08 mL, 0.44 mmol) at room temperature. The resulting solution was stirred for 14 h at room temperature and then partitioned between ethyl acetate and water. After separation of the layers, the aqueous layer was extracted with ethyl acetate (2 $\times$ 75 mL), and the combined organic layers were washed successively with water, 5% aqueous

KHSO_4 , water, half-saturated NaHCO_3 , and brine. After being dried (MgSO_4), filtered, and concentrated in vacuo, the residue was purified by flash chromatography (ethyl acetate) to give the macrocycle **17** as a colorless solid (40 mg, 50%, three steps). M.p. 258–260 $^\circ\text{C}$; R_f =0.5 (ethyl acetate); $[\alpha]_{\text{D}}^{25}$ =−14.8 (c =0.19, CH_2Cl_2); ^1H NMR (600 MHz, $[\text{D}_6]\text{DMSO}$): δ =1.01 (d, J =7.3 Hz, 5- CH_3), 1.06 (d, J =6.6 Hz, 3H; 11- CH_3), 1.11 (d, J =7.3 Hz, 3H; 5- CH_3), 1.12 (d, J =6.6 Hz, 3H; 14- CH_3), 2.50 (m, 2H; 6-H, 11-H), 2.59–2.67 (m, 2H; 10-H), 2.86–2.95 (m, 2H; 1-H, 6-H), 3.33 (dd, J =13.9, 3.7 Hz, 1H; 1-H), 3.74–3.79 (m, 1H; 17-H), 3.94–4.03 (m, 3H; 2-H, 14-H, 5-H), 6.75–6.79 (m, 2H; 4''-H, 6''-H), 6.83 (d, J =7.3 Hz, 2H; 4-NH, 13-NH), 6.94 (s, 1H; 2''-H), 6.98 (dd, J =7.3, 7.3 Hz, 1H; 5''-H), 7.15–7.19 (m, 3H; 4'-H, aryl H), 7.23–7.27 (m, 2H; aryl H), 8.17 (d, J =8.1 Hz, 1H; 19-NH), 8.34 ppm (brs, 1H; 16-NH); ^{13}C NMR (150 MHz, $[\text{D}_6]\text{DMSO}$): δ =15.8 (CH_3 -17), 17.9 (CH_3 -11), 18.3, 19.1 (CH_3 -5, CH_3 -14), 34.6 (C-1), 39.1 (C-6), 39.5 (C-10), 42.0 (C-11), 45.0 (C-5), 47.6 (C-14), 49.6 (C-17), 54.4 ppm (C-2); IR (film): $\tilde{\nu}$ =3297, 2931, 1888, 1640, 1526, 1446 cm^{-1} ; HRMS (EI): m/z : calcd for $\text{C}_{28}\text{H}_{36}\text{N}_4\text{O}_4$: 492.273621; found: 492.271404 [M] $^+$.

Methyl *N*-(*tert*-butoxycarbonyl)-*N*-methyl-D-alanyl-L-phenylalaninate (19): A solution of DCC (2.3 g, 11.1 mmol), dissolved in THF (11 mL) was added at 0°C to a stirred solution of Boc-*N*-Me-D-Ala-OH (**18**) (1.5 g, 7.4 mmol), H-L-Phe-OMe (**12**) (1.3 g, 7.4 mmol), hydroxybenzotriazole (0.99 g, 7.4 mmol) in dry THF (60 mL). Stirring was continued for 7 h at room temperature. The dicyclohexylurea was filtered off, washed with cold diethyl ether, and the filtrate concentrated. Purification of the residue by flash chromatography (ethyl acetate/petroleum ether 1:5) gave a gel-like compound (2.10 g, 75%). R_f =0.33 (ethyl acetate/petroleum ether 1:5); $[\alpha]_{\text{D}}^{25}$ =+62.8 (c =0.89, CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3): δ =1.23 (d, J =6.8 Hz, 3H; CH_3), 1.37 (s, 9H; C-(CH_3)₃), 2.64 (s, 3H; NCH_3), 2.99–3.07 (m, 2H; benzylic H), 3.63 (s, 3H; OCH_3), 4.71–4.77 (m, 2H; CHCO_2Me , CHMe), 6.50 (brs, 1H; NHCHCO_2Me), 7.03 (d, J =7.3 Hz, 2H; H_α , aryl H), 7.15–7.23 ppm (m, 3H; aryl H); ^{13}C NMR (100 MHz, CDCl_3): δ =13.2 (CH_3), 28.3 (C-(CH_3)₃), 29.7 (NCH_3), 37.8 (benzylic), 52.2 (OCH_3 , CHCO_2Me), 52.9 (CHNHBoc), 80.5 (Boc C), 127.1, 128.6, 129.1, 135.7, 171.3 (CO_2Me), 171.7 ppm (CONH); IR (film): $\tilde{\nu}$ =3327, 2977, 2934, 2118, 1746, 1688, 1515, 1455, 1390, 1154 cm^{-1} ; HRMS (EI): m/z : calcd for $\text{C}_{19}\text{H}_{28}\text{N}_2\text{O}_5$: 364.199791, found 364.198514 [M] $^+$.

Methyl *N*-(*tert*-Butoxycarbonyl)-L-alanyl-*N*-methyl-D-alanyl-L-phenylalaninate (20): A solution of peptide **19** (1.0 g, 2.8 mmol) in CH_2Cl_2 (22 mL) was treated with $\text{CF}_3\text{CO}_2\text{H}$ (2.2 mL), and the mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo, and the residue dried by azeotropic removal of H_2O with toluene. The crude material was subjected to the next reaction without further purification.

To a stirred solution of the crude amine salt, Boc-L-Ala-OH (0.53 g, 2.8 mmol), PyBroP (1.3 g, 2.8 mmol) in CH_2Cl_2 (3 mL) was added DIPEA (1.4 mL, 8.4 mmol) at 0°C and the mixture stirred at room temperature for 3 h. The solvent was removed in vacuo and the residue purified by flash chromatography (ethyl acetate/petroleum ether 1:1) to provide a gel-like compound (0.69 g, 58%). R_f =0.45 (ethyl acetate/petroleum ether 1:1); $[\alpha]_{\text{D}}^{25}$ =+62.4 (c =1.79, CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3): δ =1.21 (d, J =6.8 Hz, 3H; $\text{CH}_3\text{CHNCH}_3$), 1.23 (d, J =7.8 Hz, 3H; $\text{CH}_3\text{CHNHBoc}$), 1.37 (s, 9H; C-(CH_3)₃), 2.85 (s, 3H; NCH_3), 2.97 (dd, J =13.9, 7.1 Hz, 1H; benzylic H), 3.04–3.09 (m, 1H; benzylic H), 3.60 (s, 3H; OCH_3), 4.44–4.50 (m, 1H; CHNHBoc), 4.65–4.70 (m, 1H; CHCO_2Me), 5.11 (q, J =6.3 Hz, 1H; CHNCH_3), 5.31 (d, J =6.8, 1H; NHBOC), 6.70 (d, J =7.8 Hz, 1H; NHCHCO_2Me), 7.05 (d, J =7.3, 2H; H_α , aryl H), 7.14–7.24 ppm (m, 3H; aryl H); ^{13}C NMR (100 MHz, CDCl_3): 13.5 (CH_3CHCO), 17.9 ($\text{CH}_3\text{CHNHBoc}$), 28.2 (C-(CH_3)₃), 30.3 (NCH_3), 37.4 (benzylic), 46.6 (CHNHBoc), 52.1 (OCH_3 , CHNMe), 53.0 (CHCO_2Me), 79.6 (Boc C), 127.0, 128.4, 129.0, 135.9 (aryl), 155.3 (Boc C=O), 170.4 (CO_2Me), 171.7 (CONMe), 173.7 ppm (CONH); IR (film): $\tilde{\nu}$ =3327, 2979, 1742, 1682, 1642, 1520, 1455, 1249, 1169 cm^{-1} ; HRMS (EI): m/z : calcd for $\text{C}_{22}\text{H}_{33}\text{N}_3\text{O}_6$: 435.236897; found: 435.240391 [M] $^+$.

Methyl *N*-[(2*S*)-3-(3-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]propyl]phenyl)-2-methylpropanoyl]-L-alanyl-*N*-methyl-D-alanyl-L-phenylalaninate (21): A solution of tripeptide **20** (180 mg, 0.413 mmol) in CH_2Cl_2 (3.5 mL) was treated with $\text{CF}_3\text{CO}_2\text{H}$ (0.32 mL), and the mixture stirred at room temperature for 1 h. The solvent was removed in vacuo, and the residue

dried by azeotropic removal of H₂O with toluene. The crude material was subjected to the next reaction without further purification.

To a cooled (0°C) solution of the crude amine salt and amino acid **4** (133 mg, 0.413 mmol) in THF (7 mL) and CH₂Cl₂ (1.5 mL) were added 1-hydroxybenzotriazole (56.2 mg, 0.413 mmol), Et₃N (0.15 mL, 1.03 mmol), and EDCI (103 mg, 0.54 mmol), followed by stirring of the mixture at room temperature for 16 h. The solvent was removed in vacuo, and the residue purified by flash chromatography (ethyl acetate/petroleum ether 1:1) to provide a gel-like compound (0.14 g, 55%). R_f = 0.33 (ethyl acetate/petroleum ether 1:1); $[\alpha]_D^{25}$ = +61.5 (c = 0.52, CH₂Cl₂); ¹H NMR (400 MHz, CD₃OD): δ = 1.04 (d, J = 6.6, 6H; CH₃CHNH, CH₂CH(CH₃)), 1.23 (d, J = 7.3 Hz, 3H; CH₃CHN(CH₃)), 1.30 (d, J = 7.1 Hz, 3H; CH₃CHNHBoc), 1.36 (s, 9H; C(CH₃)₃), 2.40–2.72 (m, 4H; benzylic H), 2.86 (s, 3H; NCH₃), 3.09–3.16 (m, 2H; PhCH₂), 3.58 (s, 3H; OCH₃), 3.68 (m, 1H; NHBoc), 4.50–4.60 (m, 2H; CH(CH₃)NH, CH(CH₃)NHBoc), 5.03 (q, J = 7.1 Hz, 1H; CH(CH₃)NCH₃), 6.89–7.20 (m, 11H; aryl H, PhCH₂CHNH), 7.90 ppm (broad, 1H; COCHNH); ¹³C NMR (100 MHz, CD₃OD): δ = 14.0 (CH₃CHN(CH₃)), 16.7 (CH₂CH(CH₃)), 17.4 (CH₃CHNH), 20.6 (CH₃CHNHBoc), 28.8 (C(CH₃)₃), 31.4 (NCH₃), 38.1 (PhCH₂), 38.9 (CH(CH₃)CO), 40.7 (CH₂CH(CH₃)CO), 43.0 (CH₂CH(CH₃)NH), 47.3 (CH(CH₃)NH), 52.7 (CH(CH₃)NCH₃), 53.8 (OCH₃), 55.51 (CHCO₂Me), 79.8 (Boc C), 127.8, 128.0, 128.3, 129.2, 129.4, 130.2, 138.4, 140.4, 140.8 (aryl), 157.7 (Boc C=O), 173.1 (N-(CH₃)CO), 173.4 (CO₂Me), 175.2 (COCHN(CH₃)), 178.7 ppm (NHCOCH); IR (film): $\tilde{\nu}$ = 3315, 2975, 2932, 1742, 1644, 1526, 1455, 1391, 1247, 1172 cm⁻¹; HRMS (ESI): m/z : calcd for C₃₅H₃₀N₄O₇Na: 661.35717; found: 661.34712 [M +Na]⁺.

(3S,6S,9R,12S,15S)-6-Benzyl-3,9,10,12,1-pentamethyl-4,7,10,13-tetraazabicyclo[15.3.1]henicosa-1,17,19-trien-5,8,11,14-tetrone (22): NaOH (7.5 mg) in H₂O (0.5 mL) was added to a stirred solution of tetrapeptide **21** (100 mg, 0.156 mmol) in THF (3 mL). The reaction mixture was stirred for 1 h at room temperature before being poured into saturated NaHCO₃ solution (5 mL) and extracted with diethyl ether (3 × 5 mL). The aqueous layer was acidified to pH 2–3 with 1 N HCl and extracted with ethyl acetate (3 × 5 mL). The combined organic layers were dried (Na₂SO₄), filtered, and concentrated in vacuo to give the free acid in almost quantitative yield. This acid was used for the next step without further purification. To a solution of above N-Boc protected tetrapeptide acid (90 mg, 0.144 mmol) in CH₂Cl₂ (1.2 mL) was added CF₃CO₂H (0.11 mL, 1.45 mmol), and the mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo, and the residue dried by azeotropic removal of H₂O with toluene. The crude material was subjected to the macrolactamization without any further purification.

The residue was dissolved in DMF (140 mL) and the stirred solution was treated successively with TBTU (140 mg, 0.435 mmol), HOBt (59 mg, 0.435 mmol), and *i*Pr₃NEt (0.08 mL, 0.435 mmol) at room temperature. The solution was stirred at room temperature for 14 h and then partitioned between ethyl acetate and water. After separation of the layers, the aqueous layer was extracted with ethyl acetate (2 × 75 mL). The combined organic layers were washed successively with water, 5% aqueous KHSO₄, water, half-saturated NaHCO₃ and brine, dried (MgSO₄), filtered, and concentrated in vacuo. The residue was purified by flash chromatography (ethyl acetate) to provide a colorless sticky solid (50 mg, 62%, three steps). R_f = 0.55 (ethyl acetate); $[\alpha]_D^{28}$ = +18.0 (c = 0.30, CH₂Cl₂); ¹H NMR (600 MHz, [D₆]DMSO): δ = 0.97 (d, J = 7.3 Hz, 17-CH₃), 1.07 (d, J = 7.3 Hz, 3H; CH₃), 1.09 (d, J = 5.9 Hz, 3H; 11-CH₃), 1.10 (d, J = 5.9 Hz, 3H; CH₃), 2.36–2.43 (m, 1H; 11-H), 2.48 (m, 1H; 10-H), 2.49 (m, 1H; 6-H), 2.60–2.70 (m, 2H; 10-H, 1-H), 2.84 (s, 3H; NCH₃), 2.95 (dd, J = 13.2, 3.7 Hz, 1H; 6-H), 3.35 (dd, J = 13.6, 3.3 Hz, 1H; 1-H), 4.04–4.08 (m, 1H; 5-H), 4.22–4.29 (m, 2H; 17-H, 2-H), 4.39–4.44 (m, 1H; 14-H), 6.46 (brs, 3H; 13-NH), 6.50 (d, J = 8.1 Hz, 4-NH), 6.65, 6.70 (2d, J = 7.3 Hz, 2H; 4'-H, 6'-H), 6.90 (dd, J = 7.3, 7.3 Hz, 1H; 5'-H), 6.93 (s, 1H; 2'-H), 7.15–7.19 (m, 1H; 4'-H), 7.21–7.27 (m, 4H; 2'-H, 4'-H), 8.31 ppm (d, J = 8.8 Hz, 1H; 19-NH); ¹³C NMR (150 MHz, [D₆]DMSO): δ = 14.3 (CH₃-17), 17.5 (CH₃-11), 18.4 (CH₃-5, CH₃-14), 30.5 (NCH₃), 35.4 (C-1), 38.6 (C-6), 40.5 (C-10), 43.6 (C-11), 44.3 (C-5), 44.7 (C-14), 53.3 (C-17), 53.4 ppm (C-2); IR (film): $\tilde{\nu}$ = 3302, 2933, 1633, 1520,

1455 cm⁻¹; HRMS (ESI): m/z : calcd for C₂₉H₃₈N₄O₄Na: 529.27853; found: 529.27827 [M +Na]⁺.

***tert*-Butyl (3R)-4-[(4R)-4-Benzyl-2-oxo-1,3-oxazolidin-3-yl]-3-(4-methoxyphenyl)-4-oxobutanoate (24)**: A solution of compound **23** (5.20 g, 0.016 mol) in anhydrous THF (32 mL) was treated at –78°C with NaHMDS in THF (2 mL, 8.7 mL, 0.017 mol). After stirring for 2.5 h at the same temperature, *tert*-butyl bromoacetate (6.5 mL, 0.048 mol) was added and the mixture stirred for additional 3 h at this temperature. The mixture was allowed to warm to 0°C, before saturated NH₄Cl solution (100 mL) was added. Most of the THF was then removed on the rotary evaporator and the resulting slurry extracted with ethyl acetate (3 × 90 mL). The combined organic layers were dried with MgSO₄, filtered, and concentrated to give the crude product as a yellow solid. Purification by flash chromatography (petroleum ether/ethyl acetate 4:1) and recrystallization from a mixture of hexane/ether gave the pure alkylation product as white needles (5.1 g, 71%). M.p. 139.5–140.5°C; R_f = 0.23 (petroleum ether/ethyl acetate 4:1); $[\alpha]_D^{25}$ = +149.0 (c = 1.02, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃): δ = 1.36 (s, 9H; C(CH₃)₃), 2.51 (dd, J = 17.1, 4.5 Hz, 1H; CH₂CO), 2.72 (dd, J = 13.3, 9.8 Hz, 1H; PhCH₂), 3.19 (dd, J = 16.9, 11.3 Hz, 1H; CH₂CO), 3.28 (dd, J = 13.3, 2.7 Hz, 1H; PhCH₂), 3.69 (s, 3H; OCH₃), 3.91 (t, J = 8.0 Hz, 1H; CH₂O), 3.99–4.02 (m, 1H; CH₂O), 4.47–4.52 (m, 1H; NCH), 5.36 (dd, J = 11.3, 4.5 Hz, 1H; CHCO), 6.75 (d, J = 8.5 Hz, 2H; aryl H), 7.17–7.28 ppm (m, 7H; aryl H); ¹³C NMR (100 MHz, CDCl₃): δ = 27.9 (C(CH₃)₃), 37.4 (PhCH₂), 40.4 (CH₂CO), 43.8 (CHCO), 55.1 (OCH₃), 55.6 (NCH), 65.5 (CH₂O), 80.7 (C(CH₃)₃), 114.0, 127.1, 128.8, 128.9, 129.4, 129.5, 135.5, 152.6 (NCO₂), 158.9 (aryl), 170.9 (CHCO), 173.4 ppm (CH₂CO₂); IR (film): $\tilde{\nu}$ = 2356, 1697, 1646, 1519, 1045 cm⁻¹; MS (EI): m/z (%): 383.1 (6), 206.0 (15), 178.1 (100), 83.9 (20); HRMS (EI): m/z : calcd for C₂₁H₂₁NO₆: 383.140500; found: 383.136857 [M –C(CH₃)₃]⁺.

(2R)-4-*tert*-Butoxy-2-(4-methoxyphenyl)-4-oxobutanoic acid (25): A solution of H₂O₂ (30% in H₂O, 8.3 mL, 0.069 mol) at 0°C was added dropwise to a solution of compound **24** (5.10 g, 0.0133 mol) in THF (196 mL) followed by a solution of LiOH in H₂O (0.3 M in H₂O, 66 mL, 0.023 mol). The reaction mixture was stirred at the same temperature for 5 h, at which point TLC indicated the complete consumption of the starting material. Then saturated aqueous solutions of Na₂SO₃ and NaHCO₃ (40 mL of each) were added. The mixture was partly concentrated on the rotary evaporator to remove THF, then diluted with H₂O (85 mL), and extracted with CH₂Cl₂ (3 × 100 mL). The combined organic layers were dried with MgSO₄, filtered, and concentrated in vacuo to afford recovered chiral auxiliary (1.5 g, 78% yield). The aqueous layer was acidified to pH 1.5–2.5 with 6 M HCl at 0°C and extracted with ethyl acetate (3 × 85 mL). The combined organic layers were dried over anhydrous MgSO₄, filtered, and concentrated in vacuo to provide the carboxylic acid **25** (2.5 g, 78%). This acid was used in the next step without further purification. ¹H NMR (400 MHz, CDCl₃): δ = 1.38 (s, 9H; C(CH₃)₃), 2.56 (dd, J = 16.6, 5.5 Hz, 1H; CH₂), 3.03 (dd, J = 16.4, 9.8 Hz, 1H; CH₂), 3.77 (s, 3H; OCH₃), 3.99 (dd, J = 10.1, 5.8 Hz, 1H; CH), 6.83 (d, J = 8.8 Hz, 2H; aryl H), 7.19 (d, J = 8.5 Hz, 2H; aryl H), 10.53 ppm (s, 1H; CO₂H); ¹³C NMR (100 MHz, CDCl₃): δ = 27.8 (C(CH₃)₃), 38.6 (CH₂), 46.4 (CH), 55.2 (OCH₃), 81.1 (C(CH₃)₃), 114.0, 128.9, 129.1, 159.0, 170.5, 179.2 ppm (CHCO₂); IR (film): $\tilde{\nu}$ = 2977, 1720, 1253, 1160 cm⁻¹; HRMS (ESI): m/z : calcd for C₁₅H₂₀O₅Na: 303.12029; found: 303.11986 [M +Na]⁺.

***tert*-Butyl (3R)-3-[(9H-fluoren-9-ylmethoxy)carbonyl]amino-3-(4-methoxyphenyl)propanoate (26)**: Et₃N (0.5 mL, 3.6 mmol) and DPPA (0.71 mL, 3.2 mmol) were added to a solution of carboxylic acid **25** (0.92 g, 3.2 mmol) in toluene (18 mL). The reaction mixture was stirred at room temperature for 30 min and then heated to reflux. Evolution of N₂ was observed between 70–80°C. The reaction progress was monitored by HPLC-MS. After complete conversion of the starting material to the isocyanate (3.5–4 h), the reaction mixture was cooled to 50°C and then treated carefully with FmocOH (1.93 g, 0.009 mol). The mixture was refluxed for 3 h. After the complete conversion of the isocyanate to the carbamate **26**, the mixture was cooled to room temperature and diluted with saturated NaHCO₃ solution (32 mL). Most of the toluene was removed on the rotary evaporator before the mixture was extracted with diethyl ether (3 × 30 mL). The combined organic layers were dried over anhy-

drous MgSO_4 , filtered, and concentrated in vacuo. Flash chromatography (petroleum ether/ethyl acetate 85:15) of the residue afforded pure carbamate **26** as a colorless oil (0.61 g, 45%). R_f =0.45 (petroleum ether/ethyl acetate 70:30); $[\alpha]_D^{25}$ =+51.9 (c =1.02, CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3): δ =1.35 (s, 9H; $\text{C}(\text{CH}_3)_3$), 2.71 (dd, J =14.1, 4.8 Hz, 1H; CH_2CO), 2.77–2.85 (m, 1H; CH_2CO), 3.78 (s, 3H; OCH_3), 4.19 (t, J =6.8 Hz, 1H; CHCH_2O), 4.38 (d, J =3.7 Hz, 2H; CH_2O), 5.07 (d, J =4.5 Hz, 1H; NHCH), 5.73 (d, J =6.3 Hz, 1H; NH), 6.85 (d, J =8.8 Hz, 2H; aryl H), 7.21 (d, J =7.8 Hz, 2H; aryl H), 7.28 (brs, 2H; aryl H), 7.38 (t, J =7.3 Hz, 2H; aryl H), 7.57 (brs, 2H; aryl H), 7.74 ppm (d, J =7.5, Hz, 2H; aryl H); ^{13}C NMR (100 MHz, CDCl_3): δ =27.9 ($\text{C}(\text{CH}_3)_3$), 41.8 (CH_2CO), 47.1 (CHCH_2O), 51.3 (NHCH), 55.2 (OCH_3), 66.6 (CH_2O), 81.2 ($\text{C}(\text{CH}_3)_3$), 113.9, 119.9, 126.9, 127.3, 127.6, 133.0, 141.2, 143.8, 155.5 (NHCO), 158.9, 170.1 ppm (CO_2tBu); IR (film): $\tilde{\nu}$ =3332, 2973, 2256, 1720, 1527 cm^{-1} ; HRMS (EI): m/z : calcd for $\text{C}_{20}\text{H}_{31}\text{NO}_5$: 474.22750; found: 474.22776 [$M+\text{H}$] $^+$.

tert-Butyl (3R)-3-amino-3-(4-methoxyphenyl)propanoate (27): Et_3NH (15 mL) at 0°C was slowly added to a solution of the Fmoc-protected amino acid ester **26** (0.271 g, 0.57 mmol) in dry THF (15 mL). Stirring was continued for 10 min at 0°C and then at room temperature for 12 h. The solution was concentrated in vacuo and the resulting oil purified by flash chromatography (methanol/dichloromethane/diethylamine 5:94.9:0.1). The pure amine **27** was obtained as colorless oil (0.086 g, 60%). R_f =0.5 (methanol/dichloromethane/diethylamine 5:94.9:0.1); $[\alpha]_D^{25}$ =+12.3 (c =1.00, CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3): δ =1.42 (s, 9H; $\text{C}(\text{CH}_3)_3$), 1.81 (s, 2H; NH_2), 2.54 (s, 1H; CH_2), 2.56 (d, J =2.7 Hz, 1H; CH_2), 3.79 (s, 3H; OCH_3), 4.32 (dd, J =7.5, 6.0 Hz, 1H; CH), 6.86 (d, J =8.5 Hz, 2H; aryl H), 7.27 ppm (d, J =8.5 Hz, 2H; aryl H); ^{13}C NMR (100 MHz, CDCl_3): δ =28.0 ($\text{C}(\text{CH}_3)_3$), 45.3 (CH_2), 52.0 (CH), 55.1 (OCH_3), 80.5 ($\text{C}(\text{CH}_3)_3$), 113.7, 127.3, 136.8, 158.7, 171.3 ppm (C=O); IR (film): $\tilde{\nu}$ =3378, 2973, 2842, 1724, 1157 cm^{-1} ; HRMS (EI): m/z : calcd for $\text{C}_{14}\text{H}_{21}\text{NO}_3$: 252.15942; found: 252.15923 [$M+\text{H}$] $^+$.

tert-Butyl (3R)-3-((N-[(9H-fluoren-9-ylmethoxy)carbonyl]-D-tryptophyl)-amino)-3-(4-methoxyphenyl)propanoate (28):

To a solution of amine **27** (0.080 g, 0.31 mmol) and N-Fmoc-D-tryptophan (0.135 g, 0.31 mmol) in anhydrous THF (2.2 mL) were added HOBt (0.043 g, 0.31 mmol) and DCC (0.095 g, 0.46 mmol) at -10°C followed by stirring of the mixture at 0°C overnight. The mixture was filtered through Celite and concentrated. The crude product was purified by flash chromatography (dichloromethane/methanol 98:2) to yield pure dipeptide **28** (0.192 g, 91%). M.p. 88.9–90.8°C; R_f =0.18 (methanol/dichloromethane 2:98); $[\alpha]_D^{25}$ =+7.64 (c =1.03, CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3): δ =1.22 (s, 9H; $\text{C}(\text{CH}_3)_3$), 2.50 (dd, J =11.6, 5.3 Hz, 1H; Tyr CH_2), 2.65 (dd, J =14.6, 4.3 Hz, 1H; Tyr CH_2), 3.14 (dd, J =14.4, 7.8 Hz, 1H; Trp CH_2), 3.31 (dd, J =12.1, 0.5 Hz, 1H; Trp CH_2), 3.71, 3.75 (2s, 3H; OCH_3), 4.18 (brs, 1H; Fmoc CH), 4.31–4.44 (m, 2H; Fmoc CH_2), 4.53 (d, J =3.0 Hz, 1H; Trp CH), 5.22 (dd, J =14.4, 6.3 Hz, 1H; Tyr CH), 5.60 (d, J =5.0 Hz, 1H; Trp NH), 6.57 (d, J =7.8 Hz, 1H; Tyr NH), 6.71 (d, J =7.5 Hz, 2H; Tyr aryl H), 6.78 (s, 1H; Ind NHCH), 6.89 (d, J =8.5 Hz, 2H; Fmoc CH), 7.11 (t, J =7.4 Hz, 1H; Trp aryl H), 7.19 (t, J =7.5 Hz, 1H; Trp aryl H), 7.26–7.33 (m, 3H; Fmoc aryl H, Trp aryl H), 7.38 (t, J =7.4 Hz, 2H; Fmoc aryl H), 7.53 (d, J =6.8 Hz, 2H; Fmoc aryl H), 7.68 (d, J =6.5 Hz, 1H; Trp aryl H), 7.75 (d, J =7.5 Hz, 2H; Tyr aryl H), 8.03, 8.19 ppm (2s, 1H; Trp NH); ^{13}C NMR (100 MHz, CDCl_3): δ =27.6 ($\text{C}(\text{CH}_3)_3$), 28.6 (Trp CH_2), 41.2 (Tyr CH_2), 46.9 (Fmoc CH), 49.2 (Tyr CH), 55.0 (OCH_3), 60.3 (Trp CH), 67.0 (Fmoc CH_2), 81.0 ($\text{C}(\text{CH}_3)_3$), 109.8 (Trp aryl CH), 111.2 (Trp aryl CH), 113.6 (Tyr aryl CH), 118.5, (Trp aryl CH), 119.5 (Trp aryl CH), 119.8 (Fmoc aryl CH), 121.9 (Trp aryl CH), 123.2 (Trp NHCH), 125.0 (Fmoc aryl CH), 126.9 (Fmoc aryl CH), 127.1 (Trp aryl C), 127.3 (Fmoc aryl CH), 127.5 (Tyr aryl CH), 132.2 (Tyr aryl C), 136.1 (Trp aryl C), 141.1, (Fmoc aryl C), 143.6 (Fmoc aryl C), 155.9 (Fmoc CO), 158.6 (aryl C- OCH_3), 169.9 (Tyr CO), 170.4 ppm (Trp CO); IR (film): $\tilde{\nu}$ =3313, 2935, 1716, 1515, 1245 cm^{-1} ; HRMS (ESI): m/z : calcd for $\text{C}_{40}\text{H}_{41}\text{N}_5\text{O}_8\text{Na}$: 682.28876; found: 682.28960 [$M+\text{Na}$] $^+$.

N-[(9H-Fluoren-9-ylmethoxy)carbonyl]-L-alanyl-N-[(1R)-3-tert-butoxy-1-(4-methoxyphenyl)-3-oxopropyl]-D-tryptophanamide (29)

a) tert-Butyl (3R)-3-(4-methoxyphenyl)-3-(D-tryptophylamino)propanoate: To a solution of the dipeptide **28** (0.134 g, 0.203 mmol) in dry

THF (5 mL) was added Et_3NH (15 mL) at 0°C and the mixture was stirred at 0°C for 15 min and then at room temperature for 1 h. Thereafter, the reaction mixture was concentrated in vacuo and the crude product purified by flash chromatography (methanol/dichloromethane/diethylamine 2:97.9:0.1) to afford the free amine as a colorless solid (0.069 g, 78%). M.p. 138.7–140.5°C; R_f =0.2 (methanol/dichloromethane/diethylamine 2:97.9:0.1); $[\alpha]_D^{25}$ =+43.7 (c =0.21, CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3): δ =1.32 (s, 9H; $\text{C}(\text{CH}_3)_3$), 1.52 (s, 2H; NH_2), 2.59 (dd, J =14.9, 6.5 Hz, 1H; Tyr CH_2), 2.76 (dd, J =14.9, 6.5 Hz, 1H; Tyr CH_2), 2.91 (dd, J =14.6, 9.0 Hz, 1H; Trp CH_2), 3.38 (dd, J =14.4, 4.0 Hz, 1H; Trp CH_2), 3.69 (dd, J =9.1, J =4.3 Hz, 1H; Trp CH), 3.76 (s, 3H; OCH_3), 5.33 (dd, J =14.9, 6.5 Hz, 1H; Tyr CH), 6.81 (d, J =8.5 Hz, 2H; Tyr aryl H), 6.99 (d, J =2.0 Hz, 1H; Ind NHCH), 7.09 (t, J =7.0 Hz, 1H; Trp aryl H), 7.17 (t, J =6.8 Hz, 1H; Trp aryl H), 7.17 (d, J =8.5 Hz, 2H; Tyr aryl H), 7.33 (d, J =8.0 Hz, 1H; Trp aryl H), 7.64 (d, J =7.8 Hz, 1H; Trp aryl H), 8.02 (d, J =8.5 Hz, 1H; Tyr NH CH), 8.50, 8.54 ppm (2s, 1H; Trp NH); ^{13}C NMR (100 MHz, CDCl_3): δ =27.8 ($\text{C}(\text{CH}_3)_3$), 30.8 (Trp CH_2), 41.7 (Tyr CH_2), 48.9 (Tyr CH), 55.1 (OCH_3), 55.5 (Trp CH), 81.0 ($\text{C}(\text{CH}_3)_3$), 111.2, 111.4, 113.8, 118.8, 119.4, 122.0, 123.1 (Trp NHCH), 127.3, 127.5, 132.9, 136.4 (Trp aryl C), 158.7, 170.0 (Tyr CO), 173.9 ppm (Trp CO); IR (film): $\tilde{\nu}$ =3309, 2923, 1720, 1658, 1249 cm^{-1} ; HRMS (EI): m/z : calcd for $\text{C}_{25}\text{H}_{31}\text{N}_3\text{O}_4$: 438.23873; found: 438.23879 [$M+\text{H}$] $^+$.

b) Peptide coupling: To a solution of the amine described above (66 mg, 0.151 mmol) in dry THF (2 mL, 0.07 M) were added Fmoc-L-Ala-OH (46 mg, 0.151 mmol), HOBt (20.3 mg, 0.151 mmol), and DCC (46 mg, 0.22 mmol) at 0°C and the mixture was stirred at 0°C for 12 h. Thereafter, the mixture was filtered and concentrated. Flash chromatography (methanol/dichloromethane 2:98) yielded the desired tripeptide **29** (0.110 g, 97%) as a slightly yellow solid. M.p. 116.2–123.5°C; R_f =0.4 (methanol/dichloromethane 4:96); $[\alpha]_D^{25}$ =+10.17 (c =1.03, CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3): δ =1.17–1.21 (m, 12H; $\text{C}(\text{CH}_3)_3$, Ala CH_3), 2.42 (dd, J =14.9, 6.3 Hz, 1H; Tyr CH_2), 2.56 (dd, J =15.1, 6.5 Hz, 1H; Tyr CH_2), 3.08 (dd, J =14.2, 6.9 Hz, 1H; Trp CH_2), 3.20 (dd, J =14.6, 5.3 Hz, 1H; Trp CH_2), 3.61 (s, 3H; OCH_3), 4.03–4.11 (m, 2H; Ala CH, Fmoc CH), 4.15–4.29 (m, 2H; Fmoc CH_2), 4.69 (brd, J =6.5 Hz, 1H; Trp CH), 5.15 (dd, J =13.9, 6.5 Hz, 1H; Tyr CH), 5.54 (d, J =6.8 Hz, 1H; Trp NH), 6.62 (d, J =8.5 Hz, 2H; Tyr aryl H), 6.78 (s, 1H; Ind NHCH), 6.82 (d, J =7.5 Hz, 1H; Ala NH), 6.88 (d, J =8.3 Hz, 2H; Tyr aryl H), 6.94 (d, J =8.0 Hz, 1H; Tyr NH), 6.99 (t, J =7.5 Hz, 1H; Trp aryl H), 7.05 (t, J =7.3 Hz, 1H; Trp aryl H), 7.16–7.22 (m, 3H; Fmoc aryl H, Trp aryl H), 7.30 (t, J =7.3 Hz, 2H; Fmoc aryl H), 7.45 (d, J =6.1 Hz, 2H; Fmoc aryl H), 7.53 (d, J =7.3 Hz, 1H; Trp aryl H), 7.67 (d, J =7.3 Hz, 2H; Fmoc aryl H), 8.04, 8.11 ppm (2s, 1H; Ind NH); ^{13}C NMR (100 MHz, CDCl_3): δ =18.3 (Ala CH_3), 27.6 (Trp CH_2), 27.7 ($\text{C}(\text{CH}_3)_3$), 41.4 (Tyr CH_2), 46.9 (Fmoc CH), 49.5 (Tyr CH), 50.8 (Ala CH), 53.6 (Trp CH), 55.1 (OCH_3), 67.0 (Fmoc CH_2), 81.1 ($\text{C}(\text{CH}_3)_3$), 110.1 (Trp aryl C), 111.1 (Trp aryl CH), 113.7 (Tyr aryl CH), 118.6, (Trp aryl CH), 119.6 (Trp aryl CH), 119.9 (Fmoc aryl CH), 122.1 (Trp aryl CH), 123.2 (Trp NHCH), 125.0 (Fmoc aryl CH), 127.0 (Fmoc aryl CH), 127.3 (Trp aryl C), 127.5 (Fmoc aryl CH), 127.7 (Tyr aryl CH), 132.5 (Tyr aryl C), 136.0 (Trp aryl C), 141.2 (Fmoc aryl C), 143.6 (Fmoc aryl C), 156.0 (Fmoc CO), 158.7 (aryl C), 170.0 (Tyr CO), 170.2 (Ala CO), 172.3 ppm (Trp CO); IR (film): $\tilde{\nu}$ =3401, 3289, 3062, 2931, 2117, 1697, 1646, 1245 cm^{-1} ; HRMS (ESI): m/z : calcd for $\text{C}_{45}\text{H}_{46}\text{N}_4\text{O}_7\text{Na}$: 753.32587; found: 753.32533 [$M+\text{Na}$] $^+$.

N-[(2S)-3-((2R)-2-[(tert-Butoxycarbonyl)amino]propyl)phenyl)-2-methylpropanoyl]-L-alanyl-N-[(1R)-3-tert-butoxy-1-(4-methoxyphenyl)-3-oxopropyl]-D-tryptophanamide (30)

a) L-Alanyl-N-[(1R)-3-tert-butoxy-1-(4-methoxyphenyl)-3-oxopropyl]-D-tryptophanamide: Et_3NH (6.5 mL) was added dropwise at 0°C to a solution of tripeptide **29** (162 mg, 0.222 mmol) in dry THF (6.5 mL, 0.034 M). The mixture was then stirred for 15 min at 0°C and then at room temperature for 1 h. Thereafter, the mixture was concentrated and the oily residue purified by flash chromatography (methanol/dichloromethane/diethylamine 2:97.9:0.1) yielding the free amine (0.096 g, 85%) as an oil. R_f =0.11 (methanol/dichloromethane/diethylamine 2:97.9:0.1); $[\alpha]_D^{25}$ =+23.9 (c =1.05, CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3): δ =1.24–1.28 (m, 12H; $\text{C}(\text{CH}_3)_3$, Ala CH_3), 1.84 (s, 2H; NH_2), 2.48 (dd, J =15.1, 6.5 Hz, 1H; Tyr CH_2), 2.65 (dd, J =15.1, 6.3 Hz, 1H; Tyr CH_2), 3.17 (dd, J =14.6, 7.3 Hz,

1H; Trp CH₂), 3.25 (dd, $J=14.6$, 6.3 Hz, 1H; Trp CH₂), 3.40 (q, $J=6.9$ Hz, 1H; Ala CH), 3.72, 3.75 (2s, 3H; OCH₃), 4.72 (q, $J=7.2$ Hz, 1H; Trp CH), 5.21 (dd, $J=14.4$, 6.5 Hz, 1H; Tyr CH), 6.74 (d, $J=8.8$ Hz, 2H; Tyr aryl H), 6.83 (s, 1H; Ind NHCH), 6.97 (d, $J=8.5$ Hz, 3H; Tyr aryl H, Tyr NH), 7.07 (t, $J=7.2$ Hz, 1H; Trp aryl H), 7.14 (t, $J=7.2$ Hz, 1H; Trp aryl H), 7.29 (d, $J=8.08$ Hz, 1H; Trp aryl H), 7.64 (d, $J=7.8$ Hz, 1H; Trp aryl H), 7.83 (d, $J=8.08$ Hz, 1H; Trp NH), 8.29, 8.43 ppm (2s, 1H; Ind NH); ¹³C NMR (100 MHz, CDCl₃): $\delta=21.3$ (Ala CH₃), 27.7 (C(CH₃)₃), 27.9 (Trp CH₂), 41.2 (Tyr CH₂), 49.3 (Tyr CH), 50.5 (Ala CH), 53.4 (Trp CH), 55.1 (OCH₃), 81.1 (C(CH₃)₃), 110.2 (Trp aryl C), 111.2 (Trp aryl CH), 113.6 (Tyr aryl CH), 118.6 (Trp aryl CH), 119.3 (Trp aryl CH), 121.8 (Trp aryl CH), 123.2 (Trp NHCH), 127.4 (Tyr aryl CH, Trp aryl C), 132.4 (Tyr aryl C), 136.1 (Trp aryl C), 158.6 (aryl C-OCH₃), 170.1 (Tyr CO), 170.3 (Ala CO), 175.9 ppm (Trp CO); IR (film): $\tilde{\nu}=3297$, 3054, 2969, 2927, 1720, 1153 cm⁻¹; HRMS (EI): m/z : calcd for C₂₈H₃₆N₄O₅: 509.27585; found: 509.27563 [$M+H$]⁺.

b) Peptide coupling: DCC (19.2 mg, 0.093 mmol) was added at -20°C to a solution of amino acid **4** (19.5 mg, 0.061 mmol), the amine prepared above (31.0 mg, 0.061 mmol), and HOBt (8.2 mg, 0.061 mmol) in dry THF (0.8 mL). The mixture was stirred at -20°C for 30 min and then at room temperature for 8 h. Subsequently, the reaction mixture was filtered through Celite and the filtrate concentrated in vacuo. Purification of the residue by flash chromatography (methanol/dichloromethane 2:98) gave the tetrapeptide **30** as a white solid (0.046 g, 92%). M.p. 109.5–116.5°C; $R_f=0.10$ (methanol/dichloromethane 2:98); $[\alpha]_D^{25}=+33.1$ ($c=1.00$, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃): $\delta=0.96$ (d, $J=6.3$ Hz, 3H; CH₃), 1.03 (d, $J=5.8$ Hz, 3H; Ala CH₃), 1.12 (d, $J=7.0$ Hz, 3H; CH₃), 1.33–1.36 (2s, 18H; C(CH₃)₃), 2.39 (dd, $J=12.5$, 9.2 Hz, 1H; CH₂), 2.55–2.57 (m, 2H; CH₂, Tyr CH₂), 2.67–2.79 (m, 4H; CH₂, CHCH₃, Tyr CH₂, Trp CH₂), 2.85 (dd, $J=13.3$, 5.8 Hz, 1H; CH₂), 3.06 (dd, $J=14.4$, 8.4 Hz, 1H; Trp CH₂), 3.33–3.34 (2s, 3H; OCH₃), 3.72–3.76 (m, 1H; CHCH₃), 4.11–4.17 (m, 1H; Ala CH), 4.57–4.62 (m, 1H; Trp CH), 5.25 (dd, $J=15.4$, 7.2 Hz, 1H; Tyr CH), 6.74 (d, $J=8.5$ Hz, 2H; Tyr aryl H), 6.93–7.02 (m, 5H; Ind NHCH, Trp aryl H, xyllyl H), 7.06 (t, $J=7.4$ Hz, 1H; Trp aryl H), 7.08–7.12 (m, 3H; Tyr aryl H, xyllyl H), 7.30 (d, $J=8.0$ Hz, 1H; Trp aryl H), 7.51 (d, $J=7.8$ Hz, 1H; Trp aryl H), 7.82 (d, $J=7.5$ Hz, 1H; Trp NH), 8.07 (brs, 1H; Ala NH), 8.28 (d, $J=8.3$ Hz, 1H; Tyr NH), 10.27 ppm (s, 1H; Ind NH); ¹³C NMR (100 MHz, CDCl₃): $\delta=17.4$ (CH₃, Ala CH₃), 20.6 (CH₃), 28.4, 28.6, 28.9 (C(CH₃)₃, Trp CH₂), 40.9 (CH₂), 43.1 (CHCH₃), 43.2 (Tyr CH₂), 43.9 (CH₂), 51.3 (CHNBoc), 51.4 (Ala CH), 55.6 (Tyr CH), 55.6 (Trp CH), 55.7 (OCH₃), 79.9, 82.1 (C(CH₃)₃), 111.1 (Trp aryl C), 112.4 (Trp aryl CH), 114.8 (Tyr aryl CH), 119.5, 119.9, 122.5 (Trp aryl CH), 124.7 (Ind NHCH), 128.0, 128.4 (xyllyl CH), 128.8 (Trp aryl C), 129.2 (Tyr aryl CH), 129.3, 131.3 (xyllyl CH), 134.4 (Tyr aryl C), 138.2 (Trp aryl C), 140.4, 140.1 (xyllyl C), 157.8 (Boc CO), 160.5 (Tyr aryl C-OMe), 171.7, 172.9, (Ala CO, Tyr CO), 176.0 (Trp CO), 179.0 ppm (CO); IR (film): $\tilde{\nu}=3293$, 2923, 1681, 1531 cm⁻¹; HRMS (ESI): m/z : calcd for C₄₆H₆₁N₅O₈Na: 834.44124; found: 834.43921 [$M+Na$]⁺.

(3S,7R,10R,13S,16S)-10-(1H-Indol-3-ylmethyl)-7-(4-methoxyphenyl)-3,13,16-trimethyl-4,8,11,14-tetraazabicyclo[16.3.1]docosa-1(22),18,20-triene-5,9,12,15-tetrone (31): TFA (1.26 mL) was added dropwise at 0°C to a solution of tetrapeptide **30** (0.063 g, 0.0776 mmol) in dry CH₂Cl₂ (1.26 mL) and the mixture stirred at room temperature. After HPLC-MS showed the complete deprotection (ca. 3 h), the mixture was concentrated on the rotary evaporator. The crude amino acid was used in the next step without further purification. It was dissolved in dry DMF (59 mL, 0.00098 M) and treated with TBTU (56.0 mg, 0.17 mmol), HOBt (22.9 mg, 0.17 mmol), and *i*Pr₂NEt (0.040 mL, 0.23 mmol) at room temperature followed by stirring at this temperature for 14 h. The mixture was partitioned between ethyl acetate and water. The organic layer was washed with H₂O, 5% aqueous KHSO₄ solution, H₂O, half saturated NaHCO₃ solution, brine, dried over MgSO₄, filtered, and concentrated in vacuo. Purification was achieved by flash chromatography (methanol/dichloromethane 2:98). The pure compound **31** was obtained as a colorless solid (0.046 g, 92%). M.p. 274.5–274.8°C (decomp); $R_f=0.10$ (methanol/dichloromethane 2:98); $[\alpha]_D^{25}=5.5$ ($c=0.45$, DMSO); ¹H NMR (600 MHz, [D₆]DMSO): $\delta=0.87$ (d, $J=6.1$ Hz, 3H; 5-CH₃), 0.91 (d, $J=7.0$ Hz, 3H; 14-CH₃), 1.04 (d, $J=7.0$ Hz, 3H; 11-CH₃), 2.22 (dd, $J=12.7$, 11.0 Hz, 1H; 6-H), 2.35–2.49 (m, 4H; 2-H, 10-H, 2-H, 11-H), 2.78–2.85 (m, 2H; 6-H,

20-H), 2.95 (dd, $J=13.2$, 10.5 Hz, 1H; 10-H), 3.01 (dd, $J=14.0$, 5.3 Hz, 1H; 20-H), 3.73 (s, 1H; OCH₃), 3.74 (m, 1H; 5-H), 4.46–4.51 (m, 1H; 14-H), 4.62–4.66 (m, 1H; 17-H), 5.13–5.17 (m, 1H; 1-H), 6.82–6.86 (m, 2H; 2'-H, 2'-H), 6.88 (d, $J=7.0$ Hz, 1H; 6'-H), 6.91–6.95 (m, 2H; 4'-H, 7'''-H), 6.96–6.99 (m, 1H; 2'''-H), 7.03 (dd, $J=7.5$, 7.5 Hz, 1H; 8'''-H), 7.06–7.12 (m, 2H; 5'''-H, 3'-H), 7.31 (d, $J=7.5$ Hz, 1H; 9'''-H), 7.54 (d, $J=7.9$ Hz, 2H; 6'''-H, 4-NH), 7.82 (d, $J=7.9$ Hz, 1H; 13-NH), 8.21 (d, $J=8.8$ Hz, 1H; 16-NH), 8.50 (d, $J=8.8$ Hz, 1H; 19-NH), 10.77 ppm (s, 1H; 1'''-NH); ¹³C NMR (150 MHz, [D₆]DMSO): $\delta=18.7$ (CH₃-5), 18.8 (CH₃-14), 19.4 (CH₃-11), 28.4 (C-20), 38.7 (C-10), 41.1 (C-11), 42.3 (C-6), 42.7 (C-2), 46.0 (C-5), 47.0 (C-14), 49.3 (C-1), 52.6 (C-17), 54.8 (OCH₃), 110.9 (C-9'''), 117.8 (C-7'''), 118.0 (C-6'''), 120.5 (C-8'''), 123.3 ppm (C-2'''); IR (film): $\tilde{\nu}=3286$, 3062, 2965, 2927, 1643, 1542 cm⁻¹; HRMS (ESI): m/z : calcd for C₃₇H₄₃N₅O₅: 660.31564; found: 660.31495 [$M+Na$]⁺.

Methyl *N*-(*tert*-butoxycarbonyl)-*L*-alanyl-*N*-methyl-*D*-tryptophanate (**33**)

a) N-Me-D-Trp-OMe: A solution of *N*-Boc-protected amino acid ester^[34] **32** (2.042 g, 6.15 mmol) in dry CH₂Cl₂ (50 mL) was treated dropwise with TFA (5.00 mL, 0.0615 mol) at 0°C and the mixture stirred at room temperature for 1 h. The mixture was concentrated and the residue dissolved in ethyl acetate, then washed with water. The aqueous layer was basified with 1 N NaOH to pH 8–9 keeping the temperature at 0°C. Both layers were then extracted with ethyl acetate. The organic layer was dried over anhydrous MgSO₄, filtered, and concentrated to yield the free amine (0.975 g, 68%). It was used in the next step without further purification. ¹H NMR (400 MHz, CDCl₃): $\delta=2.37$ (s, 3H; NCH₃), 3.12 (dd, $J=14.4$, 7.0 Hz, 1H; CH₂), 3.21 (dd, $J=14.4$, 5.8 Hz, 1H; CH₂), 3.59 (t, $J=6.0$ Hz, 1H; CH), 3.66 (s, 3H; OCH₃), 6.96 (d, $J=2.02$ Hz, 1H; Ind NHCH), 7.11 (t, $J=7.8$ Hz, 1H; aryl H), 7.17 (t, $J=7.8$ Hz, 1H; aryl H), 7.29 (d, $J=7.8$ Hz, 1H; aryl H), 7.60 (d, $J=7.8$ Hz, 1H; aryl H), 8.70 ppm (s, 1H; Ind NH); ¹³C NMR (100 MHz, CDCl₃): $\delta=28.9$ (CH₂), 34.6 (NCH₃), 51.6 (OCH₃), 63.6 (CH₂CH), 110.4 (aryl C), 111.1, 118.4, 119.2, 121.8, 123.0, (aryl CH), 127.2, 136.1, (aryl C), 174.9 ppm (CO₂); IR (film): $\tilde{\nu}=3405$ –2803 (broad), 1731, 1446, 1203 cm⁻¹.

b) Peptide coupling: Boc-L-Ala-OH (0.305 g, 1.61 mmol), HOBt (0.218 g, 1.61 mmol), and DCC (0.50 g, 2.42 mmol) were added at 0°C to a solution of the foregoing amine (0.375 g, 1.61 mmol) in dry THF (14 mL). The reaction mixture was stirred at 0°C overnight and then filtered through Celite, and concentrated. Purification of the residue by flash chromatography (dichloromethane/methanol 98:2) gave the dipeptide **33** as a colorless solid (0.384 g, 59%). M.p. 73.5–77.4°C; $R_f=0.16$ (dichloromethane/methanol 98:2); $[\alpha]_D^{26}=+54.33$ ($c=1.05$, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃): $\delta=0.88$ (d, $J=6.8$ Hz, 3H; Ala CH₃), 1.41 (s, 9H; C-(CH₃)₃), 2.81 (s, 3H; NCH₃), 3.28 (dd, $J=15.4$, 11.1 Hz, 1H; CH₂), 3.45 (dd, $J=15.4$, 4.8 Hz, 1H; CH₂), 3.73 (s, 3H; OCH₃), 4.44–4.52 (m, 1H; Ala CH), 5.26 (dd, $J=10.9$, 4.9 Hz, 1H; Trp CH), 5.50 (d, $J=7.8$ Hz, 1H; NH), 6.98 (d, $J=1.7$ Hz, 1H; Ind NHCH), 7.10 (t, $J=7.4$ Hz, 1H; aryl H), 7.16 (t, $J=7.0$ Hz, 1H; aryl H), 7.31 (d, $J=7.8$ Hz, 1H; aryl H), 7.56 (d, $J=7.8$ Hz, 1H; aryl H), 8.34 ppm (s, 1H; IndNH); ¹³C NMR (100 MHz, CDCl₃): $\delta=18.1$ (Ala CH₃), 24.4 (CH₂), 28.3 (C(CH₃)₃), 32.8 (NCH₃), 46.5 (Ala CH), 52.3 (OCH₃), 58.1 (Trp CH), 79.5 (C(CH₃)₃), 110.6 (aryl C), 111.2, 118.2, 119.4, 122.0 (aryl CH), 122.4 (Ind NHCH), 127.0, 136.0 (aryl C), 155.1 (NHCO₂), 171.1 (Ala CO), 173.5 ppm (Trp CO); IR (film): $\tilde{\nu}=3926$, 2973, 1704, 1646, 1488 cm⁻¹; HRMS (EI): m/z : calcd for C₂₁H₂₉N₃O₃Na: 426.19994; found: 426.2000 [$M+Na$]⁺.

***N*-(*tert*-Butoxycarbonyl)-*L*-alanyl-*N*-methyl-*D*-tryptophan (**34**):** NaOH solution (0.4 M in H₂O, 0.024 g, 0.60 mmol) was added to a solution of the dipeptide **33** (0.201 g, 0.48 mmol) in THF (3 mL). The reaction mixture was stirred for 2 h at room temperature, and then diluted with saturated NaHCO₃ solution (7.5 mL). The mixture was then extracted with (3 × 4 mL) of diethyl ether. The aqueous layer was acidified to pH 1–2 with 0.5 N HCl at 0°C. The acidified layer was extracted with ethyl acetate (3 × 15 mL). The combined organic layers were dried over anhydrous MgSO₄, filtered, and concentrated in vacuo. The crude acid was purified by flash chromatography (ethyl acetate/petroleum ether/acetic acid 1:1:0.1) yielding **34** as a pale colorless solid (0.141 g, 74%). M.p. 106.7–107.4°C; $R_f=0.12$ (ethyl acetate/petroleum ether/acetic acid 1:1:0.1); $[\alpha]_D^{25}=+44.84$ ($c=1.17$, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃): $\delta=0.86$ (d, $J=6.8$ Hz, 3H; Ala CH₃), 1.42 (s, 9H; C(CH₃)₃), 2.80 (s, 3H; NCH₃),

3.32 (dd, $J=15.1$, 11.3 Hz, 1H; CH₂), 3.48 (dd, $J=15.6$, 4.8 Hz, 1H; CH₂), 4.49–4.56 (m, 1H; Ala CH), 5.23 (dd, $J=10.4$, 4.4 Hz, 1H; Trp CH), 5.71 (d, $J=8.0$ Hz, 1H; NH), 6.99 (brs, 1H; Ind NHCH), 7.10 (t, $J=7.3$ Hz, 1H; aryl H), 7.16 (t, $J=7.3$ Hz, 1H; aryl H), 7.34 (d, $J=7.8$ Hz, 1H; aryl H), 7.56 (d, $J=7.8$ Hz, 1H; aryl H), 8.55, 8.61 (s, 1H; Ind NH), 10.62 ppm (s, 1H; CO₂H); ¹³C NMR (100 MHz, CDCl₃): $\delta=17.6$ (Ala CH₃), 24.2 (CH₂), 28.3 (C(CH₃)₃), 33.3 (NCH₃), 46.6 (Ala CH), 58.9 (Trp CH), 79.8 (C(CH₃)₃), 110.4 (aryl C), 111.3, 118.1, 119.3, 121.9 (aryl CH), 122.7 (Ind NHCH), 127.0, 136.0 (aryl C), 155.4 (Boc CO), 173.8 (Ala CO), 174.2 ppm (Trp CO); IR (film): $\tilde{\nu}=3012$, 1762, 1700, 1463, 1373, 1245 cm⁻¹; HRMS (ESI): m/z : calcd for C₂₀H₂₇N₃O₅Na: 412.18429; found: 412.18462 [M +Na]⁺.

N-(tert-Butoxycarbonyl)-L-alanyl-N-[(1R)-3-tert-butoxy-1-(4-methoxyphenyl)-3-oxopropyl]-N-methyl-D-tryptophanamide (35): HOBt (0.135 g, 0.33 mmol) and DCC (0.101 g, 0.49 mmol) at -20°C were added to a solution of acid **34** (0.128 g, 0.33 mmol) and amine **27** (0.082 g, 0.33 mmol) in dry THF (1.5 mL). The mixture was stirred at 0°C overnight, filtered, and the filtrate concentrated. The crude tripeptide was purified by flash chromatography (ethyl acetate/petroleum ether 50:50) providing **35** as a colorless solid (0.196 g, 96%). M.p. 109.2–112.6°C; $R_f=0.25$ (ethyl acetate/petroleum ether 1:1); [α]_D²⁵ = +21.85 ($c=1.02$, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃): $\delta=0.86$ (d, $J=6.8$ Hz, 3H; Ala CH₃), 1.33 (s, 9H; C(CH₃)₃), 1.39 (s, 9H; C(CH₃)₃), 2.65 (dd, $J=15.4$, 6.0 Hz, 1H; Tyr CH₂), 2.78 (dd, $J=15.6$, 8.0 Hz, 1H; Tyr CH₂), 2.91 (s, 3H; NCH₃), 3.18 (dd, $J=15.6$, 10.6 Hz, 1H; Trp CH₂), 3.42 (dd, $J=15.1$, 5.5 Hz, 1H; Trp CH₂), 3.74 (s, 3H; OCH₃), 4.33–4.39 (m, 1H; Ala CH), 5.29–5.34 (m, 2H; Tyr CH, NH), 5.60 (dd, $J=10.3$, 5.5 Hz, 1H; Trp), 6.77 (d, $J=8.5$ Hz, 2H; Tyr aryl H), 6.92 (brs, 1H; NHCH), 7.04 (d, $J=8.0$ Hz, 1H; NH), 7.08 (t, $J=7.4$ Hz, 1H; Trp aryl H), 7.15–7.16 (m, 3H; Tyr aryl H, Trp aryl H), 7.29 (d, $J=8.1$ Hz, 1H; Trp aryl H), 7.56 (d, $J=7.5$ Hz, 1H; Trp aryl H), 8.52 ppm (s, 1H; Ind NH); ¹³C NMR (100 MHz, CDCl₃): $\delta=16.9$ (Ala CH₃), 23.16 (Trp CH₂), 27.8, 28.2 (C(CH₃)₃), 30.6 (NCH₃), 41.5 (Tyr CH₂), 46.5 (Ala CH), 49.5 (Tyr CH), 55.1 (OCH₃), 56.5 (Trp CH), 79.6, 80.8 (C(CH₃)₃), 110.7 (Trp aryl C-8), 111.0 (Trp aryl CH), 113.7 (Tyr aryl CH), 118.4, 119.1, 121.7 (Trp aryl CH), 122.0 (Ind NHCH), 127.3 (Trp aryl C), 127.5 (Tyr aryl CH), 132.9 (Tyr aryl C), 136.0 (Trp aryl C), 155.4 (Boc CO), 158.7 (Tyr aryl C-OCH₃), 169.1 (Tyr CO), 169.8 (Ala CO), 174.2 ppm (Trp CO); IR (film): $\tilde{\nu}=3332$, 2977, 1666, 1515, 1160 cm⁻¹; HRMS (ESI): m/z : calcd for C₃₄H₄₆N₄O₇Na: 645.32587; found: 645.32523 [M +Na]⁺.

N-[(2S)-3-(3-[(2R)-2-[(tert-Butoxycarbonyl)amino]propyl)phenyl)-2-methylpropanoyl]-L-alanyl-N-[(1R)-3-tert-butoxy-1-(4-methoxyphenyl)-3-oxopropyl]-N-methyl-D-tryptophanamide (36)

a) L-Alanyl-N-[(1R)-3-tert-butoxy-1-(4-methoxyphenyl)-3-oxopropyl]-N-methyl-D-tryptophanamide: A solution of tripeptide **35** (0.120 g, 0.19 mmol) and 2,6-lutidine (0.134 mL, 1.16 mmol) in dry CH₂Cl₂ (2.8 mL) was treated with TBDMSOTf (0.17 mL, 0.76 mmol) at 0°C. After 1 h of stirring at 0°C, HPLC-MS analysis showed that the *tert*-butyl residue was replaced by the *tert*-butyldimethylsilyl group. The reaction was then quenched by adding H₂O (8 mL). The mixture was extracted with ethyl acetate (3×15 mL). The combined organic layers were dried over anhydrous MgSO₄, filtered, and concentrated. The crude product was purified by flash chromatography using a stepwise gradient (dichloromethane/methanol, 100:0; 98:2; 97:3; 90:10). The free amine was obtained as yellow oil (0.057 g, 56%). $R_f=0.12$ (dichloromethane/methanol/diethyl amine 95.9:4:0.1); [α]_D²⁶ = +16.65 ($c=0.65$, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃): $\delta=0.68$ (d, $J=6.8$ Hz, 3H; Ala CH₃), 1.30–1.36 (m, 11H; C(CH₃)₃, NH₂), 2.67 (dd, $J=15.4$, 5.5 Hz, 1H; Tyr CH₂), 2.78 (dd, $J=15.1$, 7.1 Hz, 1H; Tyr CH₂), 2.83 (s, 3H; NCH₃), 3.19 (dd, $J=15.6$, 11.1 Hz, 1H; Trp CH₂), 3.41 (dd, $J=15.5$, 5.0 Hz, 1H; Trp CH₂), 3.72 (s, 3H; OCH₃), 3.88–3.93 (m, 1H; Ala CH), 5.31–5.36 (m, 1H; Tyr CH), 5.55 (dd, $J=10.9$, 5.4 Hz, 1H; Trp CH), 6.76 (d, $J=8.6$ Hz, 2H; Tyr aryl H), 6.90 (brs, 1H; Ind NHCH), 7.05 (d, $J=7.4$ Hz, 1H; Tyr aryl H), 7.12 (t, $J=7.2$ Hz, 1H; Trp aryl H), 7.20 (d, $J=8.5$ Hz, 2H; Tyr aryl H), 7.27 (d, $J=7.6$ Hz, 1H; Trp aryl H), 7.45 (d, $J=8.1$ Hz, 1H; NH), 8.52 ppm (s, 1H; Ind NH); ¹³C NMR (100 MHz, CDCl₃): $\delta=17.0$ (Ala CH₃), 23.4 (Trp CH₂), 27.8 (C(CH₃)₃), 30.5 (NCH₃), 41.1 (Tyr CH₂), 47.3 (Ala CH), 49.8 (Tyr CH), 55.2 (OCH₃), 57.3 (Trp CH), 81.2 (C(CH₃)₃), 110.0 (Trp

aryl C), 111.3 (Trp aryl CH), 113.8 (Tyr aryl CH), 118.1, 119.2, 121.8 (Trp aryl CH), 122.5 (Ind NHCH), 127.3 (Trp aryl C), 127.8 (Tyr aryl CH), 133.0 (Tyr aryl C), 135.9 (Trp aryl C), 158.8 (Tyr aryl C-OCH₃), 169.0 (Tyr CO), 170.7 (Ala CO), 174.2 ppm (Trp CO); IR (film): $\tilde{\nu}=3324$, 2965, 1666, 1515 cm⁻¹; HRMS (EI): m/z : calcd for C₂₉H₃₈N₄O₅: 523.29150; found: 523.29154 [M +H]⁺.

b) Peptide coupling: A solution of acid **4** (0.029 g, 0.056 mmol), the amine prepared above (0.018 g, 0.056 mmol), and HOBt (0.0075 g, 0.056 mmol) in dry THF (0.7 mL) was treated with DCC (0.017 g, 0.084 mmol) at -20°C. The reaction mixture was first stirred at the same temperature for 30 min and then at room temperature for 18 h. The precipitate of urea was then filtered off and the filtrate concentrated on the rotary evaporator. Purification of the residue by flash chromatography (dichloromethane/methanol 96:4) gave tetrapeptide **36** as a colorless solid (0.028 g, 60%). M.p. 78.8–85.2°C; $R_f=0.12$ (dichloromethane/methanol 94:6); [α]_D²⁵ = +30.45 ($c=0.57$, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃): $\delta=0.84$ (d, $J=6.8$ Hz, 3H; Ala CH₃), 1.00 (d, $J=6.8$ Hz, 3H; CH₃), 1.04 (d, $J=6.3$ Hz, 3H; CH₃), 1.35–1.37 (2s, 18H; C(CH₃)₃), 2.39 (dd, $J=13.3$, 8.6 Hz, 1H; CH₂), 2.54–2.61 (m, 2H; CH₂, Tyr CH₂), 2.63–2.72 (m, 1H; CHCH₃), 2.75 (dd, $J=15.5$, 6.1 Hz, 1H; Tyr CH₂), 2.90–3.03 (m, 5H; NCH₃, CH₂), 3.12 (dd, $J=15.5$, 11.2 Hz, 1H; Trp CH₂), 3.47 (dd, $J=15.4$, 5.1 Hz, 1H; Trp CH₂), 3.66 (s, 3H; OCH₃), 3.70–3.76 (m, 1H; CHCH₃), 4.42–4.49 (m, 1H; Ala CH), 5.30–5.35 (m, 1H; Tyr CH), 5.58 (dd, $J=11.1$, 5.0 Hz, 1H; Trp CH), 6.75 (d, $J=8.6$ Hz, 2H; Tyr aryl H), 6.95 (s, 1H; Ind NHCH), 6.97 (s, 1H; xylyl H), 6.99–7.01 (m, 3H; Trp aryl H, xylyl H), 7.05 (t, $J=7.5$ Hz, 1H; Trp aryl H), 7.14 (t, $J=7.5$ Hz, 1H; xylyl H), 7.19 (d, $J=8.6$ Hz, 2H; Tyr aryl H), 7.29 (d, $J=8.1$ Hz, 1H; Trp aryl H), 7.53 (d, $J=7.8$ Hz, 1H; Trp aryl H), 8.06 (d, $J=8.3$ Hz, 2H; Tyr NH, Ala NH), 10.26 ppm (s, 1H; Ind NH); ¹³C NMR (100 MHz, CDCl₃): $\delta=16.1$ (Ala CH₃), 17.8 (CH₃), 20.9 (CH₃), 24.8 (Trp CH₂), 28.4, 28.9 (C(CH₃)₃), 32.0 (NCH₃), 40.9 (CH₂), 43.0 (CH₂), 43.1 (Tyr CH₂), 44.0 (CH₂), 47.3 (Ala CH), 51.3 (CHNBoc), 51.4 (Tyr CH), 55.8 (OCH₃), 58.7 (Trp CH), 79.8, 82.0 (C(CH₃)₃), 111.3 (Trp aryl C), 112.3 (Trp aryl CH), 114.9 (Tyr aryl CH), 119.3, 119.8, 122.5 (Trp aryl CH), 124.0 (Trp NHCH), 128.0, 128.4 (xylyl CH), 128.7 (Trp aryl C), 129.1 (Tyr aryl CH), 129.4, 131.3 (xylyl CH), 134.7 (Tyr aryl C), 138.1 (Trp aryl C), 140.5, 141.3 (xylyl C), 157.8 (Boc CO), 160.5 (Tyr aryl C-OMe), 171.7, 171.8, (Ala CO, Tyr CO), 176.2 (Trp CO), 179.0 ppm (CO); IR (film): $\tilde{\nu}=3309$, 2973, 1650, 1519, 1160 cm⁻¹; HRMS (EI): m/z : calcd for C₄₇H₆₃N₅O₈: 848.45689; found: 848.45733 [M +Na]⁺.

(3S,7R,10R,13S,16S)-10-(1H-Indol-3-ylmethyl)-7-(4-methoxyphenyl)-3,11,13,16-tetramethyl-4,8,11,14-tetraazabicyclo[16.3.1]docosa-

1(22),18,20-triene-5,9,12,15-tetrone (37): TFA (0.5 mL, 6.13 mmol) was added at 0°C to a solution of tetrapeptide **36** (0.025 g, 0.0303 mmol) in dry CH₂Cl₂ (0.5 mL). The reaction mixture was stirred at room temperature for 1.5 h, before the solvents were evaporated on the rotary evaporator. The crude amine salt was used in the next step without further purification.

To a solution of the crude amino acid salt (0.0303 mmol) in dry DMF (30 mL, 0.001 M) were added TBUTU (0.029 g, 0.090 mmol), HOBt (0.0122 g, 0.090 mmol), and *i*Pr₂NEt (0.020 mL, 0.121 mmol) followed by stirring of the mixture at room temperature for 14 h. The mixture was partitioned between ethyl acetate and water. The combined organic layers were washed with water, 5% aqueous KHSO₄ solution, water, half saturated NaHCO₃ solution, water, brine, dried over anhydrous MgSO₄, filtered, and concentrated. Purification of the residue by flash chromatography (dichloromethane/methanol 96:4) furnished the desired macrocycle **37** as a white powder (8.9 mg, 45%). M.p. 169.7–177°C (decomp); $R_f=0.41$ (dichloromethane/methanol 94:6); [α]_D²⁵ = 6.5 ($c=0.45$, DMSO); ¹H NMR (600 MHz, [D₆]DMSO): $\delta=0.60$ (d, $J=7.0$ Hz, 3H; Ala CH₃), 0.94 (d, $J=6.1$ Hz, 3H; C-5 CH₃), 1.04 (d, $J=7.0$ Hz, 3H; C-11 CH₃), 2.33 (dd, $J=12.7$, 9.2 Hz, 1H; 6-H), 2.37 (dd, $J=14.0$, 2.6 Hz, 1H; 10-H), 2.41–2.47 (m, 2H; 2-H), 2.49 (m, 1H; 11-H), 2.76 (dd, $J=13.1$, 4.4 Hz, 1H; 6-H), 2.92 (s, 3H; NMe), 2.96 (dd, $J=14.0$, 10.5 Hz, 1H; 10-H), 3.04 (dd, $J=14.9$, 11.4 Hz, 1H; 20-H), 3.13–3.17 (m, 1H; 20-H), 3.72 (s, 3H; OCH₃), 3.72–3.78 (m, 1H; 5-H), 4.56 (ddd, $J=13.8$, 6.8, 6.6 Hz, 1H; 14-H), 5.15–5.19 (m, 1H; 1-H), 5.49 (dd, $J=11.0$, 4.8 Hz, 1H; 17-H), 6.85–6.90 (m, 3H; 2'-H, 2'-H, 6''-H), 6.91–6.96 (m, 2H; 4''-H, 7'''-H), 6.99–7.00

(m, 1H; 2''-H), 7.02 (dd, $J=7.5$, 7.5 Hz, 1H; 8''-H), 7.08 (dd, $J=7.5$, 7.5 Hz, 1H; 5''-H), 7.18 (d, $J=8.8$ Hz, 1H; 3'-H), 7.29 (d, $J=7.9$ Hz, 1H; 9''-H), 7.56 (d, $J=7.9$ Hz, 1H; 6''-H), 7.63 (d, $J=7.0$ Hz, 1H; 4-NH), 7.86 (d, $J=7.9$ Hz, 1H; 13-NH), 8.43 (d, $J=7.9$ Hz, 1H; 19-NH), 10.77 ppm (brs, 1H; 1''-NH); ^{13}C NMR (150 MHz, $[\text{D}_6]\text{DMSO}$): $\delta=16.5$ (Ala CH_3), 18.9 (5 CH_3), 19.6 (11 CH_3), 24.3 (C-20), 30.0 (NCH $_3$), 38.4 (C-10), 41.1 (C-11), 41.7 (C-2), 42.0 (C-6), 44.6 (C-14), 46.2 (C-5), 48.7 (C-1), 54.8 (OCH $_3$), 55.1 (C-17), 110.9 (C-9''), 117.8 (C-7''), 117.9 (C-6''), 120.5 (C-8''), 122.7 ppm (C-8''); HRMS (ESI): m/z : calcd for $\text{C}_{38}\text{H}_{45}\text{N}_5\text{O}_5$: 674.33129; found: 674.33154 $[\text{M}+\text{Na}]^+$.

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