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Chiral, nonracemic (piperazin-2-yl)methanol derivatives with σ-receptor affinity

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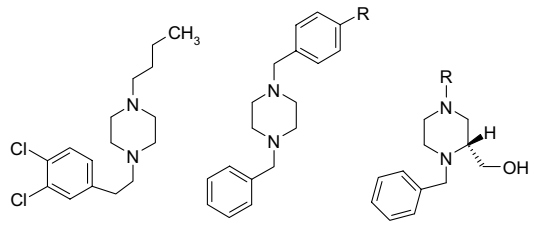
Abstract—Starting with the proteinogenic amino acid (*S*)-serine a series of chiral nonracemic (piperazin-2-yl)methanols **3** with various N-4 substituents is described. The key step in the synthesis of **3** is the reaction of the chloroacetamide **5** with various primary amines to yield the diastereomeric bicyclic piperazinediones *cis*-**6** and *trans*-**6**. The scope and limitation of this transformation is thoroughly investigated. The σ_1- and σ_2-receptor affinities of the piperazines **3** are determined in receptor binding studies with guinea pig brain and rat liver membrane preparations using [^{3}H]-labeled (+)-pentazocine and ditolylguanidine, respectively. It was found, that an additional phenyl residue in the N-4 substituent is favorable to high σ_1-receptor affinity. In this series the *p*-methoxybenzyl substituted piperazine **3d** reveals the highest σ_1-receptor affinity ($K_i = 12.4$ nM) with selectivity toward σ_2-, NMDA-, κ-opioid, and μ-opioid receptors.

1. Introduction

It is well accepted that σ-receptors represent a unique class of receptors in the central nervous system and the periphery. The σ-receptor is subdivided into σ_1- and σ_2-receptor subtypes. Recently the σ_1-receptor of various tissues and species has been cloned.[1] The corresponding amino acid sequence is not related to any known mammalian receptor or even protein. The exact structure of the σ_1-receptor protein, the amino acid sequence of the σ_2-receptor as well as a thorough understanding of the physiological role of both σ-receptor subtypes still remain to be elucidated.[2] However, it is well established, that σ-receptors are involved in several physiological and pathophysiological events. Therefore, ligands interacting with σ-receptors possess a potential as antipsychotics,[3] antidepressants,[4] anticocaine agents,[5,6] and antitumor agents.[7]

In the literature 1,4-disubstituted piperazine derivatives are described as high affinity σ-receptor ligands. Among these piperazine derivatives compounds **1**[8] and **2**[5] display very high σ_1-receptor affinity (see Fig. 1). Recently, we have reported on the synthesis and receptor binding of enantiomerically pure 1,4-diazabicyclo[4.3.0]nonan-9-ones.[9] The synthesis of these ligands proceeded via 2-(hydroxymethyl)piperazine intermediates **3** (see Fig. 1), which are structurally related to the σ_1-ligands **1** and **2**. Therefore, the σ-receptor affinity of the benzyl derivative **3c** was investigated, which resulted in a considerable σ_1-receptor affinity (see Table 2, entry 3). This result encouraged a systematic structure–affinity relationship study of hydroxymethyl substituted piperazine derivatives **3**. Scope and limitations of the synthesis of piperazines **3** is also included in this study and described in this communication.



1 2 3

Figure 1. Comparison of new piperazine derivatives **3** with known σ-active ligands **1** and **2**.

Keywords: Bicyclic piperazinediones; (Piperazin-2-yl)methanols; σ_1-receptor ligands; σ_2-receptor ligands; Structure–affinity relationships.
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2. Chemistry

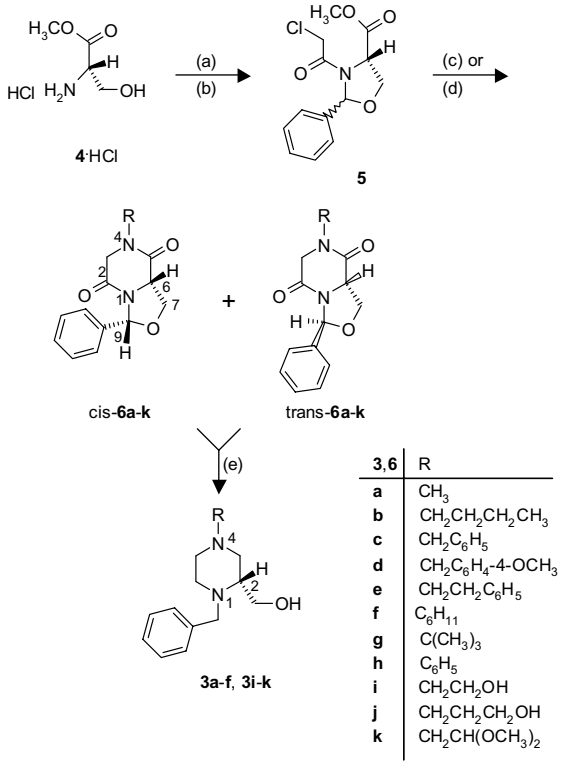
Starting with the methyl ester of (*S*)-serine (**4**·HCl) the oxazolidine derivative **5** was obtained by acylation with chloroacetyl chloride and subsequent condensation with benzaldehyde dimethyl acetal (see Scheme 1).[9] The diastereomeric ratio *cis*-**5**/*trans*-**5** is dependent on the reaction conditions during acetalization with benzaldehyde dimethyl acetal.[9] The diastereomeric mixture of **5** was reacted with various primary amines to yield the bicyclic piperazinediones **6**. The ratio of diastereomers *cis*-**6** and *trans*-**6** corresponded with the *cis*/*trans* ratio of **5**, since no epimerization occurred during aminolysis. With exception of the *tert*-butyl derivative **6g**, the diastereomers *cis*-**6**, and *trans*-**6** were separated by flash chromatography and their relative configuration was determined by NOE difference spectroscopy. The stereodescriptors *cis* and *trans* are used to characterize the arrangement of the protons in position 6 and 9 of the bicyclic ring system.

In order to investigate the scope and limitations of this key transformation various primary amines were reacted with the chloroacetamide **5**. Heating of an acetonitrile solution of **5** and methylamine, the primary amine with the smallest substituent attached to the NH_2-group, provided the bicyclic piperazinedione **6a** in a total yield of 89% (see Table 1). Comparable high yields were obtained with primary amines bearing a methylene group at the amino moiety (R-CH_2-NH_2: butan-1-amine, benzylamine, 4-methoxybenzylamine, 2-phenylethylamine). Even cyclohexylamine with a branched residue gave the bicyclic piperazinedione **6f** in 58% yield. However, *tert*-butylamine required boiling DMF for the reaction with **5** to afford the *tert*-butyl derivative **6g** in only 3% yield. The electronically deactivated primary aromatic amine aniline reacted with **5** in boiling DMF to provide **6h** in 9% yield. The products **6i**–**k** demonstrate, that primary amines substituted with additional functional groups (primary alcohol, acetal) are also successfully employed in this transformation.

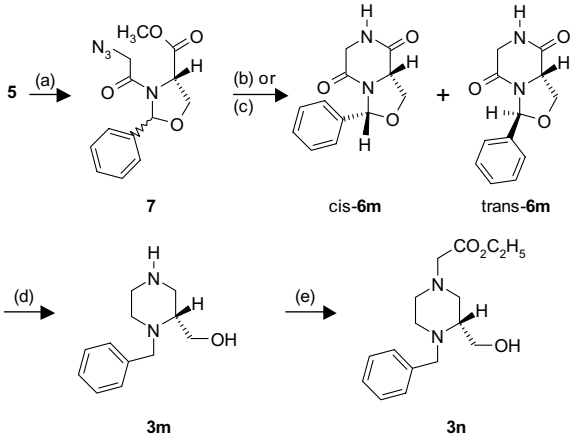
Table 1. Yield of the aminolysis of **5** and the subsequent $LiAlH_4$ reduction

Compound	Yield (%)	Compound	Yield (%)
6a	89	**3a**	44
6b	95	**3b**	74
6c	95	**3c**	57
6d	69	**3d**	73
6e	90	**3e**	82
6f	58	**3f**	74
6i	93	**3i**	55
6j	86	**3j**	53
6k	86	**3k**	71

Heating of the bicyclic piperazinediones **6** with an excess of $LiAlH_4$[10] afforded 1-benzyl-2-(hydroxymethyl)piperazines **3** bearing various substituents in position 4. Thereby, both diastereomers *cis*-**6** and *trans*-**6** led to the same product, respectively.

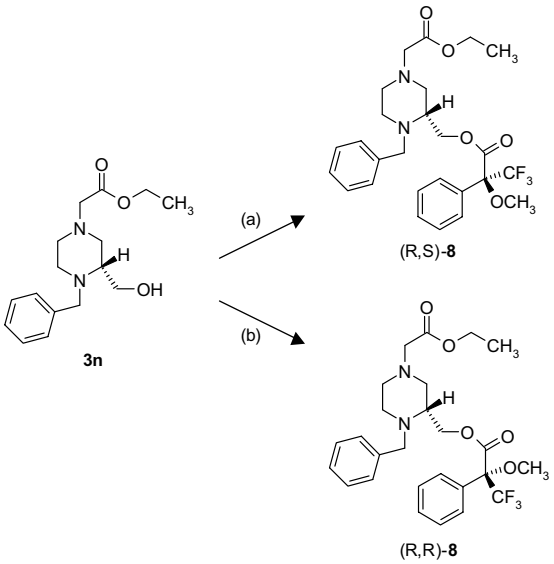


Scheme 1. Reagents and conditions: (a) $ClCH_2COCl$, NEt_3, CH_2Cl_2, 1 h, −5 °C, 1 h rt, 93%; (b) $PhCH(OCH_3)_2$, *p*-$TolSO_3H$, toluene, 2 h, 110 °C, 71%. (c) R-NH_2, NEt_3, CH_3CN, 82 °C, yields: see Table 1; (d) R-NH_2, NEt_3, DMF, 153 °C, yields: **6g** 3%; **6h** 9%; (e) $LiAlH_4$, THF, 66 °C, yields: see Table 1.



Scheme 2. Reagents and conditions: (a) NaN_3, aetone, 56 °C, 99%; (b) $NaBH_4$, $CuSO_4$, CH_3OH, rt, 76%; (c) H_2, 1 bar, Pd/C, CH_3OH, rt, 75%; (d) $LiAlH_4$, THF, 66 °C, 99%; (e) Br-CH_2CO_2Et, Na_2CO_3, THF, 66 °C, 61%.

In order to obtain the ester substituted piperazine derivative **3n** the reaction sequence was changed. The chloroacetyl derivative **5** was reacted with sodium azide to afford the azidoacetyl substituted oxazolidine **7** (mixture of diastereomers), which was reduced and cyclized with $NaBH_4/CuSO_4$[11] to yield the bicyclic piperazinediones *cis*-**6m** and *trans*-**6m** (see Scheme 2). A



Scheme 3. Reagents and conditions: (a) (*R*)-Mosher's acid chloride, NEt_3, DMAP, H_2Cl_2, 41 °C; (b) (*S*)-Mosher's acid chloride, NEt_3, DMAP, CH_2Cl_2, 41 °C.

mixture of *cis*-**6m** and *trans*-**6m** was heated with an excess of $LiAlH_4$ to yield the 1-benzyl-2-(hydroxymethyl)piperazine (**3m**) without substituent at N-4. Alkylation[12] of **3m** with ethyl 2-bromoacetate furnished the ester substituted piperazine derivative **3n**.

3. Investigation of the enantiomeric purity

In order to determine the enantiomeric purity the ester substituted piperazine derivative **3n** was acylated with (*R*)- and (*S*)-configured Mosher's acid chloride [(*R*)-(−)- and (*S*)-(+)-3,3,3-trifluoro-2-methoxy-2-phenylpropionyl chloride][13] in the presence of triethylamine and 4-(dimethylamino)pyridine to yield the esters (*R*,*S*)-**8** and (*R*,*R*)-**8**, respectively (see Scheme 3). The diastereomeric composition of (*R*,*S*)-**8** and (*R*,*R*)-**8** was determined by ^{19}F NMR spectroscopy and HPLC. The HPLC analysis was performed with a LiChrospner® 100 RP-18 endcapped column using methanol/H_2O (75:25) as eluent and UV-detection at 254 nm. The experiments revealed a diastereomeric ratio of greater than 96.7:3.3. Therefore, the enantiomeric purity of **3n** is higher than 93.4% ee. Taking the enantiomeric purity of (*S*)-serine (Sigma–Aldrich, 99%, 97% ee by GLC) into consideration it can be concluded that no racemization had occurred during the synthesis of (piperazin-2-yl)methanol derivatives **3**.

4. Receptor binding studies

The σ-receptor affinities of the (piperazin-2-yl)methanols **3a**–**f** and **3i**–**n** were determined in competition experiments with radioligands. In the σ_1-assay, homogenates of guinea pig brain preparations were used as receptor material. The σ_1-selective ligand [^3H]-(+)-pentazocine was employed as radioligand, and the nonspecific binding was determined in the presence of a large excess of haloperidol.[14] Homogenates of rat liver served as source for σ_2-receptors in the σ_2-assay. Since a σ_2-selective radioligand is not available, the nonselective radioligand [^3H]-ditolylguanidine was employed in the presence of an excess of nontritiated (+)-pentazocine (100 nM) for blocking the σ_1-receptors. Performing the σ_2-assay in the presence of an excess of nontritiated ditolylguanidine led to the nonspecific binding of the radioligand.[15,16] In order to determine receptor selectivity the most promising compounds were screened for their affinity at NMDA-, k-opioid, and μ-opioid receptors.[17]

5. Results and discussion

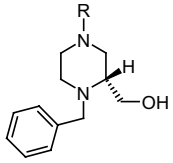
The σ-receptor affinities of the (1-benzylpiperazin-2-yl)methanols **3** are summarized in Table 2.

Piperazines with a small N-4 substituent (**3a**, $R = CH_3$, entry 1) and functionalized N-4 residues (**3i**,**j**,**k**,**n**, entries 7–10) do not interact significantly with σ_1- and σ_2-receptors. Larger aliphatic N-4 substituents, for example, butyl (**3b**) and cyclohexyl (**3f**) residues, enhance the σ_1-receptor affinity (entries 2 and 6). However, the highest σ_1-receptor affinities were found for piperazines with an additional aromatic residue in the N-4-substituent (entries 3–5). The number of methylene groups between N-4 and the phenyl residue makes no difference for the σ_1-receptor affinity, since the benzyl derivative **3c** ($K_i = 38.3$ nM) and the 2-phenylethyl derivative **3e** ($K_i = 36.8$ nM) reveal the same σ_1-receptor affinities (entries 3 and 5). A further enhancement of the σ_1-receptor affinity was achieved by introduction of a methoxy residue in 4-position of the N-4 benzyl substituent. The *p*-methoxybenzyl derivative **3d** binds with a K_i-value of 12.4 nM at σ_1-receptors being three times more active than the benzyl derivative **3c** (entry 4).

Generally, the σ_2-receptor affinities of the (2-hydroxymethyl)piperazines **3** are lower than their σ_1-receptor affinities. The relationships between structure and σ_2-receptor affinity correlate with the structure/σ_1-receptor affinity relationships: Enhancement of σ_1-receptor affinity leads to an increase of σ_2-receptor affinity. However, the piperazines **3c**–**e** binding with high affinity at σ_1-receptors show the highest σ_1/σ_2-selectivity factors.

In Table 3 the NMDA-, k-opioid, and μ-opioid receptor affinities of piperazines **3** binding with high affinity toward σ_1-receptors are shown. The residual binding of the radioligand is given at a concentration of 10 μM of the test compounds. In all cases the residual binding is greater than 50% indicating IC_{50}-values of at least 10 μM. These results lead to the conclusion that the novel σ_1-receptor ligands described in this report display high selectivity toward NMDA-, k-opioid, and μ-opioid receptors.

Table 2. σ-receptor affinities of (1-benzylpiperazin-2-yl)methanols **3** heaving various substituents in position 4



3

Entry	Compound	R	$K_i \pm$ SEM (3) (nM)		σ_1/σ_2-selectivity
			σ_1 ([^{3}H]-(+)-pentazocine)	σ_2 ([^{3}H]-ditolyl-guanidine)	
1	**3a**	CH_3	>10,000	>10,000	
2	**3b**	$CH_2CH_2CH_2CH_3$	296 ± 31	513 ± 96	2
3	**3c**	$CH_2C_6H_5$	38.3 ± 2.4	177 ± 16	5
4	**3d**	$CH_2C_6H_4$-4-OCH_3	12.4 ± 1.4	70.3 ± 10	6
5	**3e**	$CH_2CH_2C_6H_5$	36.8 ± 3.0	132 ± 32	4
6	**3f**	C_6H_{11}	58.1 ± 3.2	108 ± 21	2
7	**3i**	CH_2CH_2OH	>10,000	>10,000	
8	**3j**	$CH_2CH_2CH_2OH$	>10,000	>10,000	
9	**3k**	$CH_2CH(OCH_3)_2$	>10,000	>10,000	
10	**3n**	$CH_2CO_2C_2H_5$	1578 ± 203	>10,000	
11		Haloperidol	2.20 ± 0.31	34.2 ± 2.3	16
12		Ditolylguanidine	164 ± 47.4	63.9 ± 10.8	0.39
13		BMY 14802	265 ± 32.0	391 ± 62.2	1.5

Table 3. Residual binding (%) of the respective radioligand at a test compound concentration of 10 μM

Compound	NMDA ([^{3}H]-(+)-MK-801) (%)	κ ([^{3}H]-U-69593) (%)	μ ([^{3}H]-DAM-GO) (%)
3b	78	>100	>100
3c	86	84	85
3d	86	79	59
3e	79	>100	52
3f	77	90	98
3n	83	90	100

6. Conclusion

In summary the 4-methoxybenzyl piperazine **3d** is the most σ_1-active compound in this series. Whereas **3d** has only moderate selectivity toward σ_2-receptors (factor 6) excellent selectivity with respect to NMDA-, -opioid, and μ-opioid receptors was found.

7. Experimental

7.1. 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):[18] 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); temperature 20 °C. Elemental analyses: 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 1605 FT-IR (Perkin–Elmer). (br = broad, m = medium, s = strong). 1H NMR (300 MHz), ^{13}C NMR (75 MHz), ^{19}F NMR (282 MHz): Unity 300 FT NMR spectrometer (Varian), δ in ppm related to tetramethylsilane, coupling constants are given with 0.5 Hz resolution; the assignments of ^{13}C and ^{1}H NMR signals were supported by 2D NMR techniques. HPLC: Gradientpump 2249 Pharmacia, UV-detector VWM 2141 Pharmacia $\lambda = 254$ nm, Integrator Chromatopac C-R6A Shimadzu, column: LiChroCART® 250-4 Merck with LiChrospher® 100 RP-18 endcapped, injection volume: 20 μL. The data of compounds **5**, **6b**,**c**,**k**, **3b**,**c**, and **3k** are described in Ref. 9.

7.2. (–)-(6*S*,9*R*)-4-Methyl-9-phenyl-8-oxa-1,4-diazabicyclo[4.3.0]nonane-2,5-dione (*cis*-6a) and (–)-(6*S*,9*S*)-4-methyl-9-phenyl-8-oxa-1,4-diazabicyclo[4.3.0]nonane-2,5-dione (*trans*-6a)

A solution of methylamine in ethanol (8 mol/L, 0.11 mL, 0.88 mmol) was added to a solution of **5** (*cis*-**5**/*trans*-**5** = 30:70, 0.25 g, 0.88 mmol), and triethylamine (0.12 mL, 0.88 mmol) in acetonitrile (50 mL) and the mixture was heated to reflux for 96 h. Removal of the solvent in vacuo followed by flash chromatography (3 cm, ethyl acetate/ethanol 5:1, 5 mL) gave *cis*-**6a**, *cis*-**6a**/*trans*-**6a** (yield 0.13 g, 60%) and *trans*-**6a**.

cis-**6a** ($R_f = 0.45$): Colorless solid, mp 166–168 °C (diethyl ether), yield 0.056 g (26%). $[\alpha]_{589}$ −171.7 (*c* 0.34, CH_2Cl_2). $C_{13}H_{14}N_2O_3$ (246.27). HRMS: calcd 246.1004, found 246.1000 (−0.4 ppm). MS (EI): *m/z* (%) = 246 (M, 100), 231 (M−methyl, 1), 169 (M−phenyl, 7), 141 (M−PhCHO, 24), 105 (PhCHO, 74), 91 (benzyl, 15). IR (KBr): *ν* (cm^{-1}) = 2896 and 2889 (m, C–H), 1654 (s, C=O), 1441 (m, C–H), 750 and 699 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 3.01$ (s, 3H, CH_3), 3.83 (d, $J = 16.8$ Hz, 1H, 3-H), 4.14 (t, $J = 8.2$ Hz, 1H, 7-H), 4.25 (dd, $J = 16.6/1.7$ Hz, 1H, 3-H), 4.40 (t, $J = 7.6$ Hz, 1H, 6-H), 4.54 (dd, $J = 8.6/7.2$ Hz, 1H, 7-H), 6.48 (s, 1H, 9-H), 7.34–7.41 (m, 3H, arom), 7.46–7.49 (m, 2H, arom).

trans-**6a** ($R_f = 0.37$): Colorless solid, mp 162–164 °C (diethyl ether), yield 0.006 g (3%). $[\alpha]_{589}$ −54.8 (*c* 0.315, CH_2Cl_2). $C_{13}H_{14}N_2O_3$ (246.27). HRMS: calcd 246.1004, found 246.1005 (+0.1 ppm). MS (EI): *m/z* (%) = 246 (M, 100), 231 (M−methyl, 1), 169 (M−phenyl, 13), 141 (M−PhCHO, 28), 105 (PhCHO, 66), 91 (benzyl, 17). IR (KBr): *ν* (cm^{-1}) = 2901 and 2883 (m, C–H), 1668 (s, C=O), 1439 (m, C–H), 758 and 697 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 3.07$ (s, 3H, CH_3), 3.84 (d, $J = 17.0$ Hz, 1H, 3-H), 4.26 (d, $J = 17.2$ Hz, 1H, 3-H), 4.28 (t, $J = 6.6$ Hz, 1H, 7-H), 4.45 (t, $J = 6.8$ Hz, 1H, 7-H), 4.49 (t, $J = 7.2$ Hz, 1H, 6-H), 6.25 (s, 1H, 9-H), 7.33–7.41 (m, 5H, arom).

7.3. (–)-(6*S*,9*R*)-4-(4-Methoxybenzyl)-9-phenyl-8-oxa-1,4-diazabicyclo[4.3.0]nonane-2,5-dione (*cis*-6d) and (+)-(6*S*,9*S*)-4-(4-methoxybenzyl)-9-phenyl-8-oxa-1,4-diazabicyclo-[4.3.0]nonane-2,5-dione (*trans*-6d)

A solution of **5** (*cis*-**5**/*trans*-**5** = 30:70, 0.25 g, 0.88 mmol), 4-methoxybenzylamine (0.11 mL, 0.88 mmol) and triethylamine (0.12 mL, 0.88 mmol) in acetonitrile (50 mL) was heated to reflux for 40 h. Removal of the solvent in vacuo followed by flash chromatography (3 cm, ethyl acetate/ethanol 5:1, 5 mL) gave *cis*-**6d**, *cis*-**6d**/*trans*-**6d** (yield 0.165 g, 53%), and *trans*-**6d**.

cis-**6d** ($R_f = 0.73$): Colorless solid, mp 124 °C (diethyl ether), yield 0.045 g (15%). $[\alpha]_{589}$ −125.8 (*c* 0.545, CH_2Cl_2). $C_{20}H_{20}N_2O_4$ (352.39). Calcd C 68.17, H 5.72, N 7.95. Found C 67.9, H 5.75, N 7.56. HRMS: calcd 352.1423, found 352.1431 (+0.8 ppm). MS (EI): *m/z* (%) = 352 (M, 51), 231 (M−4-methoxybenzyl, 10), 121 (4-methoxybenzyl, 100), 105 (PhCHO, 16), 91 (benzyl, 15). IR (KBr): (cm^{-1}) = 2899 and 2837 (w, C–H), 1661 (s, C=O), 1441 (m, C–H), 757 and 699 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 3.79$ (d, $J = 16.6$ Hz, 1H, 3-H), 3.80 (s, 3H, OCH_3), 4.05 (dd, $J = 16.7/1.7$ Hz, 1H, 3-H), 4.21 (t, $J = 8.2$ Hz, 1H, 7-H), 4.41 (d, $J = 14.3$ Hz, 1H, H_3COPhCH_2N), 4.45 (td, $J = 7.6/1.3$ Hz, 1H, 6-H), 4.58 (dd, $J = 8.4/7.2$ Hz, 1H, 7-H), 4.71 (d, $J = 14.4$ Hz, 1H, H_3COPhCH_2N), 6.49 (s, 1H, 9-H), 6.87 (d, $J = 8.9$ Hz, 2H, 3′-H and 5′-H [H_3COPh]), 7.19 (d, $J = 8.9$ Hz, 2H, 2′-H and 6′-H [H_3COPh]), 7.33–7.40 (m, 3H, arom), 7.44–7.47 (m, 2H, arom).

trans-**6d** ($R_f = 0.60$): Colorless solid, mp 160–162 °C (diethyl ether), yield 0.008 g (1%). $[\alpha]_{589}$ +9.2 (*c* 0.12, CH_2Cl_2). $C_{20}H_{20}N_2O_4$ (352.39). Calcd C 68.17, H 5.72, N 7.95, found C 67.84, H 5.9, N 7.74. HRMS: calcd 352.1423, found 352.1431 (+0.8 ppm). MS (EI): *m/z* (%) = 352 (M, 44), 231 (M−4-methoxybenzyl, 6), 121 (4-methoxybenzyl, 100), 105 (PhCHO, 7), 91 (benzyl, 13). IR (KBr): *ν* (cm^{-1}) = 2896 (w, C–H), 1671 (s, C=O), 1437 (m, C–H), 757 and 699 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 3.78$ (d, $J = 16.9$ Hz, 1H, 3-H), 3.81 (s, 3H, OCH_3), 4.06 (d, $J = 17.3$ Hz, 1H, 3-H), 4.29 (t, $J = 8.1$ Hz, 1H, 7-H), 4.43–4.53 (m, 2H, 6-H and 7-H), 4.53 (d, $J = 14.4$ Hz, 1H, H_3COPhCH_2N), 4.66 (d, $J = 14.0$ Hz, 1H, H_3COPhCH_2N), 6.24 (s, 1H, 9-H), 6.88 (d, $J = 8.5$ Hz, 2H, 3′-H and 5′-H [H_3COPh]), 7.20 (d, $J = 8.5$ Hz, 2H, 2′-H and 6′-H [H_3COPh]), 7.26–7.30 (m, 3H, arom), 7.34–7.37 (m, 2H, arom).

7.4. (–)-(6*S*,9*R*)-9-Phenyl-4-(2-phenylethyl)-8-oxa-1,4-diazabicyclo[4.3.0]nonane-2,5-dione (*cis*-6e) and (–)-(6*S*,9*S*)-9-phenyl-4-(2-phenylethyl)-8-oxa-1,4-diazabicyclo[4.3.0]nonane-2,5-dione (*trans*-6e)

A solution of **5** (*cis*-**5**/*trans*-**5** = 30:70, 0.25 g, 0.88 mmol), 2-phenylethylamine (0.11 mL, 0.88 mmol) and triethylamine (0.12 mL, 0.88 mmol) in acetonitrile (50 mL) was heated to reflux for 48 h. Removal of the solvent in vacuo followed by flash chromatography (3 cm, ethyl acetate/ethanol 5:1, 7 mL) gave *cis*-**6**, *cis*-**6e**/*trans*-**6e** (yield 0.243 g, 82%), and *trans*-**6e**.

cis-**6e** ($R_f = 0.66$): Pale yellow oil, yield 0.022 g (7%), $[\alpha]_{589}$ −136.1 (*c* 1.08, CH_2Cl_2). $C_{20}H_{20}N_2O_3$ (336.39). HRMS: calcd 336.1474, found 336.1475 (+0.1 ppm). MS (EI): *m/z* (%) = 336 (M, 55), 245 (M−benzyl, 6), 231 (M−phenylethyl, 15), 104 (PhCN, 100), 91 (benzyl, 21). IR (film): *ν* (cm^{-1}) = 2886 (m, C–H), 1660 (s, C=O), 1449 (m, C–H), 1219 (m, C–O), 744 and 697 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 2.91$ (t, $J = 7.4$ Hz, 2H, PhCH_2CH$_2$N), 3.59 (dt, $J = 13.7/7.1$ Hz, 1H, PhCH$_2$CH_2N), 3.70 (d, $J = 16.5$ Hz, 1H, 3-H), 3.79 (dt, $J = 13.8/7.0$ Hz, 1H, PhCH$_2$CH_2N), 4.04 (dd, $J = 16.6/1.6$ Hz, 1H, 3-H), 4.13 ('t', $J = 8.5$ Hz, 1H, 7-H), 4.35 (td, $J = 7.5/1.6$ Hz, 1H, 6-H), 4.51 (dd, $J = 8.6/7.4$ Hz, 1H, 7-H), 6.45 (s, 1H, 9-H), 7.19–7.48 (m, 10H, arom).

trans-**6e** ($R_f = 0.55$): Pale yellow oil, yield 0.002 g (1%), $[\alpha]_{589}$ −20.3 (*c* 0.1, CH_2Cl_2). $C_{20}H_{20}N_2O_3$ (336.39). HRMS: calcd 336.1474, found 336.1475 (+0.1 ppm). MS (EI): *m/z* (%) = 336 (M, 67), 245 (M−benzyl, 8), 231 (M−phenylethyl, 28), 104 (PhCN, 100), 91 (benzyl, 35). IR (film): *ν* (cm^{-1}) = 2925 (m, C–H), 1671 (s, C=O), 1433 (m, C–H), 1227 (m, C–O), 750 and 699 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 2.89$–2.96 (m, 2H, PhCH_2CH$_2$N), 3.63 (d, $J = 17.0$ Hz, 1H, 3-H), 3.66 (dt, $J = 13.4/7.0$ Hz, 1H, PhCH$_2$CH_2N), 3.80 (dt, $J = 13.5/6.9$ Hz, 1H, PhCH$_2$CH_2N), 3.99 (br d, $J = 17.0$ Hz, 1H, 3-H), 4.23 (t, $J = 12.0$ Hz, 1H, 7-H), 4.40 (ddd, $J = 12.2/6.8/1.2$ Hz, 1H, 6-H), 4.41 (dd, $J = 11.9/6.9$ Hz, 1H, 7-H), 6.20 (s, 1H, 9-H), 7.19–7.39 (m, 10H, arom).

7.5. (–)-(6*S*,9*R*)-4-Cyclohexyl-9-phenyl-8-oxa-1,4-diazabicyclo[4.3.0]nonane-2,5-dione (*cis*-6f) and (–)-(6*S*,9*S*)-4-cyclohexyl-9-phenyl-8-oxa-1,4-diazabicyclo[4.3.0]nonane-2,5-dione (*trans*-6f)

A solution of **5** (*cis*-**5**/*trans*-**5** = 30:70, 0.254 g, 0.89 mmol), cyclohexylamine (0.10 mL, 0.89 mmol), and triethylamine (0.12 mL, 0.89 mmol) in acetonitrile (50 mL) was heated to reflux for 96 h. Removal of the solvent in vacuo followed by flash chromatography (3 cm, ethyl acetate/ethanol 5:1, 5 mL) gave *cis*-**6f**, *cis*-**6f**/*trans*-**6f** (yield 0.115 g, 41%), and *trans*-**6f**.

cis-**6f** ($R_f = 0.80$): Colorless solid, mp 154 °C, yield 0.043 g (15%). $[\alpha]_{589}$ −130.3 (*c* 0.44, CH_2Cl_2). $C_{18}H_{22}N_2O_3$ (314.38). Calcd C 68.77, H 7.05, N 8.91, found C 68.36, H 7.39, N 8.66. MS (EI): *m*/*z* (%) = 314 (M, 100), 231 (M−cyclohexyl, 20), 105 (PhCHO, 36), 82 (cyclohexyl, 10). IR (KBr): *v* (cm^{-1}) = 2929 and 2856 (m, C–H), 1652 (s, C=O), 1444 (m, C–H), 741 and 698 (m, aryl-C–H). ^1H NMR ($CDCl_3$): δ = 1.05–1.19 (m, 1H, cyclohexyl), 1.32–1.49 (m, 4H, cyclohexyl), 1.68–1.73 (m, 3H, cyclohexyl), 1.83–1.87 (m, 2H, cyclohexyl), 3.89 (d, *J* = 16.5 Hz, 1H, 3-H), 4.02 (dd, *J* = 16.5/1.5 Hz, 1H, 3-H), 4.22 (t, *J* = 8.3 Hz, 1H, 7-H), 4.37–4.60 (m, 1H, NC$H_{cyclohexyl}$), 4.41 (td, *J* = 7.3/1.4 Hz, 1H, 6-H), 4.55 (dd, *J* = 8.5/7.3 Hz, 1H, 7-H), 6.49 (s, 1H, 9-H), 7.36–7.43 (m, 3H, arom), 7.47–7.50 (m, 2H, arom).

trans-**6f** ($R_f = 0.69$): Colorless solid, mp 151 °C, yield 0.005 g (2%). $[\alpha]_{589}$ −34.8 (*c* 0.25, CH_2Cl_2). $C_{18}H_{22}N_2O_3$(314.38). Calcd C 68.77, H 7.05, N 8.91, found C 68.27, H 7.27, N 8.66. MS (EI): *m*/*z* (%) = 314 (M, 100), 237 (M−phenyl, 7), 231 (M−cyclohexyl, 17), 105 (PhCHO, 29), 82 (cyclohexyl, 14). IR (KBr): *v* (cm^{-1}) = 2929 and 2856 (m, C–H), 1667 (s, C=O), 1435 (m, C–H), 757 and 698 (m, aryl-C–H). ^1H NMR ($CDCl_3$): δ = 1.07–1.13 (m, 1H, cyclohexyl), 1.26–1.48 (m, 4H, cyclohexyl), 1.62–1.87 (m, 5H, cyclohexyl), 3.90 (d, *J* = 17.1 Hz, 1H, 3-H), 3.98 (d, *J* = 17.1 Hz, 1H, 3-H), 4.25 (t, *J* = 7.3 Hz, 1H, 7-H), 4.38–4.49 (m, 2H, 6-H and NC$H_{cyclohexyl}$), 4.42 (t, *J* = 7.0 Hz, 1H, 7-H), 6.25 (s, 1H, 9-H), 7.26–7.30 (m, 2H, arom), 7.34–7.38 (m, 3H, arom).

7.6. (6*S*,9*R*) and (6*S*,9*S*)-4-*tert*-butyl-9-phenyl-8-oxa-1,4-diazabicyclo[4.3.0]nonane-2,5-dione (*cis*/*trans*-6g)

A solution of **5** (*cis*-**5**/*trans*-**5** = 90:10, 0.10 g, 0.35 mmol), *tert*-butylamine (37 μL, 0.35 mmol) and triethylamine (50 μL, 0.35 mmol) in DMF (20 mL) was heated to reflux for 72 h. Removal of the solvent in vacuo followed by flash chromatography (2 cm, ethyl acetate/petroleum ether 1:1, 3 mL) provided **6g** (*cis*-**6g**/*trans*-**6g** = 83:17).

cis-**6g**/*trans*-**6g** ($R_f = 0.67$): Colorless solid, mp 118–123 °C, yield 0.003 g (3%). $C_{16}H_{20}N_2O_3$ (288.35). MS (EI): *m*/*z* (%) = 288 (M, 100), 273 (M−methyl, 9), 231 (M−*tert*-butyl, 35), 105 (PhCHO, 45), 91 (benzyl, 23), 57 (*tert*-butyl, 37). IR (KBr): *v* (cm^{-1}) = 2925 (m, C–H), 1661 (s, C=O), 1443 (m, C–H), 756 and 699 (m, aryl-C–H). ^1H NMR ($CDCl_3$): δ = 1.48 (s, 9 × 0.83H, CH_3) *cis*), 1.54 (s, 9 × 0.17H, CH_3) *trans*), 4.06–4.08 (m, 2H, 3-H), 4.22 (dd, *J* = 8.5/7.3 Hz, 1H, 7-H), 4.36 (t, *J* = 7.2 Hz, 1H, 6-H), 4.49 (dd, *J* = 8.3/7.2 Hz, 1H, 7-H), 6.37 (s, 0.17H, 9-H *trans*), 6.48 (s, 0.83H, 9-H *cis*), 7.36–7.41 (m, 3H, arom), 7.45–7.49 (m, 2H, arom).

7.7. (–)-(6*S*,9*R*)-4,9-Diphenyl-8-oxa-1,4-diazabicyclo-[4.3.0]nonane-2,5-dione (*cis*-6h) and (–)-(6*S*,9*S*)-4,9-diphenyl-8-oxa-1,4-diazabicyclo[4.3.0]nonane-2,5-dione (*trans*-6h)

A solution of **5** (*cis*-**5**/*trans*-**5** = 90:10, 0.50 g, 1.76 mmol), aniline (0.16 mL, 1.76 mmol), and triethylamine (0.25 mL, 1.76 mmol) in DMF (30 mL) was heated to reflux for 24 h. The reaction mixture was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (15 mL), the solution was washed with HCl (0.5 N, 2 × 10 mL), NaOH (0.5 N, 2 × 10 mL) and water (10 mL), dried with Na_2SO_4, and evaporated. Purification of the residue by flash chromatography (3 cm, ethyl acetate/petroleum ether 7:3, 10 mL) gave *cis*-**6h** and *trans*-**6h**.

cis-**6h** ($R_f = 0.52$): Pale yellow solid, mp 104–108 °C, yield 0.041 g (8%). $[\alpha]_{589}$ −157.8 (*c* 0.225, CH_2Cl_2). $C_{18}H_{16}N_2O_3$ (308.34). HRMS: calcd 308.1161, found 308.1162 (+0.1 ppm). MS (EI): *m*/*z* (%) = 308 (M, 59), 231 (M−phenyl, 3), 105 (PhCHO, 100), 91 (benzyl, 32), 77 (phenyl, 51). IR (KBr): *v* (cm^{-1}) = 2924 (m, C–H), 1677 (s, C=O), 1445 (m, C–H), 742 and 698 (m, aryl-C–H). ^1H NMR ($CDCl_3$): δ = 4.21 (d, *J* = 16.1 Hz, 1H, 3-H), 4.39 (dd, *J* = 11.6/10.3 Hz, 1H, 7-H), 4.56–4.62 (m, 2H, 6-H and 7-H), 4.69 (dd, *J* = 16.2/1.3 Hz, 1H, 3-H), 6.58 (s, 1H, 9-H), 7.28–7.35 (m, 3H, arom), 7.38–7.47 (m, 5H, arom), 7.51–7.54 (m, 2H, arom).

trans-**6h** ($R_f = 0.28$): Pale yellow, viscous oil, yield 0.005 g (1%), $[\alpha]_{589}$ −47.3 (*c* 0.23, CH_2Cl_2). $C_{18}H_{16}N_2O_3$ (308.34). HRMS: calcd 308.1161, found 308.1162 (+0.1 ppm). MS (EI): *m*/*z* (%) = 308 (M, 100), 231 (M−phenyl, 2), 105 (PhCHO, 3), 77 (phenyl, 2). IR (film): *v* (cm^{-1}) = 2935 (m, C–H), 1684 (s, C=O), 1434 (m, C–H), 756 and 698 (m, aryl-C–H). ^1H NMR ($CDCl_3$): δ = 4.22 (d, *J* = 16.4 Hz, 1H, 3-H), 4.39 (t, *J* = 9.2 Hz, 1H, 7-H), 4.50 (dd, *J* = 9.2/7.0 Hz, 1H, 7-H), 4.67 (ddd, *J* = 9.5/7.0/0.9 Hz, 1H, 6-H), 4.68 (dd, *J* = 16.4/0.9 Hz, 1H, 3-H), 6.33 (s, 1H, 9-H), 7.30–7.35 (m, 4H, arom), 7.40–7.48 (m, 6H, arom).

7.8. (–)-(6*S*,9*R*)-4-(2-Hydroxyethyl)-9-phenyl-8-oxa-1,4-diazabicyclo[4.3.0]nonane-2,5-dione (*cis*-6i) and (–)-(6*S*,9*S*)-4-(2-hydroxyethyl)-9-phenyl-8-oxa-1,4-diazabicyclo[4.3.0]-nonane-2,5-dione (*trans*-6i)

A solution of **5** (*cis*-**5**/*trans*-**5** = 90:10, 0.50 g, 1.76 mmol), 2-aminoethanol (0.11 mL, 1.76 mmol), and triethylamine (0.25 mL, 1.76 mmol) in acetonitrile (100 mL) was heated to reflux for 30 h. Removal of the solvent in vacuo followed by flash chromatography (4 cm, ethyl acetate/ethanol 5:1, 15 mL) gave *cis*-**6i**, *cis*-**6i**/*trans*-**6i** (yield 0.088 g, 18%), and *trans*-**6i**.

cis-**6i** ($R_f = 0.39$): Colorless solid, mp 132–134 °C, yield 0.318 g (65%). $[\alpha]_{589}$ −177.9 (*c* 0.945, CH_2Cl_2). $C_{14}H_{16}N_2O_4$ (276.29). Calcd C 60.86, H 5.84, N 10.14, found C 60.49, H 5.68, N 9.84. MS (EI): *m/z* (%) = 276 (M, 60), 245 (M−CH_2OH, 9), 231 (M−CH_2CH_2OH, 5), 199 (M−phenyl, 6), 105 (PhCHO, 73), 91 (benzyl, 33). IR (KBr): *v* (cm^{-1}) = 3442 (br, OH), 2926 (m, C–H), 1663 (s, C=O), 1456 (m, C–H), 1226 (m, C–O), 756 and 697 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 2.65$ (br s, 1H, O*H*), 3.39–3.47 (m, 1H, CH_2CH_2OH), 3.66–3.78 (m, 3H, CH_2CH2OH), 3.97 (d, $J = 16.5$ Hz, 1H, 3-H), 4.18 (dd, $J = 8.1/7.3$ Hz, 1H, 7-H), 4.35 (dd, $J = 16.5/1.7$ Hz, 1H, 3-H), 4.42 (td, $J = 7.3/1.7$ Hz, 1H, 6-H), 4.50 (t, $J = 8.1$ Hz, 1H, 7-H), 6.45 (s, 1H, 9-H), 7.30–7.39 (m, 3H, arom), 7.42–7.45 (m, 2H, arom).

trans-**6i** ($R_f = 0.25$): Pale yellow, viscous oil, yield 0.051 g (10%). $[\alpha]_{589}$ −64.3 (*c* 1.43, CH_2Cl_2). $C_{14}H_{16}N_2O_4$ (276.29). Calcd C 60.86, H 5.84, N 10.14, found C 60.34, H 5.55, N 9.79. MS (EI): *m/z* (%) = 276 (M, 34), 245 (M−CH_2OH, 7), 231 (M−CH_2CH_2OH, 4), 199 (M−phenyl, 11), 105 (PhCHO, 55), 91 (benzyl, 33). IR (film): *v* (cm^{-1}) = 3444 (br, OH), 2944 (m, C–H), 1668 (s, C=O), 1451 (m, C–H), 1231 (m, C–O), 1070 (m, C–OH), 756 and 692 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 2.20$ (br s, 1H, O*H*), 3.38–3.49 (m, 1H, CH_2CH_2OH), 3.74–3.84 (m, 3H, CH_2CH_2OH), 3.93 (d, $J = 17.1$ Hz, 1H, 3-H), 4.24 (t, $J = 9.0$ Hz, 1H, 7-H), 4.34 (dd, $J = 16.9/1.4$ Hz, 1H, 3-H), 4.41 (t, $J = 9.0$ Hz, 1H, 7-H), 4.49 (td, $J = 9.0/1.4$ Hz, 1H, 6-H), 6.23 (s, 1H, 9-H), 7.30–7.40 (m, 5H, arom).

7.9. (–)-(6*S*,9*R*)-4-(3-Hydroxypropyl)-9-phenyl-8-oxa-1,4-diazabicyclo[4. 3.0]nonane-2,5-dione (*cis*-6j) and (–)-(6*S*,9*S*)-4-(3-hydroxypropyl)-9-phenyl-8-oxa-1,4-diazabicyclo[4.3.0]-nonane-2,5-dione (*trans*-6j)

A solution of **5** (*cis*-**5**/*trans*-**5** = 90:10, 0.362 g, 1.28 mmol), 3-aminopropan-1-ol (0.10 mL, 1.28 mmol), and triethylamine (0.18 mL, 1.28 mmol) in acetonitrile (75 mL) was heated to reflux for 36 h. Removal of the solvent in vacuo followed by flash chromatography (4 cm, ethyl acetate/ethanol 5:1, 10 mL) gave *cis*-**6j**, *cis*-**6j**/*trans*-**6j** (yield 0.034 g, 9%), and *trans*-**6j**.

cis-**6j** ($R_f = 0.39$): Colorless solid, mp 107–108 °C, yield 0.246 g (66%). $[\alpha]_{589}$ −243.6 (*c* 0.515, CH_2Cl_2). $C_{15}H_{18}N_2O_4$ (290.32). Calcd C 62.06, H 6.25, N 9.65, found C 61.76, H 6.31, N 9.50. MS (EI): *m/z* (%) = 290 (M, 69), 259 (M−CH_2OH, 2), 245 (M−CH_2CH_2OH, 3), 231 (M−$CH_2CH_2CH_2OH$, 4), 213 (M−phenyl, 8), 105 (PhCHO, 100), 77 (phenyl, 25). IR (KBr): *v* (cm^{-1}) = 3433 (br, OH), 2882 (m, C–H), 1665 (s, C=O), 1449 (m, C–H), 1223 (m, C–O), 1056 (m, C–OH), 748 and 700 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 1.77$ ('quint', $J = 6.0$ Hz, 2H, $NCH_2CH_2CH_2OH$), 2.75 (br s, 1H, O*H*), 3.49–3.66 (m, 4H, $NCH_2CH_2CH_2OH$), 3.84 (d, $J = 16.6$ Hz, 1H, 3-H), 4.17 (dd, $J = 8.2/7.8$ Hz, 1H, 7-H), 4.24 (dd, $J = 16.6/1.7$ Hz, 1H, 3-H), 4.43 (td, $J = 7.6/1.6$ Hz, 1H, 6-H), 4.53 (dd, $J = 8.2/7.3$ Hz, 1H, 7-H), 6.48 (s, 1H, 9-H), 7.36–7.40 (m, 3H, arom), 7.45–7.48 (m, 2H, arom).

trans-**6j** ($R_f = 0.27$): Colorless, viscous oil, yield 0.040 g (11%). $[\alpha]_{589}$ −33.3 (*c* 0.725, CH_2Cl_2). $C_{15}H_{18}N_2O_4$(290.32). Calcd C 62.06, H 6.25, N 9.65, found C 61.92, H 6.33, N 9.37. MS (EI): *m/z* (%) = 290 (M, 7), 259 (M−CH_2OH, 1), 245 (M−CH_2CH_2OH, 1), 231 (M−$CH_2CH_2CH_2OH$, 1), 213 (M−phenyl, 3), 105 (PhCHO, 27), 91 (benzyl, 15), 77 (phenyl, 16). IR (film): *v* (cm^{-1}) = 3420 (br, OH), 2926 (m, C–H), 1668 (s, C=O), 1444 (m, C–H), 1233 (m, C–O), 1054 (m, C–OH), 758 and 699 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 1.73$ (br s, 1 H, O*H*), 1.81 ('quint', $J = 6.0$ Hz, 2H, $NCH_2CH_2CH_2OH$), 3.57–3.72 (m, 4H, $NCH_2CH_2CH_2OH$), 3.84 (d, $J = 16.9$ Hz, 1H, 3-H), 4.26 (dd, $J = 16.9/1.3$ Hz, 1H, 3-H), 4.28 (t, $J = 8.9$ Hz, 1H, 7-H), 4.45 (dd, $J = 8.9/7.0$ Hz, 1H, 7-H), 4.52 (td, $J = 7.0/1.3$ Hz, 1H, 6-H), 6.26 (s, 1H, 9-H), 7.33–7.40 (m, 5H, arom).

7.10. (2*R*,4*S*)- and (2*S*,4*S*)-Methyl 3-(azidoacetyl)-2-phenyl-1,3-oxazolidine-4-carboxylate (*cis*-7/*trans*-7)

A solution of **5** (*cis*-**5**/*trans*-**5** = 90:10, 1.84 g, 6.48 mmol), and sodium azide (1.265 g, 19.5 mmol) in acetone (150 mL) was heated to reflux for 16 h. The solvent was removed under reduced pressure. The residue was dissolved in diethyl ether (30 mL), washed with a half-saturated solution of NaCl (30 mL) and the organic layer was separated. The aqueous layer was extracted with diethyl ether (3 × 20 mL). The organic layers were combined, dried with Na_2SO_4, and concentrated in vacuo. The residue was characterized without further purification and was converted into **6m**.

cis-**7**/*trans*-**7**: Pale yellow, viscous oil, yield 1.881 g (99%). $C_{13}H_{14}N_4O_4$ (290.28). MS (EI): *m/z* (%) = 248 (M−N_3, 95), 234 (M−CH_2N_3, 51), 206 (M−$COCH_2N_3$, 79), 148 (2-phenyl-1,3-oxazolidine, 26), 105 (PhCHO, 68). MS (CI): *m/z* (%) = 307 (M+NH_3, 100), 291 (MH^+, 23). IR (film): *v* (cm^{-1}) = 2954 (m, C–H), 2108 (s, N_3), 1745 (s, C=O ester), 1666 (s, C=O amide), 1434 (s, C–H), 1370 and 1213 (m, C–O), 733 and 701 (s, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 3.36$ (d, $J = 15.9$ Hz, 1H, CH_2N_3), 3.56 (d, $J = 15.9$ Hz, 1H, CH_2N_3), 3.83 (s, 3 × 0.9H, OCH_3 *cis*), 3.84 (s, 3 × 0.1H, OCH_3 it trans), 4.09–4.16 (m, 1H, 5-H), 4.29 (dd, $J = 9.5/7.0$ Hz, 1H, 5-H), 4.90 (dd, $J = 10.2/7.1$ Hz, 1H, 4-H), 6.00 (br s, 0.1H, 2-H *trans*), 6.25 (s, 0.7H, 2-H *cis* rotamer 1), 6.51 (s, 0.2H, 2-H, *cis* rotamer 2), 7.35–7.50 (m, 4H, arom), 7.67–7.75 (m, 1H, arom).

7.11. (–)-(6*S*,9*R*)-9-Phenyl-8-oxa-1,4-diazabicyclo-[4.3.0]nonane-2,5-dione (*cis*-6m) and (–)-(6*S*,9*S*)-9-phenyl-8-oxa-1,4-diazabicyclo[4.3.0]nonane-2,5-dione (*trans*-6m)

(a) Reductive cyclization with $NaBH_4/CuSO_4$ following Ref. 11: $NaBH_4$ (82 mg, 2.16 .mmol) was added to a stirred and cooled (−5 °C) suspension of CH_3OH (70 mL) and $CuSO_4$ (0.010 g, 0.065 mmol). Then a solution of *cis*-**7**/*trans*-**7** (*cis*-**7**/*trans*-**7** = 90:10, 1.882 g, 6.48 mmol) dissolved in CH_3OH (20 mL) was added

dropwise. Subsequently $NaBH_4$ (163 mg, 4.32 mmol) was added in five portions within 1 h. It was allowed to warm to room temperature and the reaction mixture was stirred for 24 h. The mixture was filtered through a pad of Celite® AFA and the filtrate was concentrated in vacuo. Flash chromatography of the residue (6 cm, ethyl acetate/ethanol 5:1, 15 mL) yielded *cis*-**6m**, *cis*-**6m**/*trans*-**6m** (yield 0.412 g, 27%) and *trans*-**6m**.

cis-**6m** ($R_f = 0.38$): Colorless solid, mp 174–176 °C, yield 0.668 g (44%). $[\alpha]_{589}$ −244.1 (*c* 0.37, CH_3OH). $C_{12}H_{12}N_2O_3$ (232.24). Calcd C 62.06, H 5.21, N 12.06, found C 61.84, H 5.26, N 11.90. MS (EI): *m/z* (%) = 232 (M, 100), 155 (M−phenyl, 11), 105 (PhCHO, 86), 91 (benzyl, 15), 77 (phenyl, 18). IR (KBr): *v* (cm^{-1}) = 3244 (br, NH), 2845 (m, C–H), 1667 (s, C=O), 1454 (m, C–H), 744 and 698 (m, aryl-C–H). ^1H NMR ($CDCl_3$): $\delta = 3.94$ (dd, $J = 16.9/4.3$ Hz, 1H, 3-H), 4.20 (dd, $J = 16.5/1.6$ Hz, 1H, 3-H), 4.22 (t, $J = 7.9$ Hz, 1H, 7-H), 4.42 (td, $J = 7.5/1.6$ Hz, 1H, 6-H), 4.53 (t, $J = 7.8$ Hz, 1H, 7-H), 6.48 (br s, 1H, N*H*), 6.52 (s, 1H, 9-H), 7.36–7.43 (m, 3H, arom), 7.47–7.50 (m, 2H, arom).

trans-**6m**($R_f = 0.29$) (contains 5% *cis*-**6m**): Colorless solid, mp 218–219 °C, yield 0.079 g (5%). $[\alpha]_{589}$ −63.1 (*c* 0.535, CH_3OH). $C_{12}H_{12}N_2O_3$ (232.24). Calcd C 62.06, H 5.21, N 12.06, found C 62.33, H 5.09, N 12.22. MS (EI): *m/z* (%) = 232 (M, 89), 155 (M−phenyl, 11), 105 (PhCHO, 100), 91 (benzyl, 22), 77 (phenyl, 28). IR (KBr): *v* (cm^{-1}) = 3317 (br, NH), 2871 (m, C–H), 1666 (s, C=O), 1434 (m, C–H), 764 and 698 (m, aryl-C–H). ^1H NMR ($CDCl_3$): $\delta = 3.91$ (dd, $J = 16.9/4.7$ Hz, 1H, 3-H), 4.16 (d, $J = 17.1$ Hz, 1H, 3-H), 4.26 (t, $J = 8.9$ Hz, 1H, 7-H), 4.43 (dd, $J = 8.8/7.0$ Hz, 1H, 7-H), 4.50 (br t, $J = 7.5$ Hz, 1H, 6-H), 6.27 (s, 1H, 9-H), 6.31 (br s, 1H, N*H*), 7.33–7.40 (m, 5H, arom).

(b) Catalytic hydrogenation: Pd/C (10%, 0.20 g) was added to a solution of *cis*-**7**/*trans*-**7** (*cis*-**7**/*trans*-**7** = 30:70, 2.12 g, 6.91 mmol) in CH_3OH (60 mL). The suspension was stirred under a H_2 atmosphere (balloon) at room temperature for 8 h. The mixture was filtered through a pad of Celite® AFA and the filtrate was concentrated in vacuo. Flash chromatography (6 cm, ethyl acetate/ethanol 5:1, 20 mL) of the residue yielded *cis*-**6m**/*trans*-**6m** as a colorless solid, yield 1.21 g (75%).

7.12. (+)-[(2*R*)-1-Benzyl-4-methylpiperazin-2-yl]methanol (3a)

Under N_2 $LiAlH_4$ powder (0.45 g, 11.8 mmol) was added to a stirred solution of **6a** (mixture of *cis*/*trans* isomers, 0.292 g, 1.18 mmol) in THF (50 mL). The reaction mixture was heated to reflux for 87 h. Under N_2 atmosphere and cooling (ice bath) water (0.5 mL), 3 N NaOH (0.5 mL), and again water (0.5 mL) were successively added. The suspension was refluxed for 30 min. After the mixture was cooled to room temperature, the precipitate was filtered off and the solvent removed under reduced pressure. The residue was purified by flash chromatography (2 cm, ethanol, 5 mL, $R_f = 0.17$) and gave **3a** as a colorless solid, mp 86.5–87 °C, yield 0.114 g (44%). $[\alpha]_{589}$ +25.5 (*c* 0.55, CH_2Cl_2). $C_{13}H_{20}N_2O$ (220.31). Calcd C 70.87, H 9.15, N 12.72, found C 70.68, H 9.39, N 12.70. MS (EI): *m/z* (%) = 220 (M, 1), 189 (M−CH_2OH, 100), 98 (M−CH_2OH−benzyl, 9), 91 (benzyl, 55). MS (CI): *m/z* (%) = 221 (MH^+, 100), 189 (M−CH_2OH, 14). IR (KBr): *v* (cm^{-1}) = 3390 (br, OH), 2937 (m, C–H), 2800 (s, C–H), 1450 (m, C–H), 735 and 698 (m, aryl-C–H). ^1H NMR ($CDCl_3$): $\delta = 2.27$ (s, 3H, C*H*$_3$), 2.27–2.31 (m, 1H, 5-H, or 6-H), 2.40–2.56 (m, 3H, 3-H, 5-H, and 6-H), 2.61–2.70 (m, 2H, 2-H, and 3-H), 2.90–2.98 (m, 1H, 6-H or 5-H), 3.41 (d, $J = 13.4$ Hz, 1H, PhC*H*$_2$N), 3.60 (dd, $J = 11.3/2.4$ Hz, 1H, C*H*$_2$OH), 4.02 (d, $J = 13.4$ Hz, 1H, PhC*H*$_2$N), 4.06 (dd, $J = 11.3/4.0$ Hz, 1H, C*H*$_2$OH), 7.22–7.33 (m, 5H, arom). The signal for the proton of the OH group could not be detected. ^{13}C NMR ($CDCl_3$): $\delta = 46.1$ (1C, *C*$_3$), 50.1 and 54.5 (2C, C-5, C-6), 57.97 (1C, C-3), 57.99 (1C, N*C*H$_2$Ph), 59.0 (1C, C-2), 61.9 (1C, *C*H$_2$OH), 127.1 (1C, C-4′), 128.3 (2C, C-3′ and C-5′), 128.8 (2C, C-2′ and C-6′), 138.4 (1C, C-1′).

7.13. (+)-[(2*R*)-1-Benzyl-4-(4-methoxybenzyl)piperazin-2-yl]methanol (3d)

Under N_2 $LiAlH_4$ powder (0.236 g, 6.2 mmol) was added to a stirred solution of **6d** (mixture of *cis*/*trans* isomers, 0.219 g, 0.62 mmol) in THF (50 mL). The reaction mixture was heated to reflux for 72 h. Under N_2 atmosphere and cooling (ice bath) water (0.5 mL), 3 N NaOH (0.5 mL), and again water (0.5 mL) were successively added. The suspension was heated to reflux for 30 min. After the mixture was cooled to room temperature, the precipitate was filtered off, and the solvent removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (15 mL) and the solution was washed with 1 N NaOH (2 × 7 mL) and brine (7 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The organic layers were combined, dried with Na_2SO_4 and concentrated in vacuo. Purification of the residue by flash chromatography (2 cm, ethyl acetate, 5 mL, $R_f = 0.21$) furnished **3d** as a pale yellow oil, yield 0.149 g (73%). $[\alpha]_{589}$ +30.7 (*c* 0.59, CH_2Cl_2). $C_{20}H_{26}N_2O_2$ (326.44). Calcd C 73.59, H 8.03, N 8.58, found C 73.41, H 8.59, N 7.90. MS (EI): *m/z* (%) = 295 (M−CH_2OH, 25), 121 (4-methoxybenzyl, 100), 91 (benzyl, 21). MS (ESI): *m/z* (%) = 327 (MH^+, 100). IR (film): *v* (cm^{-1}) = 3385 (br, OH), 2934 (m, C–H), 2810 (m, C–H), 1453 (m, C–H), 740 and 699 (m, aryl-C–H). ^1H NMR ($CDCl_3$): $\delta = 2.32$–2.40 (m, 1H, 5-H or 6-H), 2.42–2.51 (m, 2H, 5-H and 6-H), 2.58–2.67 (m, 3H, 2-H and 3-H), 2.92–3.00 (m, 1H, 6-H or 5-H), 3.42 (s, 2H, $H_3COPhCH_2N$), 3.47 (d, $J = 13.5$ Hz, 1H, PhC*H*$_2$N), 3.58 (br d, $J = 10.9$ Hz, 1H, C*H*$_2$OH), 3.80 (s, 3H, OC*H*$_3$), 3.97 (d, $J = 13.5$ Hz, 1H, PhC*H*$_2$N), 4.05 (br d, $J = 10.8$ Hz, 1H, C*H*$_2$OH), 6.85 (d, $J = 8.5$ Hz, 2H, 3′-H and 5′-H [H_3COPh]), 7.20 (d, $J = 8.7$ Hz, 2H, 2′-H and 6′-H [H_3COPh]), 7.21–7.32 (m, 5H, arom). The signal for the proton of the OH group could not be detected. ^{13}C NMR ($CDCl_3$): $\delta = 49.8$ and 52.4 (2C, C-5, C-6), 55.2 (1C, O*C*$_3$), 55.9

(1C, C-3), 58.0 (1C, NCH_2Ph), 58.5 (1C, C-2), 62.2 (1C, CH_2OH), 62.5 (1C, H_3COPhCH_2N), 113.7 (2C, C-3′ and C-5′ [H_3COPh]), 127.1 (1C, C-4′ [bn]), 128.3 (2C, C-3′ and C-5′ [bn]), 128.8 (2C, C-2′ and C-6′ [bn]), 129.6 (1C, C-1′ [H_3COPh]), 130.3 (2C, C-2′ and C-6′ [H_3COPh]), 138.6 (1C, C-1′ [bn]), 158.8 (1C, C-4′ [H_3COPh]).

7.14. (+)-[(2*R*)-1-Benzyl-4-(2-phenylethyl)piperazin-2-yl]methanol (3e)

Under N_2 $LiAlH_4$ powder (0.268 g, 7.1 mmol) was added to a stirred solution of **6e** (mixture of *cis*/*trans* isomers, 0.237 g, 0.71 mmol) in THF (50 mL). The reaction mixture was heated to reflux for 72 h. Under N_2 atmosphere and cooling (ice bath) water (0.5 mL), 3 N NaOH (0.5 mL) and again water (0.5 mL) were successively added. The suspension was heated to reflux for 30 min. After the mixture was cooled to room temperature, the precipitate was filtered off, and the solvent removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (15 mL) and the solution was washed with 1 N NaOH (2 × 7 mL) and brine (7 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The organic layers were combined, dried with Na_2SO_4 and concentrated in vacuo. Purification of the residue by flash chromatography (3 cm, ethyl acetate, 10 mL, $R_f = 0.24$) furnished **3e** as a pale yellow, viscous oil, yield 0.178 g (82%). $[\alpha]_{589}$ +15.5 (*c* 0.545, CH_2Cl_2). $C_{20}H_{26}N_2O$ (310.44). Calcd C 77.38, H 8.44, N 9.02, found C 76.67, H 8.37, N 8.75. MS (EI): *m*/*z* (%) = 279 (M−CH_2OH, 100), 219 (M−benzyl, 38), 189 (M−benzyl−CH_2OH, 25), 91 (benzyl, 56). MS (ESI): *m*/*z* (%) = 311 (MH^+, 100). IR (film): *v* (cm^{-1}) = 3366 (br, OH), 2942 (m, C–H), 2810 (m, CO–H), 1452 (m, C–H), 1152 (m, C–OH), 737 and 697 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 2.42$–2.51 (m, 2H, 5-H and 6-H), 2.56–2.68 (m, 5H, 2-H, 3-H, 5-H, or 6-H and NCH_2CH_2Ph), 2.75–2.83 (m, 3H, 3-H and NCH_2CH_2Ph), 2.95–3.00 (m, 1H, 6-H or 5-H), 3.45 (d, $J = 13.3$ Hz, 1H, PhCH_2N), 3.61 (dd, $J = 11.5/2.1$ Hz, 1H, CH_2OH), 4.00 (d, $J = 13.0$ Hz, 1H, PhCH_2N), 4.08 (dd, $J = 11.3/2.8$ Hz, 1H, CH_2OH), 7.18–7.33 (m, 10H, arom). The signal for the proton of the OH group could not be detected. ^{13}C NMR ($CDCl_3$): $\delta = 33.5$ (1C, NCH_2CH_2Ph), 50.0 and 52.6 (2C, C-5, C-6), 56.3 (1C, C-3), 58.0 (1C, NCH_2Ph), 58.7 (1C, C-2), 60.3 (1C, NCH_2CH_2Ph), 62.2 (1C, CH_2OH), 126.1 and 127.1 (2 C, 2 × C-4 arom), 128.3 and 128.4 (4C, 2 × C-3 and 2 × C-5 arom), 128.6 and 128.8 (4C, 2 × C-2 and 2 × C-6 arom), 138.4 and 140.0 (2C, 2 × C-1 arom).

7.15. (+)-[(2*R*)-1-Benzyl-4-cyclohexylpiperazin-2-yl]methanol (3f)

Under N_2 $LiAlH_4$ powder (0.176 g, 4.64 mmol) was added to a stirred solution of **6f** (mixture of *cis*/*trans* isomers, 0.146 g, 0.464 mmol) in THF (30 mL). The reaction mixture was heated to reflux for 72 h. Under N_2 atmosphere and cooling (ice bath) water (0.5 mL), 3 N NaOH (0.5 mL) and again water (0.5 mL) were successively added. The suspension was heated to reflux for 30 min. After the mixture was cooled to room temperature, the precipitate was filtered off, and the solvent removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (15 mL) and the solution was washed with 1 N NaOH (2 × 7 mL) and brine (7 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The organic layers were combined, dried with Na_2SO_4 and concentrated in vacuo. Purification of the residue by flash chromatography (2 cm, ethyl acetate/ethanol, 5:1, 5 mL, $R_f = 0.30$) furnished **3f** as a viscous oil, which solidified in a refrigerator, mp 63–65 °C, yield 0.099 g (74%). $[\alpha]_{589}$ +14.1 (*c* 0.50, CH_2Cl_2). $C_{18}H_{28}N_2O$ (288.43). Calcd C 74.96, H 9.78, N 9.71, found C 74.34, H 10.36, N 9.43. MS (EI): *m*/*z* (%) = 257 (M−CH_2OH, 100), 175 (M−C_6H_{10}−CH_2OH, 11), 166 (M−benzyl−CH_2OH, 12), 91 (benzyl, 52), 83 (cyclohexyl, 8). MS (CI): *m*/*z* (%) = 289 (MH^+, 100). IR (film): *v* (cm^{-1}) = 3372 (br, OH), 2927 and 2852 (s, C–H), 2811 (m, CO–H), 1451 (m, C–H), 1155 (m, C–OH), 737 and 698 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 1.15$–1.26 (m, 5H, cyclohexyl), 1.60–1,64 (m, 1H, 4-$H_{cyclohexyl}$), 1.77–1.86 (m, 4H, cyclohexyl), 2.23–2.31 (m, 1H, $NCH_{cyclohexyl}$), 2.44–2.54 (m, 2H, 5-H and 6-H), 2.59–2.67 (m, 2H, 2-H and 5-H or 6-H), 2.74–2.81 (m, 2H, 3-H), 2.94–3.02 (m, 1H, 6-H or 5-H), 3.47 (d, $J = 13.2$ Hz, 1H, PhCH_2N), 3.62 (dd, $J = 11.4/2.5$ Hz, 1H, CH_2OH), 3.95 (d, $J = 13.6$ Hz, 1H, PhCH_2N), 4.13 (dd, $J = 11.4/2.7$ Hz, 1H, CH_2OH), 7.21–7.32 (m, 5H, arom). The signal for the proton of the OH group could not be detected. ^{13}C NMR ($CDCl_3$): $\delta = 26.0$ (2C, C-3, and C-5 cyclohexyl), 26.4 (1C, C-4 cyclohexyl), 28.8 and 28.9 (2C, C-2, and C-6 cyclohexyl), 48.8 and 50.6 (2C, C-5, C-6), 52.8 (1C, C-3), 58.4 (1C, NCH_2Ph), 58.6 (1C, C-2), 63.0 (1C, CH_2OH), 63.7 (1C, C-1 cyclohexyl), 127.2 (1C, C-4 arom), 128.5 (2C, C-3 and C-5 arom), 129.1 (2C, C-2 and C-6 arom), 138.8 (1C, C-1 arom).

7.16. (+)-[(2*R*)-1-Benzyl-4-(2-hydroxyethyl)piperazin-2-yl]methanol (3i)

To a stirred solution of 1 M $LiAlH_4$ in Et_2O (15.1 mL, 15.1 mmol) and THF (10 mL) was added dropwise a solution of **6i** (mixture of *cis*/*trans* isomers, 0.417 g, 1.51 mmol) in THF (30 mL) under N_2 atmosphere. The mixture was heated to reflux for 96 h. Under N_2 atmosphere and cooling (ice bath) water (0.4 mL), 3 N NaOH (0.4 mL) and again water (0.4 mL) were successively added. The suspension was heated to reflux for 30 min. After the mixture was cooled to room temperature, the precipitate was filtered off, and the solvent removed under reduced pressure. Purification of the residue by flash chromatography (3 cm, ethanol, 7 mL, $R_f = 0.25$) gave **3i** as a pale yellow, viscous oil, yield 0.210 g (55%). $[\alpha]_{589}$ +30.0 (*c* 0.415, CH_2Cl_2). $C_{14}H_{22}N_2O_2$ (250.34). HRMS: calcd 250.1681, found 250.1682 (+0.1 ppm), HRMS: calcd for M−CH_2OH ($C_{13}H_{19}N_2O$) 219.1497, found 219.1497 (+/−0 ppm). MS (EI): *m*/*z* (%) = 219 (M−CH_2OH, 46), 174 (benzylpiperazine, 6), 128 (M−benzyl−CH_2OH, 11), 91 (benzyl, 100). MS (CI): *m*/*z* (%) = 251 (MH^+, 100). IR (film): *v* (cm^{-1}) = 3375

(br, OH), 2943 and 2820 (m, C–H), 1446 (m, C–H), 1151 (m, C–OH), 738 and 700 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 2.30$ (td, $J = 11.6/2.4$ Hz, 1H, 5-H or 6-H), 2.39 (td, $J = 11.6/2.6$ Hz, 1H, 6-H or 5-H), 2.51–2.57 (m, 3H, 3-H and NCH_2CH_2OH), 2.60–2.68 (m, 2H, 2-H and 5-H or 6-H), 2.79 (ddd, $J = 10.6/2.8/1.8$ Hz, 1H, 3-H), 2.89 (ddd, $J = 11.6/5.1/2.5$ Hz, 1H, 6-H or 5-H), 3.35 (d, $J = 13.1$ Hz, 1H, $PhCH_2N$), 3.58 (dd, $J = 11.3/2.4$ Hz, 1H, CH_2OH), 3.63 (t, $J = 5.3$ Hz, 2H, NCH_2CH_2OH), 3.99 (dd, $J = 11.3/4.0$ Hz, 1H, CH_2OH), 4.07 (d, $J = 13.1$ Hz, 1H, $PhCH_2N$), 7.24–7.36 (m, 5H, arom). The signals for the protons of the OH groups could not be detected. ^{13}C NMR ($CDCl_3$): $\delta = 50.3$ and 52.3 (2C, C-5, C-6), 55.3 (1C, C-3), 57.90 (1C, NCH_2Ph), 57.93 (1C, NCH_2CH_2OH), 59.5 (1C, NCH_2CH_2OH), 59.8 (1C, C-2), 61.2 (1C, CH_2OH), 127.2 (1C, C-4′), 128.4 (2C, C-3′ and C-5′), 128.8 (2C, C-2′ and C-6′), 138.2 (1C, C-1′).

7.17. (–)-[(2*R*)-1-Benzyl-4-(3-hydroxypropyl)piperazin-2-yl]methanol (3j)

To a stirred solution of 1 M $LiAlH_4$ in Et_2O (5.3 mL, 5.3 mmol) and THF (10 mL) was added dropwise a solution of **6j** (mixture of *cis*/*trans* isomers, 0.154 g, 0.53 mmol) in THF (30 mL) under N_2 atmosphere. The mixture was heated to reflux for 96 h. Under N_2 atmosphere and cooling (ice bath) water (0.2 mL), 3 N NaOH (0.2 mL) and again water (0.2 mL) were successively added. The suspension was heated to reflux for 30 min. After the mixture was cooled to room temperature, the precipitate was filtered off, and the solvent removed under reduced pressure. Purification of the residue by flash chromatography (2 cm, ethanol, 5 mL, $R_f = 0.27$) furnished **3j** as a colorless, viscous oil, yield 0.074 g (53%). $[\alpha]_{589}$ +34.8 (*c* 0.775, CH_2Cl_2). C_{15} $H_{24}N_2O_2$ (264.37). HRMS: calcd for M−CH_2OH ($C_{14}H_{21}N_2O$) 233.1654, found 233.1652 (−0.2 ppm). MS (EI): *m*/*z* (%) = 264 (M, 1), 233 (M−CH_2OH, 100), 174 (benzylpiperazine, 8), 142 (M−benzyl−CH_2OH, 82), 91 (benzyl, 62). MS (CI): *m*/*z* (%) = 265 (MH^+, 100), 233 (M−CH_2OH, 13). IR (film): *v* (cm^{-1}) = 3377 (br, OH), 2943 and 2817 (m, C–H), 1452 (m, C–H), 1148 (m, C–OH), 740 and 700 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 1.71$ ('quint', $J = 5.6$ Hz, 2H, $NCH_2CH_2CH_2OH$), 2.18 (td, $J = 10.1/2.3$ Hz, 1H, 5-H or 6-H), 2.33 (td, $J = 10.6/2.8$ Hz, 1H, 6-H or 5-H), 2.44 (dd, $J = 11.2/9.0$ Hz, 1H, 3-H), 2.57–2.61 (m, 3H, 2-H and $NCH_2CH_2CH_2OH$), 2.71–2.75 (m, 1H, 5-H or 6-H), 2.82–2.89 (m, 2H, 3-H and 6-H or 5-H), 3.28 (d, $J = 13.2$ Hz, 1H, $PhCH_2N$), 3.54 (dd, $J = 11.6/2.5$ Hz, 1H, CH_2OH), 3.79 (t, $J = 5.2$ Hz, 2H, $NCH_2CH_2CH_2OH$), 3.98 (dd, $J = 11.2/4.2$ Hz, 1H, CH_2OH), 4.06 (d, $J = 13.3$ Hz, 1H, $PhCH_2N$), 7.23–7.35 (m, 5H, arom). The signals for the protons of the OH groups could not be detected. ^{13}C NMR ($CDCl_3$): $\delta = 27.2$ (1C, $NCH_2CH_2CH_2OH$), 50.4 and 50.7 (2C, C-5, C-6), 55.5 (1C, C-3), 57.9 (1C, NCH_2Ph), 58.4 (1C, $NCH_2CH_2CH_2OH$), 60.1 (1C, C-2), 60.9 (1C, CH_2OH), 64.0 (1C, $NCH_2CH_2CH_2OH$), 127.2 (1C, C-4′), 128.4 (2C, C-3′ and C-5′), 128.8 (2C, C-2′ and C-6′), 138.2 (1C, C-1′).

7.18. (+)-[(2*R*)-1-Benzylpiperazin-2-yl]methanol (3m)

Three $LiAlH_4$ pellets (2.353 g, 61.9 mmol) were added to a stirred solution of **6m** (mixture of *cis*/*trans* isomers, 1.346 g, 5.8 mmol) in THF (200 mL). The suspension was heated to reflux for 96 h. Under N_2 atmosphere and cooling (ice bath) water (2 mL), 3 N NaOH (2.0 mL), and again water (2 mL) were successively added. The suspension was heated to reflux for 30 min. After the mixture was cooled to room temperature, the precipitate was filtered off, and the solvent removed under reduced pressure. The residue was characterized without further purification and was converted into **3n**.

Compound **3m**: Pale yellow solid, yield 1.196 g (>99%). $C_{12}H_{18}N_2O$ (206.29). MS (EI): *m*/*z* (%) = 175 (M−CH_2OH, 81), 91 (benzyl, 100). MS (CI): *m*/*z* (%) = 207 (MH^+, 100). IR (film): *v* (cm^{-1}) = 3362 (br, O–H), 2943 (s, C–H), 2814 (s, OCH_3), 1454 (m, C–H), 1128 (m, C–OH), 737 and 700 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 2.24$–2.32 (m, 1H, 3-H, 5-H, or 6-H), 2.44–2.50 (m, 1H, 2-H, 3-H, 5-H, or 6-H), 2.75–2.81 (m, 1H, 2-H, 3-H, 5-H, or 6-H), 2.84–2.91 (m, 2H, 2-H, 3-H, 5-H, or 6-H), 2.98–3.06 (m, 2H, 3-H, 5-H, or 6-H), 3.35 (d, $J = 13.5$ Hz, 1H, $PhCH_2N$), 3.58 (dd, $J = 11.1/2.7$ Hz, 1H, CH_2OH), 4.03 (dd, $J = 11.4/3.8$ Hz, 1H, CH_2OH), 4.06 (d, $J = 13.8$ Hz, 1H, $PhCH_2N$), 7.23–7.37 (m, 5H, arom). The signals for the protons of the OH group and the NH group could not be detected.

7.19. (+)-Ethyl 2-[(3*R*)-4-benzyl-3-(hydroxymethyl)-piperazin-1-yl]acetate (3n)

Na_2CO_3 (1.01 g, 9.54 mmol) and ethyl bromoacetate (1.1 mL, 9.54 mmol) were added to a stirred solution of **3m** (1.968 g, 9.54 mmol) in THF (180 mL). The reaction mixture was heated to reflux for 17 h. After the mixture was cooled to room temperature, Na_2CO_3 was filtered off, and the solvent removed under reduced pressure. Flash chromatography of the residue (6 cm, ethyl acetate/acetone, 8:2, 20 mL, $R_f = 0.31$) afforded **3n** as a viscous oil; yield 1.699 g (61%), $[\alpha]_{589}$ −12.2 (*c* 1.045, CH_2Cl_2). $C_{16}H_{24}N_2O_3$ (292.38). Calcd C 65.73, H 8.27, N 9.58, found C 64.95, H 8.23, N 9.38. MS (EI): *m*/*z* (%) = 292 (M, 1), 261 (M−H_2OH, 71), 219 (M−$CO_2C_2H_5$, 9), 170 (261−benzyl, 100), 91 (benzyl, 47). MS (CI): *m*/*z* (%) = 293 (MH^+, 100). IR (film): *v* (cm^{-1}) = 3418 (br, O–H), 2937 (m, C–H), 2816 (m, C–H), 1747 (s, C=O), 1455 (m, C–H), 1168 (s, C–OH), 748 and 700 (m, aryl-C–H). ^{1}H NMR ($CDCl_3$): $\delta = 1.27$ (t, $J = 7.2$ Hz, 3H, OCH_2CH_3), 2.37–2.53 (m, 2H, 5-H and 6-H), 2.63–2.73 (m, 3H, 2-H, 3-H, and 5-H or 6-H), 2.79–2.83 (m, 1H, 2-H), 2.95 (ddd, $J = 11.3/6.1/2.6$ Hz, 1H, 6-H or 5-H), 3.14 (d, $J = 16.5$ Hz, 1H, NCH_2CO_2Et), 3,21 (d, $J = 16.8$ Hz, 1H, NCH_2CO_2Et), 3.43 (d, $J = 13.3$ Hz, 1H, $PhCH_2N$), 3.60 (dd, $J = 11.4/2.4$ Hz, 1H, CH_2OH), 4.02 (d, $J = 13.2$ Hz, 1H, $PhCH_2N$), 4.06 (dd, $J = 11.5/4.0$ Hz, 1H, CH_2OH), 4.19 (q, $J = 7.2$ Hz, 2H, OCH_2CH_3), 7.23 –7.32 (m, 5H, arom). The signal for the proton of the OH group could not be detected. ^{13}C NMR ($CDCl_3$): $\delta = 14.2$ (1C, OCH_2CH_3), 49.8 and 52.4 (2C, C-5, C-6), 55.5 (1C, C-2), 57.9 (1C, $PhCH_2N$), 59.0

(1C, C-3), 59,3 (1C, NCH_2CO_2Et), 60.7 (1C, OCH_2CH_3), 61.5 (1C, CH_2OH), 127.2 (1C, C-4′), 128.4 (2C, C-3′ and C-5′), 128.9 (2C, C-2′ and C-6′), 138.1 (1C, C-1′), 170.2 (1C, C=O).

7.20. (2*S*)-{[(2*R*)-1-Benzyl-4-(ethoxycarbonylmethyl)piperazin-2-yl]methyl}-3,3,3-trifluoro-2-methoxy-2-phenylpropionate [(*R*,*S*)-8]

A solution of **3n** (33 mg, 0.114 mmol), triethylamine (15 μL, 0.114 mmol), 4-(dimethylamino)pyridine (14 mg, 0.114 mmol) and (*R*)-(−)-3,3,3-trifluoro-2-methoxy-2-phenylpropionyl chloride (20 μL, 0.108 mmol) in CH_2Cl_2 (5 mL) was heated to reflux for 7 h. After the mixture was cooled to room temperature, the mixture was washed with a saturated solution of $NaHCO_3$ (5 mL) and water (5 mL). The organic layer was dried (Na_2SO_4) and evaporated in vacuo. About 15 mg of the residue was investigated by ^1H and ^{19}F NMR and by HPLC, the remainder was purified by flash chromatography (1 cm, petroleum ether/ethyl acetate 3:7, 1 mL, $R_f = 0.52$) to provide (*R*,*S*)-**8**. $C_{26}H_{31}F_3N_2O_5$ (508.54). ^1H NMR ($CDCl_3$): $\delta = 1.25$ (t, $J = 7.2$ Hz, 3H, OCH_2CH_3), 2.32–2.47 (m, 3H, 3-H, 5-H and/or 6-H), 2.63–2.81 (m, 3H, 2-H, 3-H, 5-H, and/or 6-H), 2.86–2.88 (m, 1H, 2-H, 3-H, 5-H, or 6-H), 3.12 (d, $J = 16.8$ Hz, 1H, NCH_2CO_2Et), 3.18 (d, $J = 16.8$ Hz, 1H, NCH_2CO_2Et), 3.35 (d, $J = 13.5$ Hz, 1H, $PhCH_2N$), 3.55 (s, 3H, OCH_3), 3.95 (d, $J = 13.4$ Hz, 1H, $PhCH_2N$), 4.16 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 4.57 (br d, $J = 4.8$ Hz, 2H, $CH_2OC{=}O$), 7.22–7.27 (m, 5H, arom), 7.35–7.40 (m, 3H, arom), 7.52–7.55 (m, 2H, arom). ^{19}F NMR ($CDCl_3$): $\delta = -71.80$ (s, 3F, CF_3, 96.7% intensity), −71.83 (s, 3F, CF_3, 3.3% intensity). HPLC: $t((R,S)\text{-}\mathbf{8}) = 49$ min, $t((R,R)\text{-}\mathbf{8}) = 47.1$ min, $MeOH/H_2O$.= 75:25, 0.8 mL/min.

7.21. (2*R*)-{[(2*R*)-1-Benzyl-4-(ethoxycarbonylmethyl)piperazin-2- yl]methyl}-3,3,3-trifluoro-2-methoxy-2-phenylpropionate [(*R*,*R*)-8]

A solution of **3n** (30 mg, 0.103 mmol), triethylamine (14 μL, 0.103 mmol), 4-(dimethylamino)pyridine (13 mg, 0.103 mmol) and (*S*)-(+)-3,3,3-trifluoro-2-methoxy-2-phenylpropionyl chloride (18 μL, 0.098 mmol) in CH_2Cl_2 (5 mL) was heated to reflux for 7 h. After the mixture was cooled to room temperature, the reaction solution was washed with a saturated solution of $NaHCO_3$ (5 mL) and water (5 mL). The organic layer was dried (Na_2SO_4) and evaporated in vacuo. About 14 mg of the residue was investigated by ^1H and ^{19}F NMR and by HPLC, the remainder was purified by flash chromatography (1 cm, petroleum ether/ethyl acetate 3:7, 1 mL, $R_f = 0.52$) to provide (*R*,*R*)-**8**. $C_{26}H_{31}F_3N_2O_5$ (508.54). ^1H NMR ($CDCl_3$): $\delta = 1.25$ (t, $J = 7.2$ Hz, 3H, OCH_2CH_3), 2.33–2.46 (m, 3H, 3-H, 5-H and/or 6-H), 2.64–2.74 (m, 2H, 2-H, 3-H, 5-H, and/or 6-H), 2.79 –2.87 (m, 2H, 2-H, 3-H, 5-H, and/or 6-H), 3.12 (d, $J = 16.5$ Hz, 1H, NCH_2CO_2Et), 3.17 (d, $J = 16.4$ Hz, 1H, NCH_2CO_2Et), 3.30 (d, $J = 13.7$ Hz, 1H, $PhCH_2N$), 3.55 (s, 3H, OCH_3), 3.94 (d, $J = 13.2$ Hz, 1H, $PhCH_2N$), 4.16 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 4.56 (br s, 2H, $CH_2OC{=}O$), 7.22–7.27 (m, 5H, arom), 7.36–7.41 (m, 3H, arom), 7.51–7.55 (m, 2H, arom). ^{19}F NMR ($CDCl_3$): $\delta = -71.80$ (s, 3F, CF_3, 2.7% intensity), −71.83 (s, 3F, CF_3, 97.3% intensity). HPLC: $t((R,S)\text{-}\mathbf{8}) = 49$ min, $t((R,R)\text{-}\mathbf{8}) = 47.1$ min, $MeOH/H_2O = 75$: 25, 0.8 mL/min.

8. Receptor binding studies

8.1. General

Homogenizer: Potter®S (B. Braun Biotech International). Ultraturrax: Euroturrax® T20 (Ika Labortechnik). Centrifuge: High speed cooling 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: Rotiscint Eco Plus (Roth). Liquid scintillation analyzer: TriCarb 2100 TR (Canberra Packard), counting efficiency 66%. All experiments were carried out in triplicates. IC_{50} values were determined in competition experiments with at least six concentrations of test compounds and were calculated with the program GraphPad Prism® 3.0 (GraphPad Software) by nonlinear regression analysis. K_i values were calculated according to Cheng and Prusoff.[19] The K_i values are given as mean value ± SEM from three independent experiments.

8.2. σ_1-Receptor binding assay

For the σ_1-assay guinea pig brain membranes were prepared as described in Ref. 20. The test was performed with the radioligand [^{3}H]-(+)-pentazocine (1036 GBq/mmol; NEN™ Life Science Products). The thawed membrane preparation (about 150 μg of protein) was incubated with various concentrations of the test compound, 3 nM [^{3}H]-(+)-pentazocine, and buffer (50 mM Tris–HCl, pH 7.4) in a total volume of 500 μL for 120 min at 37 °C. The incubation was terminated by rapid filtration through Whatman GF/B filters using a cell harvester. After washing four times with 2 mL of 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.

8.3. σ_2-Receptor binding assay

For the σ_2-assay rat liver membranes were prepared as described in Ref. 20. The membrane preparation (about 60 μg of protein) was incubated with 3 nM [^{3}H]-ditolylguanidine (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 the sample was washed three times with 2 mL of cold buffer, a total volume of 3 mL of scintillation cocktail was 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 nonradiolabeled ditolylguanidine.

8.4. Investigation of the affinity for the phencyclidine binding site of the NMDA receptor

For the NMDA-assay pig brain membrane preparations were used as receptor material. The test was performed with the radioligand [^{3}H]-(+)-MK-801 (832.5 GBq/mmol; NEN™ Life Science Products). The thawed membrane preparation (about 100 μg of the protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-MK-801, and buffer (5 mM Tris–acetate, 1 mM EDTA, pH 7.5) in a total volume of 500 μL for 90 min at 25 °C. The incubation was terminated by rapid filtration through presoaked Whatman GF/C filters (1% polyethylenimine in water for 3 h at 4 °C) using a cell harvester. After washing four times with 2 mL of 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 (+)-MK-801.[17]

8.5. κ-Opiod receptor binding assay

Prepartaions of guinea pig brains without cerebellum were employed for the κ-assay. The test was performed with the radioligand [^{3}H]-U-69593 (1468.9 GBq/mmol; NEN Life Science Products). The thawed membrane preparation (about 900 μg of the protein) was incubated with various concentrations of test compounds, 1 nM [^{3}H]-U-69593, 5 mM $MgCl_2$ and buffer (50 mM Tris–HCl, pH 7.5) in a total volume of 500 μL at 25 °C for 90 min. The incubation was terminated by rapid filtration through presoaked Whatman GF/B filters (0.25% polyethylenimine in 50 mM Tris–HCl, pH 7.4 for 2 h at 4 °C) using a cell harvester. After washing four times with 2 mL of 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 1 μM-U-50488.[17]

8.6. μ-Opiod receptor binding assay

In the μ-assay the same membrane preparation was used as in the κ-assay. The test was performed with the radioligand [^{3}H]-DAMGO (2016.5 GBq/mmol; NEN™ Life Science Products). The thawed membrane preparation (about 400 μg of the protein) was incubated with various concentrations of test compounds, 1 nM [^{3}H]-DAMGO, 5 mM $MgCl_2$, 100 μM PMSF (phenylmethanesulfonyl fluoride) and buffer (50 mM Tris–HCl, pH 7.4) in a total volume of 500 μL at 25 °C for 90 min. The incubation was terminated by rapid filtration through presoaked Whatman GF/B filters (50 mM Tris–HCl, pH 7.4 for 2 h at 4 °C) using a cell harvester. After washing four times with 2 mL of 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 1 μM naloxone.[17]

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