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Conformationally Constrained Ethylenediamines: Synthesis and Receptor Binding of 6,8-Diazabicyclo[3.2.2]nonanes

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Abstract—The synthesis and receptor affinity of 6,8-diazabicyclo[3.2.2]nonanes representing conformationally constrained ethylenediamines are described. The Dieckmann analogous cyclization of the (piperazin-2-yl)propionate **9** provided the bicyclononane **10** only, when the first cyclization product was trapped with chlorotrimethylsilane. **10** was stereoselectively transformed into the bicyclic amines **19a**,**b** and amides **22a**,**b**, which were investigated in competition experiments with radioligands for their σ_1-, σ_2-, κ-, and μ-receptor affinities. The (2*R*)-configured dimethylamine **19a** showed promising σ_1-receptor affinity ($K_i = 23.8$ nM) and selectivity, whereas the (2*S*)-configured (dichlorophenyl)acetamide **22b** displayed a σ-receptor binding profile (σ_1: $K_i = 184$ nM; σ_2: $K_i = 263$ nM) very similar to the binding profile of the atypical antipsychotic BMY-14802 (**26**).

Introduction

Ligands interacting with high affinity and selectivity with central nervous system (CNS) receptors are able to modulate (patho)physiological events and to influence psychiatric disorders. Therefore, we are interested in the development of novel ligands for σ- and opioid-receptors—particularly κ-opioid-receptors.

σ-Receptors, which have been originally classified into the opioid receptor family,[1] are now well accepted as real receptors.[2,3] Their ligands possess potential as atypical antipsychotics,[4,5] antidepressants,[6] and antitumor agents.[7,8]

Agonists at each of the opioid-receptor subtypes (μ-, κ-, δ-receptors) cause strong analgesic effects. Among these analgesics κ-receptor agonists display an advantageous side effect profile with minimal physical dependence, respiratory depression and inhibition of gastrointestinal motility.[9]

The ethylenediamine substructure substituted with different residues at the nitrogen atoms represents a crucial pharmacophoric element of several σ- and κ-receptor ligands (see Fig. 1). For example, the simple ethylenediamine **1** binds with high affinity to σ-receptors ($K_i = 0.34$ nM).[10] Insertion of the ethylenediamine substructure into a piperazine ring system leads to a high affinity σ-receptor ligand as well (compound **2**, σ_1: $K_i = 0.55$ nM).[11] In the cyclohexane derivative **3**, which also comprises the ethylenediamine substructure, the stereochemistry is essential for high σ-receptor binding. Among the stereoisomers the *cis*-configured (1*R*,2*S*)-cyclohexane **3** shown in Figure 1 is the most active σ-receptor ligand ($K_i = 118$ nM).[12]

However, changing the *cis*-(1*R*,2*S*)-configuration of the cyclohexane derivative **3** into the *trans*-(1*S*,2*S*)-configuration provides the prototypical κ-receptor agonist U-50488 (**4**, $K_i = 0.89$ nM).[12] In the class of κ-receptor agonists the ethylenediamine substructure may also be inserted into a piperazine heterocycle and its side chain. Hence, the 2-(pyrrolidinylmethyl)piperazines **5** ($IC_{50} = 0.018$ nM)[13] and **6** ($K_i = 0.34$ nM)[14] belong to the most active κ-receptor agonists. The κ-receptor affinity of **5** and **6** strongly depends on the stereochemistry.

Herein, we report on the synthesis of enantiopure 6,8-diazabicyclo[3.2.2]nonanes **8** and their affinity for σ_1-, σ_2-, κ-, and μ-receptors (see Fig. 2). The bicyclic derivatives **8** comprise the ethylenediamine substructure 2-fold—in the bridged piperazine ring system and, moreover, in the side chain nitrogen atom combined with C^1, C^2 and N^8. In the bicyclic derivatives **8** the relative orientation of the pharmacophoric elements—the nitro-

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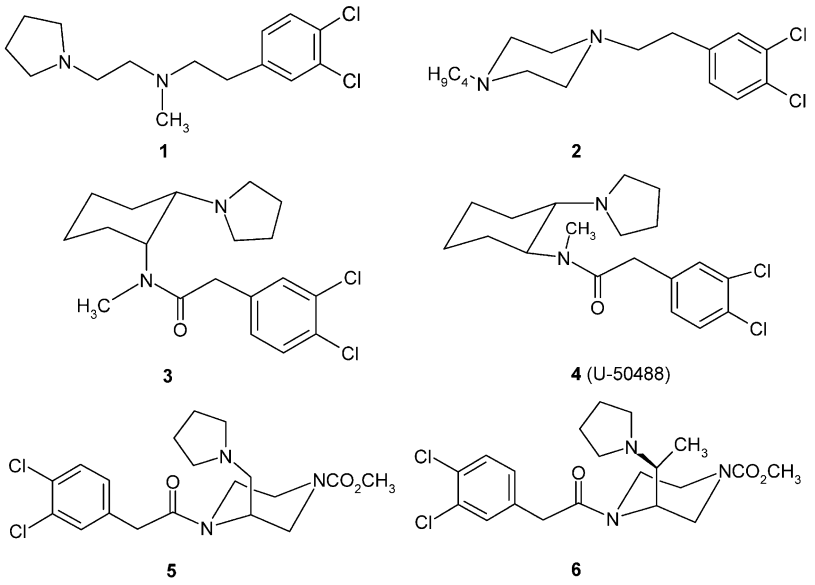


Figure 1.

gen atoms and their substituents—is fixed due to the limited conformational flexibility of the ethylenediamine substructures. The receptor affinity of conformationally constrained ligands may give insight into the pharmacologically active conformation of analogous flexible ligands.[15]

In the literature, two approaches for the synthesis of 6,8-diazabicyclo[3.2.2]nonanes are described. First, the 2-fold intramolecular aminolysis of *like*-configured 2,6-diaminopimelic acid derivatives provides racemic 6,8-diazabicyclo[3.2.2]nonanes without further substituents at the propano bridge.[16,17] In the second approach an intramolecular enolate epoxide cyclization was used as key step. However, only poor yields of a racemic 6,8-diazabicyclo[3.2.2]nonane (16%) were obtained since the epoxide opening occurred with unfavourable regioselectivity.[18]

According to our plan a *Dieckmann* analogous cyclization of 3-(dioxopiperazin-2-yl)propionic acid esters **7** should represent the key step in the synthesis of enantiomerically pure 6,8-diazabicyclo[3.2.2]nonanes **8**.[19] This strategy enables the introduction of further substituents (e.g., nitrogen containing groups) at the propano bridge. Recently, we have described a general method for the synthesis of enantiopure 3-(dioxopiperazin-2-yl)propionates **7** with various substituents at the nitrogen atoms. The proteinogenic amino acid (*S*)-glutamate has been used as enantiomerically pure educt.[20]

Chemistry

In our initial attempts the intramolecular ester condensation (*Dieckmann* analogous cyclization)[21] was investigated with the 1-benzyl-4-methyl derivative **9**.[20] However, the standard conditions usually applied for Dieckmann condensations ($NaOCH_3/CH_3OH$; KO^{t}Bu/toluene; LDA/THF; KHMDS/THF at room temperature and at reflux temperature) did not lead to the desired bicyclic ketone **11**. We presume, that the equilibrium of this cyclization is shifted towards the dioxoester **9** since the formed β-dicarbonyl compound **11** cannot be stabilized by deprotonation. Abstraction of a proton from the formed β-dicarbonyl compound in the last step is the driving force of the otherwise endergonic ester condensation (Dieckmann condensation). In the case of the bicyclic β-dicarbonyl compound **11** the proton has to be removed from a bridgehead center, which is impeded according to *Bredt's* rule (Scheme 1).[22]

Therefore, the dioxoester **9** was deprotonated quantitatively with the strong base lithium hexamethyldisilazane (LiHMDS) and the resulting anion was trapped with chlorotrimethylsilane ($ClSiMe_3$). Indeed, the bicyclic, mixed methyl silyl acetal **10** was obtained in 97% yield according to this procedure. Careful hydrolysis of the mixed methyl silyl acetal **10** with *p*-toluenesulfonic acid in a mixture of THF and water provided the bicyclic ketone **11** in 90% yield.

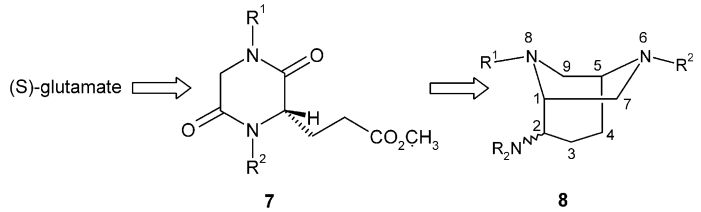
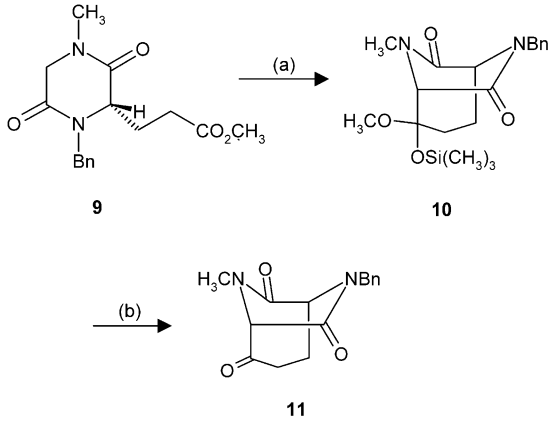


Figure 2.

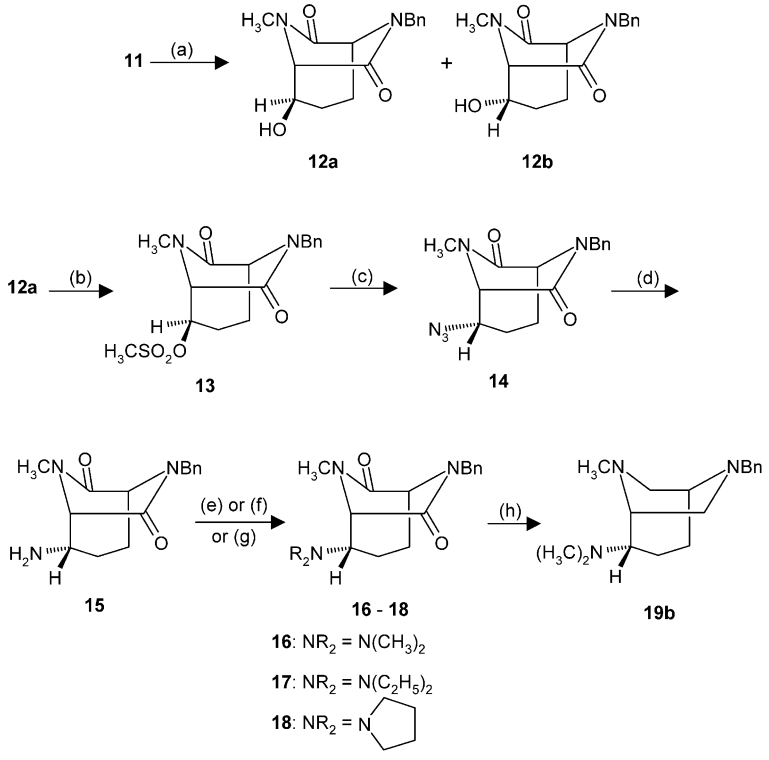
In order to support the hypothesis of suppressed deprotonation during attempted cyclization of **9**, the β-dicarbonyl compound **11** was treated with $NaOCH_3$ in methanol. Within a few minutes at room temperature, the bicyclic ketone **11** was transformed into the dioxoester **9** which could be isolated. Obviously, nucleophilic attack at the ketone carbonyl moiety followed by ring opening occurred with $NaOCH_3$ instead of deprotonation. The same observation (opening of the bicyclic ring system) was made during attempts of reductive amination of the bicyclic ketone **11**. Therefore, the amino moiety in position 2 was introduced on a different way (Scheme 2).



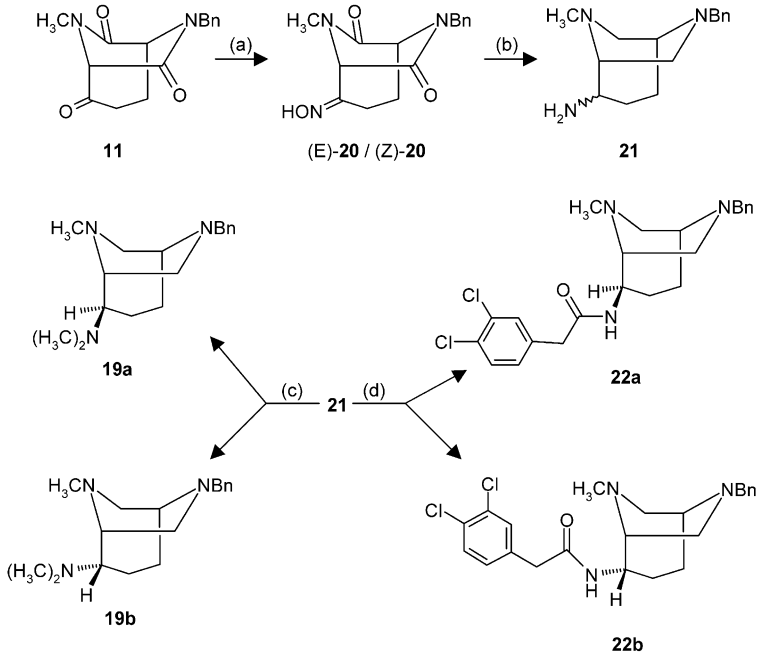
Scheme 1. Reagents and reaction conditions: (a) (1) LiHMDS, THF, 30 min, −78 °C; (2) $ClSi(CH_3)_3$, 30 min, −78 °C, 60 min, rt, 97%; (b) *p*-TosOH, THF/H_2O, 16 h, rt, 90%.

Thus, the ketone **11** was reduced carefully with $NaBH_4$ in methanol to yield the diastereomeric alcohols **12a** and **12b** in a ratio of 85:15. The main diastereomer **12a** was activated with methane sulfonyl chloride to provide the methane sulfonate **13**, which subsequently reacted in a S_N2 reaction with NaN_3 to afford the inverted (*S*)-configured azide **14**. Reduction of the azide **14** with hydrogen in the presence of the catalyst Pd/C led to the primary amine **15**. Reductive alkylation of the primary amine **15** with formaldehyde or acetaldehyde and $NaBH_3CN$[23] yielded the tertiary amines **16** and **17**, respectively. The pyrrolidine derivative **18** was obtained only in a low yield by 2-fold alkylation of the primary amine **15** with 1-bromo-4-chlorobutane. In the last step the piperazinedione substructure of **16** was reduced with $LiAlH_4$[24,25] to provide the bridged piperazine **19b** (Scheme 2).

Alternatively, the ketone **11** was condensed with hydroxylamine to yield the diastereomeric oximes (*E*)-**20** and (*Z*)-**20** in a ratio of 1:1. The isomeric oximes (*E*)-**20** and (*Z*)-**20** could be separated partly providing the pure (*E*)-isomer (20%) and enriched (*Z*)-isomer. The diastereomeric mixture of (*E*)-**20** and (*Z*)-**20** was reduced with an excess of $LiAlH_4$. Thereby the oxime moiety as well as both lactam carbonyl groups were reduced to furnish the primary amine **21**, which was employed for further transformations without purification. Reductive methylation[23] of the primary amine **21** with formaldehyde and $NaBH_3CN$ yielded the dimethylamines **19a** and **19b**, which were isolated in a ratio of 2:3. In this sequence the dimethylamine **19b** with (*S*)-configuration in position 2 was obtained in 7.4% yield in three steps from the



Scheme 2. Reagents and reaction conditions: (a) $NaBH_4$, THF/propan-2-ol/H_2O, 16 h, rt, 77%; (b) CH_3SO_2Cl, NEt_3, CH_2Cl_2, 30 min, 0 °C, 2.5 h, rt, 92%; (c) NaN_3, DMF, 2 h, 155 °C, 79%; (d) H_2, Pd/C, CH_3OH, 4.5 h, rt, 99%; (e) CH_2=O, $NaBH_3CN$, CH_3CN, 3 h, rt, 66% (**16**); (f) CH_3CH=O, $NaBH_3CN$, CH_3CN, 3 h, 0 °C, 77% (**17**); (g) $Br(CH_2)_4Cl$, NEt_3, DMF, 16 h, 155 °C, 11% (**18**). (h) $LiAlH_4$, THF, 88 h, 66 °C, 97%.



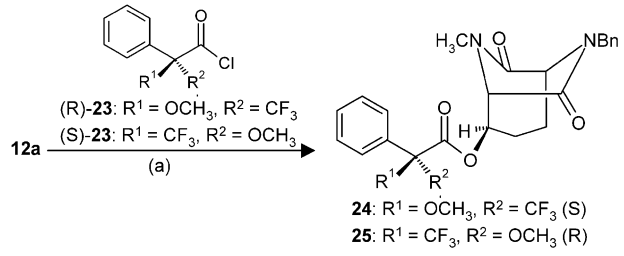
Scheme 3. Reagents and reaction conditions: (a) $NH_2OH{\cdot}HCl$, CH_3OH, NEt_3, 16 h, rt, 83%; (b) $LiAlH_4$, THF, 20 h, 66 °C, 65%; (c) $CH_2{=}O$, $NaBH_3CN$, CH_3CN, 21 h, rt, 6.3% (**19a**), 9.4% (**19b**); (d) (3,4-dichlorophenyl)acetic acid, CDI, CH_2Cl_2, 30 min, 0 °C, 40 h, rt, 11% (**22a**), 15%.

ketone **11** (reaction with hydroxylamine, $LiAlH_4$-reduction, $CH_2{=}O/NaBH_3CN$ reductive alkylation). Although the synthesis of **19b** outlined in Scheme 2 comprises six steps the overall yield of **19b** is significantly higher (30%). However, the diastereomeric dimethylamine **19a** is only available via the oxime route (6.3%). Thus, the two routes complement one another.

Additionally, the crude primary amine **21** was acylated with (3,4-dichlorophenyl)acetic acid and the coupling reagent 1,1′-carbonyldiimidazole (CDI) to afford the diastereomeric amides **22a** and **22b** in a ratio of 2:3. The (3,4-dichlorophenyl)acetyl moiety was introduced, because several σ- and κ-ligands include this acyl residue as pharmacophoric element (cf., Fig. 1).

The *Dieckmann* analogous cyclization of the dioxoester **9** was performed using the strong base LiHMDS. In the presence of LiHMDS, the asymmetric center in position 2 bearing the propionate side chain might be unstable. Therefore, the enantiomeric purity of the bicyclic products has to be controlled. For this purpose, the alcohol **12a**, the main diastereomer formed during $NaBH_4$ reduction of the ketone **11**, was acylated with (*R*)- and (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropionyl chloride [(*R*)-**23** and (*S*)-**23**, *Mosher's* acid chlorides][26,27] to yield the diastereomeric *Mosher* esters **24** and **25**, respectively. The analytical methods were elaborated with purified esters **24** and **25**, the determination of the enantiomeric purity, however, was performed with unpurified esters **24** and **25** (Scheme 4).

In the ^1H NMR spectra of **24** and **25** the signal for the methoxy group is found at 3.52 and 3.64 ppm, respectively. Integration of these signals results in a ratio of 99:1 (ee = 98%). The ^{19}F NMR spectra of **24** and **25** reveal the signals for the F_3C-moiety at −72.2 and −71.8 ppm, respectively. The ratio of the signal intensities was determined to be 98.8:1.2 (ee = 97.6%) and 1.6:98.4 (ee = 96.8%), respectively. The HPLC analysis of the unpurified *Mosher* esters **24** and **25** succeeded using a LiChrospher® 100 RP-18, endcapped stationary phase. With the eluent acetonitrile/water = 1:1 a baseline separation of the diastereomeric Mosher esters **24** and **25** was achieved, providing an enantiomeric excess of 94.8% (peak ratio 97.4:2.6). In conclusion, in the worst case (HPLC analysis) the ratio of diastereomeric Mosher esters **24** and **25** and thus the ratio of enantiomers of the alcohol **12a** amounts to at least 97.4:2.6. Therefore, a significant racemization of the dioxoester **9** during LiHMDS cyclization can be excluded.



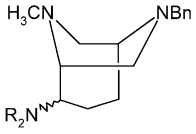
Scheme 4. Reagents and reaction conditions: (a) (*R*)-**23** or (*S*)-**23**, NEt_3, 4-dimethylaminopyridine, CH_2Cl_2, 9 h, 41 °C.

Receptor binding studies

The σ_1-, σ_2-, κ-, and μ-receptor affinities of the diastereomeric dimethylamines **19a** and **19b** and the phenylacetamides **22a** and **22b** were determined in competition experiments with radioligands.

In the σ_1-assay homogenates of guinea pig brains were used as receptor material. The σ_1-selective ligand [^3H]-(+)-pentazocine was employed as radioligand, and the

Table 1. Affinities of the diazabicyclo[3.2.2]nonanes **19** and **22** for σ_1-, σ_2-, κ- and μ-receptors



Compd	σ_1 ([^{3}H]-(+)-pentazocine)	σ_2 ([^{3}H]-ditolylguanidine)	κ ([^{3}H]-U69593)	μ ([^{3}H]-DAMGO)
	K_i (nM) SEM			
19a	23.8 ± 5.3	353 ± 9.0	13930 ± 570	> 10 μM
19b	240 ± 39	1400 ± 480	15900 ± 1670	> 10 μM
22a	131 ± 27	1110 ± 560	2650 ± 360	5220 ± 2240
22b	184 ± 22	263 ± 52	2600 ± 360	6300 ± 520
BMY-14802 (**26**)	265 ± 32	391 ± 62	—	—
Ditolylguanidine	164 ± 47	63.9 ± 10.8	—	—
(+)-Pentazocine	3.58 ± 0.20	—	—	—
U-50488 (**4**)	—	—	0.49 ± 0.16	—
Naloxone	—	—	3.17	0.68 ± 0.04

nonspecific binding was determined in the presence of a large excess of haloperidol.[28] 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 [^{3}H]-ditolylguanidine was employed in the presence of an excess of non-radiolabeled (+)-pentazocine (100 nM) for selective labeling of σ_1-receptors. Performing the σ_2-assay in the presence of an excess of non-tritiated ditolylguanidine led to the non-specific binding of the radioligand.[28] The κ-receptor binding of the test compounds was determined with homogenates of guinea pig brain (without cerebellum) membranes as receptor material using the κ-selective radioligand [^{3}H]-U-69593.[14] The non-specific binding was determined with an excess of U-50488 (**4**). In the μ-receptor assay the same membrane preparation as described for the κ-assay was used. The radioligand [^{3}H]-DAMGO was employed for labeling the μ-receptors and the non-specific binding was determined in the presence of 1 μM naloxone.[14]

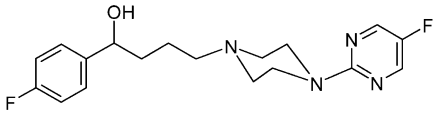
Results and Discussion

The results of the receptor binding studies are summarized in Table 1. It can be seen that the dimethylamine **19a** with (*R*)-configuration in position 2 displays the highest affinity for σ_1-receptors (K_i = 23.8 nM), whereas the σ_1-receptor affinity of the diastereomer **19b** is 10-fold lower. Although the (2*R*)-configured phenylacetamide **22a** contains the pharmacophoric dichlorophenylacetamide substructure of lead structure **3** it reveals lower σ_1-receptor affinity (K_i = 131 nM) than the analogous dimethylamine **19a**. Again the (2*S*)-diastereomer **22b** is less active at σ_1-receptors. However, the difference between the K_i-values of the diastereomeric phenylacetamides **22a** and **22b** is very small.

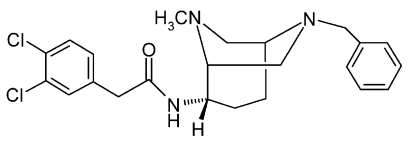
The σ_2-receptor affinities of both diastereomeric dimethylamines **19a** and **19b** are significantly lower than their σ_1-receptor affinities (factors 15 and 6). The (2*R*)-configured diastereomer **19a** is more active than the (2*S*)-diastereomer **19b** by the factor 4. In analogy to the dimethylamines **19**, the phenylacetamides **22** bind with lower affinity at σ_2-receptors than at σ_1-receptors. However, in the phenylacetamide series the (2*S*)-configured diastereomer **22b** displays higher σ_2-receptor affinity than its (2*R*)-diastereomer **22a** (factor 4). Altogether, the σ-receptor binding profile of **22b** is very similar to the σ-binding profile of the reference compound BMY-14802 (**26**, cf., Table 1), which has been evaluated as atypical antipsychotic in clinical trials.

In Figure 3, the structure of the phenylacetamide **22b** is compared with the structure of the reference compound BMY-14802 (**26**). We presume, that the aryl moieties and the nitrogen atoms of the piperazine ring systems occupy similar positions at σ_1- and σ_2-receptors.

The dimethylamines **19a** and **19b** display very low affinities in the κ- and μ-receptor assays (K_i > 10 μM). Considerable κ-receptor affinities were found for the compounds **22** containing the κ-pharmacophoric phenylacetamide substructure. Surprisingly, the κ-receptor does not discriminate between the diastereomers **22a** and **22b**. Since the K_i-values of the phenylacetamides **22a** and **22b** are much higher than the K_i-values of the lead compounds **4**–**6** we suppose that the absolute configuration of the bicycles **22** has to be changed for high κ-receptor affinity.



26 (BMY-14802)



22b

Figure 3.

Conclusion

Within the novel class of 6,8-diazabicyclo[3.2.2]nonane derivatives we have found the (2*R*)-configured dimethylamine **19a** with high affinity for σ_1-receptors and good selectivity towards σ_2- (factor 15), κ- (factor 580) and μ-receptors (factor > 400). The (2*S*)-configured phenylacetamide **22b** has almost the same affinity for σ_1- and σ_2-receptors resulting in a receptor binding profile similar to that of the atypical antipsychotic BMY-14802 (**26**). The κ-receptor affinity of the phenylacetamides **22** is low presumably by reason of the stereochemistry.

Experimental

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):[29] Silica gel 60, 0.040–0.063 mm (Merck); parentheses include: Diameter of the column (cm), eluent, fraction size (mL), R_f. Melting points: Melting point apparatus SMP 2 (Stuart Scientific), uncorrected. Optical rotation: Polarimeter 241 (Perkin-Elmer); 1.0 dm tube; concentration *c* (g/100 mL). Elemental analyses: CHN-Elementaranalysator Rapid (Heraeus), Elemental Analyzer 240 (Perkin-Elmer) and Vario EL (Elementaranalysesysteme GmbH). MS: MAT 312, MAT 8200, MAT 44, and TSQ 7000 (Finnigan); EI = electron impact, CI = chemical ionization. High resolution MS (HRMS): MAT 8200 (Finnigan). IR: IR spectrophotometer 1600 FT-IR and 2000 FT-IR (Perkin-Elmer); s = strong, m = medium, w = weak. ^{1}H NMR (300 MHz), ^{13}C NMR (75 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 of ^{1}H NMR signals were supported by 2D NMR techniques. HPLC: Gradient pump 2249 (Pharmacia); UV-detector VWM 2141 (Pharmacia); integrator Chromatopac C-r6A (Shimadzu); column LiChroCart® 250–4 (Merck); stationary phase LiChroSpher® 100 RP-18 endcapped; injection volume 20 μL.

(1*S*,5*S*)-6-Benzyl-2-methoxy-8-methyl-2-(trimethylsiloxy)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (10). Under nitrogen a solution of lithium bis(trimethylsilyl)amide (1 M in THF; 3.30 mL, 3.30 mmol) was added dropwise to a solution of **9**[20] (877 mg, 2.88 mmol) in THF (50 mL) at −78 °C. After a reaction time of 30 min at −78 °C a solution of chlorotrimethylsilane (1.15 mL, 8.97 mmol) in THF (6.0 mL) was added and the reaction mixture was stirred for 30 min at −78 °C, then for 60 min at room temperature. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate (80 mL). The solution was washed with NaOH (0.5 N, 2×50 mL), with HCl (0.5 N, 2×50 mL) and with brine (50 mL), dried (Na_2SO_4) and concentrated in vacuo. The resulting product **10** was characterized and applied for the next reaction without further purification. Colorless solid, yield 1.05 g (97%); tlc: ethyl acetate, R_f 0.50. $C_{19}H_{28}N_2O_4Si$ (376.4). MS (EI): *m*/*z* (%) = 376 (M, 48), 361 (M−CH_3, 15), 345 (M−OCH_3, 12), 285 (M−CH_2Ph, 4). $[\alpha]_{589}^{25} = +60.4$ (*c* 0.56, CH_2Cl_2). IR (film): ν (cm^{-1}) = 3032 (w, $\nu_{CH\ arom.}$), 2959 (m, $\nu_{CH\ aliph.}$), 1682 (s, $\nu_{C=O,\ tert.\ amides}$), 1452 (s, $\delta_{CH\ aliph.}$), 1254, 1017 (each m, ν_{COC}), 1105, 875 (each m, O–Si), 843 (m, $Si(CH_3)_3$), 733, 701 (each m, $\gamma_{monosubst.\ aromate}$). ^{1}H NMR ($CDCl_3$): δ = 0.21 (s, 9H, $Si(CH_3)_3$), 1.40–1.51 (m, 1H, 4-H), 1.65–1.89 (m, 3H, 4-H, 3-H), 3.00 (s, 3H, NCH_3), 3.25 (s, 3H, OCH_3), 3.82 (dd, *J* = 5.5/2.4 Hz, 1H, 5-H), 3.93 (s, 1H, 1-H), 4.30 (d, *J* = 14.6 Hz, 1H, NCH_2Ph), 4.72 (d, *J* = 14.6 Hz, 1H, NCH_2Ph), 7.20–7.36 (m, 5H, aromat. H). ^{13}C NMR ($CDCl_3$): δ = 1.3 (3 C, $Si(CH_3)_3$), 24.2 (1 C, C-4), 32.7 (1 C, C-3), 33.1 (1 C, NCH_3), 48.6 (1 C, NCH_2Ph), 48.8 (1 C, OCH_3), 59.0 (1 C, C-5), 69.5 (1 C, C-1), 98.3 (1 C, C-2), 127.7 (1 C, aromat CH), 128.3 (2 C, aromat. CH), 128.6 (2 C, aromat. CH), 135.8 (1 C, aromat. C), 166.0 (1 C, C=O), 168.4 (1 C, C=O).

(1*S*,5*S*)-6-Benzyl-8-methyl-6,8-diazabicyclo[3.2.2]nonane-2,7,9-trione (11). As described for **10** the piperazinedione **9** (8.28 g, 27.2 mmol) was reacted with lithium bis(trimethylsilyl)amide (30.0 mL, 30.0 mmol) and chlorotrimethylsilane (11.2 mL, 87.4 mmol, in 8 mL THF) to yield the mixed methyl silyl acetal **10**. Without purification **10** was dissolved in a mixture of THF (100 mL) and water (10 mL), *p*-toluenesulfonic acid (1.00 g, 5.25 mmol) was added and the mixture was stirred for 16 h at room temperature. The solvent was removed under reduced pressure and the residue was purified by fc (8 cm, ethyl acetate, 100 mL, R_f 0.41). Colorless solid, mp 183–184 °C, yield 6.70 g (90%, with regard to **9**). $C_{15}H_{16}N_2O_3$ (272.3) calcd C 66.2H 5.92 N 10.3, found C 65.9H 6.41 N 10.4. MS (EI): *m*/*z* (%) = 272 (M, 49), 215 (M−CO–NCH_3, 8), 181 (M−CH_2Ph, 97). MS (CI): *m*/*z* (%) = 273 (MH^+, 100), 181 (MH^+−$PhCH_3$, 2). $[\alpha]_{589}^{25} = +5.2$ (*c* 0.55, CH_2Cl_2). IR (film): ν (cm^{-1}) = 3030 (w, $\nu_{CH\ arom.}$), 2975, 2935 (each m, $\nu_{CH\ aliph.}$), 1728 (s, $\nu_{C=O,\ ketone}$), 1682 (s, $\nu_{C=O,\ tert.\ amides}$), 1452 (s, $\delta_{CH\ aliph.}$), 1253, 1177 (each m, ν_{COC}), 732 (m, $\gamma_{monosubst.\ aromate}$). ^{1}H NMR ($CDCl_3$): δ = 1.84 (dddd, *J* = 14.5, 8.8, 7.2, 3.3 Hz, 1H, 4-H), 2.28 (ddt, *J* = 14.5/8.4/4.1 Hz, 1H, 4-H), 2.46 (ddd, *J* = 15.5, 7.3, 4.3 Hz, 1H, 3-H), 2.67 (dt, *J* = 15.5, 8.4 Hz, 1H, 3-H), 3.03 (s, 3H, NCH_3), 4.03 (t, *J* = 3.7 Hz, 1H, 5-H), 4.18 (s, 1H, 1-H), 4.53 (d, *J* = 14.6 Hz, 1H, NCH_2Ph), 4.66 (d, *J* = 14.6 Hz, 1H, NCH_2Ph), 7.21–7.36 (m, 5H, aromat. H). ^{13}C NMR ($CDCl_3$): δ = 29.2 (1 C, C-4), 32.9 (1 C, NCH_3), 36.9 (1 C, C-3), 49.0 (1 C, NCH_2Ph), 59.1 (1 C, C-5), 73.5 (1 C, C-1), 128.3 (2 C, aromat. CH), 128.4 (1 C, aromat. CH), 129.1 (2 C, aromat. CH), 135.3 (1 C, aromat. C), 163.3 (1 C, C=O), 167.4 (1 C, C=O), 199.2 (1 C, $C=O_{ketone}$).

(1*S*,2*R*,5*S*)-6-Benzyl-2-hydroxy-8-methyl-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (12a) and (1*S*,2*S*,5*S*)-6-benzyl-2-hydroxy-8-methyl-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (12b). Sodium borohydride (0.83 g, 21.9 mmol) was added to a cooled (ice bath) solution of **11** (1.95 g, 7.16 mmol) in a mixture of THF/propan-2-ol/water

(100 mL, 20 mL, 5 mL). After stirring for 16 h at room temperature the solvent was removed in vacuo, the residue was dissolved in ethyl acetate (80 mL) and washed with water (2×50 mL) and HCl (0.5 N, 1×50 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo to yield a mixture of the diastereomeric alcohols **12a** and **12b** as a colorless solid. The diastereomers **12a**/**12b** (ratio 85:15 according to the ^1H NMR spectrum) were separated by fc (4 cm, ethyl acetate/acetone = 1:1, 20 mL).

12a (R_f 0.38): Colorless solid, mp 165–167 °C yield 0.53 g (27%). $C_{15}H_{18}N_2O_3$ (274.3) calcd C 65.7H 6.61 N 10.2, found C 65.7H 6.55 N 10.1. MS (EI): *m/z* (%) = 274 (M, 35), 217 (M−CH_3NCO, 9), 183 (M−CH_2Ph, 39). $[\alpha]_{589}^{25}$ = +166 (*c* 1.06, CH_2Cl_2). IR (film): ν (cm^{-1}) = 3419 (m, ν_{OH}), 3055 (w, $\nu_{CH\ arom.}$), 2976 (w, $\nu_{CH\ aliph.}$), 1678 (s, $\nu_{C=O,\ tert.\ amides}$), 1516, 1456 (each w, $\delta_{CH\ aliph.}$), 1265, 1073 (each m, ν_{COC}), 737, 700 (each m, $\gamma_{monosubst.\ aromate}$). ^1H NMR ($CDCl_3$): δ = 1.48–1.75 (m, 3H, 4-H, 3-H), 1.90–2.02 (m, 1H, 3-H), 2.99 (s, 3H, NC*H*$_3$), 3.35–3.45 (s broad, 1H, CHO*H*), 3.82 (dt, *J* = 4.6, 3.3 Hz, 1H, 2-H), 3.86 (dd, *J* = 5.4, 2.4 Hz, 1H, 5-H), 4.00 (d, *J* = 3.1 Hz, 1H, 1-H), 4.50 (d, *J* = 14.3 Hz, 1H, NC*H*$_2$Ph), 4.68 (d, *J* = 14.6 Hz, 1H, NC*H*$_2$Ph), 7.22–7.36 (m, 5H, aromat. H); ^{13}C NMR ($CDCl_3$): δ = 23.4 (1 C, C-4), 29.1 (1 C, C-3), 32.7 (1 C, N*C*H$_3$), 48.7 (1 C, N*C*H$_2$Ph), 59.0 (1 C, C-5), 65.8 (1 C, C-2), 67.7 (1 C, C-1), 128.1 (1 C, aromat. CH), 128.4 (2 C, aromat. CH), 128.9 (2 C, aromat. CH), 135.6 (1 C, aromat. C), 167.7 (1 C, C=O), 169.5 (1 C, C=O).

12b (R_f 0.45): Colorless solid, mp 171–173 °C, yield 0.20 g (10%).$C_{15}H_{18}N_2O_3$ (274.3) calcd C 65.7H 6.61 N 10.2, found C 65.4H 6.61 N 10.2. MS (EI): *m/z* (%) = 274 (M, 79), 246 (M−CO, 6), 217 (M−CH_3NCO, 23), 183 (M−CH_2Ph, 52). $[\alpha]_{589}^{23}$ = +88.1 (*c* 0.50, CH_2Cl_2). IR (film): ν (cm^{-1}) = 3394 (m, ν_{OH}), 3063, 3030 (w, $\nu_{CH\ arom.}$), 2934 (m, $\nu_{CH\ aliph.}$), 1668 (s, $\nu_{C=O,\ tert.\ amides}$), 1455 (m, $\delta_{CH\ aliph.}$), 1254, 1063 (each m, ν_{COC}), 737, 701 (each m, $\gamma_{monosubst.\ aromate}$). ^1H NMR ($CDCl_3$): δ = 1.23–1.38 (m, 1H, 4-H), 1.49–1.71 (m, 1H, 3-H), 1.85–1.98 (m, 2H, 3-H, 4-H), 3.11 (s, 3H, NCH_3), 3.36–3.54 (s broad, 1H, CHO*H*), 3.82 (dd, *J* = 4.9, 2.4 Hz, 1H, 5-H), 4.05 (d, *J* = 1.8 Hz, 1H, 1-H), 4.14 (ddd, *J* = 8.5, 4.8, 1.9 Hz, 1H, 2-H), 4.43 (d, *J* = 14.6 Hz, 1H, NC*H*$_2$Ph), 4.58 (d, *J* = 14.6 Hz, 1H, NC*H*$_2$Ph), 7.11–7.35 (m, 5H, aromat. H). ^{13}C NMR ($CDCl_3$): δ = 24.5 (1 C, C-4), 29.1 (1 C, C-3), 34.5 (1 C, N*C*H$_3$), 48.5 (1 C, N*C*H$_2$Ph), 59.0 (1 C, C-5), 67,2 (1 C, C-2), 69.6 (1 C, C-1), 128.1 (1 C, aromat. CH), 128.2 (2 C, aromat. CH), 128.9 (2 C, aromat. CH), 135.4 (1 C, aromat. C), 167.9 (1 C, C=O), 168.3 (1 C, C=O).

Additionally, a mixture of **12a** and **12b** was isolated. Colorless solid (R_f 0.45/0.38) yield 0.78 g (40%). Total yield 1.51 g (77%).

[(1*S*,2*R*,5*S*)-(6-Benzyl-8-methyl-7,9-dioxo-6,8-diazabicyclo[3.2.2]nonan-2-yl)]methane sulfonate (13). To a cooled (ice bath) solution of **12a** (209 mg, 0.76 mmol) in CH_2Cl_2 (20 mL) triethylamine (0.32 mL, 2.30 mmol) and methanesulfonyl chloride (0.10 mL, 1.28 mmol), dissolved in CH_2Cl_2 (0.9 mL), were added successively. After stirring for 30 min at 0 °C and 2.5 h at room temperature NaOH (0.5 N, 20 mL) was added, followed by stirring for another 30 min. The aqueous layer was separated and extracted with CH_2Cl_2 (10 mL) once. The combined organic layers were washed with 0.5 N NaOH (1×20 mL), 0.5 N HCl (1×20 mL) and brine (1×20 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by fc (2 cm, ethyl acetate/acetone = 8:2, 10 mL, R_f 0.56). Colorless solid, mp 159–161 °C, yield 246 mg (92%). $C_{16}H_{20}N_2O_5S$ (352.4) calcd C 54.5H 5.76 N 7.85 S 9.10, found C 54.6H 5.72 N 7.95 S 8.87. MS (EI): *m/z* (%) = 352 (M, 44), 261 (M−CH_2Ph, 2), 256 (M−CH_3SO_2OH, 15), 228 (M−$CH_3CH_2OSO_2CH_3$, 58), 137 (228−CH_2Ph, 10). $[\alpha]_{589}^{24}$ = +147 (*c* 1.16, CH_2Cl_2). IR (film): ν (cm^{-1}) = 3024 (w, $\nu_{CH\ arom.}$), 2935 (w, $\nu_{CH\ aliph.}$), 1682 (s, $\nu_{C=O,\ tert.\ amides}$), 1451 (m, $\delta_{CH\ aliph.}$), 1354, 1173 (each m, CH_3–SO_2O–R), 1253 (w, ν_{COC}), 733, 701 (each m, $\gamma_{monosubst.\ aromate}$). ^1H NMR ($CDCl_3$): δ = 1.52–1.64 (m, 1H, 4-H), 1.70–1.87 (m, 2H, 4-H, 3-H), 2.02 (ddt, *J* = 13.7, 9.4, 4.8 Hz, 1H, 3-H), 3.03 (s, 3H, NC*H*$_3$), 3.15 (s, 3H, OSO_2CH_3), 3.91 (dd, *J* = 6.1, 2.4 Hz, 1H, 5-H), 4.20 (d, *J* = 3.4 Hz, 1H, 1-H), 4.39 (d, *J* = 14.3 Hz, 1H, NC*H*$_2$Ph), 4.73 (ddd, *J* = 8.6, 5.2, 3.4 Hz, 1H, 2-H), 4.83 (d, *J* = 14.3 Hz, 1H, NC*H*$_2$Ph), 7.25–7.39 (m, 5H, aromat. H). ^{13}C NMR ($CDCl_3$): δ = 23.4 (1 C, C-4), 27.1 (1 C, C-3), 32.7 (1 C, N*C*H$_3$), 39.0 (1 C, OSO_2CH_3), 49.0 (1 C, N*C*H$_2$Ph), 58.7 (1 C, C-5), 64.8 (1 C, C-1), 72.0 (1 C, C-2), 128.3 (1 C, aromat. CH), 128.5 (2 C, aromat. CH), 128.9 (2 C, aromat. CH), 135.6 (1 C, aromat. C), 165.1 (1 C, C=O), 169.0 (1 C, C=O).

(1*S*,2*S*,5*S*)-2-Azido-6-benzyl-8-methyl-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (14). A solution of **13** (982 mg, 2.79 mmol) and sodium azide (920 mg, 14.1 mmol) in DMF (60 mL) was heated to reflux for 2 h. Then it was concentrated in vacuo, the oily residue was dissolved in ethyl acetate (120 mL) and washed with water (3×80 mL). The organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (3 cm, ethyl acetate, 10 mL, R_f 0.55). Colorless solid, mp 142–144 °C, yield 656 mg (79%). $C_{15}H_{17}N_5O_2$ (299.3) calcd C 60.2H 5.73 N 23.39, found C 60.3H 5.82 N 23.21. MS (EI): *m/z* (%) = 299 (M, 8), 271 (M−N_2, 4), 256 (M−N_3H, 3), 208 (M−CH_2Ph, 2), 180 (M−N_2–CH_2Ph, 51). $[\alpha]_{589}^{22}$ = +92.0 (*c* 0.80, CH_2Cl_2). IR (film): ν (cm^{-1}) = 3031 (w, $\nu_{CH\ arom.}$), 2936 (w, $\nu_{CH\ aliph.}$), 2101 (s, ν_{N3}), 1682 (s, $\nu_{C=O,\ tert.\ amides}$), 1453 (m, $\delta_{CH\ aliph.}$), 1251 (m, ν_{COC}), 733, 702 (each m, $\gamma_{monosubst.\ aromate}$). ^1H NMR ($CDCl_3$): δ = 1.26–1.39 (m, 1H, 4–H), 1.59–1.74 (m, 1H, 3-H), 1.87–2.02 (m, 2H, 4-H, 3-H), 3.07 (s, 3H, NC*H*$_3$), 3.85 (dd, *J* = 4.6, 3.0 Hz, 1H, 5-H), 3.90 (ddd, *J* = 8.6, 4.9, 2.1 Hz, 1H, 2-H), 3.97 (d, *J* = 2.1 Hz, 1H, 1-H), 4.48 (d, *J* = 14.6 Hz, 1H, NC*H*$_2$Ph), 4.56 (d, *J* = 14.3 Hz, 1H, C*H*$_2$Ph), 7.19–7.36 (m, 5H, aromat. H). ^{13}C NMR ($CDCl_3$): δ = 24.2 (1 C, C-4), 26.0 (1 C, C-3), 34.1 (1 C, N*C*H$_3$), 48.8 (1 C, N*C*H$_2$Ph), 57.3 (1 C, C-2), 58.8 (1 C, C-5), 66.4 (1 C, C-1), 128.3 (1 C, aromat. CH), 128.4 (2 C, aromat. CH), 129.0 (2 C, aromat. CH), 135.3 (1 C, aromat. C), 166.7 (1 C, C=O), 168.0 (1 C, C=O).

(1*S*,2*S*,5*S*)-2-Amino-6-benzyl-8-methyl-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (15). Pd/C catalyst (10%, 30 mg) was added to a solution of **14** (180 mg, 0.60 mmol) in methanol (12 mL). The suspension was stirred under a H_2 atmosphere (1 bar, balloon) at room temperature for 4.5 h. The mixture was filtered through a pad of Celite® AFA and the filtrate was concentrated in vacuo. Colorless solid, mp 171–174 °C, yield 163 mg (99%). $C_{15}H_{19}N_3O_2$ (273.3) calcd C 65.9H 7.01 N 15.37, found C 65.5H 7.36 N 14.92. MS (EI): *m*/*z* (%) = 273 (M, 7), 230 (M−CH_2CHNH_2, 28), 182 (M−CH_2Ph, 51). $[\alpha]_{589}^{23} = +71.7$ (*c* 0.79, CH_2Cl_2). IR (film): ν (cm^{-1}) = 3445 (m, ν_{NH2}), 3058 (w, $\nu_{CH\ arom.}$), 2933 (w, $\nu_{CH\ aliph.}$), 1671 (s, $\nu_{C=O,\ tert.\ amides}$), 1455 (m, $\delta_{CH\ aliph.}$), 1261, 1181 (each w, ν_{COC}), 735, 702 (each m, $\gamma_{monosubst.\ aromate}$). ^{1}H NMR ($CDCl_3$): δ = 1.24–1.48 (m, 4H, 3-H, 4-H, N*H*$_2$), 1.75–1.92 (m, 2H, 3-H, 4-H), 3.13 (s, 3H, NC*H*$_3$), 3.34 (ddd, *J* = 8.5, 4.9, 1.6 Hz, 1H, 2-H), 3.77–3.84 (m, 2H, 1-H, 5-H), 4.44 (d, *J* = 14.6 Hz, 1H, NC*H*$_2$Ph), 4.55 (d, *J* = 14.6 Hz, 1H, NC*H*$_2$Ph), 7.17–7.33 (m, 5H, aromat. H). ^{13}C NMR ($CDCl_3$): δ = 24.3 (1 C, C-4), 29.7 (1 C, C-3), 35.0 (1 C, N*C*H$_3$), 48.4 (1 C, N*C*H$_2$Ph), 48.6 (1 C, C-2), 59.0 (1 C, C-5), 69.7 (1 C, C-1), 128.0 (1 C, aromat. CH), 128.2 (2 C, aromat. CH), 128.9 (2 C, aromat. CH), 135.6 (1 C, aromat. C), 168.3 (1 C, C=O), 168.5 (1 C, C=O).

(1S,2S,5S)-6-Benzyl-2-(dimethylamino)-8-methyl-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (16). A solution of formaldehyde (37% in water; 1.2 mL, 15 mmol) and subsequently $NaBH_3CN$ (62 mg, 1.0 mmol) were added to a solution of **15** (104 mg, 0.38 mmol) in acetonitrile (10 mL). After stirring for 3 h at room temperature the solvent was removed and the residue was dissolved in ethyl acetate (50 mL). The organic solution was washed with NaOH (0.5 N, 2×30 mL), dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (2 cm, acetone/C_2H_5OH = 9:1, 8 mL, R_f 0.33). Colorless oil, yield 76 mg (66%). $C_{17}H_{23}N_3O_2$ (301.2). HRMS: calcd 301.1790, found 301.1792 (+0.5 ppm). MS (EI): *m*/*z* (%) = 301 (M, 9), 256 (M−$HN(CH_3)_2$, 2), 230 (M−$CH_2CHN(CH_3)_2$, 1), 165 (256−CH_2Ph, 1). MS (CI): *m*/*z* (%) = 302 (MH^+, 78), 256 (M−$HN(CH_3)_2$, 3), 167 (MH^+−CH_2Ph–$N(CH_3)_2$, 14). $[\alpha]_{589}^{20} = +80.4$ (*c* 0.53, CH_2Cl_2). IR (film): ν (cm^{-1}) = 3030 (w, $\nu_{CH\ arom.}$), 2946 (m, $\nu_{CH\ aliph.}$), 2782 (w, ν_{NCH3}), 1677 (s, $\nu_{C=O,\ tert.\ amides}$), 1455 (m, $\delta_{CH\ aliph.}$), 1256, 1038 (each m, ν_{COC}), 732, 700 (each m, $\gamma_{monosubst.\ aromate}$). ^{1}H NMR ($CDCl_3$): δ = 1.23 (dddd, *J* = 14.0, 12.5, 5.2, 1.5 Hz, 1H, 4-H), 1.53 (dtd, *J* = 13.6, 11.9, 5.5 Hz, 1H, 3-H), 1.73 (dtd, *J* = 13.6, 4.9, 3.1 Hz, 1H, 3-H), 1.89 (dtd, *J* = 14.3, 5.8, 3.0 Hz, 1H, 4-H), 2.22 (s, 6H, N(C*H*$_3$)$_2$), 2.55 (dd, *J* = 11.3, 4.6 Hz, 1H, 2-H), 3.00 (s, 3H, NC*H*$_3$), 3.74 (dd, *J* = 6.1, 1.5 Hz, 1H, 5-H), 3.95 (s, 1H, 1-H), 4.40 (d, *J* = 14.6 Hz, 1H, NC*H*$_2$Ph), 4.50 (d, *J* = 14.6 Hz, 1H, NC*H*$_2$Ph), 7.14–7.29 (m, 5H, aromat. H).

(1S,2S,5S)-6-Benzyl-2-(diethylamino)-8-methyl-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (17). Acetaldehyde (0.45 mL, 8.0 mmol) was added to a cooled (ice bath) solution of **15** (55 mg, 0.20 mmol) in acetonitrile (6 mL). After 15 min $NaBH_3CN$ (35 mg, 0.56 mmol) was added and the reaction mixture was stirred for 3 h at 0 °C. Removal of the solvent in vacuo furnished a yellow oil, which was dissolved in ethyl acetate (30 mL) and washed with NaOH (0.5 N, 2×20 mL). The dried organic layer (Na_2SO_4) was evaporated in vacuo and purified by fc (2 cm, ethyl acetate/acetone = 8:2, 5 mL, R_f 0.32). Pale yellow oil, yield 51 mg (77%). $C_{19}H_{27}N_3O_2$ (329.2). HRMS: calcd 329.2103, found 329.2104 (+0.3 ppm). MS (EI): *m*/*z* (%) = 329 (M, 4), 167 (M−CH_2Ph–NC_4H_9, 1), 139 (167−NCH_3, 3), 112 ($CH_2CHCHN(C_2H_5)_2$, 100). MS (CI): *m*/*z* (%) = 330 (MH^+, 100), 302 (MH^+−NCH_3, 5), 271 (M−2×C_2H_5, 2), 243 (M−$CH_2N(C_2H_5)_2$, 4). $[\alpha]_{589}^{21} = +37.3$ (*c* 0.36, CH_2Cl_2). IR (film): ν (cm^{-1}) = 3029 (w, $\nu_{CH\ arom.}$), 2970, 2934 (each m, $\nu_{CH\ aliph.}$), 2815 (w, ν_{NCH3}), 1676 (s, $\nu_{C=O,\ tert.\ amides}$), 1456 (s, $\delta_{CH\ aliph.}$), 1232, 1060 (each m, ν_{COC}), 733, 702 (each m, $\gamma_{monosubst.\ aromate}$). ^{1}H NMR ($CDCl_3$): δ = 1.03 (t, *J* = 7.0 Hz, 6H, N(CH_2CH_3)$_2$), 1.23–1.38 (m, 1H, 4-H), 1.62–1.78 (m, 2H, 3-H, 4-H), 1.96 (dtd, *J* = 14.6, 5.6, 3.5 Hz, 1H, 3-H), 2.45 (dq, *J* = 13.7, 6.8 Hz, 2H, N(CH_2CH_3)$_2$), 2.63 (dq, *J* = 14.3, 7.0 Hz, 2H, N(CH_2CH_3)$_2$), 2.97–3.07 (m, 1H, 2-H), 3.05 (s, 3H, NC*H*$_3$), 3.79 (dd, *J* = 6.1, 1.5 Hz, 1H, 5-H), 3.92 (s, 1H, 1-H), 4.43 (d, *J* = 14.6 Hz, 1H, NC*H*$_2$Ph), 4.58 (d, *J* = 14.6 Hz, 1H, NC*H*$_2$Ph), 7.17–7.35 (m, 5H, aromat. H).

(1*S*,2*S*,5*S*)-6-Benzyl-8-methyl-2-(pyrrolidin-1-yl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (18). Triethylamine (0.4 mL, 2.9 mmol) and a solution of 1-bromo-4-chlorobutane (0.3 mL, 2.6 mmol) in DMF (0.7 mL) were added to a solution of **15** (63 mg, 0.23 mmol) in DMF (20 mL). The reaction mixture was heated to reflux for 16 h. Then, the mixture was concentrated in vacuo, the resulting brown oil was dissolved in ethyl acetate (50 mL), washed with NaOH (2×30 mL) and water (1×30 mL) and dried (Na_2SO_4). The solvent was evaporated in vacuo and the residue was purified by fc (2 cm, acetone, 4 mL, R_f 0.38). Pale yellow oil, yield 8 mg (11%). $C_{19}H_{25}N_3O_2$ (327.2). HRMS: calcd 327.1947, found 327.1946 (−0.2 ppm). MS (EI): *m*/*z* (%) = 327 (M, 2), 236 (M−CH_2Ph, 1), 217 (M−$CH_2CHCHN(CH_2)_4$, 2). $[\alpha]_{589}^{21} = +73.2$ (*c* 0.38, CH_2Cl_2). IR (film): ν (cm^{-1}) = 2965 (w, $\nu_{CH\ aliph.}$), 1669 (s, $\nu_{C=O,\ tert.\ amides}$), 1457 (m, $\delta_{CH\ aliph.}$), 1261 (w, ν_{COC}), 733, 699 (each m, $\gamma_{monosubst.\ aromate}$). ^{1}H NMR ($CDCl_3$): δ = 1.22–1.34 (m, 1H, 4-H), 1.48–1.66 (m, 1H, 3-H), 1.68–1.82 (m, 4H, $CH_2CH_2NCH_2CH_2$), 1.86–2.00 (m, 2H, 3-H, 4-H), 2.50–2.68 (m, 5H, $CH_2CH_2NCH_2CH_2$, 2-H), 3.10 (s, 3H, NC*H*$_3$), 3.80 (dd, *J* = 6.4, 1.4 Hz, 1H, 5-H), 4.15 (s, 1H, 1-H), 4.46 (d, *J* = 14.6 Hz, 1H, NC*H*$_2$Ph), 4.59 (d, *J* = 14.6 Hz, 1H, NC*H*$_2$Ph), 7.20–7.38 (m, 5H, aromat. H).

(*E*)- and (*Z*)-(1*S*,5*S*)-6-Benzyl-2-(hydroxyimino)-8-methyl-6,8-diazabicyclo[3.2.2]nonane-7,9-dione ((*E*)-20 and (*Z*)-20). The bicyclic ketone **11** (2.05 g, 7.51 mmol) was added to a solution of hydroxylamine hydrochloride (2.64 g, 38 mmol) and triethylamine (5.3 mL, 38 mmol) in methanol (25 mL). After stirring for 16 h at room temperature the solvent was evaporated in vacuo. The residue was dissolved in ethyl acetate (120 mL), washed with water (2×100 mL) and dried (Na_2SO_4). After removal of the solvent under reduced pressure the resi-

due was purified by fc (4 cm, ethyl acetate, 20 mL, R_f 0.38/0.30) providing a mixture of (*E*)-**20** and (*Z*)-**20** (ratio 1:1 according to the ^{1}H NMR spectrum), colorless solid, yield 1.79 g (83%). The diastereomers were separated by a further fc (4 cm, ethyl acetate, 10 mL).

(*E*)-**20** (R_f 0.38): Colorless solid, mp 155–158 °C, yield 0.43 g (20%). $C_{15}H_{17}N_3O_3$ (287.1). HRMS: calcd 287.1270, found 287.1279 (+3.2 ppm). MS (EI): *m*/*z* (%) = 287 (M, 11), 270 (M−OH, 48), 196 (M−CH_2Ph, 48). $[\alpha]_{589}^{20}$ = +128 (*c* 0.23, CH_2Cl_2). IR (film): ν (cm^{-1}) = 3300 (m, ν_{N-OH}), 3085 (w, $\nu_{CH\ arom.}$), 2932 (w, $\nu_{CH\ aliph.}$), 1682 (s, $\nu_{C=O,\ tert.\ amides\ +\ C=N,\ oxime}$), 448 (m, $\delta_{CH\ aliph.}$), 1254 (m, ν_{COC}), 732, 699 (each m, $\gamma_{monosubst.\ aromate}$). ^{1}H NMR ($CDCl_3$): δ = 1.61 (dddd, *J* = 14.3, 7.6, 6.6, 4.7 Hz, 1H, 4-H), 1.95 (dddd, *J* = 14.6, 7.7, 6.7, 3.1 Hz, 1H, 4-H), 2.60 (ddd, *J* = 17.1, 7.6, 6.7 Hz, 1H, 3-H), 2.70 (ddd, *J* = 17.1, 7.4, 6.6 Hz, 1H, 3-H), 2.98 (s, 3H, NCH_3), 3.99 (dd, *J* = 4.6, 3.0 Hz, 1H, 5-H), 4.38 (s, 1H, 1-H), 4.53 (d, *J* = 14.6 Hz, 1H, NCH_2Ph), 4.70 (d, *J* = 14.6 Hz, 1H, NCH_2Ph), 7.24–7.38 (m, 5H, aromat. H), 8.59 (s broad, 1H, N–O*H*).

(Z)-**20**: In a second fraction (R_f 0.34–0.28), the (*Z*)-isomer (*Z*)-**20** predominated [(*E*)-**20**/(*Z*)-**20** = 33:67 according to the ^{1}H NMR spectrum]. Colorless solid, mp 158–164 °C, yield 1.28 g (59%). $C_{15}H_{17}N_3O_3$ (287.1). HRMS: calcd 287.1270, found 287.1276 (+2.2 ppm). MS (EI): *m*/*z* (%) = 287 (M, 12), 270 (M−OH, 30), 196 (M−CH_2Ph, 42). $[\alpha]_{589}^{20}$ = +125 (*c* 0.59, CH_2Cl_2). IR (film): ν (cm^{-1}) = 3301 (m, ν_{N-OH}), 3087 (w, $\nu_{CH\ arom.}$), 2930 (w, $\nu_{CH\ aliph.}$), 1680 (s, $\nu_{C=O,\ tert.\ amides\ +\ C=N,\ oxime}$), 1449 (m, $\delta_{CH\ aliph.}$), 1254 (m, ν_{COC}), 732, 700 (each m, $\gamma_{monosubst.\ aromate}$). ^{1}H NMR ($CDCl_3$): δ = 1.55–1.68 (m, 0.33H, 4-H^E), 1.59 (ddd, *J* = 13.7, 11.3, 6.9 Hz, 0.67H, 3-H^Z), 1.88–2.03 (m, 1.67H, 4-H^{Z+E}), 2.33–2.42 (m, 0.67H, 3-H^Z), 2.54–2.73 (m, 2×0.33H, 3-H^E), 2.98 (s, 3×0.33H, NCH_3^E), 3.04 (s, 3×0.67H, NCH_3^Z), 3.99 (dd, *J* = 8.1, 4.5 Hz, 1H, 5-H^{Z+E}), 4.37 (s, 0.33H, 1-H^E), 4.49 (d, *J* = 14.5 Hz, 0.67H, NCH_2Ph^Z), 4.52 (d, *J* = 14.5 Hz, 0.33H, NCH_2Ph^E), 4.67 (d, *J* = 14.5 Hz, 0.33H, NCH_2Ph^E), 4.69 (d, *J* = 14.5 Hz, 0.67H, NCH$_2$Ph^Z), 5.39 (s, 0.67H, 1-H^Z), 7.21–7.37 (m, 5H, aromat. H), 9.57 (s broad, 0.67H, N–OH^Z). A signal for the OH-proton of (E)-**20** was not found.

(1*R*,2*R*,5*S*)- and (1*R*,2*S*,5*S*)-6-Benzyl-8-methyl-6,8-diazabicyclo[3.2.2]nonan-2-amine (21). A solution of $LiAlH_4$ (1 M in Et_2O; 10.0 mL, 10.0 mmol) was added to a cooled (ice bath) suspension of **20** (mixture of diastereomers, 606 mg, 2.11 mmol) in THF (70 mL) and the reaction mixture was heated to reflux for 20 h. The excess of $LiAlH_4$ was carefully destroyed by successive addition of $Na_2SO_4\times10H_2O$ (2.5 g). Then the suspension was heated to reflux for 60 min. After cooling to room temperature, the precipitate was filtered off and thoroughly extracted with ethyl acetate. The organic layer was concentrated in vacuo. With regard to the instability of the primary amine **21**, the resulting colorless, transparent oil (yield: 338 mg, 65%) was neither purified nor characterized, but directly reacted further on.

(1*R*,2*R*,5*S*)-6-Benzyl-*N*,*N*,8-trimethyl-6,8-diazabicyclo[3.2.2]nonan-2-amine (19a) and (1*R*,2*S*,5*S*)-6-benzyl-*N*,*N*,8-trimethyl-6,8-diazabicyclo[3.2.2]nonan-2-amine (19b). Method A. Synthesis of both diastereomers **19a** and **19b** via reduction of the oxime **20** and reductive methylation. A cooled (ice bath) solution of the primary amine **21** (338 mg, 1.38 mmol) in acetonitrile (25 mL) was reacted with an aqueous solution of formaldehyde (37%, 4.2 mL, 56 mmol) and after 15 min $NaBH_3CN$ (202 mg, 3.2 mmol) was added. After stirring for 1 h at room temperature, the pH of the solution was brought to pH 7 by dropwise addition of glacial acetic acid. The reaction mixture was stirred for 20 h at room temperature, then the solvent was evaporated in vacuo. The residue was dissolved in ethyl acetate (100 mL), the solution was washed with NaOH (0.5 N, 2×70 mL) and water (1×70 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by fc (2 cm, 24 cm, acetone/C_2H_5OH = 8:2 + 2% ethyldimethylamine, 2 mL).

19a (R_f 0.16): Pale yellow oil, yield 36 mg (6.3% with regard to the oxime **20**). $C_{17}H_{27}N_3$ (273.2). HRMS: calcd 273.2205, found 273.2205 (+0.2 ppm). MS (EI): *m*/*z* (%) = 273 (M, 20), 228 (M−HN$(CH_3)_2$, 4), 203 (M−H_2CCHN$(CH_3)_2$, 47), 187 (M−$CH_2CH_2CH_2$N$(CH_3)_2$, 12), 137 (228−CH_2Ph, 45). $[\alpha]_{589}^{16}$ = +13.1 (*c* 0.38, CH_2Cl_2). IR (film): ν (cm^{-1}) = 3028 (w, $\nu_{CH\ arom.}$), 2934 (s, $\nu_{CH\ aliph.}$), 2782 (s, ν_{NCH3}), 1452 (m, $\delta_{CH\ aliph.}$), 730, 698 (each m, $\gamma_{monosubst.\ aromate}$). ^{1}H NMR ($CDCl_3$): δ = 1.41–1.54 (m, 1H, 4-H), 1.65–1.77 (m, 1H, 3-H), 1.86–2.14 (m, 2H, 3-H, 4-H), 2.28 (s, 6H, N(CH_3)$_2$), 2.43 (s, 3H, NCH_3), 2.48–2.57 (m, 1H, 2-H), 2.52 (dd, *J* = 10.7, 2.1 Hz, 1H, 9-H), 2.72–2.76 (m, 1H, 1-H), 2.84 (ddt, *J* = 5.8, 4.2, 2.0 Hz, 1H, 5-H), 2.91 (dd, *J* = 14.6, 3.9 Hz, 1H, 7-H), 2.96 (dd, *J* = 14.5, 3.8 Hz, 1H, 7-H), 2.99 (dd, *J* = 11.3, 1.8 Hz, 1H, 9-H), 3.68 (s, 2H, NCH_2Ph), 7.15–7.37 (m, 5H, aromat. H). ^{13}C NMR ($CDCl_3$): δ = 23.8 (1 C, C-3), 30.4 (1 C, C-4), 42.6 (2 C, N(*C*H_3)$_2$), 43.6 (1 C, N*C*H_3), 50.2 (1 C, C-7), 52.4 (1 C, C-5), 55.0 (1 C, C-9), 58.8 (1 C, C-1), 60.3 (1 C, N*C*H_2Ph), 67.6 (1 C, C-2), 126.7 (1 C, aromat. CH), 128.1 (2 C, aromat. CH), 128.4 (2 C, aromat. CH), 139.9 (1 C, aromat. C).

19b (R_f 0.30): Pale yellow oil, yield 54 mg (9.4% with regard to the oxime **20**). $C_{17}H_{27}N_3$ (273.2). HRMS: calcd 273.2205, found 273.2205 (+0.2 ppm). MS (EI): *m*/*z* (%) = 273 (M, 20), 228 (M−HN$(CH_3)_2$, 5), 203 (M−H_2CCHN$(CH_3)_2$, 48), 187 (M−$CH_2CH_2CH_2$N$(CH_3)_2$, 13), 137 (228−CH_2Ph, 48). $[\alpha]_{589}^{16}$ = +32.4 (*c* 0.49, CH_2Cl_2). IR (film): ν (cm^{-1}) = 3028 (w, $\nu_{CH\ arom.}$), 2927 (s, $\nu_{CH\ aliph.}$), 2788 (s, ν_{NCH3}), 1451 (m, $\delta_{CH\ aliph.}$), 727, 697 (each m, $\gamma_{monosubst.\ aromate}$). ^{1}H NMR ($CDCl_3$): δ = 1.48–1.57 (m, 1H, 3-H), 1.62 (dddd, *J* = 13.7, 8.8, 4.3, 2.1 Hz, 1H, 4-H), 1.72 (dddd, *J* = 13.6, 8.8, 4.6, 1.6 Hz, 1H, 4-H), 2.01–2.16 (m, 1H, 3-H), 2.24 (s, 6H, N(CH_3)$_2$), 2.29 (s, 3H, NCH_3), 2.60–2.69 (m, 4H, 1-H, 5-H, 7-H, 9-H), 2.76 (dd, *J* = 9.2, 1.5 Hz, 1H, 7-H), 2.90 (dd, *J* = 10.4, 2.0 Hz, 1H, 9-H), 2.94 (dd, *J* = 12.2, 3.4 Hz, 1H, 2-H), 3.64 (d, *J* = 13.4 Hz, 1H, NCH_2Ph), 3.71 (d, *J* = 13.4 Hz, 1H, NCH_2Ph), 7.18–7.38 (m, 5H, aromat. H). ^{13}C NMR ($CDCl_3$): δ = 21.1 (1 C, C-3), 33.5

(1 C, C-4), 40.8 (2 C, N(CH_3)$_2$), 43.4 (1 C, NCH_3), 49.8 (1 C, C-7), 52.9 (1 C, C-9), 54.8 (1 C, C-5), 60.1 (1 C, C-1), 61.0 (1 C, NCH_2Ph), 67.6 (1 C, C-2), 126.7 (1 C, aromat. CH), 128.1 (2 C, aromat. CH), 128.6 (2 C, aromat. CH), 139.9 (1 C, aromat. C).

Method B. Diastereoselective synthesis of **19b** via reduction of the dimethylamine **16**. A solution of $LiAlH_4$ (1 M in Et_2O; 3.0 mL, 3.0 mmol) was slowly added to an ice-cold solution of **16** (190 mg, 0.63 mmol) in THF (45 mL). The reaction mixture was heated to reflux for 88 h, then the excess of $LiAlH_4$ was carefully hydrolyzed with $Na_2SO_4\times10H_2O$ (5 g). The suspension was stirred for 30 min under reflux, the precipitate was filtered off and extracted with ethyl acetate. Removal of the solvent in vacuo directly provided the dimethylamine **19b** without further purification. Colorless oil, yield 166 mg (97%).

***N*-[(1*R*,2*R*,5*S*)-6-Benzyl-8-methyl-6,8-diazabicyclo[3.2.2]-nonan-2-yl]-2-(3,4-dichlorophenyl)acetamide (22a) and *N*-[(1*R*,2*S*,5*S*)-6-benzyl-8-methyl-6,8-diazabicyclo[3.2.2]-nonan-2-yl]-2-(3,4-dichlorophenyl)acetamide (22b).** As described for the synthesis of **21** a solution of $LiAlH_4$ in Et_2O (1 M, 10.0 mL, 10.0 mmol) was cautiously added to a cooled (ice-bath) suspension of the oxime **20** (mixture of diastereomers; 585 mg, 2.04 mmol) in THF (75 mL). After stirring for 15 min at 0 °C, the reaction mixture was heated to reflux for 36 h. The mixture was cooled and the excess of $LiAlH_4$ was carefully destroyed by addition of $Na_2SO_4\times10H_2O$ (4.5 g). The suspension was refluxed for another 30 min, then the precipitate was filtered off and washed with ethyl acetate. The filtrate was concentrated in vacuo to yield the primary amine **21** (colorless oil, yield 384 mg, 77%), which was directly reacted further on. The residue (384 mg) was dissolved in CH_2Cl_2 (25 mL) and treated with (3,4-dichlorophenyl)acetic acid (656 mg, 3.2 mmol) and carbonyldiimidazole (CDI, 520 mg, 3.2 mmol). After stirring for 30 min at 0 °C and for 40 h at room temperature, the reaction mixture was washed with a saturated solution of $NaHCO_3$ (20 mL) and with brine (20 mL). The organic layers were dried ($MgSO_4$), concentrated in vacuo and the residue was purified by fc (4 cm, petroleum ether/ethyl acetate 2:8 + 2% ethyldimethylamine, 5 mL).

22a (R_f 0.23): Pale yellow oil, yield: 94 mg (11% with regard to **20**). $C_{23}H_{27}Cl_2N_3O$ (431.2). HRMS: calcd 431.1531, found 431.1530 (−0.2 ppm). MS (EI): *m*/*z* (%) = 435/433/431 (M, 8/41/59), 344/342/340 (M−CH_2Ph, 4/21/35), 284 (M−$C_6H_3Cl_2$, 5). $[\alpha]_{589}^{16}$ = +24.8 (*c* 0.99, CH_2Cl_2). IR (film): ν (cm^{-1}) = 3303 (m, $\nu_{NH,\ s.\ amide}$), 3061, 3029 (each w, $\nu_{CH\ arom.}$), 2931 (m, $\nu_{CH\ aliph.}$), 2801 (m, ν_{NCH3}), 1647 (s, $\nu_{C=O,\ s.\ amide}$), 1545 (w, amide II), 1469 (m, $\delta_{CH\ aliph.}$), 1257, 1136 (each m, ν_{COC}), 910 (m, C–Cl), 818 (w, $\gamma_{dichlorophenyl}$), 732, 698 (each m, $\gamma_{monosubst.\ aromate}$). ^{1}H NMR ($CDCl_3$): δ = 1.68 (dq, *J* = 13.1, 6.6 Hz, 1H, 3-H), 1.78–1.93 (m, 2H, 4-H), 2.21 (dq, *J* = 13.4, 6.6 Hz, 1H, 3-H), 2.49 (s, 3H, NCH_3), 2.78 (d, *J* = 2.7 Hz, 2H, 7-H), 2.82 (dd, *J* = 11.0, 2.8 Hz, 1H, 9-H), 2.88 (dd, *J* = 11.0, 2.4 Hz, 1H, 9-H), 2.90 (dt, *J* = 5.8, 2.6 Hz, 1H, 1-H), 2.95 (dt, *J* = 7.9, 2.7 Hz, 1H, 5-H), 3.48 (d, *J* = 15.6 Hz, 1H, COCH_2Aryl), 3.55 (d, *J* = 15.6 Hz, 1H, COCH_2Aryl), 3.76 (s, 2H, NCH_2Ph), 4.46 (dtd, *J* = 8.9, 6.7, 5.8 Hz, 1H, 2-H), 6.20 (d broad, *J* = 8.9 Hz, 1H, N*H*), 7.17 (dd, *J* = 8.2, 2.1 Hz, 1H, aromat. 6-$H_{dichlorophenyl}$), 7.34–7.50 (m, 7H, aromat. H). ^{13}C NMR ($CDCl_3$): δ = 26.5 (1 C, C-3), 31.6 (1 C, C-4), 42.8 (1 C, COCH_2Aryl), 44.2 (1 C, NCH_3), 46.6 (1 C, C-7), 52.6 (1 C, C-2), 52.7 (1 C, C-9), 54.6 (1 C, C-5), 60.4 (1 C, C-1), 61.0 (1 C, NCH_2Ph), 127.2 (1 C, aromat. CH), 128.4 (2 C, aromat. CH), 128.5 (2 C, aromat. CH), 128.6 (1 C, aromat. $CH_{dichlorophenyl,\ C-6}$), 130.6 (1 C, aromat. $CH_{dichlorophenyl,\ C-2\ or\ C-5}$), 131.1 (1 C, aromat. $CH_{dichlorophenyl,\ C-5\ or\ C-2}$), 131.3 (1 C, aromat. *C*-Cl), 132.6 (1 C, aromat. *C*-Cl), 135.4 (1 C, aromat. $C_{dichlorophenyl}$), 139.2 (1 C, aromat. C), 168.7 (1 C, C=O).

22b (R_f 0.34): Pale yellow oil, yield 133 mg (15% with regard to **20**). $C_{23}H_{27}Cl_2N_3O$ (431.2) calcd C 63.9H 6.29 N 9.7, found C 63.4H 6.27 N 9.1. HRMS: calcd 431.1531, found 431.1530 (−0.2 ppm). MS (EI): *m*/*z* (%) = 435/433/431 (M, 1/4/7), 342/340 (M−CH_2Ph, 3/5), 285 (M−$C_6H_3Cl_2$, 77). $[\alpha]_{589}^{16}$ = +53.6 (*c* 0.55, CH_2Cl_2). IR (film): ν (cm^{-1}) = 3324 (m, $\nu_{NH,\ s.\ amide}$), 3061, 3028 (each w, $\nu_{CH\ arom.}$), 2933 (m, $\nu_{CH\ aliph.}$), 2801 (m, ν_{NCH3}), 1649 (s, $\nu_{C=O,\ s.\ amide}$), 1550 (w, amide II), 1497, 1471 (each m, $\delta_{CH\ aliph.}$), 1257, 1135 (each m, ν_{COC}), 910 (m, C-Cl), 819 (w, $\gamma_{dichlorophenyl}$), 732, 698 (each m, $\gamma_{monosubst.\ aromate}$). ^{1}H NMR ($CDCl_3$): δ = 1.57 (td broad, *J* = 12.8, 4.3 Hz, 1H, 4-H), 1.69 (dq, *J* = 13.6, 3.2 Hz, 1H, 3-H), 1.87 (ddt, *J* = 13.3, 8.6, 4.3 Hz, 1H, 4-H), 2.25–2.39 (m, 1H, 3-H), 2.35 (s, 3H, NCH_3), 2.57 (dt, *J* = 4.0, 3.0 Hz, 1H, 1-H), 2.76 (d, *J* = 2.7 Hz, 2H, 9-H), 2.90 (dd, *J* = 11.6, 3.6 Hz, 1H, 7-H), 2.93–2.97 (m, 1H, 5-H), 3.01 (dd, *J* = 12.2, 3.2 Hz, 1H, 7-H), 3.62 (s, 2H, NCH_2Ph), 3.78 (s, 2H, COCH_2Aryl), 4.18 (tt, *J* = 7.9, 2.7 Hz, 1H, 2-H), 7.05 (d broad, *J* = 8.2 Hz, 1H, N*H*), 7.25 (dd, *J* = 8.2, 2.1 Hz, 1H, aromat. 6-$H_{dichlorophenyl}$), 7.30–7.45 (m, 5H, aromat. H), 7.51 (d, *J* = 2.1 Hz, 1H, aromat. 2-$H_{dichlorophenyl}$), 7.52 (d, *J* = 7.9 Hz, 1H, aromat. 5-$H_{dichlorophenyl}$). ^{13}C NMR ($CDCl_3$): δ = 27.2 (1 C, C-3), 28.8 (1 C, C-4), 43.0 (1 C, COCH_2Aryl), 44.7 (1 C, NCH_3), 48.7 (1 C, C-7), 51.6 (1 C, C-2), 53.5 (1 C, C-5), 53.6 (1 C, C-9), 60.5 (1 C, C-1), 60.6 (1 C, NCH_2Ph), 126.8 (1 C, aromat. CH), 128.1 (2 C, aromat. CH), 128.2 (2 C, aromat. CH), 128.7 (1 C, aromat. $CH_{dichlorophenyl,\ C-6}$), 130.5 (1 C, aromat. $CH_{dichlorophenyl,\ C-2\ or\ C-5}$), 131.0 (1 C, aromat. *C*–Cl), 131.1 (1 C, aromat. $CH_{dichlorophenyl,\ C-5\ or\ C-2}$), 132.5 (1 C, aromat. C–Cl), 135.8 (1 C, aromat. $C_{dichlorophenyl}$), 139.6 (1 C, aromat. C), 168.1 (1 C, C=O).

[(1*S*,2*R*,5*S*)-6-Benzyl-8-methyl-7,9-dioxo-6,8-diazabicyclo[3.2.2]nonan-2-yl] (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (24). A solution of **12a** (22 mg, 0.080 mmol) in CH_2Cl_2 (4 mL) was treated with (*R*)-(−)-3,3,3-trifluoro-2-methoxy-2-phenylacetyl chloride [(*R*)-Mosher's acid chloride; (*R*)-**23**, 13 μL, 0.070 mmol], triethylamine (0.3 mL, 2.1 mmol) and dimethylaminopyridine (17 mg, 0.14 mmol). The reaction mixture was heated to reflux for 9 h, then it was cooled to room temperature, diluted with CH_2Cl_2 (6 mL) and washed with saturated solutions of $NaHCO_3$ (5 mL) and NH_4Cl

(5 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The diastereomeric ratio of the non-purified residue was investigated by ^{19}F and ^{1}H NMR spectroscopy and by HPLC. A sample of the residue was purified by fc (2 cm, petroleum ether/ethyl acetate = 3:7, 2 mL, R_f 0.38). Colorless, viscous oil. $C_{25}H_{25}F_3N_2O_5$ (490.5). ^{1}H NMR ($CDCl_3$): δ = 1.55–1.67 (m, 1H, 4-H), 1.70–1.86 (m, 2H, 4-H, 3-H), 1.91–2.05 (m, 1H, 3-H), 3.06 (s, 3H, NCH_3), 3.52 (s, 3H, OCH_3), 3.88 (dd, J = 5.9, 1.9 Hz, 1H, 5-H), 4.06 (d, J = 3.2 Hz, 1H, 1-H), 4.42 (d, J = 14.8 Hz, 1H, NCH_2Ph), 4.67 (d, J = 14.4 Hz, 1H, NCH_2Ph), 4.96 (ddd, J = 8.5, 5.2, 3.3 Hz, 1H, 2-H), 7.20–7.60 (m, 10H, aromat. H). ^{19}F NMR ($CDCl_3$): δ = −72.2 (s, 3 F, CF_3, 98.8% intensity), −71.8 (s, 3 F, CF_3, 1.2% intensity). HPLC (acetonitrile/water = 1:1; flow rate: 0.80 mL/min): retention time: 30.6 min (97.6% intensity).

[(1*S*,2*R*,5*S*)-6-Benzyl-8-methyl-7,9-dioxo-6,8-diazabicyclo[3.2.2]nonan-2-yl)] (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (25). As described for **24** the alcohol **12a** (23 mg, 0.084 mmol) was acylated with (*S*)-(+)-Mosher's acid chloride [(*S*)-**23**, 15 μL, 0.080 mmol], triethylamine (0.3 mL, 2.1 mmol) and dimethylaminopyridine (19 mg, 0.15 mmol) in CH_2Cl_2 (4 mL). The diastereomeric ratio of the non-purified residue was investigated by ^{19}F and ^{1}H NMR spectroscopy and by HPLC. A sample of the residue was purified by fc (2 cm, petroleum ether/ethyl acetate = 3:7, 2 mL, R_f 0.41). Colorless, viscous oil. $C_{25}H_{25}F_3N_2O_5$ (490.5). ^{1}H NMR ($CDCl_3$): δ 1.47–1.60 (m, 1H, 4-H), 1.61–1.78 (m, 2H, 4-H, 3-H), 1.81–1.91 (m, 1H, 3-H), 3.08 (s, 3H, NCH_3), 3.64 (s, 3H, OCH_3), 3.87 (dd, J = 4.9, 1.5 Hz, 1H, 5-H), 4.10 (d, J = 2.7 Hz, 1H, 1-H), 4.43 (d, J = 14.6 Hz, 1H, NCH_2Ph), 4.69 (d, J = 14.6 Hz, 1H, NCH_2Ph), 4.89 (ddd, J = 8.7, 5.2, 2.7 Hz, 1H, 2-H), 7.20–7.63 (m, 10H, aromat. H). ^{19}F NMR ($CDCl_3$): δ = −72.2 (s, 3 F, CF_3, 1.6% intensity), −71.8 (s, 3 F, CF_3, 98.4% intensity). HPLC (acetonitrile/water = 1:1; flow rate: 0.80 mL/min): retention time: 33.0 min (97.4% intensity).

Receptor binding studies, general

Teflon-glass-homogenizer: Potter® S (B. Braun Biotech International). Rotor/stator homogenizer: Ultraturrax® T25 basic (Ika Labortechnik). Centrifuge: High speed refrigerating centrifuge model J2-HS (Beckman). Filter: Whatman glass fibre filters GF/B, presoaked in 0.5% polyethylenimine (in water) for 2 h at 4 °C before use. Filtration was performed with a Brandel 24-well cell harvester. Scintillation cocktail: Rotiszint eco plus (Roth). Liquid scintillation analyzer: Tri-Carb 2100 TR (Canberra Packard), counting efficiency 66%. All experiments were carried out in triplicate. IC_{50}-values were determined from competition experiments with at least six concentrations of test compounds and were calculated with the curve-fitting program GraphPad Prism® 3.0 (GraphPad Software) by nonlinear regression analysis. K_i-values were calculated according to Cheng and Prusoff.[30] K_D values for the radioligands were taken from the literature. For compounds with high affinity (low K_i-values) mean values ± SEM from at least three independent experiments are given.

Investigation of σ_1-receptor-affinity

[^{3}H]-(+)-Pentazocine binding to guinea pig brain membrane preparations was performed according to the procedure described in ref 28.

Membrane preparation. Thawed guinea pig brains (Dunkin Hartley, Harlan-Sera-Lab) were homogenized with an Ultraturrax (8000 rpm) in 10 volumes of cold 0.32 M sucrose. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 22,000*g* for 20 min at 4 °C. The pellet was resuspended in 10 volumes of buffer (50 mM Tris–HCl, pH 7.4) with an ultraturrax (8000 rpm), incubated for 30 min at 25 °C and centrifuged at 22,000*g* (20 min, 4 °C). The pellet was resuspended in buffer, the protein concentration was determined according to the method of Bradford[31] using bovine serum albumin as standard, and subsequently the preparation was frozen (−83 °C) in 5 mL portions of about 2 mg protein/mL.

Performance of the σ_1-receptor binding assay. The test was performed with the radioligand [ring-1,3-^{3}H]-(+)-pentazocine (1036 GBq/mmol; NEN Life Science Products). The thawed membrane preparation (about 150 μg of the protein) was incubated with various concentrations of test compounds, 3 nM [^{3}H]-(+)-pentazocine and buffer (50 mM Tris–HCl, pH 7.4) in a total volume of 500 μL for 150 min at 37 °C. The incubation was terminated by rapid filtration through presoaked Whatman GF/B filters using a cell harvester. After washing four times with 2 mL of 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. Non-specific binding was determined with 10 μM haloperidol.

Investigation of σ_2-receptor-affinity

σ_2-Receptor-affinity was determined using rat liver membranes with [^{3}H]-ditolylguanidine in the presence of 100 nM (+)-pentazocine to mask σ_1-binding sites. The assay was performed according to the procedure described in ref 28.

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

quently the preparation was frozen (−83 °C) in 5 mL portions of about 2.5 mg protein/mL.

Performance of the σ_2-receptor binding assay. The membrane preparation (about 60 μg protein) was incubated with 3 nM [^{3}H]-ditolylguanidine (di-[p-ring-^{3}H]-1,3-di-*o*-tolylguanidine, 2220 GBq/mmol; American Radiolabeled Chemicals Inc.) and different concentrations of test compounds in buffer (50 mM Tris–HCl, pH 8.0) in the presence of 100 nM (+)-pentazocine. The total volume was 250 μL. The incubation (120 min, 25 °C) was stopped by addition of 2 mL of ice cold buffer (10 mM Tris–HCl, pH 8.0) followed by rapid filtration through presoaked Whatman GF/B filters using a cell harvester. After washing three times with 2 mL of 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. Non-specific binding was determined with 10 μM nonradiolabeled ditolylguanidine.

Investigation of κ-receptor-affinity

[^{3}H]-U-69593 binding to guinea pig brain membrane preparations was performed according to standard procedure described in ref 14.

Membrane preparation. The cerebellum was removed from guinea pig brains (Dunkin Hartley, Harlan) and the brains were bisected. Three brain halves were homogenised in 50 mL of buffer (50 mM Tris–HCl pH 7.4) with a potter (800 rpm, 10 up-and-down strokes). The suspension was centrifuged at 49,000*g* for 10 min at 4 °C. The supernatant was removed and the pellet was resuspended in buffer (30 mL) with an Ultraturrax (8000 rpm). Subsequently, it was centrifuged at 49,000*g* for 10 min at 4 °C. The pellet was resuspended in buffer, incubated for 45 min at 37 °C and centrifuged (49,000*g*, 10 min, 4 °C). Again the pellet was resuspended and centrifuged. Then, the pellet was resuspended in buffer (30 mL), the protein concentration was determined according to the method of Bradford[31] using bovine serum albumin as standard, and subsequently the preparation was frozen (−83 °C) in 5 mL portions of about 3 mg protein/mL.

Performance of the κ-receptor binding 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 analyser. Non-specific binding was determined with 1 μM U-50488.

Investigation of μ-receptor-affinity

[^{3}H]-DAMGO binding to guinea pig brain membrane preparations was performed according to standard procedure described in ref 14.

Membrane preparation. Described under Investigation of κ-receptor-affinity.

Performance of the μ-receptor binding 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 analyser. Non-specific binding was determined with 1 μM naloxone.

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