Tripodal Synthetic Peptide Bundles

Gunter Trojandt, Ulrike Herr, Kurt Polborn and Wolfgang Steglich*

Dedicated to Professor Wolfgang Beck on the occasion of his 65th birthday

Abstract: The stereochemical course of the formation of the two diastereomers of trimethyl 2,2′,2″-nitrilotris[2-(benzoylamino)acetate], **2a** and **2b**, is described. The structures of both isomers were confirmed by X-ray diffraction studies. Diastereomer **2b** could be obtained in larger quantities by epimerisation of **2a** to **2b** with catalytical amounts of NaOMe. The (*RRR*/*SSS*)-triester **2b** is a suitable template for the synthesis of tripodal peptide bundles. Saponification of **2b** yielded the C_3-symmetrical racemic triacid **4b**, which was coupled with amino acid methyl esters and dipeptide esters to give pseudo-hexapeptides and pseudo-nonapeptides, respectively. The resulting mixtures of diastereomers were easily separated by crystallisation. Their absolute configuration at the template unit (*RRR or SSS*) was established by means of the CD spectra. The pseudo-hexapeptide (*SSS*)-N(BzGly*ValOMe)$_3$ (**14**) was saponified to yield the optically pure triacid (*SSS*)-N(BzGly*ValOH)$_3$ (**23**). Compound **23** is an ideally preorganised template for the production of longer tripodal peptides. This was illustrated by the synthesis of two pseudo-pentadecapeptides. Peptide bundles with polar side chains (histidine and serine) or end groups (catechol or hydroxamate units) were synthesised by using the templates **4b**, **22** and **23** as anchors.

Keywords
peptide bundles · peptides · pseudo-peptides · template synthesis · tripodal ligands

Introduction

In the last decade, organic chemists have been increasingly inspired by the concept of biomimetic chemistry[1] and have tried to imitate the remarkable features of biological systems with synthetic compounds. The rational design and synthesis of novel molecular devices with defined structural and functional features is the ambitious aim of numerous research groups and has provided a large variety of molecules with outstanding characteristics.[2] In recent years, several systems have been built up from amino acids, which function as receptors,[3] transmembrane channels[4] or molecular switches.[5] Libman and Shanzer[6] have described a new class of tripodal compounds in which three peptide strands are attached to a common C_3-symmetric anchor ending with catechol or hydroxamate units. Such peptide derivatives, stabilised by intramolecular hydrogen bonds, are capable of binding ferric ions with very high formation constants. They are of particular interest as model compounds for metalloenzymes[7] and as synthetic analogues of natural siderophores.[8]

As a part of our continuing investigations on electrophilic glycine equivalents, we recently described the synthesis of novel C_3-symmetric peptide derivatives with three peptide chains connected by a nitrogen atom.[9] A particular feature of these molecules is their uniform basic structure with three parallel peptide chains stabilised by interstrand hydrogen bonds. Hence, these compounds are small synthetic peptides with a defined three-dimensional structure.

Here, we describe the preparation and structural properties of peptide bundles of this type. In addition, amino acids with polar side chains or end groups have been incorporated into the peptides, in order to make them suitable for the coordination of biologically important metal ions (e.g. Fe^{3+} or Zn^{2+}). The coordination behaviour of the ligands is either already published[10] or under active investigation.

Results and Discussion

Strategy: Our strategy for building up derivatives with three covalently linked peptide chains is depicted in Scheme 1. The synthesis was accomplished by a stepwise procedure: a) preparation of trimethyl 2,2′,2″-nitrilotris[2-(benzoylamino)acetate][9] from methyl *N*-benzoyl-2-bromoglycinate[11] and ammonia, b) deprotection of the *C*-terminus and c) coupling of the racemic template[12] with amino acid or peptide esters.

Template Synthesis and Stereochemistry: Dropwise addition of a NH_3/THF solution to a mixture of bromoglycinate **1** and triethylamine in THF afforded N(BzGly*OMe)$_3$ (**2**) in 78% yield (Scheme 2). After standard workup the crude product was

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[+] X-ray structure investigations

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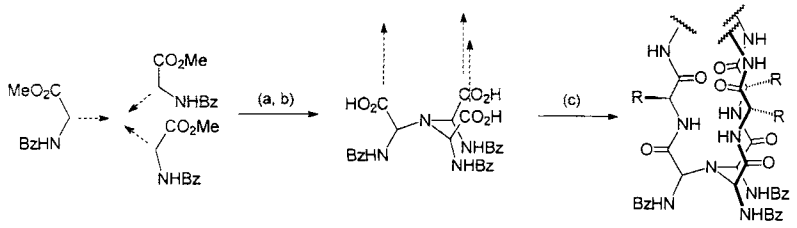
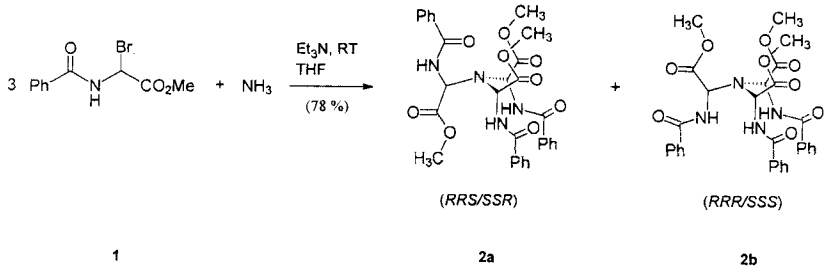


Scheme 1. Strategy used for synthesis of peptide bundles.



Scheme 2. Synthesis of pseudo amino acids **2**.

product was diluted with methanol. The (*RRS*/*SSR*) pair of enantiomers **2a** (68%) precipitated immediately and was filtered off. The (*RRR*/*SSS*) pair **2b** (10%) crystallised from the mother liquor by standing overnight.

The relative stereochemistries in (*RRS*/*SSR*)-**2a** and (*RRR*/*SSS*)-**2b** were determined from the crystal structures (Figure 1).[9] In the symmetrical isomer **2b**, the three α-substituted glycine residues are of the same absolute configuration and are aligned parallel. The structure is stabilised by three intramolecular hydrogen bonds between the helically arranged benzoylamino groups. The central nitrogen atom lies 0.219 Å above the plane defined by the three α-carbon atoms of the glycine residues.

The C_3 symmetry of **2b** found in the solid state is also reflected in the ^{1}H NMR spectrum. As expected, only one set of signals is observed for the three α-substituted glycine residues. The signal of the amide NH protons of **2b** is found downfield ($\delta = 8.38$ in $CDCl_3$) of the corresponding signal for the single-chain reference molecule **3** ($\delta = 6.85$ in $CDCl_3$) (Figure 2).[13] The fact that this downfield shift is preserved upon dilution suggests that the stabilisation of the pseudo amino acid **2b** is maintained in solution.

In the unsymmetrical isomer **2a**, one of the three α-substituted glycine residues shows the opposite configuration and is aligned antiparallel to the others. The structure is stabilised by three intramolecular hydrogen bonds: one between a pair of benzoylamino groups and two further intrachain hydrogen bonds between the amide NH and the ester carbonyl groups of the glycine residues. The central nitrogen atom lies 0.093 Å over the plane defined by the three α-carbon atoms of the glycine residues. The ^{1}H NMR spectrum shows a double set of signals in a ratio of 1:2. The resonance of one methyl ester is shifted to higher field ($\Delta\delta = 0.48$) compared to the signal of the

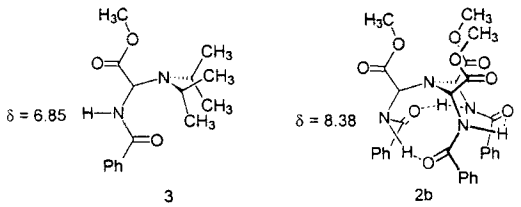


Figure 2. Downfield shift of amide NH signal of **2b** relative to the corresponding signal in **3**.

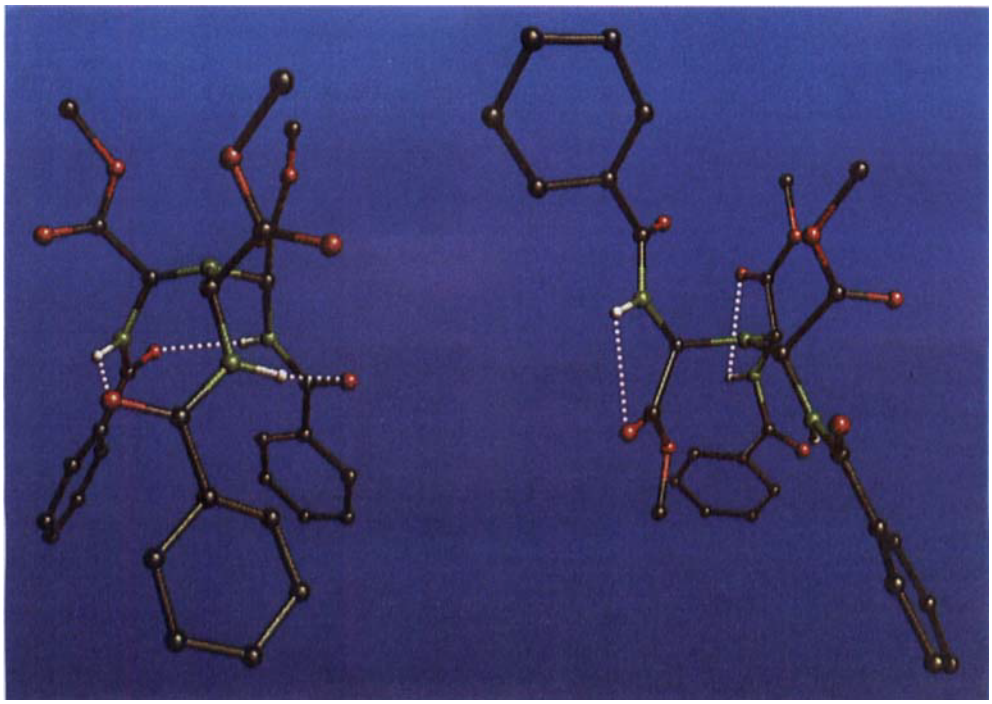
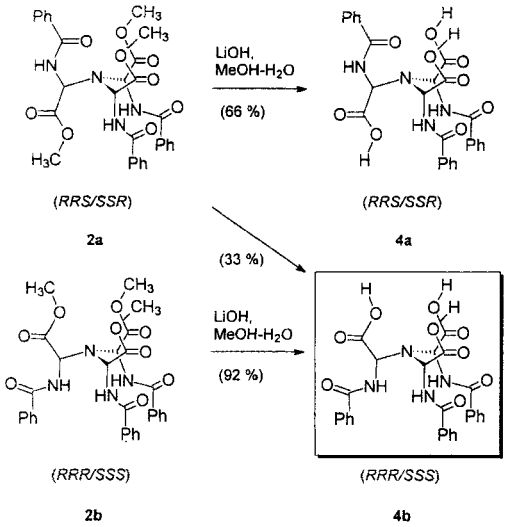


Figure 1. Crystal structure of **2a** (right) and **2b** (left).

other two. This observation is consistent with a structure in the solid state in which one methyl ester is positioned near the shielding face of an aromatic ring (Figure 1). Further information about the structure in solution was obtained from NOE studies. In the unsymmetrical isomer **2a**, a NOE cross-peak between the *ortho* protons of one aromatic ring and two methyl ester groups supports the conformation found in the solid state. The data recorded suggest that the conformations of (*RRR*/*SSS*)-**2b** and (*RRS*/*SSR*)-**2a** in solution correspond to those in the solid state.

The racemic template **4b** [(*RRR*/*SSS*)-N(BzGly*OH)$_3$] was synthesised in 92% yield

by saponification of (*RRR*/*SSS*)-N(BzGly*OMe)$_3$ (**2b**) with lithium hydroxide (Scheme 3).[14] Surprisingly, upon hydrolysis of (*RRS*/*SSR*)-**2a**, a mixture of (*RRS*/*SSR*)-**4a** and (*RRR*/*SSS*)-**4b** was obtained in a ratio 2:1, which can be rationalised by a partial isomerisation during saponification. This is a crucial observation since the symmetrical triacid **4b** is required on a large scale.



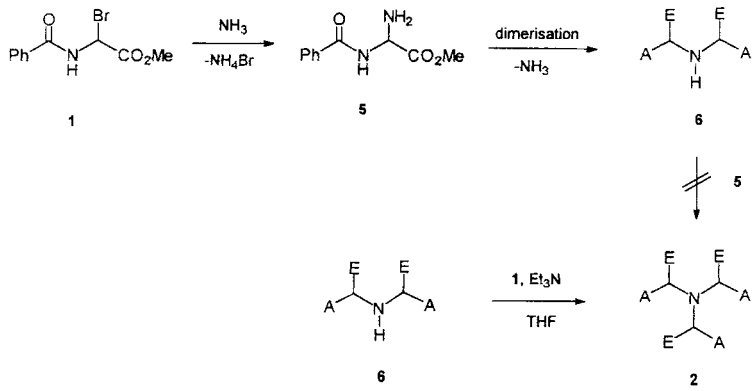
Scheme 3. Preparation of symmetrical triacid **4b**.

Stereochemical Course of the Template Synthesis: The template synthesis was studied in further detail. As previously stated the reaction of bromoester **1** with ammonia afforded 68% (*RRS*/*SSR*)-**2a** and 10% (*RRR*/*SSS*)-**2b**. However, HPLC analysis[15] of the crude reaction mixture yielded a ratio of 5:1 for (*RRS*/*SSR*)-**2a** and (*RRR*/*SSS*)-**2b**.

This ratio can be understood by comparing the possible configurations of the different isomers. The isomers with the configuration (*RRS*), (*RSR*) and (*SRR*) are identical as well as the corresponding mirror images (*SSR*), (*SRS*) and (*RSS*). Consequently, (*RRS*/*SSR*)-**2a** is formed three times more often than (*RRR*/*SSS*)-**2b** in the reaction. Although a 3:1 mixture of (*RRS*/*SSR*)-**2a** and (*RRR*/*SSS*)-**2b** is expected, the experimentally observed ratio is 5:1. This observation implies that one or more steps of the template synthesis are stereochemically controlled. Intrigued by this fact, we undertook further experiments in order to explain the stereochemical outcome of the formation of **2**.

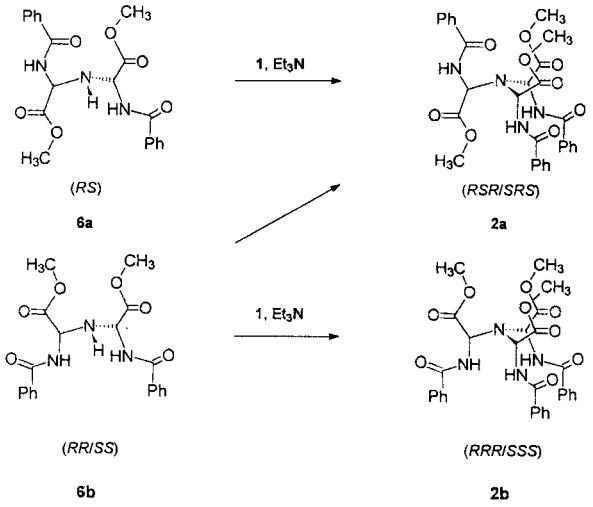
Easton et al.[16] have reported that a 1:1 mixture of the secondary amines (*RS*)-**6a** and (*RR*/*SS*)-**6b** was formed by passing a stream of ammonia through a solution of **1** in dichloromethane. Neither (*RRS*/*SSR*)-**2a** nor (*RRR*/*SSS*)-**2b** could be detected.[16] This result can be explained by a rapid conversion of the α-bromo derivative **1** into the α-amino derivative **5** by excess ammonia, followed by dimerisation of **5** to the secondary amines **6** (Scheme 4). Further reaction of **6** to **2** does not occur because the α-aminoglycine derivative **5** is too unreactive for further alkylation—in contrast to **1**.

As a part of our investigation we prepared (*RS*)-**6a** and (*RR*/*SS*)-**6b** according to the literature procedure.[16] Both isomers



Scheme 4. Dimerisation of **1** by reaction with ammonia (E = CO_2Me, A = NHBz).

were separately dissolved in THF and then treated with an excess of **1** (Scheme 5). It can be concluded from the ^{1}H NMR spectra of the crude product that the reaction of (*RS*)-**6a** with



Scheme 5. Reactions of (*RS*)-**6a** and (*RR*/*SS*)-**6b** with **1**.

the bromo compound **1** leads to (*RRS*/*SSR*)-**2a**, exclusively. Interestingly, treatment of (*RR*/*SS*)-**6b** with **1** affords a 2:1 mixture of (*RRS*/*SSR*)-**2a** and (*RRR*/*SSS*)-**2b**.

The mechanism of the one-pot synthesis of [N(BzGly*OMe)$_3$] (**2**) can be rationalised as outlined in Scheme 6:

a) α-Bromo derivative **1** is converted into the acylimine **7** by triethylamine.
b) Reaction of ammonia with **7** gives a 1:1 mixture of the enantiomers **5a** and **5b**.
c) Both isomers react with further acylimine **7** to give the secondary amines (*RS*)-**6a** and (*RR*/*SS*)-**6b** without stereochemical control (1:1 mixture).
d) The reaction of (*RS*)-**6a** with **7** leads only to (*RRS*/*SSR*)-**2a**, but (*RR*/*SS*)-**6b** affords a 1:2 mixture of (*RRR*/*SSS*)-**2b** and (*RRS*/*SSR*)-**2a**.

This reaction course is consistent with the ratio of 5:1 found experimentally.[15]

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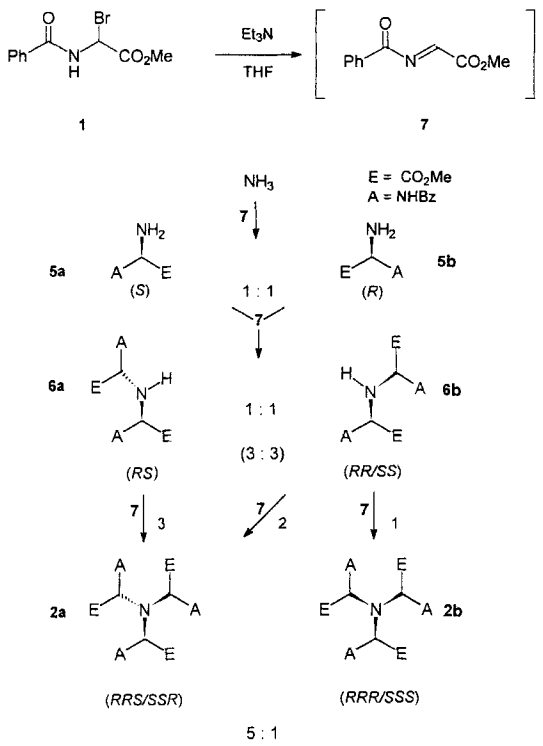
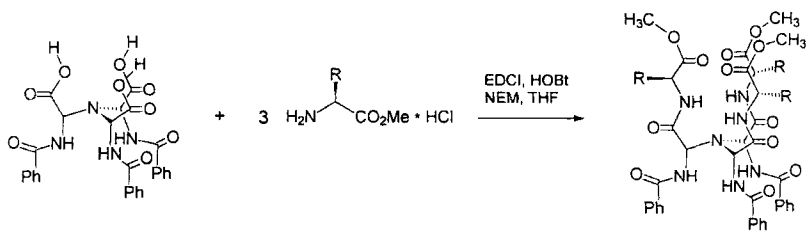


Scheme 6. Reaction course explaining the ratio of 5:1 for (*RRS*/*SSR*)-**2a** and (*RRR*/*SSS*)-**2b**.



Scheme 7. Synthesis of peptide bundles **12**–**15** (EDCI = *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride; HOBt = 1-hydroxybenzotriazole; NEM = *N*-ethylmorpholine).

Table 1. Synthesis of peptide bundles **12**–**15**.

Amino acid	Yield (%) [a]	Compound
Gly	81	**12**
Ala	76	**13**
Val	81	**14**
Ser	72	**15**

[a] Yield of diastereomeric mixture.

Epimerisation of Esters 2a and 2b: As previously mentioned, isomerisation and enrichment of the symmetric triacid **4b** [(*RRR*/*SSS*)-N(BzGly*OH)$_3$] is observed during saponification of (*RRS*/*SSR*)-N(BzGly*OMe)$_3$ (**2a**) with aqueous LiOH. Thus, treatment of (*RRS*/*SSR*)-**2a** with a catalytic amount of NaOMe in methanol should yield the symmetrical triester **2b** [(*RRR*/*SSS*)-N(BzGly*OMe)$_3$] by epimerisation accompanied by repeated transesterfication. Indeed, upon treating a solution of (*RRS*/*SSR*)-**2a** in THF/MeOH [(*RRS*/*SSR*)-**2a** is insoluble in MeOH] with 15 mol% of NaOMe, a mixture of (*RRS*/*SSR*)-**2a** and (*RRR*/*SSS*)-**2b** in a ratio of 2:1 was isolated after 24 h of stirring.[15] The same ratio was obtained from the symmetric triester (*RRR*/*SSS*)-**2b**. These results indicate that a thermodynamic equilibration had taken place.

The two diastereomers are easily separated by HPLC,[15] but we have not yet found a suitable method for the separation on a large scale. Separation of (*RRR*/*SSS*)-**2b** and (RRS/SSR)-**2a** by crystallisation has not yet been achieved.

The ratio of 2:1 (**2a**:**2b**) was inversed to 1:2 (**4a**:**4b**) upon hydrolysis of the crude product with lithium hydroxide. Thus, epimerisation of the unsymmetric triester (*RRS*/*SSR*)-**2a** followed by hydrolysis yielded a reaction mixture which contained 62% of the symmetric triacid (*RRR*/*SSS*)-**4b**. This mixture could not be separated. However, it could be used as starting material for the preparation of the pure pseudo-hexapeptides **12** and **14** on a larger scale (see Experimental Section).

Synthesis of Peptide Bundles and of Optically Active Templates: Pseudo-hexapeptides **12**–**15** were obtained by coupling of (*RRR*/*SSS*)-N(BzGly*OH)$_3$ (**4b**) with L-amino acid methyl esters **8**–**11** (EDCI/HOBt)[17] (Scheme 7, Table 1). The resulting peptides were isolated as 1:1 mixtures of diastereomers varying in the configuration of the template unit [(*RRR*) or (*SSS*)]. The separation of the highly crystalline (*SSS*)-pseudo-hexapeptides was easily achieved by recrystallisation from methanol.

The stereochemistry of the glycine adduct **12** was determined by means of its X-ray crystal structure (Figure 3). Three covalently linked peptide chains are aligned in a parallel fashion. The structure is stabilised by six intramolecular hydrogen bonds: three between the helically arranged benzoylamino groups, which force the peptide chains in one direction and three between the amide NH and ester carbonyl groups.

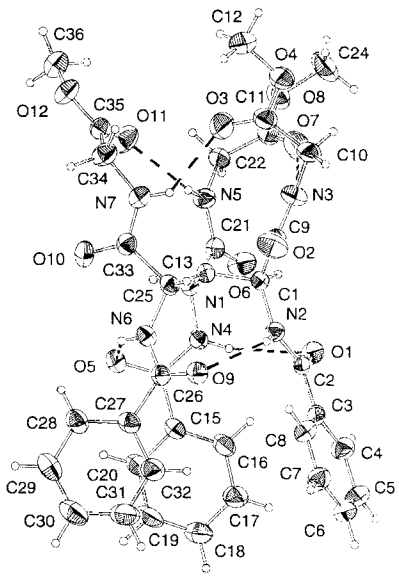


Figure 3. Crystal structure of **12**.

In the crystal we chose for the structure determination, only one single isomer could be observed. This suggests that the pseudo-hexapeptide **12** crystallises as a conglomerate.[18] To confirm these results we dissolved several single crystals of the conglomerate separately in CH_3CN and analysed the solutions by CD spectroscopy. As expected, the solutions show split CD curves[19] with positive or negative signs obviously due to the

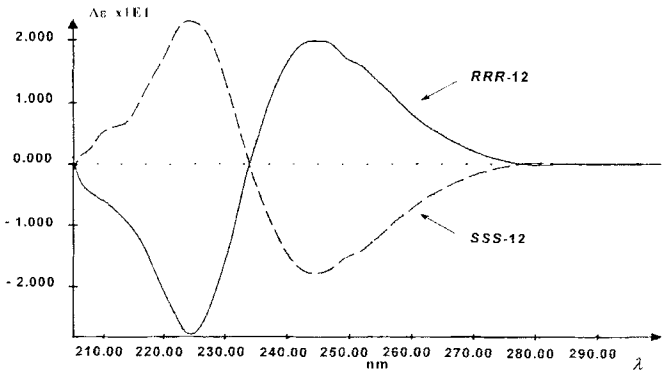


Figure 4. CD spectrum of the two enantiomers of **12** in CH_3CN.

spatial interaction of the helically arranged benzoylamino groups (Figure 4).

The absolute configuration at the template unit [(*RRR*) or (*SSS*)] and the sense of orientation of the benzoylamino groups as right- or left-handed helices—can be determined by means of the CD spectra. According to the X-ray crystal structures of (*SSS*)-**14**[20] and (*SSS*)-**15**[21] depicted in Figures 5 and 6, the three peptide chains are aligned in form of a left-handed helix.

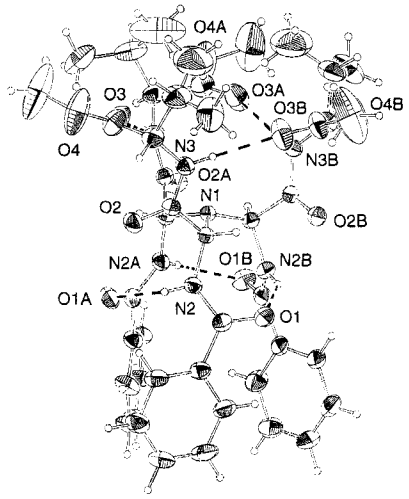


Figure 5. Crystal structure of **14**.

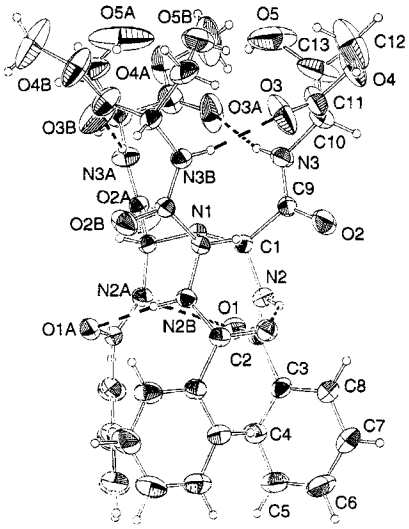


Figure 6. Crystal structure of **15**.

Solutions of (*SSS*)-**14** and (*SSS*)-**15** show split CD curves with negative sign (Figures 7 and 8). This suggests the following rule: pseudo-peptides of type **12**–**15** with a right-handed helical

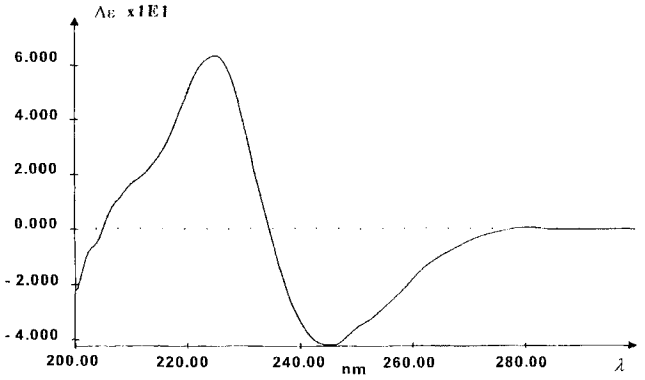


Figure 7. CD spectrum of **14**.

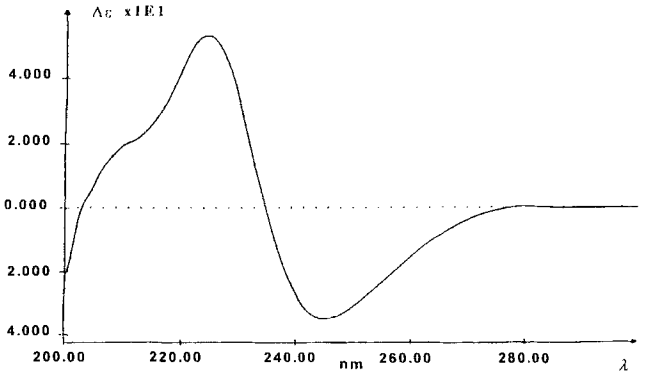
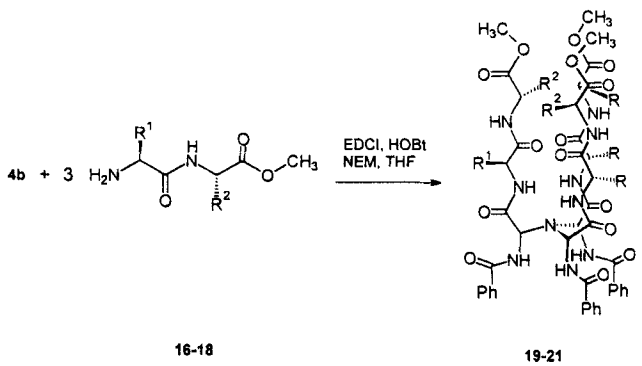


Figure 8. CD spectrum of **15**.

structure [(*RRR*) configuration at the template unit] exhibit a split CD curve with positive sign; pseudo-peptides with a left-handed helical structure [(*SSS*) configuration at the template unit] show a split CD curve with negative sign. This is in accord with the dibenzoate rule of Nakanishi.[19]

In order to verify this rule the racemic template **4b** was coupled with several dipeptide esters (Scheme 8). After standard workup, the 1:1 mixtures of diastereomers **19**–**21** were separated by fractional crystallisation (Table 2).



Scheme 8. Synthesis of pseudo-nonapeptides **19**–**21** (for abbreviations, see Scheme 7).

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Table 2. Synthesis of pseudo-nonapeptides **19**–**21**.

R^1	R^2	aa^1	aa^2	Yield (%)	Compound
*i*Pr	Bn	Leu	Phe	40	**19**
*i*Pr	H	Leu	Gly	58	**20**
H	*i*Pr	Gly	Leu	74	**21**

According to the X-ray structure determination of the crystalline isomer of **21**, the three peptide chains adopt a C_3-symmetrical helical structure (Figure 9). The structure is stabilised

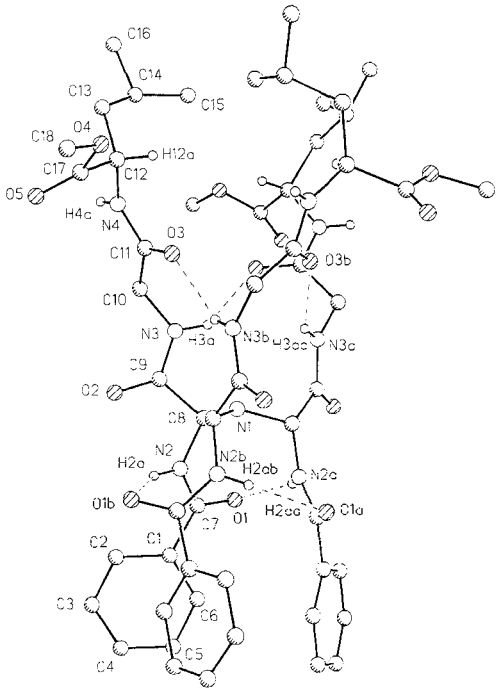


Figure 9. Crystal structure of **21**.

by six intramolecular hydrogen bonds: as expected, three between the benzoylamino groups and three between the amide NH and amide carbonyl groups. The X-ray structural analysis also revealed the stereochemistry at the template unit to be (*RRR*). This result agrees with the configuration determined by CD spectroscopy (positive sign) (Figure 10).

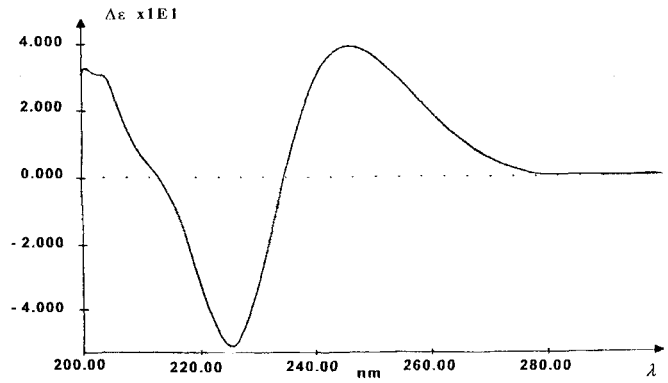


Figure 10. CD spectrum of **21**.

In summary, the symmetric triacid N(BzGly*OH)$_3$ (**4b**) was found to be a suitable template for the formation of peptide bundles up to pseudo-nonapeptides. The absolute configuration of these derivatives can be determined by recording their CD spectra.

The pseudo-hexapeptides (*RRR*/*SSS*)-N(BzGly*GlyOMe)$_3$ (**12**) and (*SSS*)-N(BzGly*ValOMe)$_3$ (**14**) are available in 81% yield from **4b** or from the 1:2 mixture of the two isomeric triacids **4a** and **4b** on a larger scale. The X-ray crystal structures of **12** and **14** suggest that both molecules are stabilised by six intramolecular hydrogen bonds (Figures 3 and 5). Comparison of the ^{1}H NMR chemical shifts of the NH groups of **12** and **14** as a function of temperature provides further evidence for intramolecular hydrogen bonding (Figure 11). For the ^{1}H NMR chemical shifts of the N*H*-Gly and N*H*-Val protons, we

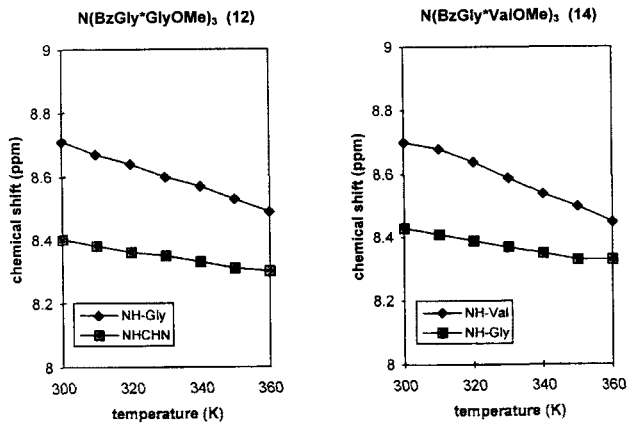
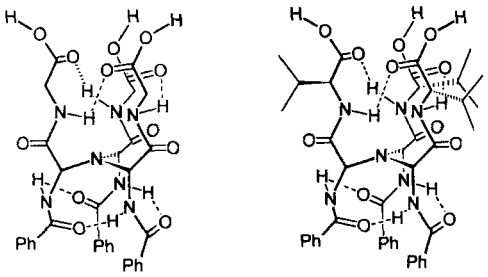


Figure 11. ^{1}H NMR chemical shifts of the NH groups of **12** and **14** as a function of temperature (5.0 mM, [D$_6$]DMSO).

observed temperature gradients ($\Delta\delta/T$) between 3.5 to 4.5×10^{-3} ppm K^{-1}. From these data the final orientation of the hydrogen bonds cannot be determined. The corresponding ^{1}H NMR chemical shifts of the N*H*CHN protons, however, are fairly constant over a range of 300 to 360 K ($< 2 \times 10^{-3}$ ppm K^{-1}). It can be concluded that they are shielded from the solvent and are involved in intramolecular hydrogen bonding.[22] Pseudo-hexapeptides **12** and **14** are therefore strongly stabilised and preorganised peptide bundles.

By saponification of **12** and **14** with LiOH in THF/H$_2$O, two new template molecules (*RRR*/*SSS*)-N(BzGly*GlyOH)$_3$ (**22**) (95% yield) and (*SSS*)-N(BzGly*ValOH)$_3$ (**23**) (93% yield) are accessible. The stereochemistry of **22** was confirmed by a single-crystal X-ray diffraction study (Figure 12).[23] As expected, the



22 **23**

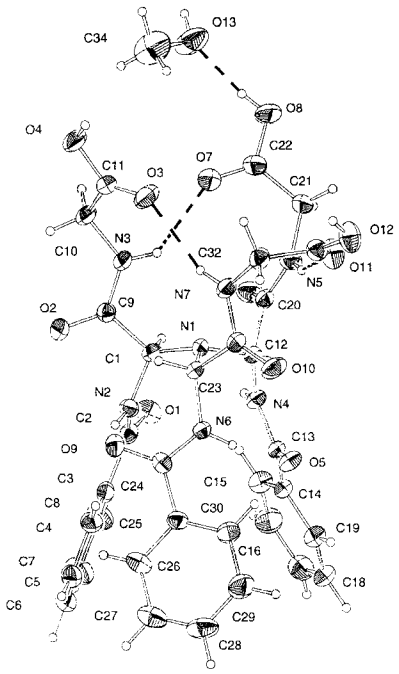


Figure 12. Crystal structure of **22**.

three peptide chains are aligned parallel and are stabilised by six intramolecular hydrogen bonds. There is one further intermolecular hydrogen bond between a carboxyl OH group and a methanol molecule present in the crystal, as a result of which the perfect C_3-symmetric arrangement of the peptide chains is lost.

(*SSS*)-N(BzGly*ValOH)$_3$ {$[\alpha]_D^{25} = +18$}} (**23**) is available in high purity by saponification of (*SSS*)-**14** as shown by the ^{1}H NMR spectrum of the crude reaction product (Figure 13).

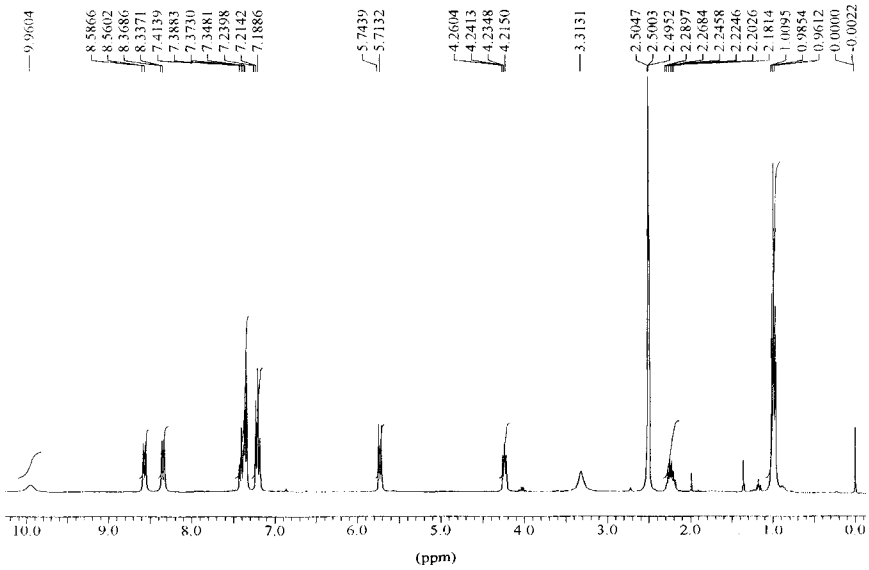
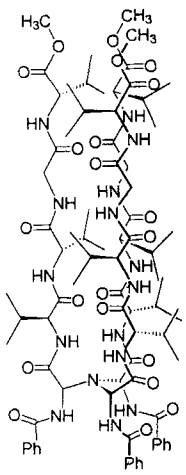


Figure 13. ^{1}H NMR spectrum ([D$_6$]DMSO) of the crude reaction product obtained by saponification of **14**.

Pseudo-hexapeptide **23** should be an ideal template for building up longer peptide bundles and derivatives with functionalised side chains or end groups. This was illustrated by the synthesis of the pseudo-pentadecapeptides **24** and **25**, which was achieved



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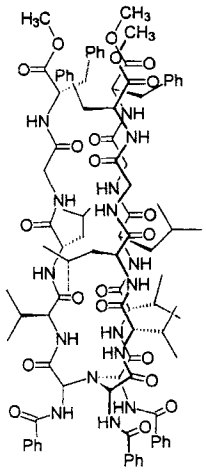
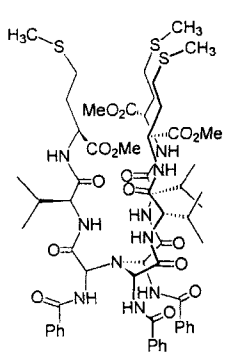


25

by coupling **23** with H-Val-Gly-ValOMe and H-Leu-Gly-PheOMe in 86% and 80% yield, respectively.

Peptide Bundles with Polar Side Chains or End Groups: Peptide bundles deprotected at the *C*-terminus have proven to be ideally preorganised for the formation of macrobicyclic metal complexes.[10] In order to design novel synthetic siderophores[6, 7, 24] or model compounds for metalloenzymes,[7] we incorporated amino acids with polar side chains in our bundles making them suitable for the coordination of biologically important metal ions.

Pseudo-peptides **26**–**28** are available by coupling the template (*SSS*)-**23** with H-Met-OMe, H-Phe-Ser-OMe or H-Trp-



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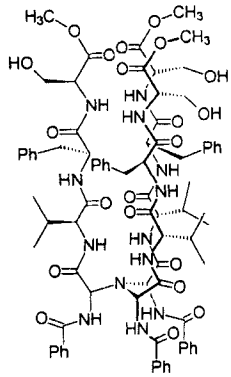

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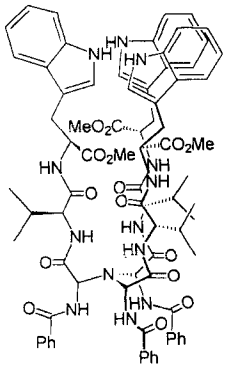

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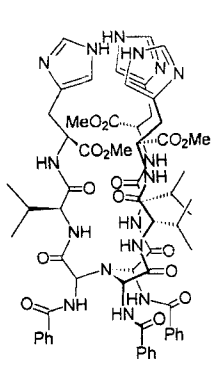

29

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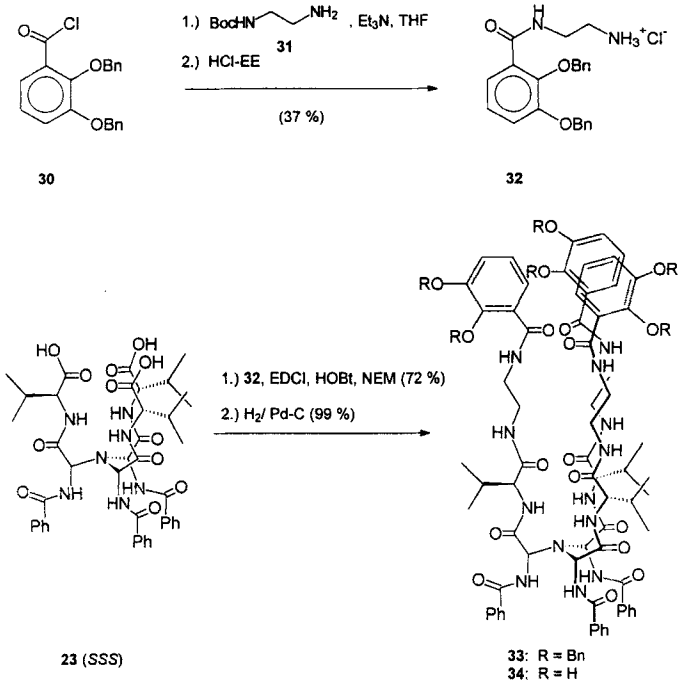
OMe, respectively [EDCI/HOBt].[17] The synthesis of peptide **29** required the use of a side chain protected histidine ester, which was obtained by deprotecting commercially available Boc-His(π-Bom)OMe · HCl with HCl in ethyl acetate[25a] and coupling H-His(π-Bom)OMe with template (*SSS*)-**23**. The cleavage of the Bom groups to generate the free imidazole groups was achieved by hydrogenolysis in 80% acetic acid[25b,c] to give **29** in 53% yield. The unique structural features of **29** with three C_3-symmetrically arranged histidine residues make this ligand particularly attractive for coordination studies with metal ions, especially with Zn^{2+} since several enzymes exploit the spatial proximity of three histidine imidazole side chains for the coordination of one Zn^{2+} ion.[26] Details of these investigations will be described separately.

For the preparation of artificial analogues of siderophores like enterobactin[6,8] or desferrioxamin,[6c,27] template **23** was coupled with catechol and hydroxylamine derivatives. The synthesis of a peptide bundle with three catechol residues was accomplished as follows (Scheme 9): a) Coupling of mono-Boc-

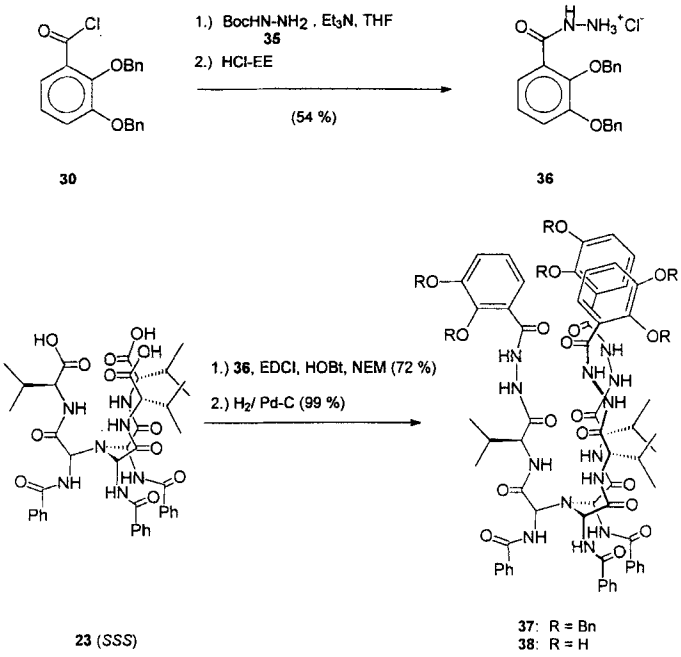


Scheme 9. Preparation of catechol ligand **34**.

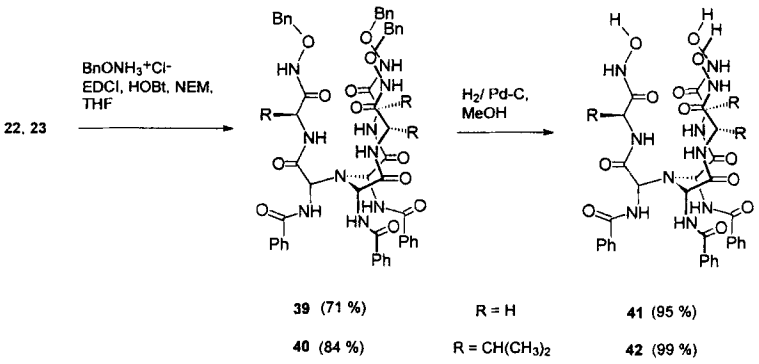
ethylenediamine (**31**)[28] with acid chloride **30**,[24] b) cleavage of the Boc group with HCl in ethyl acetate[25b] and c) coupling of amine **32** with template (*SSS*)-**23**. The ligand **34** was obtained by hydrogenation[25] of the benzyl-protected derivative **33**. The air-sensitive ligand **34** was characterised by ^1H and ^{13}C NMR spectroscopy and FAB MS.

To create a catechol ligand with a more rigid structure, **38** was synthesised from **30** and mono-Boc-hydrazine (**35**) according to the same procedure (Scheme 10). The air-sensitive ligand **38** was characterised by ^1H NMR spectroscopy and FAB MS.

Ligands **41** and **42** were prepared analogously by coupling template (*RRR*/*SSS*)-**22** and (*SSS*)-**23**, respectively, with *O*-benzylhydroxylamine and removing the benzyl groups by hydrogenation (Scheme 11).



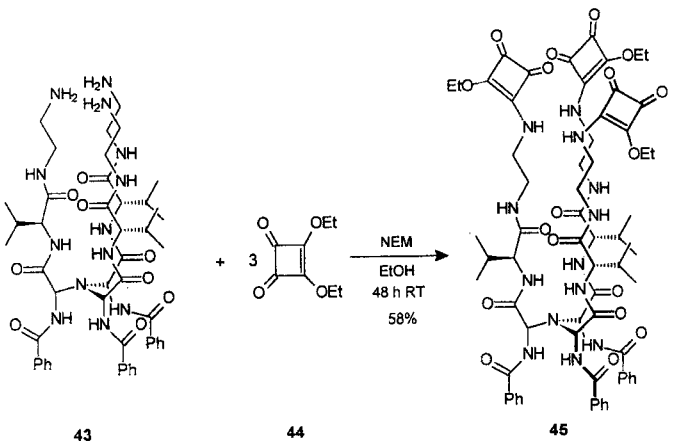
Scheme 10. Preparation of catechol ligand **38**.



Scheme 11. Preparation of hydroxamic acid ligands **41** and **42**.

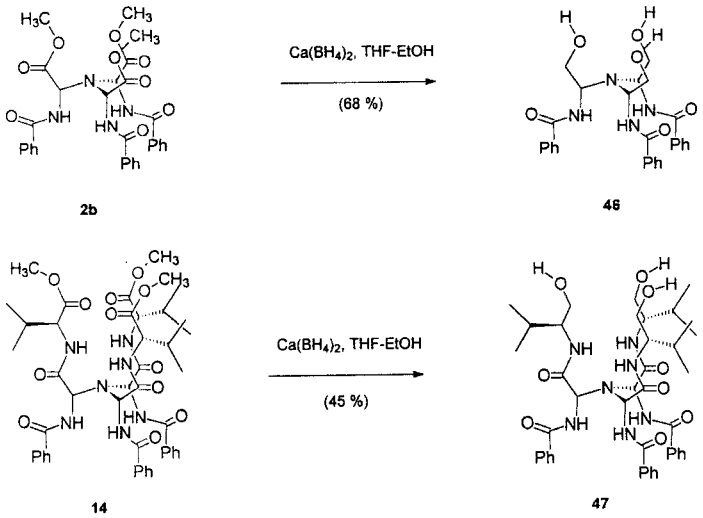
So far, we have shown that peptide bundles with well-defined metal binding sites can be obtained by coupling amines with template molecules activated at the *C*-terminus. In contrast, template **43**, which is accessible in a two-step procedure from (*SSS*)-**23** and **31**, allows coupling with *C*-activated derivatives or electrophiles. This is demonstrated by the synthesis of a peptide bundle with three chains ending with squaric acid derivatives as depicted in Scheme 12. This compound could be useful as receptor for the recognition of onium compounds.[29]

Synthesis of Tripodal Alcohols: Chiral C_2-symmetric alcohols have found wide application as catalyst ligands in asymmetric synthesis.[30] It is possible that the stereodifferentiation might be increased by using C_3-symmetric triols as ligands for the synthesis of catalysts, since the stereochemical information is concentrated into 120° compared to 180° for C_2-symmetric diols.[31] The reduction of the pseudo amino acid **2b** and the pseudohexapeptide **14** with $Ca(BH_4)_2$[32] allowed a straightforward access to the racemic trialcohol **46** and the optically active triol **47**, respectively (Scheme 13). The structure of **46**, confirmed by single-crystal X-ray diffraction study (Figure 14),[33] reveals



43 44 45

Scheme 12. Coupling of nucleophilic template **43** with electrophile **44**.



2b 46 14 47

Scheme 13. Synthesis of tripodal alcohol **46** and **47**.

that the three modified glycine residues are of the same absolute configuration and aligned parallel. The structure is stabilised by intramolecular hydrogen bonds: three between the helically arranged benzoylamino groups and one between the central nitrogen atom and a hydroxyl group. In addition, two molecules are connected by intermolecular hydrogen bonds as a result of which the expected C_3-symmetric orientation is disturbed. The close spatial proximity of the hydroxyl groups in **46** suggests an ideal preorganisation for the coordination of metal ions. Work aimed at complexing **46** and **47** with early transition metal ions is in progress.

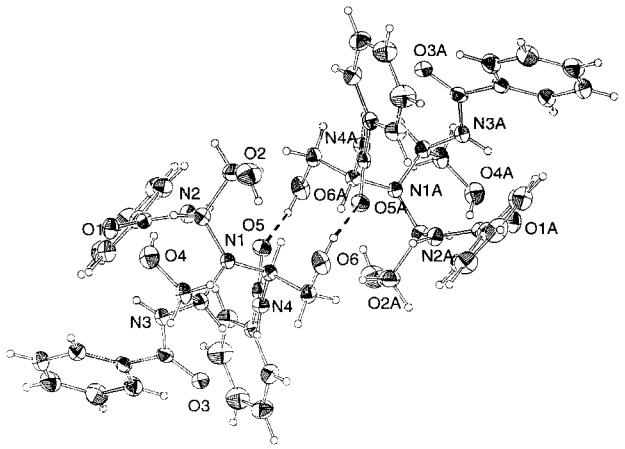


Figure 14. Crystal structure of **46**.

Conclusion

In this paper, we have shown that electrophilic glycine equivalents can be used to synthesise C_3-symmetric pseudo amino acids, which are suitable templates for the formation of peptide bundles of defined geometry. The stereochemistry of these derivatives can be easily assigned by CD spectroscopy. The incorporation of amino acids with polar side chains (histidine, tryptophan and serine) or end groups (catechol or hydroxamate units) leads to a novel series of ligands which are of particular interest as model substances for metalloenzymes and as synthetic analogues of siderophores. The coordination behaviour of these peptide bundles with biologically important metal ions is under active investigation.

Experimental Section

THF was distilled from potassium under an atmosphere of dry argon. All common reagents and solvents were obtained from commercial suppliers and used without further purification unless otherwise stated. TLC was performed on aluminium-backed plates coated with silica gel 60 with F_{254} indicator. Column chromatography was carried out using silica gel 60 (Merck, 230-400 mesh). Optical rotations were determined with a Perkin-Elmer 241 polarimeter using a Na lamp; data are reported as follows: $[\alpha]_D^{25}$ (concentration in g per 100 mL solvent). CD spectra were collected on a Jobin Yvon CD 6 spectropolarimeter. Melting points were determined with a Reichert Thermovar apparatus and a Büchi 350 and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1420 spectrometer and a Bruker IFS 45 FT-IR spectrometer. NMR spectra were measured on a Bruker ARX300 at 300 MHz (^{1}H) or 75.44 MHz (^{13}C). All chemical shifts were recorded in ppm downfield from TMS on the δ scale. Mass spectra were recorded on a Finnigan MAT90 and a Finnigan MAT95Q (high-resolution mass spectra). Elemental analyses were performed by the University of Munich Microanalytical Laboratory. X-Ray crystallographic analyses were carried out as described in the appropriate compound characterisation sections.

Trimethyl 2,2′,2″-nitrilotris[2-(benzoylamino)acetate] (2): To a vigorously stirred solution of methyl *N*-benzoyl-2-bromoglycinate (91.4 g, 336 mmol) in dry THF (600 mL) was added triethylamine (60.9 mL, 439 mmol) and a freshly prepared solution of ammonia in dry THF (67.2 mmol) [34] at room temperature. Stirring was continued for 24 h at ambient temperature. Ethyl acetate (350 mL) was added, and the solution was washed with 2N HCl (250 mL), saturated $NaHCO_3$ (250 mL) and brine (300 mL), dried ($MgSO_4$), filtered, and then evaporated under reduced pressure. After addition of methanol (230 mL), the unsymmetrical isomer **2a** crystallised immediately. The mixture was left for 10 min at room temperature before **2a** was filtered off. The symmetrical isomer **2b** was obtained from the mother liquor on standing overnight.

(*RRS*/*SSR*)-**2a**: Yield 27.0 g (68%) as colourless crystals: M.p. 200°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.41 (s, 3H, OCH_3), 3.74 (s, 6H, OCH_3), 5.73 (d, J = 6 Hz, 2H, NHC*H*N), 5.75 (d, J = 7 Hz, 1H, NHC*H*N), 7.44–7.59 (m, 9H, Ph), 7.62 (d, J = 7 Hz, 1H, N*H*CHN), 7.88 (d, J = 8 Hz, 2H, *o*-Ph), 8.05 (d, J = 8 Hz, 4H, *o*-Ph), 8.86 (d, J = 6 Hz, 2H, N*H*CHN); ^{13}C NMR (75.44 MHz, $CDCl_3$): δ = 53.12, 53.17, 62.08, 63.55, 127.26, 127.54, 128.65, 128.75, 132.18, 132.95, 133.29, 166.86, 167.74, 168.76, 169.30; IR (KBr): $\tilde{\nu}$ = 3410, 3360, 3256, 3060, 3040, 2950, 1755, 1680, 1649, 1603 cm^{-1}; FAB MS (NBA); *m*/*z* (%): 591.2 (0.75) [M^+ + H]; $C_{30}H_{30}N_4O_9$ (590.59): calcd C 61.01, H 5.11, N 9.48; found C 61.18, H 5.13, N 9.38.

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(*RRR*/*SSS*)-**2b**: Yield 4.0 g (10%) as colourless crystals. M.p. 204 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.89 (s, 9H, OCH_3), 6.05 (d, J = 10 Hz, 3H, NHC*H*N), 7.17 (dd, J = 7, 7 Hz, 6H, *m*-Ph), 7.35 (m, 9H, Ph), 8.37 (d, J = 9.6 Hz, 3H, N*H*CHN); ^{13}C NMR (75.44 MHz, $CDCl_3$): δ = 53.16, 61.84, 127.40, 128.16, 131.83, 1132.61, 167.41, 168.71; IR (KBr): $\tilde{\nu}$ = 3420, 3363, 2950, 1748, 1649, 1602, 1580, 1525, 1489 cm^{-1}; FAB MS (NBA); *m*/*z* (%): 613 (0.55) [M^+ + Na], 591.2 (0.75) [M^+ + H]; $C_{30}H_{30}N_4O_9$ (590.59): calcd C 61.01, H 5.11, N 9.48; found C 60.91, H 5.17, N 9.29.

(*RRR*/*SSS*)-2,2′,2″-Nitrilotris[2-(benzoylamino)acetic acid] (4b): To a cooled (0 °C) solution of (*RRR*/*SSS*)-**2b** (3.0 g, 5.08 mmol) in methanol (60 mL) and H_2O (20 mL) was added lithium hydroxide (487 mg, 20.3 mmol). After 3 h at 0 °C stirring was continued for 15 h at ambient temperature. After the addition of 2N HCl (80 mL), the mixture was extracted with ethyl acetate (3 × 120 mL). The combined organic layers were washed with brine (200 mL) and dried ($MgSO_4$), and the solvent was evaporated. Yield: 2.56 g (92%) as a colourless solid; M.p. 110 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 5.90 (d, J = 9 Hz, 3H, NHC*H*N), 7.16–7.27 (m, 6H, *m*-Ph), 7.35–7.51 (m, 9H, Ph), 8.55 (d, J = 9 Hz, 3H, N*H*CHN); ^{13}C NMR (75.44 MHz, [D$_6$]DMSO): δ = 63.25, 127.17, 128.05, 131.54, 133.15, 166.49, 170.55; IR (KBr): $\tilde{\nu}$ = 3377, 3332, 3064, 2943, 2598, 1745, 1649, 1533, 1490 cm^{-1}; FAB HRMS (NBA) calcd for $C_{27}H_{23}N_4O_9$ (M^-): 547.146503; found *m*/*e* 547.146740; $C_{27}H_{24}N_4O_9 \cdot H_2O$ (566.52): calcd C 57.24, H 4.63, N 9.89; found C 57.47, H 4.73, N 10.01.

Equilibration and hydrolysis of 2a: Compound **2a** (10.0 g, 17 mmol) was dissolved in THF/methanol (360 mL, 5:1) and treated with 15 mol% NaOMe (138 mg, 2.55 mmol). After 24 h of stirring at room temperature, 2N HCl (200 mL) and ethyl acetate (300 mL) were added. The aqueous layer was separated and extracted with ethyl acetate (2 × 100 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated to dryness to give a mixture of the two isomers in 95% yield (9.44 g, 16 mmol, **2a**:**2b** = 63:37, ratio determined by NMR and HPLC [15]). The crude product was dissolved in methanol (90 mL) and water (30 mL), and treated with lithium hydroxide (1.92 g, 80 mmol). After 24 h of stirring at room temperature, 2N HCl (150 mL) and ethyl acetate (150 mL) were added. The aqueous layer was separated and extracted with ethyl acetate (2 × 100 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated to dryness to give a mixture of the two isomers in quantitative yield (8.78 g, 16 mmol, **4a**:**4b** = 38:62, ratio determined by NMR and HPLC [15]).

***N*,*N*′,*N*″-[(*RRR*/*SSS*)-Nitrilotris[2-(benzoylamino)-1-oxo-2,1-ethanediyl]]-tris[glycine] trimethyl ester (12)**: To a stirred solution of **4b** (300 mg, 0.55 mmol) in THF (30 mL) were added consecutively H-Gly-OMe·HCl (276 mg, 2.20 mmol), NEM (0.56 mL, 4.40 mmol) and HOBt (595 mg, 4.40 mmol) (for abbreviations, see legend of Scheme 7). The reaction mixture was cooled to 0 °C, treated with EDCI (422 mg, 2.20 mmol) and then allowed to warm slowly to room temperature (18 h), diluted with ethyl acetate (30 mL) and washed with saturated $NaHCO_3$ (30 mL), 2N HCl (30 mL), $NaHCO_3$ (30 mL) and brine. The organic solution was dried ($MgSO_4$) and evaporated to dryness. The crude material was chromatographed (SiO_2, $CHCl_3$:MeOH 30:1) to afford a 1:1 mixture of enantiomers. Yield: 339 mg (81%) as a colourless solid; M.p. 220–224 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.78 (s, 9H, OCH_3), 3.87 (dd, J = 18, 5 Hz, 3H, CH_2-Gly), 4.51 (dd, J = 18, 7 Hz, 3H, CH_2-Gly), 5.90 (d, J = 9 Hz, 3H, NHC*H*N), 7.13 (t, J = 8 Hz, 6H, *m*-Ph), 7.32 (t, J = 8 Hz, 3H, *p*-Ph), 7.42 (d, J = 8 Hz, 6H, *o*-Ph), 8.26 (d, J = 7 Hz, 1.5H, NH-Gly), 8.27 (d, J = 5 Hz, 1.5H, NH-Gly), 8.56 (d, J = 9 Hz, 3H, N*H*CHN; ^{13}C NMR (75.44 MHz, $CDCl_3$): δ = 41.46, 52.62, 62.95, 127.45, 128.06, 131.59, 132.94, 167.44, 168.84, 171.78; IR (KBr): $\tilde{\nu}$ = 3350, 1740, 1690, 1650, 1520, 1480, 1430, 1220 cm^{-1}; FAB MS (NBA); *m*/*z* (%): 763 (6.41) [M^+ + H]; $C_{36}H_{39}N_7O_{12}$ (761.75): calcd C 56.76, H 5.16, N 12.87; found C 56.63, H 5.04, N 12.81.

Large-scale preparation of 12: To a stirred solution of the mixture of the two isomers **4** (**4a**:**4b** = 38%:62%, 8.76 g, 16.0 mmol) in THF (400 mL) were added consecutively H-Gly-OMe·HCl (8.04 g, 64.0 mmol), NEM (10.1 mL, 80.0 mmol) and HOBt (10.8 g, 80.0 mmol). The reaction mixture was cooled to 0 °C, treated with *N*,*N*′-dicyclohexylcarbodiimide (13.2 g, 64.0 mmol) and then allowed to warm slowly to room temperature (18 h). The resulting suspension was filtered, diluted with ethyl acetate (300 mL) and washed with saturated $NaHCO_3$ (300 mL), 2N HCl (300 mL), $NaHCO_3$ (300 mL) and brine. The organic solution was dried ($MgSO_4$) and evaporated to dryness. The crude material was dissolved in acetonitrile (200 mL) and filtered. The mother liquor was left for 24 h at room temperature and filtered again. After evaporation of the solvent, the residue was taken up in methanol (200 mL). A white powder, the 1:1 mixture of enantiomers, crystallised immediately and was filtered off. Yield: 5.46 g (45% relative to the mixture of the two isomers, 72% relative to the symmetric triacid **4b**).

***N*,*N*′,*N*″-[(*RRR*/*SSS*)-Nitrilotris[2-(benzoylamino)-1-oxo-2,1-ethanediyl]]-tris[L-alanine] trimethyl ester (13)** was prepared according to the procedure used for the preparation of **12** from H-Ala-OMe·HCl (307 mg, 2.20 mmol). Purification by flash chromatography (SiO_2, $CHCl_3$:MeOH 30:1) gave **13** as a 1:1 mixture of diastereomers. Yield: 336 mg (76%) as a colourless solid. The (*SSS*) isomer could be separated by fractional crystallisation from methanol. (*SSS*)-**13**: M.p. 279 °C, $[\alpha]_D^{25}$ = −23 (*c* = 1, DMSO); (*RRR*)-**13**: oil; (*RRR*/*SSS*)-**13**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.53 (d, J = 7 Hz, 9H, CH_3), 3.78 (s, 9H, OCH_3), 4.59–4.71 (m, 3H, CH-Ala), 5.75* (d, J = 9 Hz, 1.5H, NHC*H*N), 5.89 (d, J = 9 Hz, 1.5H, NHC*H*N), 7.11 (t, J = 8 Hz, 6H, *m*-Ph), 7.30 (t, J = 8 Hz, 3H, *p*-Ph), 7.41 (d, J = 8 Hz, 6H, *o*-Ph), 7.94* (d, J = 7 Hz, 1.5H, NH-Ala), 8.10 (d, J = 7 Hz, 1.5H, NH-Ala), 8.43* (d, J = 9 Hz, 1.5H, N*H*CHN), 8.60 (d, J = 9 Hz, 1.5H, N*H*CHN); (*SSS*) isomer: ^{13}C NMR (75.44 MHz, $CDCl_3$): δ = 16.60, 48.73, 52.72, 62.63, 127.48, 127.99, 131.42, 133.18, 167.22, 168.20, 174.85; IR (KBr): $\tilde{\nu}$ = 3300, 2950, 1730, 1680, 1650, 1525, 1475, 1450 cm^{-1}; FAB MS (NBA); *m*/*z* (%): 826 (7.37); $C_{39}H_{45}N_7O_{12}$ (803.83): calcd C 58.27, H 5.64, N 12.20; found C 58.59, H 5.62, N 12.18.

***N*,*N*′,*N*″-[(*RRR*/*SSS*)-Nitrilotris[2-(benzoylamino)-1-oxo-2,1-ethanediyl]]-tris[L-valine] trimethyl ester (14)** was prepared according to the procedure used for the preparation of **12** from H-Val-OMe·HCl (369 mg, 2.20 mmol). Yield: 396 mg (81%) as a colourless solid. The (*SSS*) isomer could be separated by fractional crystallisation from methanol. (*SSS*)-**14**: M.p. 202–204 °C, $[\alpha]_D^{25}$ = +23 (*c* = 1, $CHCl_3$); (*RRR*)-**14**: oil; (*RRR*/*SSS*)-**14**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.96–1.02* [m, 9H, CH(CH_3)$_2$], 1.04 [d, J = 7 Hz, 4.5H, CH(CH_3)$_2$], 1.05 [d, J = 7 Hz, 4.5H, CH(CH_3)$_2$], 2.23–2.37 [m, J = 7 Hz, 3H, CH(CH$_3$)$_2$], 3.75 (s, 4.5H, OCH_3), 3.77* (s, 4.5H, OCH_3), 4.51 (dd, J = 7, 7 Hz, 1.5H, CH-Val), 4.56* (dd, J = 7, 7 Hz, 1.5H, CH-Val), 5.81* (d, J = 9 Hz, 1.5H, NHC*H*N), 5.87 (d, J = 9 Hz, 1.5H, NHC*H*N), 7.09–7.18 (m, 6H, *m*-Ph), 7.28–7.35 (m, 3H, *p*-Ph), 7.39–7.46 (m, 6H, *o*-Ph), 7.71* (d, J = 8 Hz, 1.5H, NH-Val), 8.02 (d, J = 8 Hz, 1.5H, NH-Val), 8.48* (d, J = 9 Hz, 1.5H, N*H*CHN), 8.58 (d, J = 9 Hz, 1.5H, N*H*CHN); (*SSS*) isomer: ^{13}C NMR (75.44 Hz, $CDCl_3$): δ = 18.39, 19.21, 30.36, 52.25, 58.85, 63.69, 127.42, 128.11, 131.69, 132.89, 167.84, 168.72, 172.31; IR (KBr): $\tilde{\nu}$ = 3340, 3040, 2960, 1730, 1680, 1650, 1580 cm^{-1}; FAB MS (NBA); *m*/*z* (%): 910 (4.70) [M^+ + Na]; $C_{45}H_{57}N_7O_{12}$ (887.99): calcd C 60.87, H 6.47, N 11.04; found C 60.71, H 6.51, N 11.09.

Large-scale preparation of 14: **14** was prepared according to the procedure used for the preparation of **12** from H-Val-OMe·HCl (10.73 g, 64.0 mmol). After evaporation of the solvent the residue was taken up in methanol (200 mL) and left at −20 °C for crystallisation. Yield: 1.74 g (12%) (*SSS*)-**14** (20% relative to the symmetric triacid **4b**). The (*RRR*) isomer was not isolated.

***N*,*N*′,*N*″-[(*RRR*/*SSS*)-Nitrilotris[2-(benzoylamino)-1-oxo-2,1-ethanediyl]]-tris[L-serine] trimethyl ester (15)** was prepared according to the procedure used for the preparation of **12** from H-Ser-OMe·HCl (342 mg, 2.20 mmol). Yield: 338 mg (72%) as a colourless foam. The (*SSS*) isomer could be separated by fractional crystallisation from methanol. (*SSS*)-**15**: M.p. 273 °C; $[\alpha]_D^{25}$ = +16 (*c* = 1, DMSO); (*RRR*)-**15**: foam; (*RRR*/*SSS*)-**15**: ^{1}H NMR (300 MHz, $CDCl_3$:[D$_6$]DMSO 10:1): δ = 3.79 (s, 4.5H, OCH_3), 3.82* (s, 4.5H, OCH_3), 3.85–4.10 (m, 6H, CH_2OH), 4.81 (t, J = 6 Hz, 3H, CH_2OH), 5.87 (d, J = 10 Hz, 1.5H, NHC*H*N), 5.93* (d, J = 10 Hz, 1.5H, NHC*H*N), 7.08–7.20 (m, 6H, *m*-Ph), 7.30–7.45 (m, 9H, Ph), 8.30 (d, J = 8 Hz, 1.5H, NH-Ser), 8.35 (d, J = 6 Hz, 1.5H, NH-Ser), 8.47 (d, J = 10 Hz, 1.5H, N*H*CHN), 8.56 (d, J = 10 Hz, 1.5H, N*H*CHN); (*SSS*) isomer: ^{13}C NMR (75.44 MHz, $CDCl_3$:[D$_6$]DMSO 10:1): δ = 52.45, 55.80, 61.04, 63.15, 127.10, 127.92, 131.53, 132.89, 166.99, 168.43, 171.23; IR (KBr): $\tilde{\nu}$ = 3500, 3300, 1720, 1690, 1650, 1520, 1350, 1230 cm^{-1}; FAB MS (NBA); *m*/*z* (%): 852 (6.27) [M^+ + H]; $C_{39}H_{45}N_7O_{15}$ (851.82): calcd C 54.99, H 5.33, N 11.46; found C 54.62, H 5.37, N 11.51.

***N*,*N*′,*N*″-[(*RRR*/*SSS*)-Nitrilotris[2-(benzoylamino)-1-oxo-2,1-ethanediyl]]-tris-[leucylphenylalanine] trimethyl ester (19)** was prepared according to the

procedure used for the preparation of **12** from H-Leu-Phe-OMe (643 mg, 2.20 mmol). Yield: 302 mg (40%) as a colourless foam. The (*SSS*) isomer could be separated by fractional crystallisation from methanol. (*SSS*)-**19**: M.p. 278 °C; $[\alpha]_D^{25} = +61$ ($c = 1$, $CHCl_3$); (*RRR*)-**19**: colourless foam; (*SSS*)-**19**: ^{1}H NMR (300 MHz, $CDCl_3$) [35]: $\delta = 0.90$ [d, $J = 7$ Hz, 9H, $CH(CH_3)_2$], 0.91 [d, $J = 7$ Hz, 9H, $CH(CH_3)_2$], 1.54–1.85 [m, 9H, $CH(CH_3)_2$ and CH_2-Leu], 3.13 (dd, $J = 14$, 6 Hz, 3H, CH_2-Phe), 3.19 (dd, $J = 14$, 6 Hz, 3H, CH_2-Phe), 3.66 (s, 9H, OCH_3), 4.28–4.46 (m, 3H, CH-Phe), 4.75–4.92 (m, 3H, CH-Leu), 5.72 (d, $J = 9$ Hz, 3H, NHCHN), 6.44 (d, $J = 8$ Hz, 3H, NH-Phe), 7.07–7.45 (m, 30H, Ph), 8.29 (d, $J = 7$ Hz, 3H, NH-Leu), 8.59 (d, $J = 9$ Hz, 3H, NHCHN); ^{13}C NMR (75.44 MHz, $CDCl_3$): $\delta = 22.02$, 22.81, 24.74, 37.83, 40.78, 52.33, 53.35, 63.18, 127.17, 127.46, 128.07, 128.69, 129.36, 131.57, 133.06, 135.84, 167.55, 168.63, 171.74, 172.15; IR (KBr): $\tilde{\nu} = 3290$, 2950, 1740, 1650, 1650, 1520, 1490 cm^{-1}; FAB MS (NBA); *m/z* (%): 1393 (0.01) [M^+ + Na]; $C_{75}H_{90}N_{10}O_{15} \cdot 0.5\,H_2O$ (1380.61): calcd C 65.25, H 6.64, N 10.15; found C 65.25, H 6.75, N 10.43.

***N,N′,N″*-[(*RRR*/*SSS*)-Nitrilotris[2-(benzoylamino)-1-oxo-2,1-ethanediyl]]-tris[leucylglycine] trimethyl ester (20)** was prepared according to the procedure used for the preparation of **12** from H-Leu-Gly-OMe (338 mg, 2.20 mmol). Yield: 352 mg (58%); The (*SSS*) isomer could be separated by fractional crystallisation from methanol. (*SSS*)-**20**: M.p. 264 °C; (*RRR*)-**20**: colourless foam; (*SSS*)-**20**: ^{1}H NMR (300 MHz, $CDCl_3$:[D$_6$]DMSO 8:2) [36]: $\delta = 0.93$ [d, $J = 7$ Hz, 9H, $CH(CH_3)_2$], 0.97 [d, $J = 7$ Hz, 9H, $CH(CH_3)_2$], 1.66–1.78 (m, 6H, CH_2-Leu), 1.81–1.92 [m, 3H, $CH(CH_3)_2$], 3.70 (s, 9H, OCH_3), 3.89 (dd, $J = 18$, 6 Hz, 3H, CH_2-Gly), 3.97 (dd, $J = 18$, 6 Hz, 3H, CH_2-Gly), 4.46–4.58 (m, 3H, CH-Leu), 5.72 (d, $J = 9$ Hz, 3H, NHCHN), 7.14 (t, $J = 8$ Hz, 6H, *m*-Ph), 7.31–7.40 (m, 9H, Ph), 8.10 (t, $J = 6$ Hz, 3H, Gly-NH), 8.45 (d, $J = 9$ Hz, 3H, NHCHN), 8.78 (d, $J = 7$ Hz, 3H, Leu-NH); ^{13}C NMR (75.44 MHz, $CDCl_3$:[D$_6$]DMSO 8:2): $\delta = 20.95$, 22.79, 51.47, 52.27, 62.84, 126.94, 127.58, 131.19, 132.57, 166.71, 168.39, 169.60, 172.88; IR (KBr): $\tilde{\nu} = 3320$, 2940, 1750, 1680, 1650, 1520, 1480 cm^{-1}; FAB MS (NBA); *m/z* (%): 1100 (0.01) [M^+ + H]; $C_{54}H_{72}N_{10}O_{15}$ (1101.23): calcd C 58.90, H 6.59, N 12.72; found C 58.58, H 6.70, N 12.60.

***N,N′,N″*-[(*RRR*/*SSS*)-Nitrilotris[2-(benzoylamino)-1-oxo-2,1-ethanediyl]]-tris[glycylleucine] trimethyl ester (21)** was prepared according to the procedure used for the preparation of **12** from H-Gly-Leu-OMe (338 mg, 2.20 mmol). Yield: 460 mg (76%); The (*RRR*) isomer could be separated by slow diffusion of pentane into a solution of **21** in acetone. (*RRR*)-**21**: M.p. 221 °C; $[\alpha]_D^{25} = -9$ ($c = 1$, MeOH);(*SSS*)-**21**: colourless foam; (*RRR*)-**21**: ^{1}H NMR (300 MHz, $CDCl_3$) [36]: $\delta = 0.90$–0.95 [m, 18H, $CH(CH_3)_2$], 1.55–1.75 [m, 9H, $CH(CH_3)_2$ and Leu-CH_2], 3.71 (s, 9H, OCH_3), 3.79 (dd, $J = 17$, 5 Hz, 3H, CH_2-Gly), 4.36 (dd, 17, 7 Hz, 3H, CH_2-Gly), 4.55 (td, $J = 8$, 7 Hz, 3H, CH-Val), 5.83 (d, $J = 9$ Hz, 3H, NHCHN), 7.13 (t, $J = 8$ Hz, 6H, *m*-Ph), 7.33 (t, $J = 8$ Hz, 3H, *p*-Ph), 7.39 (d, $J = 9$ Hz, 6H, *o*-Ph), 7.85 (d, $J = 8$ Hz, 3H, Leu-NH), 8.51 (d, $J = 9$ Hz, 3H, NHCHN), 8.87 (dd, $J = 7$, 5 Hz, 3H, Gly-NH); ^{13}C NMR (75.44 MHz, $CDCl_3$): $\delta = 21.95$, 22.81, 24.85, 41.63, 43.21, 50.94, 52.42, 63.34, 127.42, 128.10, 131.70, 132.50, 167.46, 169.03, 169.29, 173.10; IR (KBr): $\tilde{\nu} = 3200$, 3080, 2960, 1740, 1680, 1630, 1580, 1520, 1490 cm^{-1}; FAB MS (NBA); *m/z* (%): 1123 (0.92) [M^+ + Na]; $C_{54}H_{72}N_{10}O_{15}$ (1101.23): calcd C 58.90, N 6.59, N 12.72; found C 58.63, H 6.77, N 12.66.

***N,N′,N″*-[(*RRR*/*SSS*)-Nitrilotris[2-(benzoylamino)-1-oxo-2,1-ethanediyl]]-tris[L-glycine] (22)**: To a cooled (0 °C) solution of (*RRR*/*SSS*)-**12** (1.52 g, 2.0 mmol) in THF (60 mL) and H_2O (20 mL) was added lithium hydroxide (240 mg, 10 mmol). After 3 h at 0 °C, stirring was continued for 15 h at ambient temperature. After the addition of 2N HCl (80 mL), the mixture was extracted with ethyl acetate (3 × 120 mL). The combined organic layers were washed with brine (200 mL), dried ($MgSO_4$), and the solvent was evaporated. Yield: 1.38 g (95%) as a colourless solid; M.p. 218–224 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 3.82$ (dd, $J = 18$, 6 Hz, 3H, CH_2-Gly), 4.10 (dd, $J = 18$, 6 Hz, 3H, CH_2-Gly), 5.74 (d, $J = 9$ Hz, 3H, NHCHN), 7.16 (t, $J = 8$ Hz, 6H, *m*-Ph), 7.33–7.38 (m, 9H, Ph), 8.38 (d, $J = 9$ Hz, 3H, NHCHN), 8.54 (t, $J = 6$ Hz, 3H, NH-Gly); ^{13}C NMR (75.44 MHz, [D$_6$]DMSO): $\delta = 42.20$, 63.55, 127.74, 128.71, 132.36, 133.56, 167.31, 169.10, 172.31; IR (KBr): $\tilde{\nu} = 3390$, 3064, 2937, 2625, 1736, 1670, 1652, 1579, 1526, 1488 cm^{-1}; FAB MS (NBA); *m/z* (%): 720 (8.37) [M^+ + H]; $C_{33}H_{33}N_7O_{12} \cdot 0.5\,H_2O$ (728.67): calcd C 54.40, H 4.70, N 13.46; found C 54.13, H 4.99, N 13.49.

***N,N′,N″*-[(*SSS*)-Nitrilotris[2-(benzoylamino)-1-oxo-2,1-ethanediyl]]-tris[L-valine] (23)** was prepared according to the procedure used for the preparation of **22** from (*SSS*)-**14** (1.95 g, 2.20 mmol). Yield: 1.73 mg (93%) as a colourless solid; M.p. 217 °C; $[\alpha]_D^{25} = +18$ ($c = 1$, MeOH); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 0.73$–0.77 [m, 18H, $CH(CH_3)_2$], 1.93–2.08 (m, 3H, $CH(CH_3)_2$), 4.03 (dd, $J = 8$, 6 Hz, 3H, CH-Val), 5.49 (d, $J = 9$ Hz, 3H, NHCHN), 6.83–6.88 (m, 6H, *m*-Ph), 7.03–7.09 (m, 9H, Ph), 8.06 (d, $J = 8$ Hz, 3H, NH-Val), 8.11 (d, $J = 9$ Hz, 3H, NHCHN); ^{13}C NMR (75.44 MHz, [D$_6$]DMSO): $\delta = 19.01$, 19.00, 30.32, 59.24, 64.06, 127.66, 128.55, 132.17, 133.53, 167.76, 168.95, 173.53; IR (KBr): $\tilde{\nu} = 3334$, 2967, 1729, 1680, 1667, 1656, 1630, 1530, 1486 cm^{-1}; FAB MS (NBA); *m/z* (%): 868 (2.16) [M^+ + Na]; $C_{42}H_{51}N_7O_{12}$ (845.91): calcd C 59.64, H 6.08, N 11.59; found C 59.98, H 6.35, N 11.69.

***N,N′,N″*-[(*SSS*)-Nitrilotris[2-(benzoylamino)-1-oxo-2,1-ethanediyl]]-tris[valylvalylglycylvaline] trimethyl ester (24)** was prepared according to the procedure used for the preparation of **12** from (*SSS*)-**23** (300 mg, 0.35 mmol) and H-Val-Gly-Val-OMe (503 mg, 1.75 mmol). Yield: 342 mg (59%) as a colourless solid; M.p. 150–152 °C; $[\alpha]_D^{25} = -2$ ($c = 0.7$, DMSO); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.89$ [d, $J = 7$ Hz, 9H, $CH(CH_3)_2$], 0.90 [d, $J = 7$ Hz, 9H, $CH(CH_3)_2$], 0.95 [d, 7 Hz, 9H, $CH(CH_3)_2$], 0.96 (d, $J = 7$ Hz, 9H, $CH(CH_3)_2$], 1.01 [d, $J = 7$ Hz, 18H, $CH(CH_3)_2$], 2.10–2.23 [m, 6H, $CH(CH_3)_2$], 2.24–2.41 [m, 3H, $CH(CH_3)_2$], 3.68 (s, 9H, OCH_3), 3.94 (dd, $J = 19$, 6 Hz, 3H, CH_2-Gly), 4.01 (dd, $J = 17$, 6 Hz, 3H, CH_2-Gly), 4.25–4.36 (m, 6H, CH-Val), 4.43 (dd, $J = 8$, 8 Hz, 3H, CH-Val), 5.69 (d, $J = 9$ Hz, 3H, NHCHN), 7.14 (t, $J = 8$ Hz, 6H, *m*-Ph), 7.31–7.39 (m, 9H, Ph), 7.51 (d, $J = 9$ Hz, 3H, Val-NH), 7.72 (d, $J = 8$ Hz, 3H, Val-NH), 7.89–7.98 (m, 3H, Gly-NH), 8.45 (d, $J = 8$ Hz, 3H, Val-NH), 8.50 (d, $J = 9$ Hz, 3H, NHCHN); ^{13}C NMR (75.44 MHz, $CDCl_3$): $\delta = 17.99$, 18.34, 18.48, 18.90, 19.32, 19.81, 30.51, 30.58, 30.79, 42.91, 51.83, 57.34, 59.12, 59.96, 63.84, 127.22, 128.05, 131.69, 132.85, 167.64, 168.39, 169.23, 171.37, 171.89, 172.06; IR (KBr): $\tilde{\nu} = 3300$, 3060, 2980, 1730, 1650, 1500 cm^{-1}; FAB MS (NBA); *m/z* (%): 1676 (5.18) [M^+ + Na], 1654 (2.98) [M^+ + H]; $C_{81}H_{120}N_{16}O_{21}$ (1653.94): calcd C 58.82, H 7.31, N 13.55; found C 58.72, H 7.14, N 13.52.

***N,N′,N″*-[(*SSS*)-Nitrilotris[2-(benzoylamino)-1-oxo-2,1-ethanediyl]]-tris[valylleucylglycylphenylalanine] trimethyl ester (25)** was prepared according to the procedure used for the preparation of **12** from (*SSS*)-**23** (300 mg, 0.35 mmol) and H-Leu-Gly-Phe-OMe (583 g, 1.75 mmol). Yield: 341 mg (53%), M.p. 154 °C, $[\alpha]_D^{25} = +16$ ($c = 1.9$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.88$–0.98 (m, 18H, $CH(CH_3)_2$), 1.02 [d, $J = 7$ Hz, 18H, $CH(CH_3)_2$], 1.59–1.80 [m, 9H, CH_2-Leu and $CH(CH_3)_2$], 2.17–2.34 [m, 3H, $CH(CH_3)_2$], 2.96 (dd, $J = 14$, 7 Hz, 3H, CH_2-Phe), 3.02 (dd, $J = 14$, 7 Hz, 3H, CH_2-Phe), 3.57 (s, 9H, OCH_3), 3.83 (dd, $J = 17$, 6 Hz, 3H, CH_2-Gly), 3.94 (dd, $J = 17$, 6 Hz, 3H, CH_2-Gly), 4.21–4.42 (m, 6H, CH), 4.60–4.75 (m, 3H, CH), 5.71 (d, $J = 9$ Hz, 3H, NHCHN), 7.08–7.28 (m, 24H, Ph), 7.30–7.40 (m, 6H, Ph), 7.53 (d, $J = 8$ Hz, 3H, NH), 7.70 (t, $J = 6$ Hz, 3H, NH-Gly), 7.84 (d, $J = 7$ Hz, 3H, NH-Phe), 8.48 (d, 6 Hz, 3H, NH), 8.50 (d, $J = 9$ Hz, 3H, NHCHN); ^{13}C NMR (75.44 MHz, $CDCl_3$): $\delta = 18.41$, 19.64, 21.59, 23.07, 24.65, 30.75, 37.66, 42.91, 51.96, 52.60, 53.51, 60.35, 63.60, 126.72, 127.25, 128.08, 128.34, 129.17, 131.80, 132.60, 136.35, 167.56, 169.01, 169.09, 171.58, 171.75, 172.48; IR (KBr): $\tilde{\nu} = 3290$, 3060, 2960, 1740, 1680, 1650, 1540, 1500, 1340, 1220 cm^{-1}; FAB MS (NBA); *m/z* (%): 1861 (0.16) [M^+ + Na], 1839 (0.06) [M^+ + H]; $C_{96}H_{126}N_{16}O_{21}$ (1840.16): calcd C 62.66, H 6.90, N 12.18; found C 62.44, H 6.95, N 11.88.

(*SSS*)-N(BzGly*ValMetOMe)$_3$ (26) was prepared according to the procedure used for the preparation of **12** from (*SSS*)-**23** (300 mg, 0.35 mmol), methionine methyl ester hydrochloride (280 mg, 1.40 mmol), HOBt (378 mg, 2.80 mmol), NEM (0.35 mL, 2.80 mmol) and EDCI (268 mg, 1.40 mmol). Yield: 399 mg (89%); M.p. 260 °C (decomp.), $[\alpha]_D^{25} = +16$ ($c = 0.5$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$/[D$_6$]DMSO 8:2): $\delta = 0.96$ [d, $J = 7$ Hz, 9H, $CH(CH_3)_2$], 0.97 [d, $J = 7$ Hz, 9H, $CH(CH_3)_2$], 1.87–2.13 (m, 15H, SCH_3 and CH_2), 2.13–2.31 [m, 3H, $CH(CH_3)_2$], 2.40–2.60 (m, 6H, CH_2S), 3.57 (s, 9H, OCH_3), 4.28 (t, $J = 7$ Hz, 3H, CH-Val), 4.44–4.55 (m, 3H, CH-Met), 5.59 (d, $J = 9$ Hz, 3H, NHCHN), 7.07 (t, $J = 9$ Hz, 6H, *m*-Ph), 7.23–7.33 (m, 9H, *o*-,*p*-Ph), 8.11 (d, $J = 8$ Hz, 3H, NH-Met), 8.38 (d, $J = 7$ Hz, 3H, NH-Val), 8.44 (d, $J = 9$ Hz, 3H, NHCHN); ^{13}C NMR (75.44 MHz, $CDCl_3$/[D$_6$]DMSO 8:2): $\delta = 14.88$, 17.71, 19.32, 29.83, 30.36, 30.41, 51.00, 51.71, 59.00, 63.20, 126.80, 127.75, 131.41, 132.50, 166.86, 168.07, 171.14, 171.65; IR (KBr): $\tilde{\nu} = 3300$, 3060 (w), 2980, 2940, 1750, 1650, 1580, 1530, 1490, 1430, 1350, 1280, 1210, 1180, 1120, 1080, 980, 805, 790, 720, 700; FAB

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MS (NBA); *m/z* (%): 1304 (0.60) [M^+ + Na]; $C_{60}H_{84}N_{10}O_{15}S_3$ (1281.59): calcd C 56.23, H 6.61, N 10.93, found C 56.03, H 6.52, N 10.73.

(*SSS*)-N(BzGly*ValPheSerOMe)$_3$ (27) was prepared according to the procedure used for the preparation of **12** from (*SSS*)-**23** (300 mg, 0.35 mmol), phenylalanylserine methyl ester (466 mg, 1.75 mmol) [37], NEM (0.35 mL, 2.80 mmol), HOBt (378 mg, 2.80 mmol) and EDCI (268 mg, 1.40 mmol). Yield: 440 mg (79%); M.p. 134 °C, $[\alpha]_D^{25} = -25$ ($c = 1$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.86$ [d, $J = 7$ Hz, 9H, $CH(CH_3)_2$], 0.91 [d, $J = 7$ Hz, 9H, $CH(CH_3)_2$], 2.13–2.27 [m, 3H, $CH(CH_3)_2$], 3.06 (dd, $J = 14$, 9 Hz, 3H, CH_2-Phe), 3.20 (dd, $J = 14$, 5 Hz, 3H, CH_2-Phe), 3.38–3.43 (m, 3H, CH_2OH), 3.69 (s, 9H, OCH_3), 3.92–4.05 (m, 6H, CH_2-Ser), 4.12–4.25 (m, 3H, CH), 4.43–4.53 (m, 3H, CH), 4.57–4.69 (m, 3H, CH), 5.68 (d, $J = 9$ Hz, 3H, NHC*H*N), 7.05–7.42 (m, 36H, Ph and NH), 8.11–8.14 (m, 3H, NH), 8.46 (d, $J = 9$ Hz, 3H, N*H*CHN); ^{13}C NMR (75.44 MHz, $CDCl_3$): $\delta = 18.71$, 19.69, 30.47, 36.49, 52.99, 55.33, 55.54, 61.49, 62.81, 64.18, 127.19, 127.83, 128.67, 128.90, 129.46, 132.49, 132.81, 137.42, 168.54, 169.81, 171.15, 171.35, 172.14; IR (KBr): $\tilde{\nu} = 3300$, 3040, 2950, 1740, 1650, 1520, 1350, 1300, 1230, 1140, 1080, 930, 700. FAB MS (NBA); *m/z* (%): 1613 (2.00) [M^+ + Na]; $C_{81}H_{99}N_{13}O_{21}$ (1590.75): calcd C 61.16, H 6.27, N 11.45, found C 60.82, H 6.37, N 11.53.

***N,N′,N″*-[(*SSS*)-Nitrilotris[2-(benzoylamino)-1-oxo-2,1-ethanediyl]]-tris[valyltryptophan] trimethyl ester (28)** was prepared according to the procedure used for the preparation of **12** from (*SSS*)-**23** (300 mg, 0.35 mmol), tryptophan methyl ester hydrochloride (446 mg, 1.75 mmol), HOBt (378 mg, 2.80 mmol), NEM (0.35 mL, 2.80 mmol) und EDCI (268 mg, 1.40 mmol). The crude product was recrystallised from methanol to give a colourless solid. Yield: 273 mg (54%); M.p. 222 °C, $[\alpha]_D^{25} = +40$ ($c = 1$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.58$ [d, $J = 7$ Hz, 9H, $CH(CH_3)_2$], 0.82 [d, $J = 7$ Hz, 9H, $CH(CH_3)_2$], 1.50–1.76 [m, 3H, $CH(CH_3)_2$], 2.88 (dd, $J = 15$, 10 Hz, 3H, CH_2-Trp), 3.43 (dd, $J = 15$, 3 Hz, 3H, CH_2-Trp), 3.54–3.63 (m, 3H, CH-Val), 3.78 (s, 9H, OCH_3), 4.72–4.85 (m, 3H, CH-Trp), 5.39 (d, $J = 9$ Hz, 3H, NHC*H*N), 6.03 (d, $J = 9$ Hz, 3H, NH-Trp), 6.86–7.09 (m, 9H, Ph), 7.20–7.29 (m, 6H, Ph), 7.36–7.54 (m, 15H, Ph), 7.85 (d, $J = 5$ Hz, 3H, NH-Val), 8.91 (d, $J = 9$ Hz, 3H, N*H*CHN), 9.54–9.55 (brs, 3H, NH); ^{13}C NMR (75.44 MHz, $CDCl_3$): $\delta = 18.68$, 20.23, 29.15, 29.60, 52.53, 61.08, 62.12, 108.70, 110.70, 110.99, 117.63, 118.63, 121.20, 125.21, 127.25, 127.53, 128.26, 131.73, 133.43, 136.25, 167.23, 167.68, 170.56, 172.61; IR (KBr): $\tilde{\nu} = 3300$, 3030, 2980, 1740, 1650, 1520, 1340, 1210, 1100, 750, 690; FAB MS (NBA); *m/z* (%): 1446 (5.01) [M^+ + H]; $C_{78}H_{87}N_{13}O_{15}$ (1446.63): calcd C 64.76, H 6.06, N 12.59, found C 65.01, H 6.24, N 12.71.

***N,N′,N″*-[(*SSS*)-Nitrilotris[2-benzoylamino)-1-oxo-2,1-ethanediyl]]-tris[L-valyl-*N*(π)-benzyloxymethyl-L-histidine] trimethyl ester (29a)**: (*SSS*)-**23** (499 mg, 0.59 mmol) and *N*(π)-benzyloxymethyl-L-histidine methyl ester dihydrochloride (851 mg, 2.35 mmol) [38] were suspended in THF (70 mL) and treated with NEM (0.74 mL, 5.90 mmol). After cooling to 0 °C, HOBt (478 mg, 3.54 mmol) and EDCI (566 mg, 2.95 mmol) were added. Stirring was continued for 1 h at 0 °C, then the reaction mixture was allowed to warm to room temperature (23 h). Ethyl acetate (75 mL) was added, and the organic layer was washed with saturated $NaHCO_3$ (2 × 70 mL) and 10% citric acid (5 × 70 mL). The combined citric acid extracts were neutralised with saturated $NaHCO_3$ and extracted with ethyl acetate (5 × 70 mL). The ethyl acetate extracts were combined and washed with brine. Drying ($MgSO_4$) and evaporation gave a colourless solid which was recrystallised from ethyl acetate/petroleum ether. Yield: 694 mg (71%); M.p. 104–106 °C; $[\alpha]_D^{25} = +10.5$ ($c = 1.00$, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.89$ (d, $J = 7$ Hz, 9H, $CH(CH_3)_2$), 0.93 (d, $J = 7$ Hz, 9H, $CH(CH_3)_2$), 2.12 (m, 3H, $CH(CH_3)_2$), 3.24 (m, 6H, CH_2-His), 3.69 (s, 9H, OCH_3), 4.26 (dd, $J = 7$ Hz, 3H, CH-Val), 4.41 (d, $J = 12$ Hz, 3H, OCH_2), 4.48 (d, $J = 12$ Hz, 3H, OCH_2), 4.98 (m, 3H, CH-His), 5.34 (d, $J = 11$ Hz, 3H, NCH_2), 5.49 (d, $J = 11$ Hz, 3H, NCH_2), 5.82 (d, $J = 9$ Hz, 3H, NHC*H*N), 6.81 (s, 3H, H_1-Im), 7.11 (dd, $J = 8$ Hz, 6H, *m*-Ph), 7.28–7.35, 7.40 (m, 18H, d, $J = 7.2$ Hz, 6H, Ph), 7.49 (s, 3H, H_2-Im), 8.08 (d, $J = 9$ Hz, 3H, NH-Val), 8.33 (d, $J = 7$ Hz, 3H, NH-His), 8.62 (d, $J = 9$ Hz, 3H, N*H*CHN); ^{13}C NMR (75.44 MHz, $CDCl_3$): $\delta = 18.59$, 19.31, 26.36, 30.73, 51.62, 52.44, 60.30, 63.49, 70.12, 73.49, 126.65, 127.42, 128.13, 128.27, 128.67, 129.94, 131.72, 132.89, 136.20, 138.69, 167.81, 169.00, 171.30, 171.36; IR (KBr): $\tilde{\nu} = 3300$, 3020, 2960, 2860, 1740, 1660, 1600, 1580, 1510, 1480, 1340, 1280, 1210, 1090, 1070, 930, 750, 700, 660; FAB MS (NBA); *m/z* (%): 1600 (96) [M^+]; $C_{87}H_{102}N_{16}O_{18}$ (1659.87), calcd C 62.95, H 6.19, N 13.50; found, C 62.81, H 6.08, N 13.58.

***N,N′,N″*-[(*SSS*)-Nitrilotris[2-benzoylamino)-1-oxo-2,1-ethanediyl]]tris[L-valyl-L-histidine] trimethyl ester (29)**: **29a** (300 mg, 0.18 mmol) was dissolved in 80% aqueous acetic acid (30 mL) and treated with palladium on carbon (10%, 300 mg). The suspension was hydrogenated for 24 h (monitored by TLC). The palladium on carbon was filtered off and the solvent was evaporated. The residue was neutralised with saturated $NaHCO_3$ and extracted with ethyl acetate (5 × 70 mL). The combined ethyl acetate extracts were washed with brine, dried ($MgSO_4$) and evaporated to give a colourless solid which was recrystallised from methanol/ether. Yield: 109 mg (47%); M.p. 145–147 °C; $[\alpha]_D^{25} = -3.2$ ($c = 1.00$, methanol); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 0.92$ (d, $J = 6$ Hz, 18H, $CH(CH_3)_2$), 2.11–2.18 (m, 3H, $CH(CH_3)_2$), 2.94 (brs, 6H, CH_2-His), 3.52 (s, 9H, OCH_3), 4.24 (dd, $J = 6$ Hz, 3H, CH-Val), 4.51 (m, 3H, CH-His), 5.65 (d, $J = 9$ Hz, 3H, NHC*H*N), 6.90 (brs, 3H, H_1-Im), 7.22 (dd, $J = 8$ Hz, 6H, *m*-Ph), 7.37–7.44 (m, 9H, Ph), 7.54 (brs, H_2-Im), 8.50 (m, br, 9H, N*H*CHN, NH-His, NH-Val), 11.8 (brs, NH-Im); ^{13}C NMR (75.44 MHz, [D$_6$]DMSO): $\delta = 17.97$, 19.06, 28.78, 29.98, 51.53, 52.62, 58.83, 59.65, 63.23, 126.81, 128.05, 131.75, 132.57, 166.41, 167.75, 170.76, 171.44; IR (KBr): $\tilde{\nu} = 3260$, 3060, 2950, 1740, 1660, 1600, 1580, 1520, 1490, 1440, 1340, 1320, 1210, 1170, 1080, 980, 930, 800, 710, 690, 620; FAB MS (NBA); *m/z* (%): 1322 (9) [M^- + Na], 1300 (53) [M^+ + H], 1299 (71) [M^+], 105 (100) [Ph-C≡O$^+$]; FAB HRMS (NBA) calcd for $C_{63}H_{79}N_{16}O_{15}$ (M^+ + H): 1299.5911, found (m/e) 1299.5987.

***N*-(*tert*-Butyloxycarbonyl)-*N′*-(2,3-dibenzyloxybenzoyl)ethylenediamine (32a)**: A solution of *N*-(*tert*-butyloxycarbonyl)ethylenediamine (**31**) (4.04 g, 25.2 mmol) and triethylamine (4.2 mL, 30.2 mmol) in THF (30 mL) was cooled to 0 °C, and a solution of 2,3-bis(benzyloxy)benzoyl chloride (**30**) (8.89 g, 25.2 mmol) in THF (25 mL) was added dropwise over 0.5 h. After an additional hour of stirring at 0 °C, ethyl acetate was added (30 mL) and the reaction mixture was washed with saturated $NaHCO_3$ (30 mL), 2N HCl (30 mL), $NaHCO_3$ (30 mL) and brine. The organic solution was dried ($MgSO_4$) and evaporated to dryness. The crude product and charcoal were suspended in ethyl acetate (50 mL), and the mixture was heated to reflux over 5 min. The suspension was filtered through Celite and was diluted with hexanes until a precipitate was formed, which was filtered off. Yield: 5.4 g (45%); M.p. 135 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.41$ [s, 9H, $C(CH_3)_3$], 3.12–3.19 (m, 2H, CH_2), 3.31–3.37 (m, 2H, CH_2), 5.10 (s, 2H, CH_2Ph), 5.16 (s, 2H, CH_2Ph), 7.05–7.09 (m, 2H, Ph), 7.27–7.41 (m, 11H, Ph), 7.60–7.64 (m, 1H, NH), 7.95–8.05 (m, 1H, NH); ^{13}C NMR (75.44 MHz, $CDCl_3$): $\delta = 28.38$, 39.60, 41.00, 71.36, 117.19, 123.28, 124.42, 127.21, 127.69, 128.30, 128.71, 128.79, 128.90, 136.40, 146.84, 151.73, 166.00; IR (KBr): $\tilde{\nu} = 3340$, 2960, 2900, 1680, 1630, 1580, 1520, 1450, 1380 cm^{-1}; FAB MS (NBA); *m/z* (%): 477 (15.2) [M^+ + H]; $C_{28}H_{32}N_2O_5$ (476.57): calcd C 70.57, H 6.77, N 5.88; found C 70.42, H 6.70, N 5.99.

***N*-(*tert*-Butyloxycarbonyl)-*N′*-(2,3-dibenzyloxybenzoyl)hydrazine (36a)** was prepared according to the procedure used for the preparation of **32a** from **30** (6.42 g, 19.2 mmol) and *N*-(*tert*-butyloxycarbonyl)hydrazine (**35**) (2.5 g, 19.2 mmol). The crude product was recrystallised from EtOAc/hexane. Yield: 5.39 g (62.6%); M.p. 151 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.49$ [s, 9H, $C(CH_3)_3$], 5.15 (s, 4H, CH_2Ph), 6.50–6.70 (m, 1H, NH), 7.10–7.52 (m, 12H, Ph), 7.68–7.74 (m, 1H, Ph), 9.56 (d, $J = 4$ Hz, 1H, NH); ^{13}C NMR (75.44 MHz, $CDCl_3$): $\delta = 28.13$, 42.50, 71.37, 81.48, 117.76, 123.37, 124.64, 125.37, 127.81, 128.31, 128.50, 128.65, 129.37, 136.23, 146.83, 151.86, 155.18, 164.80; IR (KBr): $\tilde{\nu} = 3360$, 3240, 2980, 1730, 1650, 1580, 1500, 1450, 1390, 1370, 1250, 1150; FAB MS (NBA); *m/z* (%): 449 (17.97) [M^+ + H]; $C_{26}H_{28}N_2O_5$ (448.52): calcd C 69.63, H 6.29, N 6.25; found C 70.13, H 6.61, N 6.41.

(*SSS*)-N[BzGly*ValNHCH$_2$CH$_2$NHCOC$_6$H$_3$(OBn)$_2$]$_3$ (33): Ethylenediamine **32a** (620 mg, 1.30 mmol) was treated with a solution (3 M) of HCl in EtOAc (20 mL) with vigorous stirring. After 18 h of stirring, the precipitate **32** was filtered off and dried in vacuo (70 °C). A suspension of this material (537 mg, 1.30 mmol) in ethyl acetate (15 mL) and saturated $NaHCO_3$ (15 mL) was stirred for 30 min, and then extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo to give a colourless foam. The crude product was coupled with (*SSS*)-**23** (254 mg, 0.30 mmol) according to the procedure used for the preparation of **24**. Purification by flash column chromatography (SiO_2, acetone/$CHCl_3$ 1:6) gave **33** as a colourless solid. Yield: 415 mg (72%); M.p. 136 °C; $[\alpha]_D^{25} = +20$ ($c = 0.8$, DMSO); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.94$ [d, $J = 7$ Hz, 9H, $CH(CH_3)_2$], 0.95 [d, $J = 7$ Hz,

9H, $CH(CH_3)_2$], 2.12–2.31 [m, 3H, $CH(CH_3)_2$], 3.11–3.43 (m, 12H, $NHCH_2CH_2NH$), 4.45 (dd, J = 8, 7 Hz, 3H, CH-Val), 5.06 (s, 6H, CH_2Ph), 5.11 (d, 6H, CH_2Ph), 5.75 (d, J = 9 Hz, 3H, NHC*H*N), 7.03–7.13 (m, 12H, Ph), 7.26–7.60 (m, 45H, Ph and $NHCH_2$), 8.15 (t, J = 5 Hz, 3H), 8.33 (d, J = 8 Hz, 3H, NH-Val), 8.54 (d, J = 9 Hz, 3H, NHCHN); ^{13}C NMR (75.44 MHz, [D$_6$]DMSO): δ = 18.33, 19.43, 29.94, 59.47, 70.35, 75.26, 116.12, 121.07, 124.25, 127.14, 127.14, 127.90, 128.12, 128.23, 128.30, 128.50, 128.60, 130.91, 133.02, 136.95, 137.15, 145.37, 151.77, 166.00, 166.78, 168.51, 171.03; IR (KBr): $\tilde{\nu}$ = 3300, 1650, 1570, 1520, 1460, 1310, 1270; FAB MS (NBA); *m/z* (%): 1920 (0.01) [M^+ + H]; $C_{111}H_{117}N_{13}O_{18}$ (1921.23): calcd C 69.39, H 6.14, N 9.48; found C 69.37, H 6.27, N 9.75.

(*SSS*)-N[BzGly*ValNHNHCOC$_6$H$_3$(OBn)$_2$]$_3$ (37) was prepared according to the procedure used for the preparation of **33** from hydrazine **36a** (610 mg, 1.36 mmol) and (*SSS*)-**23** (260 mg, 0.30 mmol). Yield: 435 mg (79%); M.p. 118 °C; $[\alpha]_D^{25}$ = + 2 (c = 1, DMSO); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.03 [d, J = 7 Hz, 18H, $CH(CH_3)_2$], 2.10–2.39 (m, 3H, $CH(CH_3)_2$), 4.22–4.43 (m, 3H, CH-Val), 5.03 (s, 6H, CH_2Ph), 5.16 (s, 6H, CH_2Ph), 5.77 (d, J = 9 Hz, 3H, NHC*H*N), 6.95–7.57 (m, 54H, Ph), 8.45–8.60 (m, 6H, NH-Val and N*H*CHN), 10.13 (br, 3H, N*H*NH), 10.35 (br, 3H, NHNH); ^{13}C NMR (75.44 MHz, [D$_6$]DMSO): δ = 18.52, 19.37, 30.56, 58.02, 63.90, 70.29, 116.63, 121.20, 124.34, 127.15, 128.01, 128.16, 128.21, 128.30, 128.60, 128.76, 129.01, 132.00, 132.92, 136.88, 137.09, 145.49, 151.85, 164.55, 166.96, 168.10, 169.44; IR (KBr): $\tilde{\nu}$ = 3300 (m, br), 3060 (w), 3040 (w), 2980 (w), 1660 (s), 1580 (m), 1520 (s), 1480 (s), 1450 (s), 1380 (m), 1220 (m), 1090 (m), 1050 (w), 1030 (w), 970 (w), 920 (w), 860 (w), 810 (w), 760 (m), 700 (m); FAB MS (NBA); *m/z* (%): 1860 (0.78) [M^+ + Na]; $C_{105}H_{105}N_{13}O_{18}$ (1837.07): calcd C 68.65, H 5.76, N 9.91; found C 68.91, H 5.93, N 9.92.

(*SSS*)-N(BzGly*ValNHCH$_2$CH$_2$NHC$_6$H$_3$(OH)$_2$)$_3$ (34): The protected catecholate derivative **33** (100 mg, 0.05 mmol) was dissolved in degassed methanol (15 mL), treated with palladium on carbon (10%, 10 mg) and stirred under an atmosphere of hydrogen for 24 h. The suspension was filtered under an atmosphere of argon. After evaporation of the solvent, a colourless solid was obtained. Yield: 68 mg (99%); ^{1}H NMR (300 MHz ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 0.97–1.05 (m, 18H, $CH(CH_3)_2$), 2.18–2.25 (m, 3H, $CH(CH_3)_2$), 3.26–3.54 (m, 12H, $NHCH_2CH_2NH$), 4.26 (m, 3H, CH-Val), 5.71 (d, J = 9 Hz, 3H, NHC*H*N), 6.64 (t, J = 8 Hz, 3H, Ph), 6.92 (d, J = 8 Hz, 3H, Ph), 7.18–7.23 (m, 6H, *m*-Ph), 7.29–7.65 (m, 12H, Ph), 8.55 (d, J = 9 Hz, 3H, NH-Gly), 8.74 (d, J = 8 Hz, 3H, NH-Val), 10.47, 10.83 (brs, 6H, $NHCH_2CH_2NH$); ^{13}C NMR (75.44 MHz, [D$_6$]DMSO): δ = 18.49, 19.39, 29.96, 58.44, 63.41, 68.01, 70.01, 70.36, 74.30, 74.48, 113.78, 117.53, 118.38, 119.41, 127.12, 128.31, 131.95, 133.07, 146.31, 149.61, 166.58, 168.22, 168.67, 170.58; FAB MS (NBA); *m/z* (%): 1403 (0.44) [M^+ + Na]; 1381 (0.05) [M^+ + H]; $C_{69}H_{81}N_{13}O_{18}$ (1380.48).

(*SSS*)-N(BzGly*ValNHNHC$_6$H$_3$(OH)$_2$)$_3$ (38) was prepared according to the procedure used for the preparation of **34** from **37** (100 mg, 0.05 mmol) and palladium on carbon (10%, 10 mg). Yield: 70 mg (99%), ^{1}H NMR (300 MHz, [D$_6$]DMSO:$CDCl_3$ 10:1): δ = 0.90–1.25 (m, 18H, $CH(CH_3)_2$), 2.10–2.28 (m, 3H, $CH(CH_3)_2$), 4.21 (m, 3H, CH-Val), 5.72 (d, J = 9 Hz, 3H, NHC*H*N), 6.50 (t, J = 9 Hz, 3H, Ph), 6.83 (d, J = 9 Hz, 3H, Ph), 7.05–7.50 (m, 18H, Ph), 8.41 (d, J = 9 Hz, 3H, NH-Gly), 8.89 (d, J = 7 Hz, 3H, NH-Val), 10.26 (s, 3H, OH), 10.85 (s, 3H, OH); FAB MS (NBA); *m/z* (%): 1319 (1.36) [M^+ + Na]; $C_{63}H_{69}N_{13}O_{18}$ (1296.32).

(*RRR*/*SSS*)-N(BzGly*GlyNHOBn)$_3$ (39) was prepared according to the procedure used for the preparation of **12** from N(BzGly*GlyOH)$_3$ (**22**) (300 mg, 0.42 mmol), *O*-benzylhydroxylamine hydrochloride (268 mg, 1.68 mmol), HOBt (454 mg, 3.36 mmol), NEM (0.42 mL, 3.36 mmol) and EDCI (322 mg, 1.68 mmol). The crude product was recrystallised from ethyl acetate/petroleum ether to give a colourless solid. Yield: 309 mg (71%); M.p. 128 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 3.74 (dd, J = 15, 6 Hz, 3H, CH_2-Gly), 3.95 (dd, J = 15, 6 Hz, 3H, CH_2-Gly), 4.82 (s, 6H, CH_2Ph), 5.76 (d, J = 9 Hz, 3H, NHC*H*N), 7.26 (t, J = 8 Hz, 6H, *m*-Ph), 7.30–7.54 (m, 24H), 8.55 (d, J = 9 Hz, 3H, N*H*CHN), 8.80 (m, 3H, NH-Gly), 11.42 (s, 3H, N*H*O); ^{13}C NMR (75.44 MHz, [D$_6$]DMSO): δ = 63.20, 77.25, 127.17, 128.29, 128.48, 129.05, 129.30, 131.95, 133.05, 135.91, 166.44, 166.58, 168.76, 194.13; FAB MS (NBA); *m/z* (%): 1057 (0.16) [M^+ + Na]; $C_{54}H_{54}N_{10}O_{12}$ (1035.08): calcd C 62.66, H 5.26, N 13.53; found C 62.31, H 5.35, N 13.55.

(*SSS*)-N(BzGly*ValNHOBn)$_3$ (40) was prepared according to the procedure used for the preparation of **12** from N(BzGly*ValOH)$_3$ (**23**) (300 mg, 0.35 mmol), *O*-benzylhydroxylamine hydrochloride (223 mg, 1.40 mmol), NEM (0.35 mL, 2.8 mmol), HOBt (378 mg, 2.80 mmol) and EDCI (268 mg, 1.40 mmol). The crude product was recrystallised from ethyl acetate/petroleum ether to give a colourless solid. Yield: 341 mg (84%), M.p. 257 °C; $[\alpha]_D^{25}$ = − 21 (c = 1, $CHCl_3$); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 0.91 [d, J = 7 Hz, 9H, $CH(CH_3)_2$], 0.97 [d, J = 7 Hz, 9H, $CH(CH_3)_2$], 2.04–2.25 [m, 3H, $CH(CH_3)_2$], 4.01–4.10 (m, 3H, CH-Val), 4.81 (s, 6H, CH_2Ph), 5.74 (d, J = 9 Hz, 3H, NHC*H*N), 7.23 (t, J = 8 Hz, 6H, *m*-Ph), 7.29–7.48 (m, 24H, Ph), 8.55 (d, J = 9 Hz, 3H, N*H*CHN), 8.64 (d, J = 7 Hz, 3H, NH-Val), 11.41 (s, 3H, N*H*O); ^{13}C NMR (75.44 MHz, [D$_6$]DMSO): δ = 18.68, 19.28, 29.94, 57.52, 63.69, 77.09, 127.15, 128.25, 128.39, 128.94, 131.91, 133.05, 135.97, 166.87, 167.81, 168.56; IR (KBr): $\tilde{\nu}$ = 3300, 3060, 2970, 2870, 1660, 1520, 1490, 1370, 1220, 1080, 1030, 800, 750, 670; FAB MS (NBA); *m/z* (%): 1183 (0.04) [M^+ + Na]. $C_{63}H_{72}N_{10}O_{12}$ (1161.33): calcd C 65.16, H 6.25, N 12.06; found C 64.99, H 5.96, N 11.99.

(*RRR*/*SSS*)-N(BzGly*GlyNHOH)$_3$ (41): The protected hydroxamic acid derivative (300 mg, 0.29 mmol) **39** was dissolved in methanol (30 mL), treated with palladium on carbon (10%, 30 mg) and stirred in an atmosphere of hydrogen for 24 h. The suspension was filtered through Celite under an atmosphere of argon. After evaporation of the solvent, a colourless solid was obtained. Yield: 211 mg (95%); ^{1}H NMR (300 MHz, $CDCl_3$:[D$_6$]DMSO 10:1): δ = 3.67 (dd, J = 13, 5 Hz, 3H, CH_2-Gly), 4.10 (dd, J = 16, 6 Hz, 3H, CH_2-Gly), 5.73 (d, J = 9 Hz, 3H, NHC*H*N), 7.06 (t, 8 Hz, 6H, *m*-Ph), 7.20–7.35 (m, 9H, Ph), 8.37 (d, J = 9 Hz, 3H, N*H*CHN), 8.78–8.97 (m, 3H, NH-Gly), 10.76 (br, 3H, NHOH); ^{13}C NMR (75.44 MHz, CD-Cl_3:[D$_6$]DMSO 10:1): δ = 62.35, 65.27, 126.90, 127.72, 131.25, 132.86, 166.52, 166.84, 168.48; FAB HRMS (NBA) calcd for $C_{33}H_{36}N_{10}O_{12}Na$ (M^+ + Na): 787.2347; found *m/e* 787.2412; $C_{33}H_{36}N_{10}O_{12}$ (764.71).

(*SSS*)-N(BzGly*ValNHOH)$_3$ (42) was prepared according to the procedure used for the preparation of **41** from N(BzGly*ValNHOBn)$_3$ (**40**) (300 mg, 0.26 mmol) and palladium on carbon (10%, 30 mg). Yield: 299 mg (99%); ^{1}H NMR (300 MHz, $CDCl_3$:[D$_6$]DMSO 10:1): δ = 0.72 (d, J = 7 Hz, 9H, $CH(CH_3)_2$), 0.75 (d, J = 7 Hz, 9H, $CH(CH_3)_2$), 1.87–2.01 (m, 3H, $CH(CH_3)_2$), 3.70–3.77 (m, 3H, CH-Val), 5.49 (d, J = 9 Hz, 3H, NHC*H*N), 6.83 (t, J = 7 Hz, 6H, *m*-Ph), 6.90–7.15 (m, 9H, Ph), 8.14 (d, J = 9 Hz, 3H, N*H*CHN), 8.28 (d, J = 8 Hz, 3H, NH-Val), 10.35 (br, 3H); ^{13}C NMR (75.44 MHz, $CDCl_3$:[D$_6$]DMSO 10:1): δ = 19.06, 19.37, 29.63, 58.10, 62.75, 127.20, 128.00, 131.52, 133.15, 167.47, 168.73, 169.10; IR (KBr): $\tilde{\nu}$ = 3380, 3270, 2960, 1660, 1580, 1520, 1490, 1320, 1160, 1130, 1080, 1050, 930, 890, 800, 710, 690; $C_{42}H_{54}N_{10}O_{12}$ (890.95).

***N,N′,N″*-[(*SSS*)-Nitrilotris[2-(benzoylamino)-1-oxo-2,1-ethanediyl]]tris-[*N*^{1}-[2-(*tert*-butoxycarbonylamino)ethyl]-L-valinamide] (43a)**: A solution of (*SSS*)-**23** (600 mg, 0.71 mmol) in THF (80 mL) was treated with **31** (568 mg, 3.55 mmol, in 5 mL of THF) [28] and NEM (0.36 mL, 2.84 mmol). After cooling to 0 °C, HOBt (576 mg, 4.26 mmol) and EDCI (544 mg, 2.84 mmol) were added. Stirring was continued for 1 h at 0 °C; then the reaction mixture was allowed to warm to room temperature (23 h). Ethyl acetate (40 mL) was added, and the organic layer was washed with saturated $NaHCO_3$ (40 mL), 2N HCl (40 mL), saturated $NaHCO_3$ (40 mL) and brine (40 mL). Drying ($MgSO_4$) and evaporation gave a colourless solid which was recrystallised from ethyl acetate/petroleum ether. Yield: 766 mg (85%); M.p. 235 °C; $[\alpha]_D^{25}$ = + 48.3 (c = 1.00, DMF); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 0.94 (d, J = 6 Hz, 9H, $CH(CH_3)_2$), 0.96 (d, J = 6 Hz, 9H, $CH(CH_3)_2$), 1.38 (s, 27H, $C(CH_3)_3$), 2.17 (m, 3H, $CH(CH_3)_2$), 3.02–3.06 (m, 6H, $NHCH_2CH_2NHBoc$), 3.09–3.23 (m, 6H, CH_2CH_2NHBoc), 4.17 (dd, J = 6 Hz, 3H, CH-Val), 5.71 (d, J = 9 Hz, 3H, NHC*H*N), 6.70 (m, br, 3H, $NHCH_2CH_2NHBoc$), 7.23 (dd, J = 7 Hz, 6H, *m*-Ph), 7.41–7.43 (m, 9H, Ph), 8.07 (brs, 3H, $NHCH_2CH_2NHBoc$), 8.56–8.61 (m, 6H, N*H*CHN, NH-Val); ^{13}C NMR (75.44 MHz, [D$_6$]DMSO): δ = 18.11, 19.24, 28.11, 29.75, 38.69, 39.48, 59.20, 63.38, 77.60, 126.92, 127.99, 131.66, 132.65, 155.47, 166.56, 168.25, 170.77; IR (KBr): $\tilde{\nu}$ = 3340, 3060, 2970, 2880, 1720, 1650, 1520, 1400, 1370, 1250, 1170, 1120, 1100, 1080, 1000, 930, 860, 810, 780, 720, 695; FAB MS (NBA); m/z (%) = 1295 (0.02) [M$^+$ Na], 1273 (0.05) [M^+ + H], 1272 (0.11) [M^+], 1172 (3.76) [M$^+$-NHCOC(CH$_3$)$_3$], 105 (100) [Ph-C≡O$^+$]; $C_{63}H_{93}N_{13}O_{15}$ (1272.51): calcd C 59.46, H 7.37, N 14.31, found C 59.45, H 7.39, N 14.31.

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***N,N′,N″*-[(*SSS*)-Nitrilotris[2-(benzoylamino)-1-oxo-2,1-ethanediyl]]tris-[*N*^{1}-[2-(2-ethoxy-3,4-dioxo-1-cycobuten-1-ylamino)ethyl]-L-valinamide] (45)**: **43a** (165 mg, 0.13 mmol) was dissolved in 10 mL of a 2N solution of trifluoroacetic acid in CH_2Cl_2. After 2 h of stirring at room temperature, the solution was evaporated in vacuo. The resulting oil was dried overnight on a high-vacuum line to provide a colourless solid (**43**). The crude product was taken up in ethanol (25 mL) and treated with NEM (0.08 mL, 0.64 mmol), and 3,4-diethoxy-3-cyclobutene-1,2-dione (**44**) (0.08 mL, 0.51 mmol). After 48 h of stirring at room temperature, the solution was evaporated to dryness. The resulting oil was dissolved in CH_2Cl_2 (20 mL) and washed with 2N HCl (20 mL), saturated $NaHCO_3$ (20 mL) and brine (20 mL). Drying ($MgSO_4$) and evaporation gave a colourless solid which was recrystallised from CH_2Cl_2/ether. Yield: 102 mg (58%); M.p. 195–197 °C; $[\alpha]_D^{25} = +159.16$ ($c = 1.00$, $CHCl_3$); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 0.92$ (d, $J = 6$ Hz, 18H, $CH(CH_3)_2$), 1.35 (t, $J = 7$ Hz, 9H, CH_2CH_3), 2.13 (m, $J = 6$ Hz, 3H, $CH(CH_3)_2$), 3.21–3.25, 3.26–3.37, 3.55 (m, 3H, m, 6H, m, 3H, $NHCH_2CH_2NH$), 4.18 (dd, $J = 6$ Hz, 3H, CH-Val), 4.63 (q, $J = 7$ Hz, 6H, CH_2CH_3), 5.70 (d, $J = 9$ Hz, 3H, NHCHN), 7.23 (dd, $J = 7$ Hz, 6H, *m*-Ph), 7.40–7.42 (m, 9H, Ph), 8.21, 8.68 (brs, 3H, $NHCH_2CH_2NH$), 8.49–8.58 (m, br, 6H, NHCHN, NH-Val); ^{13}C NMR (75.44 MHz, [D$_6$]DMSO): $\delta = 15.51$, 17.92, 19.23, 29.79, 38.79, 39.19, 43.04, 43.55, 59.12, 63.43, 126.90, 128.03, 131.72, 132.72, 166.62, 168.20, 170.94, 172.38, 172.72, 176.43, 176.94, 189.02, 193.74; IR (KBr): $\tilde{\nu} = 3280$, 3060, 2960, 1800, 1650, 1620, 1520, 1490, 1390, 1340, 1150, 1100, 1050, 1000, 810, 780, 720, 700; FAB MS (NBA): m/z (%) = 1367 (0.47) [$M^+ + Na$], 1345 (1.00) [$M^+ + H$], 1344 (1.55) [M^+], 105 (100) [Ph-C≡O$^+$]; $C_{66}H_{81}N_{13}O_{18} \cdot H_2O$ (1362.47): calcd C 58.18, H 6.14, N 13.36, found C 57.88, H 6.41, N 13.29.

(*RRR*/*SSS*)-N(BzGly*$CH_2OH)_3$ (46): **2b** (430 mg, 0.73 mmol) and $CaCl_2$ (486 mg, 4.38 mmol) were suspended in THF/ethanol (20 mL, 1:1), cooled to −5 °C, and treated with $NaBH_4$ (331 mg, 8.76 mmol). After 5 h of stirring at −5 °C, the suspension was carefully poured into a cooled solution (0 °C) of 10% citric acid and diluted with ethyl acetate (20 mL). The aqueous layer was extracted with ethyl acetate (2 × 10 mL). The combined organic extracts were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated to dryness. The crude product was recrystallised from methanol to give a colourless solid. Yield: 176 mg (68%); M.p. 179 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 3.60$–3.69 (m, 6H, CH_2OH), 5.00 (t, 7 Hz, 3H, CH_2OH), 5.12–5.30 (m, 3H, NHCHN), 7.20 (t, $J = 7$ Hz, 6H, *m*-Ph), 7.38 (t, $J = 8$ Hz, 3H, *p*-Ph), 7.46 (d, $J = 8$ Hz, 6H, *o*-Ph), 8.29 (d, $J = 9$ Hz, 3H, NHCHN); ^{13}C NMR (75.44 MHz, [D$_6$]DMSO): $\delta = 61.65$, 127.29, 127.99, 131.31, 133.99, 166.81. $C_{27}H_{30}N_4O_6$ (506.56): calcd C 64.02, H 5.97, N 11.06, found C 63.74, H 6.05, N 10.81.

(*SSS*)-*N*(BzGly*ValC$H_2OH)_3$ (47) was prepared according to the procedure used for the preparation of **46** from (*SSS*)-N(BzGly*ValOMe)$_3$ (**14**) (300 mg, 0.34 mmol), $CaCl_2$ (226 mg, 2.04 mg) and $NaBH_4$ (154 mg, 4.08 mmol). The crude product was chromatographed (SiO_2, acetone/CH_2Cl_2 1:2). Yield: 123 mg (45%); M.p. 211 °C, $[\alpha]_D^{25} = +26$ ($c = 0.8$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.98$ [d, $J = 7$ Hz, 18H, $CH(CH_3)_2$], 1.75–1.94 [m, 3H, $CH(CH_3)_2$], 3.45–3.52 (m, 3H, OH), 3.58–3.66 (m, 3H, CH-Val), 3.80–3.96 (m, 6H, CH_2OH), 5.86 (d, $J = 9$ Hz, 3H, NHCHN), 7.05 (t, $J = 8$ Hz, 6H, *m*-Ph), 7.22–7.32 (m, 9H, *o,p*-Ph), 7.76 (d, $J = 9$ Hz, 3H, NH), 8.46 (d, $J = 9$ Hz, 3H, NH); ^{13}C NMR (75.44 MHz, $CDCl_3$): $\delta = 19.26$, 19.76, 29.25, 58.04, 63.36, 63.77, 127.17, 127.86, 131.40, 132.83, 168.03, 169.74; IR (KBr): $\tilde{\nu} = 3340$, 3060, 2960, 2890, 1650, 1570, 1520, 1480, 1390, 1310, 1230, 1160, 1140, 1080, 1030, 780, 710, 700, 650; FAB MS (NBA): *m/z* (%): 826 (5.19) [$M^+ + Na$], 804 (2.12) [$M^+ + H$]; $C_{42}H_{57}N_7O_9$ (803.96); calcd C 62.75, H 7.15, N 12.20, found C 62.77, H 7.27, N 12.27.

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[21] Crystallographic data for (*SSS*)-**15**: $C_{39}H_{45}N_7O_{15}$, $M_r = 851.83$, space group *R*3 (no. 146), trigonal with $a = 10.319(1)$ Å, $\alpha = 100.052(7)°$, $V = 1041.2$ Å^3, $Z = 1$, $\rho_{calcd} = 1.358$ g cm^{-3}; $Mo_{K\alpha}$ radiation (23 °C); reflections collected 5802, unique reflections 1942, observed reflections 1888 [$I > 2\sigma(I)$]. Structure solution by SHELXS-86 and refinement by SHELXS-93, non-hydrogen atoms refined anisotropically, hydrogens with $U_i = 1.2\,U_{eq}$ of the adjacent atoms (C, O, N). Full-matrix refinement against F^2. Final *R*1 and *wR*2 values were 0.0363 and 0.0944 for 1888 reflections with $I > 2\sigma(I)$, 197 variables and 14 restraints. *R*1 = 0.0373 and *wR*2 = 0.0958 for all data. Weights: SHELXL-93. Maximum and minimum of the final fourier synthesis 0.177 and −0.122 e A^{-3} [39].

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[23] Crystallographic data for (*RRR*/*SSS*)-**22**: $C_{33}H_{33}N_7O_{12}\cdot 1.25\,CH_3OH$, $M_r = 759.72$, space group $P2_1/c$ (no. 14), monoclinic with $a = 20.802(7)$, $b = 9.767(2)$, $c = 18.554(9)$ Å, $\beta = 92.37(3)^\circ$, $V = 3766.5$ Å^3, $Z = 4$, $\rho_{calcd} = 1.340$ g cm^{-3}; $Mo_{K\alpha}$ radiation (23 °C); reflections collected 5151, unique reflections 4986, observed reflections 3536 [$I > 2\sigma(I)$]. Structure solution by SHELXS-86 and refinement by SHELXS-93, non-hydrogen atoms refined anisotropically, hydrogens with $U_i = 1.2\,U_{eq}$ of the adjacent atoms (C, O, N). Full-matrix refinement against F^2. Final $R1$ and $wR2$ values were 0.0595 and 0.1767 for 3536 reflections with $I > 2\sigma(I)$, 512 variables and 7 restraints. $R1 = 0.0636$ and $wR2 = 0.1393$ for all data. Weights: SHELXL-93. Maximum and minimum of the final fourier synthesis 0.554 and -0.272 e A^{-3} [39].

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[33] Crystallographic data for (*RRR*/*SSS*)-**46**: $C_{27}H_{30}N_4O_6$, $M_r = 506.55$, space group $P2_1/c$ (no. 14), monoclinic with $a = 11.068(3)$, $b = 13.730(2)$, $c = 17.257(5)$ Å, $\beta = 104.67(2)^\circ$, $V = 2536.9$ Å^3, $Z = 4$, $\rho_{calcd} = 1.326$ g cm^{-3}; $Mo_{K\alpha}$ radiation (23 °C); reflections collected 3713, unique reflections 3506, observed reflections 2784 [$I > 2\sigma(I)$]. Structure solution by SHELXS-86 and refinement by SHELXS-93, non-hydrogen atoms refined anisotropically, hydrogens with $U_i = 1.2\,U_{eq}$ of the adjacent atoms (C, O, N). Full-matrix refinement against F^2. Final $R1$ and $wR2$ values were 0.0483 and 0.1299 for 2784 reflections with $I > 2\sigma(I)$ and 337 variables. $R1 = 0.0636$ and $wR2 = 0.1393$ for all data. Weights: SHELXL-93. Maximum and minimum of the final fourier synthesis 0.360 and -0.345 e A^{-3} [39].

[34] The NH_3–THF solution was prepared by bubbling dry ammonia into 200 mL of THF over 10 min. To determine the ammonia concentration, 1 mL of the NH_3–THF solution was diluted with 10 mL water and titrated with a 1 N HCl standard solution against methyl orange.

[35] In the ^{1}H NMR spectrum only the crystalline (*SSS*) isomer is described.

[36] In the ^{1}H NMR spectrum only the crystalline (*RRR*) isomer is described.

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[38] His(π-Bom)-OMe·2 HCl was obtained by deprotecting commercially available Boc-His(π-Bom)-OMe·HCl with HCl in ethyl acetate (Boc = *tert*-butoxycarbonyl, Bom = benzyloxymethyl).[25a]

[39] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-100018. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (Fax: Int. code +(1223)336-033; e-mail: deposit@chemcrys.cam.ac.uk).

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