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Synthesis of chiral non-racemic 3-(dioxopiperazin-2-yl)propionic acid derivatives

Manuela Weigl and Bernhard Wünsch*

Pharmazeutisches Institut der Universität Freiburg, Albertstraße 25, D-79104 Freiburg i, Br., Germany

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Abstract—Starting with the proteinogenic amino acid (*S*)-glutamate (**3**) a facile, high yielding synthesis of the chiral non-racemic 3-(dioxopiperazin-2-yl)propionates **6**, **11**, and **14** is presented. Key intermediates in the synthesis of N^1-benzyl substituted (dioxopiperazin-2-yl)propionates **11** and **14** are the *N*-monobenzylglutamate **8** and the chloroacetamide **12**, which allow introduction of various substituents in position 4 of the piperazine ring. In receptor binding studies with radioligands the 3-(piperazin-2-yl)propanol **15** was found to have promising affinity for σ_1-receptors (K_i=66.1 nM). © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

In the course of our work on novel central nervous system (CNS) receptor ligands our interest has been focused on 3-(dioxopiperazin-2-yl)propionic acid derivatives **1**. The (dioxopiperazin-2-yl)propionates **1** and their derivatives are considered as homologues of known CNS active piperazines with hydroxymethyl,[1] aminomethyl,[2] aminoethyl,[3] or carboxyl[4] groups in position 2. Cyclization of the (dioxopiperazin-2-yl)propionic acid derivatives **1** may result in bicyclic lactams **2**, which are described as nootropic agents[5] and ligands for σ_1-receptors (Fig. 1).[6]

Additionally the dioxopiperazines **1** represent dipeptide mimetics, with limited conformational flexibility compared to the regular gly-glu-dipeptide. Locally constrained peptide analogues will be obtained by integration of dioxopiperazines **1** into pharmacologically active peptides.

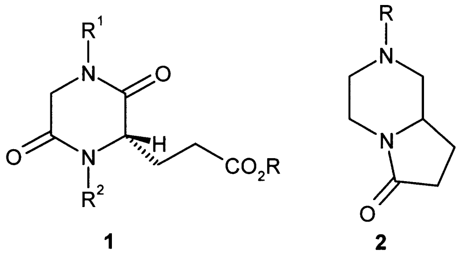

Figure 1.

In this communication we describe the synthesis of chiral non-racemic 3-(dioxopiperazin-2-yl)propionic acid esters **1** with various substituents at the piperazine nitrogen atoms. The reduction of some (dioxopiperazin-2-yl)propionates was investigated, furthermore one compound was screened concerning CNS receptor affinity.

2. Chemistry

The synthesis of the (dioxopiperazin-2-yl)propionate **6** without substituents at both nitrogen atoms has already been described in the literature.[7] Starting with the 5-monomethyl ester of (*S*)-glutamate the synthesis comprises four reaction steps with partly drastic reaction conditions. The total yield of **6** from the monomethyl ester of (*S*)-glutamate, which has to be prepared additionally from the amino acid (*S*)-glutamate, amounts to 43% (Scheme 1).

According to our novel strategy the proteinogenic amino acid (*S*)-glutamate (**3**) was treated with an excess of $ClSi(CH_3)_3$ in methanol[8] to provide the dimethyl ester hydrochloride **4**·HCl[9] in quantitative yield. Subsequently, **4**·HCl was acylated with Cbz-glycine using the coupling reagents dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt)[10] to yield the dipeptide **5** (94%). Attempts with the coupling reagents 1,1′-carbonyldiimidazole (CDI), ethyl 2-ethoxy-1,2-dihydroquinoline-1-carboxylate (EEDQ) or DCC without HOBt resulted in considerably lower yields of the dipeptide **5**. Hydrogenolytic cleavage of the Cbz protective group[11] liberated the primary amine, which cyclized spontaneously to form the dioxopiperazine **6**.

In summary, herein a three step synthesis of **6** starting with the non-derivatized proteinogenic amino acid (*S*)-glutamate

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Keywords: medicinal chemistry; (*S*)-glutamic acid derivatives; 3-(dioxopiperazin-2-yl)propionic acid derivatives; peptidomimetics; σ-receptor ligands.

* Corresponding author. Fax: +49-761-203-6321;
e-mail: wuensch@ruf.uni-freiburg.de

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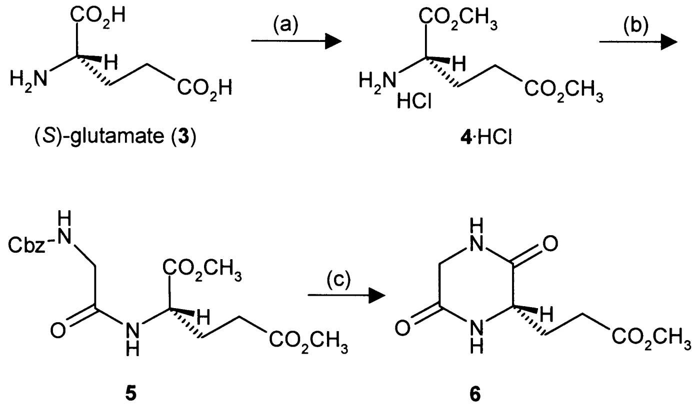
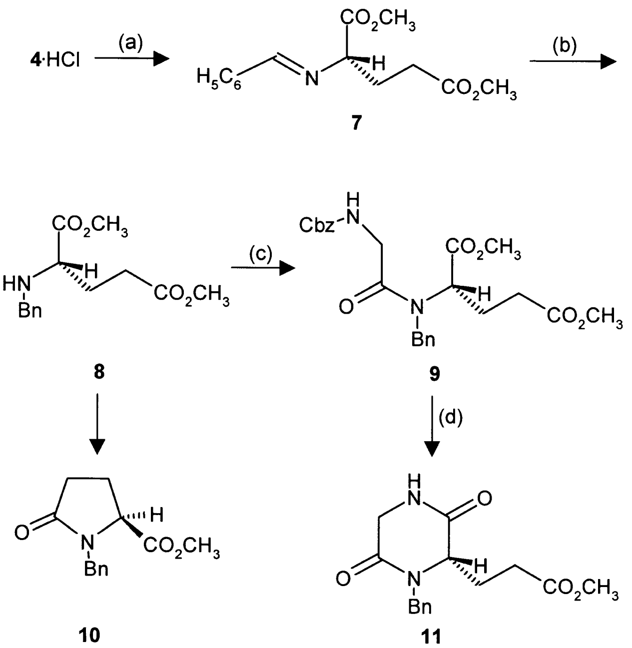


Scheme 1. *Reagents and reaction conditions*: (a) $ClSi(CH_3)_3$, CH_3OH, 16 h, rt, 100%; (b) Cbz-glycine, DCC, HOBt, 4-ethylmorpholine, THF, CH_2Cl_2, 16 h, rt, 94%; (c) H_2, 1013 mbar, Pd/C (10%), CH_3OH, 16 h, rt, 85%.

(**3**) is presented. Only very mild reaction conditions were used resulting in 80% total yield over three steps from (*S*)-glutamate (**3**).

The N^1-benzyl substituted (dioxopiperazin-2-yl)propionate **11** was accessible using the same reaction sequence starting with the *N*-monobenzylglutamate **8**. The *N*-monobenzyl amino acid derivative **8** was prepared by condensation of the dimethyl ester of (*S*)-glutamate (**4**) with benzaldehyde and subsequent reduction of the imine **7**. Hydrogenation[12] of the imine **7** with the catalyst Pd/C provided a mixture of the *N*-monobenzyl derivative **8** (25%) and the debenzylated dimethyl glutamate **4** (21%). With $NaBH_4$ in methanol[13] the imine **7** was reduced to yield the *N*-monobenzylglutamate **8**. However, the reaction conditions during the $NaBH_4$ reduction have to be carefully controlled, since the *N*-monobenzylglutamate **8** cyclized very fast to give the lactam **10**.[14] Thus, reaction of the imine **7** with exactly 2 mol equiv. of $NaBH_4$ at 0°C for 30 min furnished the *N*-monobenzylglutamate **8** in 93% yield without detectable amounts of the lactam **10**. Elongation of the reaction time, enhancement of the reaction temperature or using a greater excess of the reducing agent $NaBH_4$ resulted in increasing amounts of lactam **10**, which was generated in over 70% yield by stirring the reaction mixture for 22 h at room temperature. Because of its high tendency towards cyclization the *N*-monobenzylglutamate **8** could be stored only for a few days at −20°C (Scheme 2).

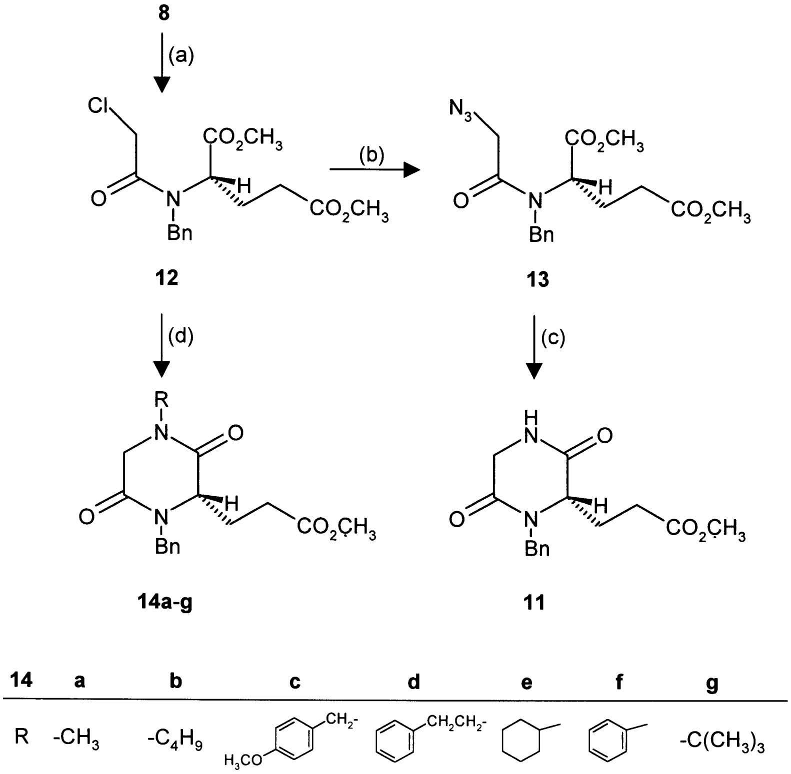


Scheme 2. *Reagents and reaction conditions*: (a) $C_6H_5CH{=}O$, NEt_3, pentane, Na_2SO_4, 16 h, rt, 91%; (b) $NaBH_4$, CH_3OH, 30 min, 0°C, 93%; (c) Cbz-glycine, DCC, THF, CH_2Cl_2, 16 h, rt, 47%; (d) H_2, 1013 mbar, Pd/C (10%), CH_3OH, 4 h, rt, 84%.

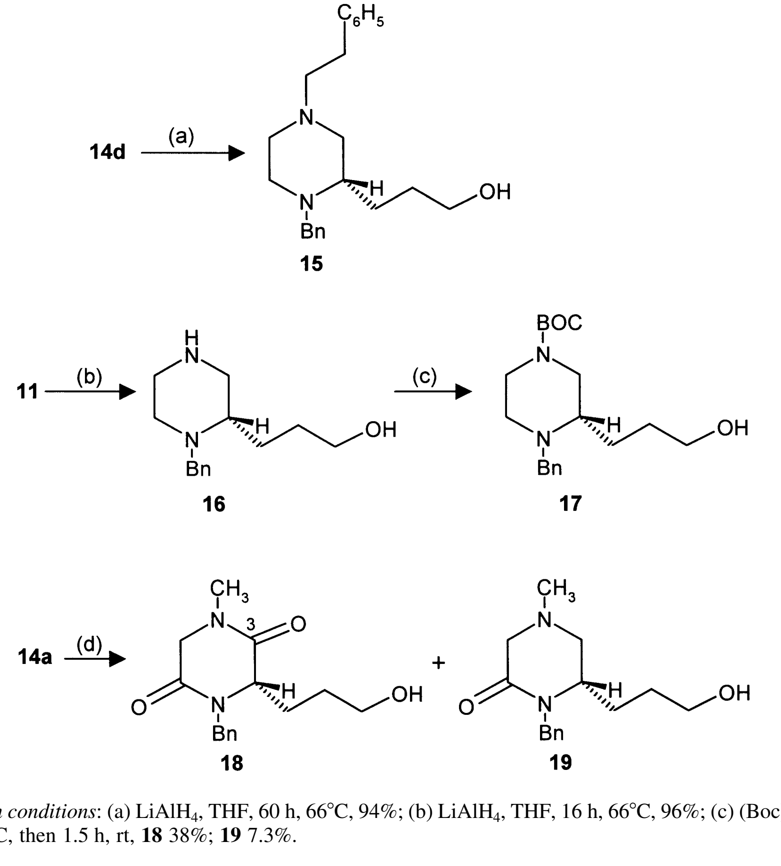
The peptide coupling of the *N*-monobenzylglutamate **8** with Cbz-glycine according to the above described method with DCC and HOBt[10] resulted in almost quantitative formation of the lactam **10**. Only when HOBt was omitted the desired dipeptide **9** could be isolated in 47% yield. Hydrogenolytic cleavage of the Cbz protective group[11] and spontaneous cyclization led to the N^1-benzyl substituted (dioxopiperazin-2-yl)propionate **11** in 84% yield. The amidic N-benzyl substituent was not affected during this hydrogenolysis.

In order to improve the total yield of the N^1-benzyl substituted (dioxopiperazin-2-yl)propionate **11** the *N*-monobenzylglutamate **8** was acylated with chloroacetyl chloride.[15] After optimization of the reaction conditions the chloroacetamide **12** was obtained in 73% yield. Formation of lactam **10** was not observed during this acylation. Nucleophilic substitution with NaN_3 led to the azidoacetamide **13** which was reduced with hydrogen in the presence of Pd/C catalyst to provide the (dioxopiperazin-2-yl)propionate **11**. Although the reaction sequence via chloroacetylation of **8** involves an additional reaction step, the total yield of **11** is raised from 39% via peptide coupling with Cbz-glycine to 63%. Moreover, the strongly allergenic coupling reagent DCC is avoided (Scheme 3).

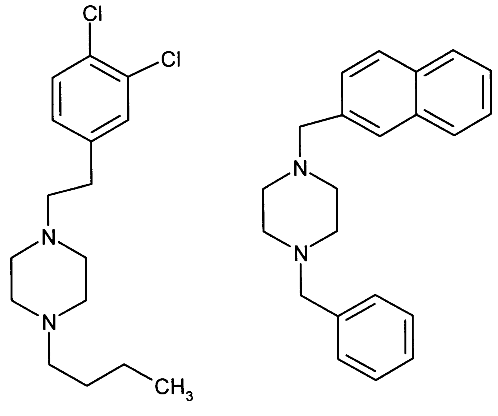
Additionally to the preparation of the (dioxopiperazin-2-yl)propionate **11** the chloroacetamide **12** served as educt for the synthesis of N^1-benzyl substituted (dioxopiperazin-2-yl)propionates **14** with various substituents in position 4. Thus, the chloroacetamide **12** reacted with primary amines in a *Domino* reaction involving nucleophilic substitution and intramolecular aminolysis to yield the (dioxopipera-



Scheme 3. *Reagents and reaction conditions*: (a) $ClCH_2COCl$, $NaHCO_3$, CH_2Cl_2, 16 h, rt, 73%; (b) NaN_3, acetone, 8 h, 56°C, 99%; (c) H_2, 1013 mbar, Pd/C (10%), CH_3OH, 16 h, rt, 87%; (d) RNH_2, CH_3CN, 16 h, rt, **14a** 93%; **14b** 94%; **14c** 92%; **14d** 97%; cyclohexylamine, CH_3CN, 48 h, 82°C, **14e** 79%; aniline, DMF, 16 h, 155°C, **14f** 52%.



Scheme 4. *Reagents and reaction conditions*: (a) $LiAlH_4$, THF, 60 h, 66°C, 94%; (b) $LiAlH_4$, THF, 16 h, 66°C, 96%; (c) $(Boc)_2O$, THF, NaOH, 16 h, rt, 59%; (d) $LiBH_4$, THF, Et_2O, 5 h, −5°C, then 1.5 h, rt, **18** 38%; **19** 7.3%.



20 **21**

Figure 2.

zin-2-yl)propionates **14**. Stirring at room temperature for 16 h was sufficient for the reaction of **12** with primary amines bearing the small methyl substituent or primary alkyl residues (**14a–d**, 92–97%), whereas heating to reflux (82°C) was necessary for a high-yielding transformation of cyclohexylamine (**14e**, 79%). The aromatic primary amine aniline required a higher temperature (DMF, 155°C) to react with the chloroacetamide **12**. Even at 155°C for 48 h the sterically demanding *tert*-butylamine did not react with the chloroacetamide **12** to furnish the *tert*-butyl derivative **14g**.

In summary, a great variety of chiral non-racemic (dioxopiperazin-2-yl)propionates **6**, **11**, and **14** were prepared starting with the proteinogenic amino acid (*S*)-glutamate (**3**) (Scheme 3).

Finally, transformation of the (dioxopiperazin-2-yl)propionates **11** and **14** into propanol derivatives was investigated. For this purpose the N^4-phenylethyl substituted piperazine derivative **14d** was heated with $LiAlH_4$.[15,16] During this reaction the ester moiety as well as the lactam carbonyl groups were reduced to provide the (piperazin-2-yl)propan-1-ol derivative **15** in 94% yield. Analogously, the N^4-unsubstituted (dioxopiperazin-2-yl)propionate **11** was reduced with $LiAlH_4$ to yield the (piperazin-2-yl)propanol **16**. Because of its high polarity **16** was transformed directly into the corresponding BOC-derivative **17**. With $LiBH_4$[17] the ester moiety of the N^4-methyl derivative **14a** was reduced selectively leading to the (dioxopiperazin-2-yl)-propanol **18**. However, a small amount of the (monooxopiperazin-2-yl)propanol **19** could be isolated by flash chromatography. In spite of many attempts the lactam reduction in position 3 could not be suppressed completely, but even became the main reaction in some cases. We assume, that the C-3-lactam carbonyl reduction is caused by complexation of the reducing agent to the developing alcohol moiety and subsequent intramolecular hydride transfer (neighboring group effect) (Scheme 4).

3. Receptor binding studies

Ligands interacting with σ-receptors possess a potential as antipsychotics,[18] antidepressants[19] and antitumor agents.[20] Among the high affinity σ-receptor ligands there are several piperazine derivatives. For example, the 1-butyl-4-phenylethylpiperazine **20** shown in Fig. 2 reveals high affinity (K_i=0.55 nM) and selectivity for σ_1-receptors.[21] Likewise, the 1-benzyl-4-(naphthylmethyl)piperazine **21** binds with high affinity at σ_1- (IC_{50}=1.3 nM) and σ_2-receptors (IC_{50}=23 nM).[22]

Therefore, we investigated the σ-receptor affinity of the 3-(1-benzyl-4-phenethylpiperazin-2-yl)propan-1-ol (**15**) in receptor binding studies with radioligands. The σ_1-assay was performed with guinea pig brain membrane preparations using [^{3}H]-(+)-pentazocine as radioligand. Rat liver homogenates were used for the determination of σ_2-receptor affinity with the non-selective radioligand [^{3}H]-ditolylguanidine in the presence of an excess of non-radiolabeled (+)-pentazocine to mask the σ_1-receptors.[23]

In the σ_1-assay the (piperazin-2-yl)propanol **15** revealed affinity in the low nanomolar range (K_i=66.1±27.4 nM). Obviously the 3-hydroxypropyl side chain attached in position 2 of the piperazine ring system does not strongly hinder the interaction of **15** with σ_1-receptors. The affinity of the (piperazin-2-yl)propanol **15** for σ_2-receptors was considerably lower (K_i=1190±130 nM) indicating high selectivity for σ_1-receptors (18 fold).

In addition to the σ-receptor affinities the (piperazin-2-yl)-propanol **15** was screened for μ-opioid-, κ-opioid- and NMDA-receptor affinity.[3b] It was shown, that **15** did not interact significantly with these receptor systems (IC_{50}>10 μM).

4. Conclusion

In this communication a facile high yielding method for the preparation of chiral non-racemic 3-(dioxopiperazin-2-yl)-propionates **6**, **11**, and **14** is presented. These (dioxopiperazin-2-yl)propionates represent valuable intermediates for the synthesis of novel ligands for CNS receptors. Exemplary, the receptor binding of the basic (1-benzyl-4-phenethylpiperazin-2-yl)propanol **15** has been investigated, revealing high affinity for σ_1-receptors.

5. Experimental

5.1. Chemistry, general

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. THF was distilled from sodium/benzophenone ketyl prior to use. Petroleum ether used refers to the fraction boiling at 40–60°C. Thin layer chromatography (tlc): Silica gel 60 F_{254} plates (Merck). Flash chromatography (fc):[24] Silica gel 60, 0.040–0.063 mm (Merck); parentheses include: Diameter of the column (cm), eluent, fraction size (ml), R_f. Melting points: Melting point apparatus SMP 2 (Stuart Scientific), uncorrected. Optical rotation: Polarimeter 241 (Perkin–Elmer); 1.0 dm tube; concentration *c* (g/100 ml). Elemental analyses: CHN-Elementaranalysator Rapid (Heraeus), Elemental Analyzer

240 (Perkin–Elmer) and Vario EL (Elementaranalysesysteme GmbH). MS: MAT 312, MAT 8200, MAT 44, and TSQ 7000 (Finnigan); EI=electron impact, CI=chemical ionization. High resolution MS (HRMS): MAT 8200 (Finnigan). IR: IR spectrophotometer 1600 FT-IR and 2000 FT-IR (Perkin–Elmer); s=strong, m=medium, w=weak. ^1H NMR (300 MHz), ^{13}C NMR (75 MHz): Unity 300 FT NMR spectrometer (Varian); ^1H NMR (400 MHz), ^{13}C NMR (100 MHz): JNM-GX 400 (Jeol); δ in ppm related to tetramethylsilane, coupling constants are given with 0.5 Hz resolution; the assignments of ^{13}C and of ^1H NMR signals were supported by 2D NMR techniques.

5.1.1. (*S*)-Glutamic acid dimethyl ester hydrochloride (4·HCl).[9] Chlorotrimethylsilane (75 ml, 594 mmol) was slowly added within 90 min to an ice-cold suspension of (*S*)-glutamate (**3**, 25.1 g, 171 mmol) in methanol (350 ml). After stirring for 16 h at room temperature the reaction mixture was concentrated in vacuo. Methanol (50 ml) was added to the residue and subsequently evaporated in vacuo. Then, Et_2O (60 ml) was added, the mixture was stirred vigorously and the solvent was removed in vacuo (3×). Colorless solid, mp 89–91°C (Ref. 9: mp 88–90°C), yield 36.1 g (100%). $C_7H_{14}ClNO_4$ (211.7). MS (EI): *m*/*z* (%)=176 (MH^+-Cl, 2), 144 ($176-CH_3OH$, 8). $[\alpha]_{589}^{20}=+26.2$ (c=1.59, H_2O) [Ref. 9: $[\alpha]_{589}^{21}=+26.0$ (c=5.0, H_2O]. IR (KBr): $\tilde{\nu}$ (cm^{-1})=2955 (m, $\nu_{CH\ aliph.}$), 1734 (s, $\nu_{C=O}$), 1507 (w, $\delta_{NH_3^+}$), 1442 (m, $\delta_{CH\ aliph.}$), 1229, 1146 (each m, ν_{COC}). ^1H NMR (D_2O): δ=2.24 (dt, J=14.3, 7.3 Hz, 1H, $C\mathit{H}_2CH_2CO_2CH_3$), 2.31 (dt, J=14.3, 7.3 Hz, 1H, $C\mathit{H}_2CH_2CO_2CH_3$), 2.64–2.68 (m, 2H, $CH_2C\mathit{H}_2CO_2CH_3$), 3.66 (s, 3H, $CO_2C\mathit{H}_3$), 3.78 (s, 3H, $CO_2C\mathit{H}_3$), 4.23 (t, J=6.8 Hz, 1H, $NH-C\mathit{H}-CO_2CH_3$).

5.1.2. Dimethyl (*S*)-2-[2-(benzyloxycarbonylamino)-acetylamino]pentanedioate (5). **4**·HCl (1.06 g, 5.01 mmol) and 4-ethylmorpholine (1.25 ml, 9.87 mmol) were added to a suspension of Cbz-glycine (1.28 g, 6.12 mmol) in a mixture of THF (25 ml) and CH_2Cl_2 (25 ml). Then, HOBt (1.50 g, 9.79 mmol) and DCC (1.23 g, 5.96 mmol) were added at 0°C. The reaction mixture was stirred for 30 min at 0°C and for 16 h at rt. The reaction mixture was filtered and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (100 ml) and washed with a saturated solution of $NaHCO_3$ (2×100 ml), 0.5N HCl (1×100 ml) and a saturated solution of NaCl (1×100 ml). The CH_2Cl_2 layer was dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was purified by fc (4 cm, CH_2Cl_2/ethyl acetate=7:3, 20 ml, R_f 0.33). Colorless viscous oil, which solidified slowly, colorless solid (petroleum ether/ethyl acetate=1:1) mp 65–67°C, yield 1.71 g (94%). $C_{17}H_{22}N_2O_7$ (366.4) calcd C 55.7 H 6.05 N 7.65 found C 55.8 H 6.18 N 7.49. MS (EI): *m*/*z* (%)=366 (M, 38), 334 ($M-CH_3OH$, 20), 259 ($M-OCH_2Ph$, 39). MS (CI): *m*/*z* (%)=367 (MH^+, 80), 335 (MH^+-CH_3OH, 10), 323 (MH^+-CONH, 100), 259 (MH^+-PhCH_2OH, 50). $[\alpha]_{589}^{20}=+13.1$ (c=1.03, $CHCl_3$). IR (KBr): $\tilde{\nu}$ (cm^{-1})=3332 (m, $\nu_{NH\ sec.\ amide+carbamate}$), 3034 (w, $\nu_{CH\ arom.}$), 2954 (m, $\nu_{CH\ aliph.}$), 1738, 1731 (each s, $\nu_{C=O,\ ester\ and\ carbamate}$), 1682 (s, $\nu_{C=O,\ sec.\ amide}$), 1538 (m, amide II), 1440 (s, $\delta_{CH\ aliph.}$), 1215, 1172 (each m, ν_{COC}), 736, 699 (each m, $\gamma_{monosubst.\ aromat}$). ^1H NMR (400 MHz, $CDCl_3$): δ=1.89–1.96 (m, 1H, $C\mathit{H}_2CH_2CO_2CH_3$), 2.10–2.19 (m, 1H, $C\mathit{H}_2CH_2CO_2CH_3$), 2.26–2.41 (m, 2H, $CH_2C\mathit{H}_2CO_2CH_3$), 3.65 (s, 3H, $CO_2C\mathit{H}_3$), 3.71 (s, 3H, $CO_2C\mathit{H}_3$), 3.82–4.12 (m, 2H, Cbz-$NHC\mathit{H}_2CONH$), 4.62 (dd, J=7.7, 2.6 Hz, 1H, $NH-C\mathit{H}-CO_2CH_3$), 5.12 (s, 2H, $OC\mathit{H}_2Ph$), 5.80 (s, 1H, $N\mathit{H}-CO_2CH_2Ph$), 7.11 (d, J=7.3 Hz, 1H, $N\mathit{H}-CO$), 7.28–7.34 (m, 5H, aromat. H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=27.1 (1C, $\mathit{C}H_2CH_2CO_2CH_3$), 30.0 (1C, $CH_2\mathit{C}H_2CO_2CH_3$), 44.3 (1C, Cbz-$NH\mathit{C}H_2CONH$), 51.6 (1C, $CO_2\mathit{C}H_3$), 51.9 (1C, $NH-\mathit{C}H-CO_2CH_3$), 52.6 (1C, $CO_2\mathit{C}H_3$), 67.1 (1C, $CO_2\mathit{C}H_2Ph$), 128.1 (1C, aromat. CH), 128.2 (2C, aromat. CH), 128.5 (2C, aromat. CH), 136.2 (1C, aromat. C), 169.3 (1C, C=O), 172.1, (1C, C=O), 173.3 (1C, C=O).

5.1.3. Methyl (*S*)-3-(3,6-dioxopiperazin-2-yl)propionate (6).[7] A mixture of **5** (423 mg, 1.15 mmol), Pd/C (10%, 100 mg) and methanol (35 ml) was stirred under a hydrogen atmosphere (balloon, 1013 mbar) for 16 h at rt. Then, it was filtered through Celite and the solvent was evaporated in vacuo. Colorless viscous oil, which solidified slowly, colorless solid (C_2H_5OH) mp 197–198°C, yield 196 mg (85%), CH_2Cl_2/CH_3OH=8:2, R_f 0.60. $C_8H_{12}N_2O_4$ (200.2) calcd C 48.0 H 6.04 N 13.99 found C 48.3 H 5.83 N 13.91. MS (EI): *m*/*z* (%)=200 (M, 14), 168 ($M-CH_3OH$, 100), 140 ($M-CO_2CH_3$, 20), 126 ($M-CH_2CO_2CH_3$, 28). $[\alpha]_{589}^{20}=+4.1$ (c=0.32, $CHCl_3$). IR (KBr): $\tilde{\nu}$ (cm^{-1})=2970 (w, $\nu_{CH\ aliph.}$), 1728 (s, $\nu_{C=O,\ ester}$), 1680 (s, $\nu_{C=O,\ sec.\ amide}$), 1668 (s, $\nu_{C=O,\ sec.\ amide}$), 1459 (m, $\delta_{CH\ aliph.}$), 1259, 1179, 1089 (each m, ν_{COC}). ^1H NMR (400 MHz, CD_3OD): δ=2.10–2.16 (m, 2H, $C\mathit{H}_2CH_2CO_2CH_3$), 2.46 (t, J=7.5 Hz, 2H, $CH_2C\mathit{H}_2CO_2CH_3$), 3.67 (s, 3H, $CO_2C\mathit{H}_3$), 3.86 (d, J=17.1 Hz, 1H, $NHC\mathit{H}_2CO$), 3.98 (d, J=17.5 Hz, 1H, $NHC\mathit{H}_2CO$), 4.01 (t, J=6.0 Hz, 1H, $NH-C\mathit{H}-CO$). The analytical data correspond to the data given in Ref. 7.

5.1.4. Dimethyl (*S*)-2-(benzylidenamino)pentanedioate (7). A suspension of **4**·HCl (19.9 g, 94.2 mmol), triethylamine (13.2 ml, 95.2 mmol), benzaldehyde (10.0 ml, 99.2 mmol) and Na_2SO_4 in pentane (250 ml) was stirred for 16 h at rt. It was filtered, the residue was carefully extracted with pentane (4×40 ml) and the organic layer was concentrated in vacuo. Colorless oil, yield 22.6 g (91%), CH_2Cl_2/CH_3OH=9:1, R_f 0.83. $C_{14}H_{17}NO_4$ (263.3). MS (EI): *m*/*z* (%)=263 (M, 45), 232 ($M-OCH_3$, 28), 204 ($M-CO_2CH_3$, 93), 190 ($M-CH_2CO_2CH_3$, 73). MS (CI): *m*/*z* (%)=264 (MH^+, 100), 232 (MH^+-CH_3OH, 5), 204 ($MH^+-HCO_2CH_3$, 3). IR (film): $\tilde{\nu}$ (cm^{-1})=3451 (broad, w, ν_{NH}), 3030 (w, $\nu_{CH\ arom.}$), 2953 (m, $\nu_{CH\ aliph.}$), 1738 (s, $\nu_{C=O}$), 1644 (m, $\nu_{C=N}$), 1437 (m, $\delta_{CH\ aliph.}$), 1203, 1170 (each m, ν_{COC}), 733, 694 (each m, $\gamma_{monosubst.\ aromat}$). ^1H NMR (400 MHz, $CDCl_3$): δ=2.22–2.45 (m, 4H, $C\mathit{H}_2C\mathit{H}_2CO_2CH_3$), 3.65 (s, 3H, $CO_2C\mathit{H}_3$), 3.75 (s, 3H, $CO_2C\mathit{H}_3$), 4.07 (dd, J=7.9, 4.9 Hz, 1H, $N-C\mathit{H}-CO_2CH_3$), 7.39–7.48 (m, 3H, aromat. H), 7.76–7.80 (m, 2H, aromat. H), 8.30 (s, 1H, $N=C\mathit{H}-Ph$).

5.1.5. Dimethyl (*S*)-2-(benzylamino)pentanedioate (8). $NaBH_4$ (4.22 g, 112 mmol) was added to a cooled solution of **7** (17.3 g, 65.6 mmol) in CH_3OH (170 ml). After stirring for 30 min at 0°C the solvent was removed in vacuo and the residue was dissolved in water (150 ml). The aqueous layer was extracted with Et_2O (5×100 ml), the organic layer was dried (Na_2SO_4), filtered and concentrated in vacuo. Colorless oil, yield 16.2 g (93.0%), petroleum ether/ethyl acetate=6:4, R_f 0.44. $C_{14}H_{19}NO_4$ (265.3) MS (EI): *m/z* (%)=265 (M, 2), 233 (M−CH_3OH, 17), 206 (M−CO_2CH_3, 62), 174 (206−CH_3OH, 100), 146 (M−2×CO_2CH_3, 24). MS (CI): *m/z* (%)=266 (MH^+, 100), 234 (MH^+−CH_3OH, 94), 206 (MH^+−HCO_2CH_3, 4), 174 (206−CH_3OH, 100). $[\alpha]_{589}^{20}$=−27.3 (*c*=1.11, $CHCl_3$). IR (film): $\tilde{\nu}$ (cm^{-1})=3450 (w, ν_{NH}), 3030 (w, $\nu_{CH\ arom.}$), 2960 (w, $\nu_{CH\ aliph.}$), 1734 (s, $\nu_{C=O}$), 1560 (w, δ_{NH}), 1460 (m, $\delta_{CH\ aliph.}$), 1220, 1185 (each m, ν_{COC}), 740, 698 (each m, $\gamma_{monosubst.\ aromat}$). ^1H NMR (400 MHz, $CDCl_3$): δ=1.62–1.85 (m, 2H, $CH_2CH_2CO_2CH_3$, NH−CH_2Ph), 1.95 (dtd, *J*=14.1, 7.3, 5.8 Hz, 1H, $CH_2CH_2CO_2CH_3$), 2.37–2.42 (m, 2H, $CH_2CH_2CO_2CH_3$), 3.21 (dd, *J*=8.3, 5.3 Hz, 1H, N−CH−CO_2CH_3), 3.54 (d, *J*=12.8 Hz, 1H, $NHCH_2Ph$), 3.58 (s, 3H, CO_2CH_3), 3.66 (s, 3H, CO_2CH_3), 3.75 (d, *J*=13.2 Hz, 1H, $NHCH_2Ph$), 7.20–7.35 (m, 5H, aromat. H).

5.1.6. Dimethyl (*S*)-2-{*N*-benzyl-*N*-[2-(benzyloxycarbonylamino)acetyl]amino}pentanedioate (9). A solution of DCC (3.47 g, 16.8 mmol) in THF (60 ml) was added slowly to an ice-cold solution of **8** (3.71 g, 14.0 mmol) and Cbz-glycine (3.52 g, 16.8 mmol) in CH_2Cl_2 (120 ml) and THF (20 ml). The reaction mixture was stirred for 45 min at 0°C and for 16 h at room temperature. Then it was filtered, the filtrate was concentrated in vacuo, the residue was dissolved in CH_2Cl_2 (150 ml) and the organic layer was washed with saturated solutions of $NaHCO_3$ (100 ml), NH_4Cl (100 ml) and NaCl (100 ml). The CH_2Cl_2 layer was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by fc (8 cm, CH_2Cl_2/ethyl acetate=8:2, 40 ml, R_f 0.38). Colorless viscous oil, yield 2.97 g (47%). $C_{24}H_{28}N_2O_7$ (456.5). MS (EI): *m/z* (%)=456 (M, 1), 321 (M−CO_2CH_2Ph, 17) 289 (321−OCH_3, 19), 264 (M−$PhCH_2OCONH$−CH_2CO, 95), 174 (264−CH_2Ph, 100). MS (CI): *m/z* (%)=457 (MH^+, 10), 349 (MH^+−$PhCH_2OH$, 35), 234 (MH^+−$PhCH_2OCONH$−$CH_2CO_2CH_3$, 100), 259 (MH^+−OCH_2Ph−CH_2Ph, 50). IR (film): $\tilde{\nu}$ (cm^{-1})=3255 (w, $\nu_{N-H\ carbamate}$), 3033 (w, $\nu_{CH\ arom.}$), 2949 (w, $\nu_{CH\ aliph.}$), 1732 (s, $\nu_{C=O,\ ester/carbamate}$), 1660 (s, $\nu_{C=O,\ tert.\ amide}$), 1442 (s, $\delta_{CH\ aliph.}$), 1218, 1173 (each m, ν_{COC}), 734, 700 (each m, $\gamma_{monosubst.\ aromate}$). ^1H NMR (300 MHz, $CDCl_3$): δ=2.00–2.40 (m, 4H, $CHCH_2CH_2CO_2CH_3$), 3.49^{mr} (s, 3H, CO_2CH_3), 3.67 (s, 6H, 2×CO_2CH_3), 3.69^{mr} (s, 3H, CO_2CH_3), 4.01–4.23 (m, 2H, $NHCH_2CO$), 4.40–4.64 (m, 3H, N−CH−CO_2CH_3, NCH_2Ph), 5.14 (s, 2H, $PhCH_2OCONH$), 5.69–5.77 (s broad, 1H, NH−CO_2CH_2Ph), $5.84–5.91^{mr}$ (s broad, 1H, NH−CO_2CH_2Ph), 7.23–7.47 (m, 10H, aromat. H). Ratio of rotational isomers 73:27. mr=minor rotamer.

5.1.7. Methyl (*S*)-1-benzyl-5-oxopyrrolidine-2-carboxylate (10).[14] A solution of **8** (244 mg, 0.93 mmol) in CH_3OH (10 ml) was added to a cooled solution of $NaBH_4$ (145 mg, 3.8 mmol) in CH_3OH (10 ml). The reaction mixture was stirred for 22 h at room temperature. Subsequently, 2N HCl (30 drops) was added at 0°C and the mixture was stirred for 30 min at room temperature. The pH was brought to 8–9 with a saturated solution of $NaHCO_3$. After addition of a saturated solution of NaCl (40 ml) the mixture was extracted with CH_2Cl_2 (4×50 ml), the CH_2Cl_2 layer was dried (Na_2SO_4) filtered, and concentrated in vacuo. The residue, containing a mixture of **9** and **10** in a ratio of 29:71, was purified by fc (3 cm, petroleum ether/ethyl acetate=6:4, 10 ml, **10**: R_f 0.56). Colorless oil, yield 104 mg (48%). $C_{13}H_{15}NO_3$ (233.3) MS (EI): *m/z* (%)=233 (M, 15), 174 (M−CO_2CH_3, 100), 146 (174−CO, 23), 142 (M−CH_2Ph, 3). MS (CI): *m/z* (%)=234 (MH^+, 100), 174 (MH^+−HCO_2CH_3, 4), 143 (MH^+−CH_2Ph, 2). IR (film): $\tilde{\nu}$ (cm^{-1})=3032 (w, $\nu_{CH\ arom.}$), 2954 (m, $\nu_{CH\ aliph.}$), 1742 (s, $\nu_{C=O,\ ester}$), 1682 (s, $\nu_{C=O,\ tert.\ amide}$), 1443 (m, $\delta_{CH\ aliph.}$), 1211, 1177 (each m, ν_{COC}), 732, 700 (each m, $\gamma_{monosubst.\ aromat}$). ^1H NMR (300 MHz, $CDCl_3$): δ=2.06 (ddt, *J*=13.2, 9.8, 3.4 Hz, 1H, CH−CH_2CH_2−CO), 2.24 (dq, *J*=13.2, 9.3 Hz, 1H, CH−CH_2CH_2−CO), 2.41 (ddd, *J*=16.8, 9.5, 3.9 Hz, 1H, CH−CH_2CH_2−CO), 2.50–2.63 (m, 1H, CH−CH_2CH_2CO), 3.66 (s, 3H, CO_2CH_3), 3.98 (dd, *J*=9.1, 3.2 Hz, 1H, N−CH−CO_2CH_3), 4.00 (d, *J*=14.6 Hz, 1H, NCH_2Ph), 5.00 (d, *J*=14.6 Hz, 1H, NCH_2Ph), 7.17–7.38 (m, 5H, aromat. H).

5.1.8. Methyl (*S*)-3-(1-benzyl-3,6-dioxopiperazin-2-yl)-propionate (11). *Method A*. Hydrogenation of the azide **13**. A suspension of **13** (1.92 g, 5.52 mmol) and Pd/C-catalyst (10%, 120 mg) in CH_3OH (80 ml) was stirred under an atmosphere of H_2 (balloon, 1013 mbar) for 16 h at room temperature. The mixture was filtered through Celite, the filtrate was concentrated in vacuo and the residue was purified by fc (4 cm, ethyl acetate/acetone=8:2, 20 ml, R_f 0.38). Colorless viscous oil, yield 1.39 g (87%). $C_{15}H_{18}N_2O_4$ (290.3) calcd C 62.06 H 6.25 N 9.65 found C 61.53 H 6.39 N 9.34. MS (EI): *m/z* (%)=290 (M, 45), 258 (M−CH_3OH, 68), 199 (M−CH_2Ph, 24), 167 (199−CH_3OH, 78). MS (CI): *m/z* (%)=291 (MH^+, 100), 259 (MH^+−CH_3OH, 5). $[\alpha]_{589}^{20}$=+31.5 (*c*=0.67, CH_2Cl_2). IR (film): $\tilde{\nu}$ (cm^{-1})=2953 (w, $\nu_{CH\ aliph.}$), 1734 (s, $\nu_{C=O,\ ester}$), 1684 (s, $\nu_{C=O,\ sec.\ amide}$), 1668 (s, $\nu_{C=O,\ tert.\ amide}$), 1453 (m, $\delta_{CH\ aliph.}$), 1209, 1172 (each w, ν_{COC}), 732, 701 (each m, $\gamma_{monosubst.\ aromate}$). ^1H NMR (300 MHz, $CDCl_3$): δ=2.05–2.30 (m, 2H, $CH_2CH_2CO_2CH_3$), 2.43 (dt, *J*=16.8, 6.7 Hz, 1H, $CH_2CH_2CO_2CH_3$), 2.50 (dt, *J*=16.8, 7.6 Hz, 1H, $CH_2CH_2CO_2CH_3$), 3.69 (s, 3H, CO_2CH_3), 3.84 (dd, *J*=8.5, 4.0 Hz, 1H, N−CH−CO), 3.97 (d, *J*=14.6 Hz, 1H, NCH_2Ph), 4.02 (d, *J*=17.5 Hz, 1H, NCH_2CO), 4.14 (d, *J*=17.1 Hz, 1H, NCH_2CO), 5.33 (d, *J*=14.6 Hz, 1H, NCH_2Ph), 6.41 (s broad, 1H, CONH), 7.26–7.40 (m, 5H, aromat. H).

Method B. Hydrogenation of the dipeptide **9**. A mixture of **9** (9.0 mg, 0.02 mmol), Pd/C (10%, 3 mg) and methanol (4 ml) was stirred under an atmosphere of H_2 (balloon, 1013 mbar) for 4 h at room temperature. The work-up was performed as described under A). Colorless viscous oil, yield 4.8 mg (84%).

5.1.9. Dimethyl (*S*)-2-[*N*-benzyl-*N*-(2-chloroacetyl)amino]-pentanedioate (12). A solution of chloroacetyl chloride (4.6 ml, 58.2 mmol) in CH_2Cl_2 (30 ml) was added to a cooled solution of **8** (5.10 g, 19.2 mmol) in CH_2Cl_2 (120 ml). After stirring for 40 min at 0°C a saturated solution of $NaHCO_3$ (100 ml) was added dropwise and subsequently the reaction mixture was stirred for 16 h at room temperature. Then, the aqueous layer was separated, the organic layer was washed with 0.5N NaOH (3×100 ml) and a saturated solution of NaCl (100 ml), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by fc (6 cm, petroleum ether/ethyl acetate=6:4, 40 ml, R_f 0.44). Colorless oil, yield 4.80 g (73%). $C_{16}H_{20}ClNO_5$ (341.8) calcd C 56.2H 5.90 N 4.10 found C 56.2H 5.93 N 4.03. MS (CI): *m/z* (%)=344, 342 (MH^+, 31, 97), 312, 310 (MH^+-CH_3OH, 5, 16), 284, 282 ($MH^+-HCO_2CH_3$, 2, 7,), 234 ($284/282-CH_2Cl$, 100). $[\alpha]_{589}{}^{20}=-30.5$ (c=1.17, $CHCl_3$). IR (film): $\tilde{\nu}$ (cm^{-1})=3031 (w, $\nu_{CH\ arom.}$), 2953 (m, $\nu_{CH\ aliph.}$), 1740, (s, $\nu_{C=O,\ ester}$), 1662 (s, $\nu_{C=O,\ \mathit{tert}.\ amide}$), 1437 (m, $\delta_{CH\ aliph.}$), 1212, 1176 (each m, ν_{COC}), 797 (w, C–Cl), 733, 700 (each m, $\gamma_{monosubst.\ aromate}$). ^1H NMR (400 MHz, d_5-nitrobenzene): δ=2.27–2.62 (m, 4H, $CHCH_2CH_2CO_2CH_3$), 3.57^{mr} (s, 3H, CO_2CH_3), 3.64 (s, 3H, CO_2CH_3), 3.67 (s, 3H, CO_2CH_3), 4.34 (d, J=12.8 Hz, 1H, NCH_2Ph), 4.40 (d, J=13.2 Hz, 1H, NCH_2Ph), 4.53 t, J=6.4 Hz, 1H, N–CH–CO_2CH_3), 4.58^{mr} (d, J=13.2 Hz, 1H, NCH_2Ph), 4.68^{mr} (d, J=12.8 Hz, 1H, NCH_2Ph), 4.77^{mr} (t, J=6.0 Hz, 1H, N–CH–CO_2CH_3), 4.78 (d, J=16.7 Hz, 1H, $COCH_2Cl$), 4.89 (d, J=17.1 Hz, 1H, $COCH_2Cl$), 7.28–7.54 (m, 5H, aromat. H). Ratio of rotational isomers 77:23. mr=minor rotamer. ^1H NMR (400 MHz, d_5-nitrobenzene, 140°C): δ=2.20–2.28 (m, 1H, $CH_2CH_2CO_2CH_3$), 2.42–2.53 (m, 3H, $CH_2CH_2CO_2CH_3$), 3.61 (s, 3H, CO_2CH_3), 3.62 (s, 3H, CO_2CH_3), 4.23 (d, J=12.8 Hz, 1H, NCH_2Ph), 4.29 (d, J=13.2 Hz, 1H, NCH_2Ph), 4.66 (t, J=6.4 Hz, 1H, N–CH–CO_2CH_3), 4.69 (d, J=16.2 Hz, 1H, $COCH_2Cl$), 4.79 (d, J=16.7 Hz, 1H, $COCH_2Cl$), 7.24–7.44 (m, 5H, aromat. H). ^{13}C NMR (100 MHz, d_5-nitrobenzene, 140°C): δ=25.9 (1C, $CHCH_2CH_2CO_2CH_3$), 31.3 (1C, $CHCH_2CH_2CO_2CH_3$), 42.4 (1C, $COCH_2Cl$), 51.2 (1C, NCH_2Ph), 51.5 (1C, OCH_3), 52.1 (1C, OCH_3), 60.2 (1C, N–CH–CO_2CH_3), 128.3 (3C, aromat. CH), 129.3 (2C, aromat. CH), 137.6 (1C, aromat. C), 168.1(1C, C=O), 171.0 (1C, C=O), 173.2 (1C, C=O). ^1H NMR (400 MHz, $CDCl_3$): δ=1.95–2.10 (m, 1H, $CH_2CH_2CO_2CH_3$), 2.12–2.37 (m, 3H, $CH_2CH_2CO_2CH_3$), 3.42^{mr} (s, 3H, CO_2CH_3), 3.56 (s, 3H, CO_2CH_3), 3.57 (s, 3H, CO_2CH_3), 3.96 (d, J=12.8 Hz, 1H, NCH_2Ph), 4.02 (d, J=12.4 Hz, 1H, NCH_2Ph), 4.20^{mr} (d, J=12.8 Hz, 1H, NCH_2Ph), 4.28^{mr} (d, J=12.8 Hz, 1H, NCH_2Ph), 4.34 (t, J=6.6 Hz, 1H, N–CH–CO_2CH_3), 4.45^{mr} (d, J=15.4 Hz, 1H, CO–CH_2–Cl), 4.54 (d, J=17.1 Hz, 1H, CO–CH_2–Cl), 4.64 (d, J=17.1 Hz, 1H, CO–CH_2–Cl), $4.52–4.67^{mr}$ (m, 2H, N–CH–CO_2CH_3, CO–CH_2–Cl), 7.23–7.33 (m, 5H, aromat. H). Ratio of rotational isomers 77:23. mr=minor rotamer.

5.1.10. Dimethyl (*S*)-2-[*N*-(2-azidoacetyl)-*N*-benzylamino]-pentanedioate (13). A solution of **12** (9.34 g, 27.3 mmol) and NaN_3 (8.89 g, 137 mmol) in acetone (250 ml) was heated to reflux for 8 h. The solvent was removed in vacuo, the residue was dissolved in Et_2O (200 ml), the Et_2O layer was washed with a saturated solution of NaCl (150 ml) and water (150 ml), dried (Na_2SO_4) and concentrated in vacuo. Colorless oil, yield 9.42 g (99%), petroleum ether/ethyl acetate 6:4, R_f 0.42. $C_{16}H_{20}N_4O_5$ (348.3). MS (EI): *m/z* (%)=348 (M, 2), 290 ($M-CO_2CH_3$, 6), 262 ($290-N_2$, 44), 233 ($M-2\times CO_2CH_3$, 9). MS (CI): *m/z* (%)=349 (MH^+, 100), 321 (MH^+-N_2, 45), 291 ($MH^+-CO_2CH_3$, 8), 261 ($321-CO_2CH_3$, 3), 202 ($321-2\times CO_2CH_3$, 3). IR (film): $\tilde{\nu}$ (cm^{-1})=3030 (w, $\nu_{CH\ arom.}$), 2954 (w, $\nu_{CH\ aliph.}$), 2109 (s, ν_{N3}), 1739 (s, $\nu_{C=O,\ ester}$), 1664 (s, $\nu_{C=O,\ \mathit{tert}.\ amide}$), 1436 (m, $\delta_{CH\ aliph.}$), 1217, 1178 (each m, ν_{COC}), 736, 700 (each m, $\gamma_{monosubst.\ aromate}$). ^1H NMR (400 MHz, $CDCl_3$): δ=1.93–2.07 (m, 1H, $CH_2CH_2CO_2CH_3$), 2.13–2.26 (m, 1H, $CH_2CH_2CO_2CH_3$), 2.29–2.38 (m, 2H, $CH_2CH_2CO_2CH_3$), 3.39^{mr} (s, 3H, CO_2CH_3), 3.57 (s, 3H, CO_2CH_3), 3.59 (s, 3H, CO_2CH_3), 3.78 (d, J=15.8 Hz, 1H, NCH_2Ph), 3.85 (d, J=15.4 Hz, 1H, NCH_2Ph), 4.07^{mr} (s, 2H, NCH_2Ph), 4.42 (d, J=17.5 Hz, 1H, $COCH_2N_3$), 4.46 (t, J=6.0 Hz, 1H, N–CH–CO_2CH_3), 4.50 (d, J=17.1 Hz, 1H, $COCH_2N_3$), 7.19–7.33 (m, 5H, aromat. H). Ratio of rotational isomers 75:25. mr=minor rotamer.

5.1.11. Methyl (*S*)-3-(1-benzyl-4-methyl-3,6-dioxopiperazin-2-yl)propionate (14a). A mixture of **12** (5.87 g, 17.2 mmol), a solution of methylamine (8 M in ethanol, 2.6 ml, 20.8 mmol), NEt_3 (2.9 ml, 20.9 mmol) and acetonitrile (80 ml) was stirred for 16 h at room temperature. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate (180 ml). The organic layer was washed with 0.5N HCl (2×120 ml), 0.5N NaOH (150 ml) and a saturated solution of NaCl (150 ml), dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was purified by fc (6 cm, ethyl acetate/acetone=8:2, 40 ml, R_f 0.43). Colorless oil, yield 4.87 g (93.3%). $C_{16}H_{20}N_2O_4$ (304.1) HR-MS: calcd 304.1423 found 304.1427 (+1.3 ppm). MS (EI): *m/z* (%)=304 (M, 37), 272 ($M-CH_3OH$, 11), 213 ($M-CH_2Ph$, 10), 181 ($213-CH_3OH$, 28). $[\alpha]_{589}{}^{21}=-5.5$ (c=0.55, CH_2Cl_2). IR (film): $\tilde{\nu}$ (cm^{-1})=3052 (w, $\nu_{CH\ arom.}$), 2954 (w, $\nu_{CH\ aliph.}$), 1732 (m, $\nu_{C=O,\ ester}$), 1658 (s, $\nu_{C=O,\ \mathit{tert}.\ amides}$), 1440 (m, $\delta_{CH\ aliph.}$), 1262, 1175 (each m, ν_{COC}), 733, 701 (each m, $\gamma_{monosubst.\ aromate}$). ^1H NMR (300 MHz, $CDCl_3$): δ=2.02−2.30 (m, 2H, $CH_2CH_2CO_2CH_3$), 2.33–2.51 (m, 2H, $CH_2CH_2CO_2CH_3$), 2.97 (s, 3H, NCH_3), 3.69 (s, 3H, CO_2CH_3), 3.90 (dd, J=8.3, 3.9 Hz, 1H, N–CH–CO), 3.93 (d, J=17.1 Hz, 1H, NCH_2CO), 4.00 (d, J=15.1 Hz, 1H, NCH_2Ph), 4.20 (d, J=17.5 Hz, 1H, NCH_2CO), 5.30 (d, J=15.1 Hz, 1H, NCH_2Ph), 7.25–7.37 (m, 5H, aromat. H).

5.1.12. Methyl (*S*)-3-(1-benzyl-4-butyl-3,6-dioxopiperazin-2-yl)propionate (14b). A mixture of **12** (467 mg, 1.37 mmol), butan-1-amine (0.15 ml, 1.52 mmol), NEt_3 (0.22 ml, 1.59) and acetonitrile (50 ml) was stirred for 16 h at room temperature. The solvent was evaporated in vacuo and the residue was dissolved in ethyl acetate (100 ml). The organic layer was washed with 0.5N HCl (2×60 ml), 0.5N NaOH (60 ml) and a saturated solution of NaCl (60 ml), dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was purified by fc (3 cm, petroleum ether/ethyl acetate=3:7, 10 ml, R_f 0.49). Colorless oil, yield 443 mg (94%). $C_{19}H_{26}N_2O_4$ (346.2) HR-MS: calcd 346.1893 found 346.1889 (−1.1 ppm). MS (EI): *m/z* (%)=346 (M, 36), 314 ($M-CH_3OH$, 6), 259 ($M-CH_2CH_2CO_2CH_3$, 16), 255 ($M-CH_2Ph$, 13), 223 ($255-CH_3OH$, 39), 195 ($255-CO_2CH_3$, 33). $[\alpha]_{589}{}^{21}=-26.3$ (c=0.43, CH_2Cl_2). IR (film): $\tilde{\nu}$ (cm^{-1})=3030 (w, $\nu_{CH\ arom.}$), 2956 (m, $\nu_{CH\ aliph.}$), 2872 (w, ν_{OCH3}), 1736 (s, $\nu_{C=O,\ ester}$), 1665 (s, $\nu_{C=O,\ \mathit{tert}.\ amides}$), 1468, 1453 (each m, $\delta_{CH\ aliph.}$), 1259, 1202, 1173 (each m, ν_{COC}), 729, 701 (each m, $\gamma_{monosubst.\ aromate}$). ^1H NMR (300 MHz, $CDCl_3$): δ=0.91 (t, J=7.3 Hz, 3H, $NCH_2CH_2CH_2CH_3$), 1.20–1.36 (m, 2H, $NCH_2CH_2CH_2CH_3$), 1.51 (‘quint’ broad, J=7.5 Hz, 2H, $NCH_2CH_2CH_2CH_3$), 1.96–2.24 (m, 2H, $CH_2CH_2CO_2CH_3$), 2.29–2.48 (m, 2H, $CH_2CH_2CO_2CH_3$), 3.20 (dt, J=14.1, 7.3 Hz, 1H, $NCH_2CH_2CH_2CH_3$), 3.47 (dt, J=14.1, 7.6 Hz, 1H, $NCH_2CH_2CH_2CH_3$), 3.65 (s, 3H, CO_2CH_3), 3.84 (dd, J=8.8, 4.0 Hz, 1H, N–CH–CO), 3.90 (d, J=17.1 Hz, 1H, NCH_2CO), 4.00 (d, J=14.6 Hz, 1H, NCH_2Ph), 4.15 (d, *J*=17.1 Hz, 1H, $\mathrm{NC}H_2\mathrm{CO}$), 5.25 (d, *J*=14.6 Hz, 1H, $\mathrm{NC}H_2\mathrm{Ph}$), 7.23–7.40 (m, 5H, aromat. H).

5.1.13. Methyl (*S*)-3-[1-benzyl-4-(4-methoxybenzyl)-3,6-dioxopiperazin-2-yl]propionate (14c). 4-Methoxybenzylamine (7.90 ml, 61.1 mmol) and $\mathrm{NEt_3}$ (8.50 ml, 61.3 mmol) were added to a solution of **12** (14.4 g, 42.2 mmol) in acetonitrile (200 ml) and the reaction mixture was stirred for 16 h at room temperature. The solvent was removed in vacuo and the residue was purified by fc (8 cm, petroleum ether/ethyl acetate =3:7, 40 ml, R_f 0.37). Pale yellow oil, yield 16.0 g (92%). $\mathrm{C_{23}H_{26}N_2O_5}$ (410.5) calcd C 67.3 H 6.38 N 6.82 found C 66.7 H 6.98 N 6.66. MS (EI): *m*/*z* (%)=410 (M, 28), 289 ($\mathrm{M-CH_2-Ph-OCH_3}$, 8), 257 ($\mathrm{289-CH_3OH}$, 9), 229 ($\mathrm{289-HCO_2CH_3}$, 5). $[\alpha]_{589}^{25}=-11.4$ (*c*=0.68, $\mathrm{CH_2Cl_2}$). IR (film): $\tilde{\nu}$ (cm^{-1})=2949 (w, $\nu_{\mathrm{CH\ aliph.}}$), 1735 (s, $\nu_{\mathrm{C{=}O,\ ester}}$), 1664 (s, $\nu_{\mathrm{C{=}O,}\ \mathit{tert.}\ \mathrm{amides}}$), 1463 (m, $\delta_{\mathrm{CH\ aliph.}}$), 1247, 1172 (each m, ν_{COC}), 821 (w, $\gamma_{p\text{-}\mathrm{disubst.\ aromate}}$), 736, 699 (each w, $\gamma_{\mathrm{monosubst.\ aromate}}$). ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$): δ=2.01 (dq, *J*=14.5, 7.3 Hz, 1H, $\mathrm{C}H_2\mathrm{CH_2CO_2CH_3}$), 2.19 (dtd, *J*=14.2, 7.2, 3.9 Hz, 1H, $\mathrm{C}H_2\mathrm{CH_2CO_2CH_3}$), 2.33 (dt, *J*=16.6, 6.8 Hz, 1H, $\mathrm{CH_2C}H_2\mathrm{CO_2CH_3}$), 2.42 (dt, *J*=16.6, 7.3 Hz, 1H, $\mathrm{CH_2C}H_2\mathrm{CO_2CH_3}$), 3.65 (s, 3H, $\mathrm{CO_2C}H_3$), 3.75 (s, 3H, Aryl–$\mathrm{OC}H_3$), 3.85–3.98 (m, 4H, $\mathrm{NC}H_2$Aryl, N–C*H*–CO, $\mathrm{NC}H_2\mathrm{Ph}$), 4.32 (d, *J*=14.6 Hz, 1H, $\mathrm{NC}H_2\mathrm{CO}$), 4.62 (d, *J*=14.6 Hz, 1H, $\mathrm{NC}H_2\mathrm{CO}$), 5.23 (d, *J*=14.6 Hz, 1H, $\mathrm{NC}H_2\mathrm{Ph}$), 6.84 (d, *J*=8.9 Hz, 2H, aromat. 3-H, 5-$\mathrm{H_{methoxybenzyl}}$), 7.14 (d, *J*=8.6 Hz, 2H, aromat. 2-H, 6-$\mathrm{H_{methoxybenzyl}}$), 7.24–7.33 (m, 5H, aromat. H).

5.1.14. Methyl (*S*)-3-[1-benzyl-3,6-dioxo-4-(2-phenylethyl)piperazin-2-yl]propionate (14d). 2-Phenylethylamine (1.40 ml, 10.9 mmol) and $\mathrm{NEt_3}$ (1.5 ml, 10.9 mmol) were added to a solution of **12** (2.57 g, 7.52 mmol) in acetonitrile (120 ml). The reaction mixture was stirred for 16 h at room temperature. The solvent was removed in vacuo and the residue was purified by fc (6 cm, petroleum ether/ethyl acetate=3:7, 20 ml, R_f 0.46). Colorless solid, mp 84°C, yield 2.87 g (97%). $\mathrm{C_{23}H_{26}N_2O_4}$ (394.5) calcd C 70.0 H 6.64 N 7.10 found C 69.9 H 6.71 N 6.99. MS (EI): *m*/*z* (%)=394 (M, 40), 362 ($\mathrm{M-CH_3OH}$, 5), 321 ($\mathrm{M-CH_2CO_2CH_3}$, 5), 303 ($\mathrm{M-CH_2Ph}$, 6), 275 ($\mathrm{M-NCH_2CH_2Ph}$, 10). $[\alpha]_{589}^{21}=+5.8$ (*c*=0.63, $\mathrm{CH_2Cl_2}$). IR (KBr): $\tilde{\nu}$ (cm^{-1})=3030 (w, $\nu_{\mathrm{CH\ arom.}}$), 2946 (w, $\nu_{\mathrm{CH\ aliph.}}$), 2856 (w, ν_{OCH3}), 1734 (s, $\nu_{\mathrm{C{=}O,\ ester}}$), 1665 (s, $\nu_{\mathrm{C{=}O,}\ \mathit{tert.}\ \mathrm{amides}}$), 1464 (m, $\delta_{\mathrm{CH\ aliph.}}$), 1325, 1173 (each w, ν_{COC}), 729, 700 (each m, $\gamma_{\mathrm{monosubst.\ aromate}}$). ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$): δ=1.82 (ddt, *J*=13.7, 9.2, 6.8 Hz, 1H, $\mathrm{C}H_2\mathrm{CH_2CO_2CH_3}$), 2.11 (dddd, *J*=14.1, 10.2, 6.9, 3.6 Hz, 1H, $\mathrm{C}H_2\mathrm{CH_2CO_2CH_3}$), 2.20–2.36 (m, 2H, $\mathrm{CH_2C}H_2\mathrm{CO_2CH_3}$), 2.85 (dt, *J*=13.9, 7.0 Hz, 1H, $\mathrm{NCH_2C}H_2\mathrm{Ph}$), 2.93 (dt, *J*=13.9, 6.6 Hz, 1H, $\mathrm{NCH_2C}H_2\mathrm{Ph}$), 3.40 (dt, *J*=13.5, 6.7 Hz, 1H, $\mathrm{NC}H_2\mathrm{CH_2Ph}$), 3.65 (s, 3H, $\mathrm{CO_2C}H_3$), 3.80 (dd, *J*=9.2, 3.7 Hz, 1H, N–C*H*–CO), 3.73 (d, *J*=17.3 Hz, 1H, $\mathrm{NC}H_2\mathrm{CO}$), 3.90 (dt, *J*=13.5, 7.1 Hz, 1H, $\mathrm{NC}H_2\mathrm{CH_2Ph}$), 3.94 (d, *J*=15.1 Hz, 1H, $\mathrm{NC}H_2\mathrm{Ph}$), 3.96 (d, *J*=17.5 Hz, 1H, $\mathrm{NC}H_2\mathrm{CO}$), 5.23 (d, *J*=15.1 Hz, 1H, $\mathrm{NC}H_2\mathrm{Ph}$), 7.18–7.35 (m, 10 H, aromat. H).

5.1.15. Methyl (*S*)-3-(1-benzyl-4-cyclohexyl-3,6-dioxopiperazin-2-yl)propionate (14e). Cyclohexylamine (0.25 ml, 2.19 mmol) and $\mathrm{NEt_3}$ (0.30 ml, 2.16 mmol) were added to a solution of **12** (580 mg, 1.70 mmol) in acetonitrile (70 ml). The reaction mixture was heated to reflux for 48 h. After removal of the solvent in vacuo the residue was purified by fc (3 cm, petroleum ether/ethyl acetate=3:7, 20 ml, R_f 0.65). Colorless solid, mp 81–82°C, yield 499 mg (79%). $\mathrm{C_{21}H_{28}N_2O_4}$ (372.5) calcd C 67.7 H 7.58 N 7.52 found C 67.4 H 7.68 N 7.15. MS (EI): *m*/*z* (%)=372 (M, 16), 340 ($\mathrm{M-CH_3OH}$, 2), 299 ($\mathrm{M-CH_2CO_2CH_3}$, 5), 281 ($\mathrm{M-CH_2Ph}$, 9), 249 ($\mathrm{281-CH_3OH}$, 16), 221 ($\mathrm{281-CO_2CH_3}$, 9). $[\alpha]_{589}^{20}=-6.1$ (*c*=2.11, $\mathrm{CH_2Cl_2}$). IR (film): $\tilde{\nu}$ (cm^{-1})=3028 (w, $\nu_{\mathrm{CH\ arom.}}$), 2930, 2857 (each s, $\nu_{\mathrm{CH\ aliph.}}$), 1734 (s, $\nu_{\mathrm{C{=}O,\ ester}}$), 1669 (s, $\nu_{\mathrm{C{=}O,}\ \mathit{tert.}\ \mathrm{amides}}$), 1451 (s, $\delta_{\mathrm{CH\ aliph.}}$), 1255, 1175 (each m, ν_{COC}), 730, 702 (each m, $\gamma_{\mathrm{monosubst.\ aromate}}$). ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$): δ= 1.00–1.17 (m, 1H, $\mathrm{C}H_{2\ \mathrm{cyclohexyl}}$), 1.21–1.48 (m, 4H, $\mathrm{C}H_{2\ \mathrm{cyclohexyl}}$), 1.59–1.73 (m, 3H, $\mathrm{C}H_{2\ \mathrm{cyclohexyl}}$), 1.76–1.88 (m, 2H, $\mathrm{C}H_{2\ \mathrm{cyclohexyl}}$), 1.97–2.21 (m, 2H, $\mathrm{C}H_2\mathrm{CH_2CO_2CH_3}$), 2.28–2.45 (m, 2H, $\mathrm{CH_2C}H_2\mathrm{CO_2CH_3}$), 3.63 (s, 3H, $\mathrm{CO_2C}H_3$), 3.85 (dd, *J*=8.4, 4.2 Hz, 1H, N–C*H*–CO), 3.93 (s, 2H, $\mathrm{NC}H_2\mathrm{CO}$), 4.01 (d, *J*=14.9 Hz, 1H, $\mathrm{NC}H_2\mathrm{Ph}$), 4.28 (tt, *J*=11.6, 3.7 Hz, 1H, $\mathrm{NCH_{cyclohexyl}}$), 5.21 (d, *J*=14.9 Hz, 1H, $\mathrm{NC}H_2\mathrm{Ph}$), 7.21–7.33 (m, 5H, aromat. H). ^{13}C NMR (75.4 MHz, $\mathrm{CDCl_3}$): δ=25.2, 25.3, 25.4 (each 1C, C-3, C-4, C-$5_{\mathrm{cyclohexyl}}$), 26.4 (1C, $C\mathrm{H_2CH_2CO_2CH_3}$), 29.0, 29.1 (each 1C, C-2, C-$6_{\mathrm{cyclohexyl}}$), 29.6 (1C, $\mathrm{CH_2}C\mathrm{H_2CO_2CH_3}$), 44.5 (1C, $\mathrm{N}C\mathrm{H_2CO}$), 47.0 (1C, $\mathrm{N}C\mathrm{H_2Ph}$), 51.8 (1C, $\mathrm{CO_2}C\mathrm{H_3}$), 52.4 (1C, C-$1_{\mathrm{cyclohexyl}}$), 58.6 (1C, N–*C*H–CO), 127.9 (1C, aromat. CH), 128.2 (2C, aromat. CH), 128.8 (2C, aromat. CH), 135.5 (1C, aromat. C), 164.4 (1C, C=O), 165.0 (1C, C=O), 172.6 (1C, C=O).

5.1.16. Methyl (*S*)-3-(1-benzyl-3,6-dioxo-4-phenylpiperazin-2-yl)propionate (14f). A solution of **12** (191 mg, 0.56 mmol), aniline (0.06 ml, 0.67 mmol), and $\mathrm{NEt_3}$ (1.0 ml, 0.72 mmol) in DMF (25 ml) was heated to reflux (155°C) for 16 h. The reaction mixture was concentrated in vacuo, the residue was dissolved in ethyl acetate (100 ml) and washed with 0.5N HCl (3×0.5 ml), 0.5N NaOH (2×50 ml) and a saturated solution of NaCl (1×50 ml). The organic layer was dried ($\mathrm{Na_2SO_4}$), filtered, concentrated in vacuo and the residue was purified by fc (3 cm, petroleum ether/ethyl acetate=3:7, 20 ml, R_f 0.65). Pale yellow oil, yield 107 mg (52%). $\mathrm{C_{21}H_{22}N_2O_4}$ (366.2) HR-MS: calcd 366.1580 found 366.1580 (±0.0 ppm). MS (EI): *m*/*z* (%)=366 (M, 22), 335 ($\mathrm{M\mp OCH_3}$, 4), 293 ($\mathrm{M-CH_2CO_2CH_3}$, 4), 275 ($\mathrm{M-CH_2Ph}$, 3), 243 ($\mathrm{275-CH_3OH}$, 20), 215 ($\mathrm{275-HCO_2CH_3}$). $[\alpha]_{589}^{21}=-9.9$ (*c*=0.33, $\mathrm{CH_2Cl_2}$). IR (film): $\tilde{\nu}$ (cm^{-1})=3061 (w, $\nu_{\mathrm{CH\ arom.}}$), 2962 (m, $\nu_{\mathrm{CH\ aliph.}}$), 1730 (m, $\nu_{\mathrm{C{=}O,\ ester}}$), 1665 (s, $\nu_{\mathrm{C{=}O,}\ \mathit{tert.}\ \mathrm{amides}}$), 1442 (m, $\delta_{\mathrm{CH\ aliph.}}$), 1099, 1027 (each s, ν_{COC}), 735, 694 (each m, $\delta_{\mathrm{monosubst.\ aromate}}$). ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$): δ=2.10–2.30 (m, 2H, $\mathrm{CHC}H_2\mathrm{CH_2CO_2CH_3}$), 2.50 (t, *J*=6.9 Hz, 2H, $\mathrm{CH_2C}H_2\mathrm{CO_2CH_3}$), 3.64 (s, 3H, $\mathrm{CO_2C}H_3$), 4.04 (dd, *J*=8.8, 4.4 Hz, 1H, N–C*H*–CO), 4.14 (d, *J*=15.1 Hz, 1H, $\mathrm{NC}H_2\mathrm{Ph}$), 4.25 (d, *J*=17.1 Hz, 1H, $\mathrm{NC}H_2\mathrm{CO}$), 4.61 (d, *J*=17.1 Hz, 1H, $\mathrm{NC}H_2\mathrm{CO}$), 5.26 (d, *J*=14.6 Hz, 1H, $\mathrm{NC}H_2\mathrm{Ph}$), 7.05–7.52 (m, 10H, aromat. H).

5.1.17. (*S*)-3-[1-Benzyl-4-(2-phenylethyl)piperazin-2-yl]-propan-1-ol (15). A mixture of **14d** (1.38 g, 3.50 mmol), $\mathrm{LiAlH_4}$ (500 mg, 13.2 mmol) and THF (80 ml) was heated to reflux for 60 h. Then, $\mathrm{Na_2SO_4{\times}10H_2O}$ (5 g) was carefully added at 0°C and the suspension was heated to reflux for 2 h. It was filtered and the filtrate was concentrated in vacuo.

Colorless oil, yield 1.10 g (94%), ethyl acetate/acetone=1:1, *R*$_f$ 0.42. C$_{22}$H$_{30}$N$_2$O (338.2) HR-MS: calcd 338.2358 found 338.2368 (+3.1 ppm). MS (EI): *m/z* (%)=338 (M, 4), 79 (M−CH$_2$CH$_2$CH$_2$OH, 23), 247 (M−CH$_2$Ph, 100), 188 (279−CH$_2$Ph, 10), 141 (247−CH$_2$CH$_2$Ph, 23). MS (ESI): *m/z* (%)=339 (MH$^+$, 100), 321 (MH$^+$−H$_2$O, 9). $[\alpha]_{589}{}^{23}$=+32.6 (*c*=0.66, CH$_2$Cl$_2$). IR (film): $\tilde{\nu}$ (cm^{-1})=3384 (m, ν_{OH}), 3061, 3028 (each w, $\nu_{\mathrm{CH\ arom.}}$), 2941, 2810 (each s, $\nu_{\mathrm{CH\ aliph.}}$), 1452 (s, $\delta_{\mathrm{CH\ aliph.}}$), 1152, 1066 (each m, ν_{CO}), 737, 700 (each m, $\gamma_{\mathrm{monosubst.\ aromate}}$). ^{1}H NMR (300 MHz, CDCl$_3$): δ=1.57–1.94 (m, 4H, C*H*$_2$C*H*$_2$CH$_2$OH), 2.16–2.30 (m, 2H, NC*H*$_2$C*H*$_2$N), 2.30–2.40 (m, 1H, N–CH–C*H*$_2$N), 2.51–2.65 (m, 3H, N–C*H*–CH$_2$N, NC*H*$_2$CH$_2$Ph), 2.68–2.84 (m, 5H, NC*H*$_2$C*H*$_2$N, N–CH–C*H*$_2$N, NCH$_2$C*H*$_2$Ph), 3.20 (d, *J*=12.8 Hz, 1H, NC*H*$_2$Ph), 3.63–3.74 (m, 2H, CH$_2$CH$_2$C*H*$_2$OH), 3.92 (s broad, 1H, CH$_2$O*H*), 4.21 (d, *J*=13.1 Hz, 1H, NC*H*$_2$Ph), 7.16–7.38 (m, 10H, aromat. H). ^{13}C NMR (75.4 MHz, CDCl$_3$): δ=27.8 (1C, *C*H$_2$CH$_2$CH$_2$OH), 28.6 (1C, CH$_2$*C*H$_2$CH$_2$OH), 33.6 (1C, NCH$_2$*C*H$_2$Ph), 50.9, 52.4 (each 1C, N*C*H$_2$*C*H$_2$N), 56.9 (1C, N–CH–*C*H$_2$N), 57.9 (1C, N*C*H$_2$Ph), 59.6 (1C, N–*C*H–CH$_2$N), 60.5 (1C, N*C*H$_2$CH$_2$Ph), 62.9 (1C, CH$_2$CH$_2$*C*H$_2$OH), 126.0 (1C, aromat. CH), 127.1 (1C, aromat. CH), 128.3 (4C, aromat. CH), 128.7 (2C, aromat. CH), 129.3 (2C, aromat. CH), 138.0 (1C, aromat. C), 140.3 (1C, aromat. C).

5.1.18. (*S*)-3-(1-Benzylpiperazin-2-yl)propan-1-ol (16). A mixture of **11** (646 mg, 2.22 mmol), a tablet of LiAlH$_4$ (0.98 g, 25.8 mmol) and THF (150 ml) was stirred for 1 h at room temperature and for 16 h at 66°C. Then, 3N NaOH (4 ml), H$_2$O (1 ml) and 3N NaOH (1 ml) were carefully added and the mixture was heated to reflux for 30 min. The mixture was dried (Na$_2$SO$_4$), filtered and concentrated in vacuo. Colorless oil, yield 502 mg (97%), CH$_2$Cl$_2$/ethyl acetate=8:2, *R*$_f$ 0.16. C$_{14}$H$_{22}$N$_2$O (234.3) MS (CI): *m/z* (%)=235 (MH$^+$, 100), 217 (MH$^+$−H$_2$O, 6), 175 (MH$^+$−CH$_3$CH$_2$CH$_2$OH, 15). $[\alpha]_{589}{}^{21}$=+33.0 (*c*=1.74, CH$_2$Cl$_2$). IR (film): $\tilde{\nu}$ (cm^{-1})=3379 (s, $\nu_{OH/NH}$), 3031 (w, $\nu_{\mathrm{CH\ arom.}}$), 2948 (m, $\nu_{\mathrm{CH\ aliph.}}$), 1450 (m, $\delta_{\mathrm{CH\ aliph.}}$), 1120, 1063 (each w, ν_{CO}), 734, 700 (each m, $\gamma_{\mathrm{monosubst.\ aromate}}$). ^{1}H NMR (300 MHz, CDCl$_3$): δ=1.50–1.65 (m, 1H, C*H*$_2$CH$_2$CH$_2$OH), 1.66–1.90 (m, 3H, C*H*$_2$C*H*$_2$CH$_2$OH), 1.95–2.05 (m, 1H, NC*H*$_2$), 2.25–2.40 (m, 1H, N–C*H*–CH$_2$N), 2.60–2.94 (m, 7H, NC*H*$_2$ (5H), N*H*, O*H*), 3.17 (d, *J*=12.7 Hz, 1H, NC*H*$_2$Ph), 3.66 (t, *J*=5.9 Hz, 2H, C*H*$_2$OH), 4.18 (d, *J*=13.1 Hz, 1H, NC*H*$_2$Ph), 7.22–7.40 (m, 5H, aromat. H). ^{13}C NMR (75.4 MHz, CDCl$_3$): δ=26.8 (1C, *C*H$_2$CH$_2$CH$_2$OH), 28.5 (1C, CH$_2$*C*H$_2$CH$_2$OH), 45.4 (1C, N*C*H$_2$), 49.6 (1C, N*C*H$_2$), 51.8 (1C, N*C*H$_2$), 58.1 (1C, N*C*H$_2$Ph), 60.6 (1C, N–*C*H–CH$_2$N), 62.7 (1C, *C*H$_2$OH), 127.0 (1C, aromat. CH), 128.3 (2C, aromat. CH), 129.2 (2C, aromat. CH), 138.0 (1C, aromat. C).

5.1.19. *tert*-Butyl (*S*)-4-benzyl-3-(3-hydroxypropyl)-piperazine-1-carboxylate (17). As described for the synthesis of **16**, compound **11** (287 mg, 0.99 mmol) was reduced with LiAlH$_4$ (400 mg, 10.5 mmol) in THF (60 ml). After hydrolysis with 3N NaOH (2.0 ml) and water (0.4 ml) the resulting mixture was cooled (0°C) and di-*tert*-butyl dicarbonaterf (Boc)$_2$O, 0.7 ml, 3.27 mmol) was added. The suspension was stirred for 16 h at room temperature, then it was dried (Na$_2$SO$_4$), filtered and concentrated in vacuo. The residue was purified by fc (3 cm, ethyl acetate, 20 ml, *R*$_f$ 0.45). Pale yellow oil, yield 194 mg (59%, from **11**). C$_{19}$H$_{30}$N$_2$O$_3$ (334.2) HR-MS: calcd 334.2256 found 334.2255 (−0.4 ppm). MS (EI): *m/z* (%)=334 (M, 4), 275 (M−CH$_2$CH$_2$CH$_2$OH, 24), 219 (275−C(CH$_3$)$_3$ and M−NCO-OC(CH$_3$)$_3$, 100). $[\alpha]_{589}{}^{21}$=+6.7 (*c*=0.58, CH$_2$Cl$_2$). IR (film): $\tilde{\nu}$ (cm^{-1})=3442 (m, ν_{OH}), 2931 (m, $\nu_{\mathrm{CH\ aliph.}}$), 1678 (s, $\nu_{\mathrm{C{=}O,\ carbamate}}$), 1430 (s, $\delta_{\mathrm{CH\ aliph.}}$), 1276, 1169 (each m, ν_{COC}), 733, 700 (each m, $\gamma_{\mathrm{monosubst.\ aromate}}$). ^{1}H NMR (300 MHz, CDCl$_3$): δ=1.43 (s broad, 9H, C(C*H*$_3$)$_3$), 1.58–1.78 (m, 5H, C*H*$_2$C*H*$_2$CH$_2$O*H*), 2.12–2.24 (m, 1H, NCH$_2$C*H*$_2$NCH$_2$Ph), 2.46 (‘quint’, *J*=3.4 Hz, 1H, N–C*H*–CH$_2$N), 2.68 (ddd, *J*=13.0, 6.5, 3.2 Hz, 1H, NCH$_2$C*H*$_2$NCH$_2$Ph), 3.18–3.60 (m, 4H, C*H*$_2$–NBOC–C*H*$_2$), 3.32 (d, *J*=13.2 Hz, 1H, NC*H*$_2$Ph), 3.60–3.68 (m, 2H, C*H*$_2$OH), 3.99 (d, *J*=13.2 Hz, 1H, NC*H*$_2$Ph), 7.20–7.45 (m, 5H, aromat. H).

5.1.20. (*S*)-1-Benzyl-6-(3-hydroxypropyl)-4-methylpiperazine-2,5-dione (18) and (*S*)-1-Benzyl-6-(3-hydroxypropyl)-4-methylpiperazin-2-one (19). A solution of LiBH$_4$ (2 M in THF, 0.15 ml, 0.3 mmol), diluted with Et$_2$O (0.85 ml), was added slowly to a cooled (−5°C) solution of **14a** (120 mg, 0.39 mmol) in Et$_2$O (15 ml). The reaction mixture was stirred for 5 h at −5°C, for 3 h at 0°C and for 1.5 h at room temperature. Then, ethyl acetate (5.0 ml) and water (1.0 ml) were carefully added, the mixture was dried (Na$_2$SO$_4$), filtered and concentrated in vacuo. The residue was purified by fc (2 cm, ethyl acetate/acetone=1:1, 10 ml).

18 (*R*$_f$ 0.41): Colorless oil, yield 21.3 mg (38%). C$_{15}$H$_{20}$N$_2$O$_3$ (276.3) MS (EI): *m/z* (%)=276 (M, 100), 58 (M−H$_2$O, 2), 231 (M−CH$_2$CH$_2$OH, 13), 217 (M−CH$_2$CH$_2$CH$_2$OH, 16), 185 (M−CH$_2$Ph, 50). $[\alpha]_{589}{}^{25}$=+3.5 (*c*=0.44, CH$_2$Cl$_2$). IR (film): $\tilde{\nu}$ (cm^{-1})=3418 (s, ν_{OH}), 3031 (w, $\nu_{\mathrm{CH\ arom.}}$), 2934 (m, $\nu_{\mathrm{CH\ aliph.}}$), 1654 (s, $\nu_{\mathrm{C{=}O,}\ \mathit{tert}\mathrm{.\ amides}}$), 1474, 1453 (each m, $\delta_{\mathrm{CH\ aliph.}}$), 1337 (m, δ_{OH}), 1183, 1060 (each m, ν_{COC}), 732, 700 (each m, $\gamma_{\mathrm{monosubst.\ aromate}}$). ^{1}H NMR (300 MHz, CDCl$_3$): δ=1.40–1.61 (m, 2H, CH$_2$C*H*$_2$CH$_2$OH), 1.79–2.02 (m, 2H, C*H*$_2$CH$_2$CH$_2$OH), 2.10–2.30 (m, 1H, CH$_2$O*H*), 2.95 (s, 3H, NC*H*$_3$), 3.57 (t, *J*=6.1 Hz, 2H, CH$_2$CH$_2$C*H*$_2$OH), 3.91 (d, *J*=17.5 Hz, 1H, NC*H*$_2$CO), 3.95 (dd, *J*=6.9, 4.1 Hz, 1H, N–C*H*–CO), 4.02 (d, *J*=15.1 Hz, 1H, NC*H*$_2$Ph), 4.15 (d, *J*=17.1 Hz, 1H, NC*H*$_2$CO), 5.23 (d, *J*=15.1 Hz, 1H, NC*H*$_2$Ph), 7.22–7.35 (m, 5H, aromat. H).

19 (*R*$_f$ 0.30): Colorless oil, yield 3.9 mg (7.3%). C$_{15}$H$_{22}$N$_2$O$_2$ (262.3). MS (EI): *m/z* (%)=262 (M, 66), 217 (M−CH$_2$CH$_2$OH, 6), 203 (M−CH$_2$CH$_2$CH$_2$OH, 25), 171 (M−CH$_2$Ph, 74). $[\alpha]_{589}{}^{250}$=−34.7 (*c*=0.75, CH$_2$Cl$_2$). IR (film): $\tilde{\nu}$ (cm^{-1})=3420 (s, ν_{OH}), 3030 (w, $\nu_{\mathrm{CH\ arom.}}$), 2945 (m, $\nu_{\mathrm{CH\ aliph.}}$), 2792 (w, ν_{NCH3}), 1636 (s, $\nu_{\mathrm{C{=}O,}\ \mathit{tert}\mathrm{.\ amide}}$), 1456 (m, $\delta_{\mathrm{CH\ aliph.}}$), 1341 (m, δ_{OH}), 157, 1050 (each m, ν_{COC}), 733, 700 (each m, $\gamma_{\mathrm{monosubst.\ aromate}}$). ^{1}H NMR (300 MHz, CDCl$_3$): δ=1.37–1.58 (m, 2H, CH$_2$C*H*$_2$CH$_2$OH), 1.64–1.89 (m, 2H, C*H*$_2$CH$_2$CH$_2$OH), 2.07–2.24 (m, 1H, CH$_2$O*H*), 2.25 (s, 3H, NC*H*$_3$), 2.38 (dd, *J*=11.7, 3.8 Hz, 1H, N–CH–C*H*$_2$N), 2.63 (dd, *J*=11.7, 3.3 Hz, 1H, N–CH–C*H*$_2$N), 2.93 (d, *J*=16.8 Hz, 1H, NC*H*$_2$CO) 3.18 (ddd, *J*=11.0, 7.2, 3.6 Hz, 1H, N–C*H*–CH$_2$N), 3.36 (d, *J*=16.8 Hz, 1H, NC*H*$_2$CO), 3.56 (t, *J*=6.4 Hz, 2H, CH$_2$CH$_2$C*H*$_2$OH), 3.92

(d, J=15.2 Hz, 1H, NCH_2Ph), 5.34 (d, J=14.9 Hz, 1H, NCH_2Ph), 7.16–7.34 (m, 5H, aromat. H).

5.2. Receptor binding studies, general

General. Teflon-glass-homogenizer: Potter®S (B. Braun Biotech International). Rotor/stator homogenizer: Ultra-turrax® T25 basic (Ika Labortechnik). Centrifuge: High speed refrigerating centrifuge model J2-HS (Beckman). Filter: Whatman glass fiber filters GF/B, presoaked in 0.5% polyethylenimine in water for 2 h at 4°C before use. Filtration was performed with a Brandel 24-well cell harvester. Scintillation cocktail: Rotiszint eco plus (Roth). Liquid scintillation analyzer: Tri-Carb 2100 TR (Canberra Packard), counting efficiency 66%. All experiments were carried out in triplicate. IC_{50}-values were determined from competition experiments with at least six concentrations of test compounds and were calculated with the curve-fitting program GraphPad Prism® 3.0 (GraphPad Software) by nonlinear regression analysis. K_i-values were calculated according to Cheng and Prusoff.[25] K_D-values for the radioligands were taken from the literature. For compounds with high affinity (low K_i-values) mean values±SEM from at least three independent experiments are given.

5.2.1. Investigation of the σ_1-receptor-affinity. [^{3}H]-(+)-Pentazocine binding to guinea pig brain membrane preparations was performed according to the procedure described in Ref. 23.

Membrane preparation. Thawed guinea pig brains (Dunkin Hartley, Harlan-Sera-Lab) were homogenized with an ultra-turrax (8000 rpm) in 10 volumes of cold 0.32 M sucrose. The homogenate was centrifuged at 1000*g* for 10 min at 4°C. The supernatant was separated and centrifuged at 22,000*g* for 20 min at 4°C. The pellet was resuspended in 10 volumes of buffer (50 mM Tris HCl, pH 7.4) with an ultraturrax (8000 rpm), incubated for 30 min at 25°C and centrifuged at 22,000*g* (20 min, 4°C). The pellet was resuspended in buffer, the protein concentration was determined according to the method of Bradford[26] using bovine serum albumin as standard, and subsequently the preparation was frozen (−83°C) in 5 ml portions of about 2 mg protein/ml.

σ_1-Receptor binding assay. The test was performed with the radioligand [ring-1,3-^{3}H]-(+)-pentazocine (1036 GBq/mmol; NEN™ Life Science Products). The thawed membrane preparation (about 150 μg of the protein) was incubated with various concentrations of test compounds, 3 nM [^{3}H]-(+)-pentazocine and buffer (50 mM Tris HCl, pH 7.4) in a total volume of 500 μl for 150 min at 37°C. The incubation was terminated by rapid filtration through presoaked Whatman GF/B filters using a cell harvester. After washing four times with 2 ml of ice-cold buffer 3 ml of scintillation cocktail were added to the filters. After at least 8 h bound radioactivity trapped on the filters was counted in a liquid scintillation analyzer. Nonspecific binding was determined with 10 μM haloperidol.

5.2.2. Investigation of the σ_2-receptor-affinity. σ_2-Receptor-affinity was determined using rat liver membranes with [^{3}H]-ditolylguanidine in the presence of 100 nM (+)-pentazocine to mask σ_1-binding sites. The assay was performed according to the procedure described in Ref. 23.

Membrane preparation. One frozen rat liver (Sprague Dawley, Harlan-Sera-Lab) was allowed to thaw slowly on ice. Then it was homogenized with a potter (800 rpm) in 10 volumes of cold buffer (10 mM Tris HCl/0.32 M sucrose, pH 7,4). The homogenate was centrifuged at 1000 *g* for 10 min at 4°C. The supernatant was separated and saved on ice. The pellet was resuspended in 30 ml of cold buffer and centrifuged again. Both supernatants were then centrifuged at 31,000*g* for 20 min at 4°C. The pellet was resuspended in 30 ml of buffer (10 mM Tris HCl, pH 7.4) by vortexing and gentle potter homogenization. Then it was incubated for 15 min at 25°C and centrifuged at 31,000*g* (20 min, 4°C). The pellet was resuspended in buffer, the protein concentration was determined according to the method of Bradford[26] using bovine serum albumin as standard, and subsequently the preparation was frozen (−83°C) in 5 ml portions of about 2.5 mg protein/ml.

σ_2-Receptor binding assay. The membrane preparation (about 60 μg protein) was incubated with 3 nM [^{3}H]-ditolylguanidine (Di-[*p*-ring-^{3}H]-1,3-di-*o*-tolylguanidine, 2220 GBq/mmol; American Radiolabeled Chemicals Inc.) and different concentrations of test compounds in buffer (50 mM Tris HCl, pH 8.0) in the presence of 100 nM (+)-pentazocine. The total volume was 250 μl. The incubation (120 min, 25°C) was stopped by addition of 2 ml of ice-cold buffer (10 mM Tris HCl, pH 8.0) followed by rapid filtration through presoaked Whatman GF/B filters using a cell harvester. After washing three times with 2 ml of ice-cold buffer 3 ml of scintillation cocktail were added to the filters. After at least 8 h bound radioactivity trapped on the filters was counted in a liquid scintillation analyzer. Nonspecific binding was determined with 10 μM non-radiolabeled ditolylguanidine.

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