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Synthesis and transformations of polysubstituted diastereomeric 5-oxomorpholin-2-carboxylic acids

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ABSTRACT

A series of *trans*- and *cis*-3,4-disubstituted 5-oxomorpholine-2-carboxylic acids **5** were prepared by a cyclocondensation between diglycolic anhydride **3** and arylideneamines **4**. Transformations of the carboxylic group leading to a peptide bond in the side chain to the morpholinone ring were effected. The relative configurations and the preferred conformations of the substituents at the morpholinone ring in some of the newly prepared derivatives were determined by means of ^{1}H NMR and X-ray analysis.

1. Introduction

Morpholines are widely used in organic synthesis, mostly as basic and nucleophilic reagents. Morpholines substituted at the ring carbon atoms, however, are fragments incorporated in the structure of both natural and synthetic compounds with antitumor [1]; endoprotease inhibiting [2]; HIV-protease inhibiting activity [3], etc. The polysubstituted morpholine **1** has shown anti-inflammatory activity [4,5], while aprepitant (**2**) is an antagonist of the neurotransmitter substance P (SP) and exerts antihistaminic and antiemetic activity [5,6]. It is known that peptides incorporating lactam rings have shown diverse biological activities [7]. Morpholin-3-ones, as δ-lactams, are considered as isosteric peptide analogs and are used as peptidomimetics [8].

In the frames of a broader program for the preparation of monocyclic lactams by means of the reactions between cyclic anhydrides and imines [9,10], we started an investigation of the reaction of diglycolic anhydride (1,4-dioxane-2,6-dione) (**3**) with various imines **4**. Unlike the reactions of glutaric and succinic anhydride with imines, which attracted more attention [9–23], there are only two examples of reaction of anhydride **3** with imines so far [19]. A reaction of **3** with *N*-benzylidene-*N*-benzylamine (**4a**) and *N*-benzylidene-*N*-phenethylamine (**4b**) affording the corresponding 5-oxo-3-aryl-*N*-substituted morpholine-2-carboxylic acids **5a,b** has been described in a patent claiming that morpholinones **5a,b** possess analgesic, anti-inflammatory, and antihistaminic activity [19]. The work of Shetty, however, does not consider the steric course of the reaction and the relative configuration of the products [19] (Fig. 1).

The aim of the present paper is to determine the steric course of the reaction of the anhydride **3** with a series of arylideneamines **4** and to establish the relative configuration of the newly prepared 3,4-disubstituted 5-oxomorpholin-2-carboxylic acids **5**. Acids **5** are the starting compounds for the introduction of amino acid residues via a peptide bond to the morpholinone heterocycle in order to obtain model compounds for biological evaluation. The carboxylic acids **5** themselves are β-amino acid

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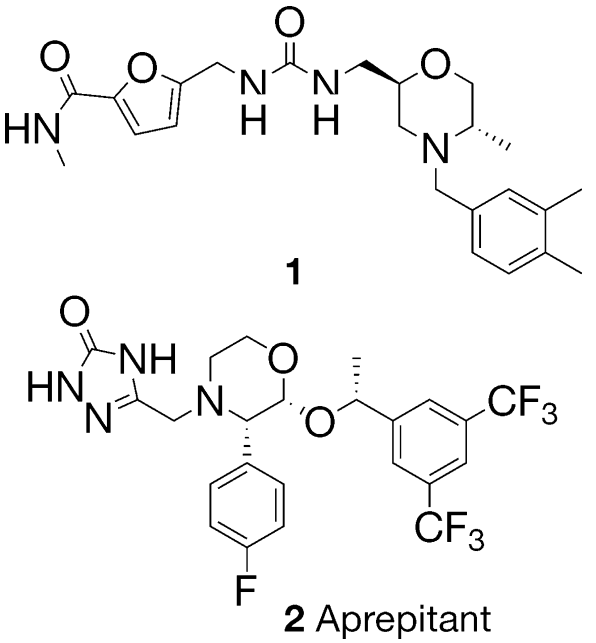


Fig. 1. Chemical structures of compounds **1** and **2**.

derivatives and as such they can be regarded as tools for medicinal chemistry [24,25]. Recently, we have shown that piperidinones containing peptide bond to the heterocycle exhibit antihistaminic activity [23], which gave us ground to continue our research with the bioisosteric morpholinones. We prepared two types of peptide derivatives of the morpholinones: type I (acylated aminomethylmorpholinones) and type II (2-carboxamides) (Fig. 2). The amino acids used were derivatives of proline, phenylalanine and tryptophan, in view to introduce pharmacophore substituents. L-Proline and L-phenylalanine moieties are incorporated in drugs with ACE inhibiting activity [26]. Protected L-tryptophan was selected, because esters of *N*-acetyl-L-tryptophan have been shown to possess high antihistaminic activity [23,27].

2. Results and discussion

The reaction of diglycolic anhydride **3** with *N*-benzylidene-*N*-benzylamine **4a** was repeated and acid **5a** was obtained in 40% yield as a single diastereomer, which was established by ^{1}H NMR spectroscopy (Scheme 1). We

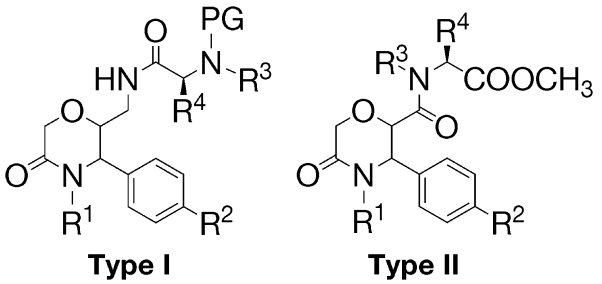
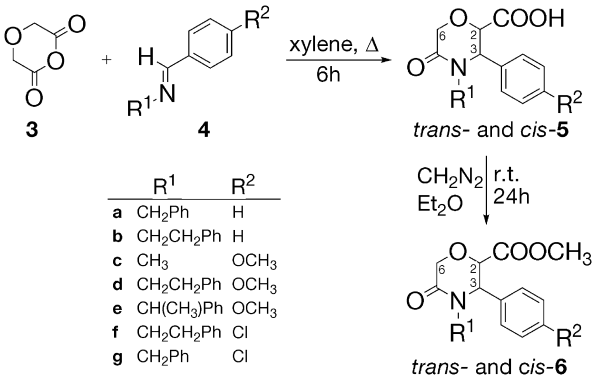


Fig. 2. Type I and type II (PG: protective group).



	R^1	R^2
a	CH_2Ph	H
b	CH_2CH_2Ph	H
c	CH_3	OCH_3
d	CH_2CH_2Ph	OCH_3
e	$CH(CH_3)Ph$	OCH_3
f	CH_2CH_2Ph	Cl
g	CH_2Ph	Cl

Scheme 1. Yields are given in Table 1.

ascribed a *trans* relative configuration to this product, having in mind that the reaction conditions, i.e. long reflux at 140 °C, would favour the formation of the thermodynamically more stable diastereomer [9,11,12]. Further on, we carried out the reactions of anhydride **3** with *N*-(arylmethylene)-*N*-alkylamines **4c-g** in *p*-xylene under reflux for 6 h (Scheme 1).

The acids **5c–g** were isolated from the reaction mixtures either by filtration (**5c,d,f**) or extraction with aq. Na_2CO_3 (**5a,e,g**), followed by recrystallization. The ^{1}H NMR spectroscopy of the crude products **5c–g** showed signals for two diastereomeric products. The ratio of the diastereomeric acids **5c,d,f,g** varied from 3:1 to 6:1. In analogy with *trans*-**5a**, we concluded that the predominant isomers of **5c–g** are also *trans* isomers. The diastereomeric mixture **5c** (*trans*:*cis* = 3:1) was separated by column chromatography and the individual diastereomers *trans*- and *cis*-**5c** were characterized. However, chromatographic separation of *trans*- and *cis*- acids **5d–g** was difficult, so the diastereomeric mixtures were analyzed by means of ^{1}H NMR spectra. This was possible, because the signals for H-2, H-3, H-6, as well as for CH_3O in acids **5d**,**e**, could be seen separately, which allowed the characterization of the two diastereomers in a mixture. The vicinal coupling constants $^3J_{2,3}$ of the *trans* isomers appear in the range of 1.7–3.6 Hz, and for the *cis*-$^3J_{2,3}$ at about 3.4 Hz (Table 1). Acid **5e**, which can be a mixture of eight enantiomers, gave a single product after recrystallization, to which a *trans* configuration was ascribed. Very small difference of $^3J_{2,3}$ for the both diastereomers within a couple is observed, which makes difficult the application of the vicinal coupling constants $^3J_{2,3}$ for the determination of the relative configuration. The recrystallization of the crude reaction products afforded only *trans*-**5** in enough pure form for further transformations. Acids *trans*- and *cis*-**5** are obtained as racemic mixtures of enantiomers and only one of them will be shown throughout the text for clarity.

In order to collect more data about the configuration of the studied compounds, we converted the crude acidic products **5** into the methyl esters **6** by means of reaction with diazomethane (Scheme 1). The mixtures of the methyl esters *trans*- and *cis*-**6** were separated by column chromatography. *Trans*-**6a,c,g** were obtained as oily products, while *trans*-**6d,e,f** were crystalline. *Cis* esters **6c,d,f** were oily compounds, and *cis*-**6g** a crystalline

Table 1
Yields of acids **5** and their methyl esters **6**.

No.	$^3J_{trans}$ (Hz)	$^3J_{cis}$ (Hz)	*Trans*/*Cis*	Yield[a] (%)
5a	2.6	–	–	40/0
5c	3.6	3.4	3:1[c]	35/12
5d	3.2	3.4	6:1[c]	51/0
5e	1.7	–	–	22/0
5f	2.8	3.4	4:1[c]	48/0
5g	2.6	–[b]	3:1[c]	35/0
6a	2.6	–	–	92/0
6c	4.2	3.5	3:1[d]	55/19
6d	3.7	3.4	6:1[d]	69/12
6e	1.5	–	–	56/0
6f	3.1	3.4	4.5:1[d]	59/13
6g	2.6	3.5	2:1[d]	55/32

[a] The values are for the isolated *trans*/*cis* product.
[b] The value of $^3J_{cis}$-**5g** could not be measured because of overlap of signals.
[c] The ratio is determined by integral intensities of the protons in the ^{1}H NMR spectra of the crude products.
[d] The ratio is determined using the yields of the esters.

product. *Cis*-**6e** could not be isolated by means of chromatography, because of the complex mixture of products. ^{1}H NMR spectroscopy analysis of the individual esters *trans*- and *cis*-**6** showed vicinal coupling constants $^3J_{2,3}$ for the *trans* esters in the range of 1.5–4.2 Hz, and for the *cis*-$^3J_{2,3}$ in the range of 3.4–3.5 Hz (Table 1). *Trans* isomers **6c,d,f,g** exhibited $COOCH_3$ singlet in a lower field than the corresponding *cis* isomers. Similar deshielding of the $COOCH_3$ singlet signal was observed in the ^{1}H NMR spectra of methyl *trans*-1,2-disubstituted 6-oxopiperidine-3-carboxylates [13,14]. The difference in the chemical shifts of $COOCH_3$ singlet signal of the *trans*- and *cis*- methyl esters **6** can be used for the determination of the relative configuration, only when the two diastereomers are available. This is important because of the close values of $^3J_{2,3}$ of the *trans* and *cis* isomers of a given diastereomeric couple.

It can be accepted that the morpholinone ring is in a half-chair conformation, due to the conjugation in the N–C=O fragment [28,29] (Fig. 3). The value of ^2J for H-6 (ca. 16.5 Hz) corresponds to a conformation where the C=O

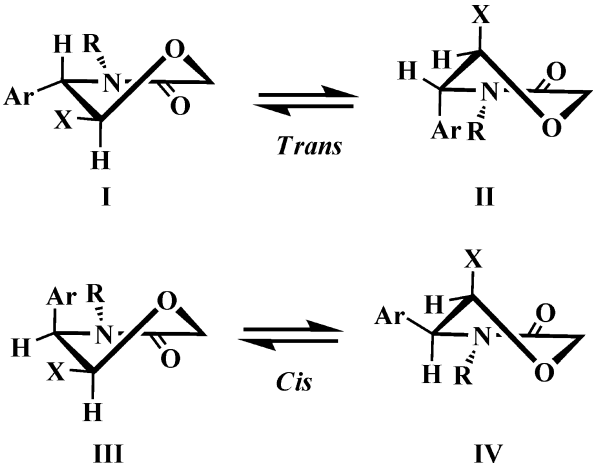


Fig. 3. Partial conformations of *trans* and *cis* morpholinones.

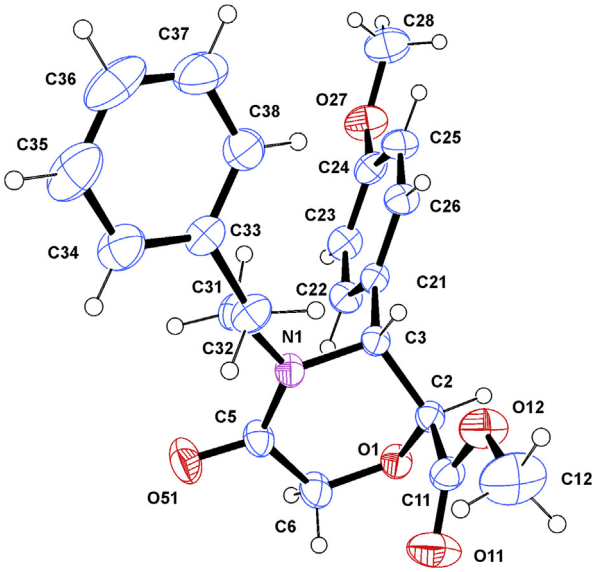
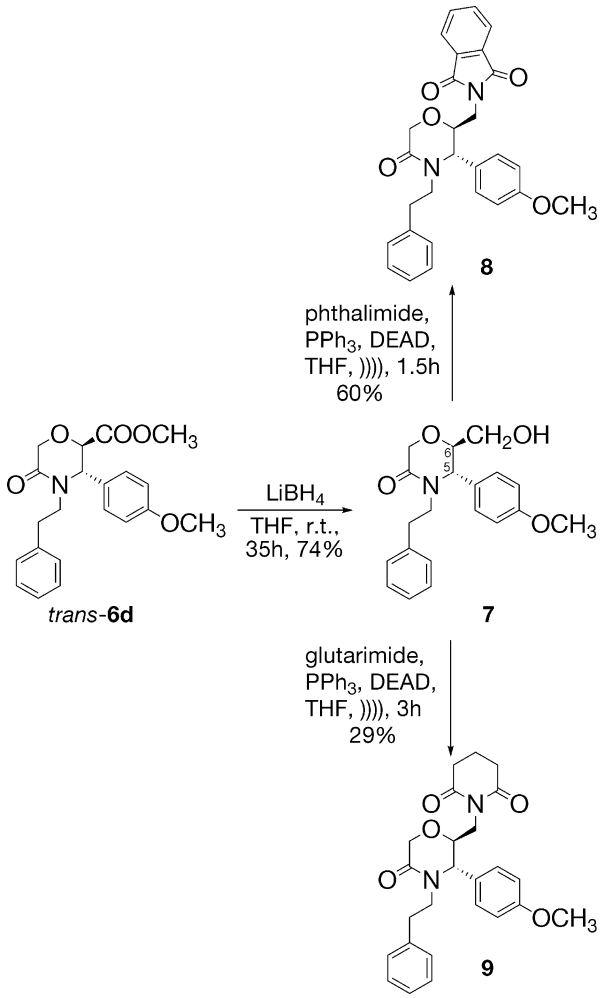


Fig. 4. *ORTEP* view of compound **6d**. Displacement ellipsoids are drawn at the 50% probability level (the hydrogen atoms are represented by circles of arbitrary radii).

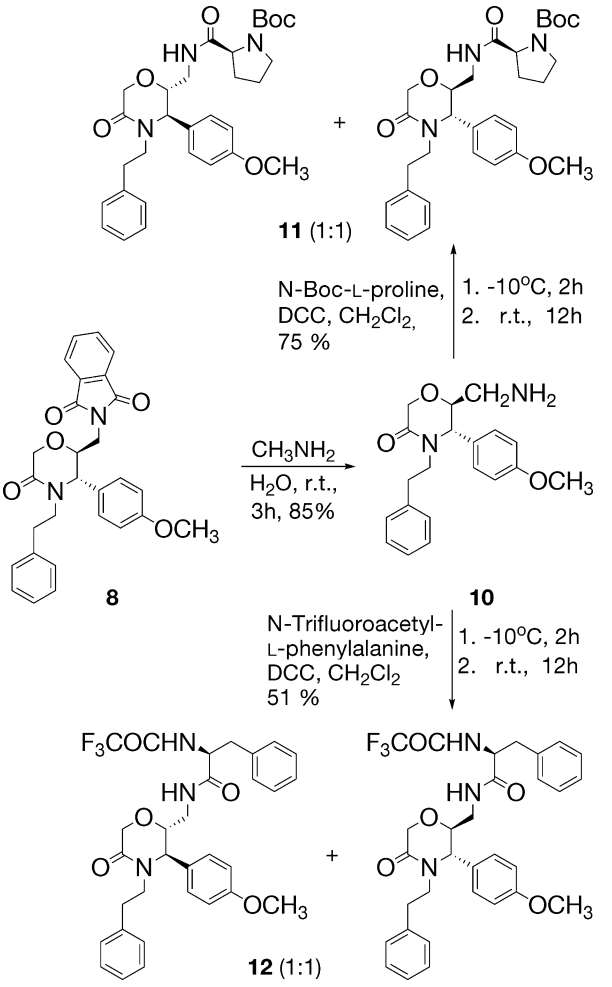
plane bisects the angle H–C(6)–H [28,29]. The $^3J_{2,3}$ values of **5** and **6** might indicate either a *trans* isomer in which the substituents at C-2 and C-3 chiral centers occupy axial, resp. pseudoaxial positions in solution, i. e. the corresponding protons are equatorial (partial conformation II, Fig. 3), or a *cis* isomer, which should exist predominantly in a conformation with axial 3-Ar, i.e. with equatorial H-3 in solution. The conformation with axial 3-Ar allows less steric $A^{1,2}$ strain between the aryl and the *N*-substituent, in analogy with other polysubstituted lactams [9,13] (partial conformation III, Fig. 3).

X-ray diffraction analysis of a single crystal of the ester *trans*-**6d** confirmed the *trans* configuration of the 2- and 3-substituents (Fig. 4). The conformation of the morpholinone ring was determined as an envelope (puckering parameters [30]: $\theta = 48.6(2)$, $\varphi = 64.7(3)^\circ$). In spite of the envelope shape of the ring, the spatial orientations of the $COOCH_3$ group at C-2 and the 4-methoxyphenyl substituent at C-3 are respectively axial and pseudoaxial, which resembles conformation II (Fig. 3). The corresponding protons at C-2 and C-3 remain to be equatorial and pseudoequatorial, respectively. The dihedral angle between the above-mentioned protons is evaluated at 60°. Thus, the ^{1}H NMR data on the conformation of compounds *trans*-**5, 6** in solution coincide with the conclusion made through X-ray single-crystal analysis (Fig. 4).

The methyl esters *trans*-**6d,f** were obtained from the crude reaction products **5d,f**, respectively, by esterification (reflux with $MeOH/H_2SO_4$) [9]. *Trans*-**6d** obtained by this method was subjected to further transformations. The planned transformations of the carboxylic group of *trans*-**6d** do not affect the stereogenic carbon atoms at the morpholinone ring, so, they are expected to give *trans* isomers.



Scheme 2. Mitsunobu substitution of OH group (only one enantiomer is shown).



Scheme 3. Peptide derivatives of type I (only one enantiomer of 8 and 10 is shown).

The preparation of 5-aminomethylmorpholin-3-one from the acid *trans*-**5d** via alcohol **7** is depicted in Scheme 2. Ester *trans*-**6d** was converted selectively into 6-hydroxymethyl-3-morpholinone **7** by the reduction with $LiBH_4$ in THF [9]. Crystalline alcohol **7** thus obtained was characterized by means of ^1H NMR, which exhibited $^3J_{5,6} = 9.4$ Hz. Alcohol **7** was treated with phthalimide and glutarimide according to the Mitsunobu protocol (Ph_3P; DEAD) [31], which gave rise to *trans*-2-((3-(4-methoxyphenyl)-5-oxo-4-phenethylmorpholin-2-yl)methyl)-isoindol-1,3-dione **8** and the respective 1-((*trans*-(3-(4-methoxyphenyl)-5-oxo-4-phenethylmorpholin-2-yl)methyl)-piperidin-2,6-dione **9**. (Scheme 2) The reaction products **8** and **9** were isolated after chromatography in 60%, resp. 29% yields as crystalline materials.

The higher yield of phthalimide **8** makes it a more suitable starting material for the Gabriel synthesis of the primary amine **10** after deprotection (Scheme 3). Best yields (85%) of sufficiently pure amine **10** were obtained by treatment of **8** with 40% aqueous methylamine at room temperature overnight [32]. The ^1H NMR spectra of alcohol **7** and amine **10** exhibited $^3J = 9.4$, resp. 9.1 Hz for the protons at the stereogenic centers. This value is consistent with the *trans* configuration of alcohol **7** and amine **10** as well as with the preferred conformation in solution with diaxial H at C-5 and C-6 (partial conformation I, Fig. 3). This conclusion about the conformation in solution of compounds **7** and **10** is in agreement with the conformation of other 5,6-disubstituted morpholin-3-ones previously described by Stefanovsky et al. [28,29]. The preference for the conformation with dipseudoequatorial substituents (I, **7** and **10**) can be attributed to the larger effective volume of the substituent at the C-6 stereocenter. The signals of H-5 and H-6 in the ^1H NMR spectra of phthalimido derivative **8** and piperidinedione **9** overlapped with other multiplet signals, which did not allow us to determine the ^3J value of the two compounds.

X-ray analysis of alcohol **7** (Fig. 5) and phthalimide **8** (Fig. 6) confirmed the *trans* configuration of the substituents at the morpholinone ring. The conformation of the morpholinone ring was determined as half-chair (puckering parameters $30\theta = 48.11(19)$ and 49.0(7), $\varphi = 17.0(3)$ and 28.1(10)° for compounds **7** and **8** respectively), with pseudoequatorial, resp. equatorial

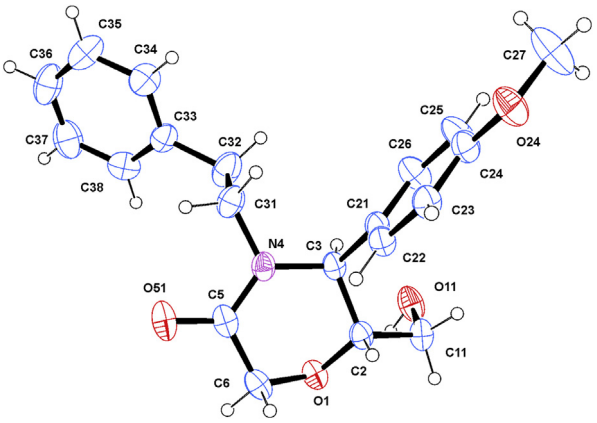


Fig. 5. *ORTEP* view of compound **7**. Displacement ellipsoids are drawn at the 50% probability level (the hydrogen atoms are represented by circles of arbitrary radii).

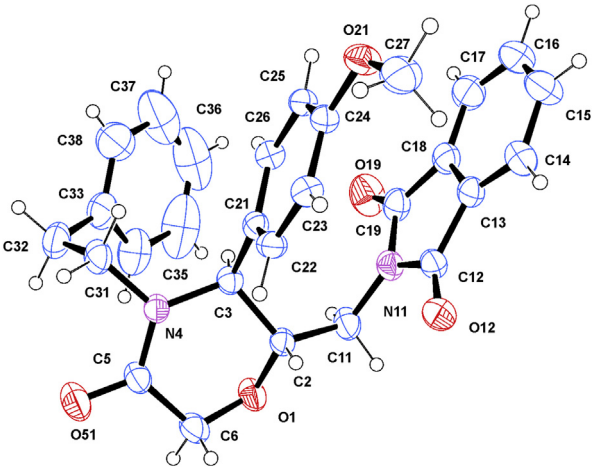


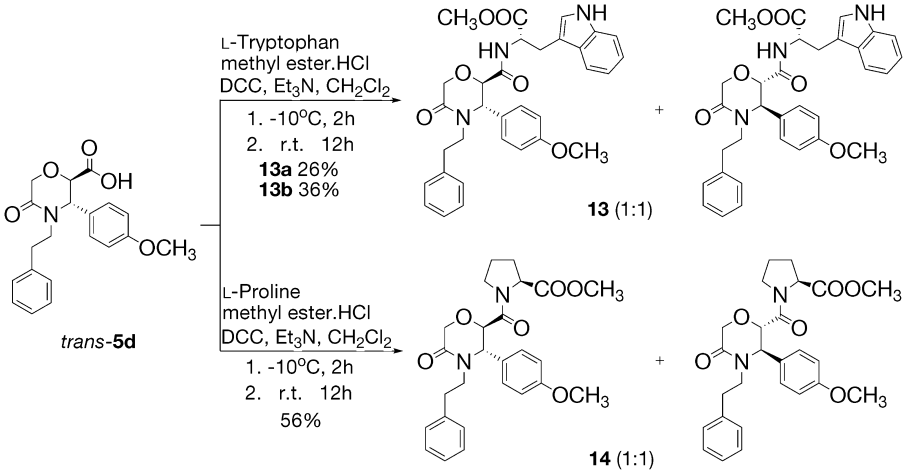
Fig. 6. *ORTEP* view of compound **8**. Displacement ellipsoids are drawn at the 50% probability level (the hydrogen atoms are represented by circles of arbitrary radii).

substituents at the chiral C-5 and C-6. The respective H-5 and H-6 protons occupy the axial positions with a dihedral angle estimated at 170°, leading to a higher value of ^3J. Thus, the conformation of compound **7** in solution as determined by ^{1}H NMR spectroscopy coincides with that pointed by means of X-ray analysis.

The introduction of a peptide bond into the side chain to the morpholinone ring was carried out from amine **10** to type-I peptides, and from acid *trans*-**5d** to type-II peptides in the presence of DCC [33], in order to obtain model compounds for the study of ACE inhibitory activity. Amine **10** was acylated by means of BOC-L-proline and *N*-trifluoroacetyl-L-phenylalanine, affording derivatives **11**, resp. **12**, in good yields after column chromatography (Scheme 3). Compounds **11** and **12** are 1:1 mixtures of α-*S*, (±)-*trans* diastereomers, according to their ^{1}H NMR spectra. In spite of our efforts, we could not separate the diastereomeric couples of the compounds **11** and **12** by means of column chromatography. This made the description of the ^{1}H NMR spectral data in the experimental part difficult, because the signals for the same protons in the different diastereomers are not shifted in one and the same direction. For this reason, in the experimental part, the signals for the same protons with higher chemical shift are noted with the "*" symbol, by analogy with a previous publication [33].

The methyl esters of L-tryptophan and L-proline (used as hydrochlorides) were *N*-acylated by means of *trans*-**5d** to the derivatives **13** and **14**, in good yields (Scheme 4). As in the case of compounds **11** and **12**, compounds **13** and **14** are 1:1 mixtures of α-*S*, (±)-*trans* diastereomers according to their ^{1}H NMR spectra. The acylated tryptophan **13** was separated by means of fractional recrystallization into the two diastereomers, which are denoted in the experimental part as **13a** (R_f 0.29) and **13b** (R_f 0.36), referring to the TLC behavior of the isomers. Proline derivative **14** could not be separated into the individual diastereomers.

The transformations done on α-amino acids to afford type-I and Type-II peptides took place with retention of their L-configuration. The *trans* configuration of the substituents at the morpholinone ring was ascribed to compounds **11**–**14**, because the reactions employed for their preparation do not affect the chiral centers of the



Scheme 4. Peptide derivatives of type II.

heterocycle. ^{1}H NMR spectra of peptide derivatives **11**–**14** are in agreement with the stereochemistry. Thus, type-I peptide compounds **11** and **12** exhibit ^3J within the 9.2–9.4 Hz range, similarly to the starting amine **10**. Type-II derivatives **13** and **14** are characterized by a ^3J value in the 3.8–4.3 Hz range, which is close to the value of ^3J of the starting acid *trans*-**5d** (3.2 Hz). In the schemes, only one enantiomer of *trans*-**5d** and **10** is shown for clarity.

3. Experimental

3.1. General

Melting points were taken on a Boetius PHMK 0.5 microhot stage apparatus and are uncorrected. IR spectra were recorded on a Specord 75 instrument in Nujol. ^{1}H NMR spectra (250.13 MHz) were obtained on a "Bruker Avance DRX-250" spectrometer; ^{1}H NMR spectra (600 MHz) were taken on a Bruker AV 600 spectrometer in $CDCl_3$ or DMSO-d_6. The chemical shifts are given in parts per million (δ ppm) relative to tetramethylsilane as an internal standard. Multiplicity is indicated by s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). Coupling constants (J) are reported in Hz.

Microanalyses were carried out at the Faculty of Chemistry and Pharmacy, University of Sofia, Bulgaria, using a Vario EL *III* Elemental analyzer.

Mass spectra (MS) were recorded on a DFS high-resolution magnetic sector instrument Thermo Fisher Scientific GmbH (Bremen, Germany), as EI-mode (electron energy 70 eV, emission current 0.250 mA, source temperature 250 °C) or as CI-mode (electron energy 120 eV, emission current 0.450 mA and source temperature 150 °C).

Thin-layer chromatography (TLC) was performed on Merck 1.05554 silica gel 60 F_{254} aluminum plates. Column chromatography was carried out using MN Kieselgel 60 (0.063–0.2 mm) and Merck silica gel 60 (0.063–0.2 mm).

The preparation of the known starting imines **4** was carried out according to the procedure for *N*-benzylidene-*N*-benzylamine **4a** [19].

3.2. X-ray structure determination

Colorless single crystals of compounds **6d, 7** and **8** were obtained by slow evaporation from ethyl acetate. Diffraction data were collected at 290 K the by ω-scan technique, on an Agilent Diffraction SuperNova Dual four-circle diffractometer equipped with an Atlas CCD detector using a mirror-monochromatized Mo Kα radiation from micro-focus source (λ = 0.7107 Å). The determination of cell parameters, data integration, scaling and absorption correction were carried out using the CrysAlis Pro program package [34]. The structures were solved by direct methods (SHELXS-97) and refined by full-matrix least-square procedures on F^2 (SHELXL-97) [35]. Non-hydrogen atoms were refined anisotropically and hydrogen atoms were placed at idealized positions and refined using the riding model. Crystallographic data (excluding structure factors) for the structural analysis were deposited with the Cambridge Crystallographic Data Centre, CCDC Nos. 905074, 905075 and 905076. Copy of this information may be obtained free of charge from: The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK. Fax: +44 1223 336–033, e-mail: deposit@ccdc.cam.ac.uk, or www.ccdc.cam.ac.uk.

3.3. General procedure for preparation of (±)-trans- and cis-3,4-disubstituted 5-oxomorpholine-2-carboxylic acids 5

To a solution of *N*-arylidene-*N*-alkylamine (**4a**, **4c**-**g**, 5 mmol) in dried *p*-xylene (10 mL), diglycolic anhydride (**3**, 0.58 g, 5 mmol) was added. The reaction mixture was refluxed for 6 h. Acids **5c,d,f** crystallized from the reaction mixtures and were obtained after filtration. Acids **5a,e,g** deposited as oily products which were extracted by means of 10% aq. Na_2CO_3 (3 × 5 mL), then once with water (5 mL) and the aqueous solutions were combined, acidified (10% HCl) and extracted with ethyl acetate (3 × 10 mL). The combined organic solutions were dried (Na_2SO_4) and the solvent was evaporated. The residue was triturated with ethyl acetate and filtered to give acids **5a,e,g**. Recrystallization of crude acids **5d**–**g** afforded *trans*-**5d**–**g** as single diastereomers. ^{1}H NMR data for *cis*-**5d,f** were extracted from the spectra of the crude acids.

3.3.1. Trans-(±)-4-benzyl-3-phenyl-5-oxomorpholine-2-carboxylic acid 5a

The reaction of anhydride **3** and imine **4a** yielded *trans*-**5a** (0.622 g, 40%) as white crystals. R_f 0.58 (light petroleum/ethyl acetate/HCOOH 2:3:0.05, two-fold development). Mp 177–179 °C. According to [19], mp of **5a** is 177–181 °C. IR (Nujol): 2400–3200 (OH); 1720 (COOH); 1620 (CON) cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$ + DMSO-d_6): δ 3.50 (d, 1H, NCHPh, J = 14.8 Hz); 4.33 (d, 1H, H-3, J = 2.6 Hz); 4.43 (d, 1H, H-6, J = 17.0 Hz); 4.79 (d, 1H, H-2, J = 2.6 Hz); 4.88 (d, 1H, H-6, J = 17.0 Hz); 5.47 (d, 1H, NCHPh, J = 14.8 Hz); 7.10–7.40 (m, 10H, arom. H); the COOH signal is not seen because of exchange. MS (EI) m/z: 311 (M, 25); 252 (14); 194 (12); 164 (30); 148 (100); 147 (48); 131 (16); 104 (12). Anal. calcd for $C_{18}H_{17}NO_4$ (311.31): C 69.44%; H 5.50%; N 4.50%; found: C 69.69%; H 5.19%; N 4.52%.

3.3.2. (±)-Trans- and cis-3-(4-methoxyphenyl)-4-methyl-5-oxomorpholine-2-carboxylic acid 5c

The reaction of anhydride **3** and imine **4c** yielded **5c** (0.833 g, 63%) as off-white crystals. Mp 173–175 °C. According to ^{1}H NMR, the *trans*/*cis* ratio was 3:1. Chromatographic purification (light petroleum/ethyl acetate/HCOOH 2:3:0.05) and recrystallization from ethyl acetate-methanol yielded:

Trans-**5c** (0.464 g, 35%) as white crystals. R_f 0.20 (light petroleum/ethyl acetate/HCOOH 2:3:0.05, two-fold development). Mp 190–192 °C. IR (Nujol): 2300–3200 (OH); 1750 (COOH); 1590 (CON) cm^{-1}. ^{1}H NMR (250 MHz, DMSO-d_6): δ 2.69 (s, 3H, NCH_3); 3.76 (s, 3H, CH_3O); 4.28 (d, 1H, H-6, J = 16.6 Hz); 4.39 (d, 1H, H-6, J = 16.6 Hz); 4.43 (d, 1H, H-3, J = 3.6 Hz); 4.82 (d, 1H, H-2, J = 3.6 Hz); 6.94–7.25 (m, 4H, arom. H); 13.31 (br s, 1H, COOH). MS (EI) m/z: 265 (M, 43); 232 (19); 221 (45); 190 (37); 177 (86); 161 (85); 146 (80); 136 (26); 121 (100); 105 (64). Anal. calcd for

$C_{13}H_{15}NO_5$ (265.26): C 58.86%; H 5.70%; N 5.28%; found: C 59.08%; H 5.51%; N 5.23%.

Cis-**5c** (0.159 g, 12%) as white crystals. R_f 0.04 (light petroleum/ethyl acetate/HCOOH 2:3:0.05, two-fold development). Mp 200–202 °C. IR (Nujol): 2300–3500 (OH); 1750 (COOH); 1590 (CON) cm^{-1}. ^1H NMR (250 MHz, DMSO-d_6): δ 2.68 (s, 3H, NCH_3); 3.74 (s, 3H, CH_3O); 4.25 (d, 1H, H-6, J = 16.3 Hz); 4.36 (d, 1H, H-6, J = 16.3 Hz); 4.65 (d, 1H, H-3, J = 3.4 Hz); 4.79 (d, 1H, H-2, J = 3.4 Hz); 6.88–7.21 (m, 4H, arom. H); 13.11 (br s, 1H, COOH). Anal. calcd for $C_{13}H_{15}NO_5$ (265.26): C 58.86%; H 5.70%; N 5.28%; found: C 59.10%; H 5.40%; N 5.35%.

3.3.3. (±)-Trans- *and* cis-*3-(4-methoxyphenyl)-5-oxo-4-phenethylmorpholine-2-carboxylic acid* **5d**

The reaction of anhydride **3** and imine **4d** yielded **5d** (1.226 g, 69%) as white crystals. Mp 168–170 °C. According to ^1H NMR, the *trans*/*cis* ratio was 6:1. The crude product was recrystallized from ethyl acetate to give *trans*-**5d** (0.908 g, 51%) as colorless crystals. R_f 0.42 (light petroleum/ethyl acetate/HCOOH 2:3:0.05, two-fold development). Mp 175–177 °C. IR (Nujol): 2400–3200 (OH); 1720 (COOH); 1600 (CON) cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): δ 2.55–2.82 (m, 3H, $NCHCH_2$); 3.76 (s, 3H, CH_3O); 3.84–3.92 (m, 1H, NCH); 4.36 (d, 1H, H-6, J = 16.5 Hz); 4.45 (d, 1H, H-6, J = 16.5 Hz); 4.54 (d, 1H, H-3, J = 3.2 Hz); 5.00 (d, 1H, H-2, J = 3.2 Hz); 6.78–7.37 (m, 9H, arom. H); 13.50 (br s, 1H, COOH). MS (EI) m/z: 355 (20, M^+); 264 (20); 251 (100); 207 (15); 177 (66); 146 (22); 121 (71); 105 (10). Anal. calcd for $C_{20}H_{21}NO_5$ (355.38): C 67.59%; H 5.96%; N 3.94%; found: C 67.10%; H 5.85%; N 3.69%.

Cis-**5d**: R_f 0.08 (light petroleum/ethyl acetate/HCOOH 2:3:0.05, two-fold development). ^1H NMR (600 MHz, DMSO-d_6): δ 2.50–2.60 (3H, m, $NCHCH_2$); 3.73 (3H, s, CH_3O); 3.84–3.92 (1H, m, NCH); 4.31 (1H, d, H-6, J = 16.6 Hz); 4.42 (1H, d, H-6, J = 16.6 Hz); 4.55 (1H, d, H-3, J = 3.4 Hz); 4.68 (1H, d, H-2, J = 3.4 Hz); 6.78–7.37 (9H, m, arom. H); 13.50 (br s, 1H, COOH).

3.3.4. (±)-Trans-*3-(4-methoxyphenyl)-5-oxo-4-(1-phenylethyl)morpholine-2-carboxylic acid* **5e**

The reaction of anhydride **3** and imine **4e** yielded **5e** (1.065 g, 60%) as oily product. Recrystallization from ethyl acetate yielded *trans*-**5e** (0.391 g, 22%) as a white powder. R_f 0.58 (light petroleum/ethyl acetate/HCOOH 2:3:0.05, two-fold development). Mp 212–216 °C. IR (Nujol): 2400–3200 (OH); 1730 (COOH); 1590 (CON) cm^{-1}. ^1H NMR (250 MHz, DMSO-d_6): δ 1.10 (d, 3H, CH_3, J = 7.2 Hz); 3.78 (s, 3H, CH_3O); 4.21 (d, 1H, H-3, J = 1.7 Hz); 4.41 (d, 1H, H-6, J = 16.7 Hz); 4.58 (d, 1H, H-2, J = 1.7 Hz); 4.61 (d, 1H, H-6, J = 16.7 Hz); 5.70 (q, 1H, NC*H*CH_3, J = 7.2 Hz); 6.94–7.36 (m, 9H, arom. H); 13.13 (br s, 1H, COOH). MS (CI) m/z: 384 (M^+ + 29, 9); 356 (M^+ + 1, 100); 312 (10); 280 (14.); 252 (74); 234 (14); 208 (12); 178 (18); 105 (29). Anal. calcd for $C_{20}H_{21}NO_5$ (355.38): C 67.59%, H 5.96%; N 3.94%; found C 67.61%; H 5.60%; N 3.96%.

3.3.5. (±)-Trans- *and* cis-*3-(4-chlorophenyl)-5-oxo-4-phenethylmorpholine-2-carboxylic acid* **5f**

The reaction of anhydride **3** and imine **4f** yielded **5f** (1.343 g, 78%) as white crystals. Mp 183–186 °C. According to ^1H NMR, the *trans*/*cis* ratio was 4:1. The crude product was recrystallized from ethyl acetate to give *trans*-**5f** (0.863 g, 48%) as colorless crystals. R_f 0.27 (light petroleum/ethyl acetate/HCOOH 2:3:0.05). Mp 222–225 °C. IR (Nujol): 2300–3200 (OH); 1720 (COOH); 1600 (CON) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.67–2.92 (m, 3H, $NCHCH_2$); 3.83–3.96 (m, 1H, NCH); 4.30 (d, 1H, H-6, J = 16.7 Hz); 4.44 (d, 1H, H-6, J = 16.7 Hz); 4.54 (d, 1H, H-3, J = 2.8 Hz); 5.10 (d, 1H, H-2, J = 2.8 Hz); 7.03–7.52 (m, 9H, arom. H); 13.10 (br s, 1H, COOH). Anal. calcd for $C_{19}H_{18}ClNO_4$ (359.81): C 63.42%; H 5.04%; N 3.89%; found C 63.34%; H 5.09%; N 3.93%.

Cis-**5f:** R_f 0.05 (light petroleum/ethyl acetate/HCOOH 2:3:0.05). ^1H NMR δ (250 MHz, DMSO-d_6): 2.51–2.62 (3H, m, $NCHCH_2$); 3.68–3.77 (m, 1H, NCH); 4.30 (d, 1H, H-6, J = 16.2 Hz); 4.41 (d, 1H, H-6, J = 16.2 Hz); 4.64 (d, 1H, H-3, J = 3.4 Hz); 4.72 (d, 1H, H-2, J = 3.4 Hz); 7.03–7.52 (m, 9H, arom. H); 13.13 (br s, 1H, COOH).

3.3.6. (±)-Trans- *and* cis-*4-benzyl-3-(4-chlorophenyl)-5-oxomorpholine-2-carboxylic acid* **5g**

The reaction of anhydride **3** and imine **4g** yielded **5g** (0.945 g, 57%) as off-white crystals. Mp 176–180 °C. According to ^1H NMR *trans*/*cis* ratio was 3:1. The crude product was recrystallized from 2-propanol to give *trans*-**5g** (0.604 g, 35%) as colorless crystals. R_f 0.30 (light petroleum/ethyl acetate/HCOOH 2:3:0.05). Mp 205–207 °C. IR (Nujol): 2300–3200 (OH); 1740 (COOH); 1620 (CON) cm^{-1}. ^1H NMR (250 MHz, DMSO-d_6): δ 3.59 (d, 1H, NCH, J = 15.1 Hz); 4.40 (d, 1H, H-6, J = 16.8 Hz); 4.48 (d, 1H, H-3, J = 2.6 Hz); 4.58 (d, 1H, H-6, J = 16.8 Hz); 4.81 (d, 1H, H-3, J = 2.6 Hz); 5.10 (d, 1H, NCH, J = 15.1 Hz); 7.01–7.59 (m, 9H, arom. H); 13.45 (br s, 1H, COOH). HRMS: 345.0762; calcd for $C_{18}H_{16}O_4N^{35}Cl$ (345.0768).

Cis-**5g**: R_f 0.07 (light petroleum/ethyl acetate/HCOOH 2:3:0.05).

3.4. General procedure for the preparation of methyl esters of (±)-trans- *and* cis-*3,4-disubstituted 5-oxomorpholine-2-carboxylic acids* **6**

3.4.1. Method A

Acid **5a,c–g** (1 mmol) was dissolved in methanol and dichloromethane 1:1 (4 mL), diazomethane (ethereal solution) was added and the mixture was left at room temperature overnight. The solution was concentrated and the residue was purified by column chromatography or recrystallization (for **6e**).

3.4.2. Method B

Acid **5d,f** (3 mmol) was dissolved in dry methanol (6 mL) and concentrated H_2SO_4 (0.16 mL, 3 mmol) was added. After 3 h under reflux, the reaction mixture was poured into a saturated aq. NaCl solution (10 mL). The solution was neutralized with 10% aq. Na_2CO_3 till pH = 8 and extracted with ethyl acetate (3 × 10 mL). The combined organic solutions were dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by means of column chromatography and/or recrystallization.

*3.4.3. Methyl (±)-*trans*-4-benzyl-3-phenyl-5-oxomorpholine-2-carboxylate **6a** (method A)*

Chromatographic purification (cyclohexane/ethyl acetate 3:2) gave *trans*-**6a** (0.299 g, 92%) as colorless oil. R_f 0.67 (light petroleum/ethyl acetate 2:3). IR ($CHCl_3$): 1730 ($COOCH_3$); 1640 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 3.38 (d, 1H, NCH, J = 14.6 Hz); 3.52 (s, 3H, $COOCH_3$); 4.37 (d, 1H, H-3, J = 2.6 Hz); 4.45 (d, 1H, H-6, J = 17.0 Hz); 4.70 (d, 1H, H-2, J = 2.6 Hz); 4.86 (d, 1H, H-6, J = 17.0 Hz); 5.60 (d, 1H, NCH, J = 14.6 Hz); 6.66–7.07 (m, 10H, arom. H). MS (CI) m/z (%): 354 (M^+ + 29, 10); 326 (M^+ + 1, 100); 294 (2); 266 (2); 248 (3); 162 (3); 119 (2). Anal. calcd for $C_{19}H_{19}NO_4$ (325.30): C 70.14%; H 5.89%; N 4.31%; found: C 70.38%; H 6.10%; N 4.67%.

*3.4.4. Methyl (±)-*trans- *and* cis*-4-methyl-3-(4-methoxyphenyl)-5-oxomorpholine-2-carboxylate **6c** (method A)*

Chromatographic purification (light petroleum/ethyl acetate 2:3) gave *trans*- and *cis*-**6c**.

Trans-**6c** (0.154 g, 55%) as colorless oil. R_f 0.28 (light petroleum/ethyl acetate 2:3). IR ($CHCl_3$): 1740 ($COOCH_3$); 1660 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 2.83 (s, 3H, NCH_3); 3.78 (s, 3H, OCH_3); 3.82 (s, 3H, $COOCH_3$); 4.34 (d, 1H, H-3, J = 4.2 Hz); 4.35 (d, 1H, H-6, J = 16.8 Hz); 4.59 (d, 1H, H-6, J = 16.8 Hz); 4.80 (d, 1H, H-2, J = 4.2 Hz); 6.91–6.94 (m, 2H, arom. H); 7.17–7.20 (m, 2H, arom. H). Anal. calcd for $C_{14}H_{17}NO_5$ (279.29): C 60.21%; H 6.14%; N 5.02%; found C 60.58%; H 6.10%; N 4.97%.

Cis-**6c** (0.080 g, 19%) as colorless oil. R_f 0.10 (light petroleum/ethyl acetate 2:3). IR ($CHCl_3$): 1760 ($COOCH_3$); 1660 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 2.86 (s, 3H, NCH_3); 3.57 (s, 3H, $COOCH_3$); 3.80 (s, 3H, OCH_3); 4.57 (d, 1H, H-3, J = 3.5 Hz); 4.64 (d, 1H, H-6, J = 16.7 Hz); 4.60 (d, 1H, H-6, J = 16.7 Hz); 4.72 (d, 1H, H-2, J = 3.5 Hz); 6.85–6.89 (m, 2H, arom. H); 7.15–7.18 (m, 2H, arom. H). Anal. calcd for: $C_{14}H_{17}NO_5$ (279.29): C 60.21%; H 6.14%; N 5.02%; found (%): C 60.82%; H 6.28%; N 4.69%.

*3.4.5. Methyl (±)-*trans- *and* cis*-3-(4-methoxyphenyl)-5-oxo-4-phenethylmorpholine-2-carboxylate **6d***

3.4.5.1. Method A. Chromatographic purification (light petroleum/ethyl acetate 3:2) afforded *trans*- and *cis*-**6d**.

Trans-**6d** (0.255 g, 69%) as colorless crystals. R_f 0.48 (light petroleum/ethyl acetate 2:3). Mp 124–126 °C (ethyl acetate). IR (Nujol): 1735 ($COOCH_3$); 1630 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 2.73–2.89 (m, 3H, $NCHCH_2$); 3.75 (s, 3H, OCH_3); 3.82 (s, 3H, $COOCH_3$); 4.10–4.19 (m, 1H, NCH); 4.37 (d, 1H, H-3, J = 3.7 Hz); 4.36 (d, 1H, H-6, J = 16.9 Hz); 4.64 (d, 1H, H-6, J = 16.9 Hz); 4.82 (d, 1H, H-2, J = 3.7 Hz); 6.90–7.30 (m, 9H, arom. H). MS (EI) m/z: (%): 369 (M^+, 15), 310 (7), 278 (20), 265 (41), 221 (48), 177 (100). Anal. calcd for $C_{21}H_{23}NO_5$ (369.16): C 68.28%; H 6.28%; N 3.79%; found: C 68.49%; H 6.65%; N 3.58%.

Cis-**6d** (0.042 g, 12%) as colorless oil. R_f 0.19 (light petroleum/ethyl acetate 2:3). IR ($CHCl_3$): 1760 ($COOCH_3$); 1640 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 2.64–3.01 (m, 3H, $NCHCH_2$); 3.55 (s, 3H, $COOCH_3$); 3.81 (s, 3H, OCH_3); 3.97–4.42 (m, 1H, NCH); 4.30 (d, 1H, H-3, J = 3.4 Hz); 4.37 (d, 1H, H-6, J = 16.7 Hz); 4.52 (d, 1H, H-2, J = 3.4 Hz); 4.61 (d, 1H, H-6, J = 16.7 Hz); 6.83–6.90 (m, 2H, arom. H); 7.08–7.15 (m, 2H, arom. H); 7.17–7.38 (m, 5H, arom. H). MS (CI) m/z: 398 (M^+ + 29); 370 (M^+, 100); 338 (20); 310 (1); 262 (16); 177 (3). Anal. calcd for $C_{21}H_{23}NO_5$ (369.16): C 68.28%; H 6.28%; N 3.79%; found: C 68.59%; H 6.55%; N 3.50%.

3.4.5.2. Method B. Recrystallization from ethyl acetate yielded *trans*-**6d** (0.609 g, 67%) of colorless crystals. Mp 124–126 °C. TLC (light petroleum/ethyl acetate 3:2) with a sample of *trans*-**6d** obtained by method A showed that the products are identical; mixed mp with the sample obtained by method A was not depressed.

*3.4.6. Methyl (±)-*trans*-3-(4-methoxyphenyl)-5-oxo-4-(1-phenylethyl)morpholine-2-carboxylate **6e** (method A)*

Chromatographic purification (cyclohexane/ethyl acetate 5:2) and recrystallization from ethyl acetate gave *trans*-**6e** (0.208 g, 56%) as colorless crystals. R_f 0.55 (light petroleum/ethyl acetate 2:3). Mp 137–139 °C. IR (Nujol): 1740 ($COOCH_3$); 1640 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 1.14 (d, 3H, CH_3CH, J = 7.3 Hz); 3.39 (s, 3H, $COOCH_3$); 3.83 (s, 3H, OCH_3); 4.14 (d, 1H, H-3, J = 1.5 Hz); 4.47 (d, 1H, H-6, J = 17.1 Hz); 4.51 (d, 1H, H-2, J = 1.5 Hz); 4.91 (d, 1H, H-6, J = 17.1 Hz); 6.14 (q, 1H, NCH, J = 7.4 Hz); 6.82–7.43 (m, 9H, arom. H). MS (EI) m/z 369 (4, M^+); 238 (4); 193 (14); 192 (100); 162 (8); 105 (9). Anal. calcd for $C_{21}H_{23}NO_5$ (369.16): C 68.28%; H 6.28%; N 3.79%; found: C 68.00%; H 6.60%; N 3.90%.

*3.4.7. Methyl (±)-*trans- *and* cis*-3-(4-chlorophenyl)-5-oxo-4-phenethylmorpholine-2-carboxylate **6f***

3.4.7.1. Method A. Chromatographic purification (cyclohexane/2-propanol 8:1) gave *trans*-and *cis*-**6f**.

Trans-**6f** (0.199 g, 59%) as colorless crystals. R_f 0.65 (light petroleum/ethyl acetate 2:3). Mp 130–133 °C (ethyl acetate). IR (Nujol): 1720 ($COOCH_3$); 1620 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 2.72–2.87 (m, 3H, $NCHCH_2$); 3.77 (s, 3H, $COOCH_3$); 4.05–4.23 (m, 1H, NCH); 4.35 (d, 1H, H-3, J = 3.1 Hz); 4.36 (d, 1H, H-6, J = 17.0 Hz); 4.64 (d, 1H, H-6, J = 17.0 Hz); 4.85 (d, 1H, H-2, J = 3.1 Hz); 7.10–7.38 (m, 9H, arom. H). MS (CI) m/z (%): 402 (M^+ + 29, 17); 374 (M^+ + 1, 100); 342 (8); 314 (1); 282 (2); 262 (2). Anal. calcd for $C_{20}H_{20}NO_4Cl$ (373.84): C 64.26%; H 5.36%; N 3.75%; found: C 64.13%; H 5.27%; N 3.77%.

Cis-**6f** (0.046 g, 13%) as an oily product. R_f 0.38 (light petroleum/ethyl acetate 2:3). IR ($CHCl_3$): 1760 ($COOCH_3$); 1630 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 2.68–3.03 (m, 3H, $NCHCH_2$); 3.56 (s, 3H, OCH_3); 4.03–4.13 (m, 1H, NCH); 4.29 (d, 1H, H-3, J = 3.4 Hz); 4.38 (d, 1H, H-6, J = 16.9 Hz); 4.52 (d, 1H, H-2, J = 3.4 Hz); 4.61 (d, 1H, H-6, J = 16.8 Hz); 7.10–7.39 (m, 9H, arom. H). MS (CI) m/z (%) 402 (M^+ + 29); 374 (M^+ +1, 100); 342 (5); 282 (2); 262(1); 181 (1). Anal. calcd for $C_{20}H_{20}NO_4Cl$ (373.84): C 64.26%; H 5.36%; N 3.75%; found: C 64.43%; H 5.05%; N 3.90%.

3.4.7.2. Method B. Recrystallization from ethyl acetate yielded *trans*-**6f** (0.649 g, 58%) as colorless crystals. Mp 130–132 °C. TLC (light petroleum/ethyl acetate 3:2) with a

sample of *trans*-**6f** obtained by method A showed that the products are identical; mixed mp with a sample obtained by method A was not depressed.

*3.4.8. Methyl (±)-*trans- *and cis-4-benzyl-3-(4-chlorophenyl)-5-oxomorpholine-2-carboxylate* ***6g*** *(method A)*

Chromatographic purification (cyclohexane/ethyl acetate 5:1) gave *trans*-and *cis*-**6g**.

Trans-**6g** (0.197 g, 55%) as colorless oil. R_f 0.63 (light petroleum/ethyl acetate 2:3). IR (film): 1750 ($COOCH_3$); 1650 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 3.39 (d, 1H, NCH, J = 14.6 Hz); 3.80 (s, 3H, $COOCH_3$); 4.35 (d, 1H, H-3, J = 2.6 Hz); 4.70 (d, 1H, H-2, J = 2.6 Hz); 4.46 (d, 1H, H-6, J = 17.1 Hz); 4.88 (d, 1H, H-6, J = 17.1 Hz); 5.61 (d, 1H, NCH, J = 14.6 Hz); 6.82–7.48 (m, 9H, arom. H). MS (EI) m/z (%): 359 (20, M^+); 300 (15); 228 (6); 198 (31); 197 (16); 196 (100), 167 (10); 162 (29); 125 (11); 104 (5); 91 (40). Anal. calcd for $C_{19}H_{18}NO_4Cl$ (359.84): C 63.42%; H 5.04%; N 3.89%; found: C 63.13%; H 5.29%; N 3.63%.

Cis-**6g** (0.115 g, 32%) as colorless crystals. R_f 0.41 (light petroleum/ethyl acetate 2:3). Mp 116–118 °C (ethyl acetate). IR (Nujol): 1760 ($COOCH_3$); 1650 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 3.36 (d, 1H, NCH, J = 14.7 Hz); 3.52 (s, 3H, $COOCH_3$); 4.45 (d, 1H, H-6, J = 16.9 Hz); 4.52 (d, 1H, H-3, J = 3.5 Hz); 4.59 (d, 1H, H-2, J = 3.5 Hz); 4.69 (d, 1H, H-6, J = 16.9 Hz); 5.49 (d, 1H, NCH, J = 14.7 Hz); 7.04–7.43 (m, 9H, arom. H). Anal. calcd for $C_{19}H_{18}NO_4Cl$ (359.84): C 63.42%; H 5.04%; N 3.89%; found: C 63.23%; H 5.32%; N 3.60%.

*3.5. (±)-*Trans*-6-(hydroxymethyl)-5-(4-methoxyphenyl)-4-phenethylmorpholin-3-one (****7****)*

To a stirred suspension of LiCl (0.509 g, 12 mmol) and KBH_4 (0.647 g, 12 mmol) in dry THF (5 mL), a solution of **6d** (1.478 g, 4 mmol) in dry THF (20 mL) was added dropwise for 20 min. The reaction mixture was stirred at room temperature for 35 h. The solvent was removed under reduced pressure and the residue was poured in water (50 mL). The suspension was extracted with ethyl acetate (3 × 10 mL) and the organic phase was dried (Na_2SO_4). After removal of the solvent, the residue was purified by recrystallization from ethyl acetate–cyclohexane to give **7** (1.008 g, 74%) as white crystals. R_f 0.35 (light petroleum/ethyl acetate 2:3). Mp 100–102 °C. IR (Nujol): 3400 (OH); 1620 (CON) cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ 2.17 (s, 1H, OH); 2.65 (ddd, 1H, CHAr, J = 5.2, 8.8, 12.9 Hz); 2.74 (ddd, 1H, NCH, J = 6.7, 8.8, 13.5 Hz); 2.87 (ddd, 1H, CHAr, J = 6.7, 9.2, 12.9 Hz); 3.37 (ddd, 1H, CH_2OH, J = 5.5, 6.2, 12.4 Hz); 3.52 (ddd, 1H, CH_2OH, J = 2.7, 6.2, 12.0 Hz); 3.63 (ddd, 1H, H-6, J = 2.8, 5.3, 9.4 Hz); 3.83 (s, 1H, OCH_3); 4.01 (ddd, 1H, NCH, J = 5.1, 9.2, 13.5 Hz); 4.26 (d, 1H, H-5, J = 9.4 Hz); 4.31 (d, 1H, H-2, J = 16.3 Hz); 4.43 (d, 1H, H-2, J = 16.3 Hz); 6.89–6.92 (m, 2H, arom. H); 7.05–7.07 (m, 2H, arom. H); 7.09–7.12 (m, 2H, arom. H); 7.19–7.22 (m, 1H, arom. H); 7.25–7.28 (m, 2H, arom. H). MS (EI) m/z (%): 341 (13, M^+); 250 (38); 237 (39); 220 (11); 188 (9); 162 (23); 135 (13); 121 (100); 105 (18). Anal. calcd for $C_{20}H_{23}NO_4$ (341.16): C 70.36%; H 6.79%; N 4.10%; found: C 70.78%; H 6.62%; N 4.00%.

*3.6. (±)-*Trans*-2-((3-(4-methoxyphenyl)-5-oxo-4-phenethylmorpholin-2-yl)methyl)isoindoline-1,3-dione (****8****)*

To a stirred solution of alcohol **7** (1.023 g, 3 mmol), Ph_3P (0.717 g, 3 mmol), and phthalimide (0.441 g, 3 mmol) in dry THF (11 mL) under argon, a solution of diethyl azodicarboxylate (1.8 mL, 40% in toluene, 4 mmol) was added dropwise for 10 min at room temperature. The reaction mixture was sonicated for 90 min. The solvent was evaporated and the residue was recrystallized from ethyl acetate to give **8** (0.846 g, 60%) as white crystals. R_f 0.48 (light petroleum/ethyl acetate 2:3). Mp 161–162 °C. IR (Nujol): 1770 (CON); 1700 (CON); 1650 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 2.37–2.99 (m, 3H, $NCHCH_2$); 3.46 (dd, 1H, CH_2Phth, J = 3.7, 14.4 Hz); 3.76 (s, 3H, OCH_3); 3.82 (dd, 1H, CH_2Phth, J = 7.6, 14.4 Hz); 3.89–4.03 (m, 1H, NCH); 4.07–4.19 (m, 2H, H-2, H-3); 4.21 (d, 1H, H-6, J = 16.5 Hz); 4.41 (d, 1H, H-6, J = 16.5 Hz); 6.82–6.91 (m, 2H, arom. H); 7.04–7.29 (m, 7H, arom. H); 7.64–7.83 (m, 4H, arom. H). MS (EI) m/z (%): 470 (13, M^+); 379 (33); 335 (32); 311 (26); 310 (100); 280 (45); 271 (12); 232 (58); 219 (11); 176 (17); 174 (42); 160 (97); 146 (24); 121 (45); 103 (31). Anal. calcd for $C_{28}H_{26}N_2O_5$ (470.52): C 71.47%; H 5.57%; N 5.95%; found: C 71.61%; H 5.50%; N 5.87%.

3.7. Trans*-1-((3-(4-methoxyphenyl)-5-oxo-4-phenethylmorpholin-2-yl)methyl)piperidine-2,6-dione (****9****)*

To a stirred solution of alcohol **7** (0.342 g, 1 mmol), Ph_3P (0.239 g, 1 mmol) and glutarimide (0.119 g, 1.05 mmol) in dry THF (4 mL) under argon, a solution of diethyl azodicarboxylate (0.59 mL, 40% in toluene, 1.3 mmol) was added dropwise for 10 min at room temperature. The reaction mixture was sonicated for 190 min. The solvent was evaporated and the residue was purified by column chromatography (cyclohexane/2-propanol 8:1) and recrystallized from ethyl acetate to give **9** (0.127 g, 29%) as white crystals. R_f 0.13 (light petroleum/ethyl acetate 2:3). Mp 134–138 °C. IR (Nujol): 1730(CON); 1680(CON); 1650(CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 1.78–1.83 (m, 2H, CH_2); 2.54 (t, 4H, CH_2, J = 6.5 Hz); 2.61–2.95 (m, 3H, $NCHCH_2$); 3.49 (dd, 1H, NCH, J = 3.4, 12.8 Hz); 3.82 (s, 3H, OCH_3); 3.86–4.20 (m, 5H, NCH, H-2, H-3, H-6); 4.40 (d, 1H, H-6, J = 16.5 Hz); 6.85–7.31 (m, 9H, arom. H). MS (CI) m/z (%): 465 (M^++ 29, 18); 437 (M^++ 1, 100); 345 (1); 324 (4); 258 (2); 216 (1). Anal. calcd for $C_{25}H_{28}N_2O_5$ (436.51): C 68.79%; H 6.47%; N 6.42%; found: C 68.93%; H 6.35%; N, 6.44.

*3.8. (±)-*Trans*-6-(aminomethyl)-5-(4-methoxyphenyl)-4-phenethylmorpholin-3-one (****10****)*

To a solution of methylamine in water (15.5 mL, 40%, 180 mmol), phthalimide derivative **8** (0.564 g, 1.2 mmol) was added and the mixture was magnetically stirred at room temperature for 3 h until homogeneous. The solution was extracted with dichloromethane (3 × 10 mL) and the combined organic solutions were dried (Na_2SO_4). The solvent was evaporated to give amine **10** (0.347 g, 85%) as an oily product, which was sufficiently pure for further experiments. R_f 0.10 (ethyl acetate/2-propanol 2:1). IR

(film): 3390 (NH_2); 3310 (NH_2); 1640 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 1.51 (br s, 2H, NH_2); 2.54–2.97 (m, 5H, $NCHCH_2$, CH_2N); 3.50–3.60 (ddd, 1H, H-6, J = 4.8, 5.0, 9.1 Hz); 3.85 (s, 3H, CH_3O); 3.97–4.06 (m, 1H, NCH); 4.10 (d, 1H, H-5, J = 9.1 Hz); 4.30 (d, 1H, H-2, J = 16.3 Hz); 4.44 (d, 1H, H-2, J = 16.3 Hz); 6.88–6.97 (m, 2H, arom. H); 7.01–7.12 (m, 2H, arom. H); 7.16 -7.33 (m, 5H, arom. H). MS (CI) m/z (%): 341 (M^+ + 1, 13); 312 (100); 279 (3); 204 (2); 162 (1). Anal. calcd for $C_{20}H_{24}N_2O_3$ (340.42): C 70.56%; H 7.11%; N 8.23%; found: C 70.28%; H 7.37%; N 8.02%.

3.9. General procedure for the synthesis of acylated derivatives of (±)-trans-6-(aminomethyl)-5-(4-methoxyphenyl)-4-phenethylmorpholin-3-one 11 and 12

To a stirred solution of amine **10** (0.170 g, 0.5 mmol) and of the corresponding N-protected L-amino acid (0.5 mmol) in dry dichloromethane (2 mL) cooled to −10 °C, a solution of dicyclohexylcarbodiimide (DCC, 0.134 g, 0.65 mmol) in dry dichloromethane (2 mL) was added dropwise. The mixture was stirred for 2 h at −10 °C and then for 12 h at room temperature. The precipitated urea derivative was filtered out and discarded. The filtrate was evaporated under reduced pressure and the resulting oil was dissolved in dichloromethane (10 mL). The solution was successively washed with 10% HCl, water, 10% Na_2CO_3 and brine. The organic layer was dried (Na_2SO_4) and the solvent was removed in vacuo. The residue was purified by means of column chromatography.

3.9.1. (2S)-tert-Butyl-2-(((±)-trans-3-(4-methoxyphenyl)-5-oxo-4-phenethylmorpholin-2-ylmethyl)carbamoyl)pyrrolidin-1-carboxylate (11)

From amine **10** and Boc-L-proline. Column chromatography purification (cyclohexane/ethyl acetate 1:2) gave **11** (0.197 g, 75%) as an oily product. R_f 0.18 (cyclohexane/ethyl acetate 1:2). IR ($CHCl_3$): 3420 (NH); 1670 ($COOC(CH_3)_3$); 1650 (CON); 1610 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 1.46 (s, 2 × 9H, $C(CH_3)_3$, $C(CH_3)_3$*); 1.86 (m, 4H, CH_2, CH_2*); 2.05 (m, 4H, CH_2N, CH_2N*); 2.49–2.94 (m, 6H, $NCHCH_2$, $NCHCH_2$*); 3.26 (m, 4H, NCH_2, NCH_2*); 3.42 (m, 4H, NCH_2, NCH_2*); 3.69 (ddd, 2H, H-6, H-6*, J = 4.3, 5.5, 8.5 Hz); 3.81 (s, 6H, OCH_3, OCH_3*); 3.85–4.02 (m, 2H, NCH, NCH*); 4.21 (m, 2H, COCHN, COCHN*); 4.25 (d, 2H, H-2, H-2*, J = 16.3 Hz); 4.37 (d, 2H, H-2, H-2*, J = 16.3 Hz); 4.20–4.35 (m, 2H, H-5, H-5*); 6.32 (m, 2H, NHCO, NHCO*); 6.98–7.33 (m, 18H, arom. H). Anal. calcd for $C_{30}H_{39}N_3O_6$ (537.28): C 67.02%; H 7.31%; N 7.82%; found: C 67.32%; H 7.21%; N 7.52%.

3.9.2. (S)-N-(((±)-trans-3-(4-methoxyphenyl)-5-oxo-4-phenethylmorpholin-2-yl)methyl)-3-phenyl-2-(2.2.2-trifluoroacetamido)-propanamide (12)

From amine **10** and *N*-trifluoroacetyl-L-phenylalanine. Column chromatography purification (cyclohexane/2-propanol 8:1) and subsequent recrystallization from ethyl acetate gave **12** (0.149 g, 51%) as white crystals. R_f 0.52 (cyclohexane/ethyl acetate 1:2). Mp 83–86 °C. IR (Nujol): 1710 (CON); 1620 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 2.53–3.06 (m, 10H, $NCHCH_2$, $NCHCH_2$*, 2 × CH_2, 2 × CH_2*); 3.08–3.24 (m, 4H, NCH_2, NCH_2*); 3.30 (ddd, 1H, H-6, J = 3.4, 7.2, 9.4 Hz); 3.54 (ddd, 1H, H-6*, J = 3.4, 7.4, 9.2 Hz); 3.82 (s, 3H, CH_3O); 3.83 (s, 3H, CH_3O*); 3.87–4.01 (m, 4H, H-5, H*-5, NCH, NCH*); 4.05 (d, 1H, H-2, J = 16.3 Hz); 4.17 (d, 1H, H-2*, J = 16.3 Hz); 4.24 (d, 1H, H-2, J = 16.3 Hz); 4.29 (d, 1H, H-2*, J = 16.3 Hz); 4.45–4.58 (m, 2H, COCHN, COCHN*); 6.87–6.95 (m, 4H, NHCO, NHCO*, N*H*CH, N*H*CH*); 6.96–7.38 (m, 18H, arom. H). MS (CI), m/z (%): 612 (M^+ + 29, 14); 584 (M^+ + 1, 100); 498 (6); 405 (6); 334 (8); 324 (19); 305 (60); 280 (10); 252 (48); 225 (58); 192 (13); 126 (36). Anal. calcd for $C_{31}H_{32}F_3N_3O_5$ (583.23): C 63.80%; H 5.53%; N 7.20%; found: C 63.55%; H 5.80%; N 7.29%.

3.10. General procedure for the preparation of 2-((±)-trans-3-(4-methoxyphenyl)-5-oxo-4-phenethylmorpholin-2-yl)carbonyl)amino derivatives 13 and 14

To a magnetically stirred solution of acid *trans*-**5d** (0.365 g, 1 mmol), L-α-amino acid methyl ester hydrochloride (1.07 mmol) and triethylamine (0.15 mL, 1.07 mmol) in dry dichloromethane (5 mL), DCC (0.275 g, 1.3 mmol) was added portionwise at −15 °C. The mixture was stirred for 2 h at −10 °C and then 12 h at room temperature. The resulting precipitate of dicyclohexylurea was filtered and discarded. The filtrate was evaporated under reduced pressure and the residue was dissolved in ethyl acetate (30 mL). The solution was successively washed with 10% HCl, water, 10% Na_2CO_3 and brine. The organic layer was dried (Na_2SO_4) and the solvent was removed in vacuo. The residue was purified by means of column chromatography and/or recrystallization.

3.10.1. Methyl (2S)-3-(1H-indol-3-yl)-2-((((±)-trans-3-(4-methoxyphenyl)-5-oxo-4-phenethylmorpholin-2-yl)carbonyl)amino)propanoate (13)

From methyl ester of L-tryptophan hydrochloride. Recrystallization from ethyl acetate gave isomer **13a**. Concentration of the mother liquor gave white crystals of **13b**. Total yield of **13** is 62%.

13a (0.143 g, 26%) as white crystals. R_f 0.29 (light petroleum/ethyl acetate 2:3). Mp 166–168 °C. IR (Nujol): 3365 (NH); 1700 ($COOCH_3$); 1660 (CON); 1640 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 2.62–2.92 (m, 3H, $NCHCH_2$); 3.30 (dd, 1H, CH_2Ind, J = 5.3, 15.1 Hz); 3.36 (dd, 1H, CH_2Ind, J = 5.5, 15.1 Hz); 3.71 (s, 3H, $COOCH_3$); 3.78 (s, 3H, OCH_3); 3.82–3.14 (m, 1H, NCH); 4.19 (d, 1H, H-6, J = 16.7 Hz); 4.20 (d, 1H, H-3, J = 4.3 Hz); 4.30 (d, 1H, H-6, J = 16.7 Hz); 4.91 (ddd, 1H, CH, J = 5.3, 5.5, 7.9 Hz); 4.96 (d, 1H, H-2, J = 4.3 Hz); 6.80–6.94 (m, 4H, arom. H); 7.04–7.25 (m, 9H, arom. H); 7.35 (d, 1H, arom. H, J = 8.0 Hz); 7.48 (d, 1H, NHCO, J = 7.9 Hz); 8.19 (br s, 1H, NH of indole ring). Anal. calcd for $C_{32}H_{33}N_3O_6$ (555.24): C 69.17%; H 5.99%; found: C 69.31%; H 6.30%.

13b (0.198 g, 36%) as white crystals. R_f 0.36 (light petroleum/ethyl acetate 2:3). Mp 108–110 °C. IR (Nujol): 3340 (NH); 1710 ($COOCH_3$); 1660 (CON); 1630 (CON) cm^{-1}. ^1H NMR (250 MHz, CDCl3): δ 2.60–2.89 (m, 3H, $NCHCH_2$); 3.31 (dd, 1H, CH_2-Ind, J = 6.4, 14.8 Hz); 3.40 (dd, 1H, CH_2-Ind, J = 5.5, 14.8 Hz); 3.72 (s, 3H, $COOCH_3$); 3.78 (s, 3H, OCH_3); 4.01 (d, 1H, H-6, J = 16.7 Hz); 4.02–4.13 (m, 1H, NCH); 4.14 (d, 1H, H-6, J = 16.7 Hz); 4.25 (d, 1H, H-3,

J = 4.1 Hz); 4.88 (ddd, 1H, CH, J = 5.5, 6.4, 7.6 Hz); 4.93 (d, 1H, H-2, J = 4.1 Hz); 6.76–6.94 (m, 4H, arom. H); 7.01–7.29 (m, 9H, arom. H); 7.36 (d, 1H, arom. H, J = 7.4 Hz); 7.53 (d, 1H, NHCO, J = 7.6 Hz); 8.27 (br s, 1H, NH of indole ring). Calcd for $C_{32}H_{33}N_3O_6$ (555.24): C 69.17%; H 5.99%; N 7.56%; found: C 69.00%; H 6.17%; N 7.91%.

3.10.2. Methyl (2S)-1-((±)-trans-*3-(4-methoxyphenyl)-5-oxo-4-phenethylmorpholine-2-carbonyl)pyrrolidine-2-carboxylate* **14**

From methyl ester of L-proline hydrochloride. Column chromatography purification (light petroleum/2-propanol 9:1) afforded 14 (0.310 g, 56%) as an uncrystallizable oil. R_f 0.33 and 0.42 (light petroleum/ethyl acetate 2:3, two-fold development). IR (Nujol): 1740 ($COOCH_3$); 1640 (CON) cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 1.75–2.25 (m, 8H, 2 × CH_2, 2 × CH_2*, proline-H); 2.71–2.97 (m, 6H, $NCHCH_2$; $NCHCH_2$*); 3.24–3.44 (m, 2H, NCH_2, proline-H); 3.54–3.67 (m, 2H, NCH_2*, proline-H); 3.71 (s, 3H, $COOCH_3$); 3.74 (s, 3H, $COOCH_3$*); 3.80 (s, 3H, OCH_3) 3.81 (s, 3H, OCH_3*); 3.92–4.58 (m, 10H, 2 × H-6, 2 × H-6*, NCH, NCH*, H-3, H-3*, proline-NCH, prolin-NCH*); 5.00 (d, 1H, H-2, J = 3.8 Hz); 5.06 (d, 1H, H-2*, J = 4.1 Hz); 6.85–6.92 (m, 4H, arom. H, arom. H*); 7.10–7.24 (m, 14H, arom. H, arom. H*). Anal. calcd for $C_{26}H_{30}N_2O_6$ (466.21): C 66.94%; H 6.48%; N 6.00%; found: C 66.82%; H 6.57%; N 5.90%.

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