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Studies on Cyclic Dipeptides, I: Aryl Modifications of *cyclo*-[Phe-His][#]

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Summary. Seven new cyclic dipeptides have been synthesized and tested for their applicability as tools to elucidate the mechanism of formation of mandelonitrile with (*SS*)-*cyclo*-[Phe-His] type catalysts. Conformational analyses based on ^{1}H NMR spectra are presented for all prepared cyclic dipeptides.

Keywords. (*SS*)-*cyclo*-[Phe-His]; Hydrocyanation; Conformational analysis.

Untersuchungen über cyclische Dipeptide, I. Mitt.: Aryl-Modifikationen von *cyclo*-[Phe-His]

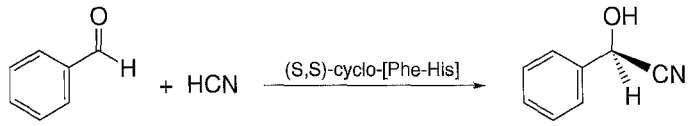
Zusammenfassung. Es wurden sieben neue cyclische Dipeptide synthetisiert und auf ihre Anwendbarkeit als (*SS*)-*cyclo*-[Phe-His]-ähnliche Katalysatoren zur Bildung von Mandelsäurenitril und zur Untersuchung des Mechanismus dieser Reaktion getestet. Konformationsanalysen aller dargestellten cyclischen Dipeptide – basierend auf ^{1}H-NMR-Spektren – werden vorgestellt.

Introduction

The broad interest in asymmetric C–C-bond-formation is due to the huge synthetic potential of such reactions which open a broad scope of compounds useful for both pharmaceutical and chemical industries. Thus, within the last decade many different approaches have been taken to develop catalysts for enantioselective syntheses of cyanohydrins: chiral *Lewis* acids [1], alkaloids [2], cyclodextrins [3], polymers [4], lipases [5], oligopeptides [6], *D*- and *L*-oxynitrilases [7], and cyclic dipeptides [8] have been reported as catalysts. Within the latter group, which first appeared at the end of the seventies, the most efficient catalysts for the synthesis of many aromatic cyanohydrins have been (*SS*)- and (*R,R*)-*cyclo*-[Phe-His] which were initially developed and introduced by *Inoue* [9,10a]. Depending on the substitution pattern of aromatic aldehydes, enantiomeric excesses of up to 97% have been reported with this catalyst. This prompted our interest to study the mechanism of formation of (*R*)-mandelonitrile using (*SS*)-cyclo-[Phe-His] (**1**) as a catalyst.

Up to 1990, no detailed work on transition state and reaction mechanism of the reaction using cyclic dipeptides was reported. The few proposed transition state models had frequently been derived from theoretical assumptions rather than from

[#] Dedicated to Prof. Dr. Dres. h.c. *Herbert Oelschläger* on the occasion of his 75th birthday



Scheme 1. Formation of (*R*)-mandelonitrile with (*SS*)-*cyclo*-[Phe-His]

experimental work [8a,10]. In these early papers, the following interactions of relevance have been established:

- π-π-interactions between the aromatic aldehyde and the aromatic part of the dipeptide;
- transfer of the cyanide anion to the aldehyde from a position in which it is attached to the base moiety; and
- H-bonding between the CO-group of the aldehyde and the piperazine-2,5-dione frame of the dipeptide (eventually).

Earlier as well as parallel to our own work, experimental studies have been initiated to investigate these interactions more thoroughly to gain a deeper insight into the reaction. The most outstanding results have been presented by *deVries* [8e] and *North* [10c]. However, in contrast to our work, these groups have tried to obtain their data on the reaction mechanism using exclusively *cyclo*-[Phe-His].

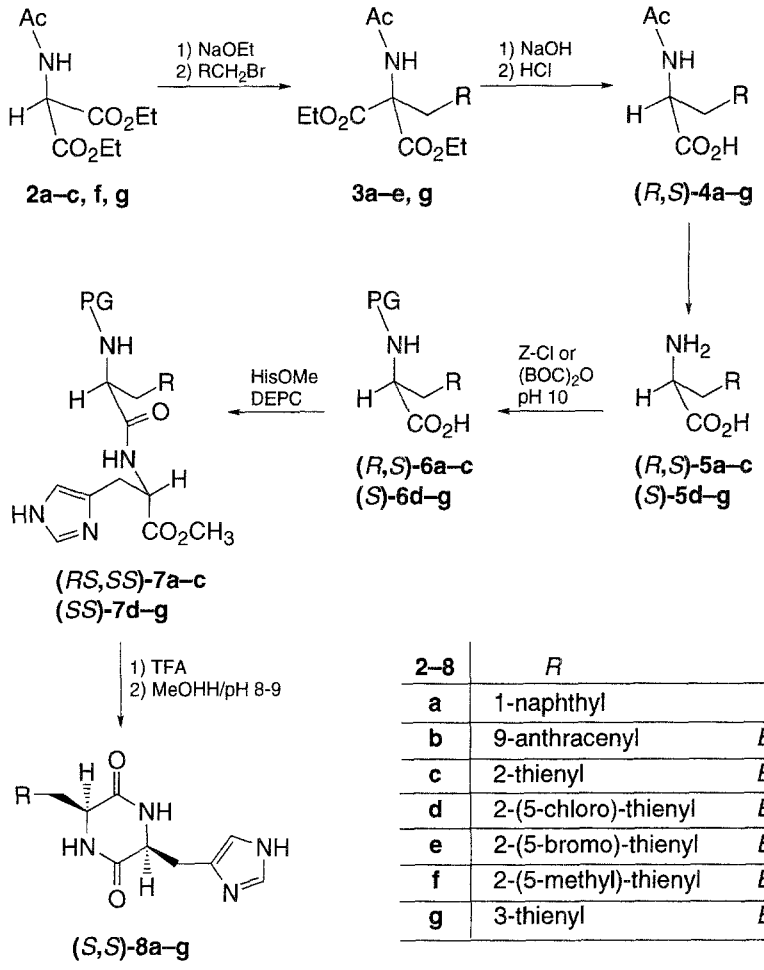
Results and Discussion

Bearing in mind the importance that has been attributed to π-π-interactions between the aromatic aldehyde and the aromatic part of the dipeptide, we decided first to study the influence of steric and isosteric modifications of the phenyl ring on the selectivity of the reaction to elucidate the role of these π-π-interactions. Therefore, seven new cyclic dipeptides have been prepared and studied (*SS*)-*cyclo*-[(1-naphthyl)-Ala-His] (**8a**), (*SS*)-*cyclo*-[(9-anthracenyl)-Ala-his] (**8b**), (*SS*)-*cyclo*-[2-Thi-His] (**8c**), (*SS*)-*cyclo*-[2-(5-chloro)-Thi-His] (**8d**), (*SS*)-*cyclo*-[2-(5- bromo)-Thi-His] (**8e**), (*SS*)-*cyclo*-[2-(5-methyl)-Thi-His] (**8f**), and (*SS*)-*cyclo*-[3-Thi- His) (**8g**).

Synthesis of Cyclic Dipeptides

The synthesis of (*SS*)-*cyclo*-[Phe-His] (**1**) was carried out according to *Inoue* [11]. All non-natural amino acids (**5a–g**) were obtained *via* malonic ester synthesis according to *Egusa* [12]. 1-Naphthylalanine (**5a**) was *Z*-protected with benzylchloroformiate [14]; amino acids **5b–g** were *BOC*-protected in aqueous solution with pyrocarbonic acid di-*tert.*-butylester at *pH* 10 [14]. Peptide coupling of protected amino acids **6a–g** with histidine methylester was carried out either by *DEPC* [15] (**6c–g**) or by the mixed anhydride method [14] (**6a, b**). The linear dipeptide **7a** was deprotected by hydrogenation under pressure and cyclized in refluxing methanol [14]. Compounds **7b–g** were deprotected with 10 equivalents of trifluoroacetic acid and – after neutralization of the reaction mixture with ammonia in methanol – also cyclized by heating in methanolic solution [14].

Different strategies were chosen for obtaining the enantiomerically pure dipeptides (*S,S*)-**8a–g**: racemic amino acids (*R,S*)-**5a–c** were N-protected and reacted with (*S*)-histidine methylester to yield the diastereomeric dipeptides (*RS,SS*)-**7a–c**. After intramolecular ring closure, (*RS*)-diastereomers were removed by repeated recrystallization from water or methanol to yield pure cyclic dipeptides (*SS*)-**8a–c** (control of diastereomeric purity by ^{1}H NMR spectra). Enantiomerically pure amino acids **5d–g** were obtained *via* enzymatic resolution of the N-acetyl-protected amino acids (*R,S*)-**4d–g** with acylase I (aspergillus acylase [E.C.3.5.1.14]) [13].



2–8	*R*	*PG*
a	1-naphthyl	*Z*
b	9-anthracenyl	*BOC*
c	2-thienyl	*BOC*
d	2-(5-chloro)-thienyl	*BOC*
e	2-(5-bromo)-thienyl	*BOC*
f	2-(5-methyl)-thienyl	*BOC*
g	3-thienyl	*BOC*

Scheme 2. Preparation of the cyclic dipeptides

Cyanohydrin Reaction

First of all, the reaction of (*SS*)-*cyclo*-[Phe-His] with benzaldehyde was studied carefully to establish a reliable basis for comparison of this catalyst to those newly prepared. The findings obtained thereby provided some new aspects in the elucidation of the mechanism of the cyanohydrin reaction. In the next step, the reaction was studied using model dipeptides.

Activation of Dipeptides

Several different methods have been proposed to activate (*SS*)-*cyclo*-[Phe-His] (**1**) to achieve optimum selectivities [8a, 8c, 8e, 10b, 16]. Most authors correlate high enantioselectivity of the reaction with amorphousness, diffuse X-ray diffraction patterns of the catalyst, and gelatineous character of the reaction mixture [8a, 8c, 8h, 10b, 17]. During the last five years, the most common technique for its preparation has been to precipitate (*SS*)-*cyclo*-[Phe-His] from a non aqueous solvent such as methanol or methanol/ether. Selectivities of up to 90–95% *ee* and even higher have been reported.

Following the activation procedures given in the literature [8a,8c,8e,10b,16], we could not achieve satisfying reproducability of selectivities in our first experiments. Only X-ray analysis or use of reaction itself as indicators being reported for activity prediction we tried to find a common analytical method to correlate catalyst quality with activity. First, we compared calculated and measured elementary analysis values of inactive and active species. This method revealed that differences in catalyst composition were not only due to crystal water or methanol, but that there was also a considerable tendency for poisoning of the catalyst by acids, *e.g. TFA* or HCl, either from laboratory air or contamination by reaction chemicals. Surprisingly, chlorine values sometimes revealed fairly high portions of HCl with inactive catalysts. Obviously, protonation of the histidine base moiety of the catalysts prevents attachement of HCN to the catalyst and thus reaction. As has been shown [8g, 10c], catalyst protonation leads to a significant shift of the methylene protons of both amino acid side chains in the ^{1}H NMR spectra. We observed such shifts with the inactive catalysts and could thus establish ^{1}H NMR spectroscopy as an analytical tool to indicate catalyst poisoning and catalyst activity. Fig. 1 shows a ^{1}H NMR spectrum of a catalytically inactive charge of (*SS*)-*cyclo*-[Phe-His] (**1**), Fig. 2 a spectrum of a catalytically active batch of **1**.

The search for a reliable and reproducible activation method for (*SS*)-*cyclo*-[Phe-His] led us to the systematic use of ion exchange resins (IER) in methanol or water to remove disturbing anions from the histidine side chain. Several strong basic IER preparations were tested for catalyst activation: OH^- form in water, CN^- form in water, OH^- form in absolute methanol, and CN^- form in absolute methanol. Application of all IERs resulted in a significant increase in reproducibility of results, however, with slight differences of activity depending on the preparation applied. Use of IER III strong basic with CN^- as counter ion produced throughout higher

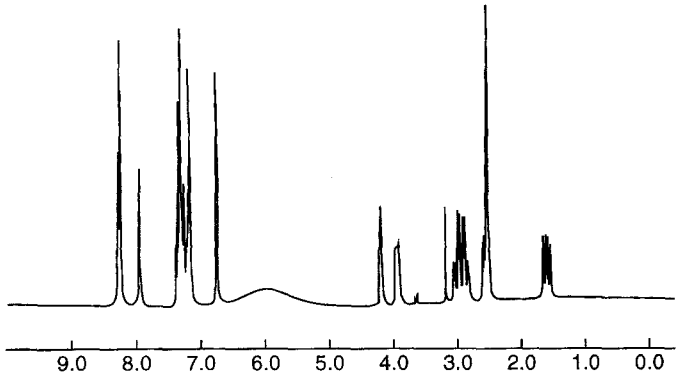


Fig. 1. ^{1}H NMR spectrum (*DMSO*-d_6, 300 MHz) of (*SS*)-*cyclo*-[Phe-His] before activation

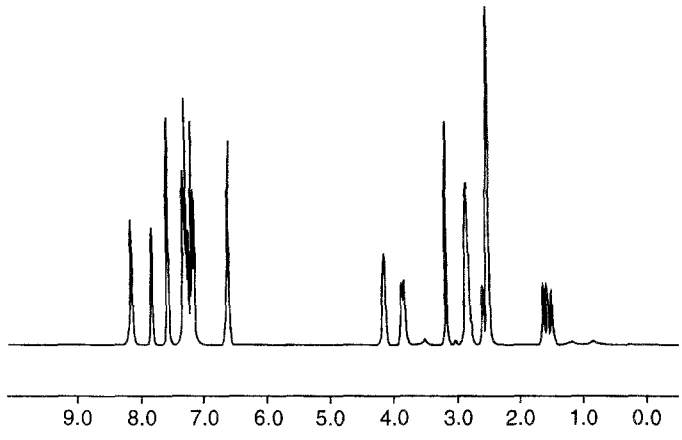
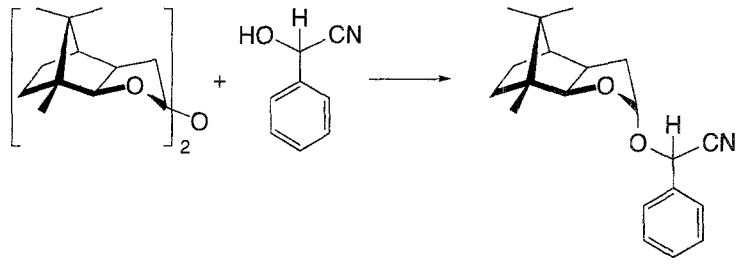


Fig. 2. ^{1}H NMR spectrum (*DMSO*-d_6, 300 MHz) of (*SS*)-*cyclo*-[Phe-His] after activation with ion exchange resins (CN^-)

selectivities in cyanohydrin formation than IER III strong basic with OH^- as counter ion. We suppose that in the first case an imidazole-base HCN association is achieved already during catalyst activation.

Conditions of the Cyanohydrin Reaction

A large variety of parameters to achieve optimum reaction conditions and handling in cyanohydrin formation have been reported. The following were adopted and kept constant in our experiments: absolute toluene as solvent, pure and waterfree HCN, reaction temperature between 0 and 4 °C, use of freshly distilled benzaldehyde, and recovery of the dipeptide by addition of absolute ether to the reaction mixture and filtration of the precipitated catalyst. Additional constant parameters in our study of the cyanohydrin reaction were: activation of the dipeptides with IER in water or absolute methanol, determination of conversion by GC, and determination of enantioselectivity by GC after acetal formation of the cyanohydrins with *exo*-[2*R*,2′*R*-(2α,2′α,3aα,3′aα,4β,4′b,7β,7′β,7aα,7′aα)]-2,2′-oxy-*bis*-(octahydro-7,8,8-trimethyl-4,7-methanobenzofurane).



Scheme 3. *MBE*-protected mandelonitrile

Table 1 summarizes enantioselectivity/conversion rate results of the reaction of benzaldehyde to (*R*)-mandelonitrile for the seven new dipeptides, in comparison to (*SS*)-*cyclo*-[Phe-His] (**1**). The conversion rate observed for (*SS*)-*cyclo*-[Phe-His] was somewhat lower than the best values reported in the literature, but the results

Table 1. Optical and chemical yields in the formation of (*R*)-mandelonitrile with cyclic dipeptides and state of reaction

catalyst	selectivity (% *ee*)/ conversion (%)	state of reaction
1	92/80	gelatinous
8a	0/<10	cristalline
8b	0/<10	cristalline
8c	72/40	gelatinous
8d	0/<20	slightly gelatinous
8e	0/<20	slightly gelatinous
8f	0/<20	gelatinous
8g	0/<20	gelatinous

were easily reproducable and the reaction time was about half of those described previously. In most of our own experiments, the maximum of selectivity was reached after about 1–2 hours, whereas other authors have described a selectivity maximum after 4 hours. At the first glance, Table 1 reveals that the exchange of the phenyl ring for other aromatic rings leads to a dramatic loss of catalytic activity. (*SS*)-*cyclo*-[2-Thi-His] (**8c**) was the only piperazin-2,5-dione which worked as an effective catalyst. An enantiomeric excess of 72% of (*R*)-cyanohydrine was obtained when this compound was applied. The conversion rate of its reaction amounted to 40% at the point of maximum selectivity. Since the conversion rates observed with the other catalysts (10–20%) may be related to simple base induced cyanohydrin formation, all other cyclic dipeptides of this study turned out to be not useful as catalysts.

The tested compounds comprised two different types of aryl modifications: naphthyl-(**8a**) and anthracenyl-dipeptides (**8b**) aimed at investigating the influence of an increased steric demand of the aryl moiety, and thienyl modifications representing the so-called isosteric approach. Replacement of the benzene ring by thiophene has become a frequently used and successful strategy in drug research. There, the concept of bio-isostery makes use of minor differences – thiophene is somewhat smaller than benzene and exhibits an increased aromatic electron density – of these otherwise very similar aromatic compounds to modulate physiological properties of benzene ring containing drugs. Alternative points of thiophene attachment (**8c**,**8g**) as well as the option to add substitutents (**8d–f**) to the smaller thiophene nucleus allow to have a set of aromatic molecules with a steric demand similar to benzene.

A gelatineous state of the reaction mixture has been correlated to good enantioselectivity of the cyanohydrin reaction by different authors [8a,8c,8h,10b]. This qualitative observation is in accordance with our negative results in the case (*SS*)-*cyclo*-[(1-naphthyl)-Ala-His] (**8a**) and (*SS*)-*cyclo*-[(9-anthracenyl)-Ala-His] (**8b**) where the reaction mixtures contained crystalline particles and appeared to be much less gelatinous compared to (*SS*)-*cyclo*-[Phe-His]. Reaction mixtures with the isosteric (*SS*)-*cyclo*-[Thi-His]-derivatives reached a more (**8c**,**8f**,**8g**) or less (**8d**,**8e**) gelatineous state; however, only for one of these cyclic dipeptides catalytic activity

was found. Obviously, the gelatineous state is a prerequisite but not sufficient to guarantee catalytic activity in this system.

Conformational Analysis of the Dipeptides

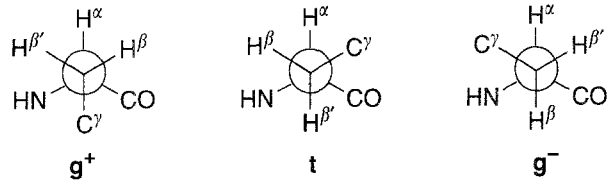
The loss of catalytic activity with steric changes of the aryl moiety might easily be explained by a change of molecule conformation. Thus, inactivity of **8a** and **8b** with their large aryl substituents was not as much surprising as the loss of activity observed with isosteric thienyl catalysts, above all the complete loss in the change from a 2-thienyl ring (**8c**) to a 3-thienyl ring (**8g**). Therefore, conformational analyses of the cyclic dipeptides were carried out using ^{1}H NMR spectroscopy in *DMSO*-d_6 in an attempt to correlate preferred solution conformations to catalytic activity. Although preferred solution conformations by no means need to be the conformations of the catalytically active species, a comparable conformational behaviour of molecules reflects similarity in an excellent way. In these ^{1}H NMR experiments, vicinal coupling constants $^3J(H_\alpha H_\beta)$ of both amino acids in the molecule were measured and entered into the *Sheinblatt* [19] modification of the *Karplus* equation for calculation of preferred conformations in cyclic dipeptides as follows:

$$^3J(H_\alpha H_\beta) = 2.6\,g^+ + 2.4\,t + 12.1\,g^- \quad (1)$$

$$^3J(H_\alpha H_{\beta'}) = 5.2\,g^+ + 12.4\,t + 2.7\,g^- \quad (2)$$

$$g^+ + t + g^- = 1 \quad (3)$$

We adjusted our data to *Sheinblatt*'s proposal assigning H_β to the methylene hydrogen of the histidine part appearing upfield and $H_{\beta'}$ to the methylene hydrogen appearing downfield. On the other hand, the upfield methylene hydrogen of the aryl side chain was assigned $H_{\beta'}$ and the downfield hydrogen H_β. The error limits amounted to $\pm 8\%$.



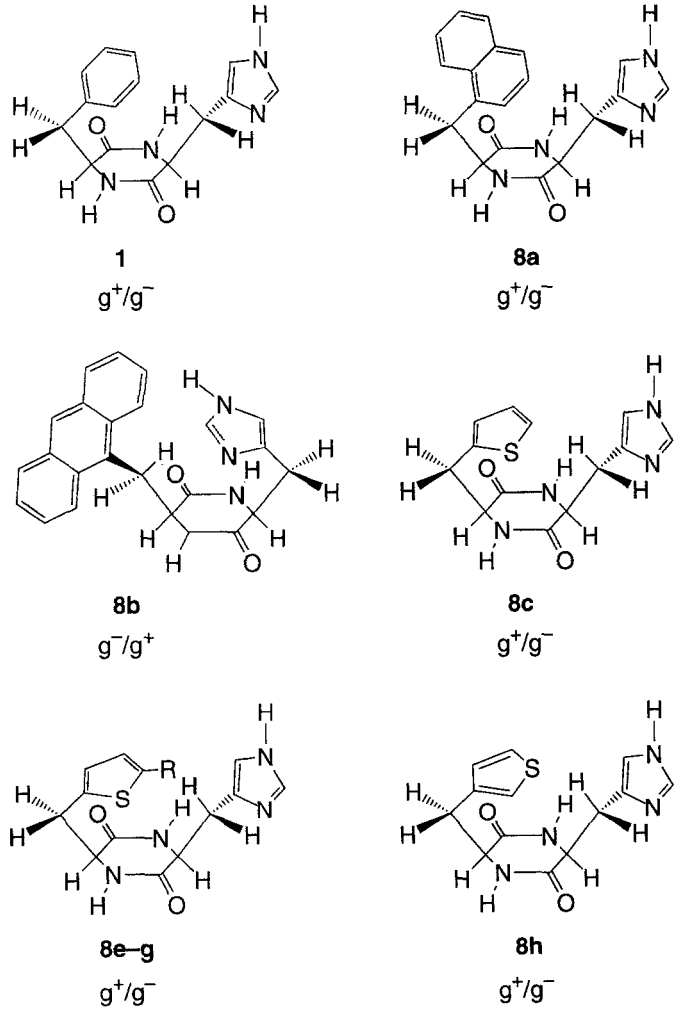
Scheme 4. *Newman* projections of the three rotamers g^+, t, and g^-

Table 2 shows the population of the different rotamers in the cyclic dipeptides. Most of them (except (*SS*)-*cyclo*-[(9-anthracenyl)-Ala-His], (**8b**)) prefer a conformation in which the aromatic side chain is folded over the piperazin-2,5-dione ring (55–90% g^+) and the histidine side chain occupies the g^--position (54–66%). This tendency has been described for many other cyclic dipeptides with aromatic residues and is explained by a π-π-interaction between the aromatic moiety and the piperazin-2,5-dione backbone.

Slightly negative values are due to differences in the theoretical assumptions to derive the equations and the equivalency criterions of the fractional populations.

Table 2. Preferred conformations of the cyclic dipeptides calculated by the modified *Karplus* equations

	aryl side chain			histidine side chain		
	g^+ (%)	t(%)	g^- (%)	g^+ (%)	t(%)	g^-(%)
1	90	−1	11	24	11	65
8a	55	7	38	42	4	54
8b	19	19	62	51	38	11
8c	90	−2	12	32	5	63
8d	60	11	29	43	−3	61
8e	80	1	19	37	4	59
8f	71	4	25	39	−5	66
8g	81	0	19	36	1	63



Scheme 5. Calculated preferred conformations of the cyclic dipeptides

Most probably because of its steric demand, the g^+-populations of the aromatic residue of (*SS*)-*cyclo*-[(9-anthracenyl)-Ala-His] (**8b**) amounted only to 19%; in consequence, the g^+-populations of the histidine side chain increased to 51%. Thus, only for this compound a changed preferred conformation could be given as the reason for its catalytic inactivity.

The results of conformational analysis confirmed the views on the effect of aryl exchange: sterically demanding aryl moieties, above all anthracenyl (**8b**), change the population pattern significantly, whereas calculated values for the isosteric thiophene dipeptide **8c**, the only compound in the series exhibiting catalytic activity, are almost identical with those of (*SS*)-*cyclo*-[Phe-His]. However, the preferred conformations of dipeptides **8e–g** are so similar that their complete inactivity is difficult to understand in the light of the present knowledge of the reaction. Considering experimental and theoretical data found in this study, it must be supposed that the formation of cyanohydrins with cyclic dipeptides cannot be described by the simple 1:1 catalyst-substrate interaction as proposed in several transition state models by different authors [10a, b, c, 8a, e].

In addition to our experimental results, the steric demand for the transition state of the reaction as derived from simple molecular modeling attempts indicated that (*SS*)-*cyclo*-[Phe-His] is not catalytically active in form of one single catalyst molecule, but that depeptide molecules aggregate to form a supramolecular catalyst structure which macroscopically appears as a gel and of which at least two catalyst molecules are directly involved into the reaction. This view is supported by the fact that even minor variations of the (*SS*)-*cyclo*-[Phe-His] molecule led to a complete loss of catalytic activity, indicating an enormous steric demand of the reacting species. This is also supported by the fact that, with sterically demanding aryls, these minor changes led to a loss of the gel character of the reaction mixture, which indicates a high degree of order of this gel. This hypothesis is based on the known ladder type molecule aggregation *via* CO-NH-hydrogen bridges [23], but certainly includes additional factors of steric order. Further evidence to support these views will be published in 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 Institut für Organische Chemie, University 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 by treatment with a solution of 5% molybdatophosphoric acid hydrate in ethanol or 0.1% ninhydrin in ethanol; CC: Merck silica gel 60 (mesh size 63–100 μm). 5-Methyl-2-thiophenealdehyde was purchased from Fluka. **2–5a** [20], **2–6b** [21], **2–4c**, **6c**, **2-4e,g** and **6g** [22] were synthesized as described in the literature. All reagents used were of standard laboratory quality.

5-Methyl-2-thiophenemethanol (**9**)

To a solution of 50 ml (456 mmol) 5-methylthiophene-2-carbaldehyde in 350 ml ethanol 13.2 g (347 mmol) sodium borohydride were added at 0 °C in small portions. After stirring the mixture for 2 h it was poured into a mixture of 400 g ice and 200 ml aqueous saturated ammonia chloride. Ethanol was removed *in vacuo*. The resulting solution was repeatedly extracted with ether; the combined organic

layers were washed with brine, dried over sodium sulfate, and evaporated *in vacuo*. The oily residue was distilled at 20 mbar. The product was unstable and was used immediatedly.

Yield: 86%; b.p.: 99–101 °C/20 mbar; TLC (petrolether/ether = 1:1): $R_f = 0.51$; ^{1}H NMR ($CDCl_3$): $\delta = 6.81$ (d; 1H, H-3-Th), 6.63 (d; 1H, H-4-Th), 4.74 (s; 2H, CH_2), 2.50 (s; 3H, CH_3), 1.75 (bs; 1H, OH) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 141.70$ (s; C-2-Th), 140.24 (s; C-5-Th), 125.54 (d; C-3-Th), 124.82 (d; C-4-Th), 59.86 (t; CH_2OH), 15.39 (q; CH_3) ppm.

2-Brommethyl-5-methylthiophene (**2f**)

The synthesis was carried out starting with compound **9** according to the literature [24]. The product is unstable and should be used immediately.

Yield: 95%; ^{1}H NMR ($CDCl_3$): $\delta = 6.85$ (d; 1H, Th-H-3, $J = 3.35$ Hz), 6.52 (d; 1H, Th-H-4, $J = 3.35$ Hz), 4.65 (s; 2H, CH_2), 2.40 (s; 3H, CH_3) ppm.

2-(Acetylamino)-2-((5-methyl-2-thienyl)methyl)propandioic acid, diethylester (**3f**)

2f was treated analogously to Ref. [25]. Yield: 55%; m.p.: 98–100 °C; TLC (petrolether/diethylether = 1:2): $R_f = 0.46$; ^{1}H NMR ($CDCl_3$): $\delta = 6.75$ (bs; 1H, NH), 6.55–6.50 (m; 2H, Th-H-3,-4), 4.25 (q; 2H, OCH_2), 3.78 (s; 2H, CH_2), 2.40 (s; 3H, CH_3), 2.05 (s; 3H, CH_3), 1.30 (t; 6H, CH_3) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 168.87$ (s; CONH), 166.97 (s; COO), 139.22 (s; H-5-Th), 133.88 (s; H-2-Th), 126.99 (d; H-3-Th), 124.60 (d; H-4-Th), 66.83 (s; C), 62.45 (t; OCH_2), 32.64 (t; CH_2), 22.80 (q; CH_3), 14.99 (q; CH_3), 13.76 (q; CH_3) ppm; $C_{15}H_{21}NO_5S$ (327.40); calcd.: C 55.03, H 6.47, N 4.28; found: C 55.06, H 6.43, N 4.34.

(R,S*)-α-(Acetylamino)-5-chloro-2-thiophenepropanioc acid* (**4d**)

6.77 g (5.07 mmol) N-chlorosuccinimid were added to a suspension of 10.0 g (46.9 mmol) **4c** in 300 ml CCl_4 and the reaction mixture was heated to reflux for 2 h. The solvent was removed *in vacuo* and the residue heated in 1 *N* HCl. The product was filtered and recrystallized from ethyl acetate/ether.

Yield: 69%; m.p.: 167–169 °C; TLC (ethyl acetate/methanol = 4:1): $R_f = 0.21$; ^{1}H NMR (*DMSO*-d_6):$\delta = 12.85$ (bs; 1H, COOH), 8.25 (d; 1H, NH, $J = 8.2$ Hz), 6.92 (d; 1H, H-3-Th, $J = 3.7$ Hz), 6.76 (d; 1H, H-4-Th, $J = 3.7$ Hz), 4.47 (m; 1H, α-CH), 3.22 (dd; 1H, H_l of CH_2, $J = 4.4$ Hz, $J = 15.0$ Hz), 3.02 (dd; 1H, H_h of CH_2, $J = 8.9$ Hz, $J = 15.0$ Hz), 1.85 (s; 3H, CH_3) ppm; ^{13}C NMR (*DMSO*-d_6); $\delta = 172.52$ (s; COOH), 169.60 (s; CONH), 139.18 (s; C-2-Th), 126.50/126.40 (d; C-3-, C-4-Th), 53.22 (d; α-CH), 31.73 (t; CH_2), 22.63 (q; CH_3) ppm, –$C_9H_{10}ClNO_3S$ (247.70); calcd.: C 43.64, H 4.07, N 5.66; found: C 43.90, H 4.11, N 5.58.

(R,S*)-α-(Acetylamino)-5-methyl-2-thiophenepropanoic acid* (**4f**)

A solution of 60 mmol **3f** in 150 aqueous sodium hydroxide (10%) was heated to reflux for 3 h, acidified to *pH* 1–2 with 3 *N* HCl, and heated again to reflux for 1 h. The reaction mixture was concentrated to a volume of 120 ml and cooled to 5 °C. Precipitating crystals were collected, washed with a small portion of water, and dried.

Yield: 93%; m.p.: 165–166 °C; TLC (ethyl acetate/methanol = 4:1): $R_f = 0.25$; ^{1}H NMR (*DMSO*-d_6): $\delta = 8.22$ (d; 1H, NH, $J = 8.1$ Hz), 6.65 (d; 1H, H-3-Th), $J = 3.4$ Hz) 6.59 (d; 1H, H-4-Th, $J = 3.4$ Hz), 4.34 (m; 1H, α-CH), 3.15 (dd; 1H, H_l of CH_2, $J = 4.8$ Hz, $J = 14.8$ Hz), 2.99 (dd; 1H, H_h of CH_2, $J = 8.8$ Hz, $J = 14.8$ Hz), 2.37 (s; 3H, CH_3), 1.84 (s; 3H, CH_3) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 172.6$ (s; COOH), 169.3 (s; CONH), 137.7 (s; C-2-Th), 137.5 (s; C-5-Th), 126.0 (d; H-3-Th), 124.8 (d; C-4-Th), 53.5 (d; α-CH), 31.3 (t; CH_2), 22.4 (q; CH_3), 14.8 (q; CH_3) ppm; $C_{10}H_{13}NO_3S$ (227.29); calcd.: C 52.85, H 5.76, N 6.16; found: C 52.83, H 5.76, N 6.06.

(S)-Amino Acids (**5c–g**), *General Procedure*

80.0 mmol racemic **4c–g** were dissolved in 900 ml 0.1 *M* potassium phosphate buffer and acidified with 2 *N* HCl to $pH = 7.5–8$. The solution was treated with 5.0 g (10000 units) Acylase I [aspergillus melleus (E.C. 3.5.1.14)] and kept at 40 °C for 24 h. The suspension was filtered, the filtrate acidified to $pH = 2–3$ with 2 N HCl, and extracted with ethyl acetate until no more N-acetyl amino acid could be detected in the organic layer. The combined organic layers were washed with brine, dried over sodium sulfate, and evaporated to dryness. Thus, 80–95% of (*R*)-**4c–g** was recovered. The acidic aqueous layer was distilled to dryness, the residue dissolved in 150 ml water and stirred slowly with 200 ml ion exchange resin (Dowex 50, H^+) for 30 min. The suspension was filtered. The ion exchange resin was washed with water until the filtrate was neutral and then stirred with 600 ml 1 *M* aqueous ammonia. After filtration of the ion exchange resin and repetition of this procedure, the filtrate was evaporated to dryness and the residue recrystallized from water.

N-Protected Amino Acids (**6a–g**)

The protection of amino acids **5a–g** was carried out analogously to the literature [14].

α-(((Phenylmethoxy)carbonyl)amino)-1-naphthalenepropanoic acid (**6a**)

Yield: 11 g; m.p.: 140–145 °C; ^{1}H NMR (*DMSO*-d_6): $\delta = 8.50–6.95$ (m; 13H, aromatic H, NH), 6.30 (bs; 1H, NH), 5.45 (s; 2H, CH_2), 4.30 (m; 1H, CH), 3.75 (m; 1H, H_1 of CH_2), 3.25 (m; 1H, H_h of CH_2) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 175.3$ (s; COOH), 155.8 (s; CONH), 137.2 (s; C-1-Ph), 135.3 (s; C-1-Naph), 133.4 (s; C-9-Naph), 132.0 (s; C-10-Naph), 128.5 (d; C-3-Ph), 128.2 (2d; C-3, -4-Naph), 127.4 (2d, C-2, -4-Ph), 126.6 (d; C-8-Naph), 125.8 (d; C-7-Naph), 125.3 (d; C-6-Naph), 123.9 (d; C-5-Naph), 65.0 (t; CH_2), 56.8 (d; CH), 35.5 (t; CH_2) ppm.

(S)-α-(((1,1-Dimethyl)ethoxycarbonyl)amino)-5-chloro-2-thiophenepropanoic acid (**6d**)

Yield: 54%; TLC (ethyl acetate): $R_f = 0.53$; ^{1}H NMR ($CDCl_3$): $\delta = 8.85$ (bs; 1H, COOH), 6.70 (d; 1H, H-4-Th), 6.55 (d, 1H, H-3-Th), 5.15 (d; 1H, NH), 4.55 (m; 1H, α-CH), 3.25 (t; 2H, CH_2), 1.20 (s; 9H, CH_3) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 174.84$ (s; COOH), 155.39 (s; CONH); 136.34 (s; H-2-Th), 128.58 (d; C-4-Th), 125.96 (d; C-3-Th), 80.64 (s; OC), 53.94 (d; α-CH), 32.52 (t; CH_2), 28.28 (q; CH_3) ppm.

(S)-α-(((1,1-Dimethyl)ethoxycarbonyl)amino)-5-bromo-2-thiophenepropanoic acid (**6e**)

Yield: 60%; $[\alpha]_D^{23} = +4.2°$ ($c = 1.0$ in CH_2Cl_2); TLC (ethyl acetate): $R_f = 0.44$; ^{1}H NMR ($CDCl_3$): $\delta = 10.05$ (bs; 1H, COOH), 6.82 (d; 1H, H-4-Th, $J = 3.7$ Hz), 6.57 (d; 1H, H-3-Th, $J = 3.7$ Hz), 5.15 (d; 1H, NH), 4.50 (m; 1H, α-CH), 3.25 (t; 2H, CH_2), 1.35 (s; 9H, CH_3) ppm; ^{13}C-NMR ($CDCl_3$): $\delta = 175.45$ (s; CO), 155.30 (s; CO), 139.13 (s; C-2-Th), 129.78 (d; C-4-Th), 127.36 (d; C-3-Th), 110.89 (s; C-5-Th), 80.68 (s; OC), 53.93 (d; α-CH), 32.52 (t; CH_2), 28.26 (q; CH_3) ppm.

(S)-α-(((1,1-Dimethyl)ethoxycarbonyl)amino)-5-methyl-2-thiophenepropanoic acid (**6f**)

Yield: 81%; $[\alpha]_D^{23} = +15°$ ($c = 0.90$ in CH_2Cl_2); TLC (ethyl acetate): $R_f = 0.48$; ^{1}H NMR ($CDCl_3$): $\delta = 6.62$ (d; 1H, H-3-Th, $J = 3.3$ Hz), 6.57 (d; 1H, H-4-Th, $J = 3.3$ Hz), 5.16 (d; 1H, NH), 5.03 (m; 1H, α-CH), 3.29 (t; 2H, CH_2, $J = 4.8$ Hz), 2.42 (s; 3H, CH_3), 1.44 (s; 9H, CH_3) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 175.8$ (s; COOH), 155.5 (s; CONH), 139.0 (s; C-2-Th), 135.3 (s; C-5-Th), 126.6 (d; C-3-Th), 125.0 (d; C-4-Th), 80.2 (s; OC), 54.5 (d; α-CH), 32.3 (t; CH_2), 28.3 (q; CH_3), 15.2 (q; CH_3) ppm.

(2S(R,S*))-N-(3-(1-Naphthalenyl)-1-oxo-2((phenylmethoxy)carbonyl)amino)-propyl)histidine methylester and (2S(R*,R*))-N-(3-(1-Naphthalenyl)-1-oxo-2-((phenylmethoxy)carbonyl) amino)-propyl)histidine methylester* (**7a**)

The peptization of **6a** with histidine methylester was carried out according to Ref. [8a]. Yield: 3.4 g (50%); TLC (ethyl acetate/methanol = 4:1): $R_f = 0.28$; ^{1}H NMR ($CDCl_3$): $\delta = 8.20$–6.90 (m; 15H, aromatic H, NH, H-5-Im), 6.50 (s; 1H, CONH), 5.95 (s; 1H, CONH), 4.95 (s; 2H, OCH_2), 4.65 (m; 2H, α-CH-Naph, α-CH-His), 3.80–3.25 (m; 5H, OCH_3 and CH_2), 3.20–2.55 (m; 2H, CH_2) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 172.2$ (s; CO), 171.3 (s; CO), 171.2 (s; CO), 170.8 (s; CO), 155.8 (2s; CONH), 136.9 (s; C-1-Ph), 135.9 (d; C-5-Im), 135.0 (s; C-4-Im), 133.6 (s; C-9-Naph), 132.6 (s; C-9-Naph), 131.8 (s; C-10-Naph), 128.5 (d; C-3-Ph), 128.1 (C-3-Naph, C-2-Naph), 127.7 (d; C-4-Naph), 127.5 (2d; C-2-Ph, C-4-Ph), 126.1 (d; C-8-Naph), 125.4 (d; C-6-Naph), 125.1 (d; C-6-Naph), 123.3 (d; C-5-Naph), 116.3 (d; C-2-Im), 66.6 (t; OCH_2), 56.2 (d; α-CH-His), 52.4 (d; α-CH-Naph), 51.9 (q; OCH_3), 35.7 (t; CH_2-Naph), 28.6 (t; CH_2-His) ppm.

Boc-Dipeptide Methylesters (**7b–g**)

Peptization of the *BOC*-protected amino acids **6b–g** with histidine methylester was carried out analogously to the literature [15].

(2S(R,S*))-N-(3-(9-Anthracenyl)-2-(((1,1-dimethylethoxy)carbonyl)amino)-1-oxopropyl)-histidine methylester and (2S(R*,R*))-N-(3-(9-Anthracenyl)-2-(((1,1-dimethylethoxy)-carbonyl)amino)-1-oxopropyl)histidine methylester* (**7b**)

Yield: 1.4 g (53%); TLC (ethyl acetate/methanol = 4:1): $R_f = 0.6$; ^{1}H NMR (*DMSO*-d_6): $\delta = 8.55$–7.30 (m; 14H, aromatic H, NH, H-5-Im), 6.95 (s; 1H, CONH), 6.75 (2s; 1H, CONH), 4.80–4.25 (m; 2H, α-CH-Ant, α-CH-His), 3.85 (m; 2H, CH_2), 3.53 (s; 3H, OCH_3), 2.85 (m; 2H, CH_2) ppm.

(2S(R,S*))-N-(2-(((1,1-Dimethylethoxy)carbonyl)amino)-1-oxo-3-(2-thienyl)propyl)-histidine methylester and (2S(R*,R*))-N-(((1,1-Dimethylethoxy)carbonyl)-amino)-1-oxo-3-(2-thienyl)-propyl)histidine methylester* (**7c**)

Yield: 8.5 g (55%); m.p.: 96–100 °C; TLC (ethyl acetate/methanol = 4:1): $R_f = 0.4$ ^{1}H NMR ($CDCl_3$): $\delta = 7.50$ (bs; 2H, H-2-Im, NH), 7.17 (m; 1H, H-5-Th), 6.94 (m; 1H, H-4-Th), 6.86 (m; 1H, H-3-Th), 6.75 (s; 1H, H-5-Im), 5.27 (d; 1H, NH), 4.78 (m; 1H, α-CH-Th), 4.34 (m; 1H, α-CH-His), 3.69 (s; 3H, OCH_3), 3.30 (m; 2H, CH_2-Th), 3.10 (m; 2H, CH_2-His) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 171.5$ (s; CO), 170.9 (s; CO), 155.6 (s; OCONH), 138.1 (s; C-2-Th), 135.3 (d; C-2-Im), 132.0 (bs; C-4-Im), 126.9 (d; C-3-Th), 126.6 (d; C-4-Th), 124.6 (d; C-5-Th), 120.0 (d; C-5-Im), 80.3 (s; OC), 55.7 (d; α-CH-Thi), 52.6 (α-Ch-His), 52.3 (q; OCH_3), 32.2 (t; CH_2-Thi), 28.6 (t; CH_2-His) 28.2 (q; CH_3) ppm; $C_{19}H_{26}N_4O_5S \cdot H_2O$ (440.52); calcd.: C 51.80, H 6.41, N 12.72; found: C 51.80, H 5.90, N 12.32.

(2S(R,R*))-N-(3-(5-chloro-2-thienyl)-2-(((1,1-dimethylethoxy)carbonyl)amino)-1-oxopropyl)histidine methylester* (**7d**)

Yield: 78%; m.p.: 77–79 °C; $[\alpha]_D^{23} = +16.4°$ ($c = 1.10$ in CH_2CL_2); TLC (ethyl acetate/methanol = 8:1): $R_f = 0.37$; ^{1}H NMR ($CDCl_3$/*DMSO*-d_6): $\delta = 7.94$ (d; 1H, NH, $J = 7.1$ Hz), 7.41 (s; 1H, H-2-Im), 6.68 (s; 1H, NH-Im), 6.63 (d; 1H, H-3-Th, $J = 3.7$ Hz), 6.55 (d; 1H, H-4-Th, $J = 3.7$ Hz), 6.61 (s; 1H, H-5-Im), 5.97 (d; 1H, NH, $J = 7.4$ Hz), 4.65 (m; 1H, α-CH-Thi), 4.29 (m; 1H, α-CH-His), 3.60 (s; 3H, OCH_3), 3.15 (dd; 2H, CH_2-Thi, $J = 5.0$ Hz, $J = 15.1$ Hz), 3.02 (dd; 2H, CH_2-His, $J = 7.8$ Hz, $J = 15.1$ Hz), 1.34 (s; 9H, CH_3) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 170.91$ (s; CO), 170.16 (s; CO), 154.88 (s; OCONH), 137.51 (s; C-2-Th), 131.85 (d; C-2-Im), 125.46/125.19 (2d; Th-C-3,-4), 117.22 (d; C-5-Im), 107.40 (s; C-5-Th), 79.13 (s; OC), 54.65 (d; α-CH-Thi), 52.07 (d; α-CH-His), 51.60 (q; OCH_3), 32.27 (t; CH_2-Thi), 28.33 (t; CH_2-Im), 27.77 (q; CH_3) ppm; $C_{19}H_{25}C1N_4O_5S \cdot 0.9\ H_2O$ (473.16); calcd.: C 48.26, H 5.71, N 11.85; found: C 48.23, H 5.71, N 11.65.

(2S(R,R*))-N-(3-(5-Bromo-2-thienyl)-2-(((1,1-dimethylethoxy)carbonyl)amino)-1-oxopropyl) histidine methylester* (**7e**)

Yield: 78%; m.p.: 75–77 °C; $[\alpha]_D^{23} = +22.4°$ ($c = 1.03$ in CH_2Cl_2); TLC (ethyl acetate/methanol = 4:1): $R_f = 0.60$; ^{1}H NMR (*DMSO*-d_6): $\delta = 7.50$ (s; 1H, CH-Im), 6.80 (d; 1H, H-4-Th), 6.55 (d; 1H, H-3-Th), 6.75 (s; 1H, CH-Im), 4.60 (m; 1H, α-CH-Thi), 4.20 (m; 1H, α-CH-His), 3.60 (s; 3H, OCH_3), 3.20–2.80 (m; 4H, CH_2-Thi, CH_2-His), 1.35 (s; 9H, CH_3); ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 170.68$ (s; CO), 170.22 (s; CO), 154.88 (s; OCONH), 139.94 (s; C-2-Th), 133.67 (d; C-2-Im), 128.15 (d; C-4-Th), 125.66 (d; C-3-Th), 115.63 (d; C-5-Im), 108.44 (s; C-5-Th), 78.26 (s; OC), 54.24 (d; α-CH-Thi), 51.29 (d; α-CH-His), 50.22 (OCH_3), 30.99 (t; CH_2-Thi), 27.27 (t; CH_2-His), 26.04 (q; CH_3) ppm; $C_{19}H_{25}BrN_4O_5S \cdot 1.55\ H_2O$ (529.32); calcd.: C 43.01, H 4.91, N 10.38; found: C 43.12, H 5.35, N 10.59.

(2S(R,R*))-N-(2-(((1,1-Dimethylethoxy)carbonyl)amino)-3-(5-methyl-2-thienyl)-1-oxopropyl) histidine methylester* (**7f**)

Yield: 45%; m.p.: 121–123 °C; $[\alpha]_D^{23} = +48.4°$ ($c = 1.0$ in CH_2Cl_2); TLC (ethyl acetate/methanol = 4:1): $R_f = 0.59$; ^{1}H NMR ($CDCl_3$): $\delta = 7.51$ (s; 1H, H-2-Im), 7.22 (d; 1H, CONH, $J = 6.1$ Hz), 6.74 (s; 1H, H-5-Im), 6.63 (d; 1H, H-3-Th, $J = 3.3$ Hz), 6.55 (d; 1H, H-4-Th, $J = 3.3$ Hz), 5.29 (d; 1H, CONH, $J = 7.2$ Hz), 4.77 (m; 1H, α-CH-Thi), 4.25 (m; 1H, α-CH-His), 3.70 (s; 3H, OCH_3), 3.25–3.10 (m; 4H,-CH_2-Thi, CH_2-His), 2.41 (s; 3H, CH_3), 1.43 (s; 9H, CH_3) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 171.6/171.3$ (s; CO), 155.8 (s; CONH), 139.1 (s; C-2-Th), 135.8 (s; C-5-Th), 135.3 (d; C-2-Im), 131.0 (bs; C-4-Im), 126.6 (d; C-3-Th), 124.9 (d; C-4-Th), 119.3 (bd; C-5-Im), 80.4 (s; OC), 55.9 (d; α-CH-Thi), 52.8 (d; α-CH-His), 52.4 (q; OCH_3), 32.4 (t; CH_2-Thi), 28.6 (t; CH_2-His), 28.3 (q; CH_3), 15.2 (q; CH_3) ppm; $C_{20}H_{28}N_4O_5S$ (436.53); calcd.: C 55.03, H 6.46, N 12.86; found: C 54.79, H 6.37, N 12.61.

(2S(R,R*))-N-(2-(((1,1-Dimethylethoxy)carbonyl)amino)-1-oxo-3-(3-thienyl)propyl)-histidine methylester* (**7g**)

Yield: 47%; m.p.: 98–101 °C; TLC (ethyl acetate/methanol = 85:4): $R_f = 0.20$; ^{1}H NMR ($CDCl_3$): $\delta = 7.55$ (d; 1H, H-2-Im), 7.35 (d; 1H, CONH), 7.30 (m; 1H, H-2-Th), 7.10 (s; 1H, H-4-Th), 6.95 (d; 1H, H-5-Th), 6.75 (s; 1H, H-5-Im), 5.40 (d; 1H, NH), 4.80 (m; 1H, α-CH-His), 4.38 (m; 1H, α-CH-Thi), 3.70 (s; 3H, O CH_3), 3.20–3.00 (m; 4H, CH_2-Im, CH_2-Thi), 1.45 (s; 9H, CH_3); ppm; ^{13}C NMR ($CDCl_3$): $\delta = 171.32$, 171.17 (2s, COOMe, CONH), 155.83 (s; OCONH), 136.45 (s; C-3-Th), 135.34 (d; C-2-Im), 131.23 (s; C-4-Im), 128.35 (d; C-2-Th), 125.87 (d; C-5-Th), 122.77 (d; C-4-Th), 119.98 (d; Im-C-5), 80.45 (s; OC), 55.45 (d; α-CH-His), 52.74 (q; O CH_3), 52.43 (d; α-CH-Thi), 32.50 (t; CH_2-Thi), 28.25 (q; CH_3), 28.20 (t; CH_2-His) ppm $C_{19}H_{26}N_4O_5S\ H_2O$ (440.52); calcd.: C 51.80, H 6.41, N 12.72; found: C 51.60, H 6.08, N 12.92.

Cyclic Dipeptides (**8a–g**), *General Procedure*

A solution of 6.00 mmol of **7** in 60.0 mmol trifluoroacetic acid and 8.00 ml dichloromethane was stirred for 15 min at 0 °C and for 2 h at room temperature. The solvent was evaporated *in vacuo*, the residue dissolved in 50 ml methanol, and alkalized with concentrated aqueous ammonia to $pH = 8$. The solution was heated to reflux for 1 d and then cooled to 0 °C. The crystals were collected, dried, and recrystallized from methanol.

(3S,cis)-1'H-3-(4-imidazolylmethyl)-6-(1-naphthalenylmethyl)piperazin-2,5-dione (**8a**)

Yield: 63%; m.p.: 260–263 °C; $[\alpha]_D^{23} = -67°$ ($c = 1.8$ in AcOH); TLC (ethyl acetate/methanol = 1:1): $R_f = 0.5$; ^{1}H NMR (*DMSO*-d_6): $\delta = 8.20$–7.25 (m; 12H, aromatic H, H-5-Im, NH), 6.45 (s; 1H, CONH), 4.15 (m; 1H, α-CH-Naph), 3.90 (m; 1H, α-CH-His), 3.35 (dd; 1H, H_1, CH_2-Naph, $J = 13$ Hz, $J = 5$ Hz),

2.95 (dd; H_h, CH_2-Naph, $J = 13$ Hz, $J = 6.4$ Hz), 2.55 (dd; 1H, H_l, CH_2-His, $J = 13.5$ Hz, $J = 4.4$ Hz), 1.95 (dd; 1H, H_h, CH_2-His, $J = 13.5$ Hz, $J = 7$ Hz) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 166.6$ (s; CO), 166.4 (s; CO), 134.8 (d; C-5-Im), 133.4 (s; C-4-Im), 132.8 (C-1-Naph), 131.9 (2s; C-9, C-10-Naph), 128.5 (d; C-3-Naph), 128.4 (d; C-4-Naph), 127.3 (C-2-Naph), 126.0 (d; C-8-Naph), 125.5 (d; C-7-Naph), 125.3 (d; C-6-Naph), 123.9 (d; C-5-Naph), 117.5 (d; C-2-Im), 55.2 (d; α-CH-Naph), 54.5 (d; α-CH-His), 36.4 (t; CH_2-Naph), 30.9 (t; CH_2-His) ppm; $C_{19}H_{18}N_4O_2 \cdot CH_3OH$ (360.0); calcd.: C 66.06, H 5.94, N 15.89; found: C 65.79, H 5.51, N 15.89.

(3S,cis)-1'H-6-(9-anthracenylmethyl)-3-(4-imidazolylmethyl)piperazin-2,5-dione (**8b**)

Yield: 33%; m.p.: 245–247 °C; $[\alpha]_D^{20} = -134°$ ($c = 1.4$ in methanol); TLC (ethyl acetate/methanol = 1:1): $R_f = 0.4$; ^{1}H NMR (*DMSO*-d_6): $\delta = 8.74$ (s; 1H, H-2-Im), 8.48 (s; 1H, H-10-anth), 8.24 (m; 2H, H-1, -8-anth), 8.10 (m; 2H, H-4, -5-anth), 7.62–7.44 (m; 4H, H-2, -3, -6, -7-anth), 6.85 (s; 1H, H-5-Im), 4.19 (m; 1H, α-CH-anth), 4.05–3.95 (m; 2H, α-CH-Im, H_l of CH_2-anth), 3.75 (dd; 1H, H_h of CH_2-anth), 2.83 (dd; 1H; H_l of CH_2-Im), 2.44 (dd; 1H, H_h of CH_2-Im) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 166.8$ (s; CONH), 165.4 (s; CONH), 134.5 (d; C-2-Im), 131.1 (s; C-11, -14-anth), 130.3 (s; C-12, -13-anth), 129.4 (s; C-4-Im), 129.0 (d; C-4, -5-anth), 126.8 (d; C-10-anth), 125.9 (d; C-2, -7-anth), 125.0 (d; C-3, -6-anth), 124.6 (d; C-1, -8-anth), 117.7 (d; C-5-Im), 56.1 (d; α-CH-anth), 54.0 (α-CH-His), 32.9 (t; CH_2-anth), 30.0 (t; CH_2-His) ppm; $C_{23}H_{20}N_4O_2 \cdot H_2O$ (400.65); calcd.: C 68.95, H 5.48, N 13.98; found: C 68.88, H 5.63, N 13.82.

(3S,cis)-1'H-3-(4-imidazolylmethyl)-6-(2-thienylmethyl)piperazin-2,5-dione (**8c**)

Yield: 28%; m.p.: 184–186 °C, $[\alpha]_D^{23} = -20°$ ($c = 1.4$ in methanol); TLC (ethyl acetate/methanol = 4:1): $R_f = 0.5$; ^{1}H NMR (*DMSO*-d_6): $\delta = 8.25$ (s; 1H, NH), 8.00 (s; 1H, H-2-Im), 7.95 (s; 1H, NH), 7.39 (dd; 1H, H-5-Th, $J = 5.1$ Hz, $J = 1.3$ Hz), 6.97 (d; 1H, H-4-Th, $J = 5.1$ Hz, $J = 3.4$ Hz), 6.84 (dd; 1H, H-3-Th, $J = 3.5$ Hz, $J = 1.3$ Hz), 6.78 (s; 1H, H-5-Im), 4.18 (m; 1H, α-CH-Thi), 3.98 (m; 1H, α-CH-His), 3.12 (dd; 1H, H_l of CH_2-Thi), 3.05 (dd; 1H, H_h of CH_2-Thi), 2.70 (dd; 1H, H_l of CH_2-His), 1.93 (dd; 1H, 1H, H_h of CH_2-His) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 166.5$ (s; CO), 166.1 (s; CO), 137.3 (s; C-2-Th), 134.6 (d; C-2-Im), 131.9 (s; C-4-Im), 127.5 (d; C-3-Th), 126.9 (d; C-4-Th), 125.2 (d; C-5-Th), 116.6 (d; C-5-Im), 55.0 (d; α-CH-Thi), 54.0 (d; α-CH-His), 32.4 (t; CH_2-Thi), 30.1 (t; CH_2-His) ppm; $C_{13}H_{14}N_4O_2S \cdot 0.5\ H_2O$ (299.34); calcd.: C 52.16, H 5.05, N 18.72; found: C 52.03, H 4.87, N 18.78.

(3S,cis)-1'H-6-((5-Chloro-2-thienyl)methyl)-3-(4-imidazolylmethyl)piperazin-2,5-dion (**8d**)

Yield: 81%; m.p.: 195–198 °C; $[\alpha]_D^{23} = -55°$ ($c = 0.10$ in methanol); TLC (ethyl acetate/methanol = 1:1): $R_f = 0.51$; ^{1}H NMR (*DMSO*-d_6) $\delta = 8.24$ (s; 1H, CONH), 8.07 (s; 1H, CONH), 7.80 (s; 1H, H-2-Im), 6.96 (d; 1H, H-3-Th, $J = 3.7$ Hz), 6.79 (s; 1H, H-5-Im), 6.68 (d; 1H, H-4-Th, $J = 3.7$ Hz), 4.15 (m; 1H, α-CH-Thi), 4.02 (m; 1H, α-CH-His), 2.99 (m; 2H, CH_2-Thi), 2.85 (dd; 1H, H_l of CH_2-Im, $J = 3.4$ Hz, $J = 14.8$ Hz), 2.22 (dd; 1H, H_h of CH_2-Im, $J = 8.4$ Hz, $J = 14.8$ Hz) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 166.81$ (s; CONH), 166.39 (s; CONH), 137.19 (s; C-2-Th), 134.50 (d; C-2-Im), 131.26 (s; C-4-Im), 127.32 (d; C-3-Th), 126.56 (s; C-5-Th), 126.35 (d; C-4-Th), 116.91 (d; C-5-Im), 54.72 (d; α-CH-Thi), 53.62 (d; α-CH-His), 32.62 (t; CH_2-Thi), 29.94 (t; CH_2-His) ppm; $C_{13}H_{13}ClN_4O_2S \cdot H_2O$ (342.81); calcd.: C 45.54, H 4.41, N 16.34; found: C 45.42, H 4.62, N 16.12.

(3S,cis)-1'H-6-((5-Bromo-2-thienyl)methyl)-3-(4-imidazolylmethyl)piperazin-2,5-dione (**8e**)

Yield: 53%; m.p.: 193–195 °C $[\alpha]_D^{23} = -54°$ ($c = 0.10$ in methanol); TLC (ethyl acetate/methanol = 1:1): $R_f = 0.54$; ^{1}H NMR (*DMSO*-d_6): $\delta = 8.24$ (s; 1H, CONH), 8.04 (s; 1H, CONH), 7.89 (s; 1H, H-2-Im), 7.06 (d; 1H, H-3-Th, $J = 3.70$ Hz), 6.81 (s; 1H, H-5-Im), 6.66 (d; 1H, H-4-Th, $J = 3.7$ Hz), 4.14 (m; 1H, α-CH-Thi), 4.09 (m; 1H, α-CH-His), 3.05 (dd; 1H, H_l of CH_2-Thi, $J = 4.4$ Hz, $J = 15.0$ Hz), 2.96 (dd; 1H, H_h of CH_2-Thi, $J = 15.0$ Hz), 2.84 (dd; 1H, H_l of CH_2-His, $J = 4.0$ Hz, $J = 14.7$ Hz), 2.20 (dd; 1H, H_h of

CH_2-His, $J = 8.2$ Hz, $J = 14.7$ Hz) ppm; ^{13}C NMR (*DMSO*-d_6); $\delta = 166.94$ (s; CONH), 166.28 (s; CONH), 139.91 (s; C-2-Th), 134.78 (d; C-2-Im), 132.24 (s; C-4-Im), 129.93 (d; C-3-Th), 128.36 (d; C-4-Th), 116.83 (d; C-5-Im), 109.57 (s; C-5-Th), 54.73 (d; α-CH-Thi), 54.20 (d; α-CH-His), 32.74 (t; CH_2-Thi), 30.15 (t; CH_2-His) ppm; $C_{13}H_{13}BrN_4O_2S \cdot 0.6\,H_2O$ (380.05); calcd.: C 41.10, H 3.76, N 14.75; found: C 41.23, H 4.05, N 14.43.

(3S,cis)-1'H-3-(4-Imidazolylmethyl)-6-((5-methyl-2-thienyl)methyl)piperazin-2,5-dione (**8f**)

Yield: 87%; m.p.: 239–241 °C, $[\alpha]_D^{23} = -120°$ ($c = 0.1$ in methanol); TLC (ethyl acetate/methanol = 1:1): $R_f = 0.54$; ^1H NMR (*DMSO*-d_6): $\delta = 8.10$ (s; 1H, CONH), 7.88 (s; 1H, CONH), 7.56 (s; 1H, H-2-Im), 6.69 (s; 1H, H-5-Im), 6.62 (d; 1H, H-3-Th), 6.60 (d; 1H, H-4-Th), 4.08 (m; 1H, α-CH-Thi), 3.94 (m; 1H, α-CH-His), 2.97 (m; 2H, CH_2-Thi), 2.78 (dd; 1H, H_l of CH_2-His, $J = 3.20$ Hz, $J = 14.6$ Hz), 2.36 (s; 3H, CH_3), 2.00 (dd; 1H, H_h of CH_2-His, $J = 8.9$ Hz, $J = 14.6$ Hz) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 166.92$ (s; CONH), 166.14 (s; CONH), 138.17 (s; C-2-Th), 135.17 (s; C-5-Th), 134.96 (d; C-2-Im), 133.48 (s; C-4-Im), 127.20 (d; C-3-Th), 125.04 (d; C-4-Th), 116.67 (d; C-5-Im), 55.11 (d; α-CH-Thi), 54.50 (d; α-CH-His), 48.60 (q; OCH_3), 32.82 (t; CH_2-Thi), 30.93 (t; CH_2-His), 14.84 (q; CH_3) ppm; $C_{14}H_{16}N_4O_2S \cdot 0.7\,H_2O$ (318.37); calcd.: C 52.95, H 5.54, N 17.36; found: C 52.97, H 5.54, N 17.64.

(3S,cis)-1'H-3-(4-Imidazolylmethyl)-6-(3-thienylmethyl)piperazin-2,5-dione (**8g**)

Yield: 36%; m.p.: 214–215 °C; $[\alpha]_D^{20} = -105.1°$ ($c = 0.392$ in methanol); TLC (ethyl acetate/methanol = 1:1): $R_f = 0.47$; ^1H NMR (*DMSO*-d_6): $\delta = 8.13$ (s; 1H, NH), 7.89 (s; 1H, NH), 7.80 (d; 1H, H-2-Im), 7.46 (m; 1H, H-2-Th), 7.40 (m; 1H, H-4-Th), 7.12 (m; 1H, H-5-Th), 6.90 (d; 1H, NH), 6.73 (d; 1H, H-5-Im), 4.13 (m; 1H, α-CH-Thi), 3.90 (m; 1H, α-CH-His), 2.92 (dd; 1H, H_l of CH_2-Thi, $J = 4.44$ Hz, $J = 14.07$ Hz), 2.86 (dd; 1H, H_h of CH_2-Thi, $J = 4.67$ Hz, $J = 14.03$ Hz), 2.70 (dd; 1H, H_l of CH_2-His, $J = 3.73$, $J = 14.71$), 1.90 (dd; 1H, H_h of CH_2-His, $J = 8.57$, $J = 14.67$) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 166.68$ (s; CONH), 166.16 (s; CONH), 136.34 (d; C-2-Im), 134.30 (s; C-3-Th), 129.80 (s; C-4-Im), 129.59 (d; C-2-Th), 125.74 (d; C-5-Th), 123.66 (d; C-4-Th), 116.99 (d; C-5-Im), 57.47 (d; α-CH-Th), 54.55 (d; α-CH-Im), 32.68 (t; CH_2-Thi), 30.88 (t; CH_2-Im) ppm; $C_{13}H_{14}N_4O_2S \cdot 1.5\,H_2O$ (317.37); calcd.: C 49.20, H 5.40, N 17.65; found: C 49.17, H 5.50, N 17.44.

Preparation of Ion Exchange Resins (IER)

100 ml Basic IER III Merck was activated with 250 ml aqueous 4% NaOH or aqueous 6% NaCN, respectively. The IER was then washed neutral with deionized water. For methanolic preparation, a portion was washed with absolute methanol and kept under methanol.

Activation of Cyclic Dipeptides

A methanolic or aqueous solution of 60–100 mg of dipeptide was passed through a small column filled with 5 ml of IER and the column was washed with the solvent. Recovery rates of 70% were reached. The solvent was evaporated *in vacuo* and the dipeptide dried *in vacuo* at 30–40 °C.

Hydrocyanation

20–25 mg (3 mol%) cyclic dipeptide were stirred for 15 minutes in 4 ml absolute toluene and 0.35 ml benzaldehyde at 0 °C 0.3–0.4 ml HCN were added, and the reaction mixture was stirred vigorously. The dipeptide could be recovered quantitatively by filtration after the reaction had been quenched with ether which caused precipitation of the catalyst.

General Procedure for the Determination of Conversion and Enantioselectivity

100 µl samples were taken and quenched with 1 ml ether. After filtration through a one way filter (Anotop 0.02 µm, Merck), the conversion could be directly determined by GC (column temperature:

150 °C) from the solution. The remaining solution was concentrated under reduced pressure until an oil was left. This oil was dissolved in absolute dichloromethane and reacted with *exo*-(2*R*,2′*R*-(2α,2′α,3aα,3′aα,4β,4′β,7β,7′β,7aα,7′aα))-2,2′-oxy-*bis*-(octahydro-7,8,8-trimethyl-4,7-methanobenzofurane) in the presence of acid IER Amberlyst 15 (Aldrich). After 30 minutes, the diastereomeric excess could be measured directly from this solution using GC (column temperature: 280 °C).

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