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Studies on Cyclic Dipeptides, II [1a]. Methylated Modifications of *cyclo*-[Phe-His]

C. R. Noe*, A. Weigand, S. Pirker, and **P. Liepert**

Institut für Pharmazeutische Chemie, Johann-Wolfgang-Goethe-Universität Frankfurt, Christian Doppler Laboratorium für Chemie chiraler Verbindungen, D-60439 Frankfurt/Main, Germany

Summary. Synthesis of seven new cyclic dipeptides and their conformational analysis based on ^{1}H NMR spectra is reported as well as their application as models to elucidate the mechanism of cyclic dipeptide catalysis in enantioselective mandelonitrile formation. Further evidence is presented to support the view that a highly ordered supramolecular complex of dipeptides acts as catalyst.

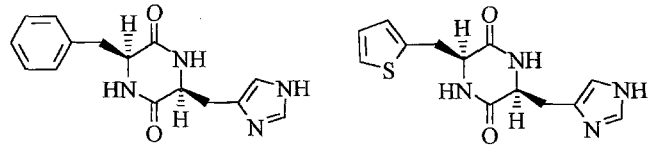
Keywords. (*S*,*S*)-cyclo-[Phe-His]; Cyanohydrin reaction; Enantioselectivity; Supramolecular catalyst complex.

Untersuchungen an cyclischen Dipeptiden, 2. Mitt. [1a]**: Methylierte Derivate von cyclo-[Phe-His]**

Zusammenfassung. Es wird die Synthese sieben neuer cyclischer Dipeptide, ihre Konformationsanalyse (basierend auf ^{1}H-NMR-Daten) und ihre Anwendung als Modelle zur Aufklärung des Mechanismus der dipeptidkatalysierten enantioselektiven Mandelsäurenitrilsynthese beschrieben. Weitere Hinweise zur Stützung der Annahme, daß ein hoch geordneter supramolekularer Komplex aus Dipeptiden für die Katalyse ausschlaggebend ist, werden vorgelegt.

Introduction

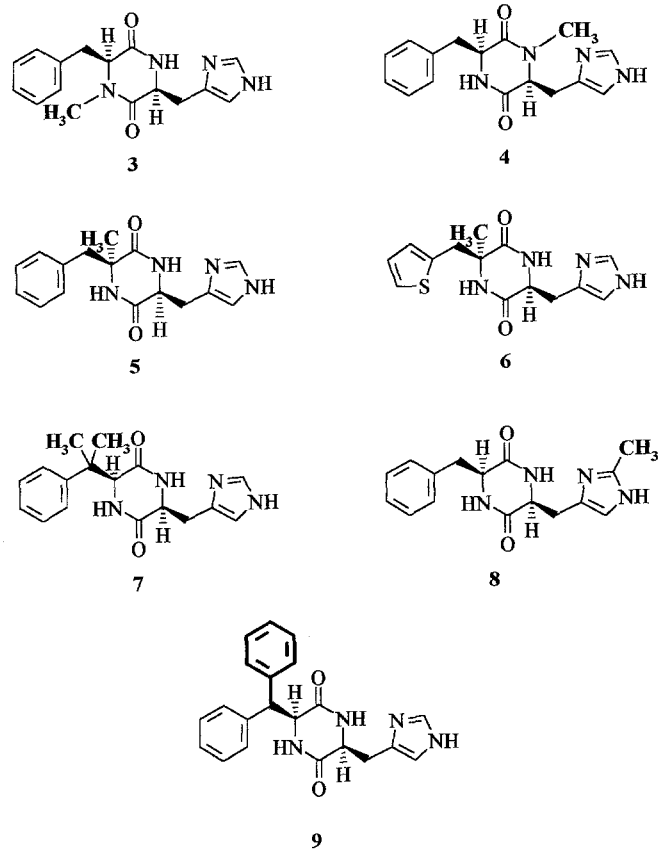
Recently we have reported the results of the application of new aryl analogues of the well known cyclic dipeptide catalyst (*S*,*S*)-*cyclo*-[Phe-His] (**1**) in enantioselective cyanohydrin formation [1a]. Surprisingly, the cyclic dipeptide system showed to be extremely sensitive towards any variation of its aromatic part, with only one of seven compounds-(*S*,*S*)-*cyclo*-[2-Thi-His] (**2**)-being at all catalytically active in the enantioselective formation of the cyanohydrin of benzaldehyde.



1 **2**

Scheme 1. *Catalyst for asymmetric cyanohydrin formation*

From several observations in these experiments we concluded – in contrast to hitherto published proposals [14] – that more than one catalyst molecule might be involved into the transition state [1a–c]. It is well known that cyclic dipeptides form supramolecular complexes *via* NH–CO bridges [13]. Therefore, in principle, it would not be surprising that a supramolecular aggregate is the active catalyst moiety. Nevertheless, it is rather difficult to prove the actual nature of such a transition state complex. To collect further evidence for the supramolecular nature of the catalyst, we have now prepared seven further cyclic dipeptide modifications of (*S,S*)-*cyclo*-[Phe-His] (**1**) – six of them methylated ones-performed conformational analysis, and tested their catalytic activity in cyanohydrin formation.



Scheme 2. *Synthesized cyclic dipeptides*

Results and Discussion

Applying general criteria for enzymatic reactions, four interaction sites would be required to accomodate optimum substrate-catalyst interaction: site 1 to arrange the aromatic ring, site 2 to orient the carbonyl group, site 3 to present the cyanide group, and site 4 to finish the reaction sequence by protonation of the cyanohydrin anion. To accomodate all these sites of interaction, two catalyst molecules in a strictly defined arrangement would be the minimum requirement. N-Methylation at

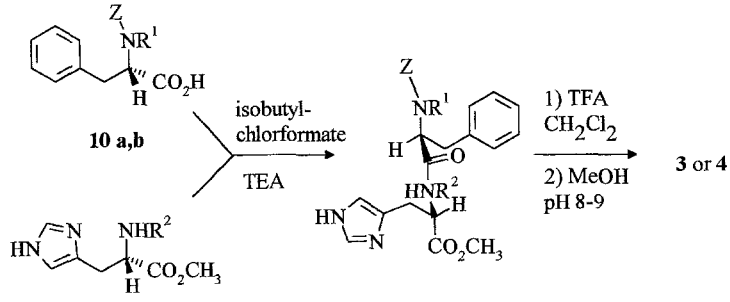
one of the ring nitrogen atoms would confine dipeptide molecule aggregation to dimer formation. Therefore, the N-methylated compounds (*S,S*)-*cyclo*-[(N-Me)-Phe-His] (**3**) and (*S,S*)-*cyclo*-[Phe-(N-Me)His] (**4**) were prepared and studied to contribute to the question whether two catalyst molecules bound *via* their CO–NH bonds might be sufficient to catalyze the cyanohydrin reaction.

Two further compounds, (*S,S*)-*cyclo*-[(α-Me)Phe-His] (**5**) and (*S,S*)-*cyclo*-[(α-Me)-2-Thi-His] (**6**), are also modifications with methyl substituents attached directly to the piperazine-2,5-dione ring. These compounds, together with (*S,S*)-*cyclo*-[(β,β-Me_2)Phe-His] (**7**) which is dimethylated at the side chain, were selected mainly to study the influence of conformational restriction of side chains on catalyst activity. Search for catalyst molecule positions which tolerate modifications was a further objective in the selection of these compounds and of (*S,S*)-*cyclo*-[Phe-(2-Me)His] (**8**) which is methylated at the imidazole ring.

Finally, positioning of a second phenyl group at the phenylalanine side chain in (*S,S*)-*cyclo*-[Dip-His] (**9**) aimed at studying the effect of introducing an alternative site for aryl interaction on cyanohydrin formation.

N-Methylated Compounds

Following the procedure given by *Inoue* [5b, 7], the N-methylated protected amino acid (*S*)-*Z*-N-methylphenylalanine (**10a**) was coupled with (*S*)-histidine methylester (**11a**) to yield the protected linear dipeptide **12a**. In the same way, (*S*)-*Z*-phenylalanine (**10b**) was reacted with (*S*)-N-methylhistidine methylester (**11b**) to yield the linear dipeptide **12b**. After hydrogenolytic cleavage of the *Z*-group, ring closure was achieved by heating **12a** and **12b** without solvent for two hours *in vacuo* at 110–120°C [8]. Recrystallization from water or methanol gave pure N-methylated cyclic dipeptides **3** and **4**, respectively.



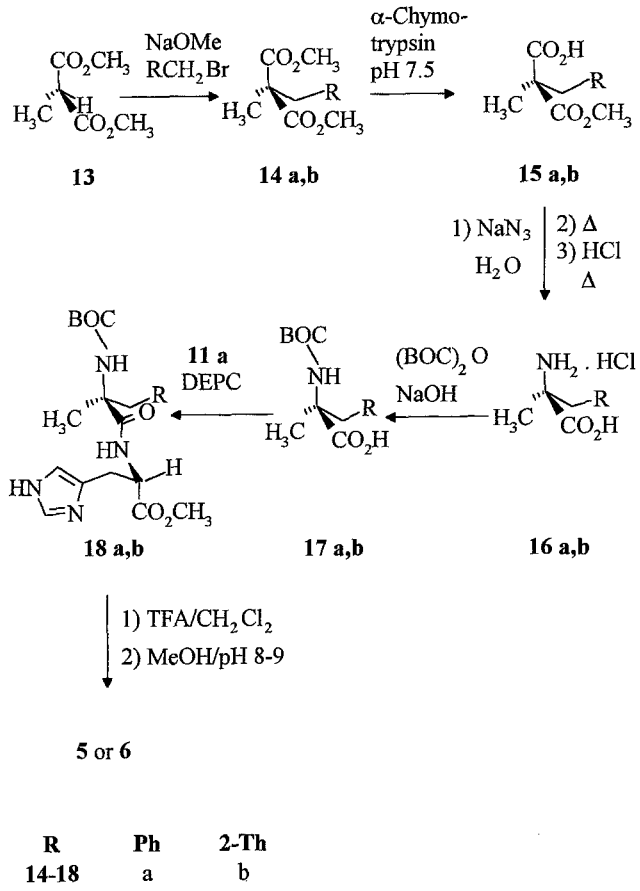
10-12	a	b
R^1	CH_3	H
R^2	H	CH_3

Scheme 3. *Synthesis of the N-methylated dipeptides* **3** *and* **4**

α-Methylated Compounds

Synthesis of dipeptides **5** and **6** involved α-methyl-amino acids **16a** and **16b** which were synthesized *via* an enzymatic approach [2]. Thus, dimethyl methylmalonate

(**13**) was deprotonated using sodium methylate in methanol and alkylated with benzyl bromide and thienyl bromide, respectively. The resulting arylmethyl substituted dimethyl methylmalonates **14a** and **14b** were subjected to enzymatic hydrolysis with α-chymotrypsin which proceeded enantiospecifically to yield the (*R*)-monoesters **15a** and **15b**. Using (*S*)-1-phenylethanamine in deuteriochloroform as an auxiliary for ^{1}H NMR spectroscopy, enantiomeric purity was found to exceed 98%.

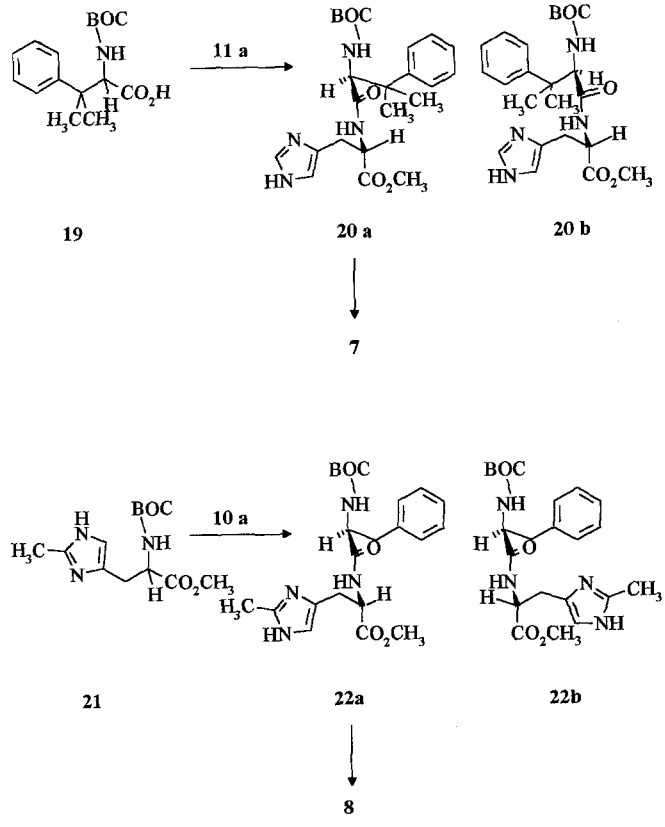


Scheme 4. *Synthesis of the α-methylated cyclic dipetides* **5** *and* **6**

Compounds **15a** and **15b** were subjected to *Curtius* rearrangement which yielded enantiopure α-methylated amino acids **16a** and **16b**. For the latter compound, this synthesis is the first one reported to apply an enzymatic method. *BOC* protection of the amino groups of **16a** and **16b** succeeded in 75% yield of **17a** and **17b** after four days reaction time, a considerable progress for this reaction which has been hitherto reported to give only bad or no yields and to require very long reaction times of up to four weeks [3]. *DEPC* (diethyl cyanophosphonate) was used as an activating reagent in the coupling reaction of **17a** and **17b** with (*S*)-histidine methylester (**11a**, [4]). After deprotection of the linear compounds **18a** and **18b** using trifluoroacetic acid, the deprotected dipeptides were refluxed in methanol for 48 hours [5] to yield the cyclic dipeptides **5** and **6**.

β-Substituted and Histidine-Methylated Compounds

In the synthesis of dipeptides **7** and **8**, a different strategy was employed. In each case, one of the amino acid derivatives was applied as a racemate. Thus, racemic (*R**,*S**)-N-*BOC*-β,β-dimethylphenylalanine (**19**, [10]) was reacted with (*S*)-histidinemethylester (**11a**) to yield the linear dipeptide **20a** in a 1:1 mixture with its diastereomer **20b**. In a similar manner, (*S*)-N-Z-phenylalanine (**10a**) was reacted with racemic (*R**,*S**)-2-methylhistidine methylester (**21**, [11]) to yield **22a** and its diastereomer **22b**. The diastereomeric mixtures of the protected linear dipeptides **20a/22b** and **22a/22b** were deprotected with trifluoroacetic acid and cyclized in methanol at $pH = 8$–9. Upon recrystallization from water, the diastereomers resulting from **20b** and **22b**, respectively, were removed, and diastereomerically pure cyclic dipeptides **7** and **8** were obtained.

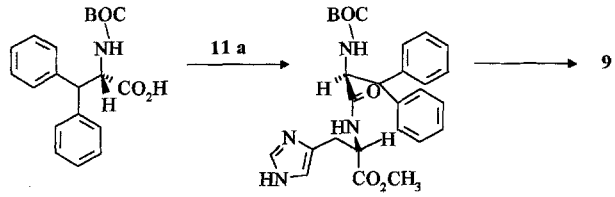


Scheme 5. *Synthesis of β,β-disubstituted cyclic dipeptides* **7** *and* **8**

Aryl modified (*S*)-N-*BOC*-β,β-diphenylalanine (**23**, [9]) was reacted with **11a** to yield the linear dipeptide **24** which was deprotected and cyclized to the cyclic dipeptide **9**.

Formation of Mandelonitrile

Mandelonitrile formation as well as determination of conversion rate and of enantioselectivity were performed as described previously [1]. Catalyst activation with



Scheme 6. *Synthesis of* **9**

Table 1. Optical and chemical yield of the formation of mandelonitrile with the cyclic dipeptides and state of reaction

Catalyst	Selectivity (%ee)/ conversion (%)	State of reaction
1	$92/80^{R}$	gelatinous
3	$0/<10^{R}$	solution
4	$0/<10^{R}$	solution
5	$15/50^{R}$	gelatinous
6	$0/<10^{R}$	gelatinous
7	$60/20^{R}$	gelatinous
8	$22/10^{S}$	gelatinous
9	$36/20^{S}$	gelatinous

R Preferred formation of (*R*)-mandelonitrile; *S* preferred formation of (*S*)-mandelonitrile

basic ion exchange resin [1] guaranteed reproducible results in the experiments concerning conversion rates and selectivities. The results are summarized in Table 1 which shows that none of the newly prepared cyclic dipeptides catalyzed mandelonitrile formation in high chemical or optical yields.

N-Methylated dipeptides **3** and **4** were insoluble in toluene, but dissolved upon addition of benzaldehyde to the reaction mixture. Only low conversion rates and no asymmetric induction were observed. In contrast, dipeptides **5** and **6** formed a gelatineous reaction mixture like catalyst **1**. Nevertheless, only **5**, which differs from **1** in only one position, exhibited very weak catalytic activity with 50% chemical yield and 15% enantiomeric excess. The thiophene isoster **6**, which is modification of **1** in two positions, did not catalyze the formation of mandelonitrile at all. Also dipeptides **7**, **8**, and **9**, which are not substituted at the piperazine-2,5-dione directly, formed a gelatinous reaction mixture and exhibited catalytic activity with some asymmetric induction but only poor conversion rates. The dimethylated compound **7** gave the best results with respect to conversion rate and selectivity catalyzing the formation of (*R*)-mandelonitrile.

Introduction of a second phenyl ring into the catalyst as in compound **9** led to the preferred formation of (*S*)-cyanohydrin. Even more surprisingly, the same inversion of selectivity was observed for the dipeptide from 2-methylhistidine (**8**).

Table 2. Preferred conformations of the cyclic dipeptides in *DMSO*-d_6 solutions calculated by the modified *Karplus* equations

	Phe side chain			His side chain		
	g^+(%)	t(%)	g^-(%)	g^+(%)	t(%)	g^-(%)
1	90	−1	11	24	11	65
3	–	–	–	24	0	75
4	–	–	–	26	−4	78
5	88	−10	22	18	3	79
6	64	10	26	47	0.5	52.5
7	–	–	–	26	−1	75
8	80	1	19	28	0	72
9	–	–	–	30	3	67

Conformational Analysis of Cyclic Dipeptides

Calculations of fractional populations were carried out using the *Sheinblatt* equations already described previously [1] with N-methylated products **3** and **4** and the 2-methylimidazole compound **8** being calculated usual. α-Methylated cyclic dipeptides **5** and **6** and β-substituted products **7** and **9** only gave indirect information about fractional populations of the aromatic side chains due to the lack of their α- or β-hydrogen atoms. Fractional populations of the histidine side chain in these dipeptides together with shift differences were nevertheless good indicators for a prediction of averaged fractional populations of the aromatic side chain.

N-Methylated cyclic dipeptides **3** and **4** show distribution patterns for the fractional populations of the aromatic and the histidine side chain very similiar to that of (*S*,*S*)-*cyclo*-[Phe-His] (**1**). The aromatic part prefers a conformation in which it is folded over the piperazine-2,5-dione ring, whereas the imidazole ring is directed toward the NH group. In **4**, the *g* population of the histidine side chain decreases to 52% which may be attributed to steric hindrance exerted by the methyl substituent at the amide group. Also, with the 2-methylimidazole cyclic dipeptide **8**, the distribution of fractional populations is very similar to that of the active catalyst **1**. With a value of 80% the aryl side chain prefers the position in which it is folded over the piperazine-2,5-dione ring. The histidine side chain occupies the *g* position to a high percentage. Conformational analysis of the α-methylated compounds **5** and **6** and the β-substituted products **7** and **9** could rely only on a calculation of the distribution of the fractional population of the histidine side chain. Again, distribution of the different conformers is similar to that of (*S*,*S*)-*cyclo*-[Phe-His] (**1**). Accordingly, it may be assumed that the distribution of the fractional population of the aromatic side chain is also similiar to that of **1**. With the conformations of all seven cyclic dipeptides **3**–**9** differing only slightly from **1**, no significant reason for the inactivity of the new compounds in the asymmetric cyanohydrin formation could be derived from conformational analysis in solution.

Conclusions

Together with the seven newly prepared cyclic dipeptides **3**–**9**, a total of 15 compounds has now been prepared and tested for catalytic activity in cyanohydrin

formation. Conformational analyses of the dipeptides revealed that most of them had a distribution pattern of fractional populations similar to that of the parent molecule **1**. Nevertheless, of all these presumptive catalysts only compounds **2** and **7** led to significant asymmetric induction, although they were by far inferior compared to **1**. This negative overall result from a series of minor modifications of a catalyst molecule supports the view that the catalytically active species in this asymmetric synthesis of cyanohydrins is not a single dipeptide molecule (in solution), but rather a highly ordered supramolecular structure requiring considerable steric demand for its formation.

It was mentioned above that, applying criteria of enzymatic action, at least two catalyst molecules in a strictly defined arrangement would be required to accomodate all interaction sites required for efficient catalysis. It is known that piperazine-2,5-diones generally exhibit a high tendency to form linear (chains) or planar (nets) supramolecular aggregates *via* their NH–CO bonds [13]. Consequently, two dipeptide molecules connected *via* their NH–CO bonds would be the minimum size catalyst species. In the N-methylated cyclic dipeptides **3** and **4**, only one of the two CO–NH moieties is suited for formation of two hydrogen bridge bonds. Therefore, their ability to form such supramolecular aggregates is reduced to dimer (or trimer in case of net type aggregation) formation. Experiments with **3** and **4** and mixtures of both compounds revealed that N-methylation of the piperazine-2,5-dione backbone resulted in a complete loss of catalytic activity. Obviously, two neighboured dipeptide molecules connected *via* their CO–NH bonds do not comply with the minimum requirement of a catalytically active species.

With active catalyst **1**, a gel is formed from its crystalline suspension in toluene upon addition of hydrocyanic acid and benzaldehyde (with improperly prepared **1**, the reaction mixture will not change from a suspension to the gelatineous state). The fact that suspensions of the cyclic dipeptides **3** and **4** in toluene did not form such a gel but a clear solution during cyanohydrin formation corresponds to some degree to the observation of other authors who described that with catalyst **1** formation of a gel during the reaction is essential for obtaining enantioselectivity [12]. Increased lipophilicity of the piperazine-2,5-dione backbone by N-methylation in **3** and **4** and their inability to form long supramolecular chains (or nets) might explain the solution state of the reaction mixture instead of the gelatineous state. The described behaviour of catalytically active **1** gives a hint to further supramolecular order caused by HCN interactions (most probably with the imidazole ring of the dipeptide). These interactions appear in addition to the multiple CO–NH hydrogen bridges and might lead to a catalytically active species, macroscopically appearing as the gelatineous state, in which two or more supramolecular dipeptide chains are associated to each other.

With α-methylated piperazine-2,5-diones **5**, the catalytic activity decreased dramatically and disappeared completely with introduction of a second modification in **6**. Both introduction of the methyl group in compound **5** and introduction of the two methyl groups in compounds **7** lead to conformational restrictions of the benzyl side chain which should favour the dominant g^+ conformation even more. Rather good results with **7** concerning enantioselectivity allow an interpretation according to which the g^+ conformation appears in fact to be the conformation of the catalytically active species, whereas introduction of the rather bulky methyl groups might disturb the contact of the reacting benzaldehyde molecule with the catalyst or catalyst molecule association leading to a reduced catalytic activity.

Inversion of enantioselectivity with catalyst **9** is not surprising in principle, because the second aryl group opens alternative options for catalyst-substrate aryl-aryl interaction. With the 2-methylimidazole modification **8**, however, an explanation for this phenomenon without assuming a role of inter-chain interactions seems to be difficult.

Further evidence for supramolecular arrangement of (*S,S*)-*cyclo*-[Phe-His]-molecules (**1**) in asymmetric cyanohydrin formation will be presented in the near future.

Experimental

Melting points: Kofler Mikroskop-Heiztisch, uncorrected; NMR: Bruker AC 300 (^{1}H: 300.13 MHz, ^{13}C: 75 MHz); elemental analyses: Microanalytical Department of the Institute für Organic Chemistry, University of Frankfurt; GC: Shimadzu GC 17a with FID, Permabond OV-1-DF-5.00 column (25 m × 0.32 mm), 1.6 ml/min (1 bar) helium carrier gas; TLC: Merck silica gel plates 60 F_{254}, detection of the compounds by treatment with a solution 5% molybdatophosphoric acid hydrate in ethanol or 0.1% ninhydrin in ethanol; CC: Merck silica gel 60 (mesh size 63–100 μm). **10a** [6b–c], **10b** [6a], **19** [10], **21** [11], and **23** [9] were prepared as described in the literature. 2-(Bromomethyl)thiophene was synthesized according to *Dittmer et al.* [15]. (*S*)-Histidine methylester (**11a**) and (*S*)-N-*Z*-phenylalanine (**10b**) were purchased from Fluka, dimethyl methylmalonate (**13**) from Aldrich, and α-chymotrypsin [E.C. 3.4.21.1] from Sigma. All reagents used in the synthesis were of standard laboratory quality.

2-Methyl-2-(2-thienylmethyl)-propanedioic acid dimethylester (**14b**)

2.8 g (121 m) clean cut sodium were dissolved in 150 ml dry methanol. 17.7 g (121 mmol) **13** were added, and the mixture was stirred at room temperature for 1 h. 14.4 g (126 mmol) 2-(Bromomethyl)thiophene were added, and the mixture was refluxed for 30 min. Most of the solvent was removed *in vacuo*, and 100 ml aqueous 0.5 *M* HCl were added. Extraction with diethyl ether (3 × 100 ml), drying over sodium sulfate, and destillation yielded a coulourless oil.

Yield: 14.6 g (50%); b.p.: 95°C/0.015 Torr; TLC (diethyl ether/petrolether = 1:2): $R_f = 0.4$; ^{1}H NMR ($CDCl_3$): $\delta = 7.18$ (d; 1H, H-5-Th), 6.96 (m; 1H, H-4-Th), 6.85 (d; 1H, H-3-Th), 3.80 (s; 6H, OCH_3), 3.48 (s; 2H, CH_2), 1.45 (s; 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 171.7$ (s; CO), 137.3 (s; C-2-Th), 127.4 (d; C-3-Th), 126.5 (d; C-4-Th), 124.6 (d; C-5-Th), 58.4 (s; C), 54.7 (q; OCH_3), 35.4 (t; CH_2), 19.5 (q; CH_3) ppm.

(R)-2-Methyl-2-(2-thienylmethyl)-propanedioic acid methylester (**15b**)

2 g (96 000 units) α-chymotrypsin [E.C.3.4.21.1.] were suspended in 400 ml buffered batches containing *DMSO* (25%) with *tris*(hydroxymethyl)aminomethane (*tris*-HCl, 0.375 *M*, *pH* = 7.5) 2.7 g (11.2 mmol) **14b** were added to this suspension, and the mixture was stirred at room temperature. Conversion was observed using TLC. Over a period of 5 days, additional **14b** was added until a total of 7.8 g (32.2 mmol) was reached. After completion of the reaction, the mixture was acidified with 2 *N* HCl, and the product was extracted with diethyl ether. Washing with sodium hydrogencarbonate, acidification with 2 *N* HCl, and extraction with diethyl ether yielded colourless crystals.

Yield: 5.9 g (80%); m.p.: 59–61°C; TLC (diethyl ether/petrolether = 1:2): $R_f = 0.1$; ^{1}H NMR ($CDCl_3$): $\delta = 7.20$ (d; H-5-Th), 6.97 (m; 1H, H-4-Th), 6.88 (d; 1H, H-3-Th), 3.82 (s; 3H, OCH_3), 3.50 (dd; 2H, CH_2), 1.49 (s; 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 177.0$ (s; CO), 171.8 (s; CO), 137.2 (s; C-2-Th), 127.7 (d; C-3-Th), 126.8 (d; C-4-Th), 124.9 (d; C-5-Th), 54.9 (s; C), 52.8 (q, OCH_3), 35.6 (t; CH_2), 19.8 (q; CH_3) ppm.

(S)-α-Methyl-2-thienylalanine hydrochloride (**16b**)

3 g (13.1 mmol) **15b** were dissolved in 3 ml water and 7 ml acetone at 0°C. 2.1 ml (15.3 mmol) triethylamine in 5 ml acetone and then 1.75 ml (18.3 mmol) ethyl chloroformate in 2 ml acetone were added slowly. After 30 min at 0°C, 1.2 g (18.3 mmol) sodium azide in 2 ml water were added dropwise. One hour later, the mixture was poured into an excess of ice water and extracted twice with diethyl ether, dried over sodium sulfate, and concentrated under reduced pressure. The crude product was dissolved in 25 ml toluene and heated to 100°C until no more nitrogen was evolved. Removal of toluene *in vacuo* gave almost pure isocyanate which was suspended in 20 ml 20% HCl and refluxed for 3 h. The solution was cooled and the crystallized product filtered.

Yield: 2.6 g (90%); m.p.: 230–232°C; ^{1}H NMR (D_2O): $\delta = 7.31$ (m; 1H, H-5-Th), 7.00 (m; 1H, H-4-Th), 6.93 (m; 1H, H-3-Th), 3.55 (d; 1H, H_l of CH_2), 3.30 (d; H_h of CH_2). 1.62 (s; 3H, CH_3) ppm; ^{13}C NMR (D_2O): $\delta = 176.2$ (s; CO), 136.6 (s; C-2-Th), 131.3 (d; C-3-Th), 130.2 (d; C-4-Th), 129.0 (d; C-5-Th), 63.3 (s; C), 38.8 (t; CH_2), 24.2 (q; CH_3) ppm.

BOC-Amino Acids (**17a,b**)

12.0 mmol **16** in 15 ml *THF*/water (2:1) were treated at 0°C with 12 ml 1 *N* NaOH and 1.35 g (6.2 mmol) pyrocarbonic acid di-*tert*.butylester. After stirring for 1 h at 0°C, the solution was warmed to room temperature, treated again with 1.35 g (9.2 mmol) $(BOC)_2O$ and 3 ml 1 *N* NaOH, and stirred for 24 h. The solution was evaporated *in vacuo*, the aqueous residue acidified at 0°C with 2 *N* HCl and extracted four times with ethyl acetate. The combined organic layers were extracted with aqueous potassium carbonate (10%), the aqueous layer once again acidified with 2 *N* HCl to $pH = 3$, and extracted with ethyl acetate. The combined ethyl acetate layers were dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo*.

(S)-2-(((1,1-Dimethyl)ethoxycarbonyl)amino)-2-methyl-3-phenylpropanoic acid (**17a**)

Yield: 60% ([2]: 65%); colourless crystals; m.p.: 156°C; TLC (ethyl acetate/methanol = 4:1); $R_f = 0.54$; ^{1}H NMR ($CDCl_3$): $\delta = 8.95$ (bs; 1H, COOH), 7.40–7.15 (m; 5H, aromatic H), 5.10 (bs; 1H, NH), 3.35 (bs; 2H, CH_2), 1.60 (bs; 3H, CH_3), 1.50 (s; 9H, CH_3) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 178.9$ (s; CO), 158.0 (s; CO), 137.8 (s; C-1-Ph), 130.2 (d; C-2, 6-Ph), 128.1 (d; C-3, 5-Ph), 126.9 (d; C-4-Ph), 79.8 (s; C), 60.0 (s; C), 41.3 (t; CH_2), 28.3 (q; CH_3), 23.5 (q; CH_3) ppm.

(S)-2-(((1,1-Dimethyl)ethoxycarbonyl)amino)-2-methyl-3-(2-thienyl)propanoic acid (**17b**)

Yield: 74%; yellow crystals; m.p.: 117–119°C; TLC (ethyl acetate/methanol = 4:1): $R_f = 0.3$; ^{1}H NMR ($CDCl_3$): $\delta = 7.19$ (d; 1H, H-5-Th), 6.96 (m; 1H, H-4-Th), 6.85 (d; 1H, H-3-Th), 5.22 (bs; 1H, NH), 3.58 (bs; 2H, CH_2), 1.57 (bs; 3H, CH_3), 1.49 (s; 9H, CH_3) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 179.5$ (s; CO), 154.9 (s; CO), 139.3 (s; C-2-Th), 126.6 (d; C-3-Th), 126.2 (d; C-4-Th), 123.9 (d; C-5-Th), 79.3 (s; C), 60.3 (s; C), 35.5 (t; CH_2), 28.3 (q; CH_3), 23.6 (q; CH_3) ppm.

N-Protected Dipeptide Methylesters (**12a,b**, **18a,b**, **22a,b**, **24**)

Procedure for **18a,b**, **20a,b**, **22a,b**, **24**

A solution of 30 mmol histidine methylester derivative dihydrochloride (**11a** or **21**), 90 mmol triethylamine, 30 mmol *DEPC*, and 26.6 mmol N-protected phenylalanine derivative (**10a**, **17a,b**, **19**, or **23**) in 60 ml *DMF* was stirred for 4 h at 0°C and for 48 h at room temperature. *DMF* was removed *in vacuo*, the residue partitioned between ethyl acetate and water (3:1), and the aqueous layer

extracted twice with ethyl acetate. The combined organic layers were washed three times with aqueous potassium carbonate (10%), dried over anhydrous sodium sulfate, and evaporated to dryness *in vacuo*. The resulting yellow oil was chromatographed over 200 g silica gel with ethylacetat/methanol (9:1). The products formed colourless foams.

Procedure for **12a,b**

a) A suspension of 3.6 g (15.2 mmol) (*S*)-histidine methylester dihydrochloride (**11a**) of (*R,S*)-N-methylhistidine methylester dihydrochloride (**11b**) and 3.1 g (30.4 mmol) dry triethylamine in 17 ml anhydrous *THF* was stirred for 3 h at room temperature.

b) 5 g (14.3 mmol) *Z*-N-methylphenylalanine (**10a**) or *Z*-phenylalanine (**10b**) were dissolved in 40 ml anhydrous *THF*. To this solution, 1.45 g (14.3 mmol) triethylamine, 1.95 g (14.3 mmol) 2-methyl-propylchloroformate, and suspension a) which was also cooled to −20°C were added at −20°C. The reaction mixture was stirred for 90 min at 0°C and then warmed to room temperature during 12 h. The solvent was removed *in vacuo*, and the residue was partitioned between 75 ml ethyl acetate and water (3:1). The organic layer was washed with 10% aqueous potassium carbonate, saturated sodium chloride, 0.5 *M* boric acid, and water and dried over sodium sulfate. Ethyl acetate was distilled off *in vacuo*, and the crude product was chromatographed over 200 g silica gel using ethyl acetate/methanol (10:1). The pure products formed colourless foams.

(S(R,R*))-N_α-(N-Methyl-((N-phenylmethoxy)carbonyl)phenylalanyl)-histidine methylester* (**12a**)

Yield: 84%; m.p.: 88–90°C; TLC (ethyl acetate/methanol = 4:1): $R_f = 0.5$; ^{1}H NMR ($CDCl_3$): $\delta = 7.90$ (d; 1H, NH), 7.40–7.10 (m; 11H, aromatic H, NH), 6.70 (s; 1H, H-5-Im), 5.10 (s; 2H, OCH_2), 4.95 (m; 1H, α-Ch-Phe), 4.75 (m; 1H, α-CH-Im), 3. 65 (s; 3H, OCH_3), 3.45/3.10 (ddd; 2H, CH_2-Phe), 3.05 (m; 2H, CH_2-Im), 2.80 (s; 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 171.4$ (s; CONH), 170.3 (s; COOMe), 156.9 (s; OCONH), 137.4 (s; C-1-Ph), 136.3 (s; C-4-Im), 135.2 (d; C-2-Im), 128.7/128.4/127.9/127.6/126.5 (d; C-2,2″,3,3″,4,4″-Ph), 116.5 (d; C-5-Im), 67.2 (t; OCH_2), 61.0 (d; α-CH-Phe), 52.6 (d; α-CH-His), 52.2 (q; OCH_3), 34.0 (t; CH_2-Phe), 31.4 (q; NCH_3), 28.6 (t; CH_2-His) ppm; $C_{25}H_{28}N_4O_5 \cdot 1.9\ H_2O$ (498.75); calcd.: C 60.21, H 6.43, N 11.23; found: C 60.25, H 6.04, N 11.42.

(S(R,R*))-N_α-Methyl-N_α-(N-((phenylmethoxy)carbonyl)phenylalanyl)histidine methylester* (**12b**)

Yield: 48%; m.p.: 78–84°C; TLC (ethyl acetate/methanol = 4:1): $R_f = 0.5$; ^{1}H NMR ($CDCl_3$): δ =7.20–7.03 (m; 11H, aromatic H, H-2-Im, NH), 6.70 (s; 1H, H-5-Im), 5.83 (d; 1H, NH), 5.22 (m; 1H, α-CH-Phe), 5.05 (s; 2H, OCH_2), 4.60 (m; 1H, α-CH-Im), 3.70 (s; 3H, OCH_3), 3.27 (dd; 1H, H_l of CH_2-Phe), 3.15–2.95 (m; 3H, H_h of CH_2-Phe and CH_2-Im), 2.78 (s; 3H, NCH_3) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 1.72.1$ (s; CONH), 170.7 (s; COOMe), 155.9 (s; OCONH), 136.1 (s; C-1-Ph), 135.9 (s, C-4-Im), 135.2 (d; C-2-Im), 129.5/128.4/126.9 (d; C-2,2″,3,3″,4,4″-Ph), 118.0 (d; C-5-Im), 66.7 (t; OCH_2), 57.7 (d; α-CH-Phe), 52.5 (d; α-CH-Im), 52.3 (q; OCH_3), 34.7 (CH_2-Phe), 32.7 (q; NCH_3), 26.1 (t; CH_2-Im) ppm; $C_{25}H_{28}N_4O_5 \cdot 1.1\ H_2O$ (495.15); calcd.: C 60.64, H 6.39, N 11.32; found: C 60.64, H 6.11, N 11.26.

(S(R,R*))-N-(2-(((1,1-Dimethyl)ethoxycarbonyl)amino)-2-methyl-1-oxo-3-phenylpropyl)histidine methylester* (**18a**)

Yield: 97%; m.p.: 151–152°C; $[\alpha]_D^{20} = -17.8°$ ($c = 1.62$ in CH_2Cl_2); TLC (ethyl acetate/methanol = 4:1): $R_f = 0.47$; ^{1}H NMR ($CDCl_3$): $\delta = 7.60$ (s; 1H, H-2-Im), 7.35–7.10 (m; 5H, aromatic H), 6.80 (s; 1H, H-5-Im), 4.80 (m; 1H, α-CH-Im), 3.75 (s; 3H, OCH_3), 3.40–3.10 (m; 4H, CH_2-Phe,

CH_2-Im), 1.50 (s, 9H, CH_3), 1.40 (s; 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 173.4$ (s; CONH), 171.3 (s; COMe), 155.7 (s; OCONH), 146.8 (s; C-2-Im), 135.8 (d; C-4-Im), 130.7 (d; C-2,6-Ph), 128.2 (d; C-3,5-Ph), 127.0 (d; C-4-Ph), 117.8 (d; C-5-Im), 81.0 (s; OC), 59.9 (s; C), 53.3 (d; α-CH-Im), 52.6 (q; OCH_3), 41.3 (t; CH_2-Phe), 28.5 (q; CH_3), 27.7 (t; CH_2-Im), 23.8 (q; CH_3) ppm; $C_{22}H_{30}N_4O_5 \cdot 0.44\ H_2O$ (430.50); calcd.: C 60.27, H 7.10, N 12.78; found: C 60.29, H 6.97, N 12.60.

(S(R,R*))-N-(2-(((1,1-Dimethyl)ethoxycarbonyl)amino)-3-(2-methyl-(2-thienylmethyl)-1-oxypropyl)-histidine methylester* (**18b**)

Yield: 64%; $[\alpha]_D^{20} = -89°$ ($c = 2$ in methanol); TLC (ethyl acetate/methanol = 4:1): $R_f = 0.6$; ^1H NMR ($CDCl_3$): $\delta = 7.53$ (s; 1H, H-2-Im), 7.40 (bs; 1H, NH), 7.21 (d; 1H, H-5-Th), 6.98 (d; 1H, H-4-Th), 6.86 (d; 1H, H-3-Th), 6.65 (s; 1H, H-5-Im), 5.04 (bs; 1H, NH), 4.78 (m; 1H, α-CH-Im), 3.74 (s; 3H, OCH_3), 3.70 (d; 1H, H_l of CH_2-Th), 3.47 (d; 1H, H_h of CH_2-Th), 3.34 (dd; 1H, H_l of CH_2-His), 3.12 (dd; 1H, H_h of CH_2-His), 1.51 (s, 9H, CH_3), 1.39 (s; 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 173.7$ (s; CONH), 171.5 (s; COMe), 154.8 (s; OCONH), 137.6 (s; C-2-Th), 135.3 (d; C-2-Im), 130.9 (d; C-4-Im), 127.6 (d; C-3-Th), 126.5 (d; C-4-Th), 124.6 (d; C-5-Th), 119.6 (d; C-5-Im), 80.4 (s; OC), 63.6 (s; C), 52.9 (d; α-CH-Im), 52.2 (q; OCH_3), 34.4 (t; CH_2-Thi), 28.3 (q; CH_3), 28.2 (t; CH_2-Im), 23.9 (q; CH_3) ppm; $C_{20}H_{28}N_4O_5S \cdot H_2O$ (456.35); calcd.: C 52.64, H 6.67, N 12.28; found: C 52.66, H 6.12, N 12.20.

(S(R,R*))-N-(2-(((1,1-Dimethyl)ethoxycarbonyl)amino)-3-methyl-3-phenyl-1-oxo-butyl)histidine methylester* and *(S(R*,S*))-N-(2-(((1,1-Dimethyl)ethoxycarbonyl)amino)-3-methyl-3-phenyl-1-oxo-butyl)histidine methylester* (**20a,b**)

Yield: 70%; m.p.: 82–84°C; TLC (ethyl acetate/methanol = 8:1): $R_f = 0.42$; ^1H NMR ($CDCl_3$): δ =7.35–7.15 (m; 14H, C_6H_5, H-Im-2, H-5-Im), 6.75–6.55 (m; 4H, CONH), 5.35 (t; 2H, NH), 4.65–4.35 (m; 4H, α-CH-Phe, α-CH-His), 3.70/3.65 (2s; 6H, OCH_3), 3.00 (2dd, 2H, H_l of CH_2-His), 2.80 (2dd, 2H, H_h of CH_2-His), 2.45/2.38 (2s; 12H, CH_3), 2.40 (s; 18H, CH_3) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 171.35/170.86$ (2s; CO), 169.95/169.92 (2s; CO), 156.13/155.87 (2s; CONH), 145.75/145.39 (2s; C-1-Ph), 135.24/135.01 (2d; C-2-Im), 128.40, 128.25, 126.46, 126.27, 126.12 (d; C-2,3,4,5,6-Ph, C-4-Im), 79.99 (s; OC), 62.93/62.73 (2d; α-CH-Phe), 52.64/52.21 (2d; α-CH-His), 52.09 (q; OCH_3), 41.75/41.14 (2s; C), 28.6 (t; CH_2-His), 28.11 (q; CH_3), 25.76/25.50 (2q; CH_3), 23.98/23.61 (2q; CH_3) ppm; $C_{23}H_{32}N_4O_5 \cdot 0.44\ H_2O$ (452.46); calcd.: 61.06, H 7.32, N 12.38; found: C 61.05, H 7.21, N 12.38.

(N-(N-((1,1-Dimethyl)ethoxycarbonyl)-L-phenylalanyl)-L-2-methylhistidine methylester and *N-(N-((1,1-Dimethyl)ethoxycarbonyl)-L-phenylalanyl)-D-2-methylhistidine methylester* (**22a, b**)

Yield: 83%; m.p.: 56–58°C; TLC (ethyl acetate/methanol = 4:1): $R_f = 0.52$. ^1H NMR ($CDCl_3$): $\delta = 11.45$ (bs; 2H, NH), 8.45 (d; 2H, CONH), 7.35–7.1 (m; 10H, aromatic H), 7.0/6.85 (2d; 2H, CONH), 6.75/6.65 (2 bs; 2H, H-5-Im), 4.55–4.40 (m; 2H, α-CH-Phe), 4.25–4.10 (m; 2H, α-CH-His), 3.65/3.60 (2s; 6H, OCH_3), 3.05–2.55 (m; 8H, CH_2-Phe, CH_2-His), 2.25/2.20 (2s; 6H, CH_3), 1.30 (s; 18H, CH_3) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 171.60/171.22/170.99$ (3s; CO), 155.90/155.53 (2s; OCONH), 144.71 (s; C-2-Im), 136.56/136.18 (2s; C-1-Ph), 129.28/129.18/128.55/128.50/126.92/126.79 (6d; C-2,3,4,5,6-Ph), 128.70 (s; C-4-Im), 118.79 (d; C-5-Im), 80.44/80.16 (2s; C), 56.40/55.69 (2d; α-CH-Phe), 52.90/52.66 (2q; OCH_3), 52.38/52.27 (2d; α-CH-His), 38.26/37.90 (2t; CH_2-Phe), 29.61/28.78 (2t; CH_2-His), 14.00 (q; CH_3) ppm; $C_{22}H_{30}N_4O_5 \cdot 0.5\ H_2O$ (439.52); calcd.: C 60.12, H 6.88, N 12.74; found: C 6.19, H 7.08, N 12.76.

(S(R,R*))-N-(2-(((1,1-Dimethyl)ethoxycarbonyl)amino)-3,3-diphenyl-1-oxo-propyl)histidine methylester* (**24**)

Yield: 81%; m.p.: 148–151°C; $[\alpha]_D^{20} = +109.7°$(c = 1.93 in CH_2Cl_2); TLC (ethyl acetate/methanol = 9:1): $R_f = 0.34$;^{1}H NMR ($CDCl_3$): $\delta = 7.58$ (s; 1H, H-2-Im), 7.55–7.10 (m; 11H, aromatic H, CONH), 6.70 (s; 1H, H-5-Im), 6.65 (d; 1H, CONH), 5.13 (d; 1H, NH), 4.78 (m; 1H, α-CH-His), 4.50 (m; 1H, α-CH-Dip), 4.39 (d; 1H, CH, $J = 10$ Hz), 3.60 (s; 3H, OCH_3), 3.18 (dd; 1H, H_l of CH_2-His), 3.10 (dd; 1H, H_h of CH_2-His), 1.43 (s; 9H, CH_3) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 170.55$ (s; CO), 170.16 (s; CO), 156.18 (s; OCONH), 140.10/139.30 (2s; C-1-Ph), 135.50 (d; C-2-Im), 128.90, 128.71, 128.65, 128.44, 128.10, 127.27, 127.15 (d, C and C′-2,3,4,5,6-Ph), 126.96 (s; C-4-Im), 123.63 (d, C-5-Im), 80.98 (s; OC), 59.20 (d; α-CH-Dip), 52.78 (q; OCH_3), 52.37 (d; α-CH-His), 28.26 (q; CH_3), 28.19 (d; CH), 27.47 (t; CH_2-His) ppm; $C_{27}H_{32}N_4O_5 \cdot 0.9\ H_2O$ (508.61); calcd.: C 63.76, H 6.69, N 11.02; found: C 63.72, H 6.45, N 11.10.

Cyclic Dipeptides (**3–9**)

Procedure for **5–9**

A solution of 6.00 mmol of **18a,b**, **20a,b**, **22a,b**, or **24** in 60.0 mmol trifluoroacetic acid and 8.00 ml dichloromethan was stirred for 15 min at 0°C and for 2 h at room temperature. The solvents were evaporated *in vacuo*. The residue was dissolved in 50 ml methanol and treated with concentrated aqueous ammonia to reach *pH* =8–9. The solution was refluxed for 1 d and then cooled to 0°C. The colourless crystals were collected, dried, and recrystallized from methanol.

Procedure for **3** and **4**

To a solution of 8.60 mmol N-methylated Z-protected dipeptide methylester (**12a** or **12b**) in 130 ml methanol, 0.7 g palladium on activated carbon (10%) were added. The suspension was hydrogenated at 5 atm for 6 h at room temperature. The catalyst was filtered off, and the filtrate was evaporated *in vacuo*. The crude product formed a foam which was not purified further. 8.10 mmol of the crude product was heated without solvent to 110–120°C/0.01 torr for 2 h. The resulting crude product was recrystallized from methanol. The colourless crystals were collected, washed with ether, and dried *in vacuo*.

(3S,cis)-3-(1H-Imidazol-4-ylmethyl)-6-methyl-6-phenylmethyl-piperazine-2,5-dione (**5**)

Yield: 71%; m.p.: 254°C; $[\alpha]_D^{20} = -32.0°(c = 1.52$ in methanol); TLC (ethyl acetate/methanol = 4:1): $R_f = 0.51$; ^{1}H NMR (*DMSO*-d_6): $\delta = 8.35$ (s; 1H, CONH), 8.25 (s; 1H, H-2-Im), 7.80 (s; 1H, CONH), 7.35–7.15 (m; 5H, aromatic H), 6.70 (1s; 1H, C-5-Im), 4.95 (m; 1H, α-CH-His), 3.10 (d; 1H, H_l of CH_2-Phe), 2.65 (d; 1H, H_h of CH_2-Phe), 2.45 (1H, H_l of CH_2-His, $J = 3.6$ Hz, $J = 14.7$Hz), 1.45 (s; 3H, CH_3), 1.35 (dd; 1H, H_h of CH_2-His, $J = 9.0$ Hz, $J = 14.9$ Hz) ppm; ^{13}C NMR (*DMSO*–d_6): $\delta = 168.7$ (s; CONH), 165.5 (s; CONH), 136.4 (s; C-2-Im), 134.5 (d; C-4-Im), 130.7 (S, C-1-Ph), 130.5 (d; C-3,5-Ph), 128.0 (d; C-2,6-Ph), 126.8 (d; C-4-Ph), 116.5 (d; C-5-Im), 59.9 (s; C), 53.6 (d; α-CH-His), 45.5 (t; CH_2-Phe), 29.7 (t; CH_2-His), 28.1 (q; CH_3) ppm; C_{16} $N_{18}N_4O_2 \cdot 0.27\ H_2O$ (303.21); calcd.: C 63.27, H 6.16, N 18.47; found: C 63.37, H 6.17, N 18.49.

(3S,cis)-3-(1H-Imidazol-4-ylmethyl)-6-methyl-6-(2-thienylmethyl)-piperazine-2,5-dione (**6**)

Yield: 35%; m.p.: 246–249°C; $[\alpha]_D^{20} = -70°(c = 1.9$ in methanol); TLC (ethyl acetate/methanol =4:1): $R_f = 0.5$; ^{1}H NMR (*DMSO*-d_6): $\delta = 8.35$ (s; 1H, CONH), 7.87 (s; 1H, CONH), 2.83 (s; 1H,

H-2-Im), 7.36 (d; 1H, H-5-Th, $J = 1.1$ Hz, $J = 5.1$ Hz), 6.98 (m; 1H, H-4-Th, $J = 5.1$ Hz, $J = 3.4$ Hz), 6.80 (d; 1H, $J = 3.4$ Hz), 6.70 (1s; 1H, C-5-Im), 4.00 (m; 1H, α-CH-His), 3.27 (d; 1H, H_l of CH_2-Thi), 2.87 (d; 1H, H_h of CH_2-Thi), 2.72 (1H, H_l of CH_2-His), 1.80 (dd; 1H, H_h of CH_2-), 1.42 (s; 3H, CH_3) ppm; ^{13}C NMR (*DMSO*-d_6): δ=168.6 (s; CONH), 166.1 (s; CONH), 137.6 (s; C-2-Th), 134.7 (s; C-2-Im), 132.7 (d; C-4-Im), 127.6 (d, C-3-Th), 126.7 (C-4-Th), 125.2 (d; 5-Th), 116.2 (d; C-5-Im), 59.7 (s; C), 54.2 (d; α-CH-His), 39.2 (t; CH_2-Thi), 30.2 (t; CH_2-His), 27.6 (q; CH_3) ppm; $C_{14}H_{16}N_4O_2S{\cdot}0.7\ H_2O$ (316.98); calcd.: 53.24, H 5.55, N 17.19; found: C 53.05, H 5.53, N 17.68.

(3S,cis)-6-(1H-Imidazol-4-ylmethyl)-1-methyl-3-(phenylmethyl)-piperazine-2,5-dione (**4**)

Yield: 22%; m.p.: 195–197°C; $[\alpha]_D^{20} = -112°$(c=1.8 in methanol); TLC (ethyl acetate/methanol = 1:1): $R_f = 0.45$; ^{1}H NMR *DMSO*-d_6 $\delta = 8.15$ (bs; 1H, CONH), 8.10 (s; 1H, H-2-Im), 7.40–7.00 (m; 5H, aromatic H), 6.75 (s; 1H, H-5-Im), 4.05 (m; 2H, α-CH-Phe, α-CH-His), 2.75 (s; 3H, NCH_3), 2.70 (dd; 1H, H_l of CH_2-His), 2.40 (dd; 1H, H_hCH_2-His), 2.30 (m; 2H, CH_2-Phe) ppm; ^{13}C NMR (*DMSO*-d_6): δ=164.77 (s; CONH), 164.0 (s; CONH), 136.1 (s; C-1-Ph), 134.3 (d; C-2-Im), 131.3 (s; C-4-Im), 129.6 (d; C-2,6-Ph), 128.1 (C-3,5-Ph), 126.4 (d, C-4-Ph), 117.0 (d; C-5-Im), 60.8 (d; α=Ch-Phe), 55.5 (d; α-CH-His), 39.8 (t; CH_2-Phe), 32.1 (q; NCH_3), 28.5 (t; CH_2-His) ppm; $C_{16}H_{18}N_4O_2 \cdot 0.8\ H_2O$ (312.74); calcd.: C 62.16, H 6.26, N 18.12; found: C 62.18, H 6.03, N 17.85.

(3S,cis)-3-(1H-Imidazol-4-ylmethyl)-1-methyl-6-(phenylmethyl)-piperazine-2,5-dione (**3**)

Yield: 99%; m.p.: 198–203°C; $[\alpha]_D^{25} = -121.6$ o ($c = 0.5$ in methanol); TLC (ethyl acetate/methanol = 1:1): $R_f = 0.5$; ^{1}H NMR (*DMSO*-d_6): $\delta = 7.85$ (s; 1H, CONH), 7.50 (s; 1H, H-2-Im), 7.40–7.05 (m; 6H, aromatic H, NH), 6.55 (s, 1H, H-5-Im), 4.25 (m; 1H, α-CH-Phe), 3.85 (m; 1H, α-CH-His), 3.15 (ddd; 2H, CH_2-Phe), 2.95 (s; 3H, NCH_3), 2.35 (dd; 1H, H_l of CH_2-His, $J = 15$ Hz, $J = 4$Hz), 1.10 (dd; 1H, H_h of CH_2-His, $J = 15$ Hz, $J = 8$ Hz) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 165.3$ (s; CONH), 165.2 (s; CONH), 135.9 (s; C-1-Ph), 134.9 (d; C-2-Im), 132.9 (s; C-4-Im), 129.9 (d; C-2,6-Ph), 128.5 (d, C-3,5-Ph), 126.9 (d; C-4-Ph), 116.9 (d; C-5-Im), 62.3 (d, α-CH-Phe), 54.6 (d; α-CH-His), 36.2 (t; CH_2-Phe), 32.3 (q; NCH_3), 32.1 (t; CH_2-His) ppm; $C_{16}H_{18}N_4O_5{\cdot}0.5\ H_2O$ (307.35); calcd.: C 62.53, H 6.23, N 18.23; found: C 62.58, H 6.11, N 18.41.

(3S,cis)-3-Diphenylmethyl-6-(1H-imidazole-4-ylmethyl)-piperazine-2,5-dione (**9**)

Yield: 70%; m.p.: 248–250°C; $[\alpha]_D^{20} = -24.9°$($c = 0.34$ in methanol); TLC (ethyl acetate/methanol =1:1): $R_f = 0.75$; ^{1}H NMR (*DMSO*-d_6): $\delta = 7.88$ (d; 1H, CONH), 7.81 (d; 1H, CONH), 7.35–7.15 (m; 11H, aromatic H, NH), 4.75 (m, 1H, α-CH-Dip), 4.30 (d; 1H, CH, $J = 5.45$ Hz), 3.95 (m; 1H, α-CH-His), 2.65 (dd; 1H, H_l of CH_2-His, $J = 3.76$ Hz, $J = 14.8$ Hz), 1.85 (dd; 1H, H_h of CH_2-His, $J = 8.96$ Hz, $J = 14.4$ Hz) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 167.18$ (s; CONH), 165.79 (s, CONH), 140.82 (s, C-1-Ph), 135.02 (d; C-2-Im), 129.57, 128.70, 128.16, 126.66, 126.44 (d; C- and C′-2,3,4,5,6-Ph), 128.42 (s; C-4-Im), 117.91 (d; C-5-Im), 57.47 (d; α-CH-Dip), 54.75 (d, CH), 54.55 (d; α-CH-His), 30.88 (t; CH_2-His) ppm; $C_{21}H_{20}N_4O_2{\cdot}1.3\ H_2O$ (383.69); caled.: C 65.71, H 5.93, N 14.60; found: C 65.70, H 5.65, N 14.42.

(3S,cis)-3-(1H-Imidazol-4-ylmethyl)-6-((1-methyl)-1-phenyl)-ethyl)-piperazine-2,5-dione (**7**)

Yield: 48%; m.p.: 255–256°C; $[\alpha]_D^{20} = -111.3°$($c = 0.528$ in methanol); TLC (ethyl acetate/methanol = 1:1): $R_f = 0.53$; ^{1}H NMR (*DMSO*-d_6): $\delta = 7.95$ (s; 1H, CONH), 7.55 (s; 1H, H-2-Im), 7.52 (s; 1H, CONH), 7.40–7.20 (m; 6H, aromatic H, NH), 6.60 (s; 1H, H-5-Im), 3.90 (s; 1H, α-CH-Phe),

3.80 (m; 1H, α-CH-His), 2.40 (dd; 1H, H_l of CH_2-His, $J = 3.25$ Hz, $J = 14.9$ Hz), 1.50 (s; 3H, CH_3), 1.40 (s; 3H, CH_3), 1.07 (dd; 1H, H_h of CH_2-His, $J = 9.7$ Hz, $J = 14.8$ Hz) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 167.01$ (s; CONH), 164.92 (s; CONH), 143.99 (d; C-2-Im), 134.96 (s; C-4-Im), 129.07 (s; C-1-Ph), 128.19 (d; C-2,6-Ph), 127.35 (d; C-3,5-Ph), 126.50 (d; C-4-Ph), 117.05 (d; C-5-Im), 63.27 (d; α-CH-Ph), 54.48 (d; α-CH-His), 42.65 (s; $C(CH_3)_2$Ph), 30.76 (t; CH_2-His), 26.69/26.20 (q; CH_3) ppm; $C_{17}H_{20}N_4O_2 \cdot 0.5\ H_2O$ (312.37); calcd.: C 63.53, H 6.59, N 17.43; found: C 63.49, H 6.73, N 17.48.

(3S,cis)-3-(2-Methyl-1H-imidazole-4-ylmethyl)-6-phenylmethyl-piperazine-2,5-dione (**8**)

Yield: 67% m.p.: 203–205°C; $[\alpha]_D^{20} = -159°$ ($c = 2$ in methanol); TLC (ethyl acetate/methanol = 1:1): $R_f = 0.745$; ^{1}H NMR (*DMSO*-d_6): $\delta = 11.45$ (bs; 1H, NH), 8.09 (s; 1H, CONH), 7.77 (s; 1H, CONH), 7.35–7.10 (m; 5H, aromatic H), 6.45 (s; 1H, H-5-Im), 4.15 (m; 1H, α-CH-Phe), 3.80 (m; 1H, α-CH-His), 2.90 (dd; 1H, H_l of CH_2-Phe, $J = 4.4$ Hz, $J = 13.4$ Hz), 2.82 (dd; 1H, H_h of CH_2-Phe, $j = 4.8$ Hz, $J = 13.4$ Hz), 2.61 (dd; 1H, H_l of CH_2-His, $J = 3.4$ Hz, $J = 14.5$ Hz), 2.19 (dd; 1H, H_h of CH_2-His, $J = 9.4$ Hz, $J = 14.5$ Hz) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 166.68$ (s; CONH), 166.00 (s; CONH), 143.27 (s; C-2-Im), 136.23 (s; C-1-Ph), 133.53 (s; C-4-Im), 130.18 (d; C-2,6-Ph), 128.11 (d; C-3,5-Ph), 126.60 (d; C-4-Ph), 115.07 (d; C-5-Im), 55.27 (d; α-CH-Phe), 54.43 (d; α-CH-His), 38.49 (t; CH_2-Phe), 31.27 (t; CH_2-His), 13.81 (q; CH_3) ppm; $C_{16}H_{18}N_4O_2 \cdot 0.5\ H_2O$ (307.36); calcd.: C 62.53, H 6.23, N 18.23; found: C 62.39, H 6.36, N 18.23.

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