New Proline Mimetics: Synthesis of Thrombin Inhibitors Incorporating Cyclopentane- and Cyclopentenedicarboxylic Acid Templates in the P2 Position. Binding Conformation Investigated by X-ray Crystallography

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With the aim to prepare nonpeptidic thrombin inhibitors, the amino acids of the thrombin-inhibiting tripeptide chain D-Phe-Pro-Arg were replaced with isosteres. Arg was replaced with the more rigid P1 truncated *p*-amidinobenzylamine (Pab), Pro with either cyclopentane-1,2-dicarboxylic acid or cyclopentene-1,5-dicarboxylic acid, and D-Phe with a series of readily available lipophilic amines. One of the most potent compounds (**25**, $pIC_{50} = 6.01$**)** in these series was cocrystallized with thrombin where the X-ray crystal structure provide insight to the structure−activity relationship (SAR).

Introduction

Proteases represent a large and varied class of biomolecules that presently are under intense investigation as targets for therapeutical intervention in disease. However, the design and discovery of orally active and selective protease inhibitors still remains a major challenge in drug discovery.[1]

Undesired blood clotting is the major culprit in a number of cardiovascular diseases, i.e., deep venous thrombosis, pulmonary embolism, unstable angina, restenosis following angioplasty, and arterial thrombosis.[2] Thrombin, a member of the trypsin family of serine proteases, plays a critical role in the blood coagulation cascade. The procoagulant properties of thrombin are exerted via the conversion of fibrinogen into a fibrin clot and from activation of zymogens upstream in the coagulation cascade.[3] Moreover, thrombin is the most potent stimulator of platelet aggregation known. Thus, small molecule thrombin inhibitors to regulate hemostasis and thrombosis are anticipated to provide powerful drugs, and indeed there has been a major focus on low-molecular-weight thrombin inhibitors in recent years.[3b,4]

The classical motif of thrombin inhibitors is the D-Phe-Pro-Arg[5] sequence mimicking thrombin's natural substrate, fibrinogen. A number of development candidates and clinical inhibitors such as inogatran[6] and melagatran[7] have been developed based on this motif (Chart 1).

The amino acid L-proline has frequently been explored as a template and starting point for peptidomimetic inhibitor design, cf. inhibitors of the human immunodeficiency virus type 1 (HIV-1) protease and angiotensin-converting enzyme (ACE). There are however notably few reports of replacing proline with five-membered carbocyclic ring isosteres in the tripeptide type motif D-Phe-Pro-Arg. Cyclohexane-1,2-dicarboxylic acid and 2-aminocyclohexanecarboxylic acid have been reported as proline isosteres in thrombin inhibitors.[8] Moreover, Turbanti et al.[9] have incorporated cyclopentane-1,2-dicarboxylic acids in ACE inhibitors. Recently a (*Z*)-alkene isostere of a *cis*-Pro dipeptide was described.[10]

We now report on the synthesis of two novel *N*-acylproline isosteres, i.e., *trans*-(1*S*,2*S*)-cyclopentane-1,2-dicarboxylic acid and (2*R*)-cyclopent-2-ene-1,2-dicarboxylic acid (Chart 2), and furthermore the incorporation of these P2 templates in a thrombin inhibitor

Chart 1

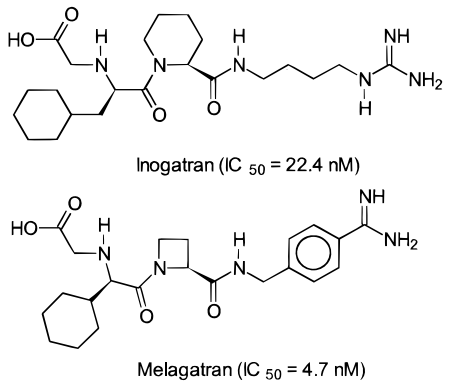
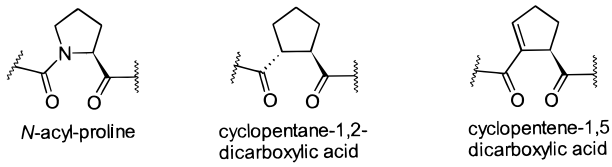


Chart 2. *N*-Acylproline in Comparison with the *N*-Acylproline Isosteres Used in This Paper



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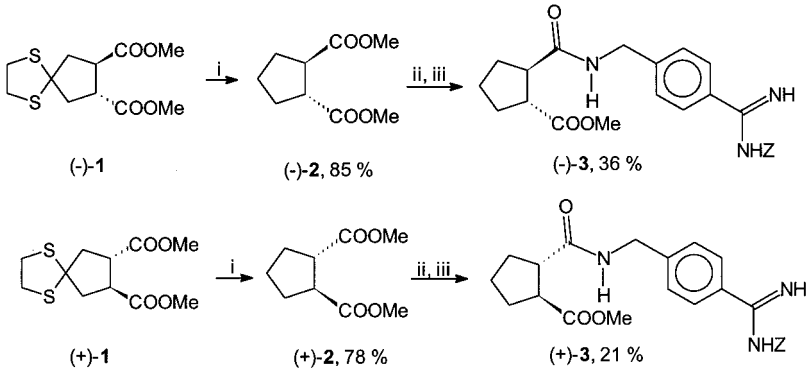
Scheme 1[a]



[a] Reagents and conditions: (i) Raney nickel, methanol, reflux; (ii) NaOH, dioxane−water, 1:1; (iii) Pab(Z)·HCl, EDC, HOBt, Et_3N, DMF.

motif having *p*-amidinobenzylamine in the P1 position and having lipophilic amines in the P3 position. Several of these compounds showed promising thrombin inhibition activity in vitro, $IC_{50} \sim 1\ \mu M$. Moreover, the X-ray crystal structure was determined for inhibitor **25** in complex with thrombin, providing additional SAR for this class of compounds.

Results and Discussion

Chemistry. Numerous synthetic routes for the desired enantiomerically pure *trans*-cyclopentane-1,2-dicarboxylic acid derivatives have been reported, including traditional resolution,[11] enzymatic resolution,[12] and asymmetric synthesis.[13] We chose to start with the enantiomerically pure thioketal (−)-*trans*-(7*R*,8*R*)-1,4-dithiaspiro[4.4]nonane-7,8-dicarboxylic acid dimethyl ester [(−)-**1**], prepared earlier in our laboratory,[14] which was desulfurized using Raney nickel in refluxing methanol to give the bisester (−)-**2** in 85% yield (Scheme 1).[15] Partial hydrolysis of (−)-**2** with sodium hydroxide in aqueous dioxane gave the corresponding monoester[16] that was coupled with *p*-(benzyloxycarbonyl)amidinobenzylamine hydrochloride (Pab(Z)·HCl)[17] using EDC [*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide]−HOBt (*N*-hydroxybenzotriazole) to give (−)-**3** in 36% overall yield. The corresponding (+)-**3** was prepared from (+)-**1** using a similar procedure. The ester function of (−)-**3** was hydrolyzed using sodium hydroxide in water−dioxane, and the resulting acid was directly coupled with various amines (i.e., benzylamine, (*R*)-phenylglycine methyl ester, cyclohexylamine, and *N*-ethylcyclohexylamine) using EDC−HOBt or Bop-Cl [bis(2-oxo-3-oxazolidinyl)-phosphinic chloride] as coupling agents (Table 1). The Z-group was removed by hydrogenolysis to give compounds (−)-**4**, **5**, **6**,[18] **7**, and **8** in overall yields ranging from 41% to 67%. The corresponding (+)-**4** was prepared from (+)-**3** using the same procedure in 67% yield.

For the synthesis of the chiral monoester of cyclopent-2-ene-1,2-dicarboxylic acid, the corresponding enantiomerically pure bismethyl esters[19] were examined as a potential precursor. Thus using the racemic compound as a model, attempts were made to selectively hydrolyze one of the esters using various metallo hydroxides or using pig liver esterase (PLE). However, only modest selectivity could be obtained. Moreover, the resulting monoesters resisted chromatographic separation. In view of these difficulties novel routes were investigated.

Table 1[a]

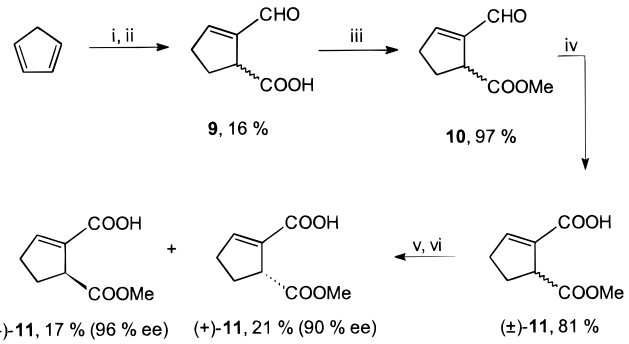
	RNHR′	Total Yield (coupling agent)	IC_{50} (μM)
(-)-**4**		41 % (EDC, HOBt)	44.7
(+)-**4** (reverse stereochemistry in cyclopentane)		67 % (EDC, HOBt)	>132
5		66 % (EDC, HOBt)	20.4
6		56 % (EDC, HOBt)	>132
7		67 % (Bop-Cl)	1.32
8		66 % (Bop-Cl)	13.2

[a] Reagents and conditions: (i) NaOH, dioxane, water; (ii) RNHR′, coupling agent, Et_3N, DMF; (iii) (only for compound **6**) NaOH, dioxane, water; (iv) H_2, Pd−C, ethanol.

Stevens et al.[20] have reported on the synthesis of racemic 1-formylcyclopentene-5-carboxylic acid (**9**) (Scheme 2). This acid was thus converted to the methyl ester using trimethoxymethane with acid catalysis to give **10** in 97% yield. The aldehyde **10** was oxidized using sodium chlorite with resorcinol as scavenger to give the acid (±)-**11** in 81% yield. This acid was coupled with benzylamine using EDC and HOBt as coupling agents to give **12** in 75% yield (Table 2). Hydrolysis of **12** followed by coupling with Pab(Z)·HCl and deprotection of the coupling product with triflic acid and anisole in dichloromethane gave racemic **13** in 60% overall yield. For the preparation of enantiomerically enriched compounds, (±)-**11** was resolved with (−)-ephedrine to give (−)-**11** and (+)-**11** in 17% and 21% yield, respectively.[21]

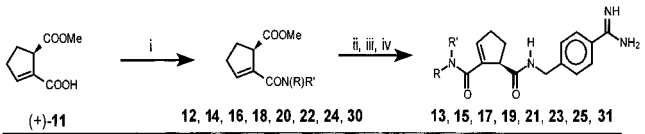
Using the same procedure, vide supra, compound (+)-**11** was coupled with piperidine, cyclohexylamine, and cyclohexylmethylamine to give compounds **15**, **17,** and

Scheme 2[a]



[a] Reagents and conditions: (i) $Cl_2CHCOCl$, Et_3N, hexane; (ii) 1 M $NaHCO_3$, 80 °C; (iii) $HC(OCH_3)_3$, TsOH; (iv) $NaClO_2$, resorcinol, NaH_2PO_4, *t*-BuOH−water, 2:1; (v) (−)-ephedrine, EtOAc, recrystallization.

Table 2[a]



	RNHR'	Yield for first peptide coupling (coupling agent)	Yield for second peptide coupling (coupling agent)	IC_{50} (μM)
13 (starting with (±)-**11**)		75 % (EDC, HOBt)	60 % (EDC, HOBt)	11.2
15		88 % (Bop-Cl)	47 % (Bop-Cl)	6.03
17		85 % (Bop-Cl)	39 % (Bop-Cl)	1.51
19		51 % (Bop-Cl)	51 % (Bop-Cl)	9.33
21		44 % (Bop-Cl)	19 % (Bop-Cl)	4.90
23		100 % (Bop-Cl)	23 % (Bop-Cl)	0.89
25		73 % (Bop-Cl)	19 % (Bop-Cl)	0.98
31		76 % (Bop-Cl)	47 % (Bop-Cl)	0.87

[a] Reagents and conditions: (i) RNHR′, coupling agent, Et_3N, DMF; (ii) NaOH, dioxane, water; (iii) Pab(Z)·HCl, coupling agent, Et_3N, DMF; (iv) TFMSA, anisole, CH_2Cl_2.

19. In view of the promising results, vide infra, obtained from **17** with cyclohexylamine in the P3 position additional amines were also investigated, i.e., aniline, *N*-ethylcyclohexylamine, *N*-ethylaniline, and *N*-ethoxycyclohexylamine resulting in compounds **21**, **23**, **25**, and **31**. *N*-Ethoxycyclohexylamine was prepared from *N*-cyclohexylhydroxylamine (**26**) in two steps. Compound **26** was Boc-protected using Boc-anhydride and Na_2CO_3 in a 1:1 mixture of water and tetrahydrofuran to give the N-protected *N*-cyclohexylhydroxylamine **27** in 41% yield along with the *O*-Boc-protected derivative **28** in 39% yield. O-Alkylation of **27** using EtI and NaOH in refluxing methanol gave **29** in 84% yield, which was

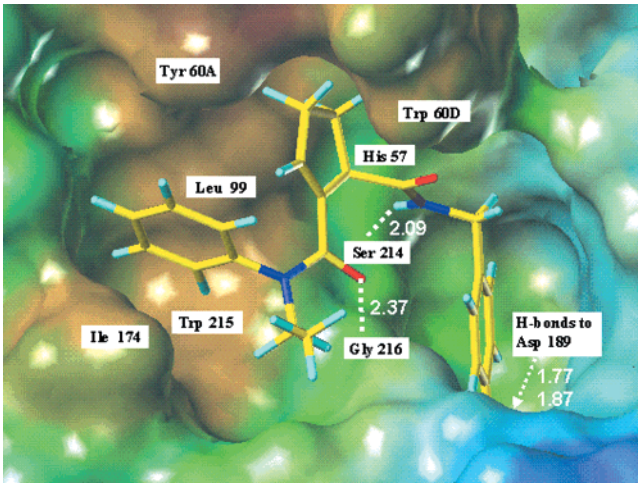


Figure 1. Lipophilic Connolly suface from the X-ray crystal structure of human α-thrombin in complex with inhibitor **25**.

Boc-deprotected prior to the coupling reaction using a 1:1 mixture of trifluoroacetic acid and dichloromethane.

Structure−Activity Relationship and X-ray Crystallographic Data. The compounds synthesized show less affinity for thrombin compared to melagatran or inogatran (Chart 1). In the following, possible explanations will be given as well as some qualitative SAR considerations for the relative affinities within each of the two series.

1. *trans*-(1*S*,2*S*)-Cyclopentane-1,2-dicarboxylic Acid Derivatives. With respect to the analogues based on the *trans*-(1*S*,2*S*)-cyclopentanedicarboxylic acid scaffold (Table 1), their low affinities as compared to classical inhibitors can be explained at least partly by the different geometry as compared to proline. The planar proline P3 amido bridge is fundamentally altered by an sp^3 carbon in cyclopentane, thereby changing the angle under which P2 is connected to P3. This obviously influences the possibility of the carbonyl group to act as a hydrogen bond acceptor for the NH of Gly216 in thrombin. On the basis of some preliminary docking studies using the Sybyl molecular modeling software, a hydrogen bond with Gly216 should be possible depending on the rest of the P3 moiety. The best compound (**7**, IC_{50} = 1.32 μM) in this series has a cyclohexyl in the P3 position. All of the phenyl analogues are less active. A possible explanation for this might be that the positioning of a phenyl group in P3 is rather critical due to its interaction with the π-electron system of Trp215. An optimal interaction between these two moieties results from either a perpendicular or a parallel relative orientation of the ring systems, whereas optimal interactions of a cyclohexane with Trp215 are based on lipophilicity only, thereby making the geometry of the interaction less critical.

2. (2*R*)-Cyclopent-2-ene-1,2-dicarboxylic Acids. These derivatives are closer mimics of proline than the above-discussed cyclopentane derivatives (Table 2). Next to the active cyclohexyl analogues **17**, **23,** and **31**, also the phenyl derivative **25** was found to have a reasonable affinity. To get a better understanding of the interaction of this compound, **25** was cocrystallized with thrombin. The structure of this complex is shown in Figure 1.

With respect to the hydrogen-onding interactions of **25**, the following observations can be made: The benz-

amidino group forms a salt bridge with the carboxylate of Asp189 (N−O distances being 2.74 and 2.78 Å). The positioning of the benzamidino moiety as a whole is identical with other X-ray structures of complexes of highly active analogues (unpublished results). The NH in the P1−P2 linker forms a neat hydrogen bond with the main chain carbonyl of the catalytic Ser256 (N−O distance is 3.07 Å). In contrast to this the hydrogen bond of the carbonyl group in the P2−P3 linker of **25** is far from optimal, the O−N distance being 3.35 Å.

The binding conformation of **25** is not optimal in the P2−P3 linker. The torsional angle of the cyclopentene double bond relative to the α-carbonyl group is not planar. The deviation from planarity is 30.1° thereby giving rise to a considerable loss in binding affinity. Besides this imperfection in the binding conformation, the P2−P3 amido group also deviates from planarity by an amount of 20°. Moreover, the P2−P3 amido group adopts a *cis*-geometry around the N−carbonyl-carbon plane, directing the phenyl group into the S3 pocket. This would favor secondary amines (tertiary amides) in the P3 position as primary amines, which preferentially adopt a *trans*-geometry, giving rise to unfavorable binding to the enzyme. Notably however, the angle between the planes of the ligand's phenyl group in P3 and the Trp215 indole plane is a near optimal 93.4°.

Conclusion

Two new proline mimetics, i.e., *trans*-(1*S*,2*S*)-cyclopentane-1,2-dicarboxylic acid and (2*R*)-cyclopent-2-ene-1,2-dicarboxylic acid, incorporated in a thrombin inhibitor motif have been prepared. The best of these thrombin inhibitors, having the proline templates in the P2 position, *p*-amidinobenzylamine in the P1 position, and lipophilic amines in the P3 position, were **23** (IC_{50} = 0.89 μM), **25** (IC_{50} = 0.98 μM), and **31** (IC_{50} = 0.87 μM) all having a secondary amine in the P3 position. The X-ray crystal structure of compound **25** complexed with thrombin shows that further optimization within this series of proline mimics should be directed toward releasing the strain in the P2−P3 linker and improving the hydrogen bonding with Gly216. Moreover, further stabilization of the *cis*-geometry around the P2−P3 amido group should lead to more potent thrombin inhibitors.

Experimental Section

X-ray Crystallography. Human α-thrombin was purchased from Enzyme Research Laboratories, Inc., South Bend, IN, and hirugen from American Diagnostica, Inc., Greenwich, CT. Hirugen−thrombin complex was prepared according to the method of Skrzypczak-Jankun et al.[22] and the sample was concentrated to 5.5 mg/mL. The crystallization was carried out at 20 °C by vapor diffusion method using 24−30% PEG 4000 and 0.1 M sodium phosphate buffer (SPB) at pH 7.3. A single crystal was used for soaking overnight in 30% PEG 4000, 0.1 M SPB, pH 7.3, containing 0.5 mg/mL of the inhibitor. The crystal belongs to monoclinic space group *C*2 with cell constants of a = 69.96 Å, b = 71.80 Å, c = 71.49 Å, $a = g$ = 90°, b = 100.2°, with one protein molecule per asymmetric unit.

The X-ray data to 1.55 Å resolution were collected on a MAR-II imaging plate system, MAR Research, Hamburg, Germany, using Cu Kα radiation from a rotating anode. The data were reduced and scaled using DENZO[23] and SCALA from the CCP4[24] package.

The hirugen−α-thrombin structure previously solved in our laboratory was used in the refinement of the (1*R*)-*N*[1]-{4-[amino(imino)methyl]benzyl}-*N*[2]-phenyl-*N*[2]-ethyl-2-cyclopentene-1,2-dicarboxamide (**25**)−thrombin structure. The refinement was performed using REFMAC (CCP4 package) with subsequent runs of X-PLOR.[25] Statistics for X-ray data collection and refinement are presented in Table 3.

Table 3. Parameters and Statistics from the Data Collection and Refinement of the Thrombin−(5*R*)-Cyclopentene-1-carbonyl-*N*-ethylphenylamide-5-carbonyl-*p*-amidinobenzylamide Complex

no. of measurements	257369
no. of unique reflections	45814
data completeness	89.7 (75.5)*
R_{merge}[a]	0.039 (0.299)*
no. of atoms in refined model	2618
protein	2329
ligands	29
solvent	260
resolution range in refinement (Å)	18.5−1.55
rms deviation	
bond length (Å)	0.015
angles (deg)	1.853
R_{cryst}[b]	0.247
R_{free}	0.285

[a] $R_{merge} = S_h S_i(I(h,i) - \langle I(h)\rangle)/S_h S_i\ I(h,i)$, where $I(h,i)$ is the intensity value of the ith measurement of h and $\langle I(h)\rangle$ is the corresponding mean value of h for all i measurements of h. [b] $R_{cryst} = S_{hkl}(|F_o - F_c|)/S_{hkl}\ |F_o|$, where $|F_o|$ and $|F_c|$ are observed and calculated structure factor amplitudes, respectively. *The numbers in parentheses are related to the data in the highest resolution shell, i.e., from 1.63 to 1.55 Å.

Plasma Coagulation Measurements. The thrombin inhibitor potency was measured with a chromogenic substrate method, in a Plato 3300 robotic microplate processor (Rosys AG, CH-8634 Hombrechtikon, Switzerland), using 96-well, half-volume microtiter plates (Costar, Cambridge, MA, cat. no. 3690). Stock solutions of test substance in DMSO (72 μL), 10 mM, were diluted serially 1:3 (24 + 48 μL) with DMSO to obtain 10 different concentrations, which were analyzed as samples in the assay, together with controls and blanks. The dilutions of each test substance were analyzed consecutively, row-wise on the microtiter plate, with wash cycles between substances to avoid cross-contamination. 2 μL of test sample was diluted with 124 μL of assay buffer (0.05 M Tris-HCl, pH 7.4, ionic strength 0.15 adjusted with NaCl, BSA 1 g/L); 12 μL of chromogenic substrate solution (S-2366, Cromogenix, Mölndal, Sweden) and finally 12 μL of α-thrombin solution (human α-thrombin, Sigma Chemical Co., St. Louis, MO, cat. no. T-6759), both in buffer, were added, and the samples were mixed. The final assay concentrations were test substance 0.00068−13.3 μM, S-2366 0.30 mM, α-thrombin 0.020 NIHU/mL. The linear absorbance increase during a 40-min incubation at 37 °C was used for calculation of percent inhibition for the test samples, as compared to blanks without inhibitor. The IC_{50} value, corresponding to the inhibitor concentration which caused 50% inhibition of the thrombin activity, was calculated from a log dose vs inhibition curve.

General Methods. ^1H and ^{13}C NMR spectra were recorded on a Bruker 250 instrument using $CDCl_3$, methanol-d_4 or D_2O. The shifts are reported in ppm (δ scale) and all J values are in Hz. TLC was performed on Merck precoated 60 F_{254} plates. The spots were visualized with UV light (254 nm) and ethanol/sulfuric acid/acetic acid/*p*-anisaldehyde (90:3:1:2). Column chromatography was performed using silica gel 60 (0.040−0.063 mm, Merck). Optical rotations were measured in $CHCl_3$ or methanol solutions at room temperature using a Perkin-Elmer 141 instrument. Organic phases were dried over anhydrous $MgSO_4$.

***trans*-(1*R*,2*R*)-Cyclopentane-1,2-dicarboxylic Acid Dimethyl Ester** [(−)-**2**]. A suspension of Raney nickel was filtered, washed with water until pH ∼ 7 and added to a solution of (−)-*trans*-(7*R*,8*R*)-1,4-dithiaspiro[4.4]nonane-7,8-dicarboxylic acid dimethyl ester [(−)-**1**][14] (1.94 g, 6.83 mmol) in methanol (80 mL). After refluxing the mixture for 4 h, it was cooled, filtered, concentrated, and purified by column

chromatography (toluene−ethyl acetate, 7:1) to give (−)-**2** (1.06 g, 85%) as a colorless liquid. (−)-**2**: $[\alpha]_D^{22}$ −73.7 (*c* 1.7, $CHCl_3$) [lit.[13b] (+)-**2**: $[\alpha]_D^{22}$ +71.9 (*c* 1.6, CCl_4)]; ^{1}H NMR (250 MHz, $CDCl_3$) identical with ref 13b; ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 25.4, 30.4, 47.1, 51.7, 175.2.

***trans*-(1*S*,2*S*)-Cyclopentane-1,2-dicarboxylic Acid Dimethyl Ester** [(+)-**2**]. Compound (+)-**2** (0.10 g, 78%) was prepared from (+)-**1** (0.20 g, 0.70 mmol)[14] according to the method for the preparation of (−)-**2**. (+)-**2**: $[\alpha]_D^{22}$ +70.9 (*c* 0.7, $CHCl_3$) [lit.[13b] $[\alpha]_D^{22}$ +71.9 (*c* 1.6, CCl_4)].

***trans*-Methyl (1*R*,2*R*)-2-{4-[Amino(benzyloxycarbonylimino)methyl]benzylaminocarbonyl}cyclopentanecarboxylate** [(−)-**3**]. To a solution of [(−)-**2**] (0.86 g, 4.6 mmol) in dioxane−water (1:1) (30 mL) was added aqueous NaOH (1.0 M, 4.6 mL) dropwise during 30 min at room temperature. After 2 h, the reaction mixture was acidified with concentrated hydrochloric acid to pH ∼ 2−3 and extracted five times with dichloromethane (10 mL). The combined organic phases were dried, filtered, concentrated and purified by column chromatography (toluene−ethyl acetate−acetic acid, 50:50:1) to give *trans*-(1*R*,2*R*)-cyclopentane-1-carboxylic acid methyl ester 2-carboxylic acid (0.38 g, 48%) as an oil.[13c] All of the monoacid was then dissolved in DMF (10 mL) containing *p*-(benzyloxycarbonyl)amidinobenzylamine hydrochloride (Pab(Z)·HCl) (0.76 g, 2.4 mmol) and *N*-hydroxybenzotriazole (HOBt) (0.475 g, 3.5 mmol). The mixture was cooled to 0 °C, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) (0.665 g, 3.5 mmol) was added and triethylamine was added dropwise until pH ∼ 8. After 1 h, the ice bath was removed and the solution was stirred at room temperature overnight. The solvent was evaporated and the residue was purified by column chromatography (toluene−ethyl acetate, 1:1) to give (−)-**3** (0.85 g, 88%, 42% overall) as white needles (ethyl acetate, hexane). (−)-**3**: $[\alpha]_D^{22}$ −22.1 (*c* 1.1, $CHCl_3$); mp 131−132 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.60−2.19 (6 H, m), 2.81−3.13 (2 H, m), 3.62 (3 H, s), 4.22−4.45 (2 H, m), 5.20 (2 H, s), 6.82−6.91 (1 H, m), 7.10−7.46 (7 H, m), 7.70−7.78 (2 H, d, J = 8.5 Hz); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 25.4, 30.2, 30.4, 43.0, 47.9, 48.4, 52.0, 67.2, 127.3, 127.7, 128.2, 128.4, 129.0, 133.4, 136.7, 143.0, 164.6, 168.1, 174.6, 176.0. Anal. ($C_{24}H_{27}N_3O_5$·0.4EtOAc) C, H, N.

***trans*-Methyl (1*S*,2*S*)-2-{4-[Amino(benzyloxycarbonylimino)methyl]benzylaminocarbonyl}cyclopentanecarboxylate** [(+)-**3**]. Compound (+)-**3** (0.30 g, 21%) was prepared from (+)-**2** (0.60 g, 3.26 mmol) according to the method for the preparation of (−)-**3**. (+)-**3**: $[\alpha]_D^{22}$ +20.1 (*c* 0.5, $CHCl_3$); mp 130−131 °C. Anal. ($C_{24}H_{27}N_3O_5$) C, H, N.

***trans*-(1*R*,2*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-benzyl-1,2-cyclopentanedicarboxamide** [(−)-**4**]. To a solution of compound (−)-**3** (84 mg, 0.19 mmol) in an ice-cold mixture of dioxane−water (1:1) (5 mL) was added NaOH (1.0 M, 0.6 mL) dropwise during 30 min. The mixture was stirred for an additional hour, neutralized with dilute aqueous hydrochloric acid, evaporated and evaporated twice with toluene. The crude carboxylic acid was dissolved in DMF (1 mL) containing benzylamine (0.10 mL, 0.86 mmol) and HOBt (40 mg, 0.29 mmol). The mixture was cooled to 0 °C and EDC (57 mg, 0.29 mmol) was added. The pH of the mixture was adjusted to ∼8 with triethylamine. After 1 h, the ice bath was removed and the solution was stirred at room temperature overnight. After evaporation of the solvent, the residue was purified by column chromatography (ethyl acetate) and dissolved in ethanol (5 mL). Palladium on carbon (3 mg) was added and the mixture was stirred under hydrogen atmosphere for 1 h. After filtration, acetic acid (0.1 mL) was added and the mixture was stirred for an additional hour. After evaporation of the solvents, the residue was purified by preparative HPLC (Kromasil C-18; water−methanol−triethylamine, 24:75:1) to give (−)-**4** (40 mg, 41%) as a white powder. (−)-**4**: $[\alpha]_D^{22}$ −22.1 (*c* 0.5, MeOH); ^{1}H NMR (250 MHz, methanol-d_4) δ 1.72−1.90 (4 H, m), 2.00−2.14 (2 H, m), 2.98−3.07 (2 H, m), 4.30−4.49 (4 H, m), 7.11−7.38 (5 H, m), 7.42−7.51 (2 H, d, J = 8.5 Hz), 7.67−7.72 (2 H, d, J = 8.5 Hz); ^{13}C NMR (62.9 MHz, methanol-d_4) δ 26.3, 32.0, 32.2, 43.6, 44.1, 50.3, 50.5, 128.1, 128.3, 128.4, 128.9, 129.1, 129.5, 140.1, 147.2, 168.2, 176.8, 176.9. Anal. ($C_{22}H_{26}N_4O_2$·4.3MeOH·0.6H_2O) C, H, N.

***trans*-(1*S*,2*S*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-benzyl-1,2-cyclopentanedicarboxamide** [(+)-**4**]. Compound (+)-**4** (44 mg, 67%) was prepared from (+)-**3** (65 mg, 0.15 mmol) according to the method for the preparation of (−)-**4**. (+)-**4**: $[\alpha]_D^{22}$ +21.9 (*c* 0.7, MeOH). Anal. ($C_{22}H_{26}N_4O_2$·4.1MeOH·1.4H_2O) C, H, N.

***trans*-(1*R*,2*R*,2′*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-[2′-(methyl)carboxylbenzyl]-1,2-cyclopentanedicarboxamide** (**5**). Compound **5** (28 mg, 66%) was prepared from (−)-**3** (48 mg, 0.110 mmol) according to the method for the preparation of (−)-**4** using phenylglycine methyl ester instead of benzylamine. **5**: $[\alpha]_D^{22}$ −37.4 (*c* 0.6, MeOH); ^{1}H NMR (250 MHz, methanol-d_4) δ 1.68−1.88 (4 H, m), 1.95−2.12 (2 H, m), 2.98−3.18 (2 H, m), 3.68 (3 H, s), 4.36−4.57 (4 H, m), 5.46 (1 H, s), 7.30−7.43 (5 H, m), 7.50−7.59 (2 H, d, J = 8.6 Hz), 7.72−7.82 (2 H, d, J = 8.6 Hz); ^{13}C NMR (62.9 MHz, methanol-d_4) δ 26.4, 32.0, 32.1, 43.7, 49.7, 50.3, 53.0, 58.4, 128.5, 128.8, 129.0, 129.1, 129.6, 129.9, 137.3, 147.1, 168.3, 172.7, 177.0, 180.9. Anal. ($C_{24}H_{28}N_4O_4$·1.5MeOH·0.7H_2O) C, H, N.

***trans*-(1*R*,2*R*,2′*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-(2′-carboxylbenzyl)-1,2-cyclopentanedicarboxamide** (**6**). Compound **6** (15 mg, 56%) was prepared from (−)-**3** (28 mg, 0.064 mmol) according to the method for the preparation of **5**, with the exception that the peptide coupling product was treated with 3 equiv of NaOH (1 M) in water−dioxane (1:1, 3 mL) at 0 °C. The mixture was stirred for 30 min, neutralized with dilute hydrochloric acid and evaporated. This product was then used in the hydrogenation step. The mobile phase in the final purification was methanol−water (25:75). **6**: $[\alpha]_D^{22}$ −8.6 (*c* 1.1, MeOH); ^{1}H NMR (250 MHz, methanol-d_4) δ 1.67−1.91 (4 H, m),1.93−2.18 (2 H, m), 2.94−3.17 (2 H, m), 4.36−4.61 (4 H, m), 5.48 (1 H, s), 7.32−7.45 (5 H, m), 7.46−7.57 (2 H, d, J = 8.6 Hz), 7.72−7.82 (2 H, d, J = 8.6 Hz); ^{13}C NMR (62.9 MHz, methanol-d_4) δ 26.3, 31.8, 31.9, 43.6, 43.7, 49.9, 50.4, 59.0, 128.0, 128.6, 128.6, 129.0, 129.1, 129.7, 136.8, 147.3, 168.1, 174.0, 176.5, 177.3. Anal. ($C_{23}H_{26}N_4O_4$·1.5MeOH·0.8H_2O) C, H, N.

***trans*-(1*R*,2*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-cyclohexyl-1,2-cyclopentanedicarboxamide (7)**. Compound **7** (51 mg, 67%) was prepared from (−)-**3** (90 mg, 0.210 mmol) according to the method for the preparation of (−)-**4** using cyclohexylamine instead of benzylamine and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (Bop-Cl) (107 mg, 0.420 mmol) in DMF (3 mL) instead of EDC and HOBt. **7**: $[\alpha]_D^{22}$ −37.4 (*c* 0.5, MeOH); ^{1}H NMR (250 MHz, methanol-d_4) δ 1.05−2.15 (16 H, m), 2.85−3.08 (2 H, m), 3.52−3.71 (1 H, m), 4.35−4.57 (2 H, m), 7.48−7.54 (2 H, d, J = 8.3 Hz), 7.72−7.80 (2 H, d, J = 8.5 Hz); ^{13}C NMR (62.9 MHz, methanol-d_4) δ 26.1, 26.2, 26.3, 26.6, 32.2, 33.8, 43.6, 45.9, 50.3, 50.5, 51.7, 65.4, 128.3, 129.0, 129.1, 147.2, 168.2, 176.0, 178.0. Anal. ($C_{24}H_{28}N_4O_4$·2.1MeOH·1.8H_2O) C, H, N.

***trans*-(1*R*,2*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-cyclohexyl-*N*^{2}-ethyl-1,2-cyclopentanedicarboxamide (8)**. Compound **8** (28 mg, 66%) was prepared from (−)-**3** (48 mg, 0.110 mmol) according to the method for the preparation of (−)-**4** using *N*-ethylcyclohexylamine instead of benzylamine and Bop-Cl instead of EDC and HOBt. **8**: $[\alpha]_D^{22}$ −18.8 (*c* 0.6, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.03−2.18 (19 H, m), 3.02−3.45 (2 H, m), 3.65−3.81 (1 H, m), 4.32−4.53 (2 H, m), 7.44−7.52 (2 H, d, J = 8.6 Hz), 7.69−7.86 (2 H, d, J = 8.6 Hz); ^{13}C NMR (62.9 MHz, methanol-d_4) δ 15.2, 26.3, 26.9, 27.1, 31.7, 32.2, 32.9, 37.9, 43.5, 48.6, 50.6, 51.2, 55.8, 58.5, 128.3, 128.9, 129.1, 147.1, 168.1, 176.7, 177.3. Anal. ($C_{24}H_{28}N_4O_4$·2.4MeOH·2.5H_2O) C, H, N.

(±)-2-Formylcyclopent-2-enecarboxylic Acid (**9**).[16] To an ice-cold, vigorously stirred solution of dichloroacetyl chloride (35.4 g, 0.24 mol) and freshly distilled cyclopentadiene (15.4 g, 0.24 mol) in hexane (250 mL) was added triethylamine (24.3 g, 0.24 mol) in hexane (200 mL) over a period of 2 h. When all the triethylamine was added, the ice bath was removed and the mixture was stirred vigorously overnight. The reaction

mixture was filtered, the filter cake was washed with hexane, the solvent was removed, and the obtained orange liquid (7,7-dichlorobicyclo[3.2.0]hept-2-en-6-one) was, without further purification, suspended in an aqueous solution of $NaHCO_3$ (1 M, 1 L). The mixture was heated to 75 °C with stirring for 5 h. After cooling, the mixture was filtered, washed three times with diethyl ether (50 mL), acidified with concentrated hydrochloric acid to pH ∼ 2, saturated with ammonium chloride and extracted several times with ethyl acetate−tetrahydrofuran (1:1). The combined organic phases were dried, filtered and evaporated. Column chromatography (toluene−ethyl acetate−acetic acid, 50:50:1) and crystallization from cyclohexane gave **9** (7.5 g, 16% overall) as white needles. **9**: mp 90−91 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.22−2.48 (2 H, m), 2.55−2.90 (2 H, m), 3.71−3.86 (1 H, m), 7.05−7.11 (1 H, dd, J = 2.5, 4.0 Hz), 9.78 (1 H, s); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 28.1, 33.1, 47.1, 144.6, 155.8, 177.9, 189.1. Anal. ($C_7H_8O_3$) C, H.

(±)-2-Formylcyclopent-2-enecarboxylic Acid Methyl Ester (**10**). To a solution of **9** (34 mg, 0.24 mmol) in trimethoxymethane (1.5 mL) was added *p*-toluenesulfonic acid monohydrate (20 mg, 0.11 mmol) and the solution was stirred overnight. Water (1 mL) was added and the mixture was stirred for an additional 30 min. The mixture was extracted twice with dichloromethane and the combined organic phases were dried, filtered and evaporated to give **10** (36 mg, 97%) as a colorless oil. **10**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.09−2.22 (1 H, m), 2.28−2.45 (1 H, m), 2.53−2.90 (2 H, m), 3.70 (3 H, s), 3.71−3.82 (1 H, m), 7.00−7.10 (1 H, m), 9.75 (1 H, s); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 28.6, 33.1, 46.9, 52.2, 145.6, 154.4, 174.4, 188.4. Anal. ($C_8H_{10}O_3$·0.35$CHCl_3$) C, H.

(±)-Cyclopent-2-ene-1,2-dicarboxylic Acid 1-Methyl Ester [(±)-**11**]. To a solution of **10** (5.6 g, 0.036 mol) in *tert*-butyl alcohol (100 mL) and water (50 mL) were added resorcinol (6.0 g, 0.063 mol), NaH_2PO_4·H_2O (7.6 g, 0.055 mol) and Na_2ClO_2 (80%, 6.3 g, 0.044 mol). The mixture was stirred for 5 h, quenched with saturated Na_2SO_3 (10 mL), washed three times with ethyl acetate, acidified with concentrated hydrochloric acid to pH ∼ 2 and extracted three times with dichloromethane. The combined organic phases were dried, filtered and evaporated and the residue was recrystallized from ether−hexane to give (±)-**11** (5.0 g, 81%) as white crystals. (±)-**11**: mp 75−76 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.02−2.80 (4 H, m), 3.70 (3 H, s), 3.71−3.92 (1 H, m), 7.01−7.11 (1 H, m), 10.50 (1 H, broad s); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 28.8, 32.8, 49.0, 52.2, 134.6, 149.6, 169.4, 174.9. Anal. ($C_8H_{10}O_4$) C, H.

Optical Resolution of (±)-**11**. Compound (±)-**11** (5.0 g, 0.029 mol) and (−)-ephedrine (2.5 g, 0.015 mol) were dissolved in ethyl acetate and the solution was allowed to stand at 0 °C overnight. The white precipitate was collected by filtration and recrystallized from ethyl acetate until the optical rotation of the precipitate became constant ($[\alpha]_D^{22}$ +19.8 (*c* 1.0, $CHCl_3$)). The precipitate was then dissolved in 40 mL of dichloromethane and 1 M $NaHSO_4$ was added until the pH of the water layer became ∼3. The organic layer was dried, filtered and concentrated to give (+)-**11** (1.07 g, 21%) as white crystals. (+)-**11**: $[\alpha]_D^{22}$ +56.0 (*c* 1.0, $CHCl_3$). The combined mother liquors were evaporated, (−)-ephedrine (1.0 g) was added and the above procedure was repeated. The optical rotation became constant at $[\alpha]_D^{22}$ −63.4 (*c* 1.0, $CHCl_3$) and the corresponding acid was shown to be (−)-**11** (0.86 g, 17%). (−)-**11**: $[\alpha]_D^{22}$ −47.2 (*c* 1.0, $CHCl_3$). The above procedures were continued until all of the starting material was resolved. Chiral HPLC (Chiralcel OD; isoheptane−2-propanol−acetic acid, 80:20:1; 0.5 mL/min) gave the following retention times: (+)-isomer = 9 min, (−)-isomer = 17 min. The following purities were recorded: (+)-isomer = 90% ee, (−)-isomer = 96% ee.

(±)-2-Benzylaminocarbonyl-2-cyclopentene-1-carboxylic Acid Methyl Ester (**12**). To an ice-cold solution of (±)-**11** (0.113 g, 0.66 mmol) in DMF (1 mL) containing benzylamine (0.107 g, 1.0 mmol) and HOBt (0.135 g, 1.0 mmol) was added EDC (0.192 g, 1.0 mmol). The pH of the reaction mixture was adjusted to ∼8 with triethylamine. After 1 h, the ice bath was removed and the solution was stirred at room temperature overnight. Hydrochloric acid (1 M) was added until pH ∼ 2 and the mixture was extracted twice with dichloromethane. The combined organic phases were dried, filtered, evaporated and purified by column chromatography (toluene−ethyl acetate, 3:1) to give **12** (0.130 mg, 75%) as white crystals (from diethyl ether−hexane). **12**: mp 93−94 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.15−2.32 (2 H, m), 2.41−2.74 Hz (2 H, m), 3.65 (3 H, s), 3.78−3.87 (1 H, m), 4.41−4.52 (1 H, dd, J = 5.5, 14.8 Hz), 4.49−4.61 (1 H, dd, J = 5.9, 15.0 Hz), 6.54 (1 H, broad s), 6.62−6.70 (1 H, m), 7.10−7.39 (5 H, m); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 28.5, 32.3, 43.5, 49.7, 52.2, 127.5, 127.8, 128.2, 128.7, 129.0, 141.0, 164.4, 174.9. Anal. ($C_{15}H_{17}NO_3$) C, H, N.

(±)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-benzyl-2-cyclopentene-1,2-dicarboxamide (**13**). To a solution of compound **12** (41 mg, 0.16 mmol) in dioxane−water (1:1, 2 mL) was added NaOH (1.0 M, 0.5 mL) dropwise during 30 min at 0 °C. The mixture was stirred for an additional hour, acidified to pH ∼ 2 with concentrated hydrochloric acid, and extracted several times with dichloromethane. The combined organic phases were dried, filtered and concentrated. The crude carboxylic acid was redissolved in DMF (1 mL) containing Pab(Z)·HCl (0.077 g, 0.24 mmol) and HOBt (0.033 g, 0.24 mmol). The mixture was cooled to 0 °C and EDC (46 mg, 0.24 mmol) was added. The pH of the reaction mixture was adjusted to ∼8 with triethylamine. After 1 h, the ice bath was removed and the solution was stirred at room temperature overnight. Hydrochloric acid (1 M) was added until pH ∼ 2 and the mixture was extracted twice with dichloromethane. The combined organic phases were dried, filtered, concentrated, purified by column chromatography (ethyl acetate) and dissolved in dichloromethane (30 mL). A mixture of trifluoromethanesulfonic acid, anisole and dichloromethane (10:3:100) was added under stirring until the solution was red (∼1 mL). After 15 min the solution was applied to a silica column and eluted with methanol/ethyl acetate/acetic acid (40:10:1). The crude product was purified by preparative HPLC (Kromasil C-18; water−methanol−triethylamine, 24:75:1) to give **13** (36 mg, 60%) as a white powder. **13**: ^{1}H NMR (250 MHz, D_2O) δ 2.02−2.17 (1 H, m), 2.41−2.59 (1 H, m), 2.65−2.57 (2 H, m), 3.91−4.03 (1 H, m), 4.42−4.63 (4 H, m), 6.83−6.92 (1 H, m), 7.27−7.43 (5 H, m), 7.46−7.55 (2 H, d, J = 8.3 Hz), 7.62−7.71 (2 H, d, J = 8.3 Hz); ^{13}C NMR (62.9 MHz, D_2O) δ 31.1, 35.1, 45.3, 54.0, 129.0, 129.4, 130.1, 130.5, 131.3, 139.4, 140.7, 145.8, 147.3, 167.0, 169.9, 184.4. Anal. ($C_{22}H_{24}N_4O_2$·0.8MeOH·1.3H_2O) C, H, N

(1*R*)-2-Piperidinylcarbonyl-2-cyclopentene-1-carboxylic Acid Methyl Ester (**14**). To an ice-cold solution of (+)-**11** (36 mg, 0.21 mmol) in DMF (1 mL) was added Bop-Cl (0.108 g, 0.42 mmol). The pH of the reaction mixture was adjusted to ∼8 with triethylamine. After stirring the mixture at 0 °C for 30 min, piperidine (36 mg, 0.42 mmol) was added and the solution was stirred at room temperature overnight. Hydrochloric acid (1 M) was added until pH ∼ 2 and the mixture was extracted twice with dichloromethane. The combined organic phases were dried, filtered, evaporated and purified by column chromatography (toluene−ethyl acetate, 3:1) to give **14** (44 mg, 88%) as a colorless syrup. **14**: $[\alpha]_D^{22}$ +22.9 (*c* 2.0, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.50−1.71 (6 H, m), 2.01−2.19 (1 H, m), 2.22−2.70 (3 H, m), 3.50−3.62 (4 H, m), 3.67 (3 H, s), 3.95−4.09 (1 H, m), 5.90−5.95 (1 H, m); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 24.7, 26.4, 27.2, 32.5, 48.1, 51.7, 51.9, 133.0, 136.4, 166.6, 174.7. Anal. ($C_{13}H_{19}NO_3$·1.05$CHCl_3$) C, H, N.

(1*R*)-*N*-{4-[Amino(imino)methyl]benzyl}-2-(1-piperidinylcarbonyl)-2-cyclopentene-1-carboxamide (**15**). To an ice-cold solution mixture of compound **14** (40 mg, 0.17 mmol) in dioxane−water (1:1) (2 mL) was added NaOH (1.0 M, 0.5 mL) dropwise during 30 min. The mixture was stirred for an additional hour, acidified to pH ∼ 2 with dilute hydrochloric acid, and extracted several times with dichloromethane. The combined organic phases were dried, filtered and concentrated. The crude carboxylic acid was redissolved in ice-cold DMF (1 mL) containing Bop-Cl (86 mg, 0.34 mmol). The pH of the mixture was adjusted to ∼8 with triethylamine. After stirring

the mixture at 0 °C for 30 min, Pab(Z)·HCl (0.108 g, 0.34 mmol) was added and the solution was stirred at room temperature overnight. Hydrochloric acid (1 M) was added until pH ∼ 2 and the mixture was extracted twice with dichloromethane. The combined organic phases were dried, filtered, concentrated, purified by column chromatography (ethyl acetate) and dissolved in dichloromethane (40 mL). A mixture of trifluoromethanesulfonic acid, anisole and dichloromethane (10:3:100) was added under stirring until the solution was red (∼1 mL). After 15 min the solution was applied to a silica column and eluted with methanol/ethyl acetate/acetic acid (40:10:1). The crude product was purified by preparative HPLC (Kromasil C-18; water−methanol−triethylamine, 24:75:1) to give **15** (33 mg, 47%) as a white powder. **15**: $[\alpha]_D^{22}$ +87.8 (*c* 0.7, MeOH); ^{1}H NMR (250 MHz, methanol-d_4) δ 1.42−1.75 (6 H, m), 1.92−2.11 (1 H, m), 2.21−2.72 (3 H, m), 2.46−3.71 (4 H, m), 3.82−3.97 (1 H, m), 4.32−4.60 (2 H, m), 6.05−6.11 (1 H, m), 7.41−7.57 (2 H, d, J = 8.3 Hz), 7.70−7.82 (2 H, d, J = 8.3 Hz); ^{13}C NMR (62.9 MHz, methanol-d_4) δ 25.5, 27.0, 27.7, 29.2, 33.7, 43.6, 54.7, 128.5, 129.0, 136.8, 138.1, 146.7, 149.9, 167.8, 168.7, 175.3. Anal. ($C_{20}H_{26}N_4O_2$·0.8MeOH·1.8H_2O) C, H, N.

(1*R*)-2-Cyclohexylaminocarbonyl-2-cyclopentene-1-carboxylic Acid Methyl Ester (**16**). Compound **16** (39 mg, 85%) was prepared from (+)-**11** (31 mg, 0.182 mmol) according to the method for the preparation of **14** using cyclohexylamine instead of piperidine. **16**: white crystals from ether−hexane; $[\alpha]_D^{22}$ +12.8 (*c* 1.2, $CHCl_3$); mp 108−109 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.05−1.48 (4 H, m), 1.50−1.79 (4 H, m), 1.84−2.00 (2 H, m), 2.17−2.30 (2 H, m), 2.39−2.72 (2 H, m), 3.70 (3 H, s), 3.70−3.90 (2 H, m), 5.97−6.13 (1 H, m), 6.60−6.68 (1 H, m); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 24.7, 25.8, 28.6, 32.2, 33.0, 48.0, 49.8, 52.2, 138.4, 140.4, 163.5, 175.0. Anal. ($C_{14}H_{21}NO_3$) C, H, N.

(1*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-cyclohexyl-2-cyclopentene-1,2-dicarboxamide (**17**). Compound **17** (21 mg, 39%) was prepared from **16** (32 mg, 0.127 mmol) according to the method for the preparation of **15**. **17**: $[\alpha]_D^{22}$ +33.4 (*c* 0.8, MeOH); ^{1}H NMR (250 MHz, methanol-d_4) δ 1.11−1.48 (6 H, m), 1.55−1.88 (6 H, m), 1.99−2.12 (1 H, m), 2.19−2.37 (1 H, m), 2.42−2.82 (2 H, m), 3.54−3.75 (1 H, m), 3.73−3.83 (1 H, m), 4.33−4.62 (2 H, m), 6.60−6.68 (1 H, m), 7.49−7.58 (2 H, d, J = 8.3 Hz), 7.70−7.80 (2 H, d, J = 8.3 Hz); ^{13}C NMR (62.9 MHz, methanol-d_4) δ 26.7, 29.5, 33.7, 33.7, 33.8, 43.6, 49.5, 52.7, 128.4, 129.0, 129.1, 140.1, 140.5, 147.1, 166.6, 168.1, 177.2. Anal. ($C_{20}H_{26}N_4O_2$·0.6MeOH·1.1H_2O) C, H, N.

(1*R*)-2-Cyclohexylmethylaminocarbonyl-2-cyclopentene-1-carboxylic Acid Methyl Ester (**18**)**.** Compound **18** (30 mg, 51%) was prepared from (+)-**11** (38 mg, 0.224 mmol) according to the method for the preparation of **14** using cyclohexylmethylamine instead of piperidine. **18**: colorless syrup; $[\alpha]_D^{22}$ +43.2 (*c* 1.1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.82−1.03 (2 H, m), 1.10−1.32 (4 H, m), 1.40−1.58 (1 H, m), 1.59−1.80 (4 H, m), 2.19−2.30 (2 H, m), 2.40−2.72 (2 H, m), 3.01−3.27 (2 H, m), 3.70 (3 H, s), 3.71−3.82 (1 H, m), 6.20−6.40 (1 H, m), 6.63−6.71 (1 H, m); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 25.9, 26.4, 28.6, 30.8, 32.2, 38.0, 45.7, 49.8, 52.3, 138.2, 140.6, 164.4, 175.0. Anal. ($C_{15}H_{23}NO_3$) C, H, N.

(1*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-cyclohexylmethyl-2-cyclopentene-1,2-dicarboxamide (**19**). Compound **19** (23 mg, 51%) was prepared from **18** (27 mg, 0.102 mmol) according to the method for the preparation of **15**. **19**: $[\alpha]_D^{22}$ +26.1 (*c* 0.9, MeOH); ^{1}H NMR (250 MHz, methanol-d_4) δ 0.81−1.02 (2 H, m), 1.12−1.35 (4 H, m), 1.40−1.59 (1 H, m), 1.60−1.80 (4 H, m), 1.94−2.13 (1 H, m), 2.20−2.39 (1 H, m), 2.42−2.74 (2 H, m), 2.96−3.13 (2 H, m), 3.75−3.90 (1 H, m), 4.35−4.60 (2 H, m), 6.61−6.68 (1 H, m), 7.50−7.59 (2 H, d, J = 8.3 Hz), 7.70−7.80 (2 H, d, J = 8.3 Hz); ^{13}C NMR (62.9 MHz, methanol-d_4) δ 27.0, 27.6, 29.6, 32.0, 33.6, 39.3, 43.6, 46.7, 52.6, 128.0, 128.7, 129.0, 140.1, 140.4, 147.3, 167.5, 168.2, 176.2. Anal. ($C_{21}H_{28}N_4O_2$·1.2MeOH·1.1H_2O) C, H, N.

(1*R*)-2-Phenylaminocarbonyl-2-cyclopentene-1-carboxylic Acid Methyl Ester (**20**). Compound **20** (57 mg, 44%) was prepared from (+)-**11** (90 mg, 0.53 mmol) according to the method for the preparation of **14** using aniline instead of piperidine. **20**: colorless syrup; $[\alpha]_D^{22}$ +4.9 (*c* 1.5, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.15−2.39 (2 H, m), 2.41−2.72 (2 H, m), 3.70 (3 H, s), 3.81−3.93 (1 H, m), 6.74−6.82 (1 H, m), 7.0−7.12 (1 H, t, J = 7.4 Hz), 7.22−7.32 (2 H, t, J = 7.8 Hz), 7.52−7.61 (2 H, d, J = 8.1 Hz), 8.43 (1 H, broad s); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 28.4, 32.6, 49.8, 52.4, 120.0, 124.1, 128.9, 129.0, 138.0, 138.5, 141.8, 162.7, 175.2. Anal. ($C_{14}H_{15}NO_3$) C, H, N.

(1*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-phenyl-2-cyclopentene-1,2-dicarboxamide (**21**). Compound **21** (13 mg, 19%) was prepared from **20** (27 mg, 0.102 mmol) according to the method for the preparation of **15**. **21**: $[\alpha]_D^{22}$ +13.5 (*c* 0.7, MeOH); ^{1}H NMR (250 MHz, methanol-d_4) δ 2.00−2.16 (1 H, m), 2.25−2.43 (1 H, m), 2.58−2.75 (2 H, m), 3.85−3.98 (1 H, m), 4.35−4.68 (2 H, m), 6.82−6.89 (1 H, m), 7.05−7.13 (1 H, t, J = 7.5 Hz), 7.25−7.35 (2 H, t, J = 7.7 Hz), 7.51−7.62 (4 H, m), 7.63−7.72 (2 H, d, J = 8.3 Hz); ^{13}C NMR (62.9 MHz, methanol-d_4) δ 29.5, 34.0, 43.6, 52.8, 121.9, 125.3 129.0, 129.1, 129.8, 139.2, 140.5, 141.7, 165.8, 168.3, 177.3. Anal. ($C_{21}H_{22}N_4O_2$) C, H, N.

(1*R*)-2-Cyclohexyl(ethyl)aminocarbonyl-2-cyclopentene-1-carboxylic Acid Methyl Ester (**22**). Compound **22** (88 mg, 100%) was prepared from (+)-**11** (51 mg, 0.30 mmol) according to the method for the preparation of **14** using *N*-ethylcyclohexylamine instead of piperidine. **22**: colorless syrup; $[\alpha]_D^{22}$ +74.1 (*c* 1.1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.92−1.82 (13 H, m), 1.95−2.32 (2 H, m), 2.33−2.65 (2 H, m), 3.15−3.41 (2 H, m), 3.58 (3 H, s), 3.75−4.13 (2 H, m), 5.77−5.95 (1 H, m); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 14.7, 21.4, 25.4, 25.8, 27.0, 31.8, 32,6, 36.3, 51.8, 52.2, 58.2, 132.5, 137.4, 167.6, 174.5. Anal. ($C_{16}H_{25}NO_3$·0.25EtOAc) C, H, N.

(1*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-cyclohexyl-*N*^{2}-ethyl-2-cyclopentene-1,2-dicarboxamide (**23**). Compound **23** (26 mg, 23%) was prepared from **22** (70 mg, 0.251 mmol) according to the method for the preparation of **15**. **23**: $[\alpha]_D^{22}$ +3.4 (*c* 0.5, MeOH); ^{1}H NMR (250 MHz, methanol-d_4) δ 1.02−1.85 (13 H, m), 1.96−2.13 (1 H, m), 2.22−2.40 (1 H, m), 2.41−2.75 (2 H, m), 3.50−3.72 (1 H, m), 3.83−3.97 (2 H, m), 3.95−4.12 (1 H, m), 4.32−4.58 (2 H, m), 5.95−6.15 (1 H, m), 7.44−7.57 (2 H, d, J = 7.7 Hz), 7.65−7.83 (2 H, d, J = 7.8 Hz); ^{13}C NMR (62.9 MHz, methanol-d_4) δ 15.1, 26.8, 29.1, 32.7, 33.8, 37.6, 43.6, 55.1, 59.9, 128.1, 129.1, 135.1, 139.0, 147.2, 168.1, 170.1, 176.4. Anal. ($C_{22}H_{30}N_4O_2$·0.4MeOH·1.8H_2O) C, H, N.

(1*R*)-2-Ethyl(phenyl)aminocarbonyl-2-cyclopentene-1-carboxylic Acid Methyl Ester (**24**). Compound **24** (60 mg, 73%) was prepared from (+)-**11** (51 mg, 0.30 mmol) according to the method for the preparation of **14** using *N*-ethylaniline instead of piperidine. **24**: colorless syrup; $[\alpha]_D^{22}$ +213.6 (*c* 0.7, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.05−1.20 (3 H, m), 1.78−1.96 (1 H, m), 2.01−2.40 (3 H, m), 3.56−3.76 (2 H, m), 3.70 (3 H, s), 3.91−4.10 (1 H, m), 5.48−5.57 (1 H, m), 7.12−7.50 (5 H, m); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 12.8, 27.2, 32.7, 45.0, 51.3, 51.7, 127.3, 128.1, 129.2, 137.4, 140.1, 143.1, 165.8, 175.0. Anal. ($C_{16}H_{19}NO_3$·0.5EtOAc) C, H, N.

(1*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-phenyl-*N*^{2}-ethyl-2-cyclopentene-1,2-dicarboxamide (**25**). Compound **25** (17 mg, 19%) was prepared from **24** (54 mg, 0.198 mmol) according to the method for the preparation of **15**. **25**: $[\alpha]_D^{22}$ +65.6 (*c* 0.4, $CHCl_3$); ^{1}H NMR (250 MHz, D_2O) δ 0.95−1.13 (3 H, t, J = 7.2 Hz), 1.62−1.82 (1 H, m), 2.04−2.43 (3 H, m), 3.50−3.73 (2 H, m), 3.81−4.00 (1 H, m), 4.32−4.53 (2 H, m), 6.10−6.25 (1 H, m), 7.05−7.25 (2 H, m), 7.33−7.49 (3 H, m), 7.49−7.59 (2 H, d, J = 8.2 Hz), 7.72−7.85 (2 H, d, J = 8.3 Hz); ^{13}C NMR (62.9 MHz, D_2O) δ 14.6, 31.2, 34.9, 45.3, 48.3, 55.3, 129.5, 130.4, 130.7, 131.4, 132.2, 132.5, 139.4, 143.7, 147.3, 169.1, 172.3, 184.1. Anal. ($C_{23}H_{26}N_4O_2$·2.2MeOH·0.5H_2O) C, H, N.

***N*-Cyclohexyl-*N*-*tert*-butyloxycarbonylhydroxylamine** (**27**). Na_2CO_3 (1.4 g, 13.2 mmol) was added slowly to a solution of *N*-cyclohexylhydroxylamine hydrochloride (**26**; 1.0 g, 6.6 mmol) and di-*tert*-butyl dicarbonate (1.6 g, 7.3 mmol) in water (20 mL) and tetrahydrofuran (10 mL). After 30 min at room temperature ethyl acetate (20 mL) was added and the organic phase was dried, filtered, concentrated and purified

by column chromatography. Elution with toluene−ethyl acetate (39:1) afforded *N*-cyclohexyl-*O*-*tert*-butyloxycarbonylhydroxylamine (**28**; 0.55 g, 39%) as a colorless syrup. Further elution with toluene−ethyl acetate (9:1) afforded **27** (0.59 g, 41%) as white crystals (from hexane). **27**: mp. 88−90 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.02−1.40 (3 H, m), 1.47 (9 H, s), 1.52−1.89 (7 H, m), 3.62−3.81 (1 H, m), 7.82 (1 H, s); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 25.4, 25.7, 28.4, 29.3, 58.3, 81.2, 157.0. Anal. ($C_{11}H_{21}NO_3$) C, H, N.

***N*-Ethoxy-*N*-*tert*-butyloxycarbonylcyclohexylamine (29).** To an ice-cold solution of **27** (0.57 g, 2.65 mmol) and potassium hydroxide (0.32 g, 5.7 mmol) was added ethyl iodide (0.42 mL, 5.25 mmol). The mixture was stirred at 0 °C for 1 h and then refluxed overnight. Pentane (30 mL) was added and the precipitated potassium iodide was removed by filtration. The mixture was then concentrated and purified by column chromatography (pentane−diethyl ether, 9:1) to afford **29** (0.54 g, 84%) as a colorless liquid. **29**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.00−1.47 (4 H, m), 1.17−1.25 (3 H, t, J = 7.1 Hz), 1.48 (9 H, s), 1.52−1.87 (6 H, m), 3.68−3.82 (1 H, tt, J = 3.5, 11.6 Hz), 3.82−3.93 (2 H, q, J = 7.1 Hz); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 13.4, 25.4, 25.7, 28.3, 29.5, 59.0, 72.0, 80.8, 156.9. Anal. ($C_{13}H_{25}NO_3$) C, H, N.

(1*R*)-2-Cyclohexyl(ethoxy)aminocarbonyl-2-cyclopentene-1-carboxylic Acid Methyl Ester (30). Compound **30** (36 mg, 76%) was prepared from (+)-**11** (30 mg, 0.18 mmol) according to the method for the preparation of **14** using *N*-ethoxycyclohexylamine instead of piperidine. *N*-Ethoxycyclohexylamine was prepared from **29**: To a solution of **29** in dichloromethane was added trifluoroacetic acid and the mixture was stirred for 5 min. After evaporation the crude organic salt was dissolved in dichloromethane and washed with $NaHCO_3$ (1 M), dried, filtered and evaporated. The resulting *N*-ethoxycyclohexylamine was used directly without further purification. **30**: colorless syrup; $[\alpha]_D^{22}$ +25.9 (*c* 0.5, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.00−1.42 (4 H, m), 1.12−1.21, (3 H, t, J = 7.1 Hz), 1.47−1.93 (6 H, m), 1.95−2.12 (1 H, m), 2.18−2.33 (1 H, m), 2.37−2.72 (2 H, m), 3.65 (3 H, s), 3.75−4.07 (3 H, m, CH), 4.05−4.27 (1 H, m), 6.50−6.57 (1 H, m); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 13.4, 25.4, 25.7, 27.5, 29.9, 30.4, 32,8, 51.4, 51.9, 58.1, 72.6, 136.8, 139.4, 166.6, 174.9. Anal. ($C_{16}H_{25}NO_4$) C, H, N.

(1*R*)-N^1-{4-[Amino(imino)methyl]benzyl}-N^2-cyclohexyl-N^2-ethoxy-2-cyclopentene-1,2-dicarboxamide (31). Compound **31** (30 mg, 47%) was prepared from **30** (36 mg, 0.13 mmol) according to the method for the preparation of **15**. **31**: $[\alpha]_D^{22}$ +9.6 (*c* 0.4, MeOH); ^{1}H NMR (250 MHz, methanol-d_4) δ 1.02−1.85 (13 H, m), 1.96−2.13 (1 H, m), 2.22−2.40 (1 H, m), 2.41−2.75 (2 H, m), 3.50−3.72 (1 H, m), 3.83−3.97 (2 H, m), 3.95−4.12 (1 H, m), 4.32−4.58 (2 H, m), 5.95−6.15 (1 H, m), 7.44−7.57 (2 H, d, J = 7.7 Hz), 7.65−7.83 (2 H, d, J = 7.8 Hz); ^{13}C NMR (62.9 MHz, methanol-d_4) δ 15.1, 26.8, 29.1, 32.7, 33.8, 37.6, 43.6, 55.1, 59.9, 128.1, 129.1, 135.1, 139.0, 147.2, 168.1, 170.1, 175.2. Anal. ($C_{22}H_{30}N_4O_3 \cdot 1.3MeOH \cdot 0.8H_2O$) C, H, N.

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