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CONCISE ARTICLE

# Homologous piperazine-alcanols: chiral pool synthesis and pharmacological evaluation

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Starting with the proteinogenic amino acids (*S*)-aspartate and (*S*)-glutamate the homologous piperazine-alcanols **3** and **4** were prepared in a five step synthesis. The diversity was introduced by N-1 alkylation of the piperazinediones **5** and **6** with various alkyl halides. Subsequent $LiAlH_4$ reduction of the dioxopiperazine-esters **7** and **8** provided the alcohols **3** and **4**. The ethanol derivatives **3** show similar $\sigma_1$ affinity as the methanol derivatives **2**, but increased selectivity over the $\sigma_2$ subtype. The corresponding propanol derivatives **4** are considerably less potent. A benzyl or dimethylallyl residue at N-1 appears to be optimal for high $\sigma_1$ affinity.



## 1. Introduction

The $\sigma_1$ receptor represents a promising target for the development of innovative therapies of various neurological and neuropsychiatric diseases. It has been shown that $\sigma_1$ receptor agonists can be used as antidepressants and antiamnesic drugs. The neuroprotective activity of $\sigma_1$ agonists permits the treatment of memory disorders (*e.g.* Alzheimer's disease, Parkinson's disease) with $\sigma_1$ agonists. In animal studies $\sigma_1$ receptor antagonists are able to reduce the unpleasant and dangerous effects after withdrawal of cocaine, amphetamine or ethanol from addicted animals. The development of $\sigma_1$ antagonists as innovative analgesics with reduced side effects is driven by their potentiation of opioid induced antinociception.[1–4] Recently the potential of $\sigma_1$ antagonists for the treatment of neuropathic pain has been demonstrated.[5,6] In addition to their CNS effects, $\sigma_1$ antagonists are investigated as novel antitumor drugs, since some human tumor cell lines express a high amount of $\sigma_1$ and $\sigma_2$ receptors.[7,8]

Since an X-ray crystal structure of the $\sigma_1$ receptor protein is not yet available, lead compounds and pharmacophore models[9,10] of $\sigma_1$ ligands are used for the design of novel ligands. The $\sigma_1$ pharmacophore models developed by Glennon,[11–13] Langer[14] and Pricl[15] for different classes of compounds show similar features including a basic amino moiety surrounded by 2–3 hydrophobic regions. The number and distances of the particular structural elements are defined, respectively.

The piperazine ring represents a privileged structural element for the development of potent $\sigma_1$ ligands, since both N-atoms can be used for the formation of ionic interactions depending on the *N*-substituents.[16–18] High $\sigma_1$ affinities were reported for 1-butyl-4-(2-phenylethyl)piperazine[19] and 1,4-dibenzylpiperazine derivatives.[20] The bicyclic compound **1a** ($R^1$ = benzyl) represents a potent $\sigma_1$ ligand ($K_i$ = 6.5 nM) with restricted conformational flexibility of the piperazine ring (Fig. 1). The stereochemistry and substitution patterns (substituent $R^1$) are responsible for the high $\sigma_1$ affinity.[21] Disconnection of the C-2/C-3-bond of **1** leads to 2-hydroxymethyl substituted piperazines **2**. The *p*-methoxybenzyl (PMB) derivative **2a** (R = PMB) interacts with high affinity with $\sigma_1$ receptors ($K_i$ = 12 nM).[22]

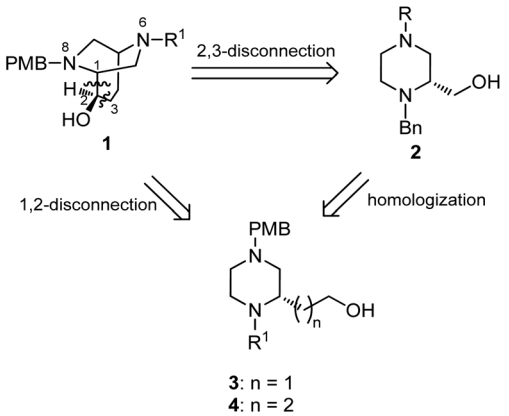


**Fig. 1** Design of novel $\sigma_1$ ligands based on monocyclic and bicyclic piperazine scaffolds.

Herein we report the synthesis and pharmacological evaluation of piperazine-alcanols **3** and **4**. On the one hand the ethanols **3** ($n$ = 1) and propanols **4** ($n$ = 2) represent homologues of the methanols **2**. On the other hand they result from disconnection of the potent bicyclic compound **1** between C-1 and C-2 (Fig. 1).

## 2. Synthesis

For the synthesis of the piperazine-alcanols **3** and **4** enantiomerically pure amino acids of nature's chiral pool were used.

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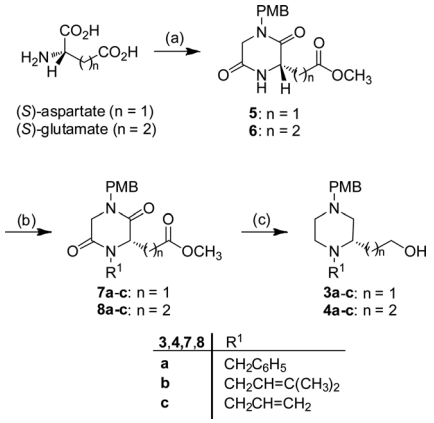



**Scheme 1** *Reagents and conditions*: (a) 1. $H_3COH$, $(H_3C)_3SiCl$, rt, 16 h; 2. $ClCH_2COCl$, $CH_2Cl_2$, $NaHCO_3$, rt, 16 h; 3. *p*-Methoxybenzylamine (PMB–$NH_2$), $H_3CCN$, $NEt_3$, $Bu_4NI$, reflux, 48 h. (b) NaHMDS, THF, $Bu_4NI$, −78 °C, 40 min, R–Br, −78 °C, 1 h. (c) $LiAlH_4$, THF, reflux, 16 h. PMB = *p*-methoxybenzyl.

(*S*)-Aspartate served as a starting compound for the preparation of ethanol derivatives **3** (Scheme 1). The piperazinedione **5** with an acetic acid ester side chain was prepared in a three step procedure comprising esterification of aspartate, chloroacetylation and cyclization with *p*-methoxybenzylamine.[23] Deprotonation of **5** with NaHMDS and subsequent alkylation with different alkyl bromides provided the piperazinediones **7a–c** with various substituents at the N-atom. Finally $LiAlH_4$ reduction of **7a–c** led to the piperazine-ethanols **3a–c**.

The piperazine-propanol derivatives **4** were prepared in a similar manner starting with (*S*)-glutamate (Scheme 1). Esterification, chloroacetylation and reaction with *p*-methoxybenzylamine yielded the piperazinedione **6**.[24] After *N*-alkylation the resulting piperazinediones **8a–c** were reduced with $LiAlH_4$ to afford the piperazine-propanol derivatives **4a–c**.

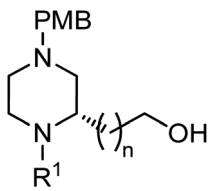
In both series of compounds the *p*-methoxybenzyl moiety was attached to the N-atom in 4-position of the piperazine ring, since this residue is the preferred substituent of the lead compounds **1** and **2**. The benzyl moiety at 1-position of **3a** and **4a** corresponds to the benzyl group at 6-position of **1a** and 1-position of **2**. Additionally the dimethylallyl residue, which is often found in $\sigma_1$ ligands, was introduced (**3b** and **4b**). According to our experience with bicyclic $\sigma_1$ ligands **1**, it was expected that the allyl group is too small to produce potent $\sigma_1$ ligands. Nevertheless, the allyl derivatives **3c** and **4c** were prepared in order to analyze the minimal size required for substituents at 1-position.

## 3. Receptor affinity

Competition experiments with radioligands were used to determine the $\sigma_1$ and $\sigma_2$ receptor affinities of the piperazine-alcanol derivatives **3** and **4**. In the $\sigma_1$ assay the test compounds competed with the radioligand [$^3$H]-(+)-pentazocine for the $\sigma_1$ receptors of a standardized preparation from guinea pig brains. Homogenates of rat liver served as the source of $\sigma_2$ receptors in the $\sigma_2$ assay. Since the radioligand [$^3$H]-1,3-di(*o*-tolyl)guanidine interacts with $\sigma_1$ and $\sigma_2$ receptors, an excess of non-tritiated (+)-pentazocine was added to mask the $\sigma_1$ receptors in the $\sigma_2$ assay.[25–27]

The σ receptor affinities of the homologous piperazine-ethanol and -propanol derivatives **3** and **4** are depicted in Table 1 and compared with the affinity data of the smaller homologue **2a** and the conformationally restricted bicyclic compound **1a**. Extension of the side chain from methanol (**2a**) to ethanol (**3a**) led to a slight reduction of $\sigma_1$ receptor affinity, but extension from ethanol (**3a**) to propanol (**4a**) led to significant reduction of $\sigma_1$ receptor

**Table 1** $\sigma_1$ and $\sigma_2$ receptor affinities of piperazine-alcanol derivatives



**2-4**

| Compound | $R^1$ | *n* | $K_i \pm$ SEM [nM] ($n = 3$)[a] $\sigma_1$ | $\sigma_2$ | $\sigma_1/\sigma_2$ selectivity |
|---|---|---|---|---|---|
| **1a** (ref. 21) | $CH_2C_6H_5$ | — | 6.5 ± 0.67 | 806 | 124 |
| **2a** (ref. 22) | $CH_2C_6H_5$ | 0 | 12 ± 1.4 | 70 ± 10 | 6 |
| **3a** | $CH_2C_6H_5$ | 1 | 20 ± 6.0 | >1000 | >50 |
| **3b** | $CH_2CH{=}C(CH_3)_2$ | 1 | 18 ± 8.0 | >1000 | >55 |
| **3c** | $CH_2CH{=}CH_2$ | 1 | 1080 | >5000 | >5 |
| **4a** | $CH_2C_6H_5$ | 2 | 188 ± 61 | >5000 | >26 |
| **4b** | $CH_2CH{=}C(CH_3)_2$ | 2 | 300 ± 21 | >5000 | >16 |
| **4c** | $CH_2CH{=}CH_2$ | 2 | 1870 | >2000 | ~1 |
| Haloperidol | | | 3.9 ± 1.5 | 78 ± 2.0 | 20 |
| Di-*o*-tolylguanidine | | | 61 ± 18 | 42 ± 15 | 0.7 |
| (+)-Pentazocine | | | 4.2 ± 1.1 | — | — |

[a] Triplicates were recorded only for high affinity compounds. The affinity of compounds, which did not reduce significantly the radioligand binding in the first assay, was recorded only once.

affinity. Piperazines **3b** and **4b** with the dimethylallyl moiety at N-1 show similar $\sigma_1$ receptor affinities as the corresponding benzyl derivatives **3a** and **4a**, respectively. However, the allyl moiety (**3c** and **4c**) appears to be too small to produce strong lipophilic interactions during binding at the $\sigma_1$ receptor protein. Generally the propanol derivatives **4** show considerably lower $\sigma_1$ affinities than the ethanol derivatives **3**.

The $\sigma_2$ affinity of the piperazine-ethanol and -propanol derivatives **3** and **4** is generally very low ($K_i > 1$ μM), which leads to high selectivity for the $\sigma_1$ receptor over the $\sigma_2$ receptor. The $\sigma_1/\sigma_2$ selectivity of the ethanol derivatives **3a** (>50-fold) and **3b** (>55-fold) is about 10-fold higher than the moderate $\sigma_1/\sigma_2$ selectivity of the methanol derivative **2a**.

## 4. Conclusion

The homologous piperazine-alcanols **3** and **4** were prepared by a five step synthesis starting with enantiomerically pure amino acids (*S*)-aspartate and (*S*)-glutamate. Homologization of the methanol side chain (**2a**) to the ethanol side chain (**3a**) retains $\sigma_1$ affinity but increases $\sigma_1/\sigma_2$ selectivity. A further extension to a propanol side chain (**4**) led to reduced $\sigma_1$ affinity indicating the ethanol side chain to be optimal for interaction with the $\sigma_1$ receptor protein. The substituent at N-1 should have a considerable size (*e.g.* benzyl, dimethylallyl), since the allyl derivative **3c** shows only very low $\sigma_1$ affinity. This observation is in good accordance with the pharmacophore models mentioned in the Introduction, which postulate at least two hydrophobic pockets at the $\sigma_1$ receptor protein accepting sterically demanding substituents.

## 5. Experimental, chemistry

### 5.1. General

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. THF was dried with sodium/benzophenone and was freshly distilled before use. Thin layer chromatography (TLC): Silica gel 60 F254 plates (Merck). Flash chromatography (FC): Silica gel 60, 40–64 μm (Merck); parentheses include: diameter of the column, eluent, fraction size, $R_f$ value. Melting point: Melting point apparatus SMP 3 (Stuart Scientific), uncorrected. Optical rotation: Polarimeter 341 (PerkinElmer); 1.0 dm tube; concentration *c* [g per 100 mL], the unit of $[\alpha]_D^{20}$ [grad mL $dm^{-1}$ $g^{-1}$]. MS: MAT GCQ (Thermo-Finnigan); IR: IR spectrophotometer 480Plus FT-ATR-IR (Jasco). $^1$H NMR (400 MHz), $^{13}$C NMR (100 MHz): Unity Mercury Plus 400 spectrometer (Varian); $\delta$ in ppm related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution.

HPLC: method 1: Merck Hitachi Equipment; UV detector: L-7400; autosampler: L-7200; pump: L-7100; degasser: L-7614; column: LiChrospher® 60 RP-select B (5 μm); LiCroCART® 250-4 mm cartridge; flow rate: 1.000 mL $min^{-1}$; injection volume: 5.0 μL; detection at $\lambda = 210$ nm; solvents: A: water with 0.05% (v/v) trifluoroacetic acid; B: acetonitrile with 0.05% (v/v) trifluoroacetic acid; gradient elution: 0.0 min: 90.0% of A, 10.0% of B; 4.0 min: 90.0% of A, 10.0% of B; 29.0 min: 0.0% of A, 100.0% of B; 31.0 min: 0.0% of A, 100.0% of B; 31.5 min: 90.0% of A, 10.0% of B; 40.0 min: 90.0% of A, 10.0% of B. Method 2: equipment: pump: HPLC pump 64 (Knauer); UV-Detector: Variable Wavelength Monitor (Knauer); data acquisition: D-2500 Chromato-Integrator (Merck Hitachi); injection volume: 20.0 μL; stop time: 2 × $t_R$; (a) column: LiChroCART® 250-4 with Superspher® 100 RP-18; flow rate: 0.6 mL $min^{-1}$; detection: wavelength: 235 nm; solvent: methanol–water = 65 : 35 + 0.1% triethylamine; (b) column: LiChroCART® 250-4 with Superspher® 100 RP-18; flow rate: 0.6 mL $min^{-1}$; detection: wavelength: 235 nm; solvent: methanol–water = 50 : 50 + 0.1% triethylamine; (c) column: Hibar® RT 250-4 with LiChrospher® 100 RP-18 (5 μm); flow rate: 1.0 mL $min^{-1}$; detection: wavelength: 254 nm; solvent: acetonitrile–water = 40 : 60 + 0.1% triethylamine; (d) column: Hibar® RT 250-4 with LiChrospher® 100 RP-18 (5 μm); flow rate: 1.0 mL $min^{-1}$; detection: wavelength: 254 nm; solvent: acetonitrile–water = 25 : 75 + 0.1% triethylamine.

### 5.2. General procedures

**5.2.1. General procedure A – alkylation of piperazinediones 5 and 6.** Under $N_2$ atmosphere a solution of piperazinedione **5** or **6** (1 eq.) and tetrabutylammonium iodide (0.2 eq.) in THF was cooled to −78 °C. Then a 2.0 M solution of sodium hexamethyldisilazane in THF (1.1 eq.) was added dropwise. After stirring the mixture at −78 °C for 40 min, the alkyl bromide (5 eq.) was added. The mixture was stirred at −78 °C for 1 h and then allowed to warm to room temperature. After stirring the mixture at ambient temperature for 2 h, water was added and the mixture extracted with $CH_2Cl_2$ (3×). The combined organic layers were dried ($Na_2SO_4$), filtered, and the solvent was removed *in vacuo*. The residue was purified by FC.

**5.2.2. General procedure B – $LiAlH_4$ reduction of piperazinediones 7 and 8.** Under $N_2$ atmosphere $LiAlH_4$ (6 eq.) was added to an ice-cooled solution of piperazinedione **7** or **8** (1 eq.) in THF. The mixture was stirred at 0 °C for 10 min and then heated to reflux for 16 h. Then water was added under ice-cooling until $H_2$ formation was stopped. The mixture was stirred at 0 °C for 10 min and then heated to reflux for 30 min. After cooling to rt, the mixture was filtered, the solvent removed *in vacuo* and the residue purified by FC.

### 5.3. Synthesis of the compounds

**5.3.1. (+)-(*S*)-2-[1-Benzyl-4-(4-methoxybenzyl)piperazin-2-yl] ethanol (3a).** According to general procedure B piperazinedione **7a** (163 mg, 0.41 mmol) was reacted with $LiAlH_4$ (94 mg, 2.47 mmol) in THF (50 mL) and the product was purified by FC (2 cm, $h$ = 15 cm, $CH_2Cl_2$–methanol = 9.5/0.5, $V$ = 10 mL, $R_f$ = 0.20). Yellow oil, yield 51 mg (36%). Purity by HPLC: method 2a: $t_R$ = 15.1 min, purity 95.1%; method 2c: $t_R$ = 11.9 min, purity 97.8%. $[\alpha]_D^{20}$ = +26.9 ($c$ = 1.76; $CH_2Cl_2$). $C_{21}H_{28}N_2O_2$ (340.5). MS (EI): *m/z* [%] = 340 (M, 9), 295 (M − $CH_2CH_2OH$, 13), 219 (M − $CH_2PhOCH_3$, 5), 121 ($CH_2PhOCH_3$, 100). $^1$H NMR ($CDCl_3$): $\delta$ [ppm] = 1.79–1.89 (m, 1H, C$H_2$$CH_2OH$), 1.98–2.08 (m, 1H, C$H_2$$CH_2OH$), 2.25–2.35 (m, 2H, piperazine-H), 2.41 (dd, $J$ = 11.7/7.0 Hz, 1H, piperazine-H), 2.45–2.52 (m, 1H, piperazine-H), 2.62 (dd, $J$ = 11.7/3.1 Hz, 1H, piperazine-H), 2.79–2.85 (m, 1H, piperazine-H), 2.87–2.94 (m, 1H, piperazine-H), 3.39 (d, $J$ = 13.3 Hz, 1H, NC$H_2$Ph), 3.41 (d, $J$ = 12.5 Hz, 1H,

NC$H_2$Ar), 3.45 (d, $J$ = 12.5 Hz, 1H, NC$H_2$Ar), 3.71–3.77 (m, 1H, $CH_2CH_2OH$), 3.79 (s, 3H, ArOC$H_3$), 3.82–3.90 (m, 1H, $CH_2CH_2OH$), 4.14 (d, $J$ = 13.3 Hz, 1H, NC$H_2$Ph), 6.85 (d, $J$ = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.20–7.27 (m, 3H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$, $NCH_2C_6H_5$ (1H)), 7.28–7.34 (m, 4H, $NCH_2C_6H_5$). The signal for the proton of the OH group is not seen in the $^1$H NMR spectrum. IR (neat): $\tilde{\nu}$ [cm$^{-1}$] = 3379 (m br, $\nu_{O-H}$), 2931 (m, $\nu_{C-H\ aliph.}$), 1034 (m, $\nu_{C-O}$), 812 (m, $\Gamma_{p\text{-subst. arom.}}$).

**5.3.2. (+)-(*S*)-2-[4-(4-Methoxybenzyl)-1-(3-methylbut-2-en-1-yl)piperazin-2-yl]ethanol (3b).** According to general procedure B piperazinedione **7b** (142 mg, 0.38 mmol) was reacted with $LiAlH_4$ (86 mg, 2.28 mmol) in THF (40 mL) and the product was purified by FC (2 cm, $h$ = 15 cm, $CH_2Cl_2$–methanol = 9.5/0.5, $V$ = 10 mL, $R_f$ = 0.13). Yellow oil, yield 30 mg (25%). Purity by HPLC: method 2a: $t_R$ = 14.7 min, purity 99.3%; method 2c: $t_R$ = 8.4 min, purity 99.5%. $[\alpha]_D^{20}$ = +16.4 ($c$ = 0.46; $CH_2Cl_2$). $C_{19}H_{30}N_2O_2$ (318.5). MS (EI): $m/z$ [%] = 318 (M, 10), 197 (M − $CH_2PhOCH_3$, 38), 121 ($CH_2PhOCH_3$, 100). $^1$H NMR ($CDCl_3$): $\delta$ [ppm] = 1.64 (s, 3H, $NCH_2CH{=}C(CH_3)_2$), 1.67–1.78 (m, 1H, $CH_2CH_2OH$), 1.72 (s, 3H, $NCH_2CH{=}C(CH_3)_2$), 1.91–2.01 (m, 1H, $CH_2CH_2OH$), 2.26–2.46 (m, 3H, piperazine-H), 2.51–2.63 (m, 2H, piperazine-H), 2.79–2.86 (m, 1H, piperazine-H), 2.97–3.13 (m, 2H, $NCH_2CH{=}C(CH_3)_2$(1H), piperazine-H), 3.39–3.52 (m, 3H, NC$H_2$Ar, $NCH_2CH{=}C(CH_3)_2$(1H)), 3.67–3.74 (m, 1H, $CH_2CH_2OH$), 3.79 (s, 3H, ArOC$H_3$), 3.82–3.90 (m, 1H, $CH_2CH_2OH$), 5.18–5.24 (m, 1H, $NCH_2CH{=}C(CH_3)_2$), 6.85 (d, $J$ = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.22 (d, $J$ = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$). A signal for the proton of the OH group is not seen in the $^1$H NMR spectrum. IR (neat): $\tilde{\nu}$ [cm$^{-1}$] = 3379 (m br, $\nu_{O-H}$), 2931 (m, $\nu_{C-H\ aliph.}$), 1034 (m, $\nu_{C-O}$), 812 (m, $\Gamma_{p\text{-subst. arom.}}$).

**5.3.3. (+)-(*S*)-2-[1-Allyl-4-(4-methoxybenzyl)piperazin-2-yl]-ethanol (3c).** According to general procedure B piperazinedione **7c** (123 mg, 0.36 mmol) was reacted with $LiAlH_4$ (81 mg, 2.13 mmol) in THF (40 mL) and the product was purified by FC (2 cm, $h$ = 15 cm, $CH_2Cl_2$–methanol = 9.5/0.5, $V$ = 10 mL, $R_f$ = 0.13). Yellow oil, yield 67 mg (65%). Purity by HPLC: method 2b: $t_R$ = 22.9 min, purity 96.9%; method 2d: $t_R$ = 13.1 min, purity 97.7%. $[\alpha]_D^{20}$ = +6.7 ($c$ = 0.19; $CH_2Cl_2$). $C_{17}H_{26}N_2O_2$ (290.4). MS (EI): $m/z$ [%] = 290 (M, 6), 169 (M − $CH_2PhOCH_3$, 22), 121 ($CH_2PhOCH_3$, 100). $^1$H NMR ($CDCl_3$): $\delta$ [ppm] = 1.67–1.78 (m, 1H, $CH_2CH_2OH$), 1.90–2.02 (m, 1H, $CH_2CH_2OH$), 2.26–2.43 (m, 3H, piperazine-H), 2.48–2.61 (m, 2H, piperazine-H), 2.75–2.82 (m, 1H, piperazine-H), 2.95–3.06 (m, 2H, $NCH_2CH{=}CH_2$ (1H), piperazine-H (1H)), 3.40 (d, $J$ = 12.5 Hz, 1H, NC$H_2$Ar), 3.44 (d, $J$ = 12.5 Hz, 1H, NC$H_2$Ar), 3.52 (dd, $J$ = 14.1/5.5 Hz, 1H, $NCH_2CH{=}CH_2$), 3.69 (ddd, $J$ = 11.7/6.3/4.7 Hz, 1H, $CH_2CH_2OH$), 3.79 (s, 3H, ArOC$H_3$), 3.84 (ddd, $J$ = 11.7/7.8/4.7 Hz, 1H, $CH_2CH_2OH$), 5.13–5.22 (m, 2H, $NCH_2CH{=}CH_2$), 5.77–5.88 (m, 1H, $NCH_2CH{=}CH_2$), 6.85 (d, $J$ = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.22 (d, $J$ = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$). The signal for the proton of the OH group is not seen in the $^1$H NMR spectrum. IR (neat): $\tilde{\nu}$ [cm$^{-1}$] = 3377 (m br, $\nu_{O-H}$), 2936 (m, $\nu_{C-H\ aliph.}$), 1033 (m, $\nu_{C-O}$), 813 (w, $\Gamma_{p\text{-subst. arom.}}$).

**5.3.4. (+)-(*S*)-3-[1-Benzyl-4-(4-methoxybenzyl)piperazin-2-yl]propan-1-ol (4a).** According to general procedure B piperazinedione **8a** (98 mg, 0.24 mmol) was reacted with $LiAlH_4$ (54 mg, 1.43 mmol) in THF (40 mL) and the product was purified by FC (2 cm, $h$ = 15 cm, $CH_2Cl_2$–methanol = 9.5/0.5, $V$ = 10 mL, $R_f$ = 0.20). Yellow oil, yield 57 mg (67%). Purity by HPLC: method 2a: $t_R$ = 16.8 min, purity 95.9%; method 2c: $t_R$ = 14.9 min, purity 97.6%. $[\alpha]_D^{20}$ = +49.9 ($c$ = 1.95; $CH_2Cl_2$). $C_{22}H_{30}N_2O_2$ (354.5). MS (EI): $m/z$ [%] = 354 (M, 3), 295 (M − $CH_2CH_2CH_2OH$, 17), 121 ($CH_2PhOCH_3$). $^1$H NMR ($CDCl_3$): $\delta$ [ppm] = 1.52–1.63 (m, 1H, $CH_2CH_2CH_2OH$), 1.67–1.94 (m, 3H, $CH_2CH_2CH_2OH$), 2.12–2.33 (m, 3H, piperazine-H), 2.49–2.61 (m, 2H, piperazine-H), 2.62–2.67 (m, 1H, piperazine-H), 2.71–2.77 (m, 1H, piperazine-H), 3.20 (d, $J$ = 12.5 Hz, 1H, NC$H_2$Ph), 3.39 (d, $J$ = 12.5 Hz, 1H, NC$H_2$Ar), 3.44 (d, $J$ = 12.5 Hz, 1H, NC$H_2$Ar), 3.61–3.71 (m, 2H, $CH_2CH_2CH_2OH$), 3.79 (s, 3H, ArOC$H_3$), 4.17 (d, $J$ = 12.5 Hz, 1H, NC$H_2$Ph), 6.84 (d, $J$ = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.19–7.27 (m, 3H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$, $NCH_2C_6H_5$ (1H)), 7.28–7.36 (m, 4H, $NCH_2C_6H_5$). The signal for the proton of the OH group is not seen in the $^1$H NMR spectrum. IR (neat): $\tilde{\nu}$ [cm$^{-1}$] = 3362 (m br, $\nu_{O-H}$), 3028 (w, $\nu_{C-H\ arom.}$), 2938 (m, $\nu_{C-H\ aliph.}$), 1035 (m, $\nu_{C-O}$), 815 (m, $\Gamma_{p\text{-subst. arom.}}$), 734 (m)/698 (m, $\Gamma_{mono\text{-subst. arom.}}$).

**5.3.5. (+)-(*S*)-3-[4-(4-Methoxybenzyl)-1-(3-methylbut-2-en-1-yl)piperazin-2-yl]propan-1-ol (4b).** According to general procedure B piperazinedione **8b** (169 mg, 0.44 mmol) was reacted with $LiAlH_4$ (99 mg, 2.61 mmol) in THF (50 mL) and the product was purified by FC (2 cm, $h$ = 15 cm, $CH_2Cl_2$–methanol = 9.5/0.5, $V$ = 10 mL, $R_f$ = 0.16). Yellow oil, yield 46 mg (32%). Purity by HPLC: method 2a: $t_R$ = 16.5 min, purity 96.8%; method 2c: $t_R$ = 10.4 min, purity 96.9%. $[\alpha]_D^{20}$ = +24.0 ($c$ = 1.42; $CH_2Cl_2$). $C_{20}H_{32}N_2O_2$ (332.5). MS (EI): $m/z$ [%] = 332 (M, 6), 211 (M − $CH_2PhOCH_3$, 37), 121 ($CH_2PhOCH_3$, 100). $^1$H NMR ($CDCl_3$): $\delta$ [ppm] = 1.47–1.56 (m, 1H, $CH_2CH_2CH_2OH$), 1.64 (s, 3H, $NCH_2CH{=}C(CH_3)_2$), 1.66–1.84 (m, 3H, $CH_2CH_2CH_2OH$), 1.73 (s, 3H, $NCH_2CH{=}C(CH_3)_2$), 2.19–2.30 (m, 2H, piperazine-H), 2.35–2.43 (m, 1H, piperazine-H), 2.51–2.58 (m, 1H, piperazine-H), 2.60–2.69 (m, 2H, piperazine-H), 2.93–3.04 (m, 2H, $NCH_2CH{=}C(CH_3)_2$(1H), piperazine-H), 3.37–3.46 (m, 3H, NC$H_2$Ar, $NCH_2CH{=}C(CH_3)_2$(1H)), 3.52–3.58 (m, 1H, $CH_2CH_2CH_2OH$), 3.60–3.67 (m, 1H, $CH_2CH_2CH_2OH$), 3.79 (s, 3H, ArOC$H_3$), 5.23–5.29 (m, 1H, $NCH_2CH{=}C(CH_3)_2$), 6.84 (d, $J$ = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.21 (d, $J$ = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$). The signal for the proton of the OH group is not seen in the $^1$H NMR spectrum. IR (neat): $\tilde{\nu}$ [cm$^{-1}$] = 3342 (m br, $\nu_{O-H}$), 2933 (m, $\nu_{C-H\ aliph.}$), 1035 (m, $\nu_{C-O}$), 814 (m, $\Gamma_{p\text{-subst. arom.}}$).

**5.3.6. (+)-(*S*)-3-[1-Allyl-4-(4-methoxybenzyl)piperazin-2-yl]propan-1-ol (4c).** According to general procedure B piperazinedione **8c** (146 mg, 0.41 mmol) was reacted with $LiAlH_4$ (92 mg, 2.43 mmol) in THF (40 mL) and the product was purified by FC (2 cm, $h$ = 15 cm, $CH_2Cl_2$–methanol = 9.5/0.5, $V$ = 10 mL, $R_f$ = 0.10). Yellow oil, yield 42 mg (34%). Purity by HPLC: method 2b: $t_R$ = 29.1 min, purity 96.8%; method 2d: $t_R$ = 16.7 min, purity 97.2%. $[\alpha]_D^{20}$ = +8.0 ($c$ = 0.38; $CH_2Cl_2$). $C_{18}H_{28}N_2O_2$ (304.4). MS (EI): $m/z$ [%] = 304 (M, 6), 183 (M − $CH_2PhOCH_3$,

28), 121 ($CH_2PhOCH_3$, 100). $^1$H NMR ($CDCl_3$): $\delta$ [ppm] = 1.48–1.58 (m, 1H, $CH_2CH_2CH_2OH$), 1.64–1.79 (m, 3H, $CH_2CH_2CH_2OH$), 2.16–2.26 (m, 2H, piperazine-H), 2.33–2.41 (m, 1H, piperazine-H), 2.46–2.52 (m, 1H, piperazine-H), 2.58–2.67 (m, 2H, piperazine-H), 2.87–2.96 (m, 2H, $NCH_2CH{=}CH_2$ (1H), piperazine-H (1H)), 3.39 (d, $J$ = 13.3 Hz, 1H, $NCH_2Ar$), 3.43 (d, $J$ = 13.3 Hz, 1H, $NCH_2Ar$), 3.49–3.59 (m, 2H, $CH_2CH_2CH_2OH$ (1H), $NCH_2CH{=}CH_2$ (1H)), 3.60–3.66 (m, 1H, $CH_2CH_2CH_2OH$), 3.79 (s, 3H, $ArOCH_3$), 5.14–5.22 (m, 2H, $NCH_2CH{=}CH_2$), 5.82–5.94 (m, 1H, $NCH_2CH{=}CH_2$), 6.84 (d, $J$ = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.21 (d, $J$ = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$). A signal for the proton of the OH group is not seen in the $^1$H NMR spectrum. IR (neat): $\tilde{\nu}$ [$cm^{-1}$] = 3393 (m br, $\nu_{O-H}$), 2935 (m, $\nu_{C-H\ aliph.}$), 1034 (m, $\nu_{C-O}$), 815 (w, $\Gamma_{p\text{-subst. arom.}}$).

**5.3.7. (+)-Methyl (*S*)-2-[1-benzyl-4-(4-methoxybenzyl)-3,6-dioxopiperazin-2-yl]acetate (7a).** According to general procedure A piperazinedione **5** (306 mg, 1.00 mmol), tetrabutylammonium iodide (74 mg, 0.20 mmol), 2 M solution of sodium hexamethyldisilazane in THF (0.55 mL, 1.10 mmol) and benzyl bromide (0.6 mL, 854 mg, 4.99 mmol) were reacted in THF (50 mL). The product was purified by FC (3 cm, $h$ = 15 cm, cyclohexane–ethyl acetate 1/1, 20 mL, $R_f$ = 0.30). Colorless solid, m.p. 95 °C, yield 130 mg (33%). $[\alpha]_D^{20}$ = +71.9 ($c$ = 0.25; $CH_2Cl_2$). $C_{22}H_{24}N_2O_5$ (396.5). MS (EI): $m/z$ [%] = 396 (M, 44), 305 (M − $CH_2Ph$, 7), 275 (M − $CH_2PhOCH_3$, 5), 121 ($CH_2PhOCH_3$, 100). $^1$H NMR ($CDCl_3$): $\delta$ [ppm] = 2.81 (dd, $J$ = 17.2/4.7 Hz, 1H, $CH_2CO_2CH_3$), 3.07 (dd, $J$ = 17.2/3.1 Hz, 1H, $CH_2CO_2CH_3$), 3.55 (s, 3H, $CO_2CH_3$), 3.80 (s, 3H, $ArOCH_3$), 3.85 (d, $J$ = 17.2 Hz, 1H, O=$CCH_2N$), 4.09–4.15 (m, 2H, O=$CCH_2N$ (1H), $CHCH_2CO_2CH_3$), 4.20 (d, $J$ = 14.9 Hz, 1H, $NCH_2Ph$), 4.43 (d, $J$ = 14.9 Hz, 1H, $NCH_2Ar$), 4.65 (d, $J$ = 14.9 Hz, 1H, $NCH_2Ar$), 5.07 (d, $J$ = 14.9 Hz, 1H, $NCH_2Ph$), 6.87 (d, $J$ = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.19–7.24 (m, 4H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$, $NCH_2C_6H_5$ (2H)), 7.29–7.35 (m, 3H, $NCH_2C_6H_5$). IR (neat): $\tilde{\nu}$ [$cm^{-1}$] = 3003 (w, $\nu_{C-H\ arom.}$), 2952 (w, $\nu_{C-H\ aliph.}$), 1734 (m, $\nu_{C=O\ ester}$), 1649 (s, $\nu_{C=O\ amide}$), 1030 (m, $\nu_{C-O}$), 817 (m, $\Gamma_{p\text{-subst. arom.}}$), 731 (m, $\Gamma_{mono\text{-subst. arom.}}$).

**5.3.8. (+)-Methyl (*S*)-2-[4-(4-methoxybenzyl)-1-(3-methylbut-2-en-1-yl)-3,6-dioxopiperazin-2-yl]acetate (7b).** According to general procedure A piperazinedione **5** (605 mg, 1.98 mmol), tetrabutylammonium iodide (146 mg, 0.40 mmol), 2 M solution of sodium hexamethyldisilazane in THF (1.1 mL, 2.17 mmol) and 1-bromo-3-methylbut-2-ene (1.16 mL, 1.47 mg, 9.88 mmol) were reacted in THF (70 mL). The product was purified by FC (4 cm, $h$ = 15 cm, cyclohexane–ethyl acetate = 1/1, 30 mL, $R_f$ = 0.30). Yellow oil, yield 225 mg (30%). $[\alpha]_D^{20}$ = +52.0 ($c$ = 0.57; $CH_2Cl_2$). $C_{20}H_{26}N_2O_5$ (374.4). MS (EI): $m/z$ [%] = 374 (M, 18), 253 (M − $CH_2PhOCH_3$, 87), 121 ($CH_2PhOCH_3$, 100). $^1$H NMR ($CDCl_3$): $\delta$ [ppm] = 1.70 (s, 3H, $NCH_2CH{=}C(CH_3)_2$), 1.73 (s, 3H, $NCH_2CH{=}C(CH_3)_2$), 2.83 (dd, $J$ = 17.2/5.5 Hz, 1H, $CH_2CO_2CH_3$), 3.13 (dd, $J$ = 17.2/3.1 Hz, 1H, $CH_2CO_2CH_3$), 3.59 (s, 3H, $CO_2CH_3$), 3.63 (dd, $J$ = 14.9/7.8 Hz, 1H, $NCH_2CH{=}C(CH_3)_2$), 3.75 (d, $J$ = 17.2 Hz, 1H, O=$CCH_2N$), 3.80 (s, 3H, $ArOCH_3$), 4.00 (d, $J$ = 17.2 Hz, 1H, O=$CCH_2N$), 4.22–4.26 (m, 1H, $CHCH_2CO_2CH_3$), 4.40 (dd, $J$ = 14.9/6.3 Hz, 1H, $NCH_2CH{=}C(CH_3)_2$), 4.47 (d, $J$ = 14.1 Hz, 1H, $NCH_2Ar$), 4.61 (d, $J$ = 14.1 Hz, 1H, $NCH_2Ar$), 5.04–5.10 (m, 1H, $NCH_2CH{=}C(CH_3)_2$), 6.86 (d, $J$ = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.21 (d, $J$ = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$). IR (neat): $\tilde{\nu}$ [$cm^{-1}$] = 2932 (m, $\nu_{C-H\ aliph.}$), 1735 (m, $\nu_{C=O\ ester}$), 1654 (s, $\nu_{C=O\ amide}$), 1031 (m, $\nu_{C-O}$), 811 (m, $\Gamma_{p\text{-subst. arom.}}$).

**5.3.9. (+)-Methyl (*S*)-2-[1-allyl-4-(4-methoxybenzyl)-3,6-dioxopiperazin-2-yl]acetate (7c).**[23] According to general procedure A piperazinedione **5** (6.24 g, 20.4 mmol), tetrabutylammonium iodide (1.50 g, 4.07 mmol), 2 M solution of sodium hexamethyldisilazane in THF (11.2 mL, 22.4 mmol) and allyl bromide (8.9 mL, 12.3 g, 102 mmol) were reacted in THF (150 mL). The product was purified by FC (8 cm, cyclohexane–ethyl acetate = 1/2, 30 mL, $R_f$ = 0.20). Colorless solid, m.p. 89 °C, yield 3.64 g (52%). Purity by HPLC: method 1: $t_R$ = 17.3 min, purity 98.2%. $[\alpha]_D^{20}$ = +57.1 ($c$ = 0.33; $CH_2Cl_2$). $C_{18}H_{22}N_2O_5$ (346.4). MS (EI): $m/z$ [%] = 346 (M, 39), 225 (M − $CH_2PhOCH_3$, 10), 121 ($CH_2PhOCH_3$, 100). $^1$H NMR ($CDCl_3$): $\delta$ [ppm] = 2.88 (dd, $J$ = 17.2/4.7 Hz, 1H, $CH_2CO_2CH_3$), 3.13 (dd, $J$ = 17.2/3.1 Hz, 1H, $CH_2CO_2CH_3$), 3.60 (s, 3H, $CO_2CH_3$), 3.63 (dd, $J$ = 15.7/7.0 Hz, 1H, $NCH_2CH{=}CH_2$), 3.78 (d, $J$ = 17.2 Hz, 1H, O=$CCH_2N$), 3.80 (s, 3H, $ArOCH_3$), 4.03 (d, $J$ = 17.2 Hz, 1H, O=$CCH_2N$), 4.23–4.27 (m, 1H, $CHCH_2CO_2CH_3$), 4.43 (ddt, $J$ = 15.7/5.5/1.6 Hz, 1H, $NCH_2CH{=}CH_2$), 4.50 (d, $J$ = 14.9 Hz, 1H, $NCH_2Ar$), 4.59 (d, $J$ = 14.9 Hz, 1H, $NCH_2Ar$), 5.20–5.27 (m, 2H, $NCH_2CH{=}CH_2$), 5.67–5.78 (m, 1H, $NCH_2CH{=}CH_2$), 6.86 (d, $J$ = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.21 (d, $J$ = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$). IR (neat): $\tilde{\nu}$ [$cm^{-1}$] = 3080 (w, $\nu_{C-H\ arom.}$), 2924 (m, $\nu_{C-H\ aliph.}$), 1735 (m, $\nu_{C=O\ ester}$), 1660 (s, $\nu_{C=O\ amide}$).

**5.3.10. (−)-Methyl (*S*)-3-[1-benzyl-4-(4-methoxybenzyl)-3,6-dioxopiperazin-2-yl]propanoate (8a).** According to general procedure A piperazinedione **6** (680 mg, 2.12 mmol), tetrabutylammonium iodide (157 mg, 0.42 mmol), 2 M solution of sodium hexamethyldisilazane in THF (1.2 mL, 2.33 mmol) and benzyl bromide (1.3 mL, 1.82 g, 10.6 mmol) were reacted in THF (70 mL). The product was purified by FC (4 cm, $h$ = 15 cm, cyclohexane–ethyl acetate = 1/1, 30 mL, $R_f$ = 0.35). Colorless oil, yield 98 mg (11%). $[\alpha]_D^{20}$ = −3.1 ($c$ = 1.09; $CH_2Cl_2$). $C_{23}H_{26}N_2O_5$ (410.5). MS (EI): $m/z$ [%] = 410 (M, 46), 289 (M − $CH_2PhOCH_3$, 11), 121 ($CH_2PhOCH_3$, 100). $^1$H NMR ($CDCl_3$): $\delta$ [ppm] = 1.99–2.08 (m, 1H, $CH_2CH_2CO_2CH_3$), 2.17–2.27 (m, 1H, $CH_2CH_2CO_2CH_3$), 2.32–2.48 (m, 2H, $CH_2CH_2CO_2CH_3$), 3.67 (s, 3H, $CO_2CH_3$), 3.80 (s, 3H, $ArOCH_3$), 3.83 (d, $J$ = 17.2 Hz, 1H, O=$CCH_2N$), 3.92–4.00 (m, 3H, O=$CCH_2N$ (1H), $CHCH_2CH_2CO_2CH_3$, $NCH_2Ph$ (1H)), 4.34 (d, $J$ = 14.1 Hz, 1H, $NCH_2Ar$), 4.65 (d, $J$ = 14.1 Hz, 1H, $NCH_2Ar$), 5.26 (d, $J$ = 14.9 Hz, 1H, $NCH_2Ph$), 6.87 (d, $J$ = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.17 (d, $J$ = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$), 7.23–7.35 (m, 5H, $NCH_2C_6H_5$). IR (neat): $\tilde{\nu}$ [$cm^{-1}$] = 2952 (m, $\nu_{C-H\ aliph.}$), 1733 (m, $\nu_{C=O\ ester}$), 1658 (s, $\nu_{C=O\ amide}$), 1029 (m, $\nu_{C-O}$), 821 (w, $\Gamma_{p\text{-subst. arom.}}$), 730 (m)/700 (m, $\Gamma_{mono\text{-subst. arom.}}$).

**5.3.11. (+)-Methyl (*S*)-3-[4-(4-methoxybenzyl)-1-(3-methylbut-2-en-1-yl)-3,6-dioxopiperazin-2-yl]propanoate (8b).** According to general procedure A piperazinedione **6** (650 mg,

2.03 mmol), tetrabutylammonium iodide (150 mg, 0.41 mmol), 2 M solution of sodium hexamethyldisilazane in THF (1.1 mL, 2.23 mmol) and 1-bromo-3-methylbut-2-ene (1.19 mL, 1.51 mg, 10.1 mmol) were reacted in THF (70 mL). The product was purified by FC (4 cm, $h = 15$ cm, cyclohexane–ethyl acetate = 1/1, 30 mL, $R_f = 0.33$). Yellow oil, yield 221 mg (28%). $[\alpha]_D^{20} = +2.1$ ($c = 0.70$; $CH_2Cl_2$). $C_{21}H_{28}N_2O_5$ (388.5). MS (EI): $m/z$ [%] = 388 (M, 23), 267 (M − $CH_2PhOCH_3$, 34), 121 ($CH_2PhOCH_3$, 100). $^1$H NMR ($CDCl_3$): $\delta$ [ppm] = 1.70 (s, 3H, $NCH_2CH{=}C(CH_3)_2$), 1.73 (s, 3H, $NCH_2CH{=}C(CH_3)_2$), 1.98–2.07 (m, 1H, $CH_2CH_2CO_2CH_3$), 2.22–2.31 (m, 1H, $CH_2CH_2CO_2CH_3$), 2.32–2.45 (m, 2H, $CH_2CH_2CO_2CH_3$), 3.56 (dd, $J = 14.9/8.6$ Hz, 1H, $NCH_2CH{=}C(CH_3)_2$), 3.67 (s, 3H, $CO_2CH_3$), 3.76 (d, $J = 17.2$ Hz, 1H, $O{=}CCH_2N$), 3.80 (s, 3H, $ArOCH_3$), 3.87 (d, $J = 17.2$ Hz, 1H, $O{=}CCH_2N$), 4.02 (dd, $J = 8.6/3.9$ Hz, 1H, $CHCH_2CH_2CO_2CH_3$), 4.38 (d, $J = 14.1$ Hz, 1H, $NCH_2Ar$), 4.47 (dd, $J = 14.9/6.3$ Hz, 1H, $NCH_2CH{=}C(CH_3)_2$), 4.61 (d, $J = 14.1$ Hz, 1H, $NCH_2Ar$), 5.07–5.13 (m, 1H, $NCH_2CH{=}C(CH_3)_2$), 6.86 (d, $J = 8.6$ Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.18 (d, $J = 8.6$ Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$). IR (neat): $\tilde{\nu}$ [$cm^{-1}$] = 2933 (m, $\nu_{C-H\ aliph.}$), 1734 (m, $\nu_{C=O\ ester}$), 1654 (s, $\nu_{C=O\ amide}$), 1031 (m, $\nu_{C-O}$), 821 (w, $\Gamma_{p\text{-subst. arom.}}$).

**5.3.12. (+)-Methyl (*S*)-3-[1-allyl-4-(4-methoxybenzyl)-3,6-dioxopiperazin-2-yl]propanoate (8c).**[24] According to general procedure A piperazinedione **6** (2.80 g, 8.74 mmol), tetrabutylammonium iodide (0.65 g, 1.75 mmol), 2 M solution of sodium hexamethyldisilazane in THF (4.81 mL, 9.61 mmol) and allyl bromide (3.8 mL, 5.29 g, 43.7 mmol) were reacted in THF (80 mL). The product was purified by FC (8 cm, $h = 15$ cm, cyclohexane–ethyl acetate = 1/2, 30 mL, $R_f = 0.29$). Yellow oil, yield 1.67 g (53%). Purity by HPLC: method 1: $t_R = 17.9$ min, purity 98.1%. $[\alpha]_D^{20} = +20.2$ ($c = 11.3$; $CH_2Cl_2$). $C_{19}H_{24}N_2O_5$ (360.4). MS (EI): $m/z$ [%] = 360 (M, 65), 239 (M − $CH_2PhOCH_3$, 54), 121 ($CH_2PhOCH_3$, 100). $^1$H NMR ($CDCl_3$): $\delta$ [ppm] = 1.97–2.07 (m, 1H, $CH_2CH_2CO_2CH_3$), 2.21–2.30 (m, 1H, $CH_2CH_2CO_2CH_3$), 2.34–2.50 (m, 2H, $CH_2CH_2CO_2CH_3$), 3.51 (dd, $J = 15.7/7.8$ Hz, 1H, $NCH_2CH{=}CH_2$), 3.68 (s, 3H, $CO_2CH_3$), 3.78 (d, $J = 17.2$ Hz, 1H, $O{=}CCH_2N$), 3.80 (s, 3H, $ArOCH_3$), 3.90 (d, $J = 17.2$ Hz, 1H, $O{=}CCH_2N$), 4.03 (dd, $J = 8.6/3.9$ Hz, 1H, $CHCH_2CH_2CO_2CH_3$), 4.42 (d, $J = 14.9$ Hz, 1H, $NCH_2Ar$), 4.52–4.61 (m, 2H, $NCH_2Ar$ (1H), $NCH_2CH{=}CH_2$ (1H)), 5.21–5.27 (m, 2H, $NCH_2CH{=}CH_2$), 5.68–5.79 (m, 1H, $NCH_2CH{=}CH_2$), 6.86 (d, $J = 8.6$ Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.18 (d, $J = 8.6$ Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$). IR (neat): $\tilde{\nu}$ [$cm^{-1}$] = 2952 (w, $\nu_{C-H\ aliph.}$), 1733 (m, $\nu_{C=O\ ester}$), 1658 (s, $\nu_{C=O\ amide}$), 1032 (m, $\nu_{C-O}$), 820 (w, $\Gamma_{p\text{-subst. arom.}}$).

## 6. Experimental, receptor binding studies

### 6.1. Materials and general procedures

Guinea pig brains and rat livers were commercially available (Harlan-Winkelmann, Germany). Homogenizer: Elvehjem Potter (B. Braun Biotech International). Centrifuge: High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Finnigan). Filter: Printed Filtermat Type A (PerkinElmer), presoaked in 0.5% aqueous polyethylenimine for 2 h at rt before use. The filtration was carried out with a MicroBeta FilterMate-96 Harvester (PerkinElmer). The scintillation analysis was performed using Meltilex (Type A) solid scintillator (PerkinElmer). The radioactivity bound to the filter was measured using a MicroBeta Trilux scintillation analyzer (PerkinElmer). The overall counting efficiency was 20%.

### 6.2. Membrane preparation for the $\sigma_1$ assay[25–27]

Five guinea pig brains were homogenized with the potter (500–800 rpm, 10 up-and-down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1200 × $g$ for 10 min at 4 °C. The supernatant was separated and centrifuged at 23 500 × $g$ for 20 min at 4 °C. The pellet was resuspended in 5–6 volumes of buffer (50 mM Tris, pH 7.4) and centrifuged again at 23 500 × $g$ (20 min, 4 °C). This procedure was repeated twice. The final pellet was resuspended in 5–6 volumes of buffer, the protein concentration was determined according to the method of Bradford[28] using bovine serum albumin as standard, and subsequently the preparation was frozen (−80 °C) in 1.5 mL portions containing about 1.5 mg protein per mL.

### 6.3. Protocol of the $\sigma_1$ assay[25–27]

The test was performed with the radioligand [$^3$H]-(+)-pentazocine (42.5 Ci mmol$^{-1}$; PerkinElmer). The thawed membrane preparation (about 75 μg of the protein) was incubated with various concentrations of test compounds, 2 nM [$^3$H]-(+)-pentazocine, and buffer (50 mM Tris, pH 7.4) in a total volume of 200 μL for 180 min at 37 °C. The incubation was terminated by rapid filtration through the presoaked filtermats by using the cell harvester. After washing each well five times with 300 μL of water, the filtermats were dried at 95 °C. Subsequently, the solid scintillator was put on the filtermat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at rt. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The non-specific binding was determined with 10 μM unlabeled (+)-pentazocine. The $K_d$-value of the radioligand [$^3$H]-(+)-pentazocine is 2.9 nM.[29]

### 6.4. Membrane preparation for the $\sigma_2$ assay[25–27]

Two rat livers were cut into small pieces and homogenized with a potter (500–800 rpm, 10 up-and-down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1200 × $g$ for 10 min at 4 °C. The supernatant was separated and centrifuged at 31 000 × $g$ for 20 min at 4 °C. The pellet was resuspended in buffer (50 mM Tris, pH 8.0) and incubated at rt for 30 min. After the incubation, the suspension was centrifuged again at 31 000 × $g$ for 20 min at 4 °C. The final pellet was resuspended in buffer, the protein concentration was determined according to the method of Bradford[28] using bovine serum albumin as standard, and subsequently the preparation was frozen (−80 °C) in 1.5 mL portions containing about 2 mg protein per mL.

### 6.5. Protocol of the $\sigma_2$ assay[25–27]

The test was performed with the radioligand [$^3$H]-di-*o*-tolylguanidine (50 Ci mmol$^{-1}$; ARC). The thawed membrane preparation (about 100 μg of the protein) was incubated with various

concentrations of test compounds, 3 nM [$^3$H]-di-*o*-tolylguanidine, 500 nM (+)-pentazocine and buffer (50 mM Tris, pH 8.0) in a total volume of 200 µL for 180 min at rt. The incubation was terminated by rapid filtration through the presoaked filtermats using a cell harvester. After washing each well five times with 300 µL of water, the filtermats were dried at 95 °C. Subsequently, the solid scintillator was put on the filtermat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at rt. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The non-specific binding was determined with 10 µM unlabeled ditolylguanidine. The $K_d$-value of the radioligand [$^3$H]-ditolylguanidine is 17.9 nM.[30]

### 6.6. Data analysis

Usually, all experiments were carried out in triplicates using standard 96-well-multiplates (Diagonal). The $IC_{50}$-values were determined in competition experiments with six concentrations of the test compounds and were calculated with the program GraphPad Prism® 3.0 (GraphPad Software) by non-linear regression analysis. The $K_i$-values were calculated according to Cheng and Prusoff.[31] The $K_i$-values of potent compounds are given as mean values ± SEM from three independent experiments.

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