Synthesis of Bicyclic σ Receptor Ligands with Cytotoxic Activity

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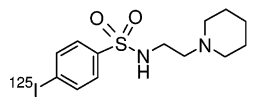
All possible stereoisomeric alcohols (6-benzyl-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonan-2-ol) and methyl ethers (6-benzyl-2-methoxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane) are prepared from (*R*)- and (*S*)-glutamate. A Dieckmann analogous cyclization, which makes use of trapping the primary cyclization product with Me_3SiCl, generates the bicyclic framework. Stereoselective $LiBH_4$ reduction and Mitsunobu inversion establish the configuration in position 2. Enantiomeric alcohols **15** (1*S*,2*S*,5*R*) and *ent*-**15** (1*R*,2*R*,5*S*) as well as diastereomeric methyl ethers *ent*-**17** (1*R*,2*R*,5*S*) and *ent*-**22** (1*R*,2*S*,5*S*) display high σ_1 receptor affinity. Cell growth inhibition of the stereoisomeric alcohols and methyl ethers against five human tumor cell lines is investigated. In particular, at a concentration of 20 μM the four methyl ethers stop completely the cell growth of the small cell lung cancer cell line A-427, indicating a specific target in this cell line. The IC_{50}-values of methyl ethers *ent*-**17** and *ent*-**22** are in the range of the antitumor drugs cisplatin and oxaliplatin. Binding assays show that the investigated tumor cell lines express considerable amounts of σ_1 and σ_2 receptors.

Introduction

Originally the sigma receptor was termed enigmatic because of the lack of an endogenous ligand and the indistinct intracellular signal transduction pathway.[1] Cloning of the σ_1 receptor about 10 years ago proved the existence of σ receptors as binding site for various exogenous and endogenous ligands.[2−5] The rat brain gene encodes for a protein of 223 amino acids with a single transmembrane domain.[4] However, further investigations revealed two transmembrane domains with the amino and carboxy termini on the intracellular side of the membrane.[6] Crucial amino acids for ligand binding are aspartate 126 and glutamate 172 located in the intracellular carboxy terminus.[6]

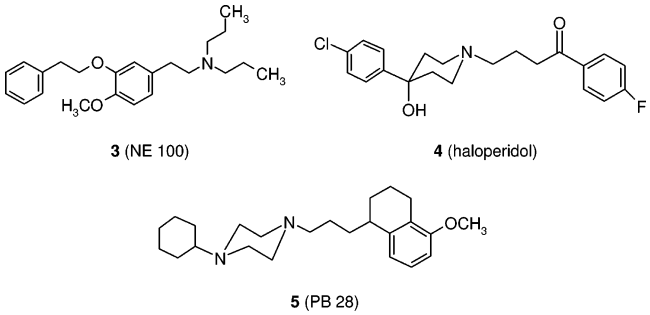
σ Receptors are considered to play a modulatory role in the activity of a variety of ion channels and neurotransmitter systems including Ca^{2+}-channels,[7] K^+-channels,[8] NMDA type glutamate receptors, dopamine receptors, and acetylcholine receptors.[9] Therefore, ligands interacting with σ receptors possess a potential for the treatment of acute and chronic neurological disorders, including schizophrenia, depression, Alzheimer's disease, pain as well as alcohol and cocaine abuse.[10] In order to disclose the in vivo relevance of σ_1 receptors σ_1 knockout mice were generated, which are viable and fertile, but show a significant decrease in motility.[11]

In addition to the CNS effects of σ receptor ligands it has been shown that some human tumor cell lines (e.g., malignant melanoma, breast carcinoma, prostate tumor, non-small cell lung cancer among others) express a large number of σ_1 and/or σ_2 receptor copies on their cell surface. The high σ receptor density can be exploited for tumor imaging (tumors and their metastasis) in diagnosis using radiolabeled highly potent σ ligands (e.g., **1**, **2**, Figure 1).[12−14]



1 **2**

Figure 1. ^{125}I-labeled potent σ receptor ligands used for tumor imagining.



3 (NE 100) **4** (haloperidol)

5 (PB 28)

Figure 2. σ_1 receptor antagonists (**3**, **4**) and σ_2 receptor agonists (**5**) with cytotoxic and antitumor activity.

Moreover, σ receptors of tumor cells can be addressed by drugs with cytotoxic potential for the treatment of cancer. In particular it was shown that the human SK−N−SH neuroblastoma and the C6 glioma cell lines, which produce large amounts of σ_1 and σ_2 receptors, are sensitive against σ_1 receptor antagonists (e.g., **3** (NE 100), **4** (haloperidol), Figure 2) and σ_2 receptor agonists (e.g., **5** (PB 28), Figure 2).[15,16] Additionally, **4** (haloperidol) inhibited B16 melanoma cell growth in single digit micromolar concentration and led to cell death at higher concentrations.[17]

Recently, we reported on the synthesis of hydroxymethyl-substituted piperazine derivatives **6**, which represent a novel class of potent σ_1 receptor ligands (e.g., **6a** with R = *p*-methoxybenzyl: K_i = 12.4 nM).[18] The piperazine ring of **6** can adopt two chair conformations with an equatorially or axially oriented, rather flexible hydroxymethyl side chain (Figure 3).

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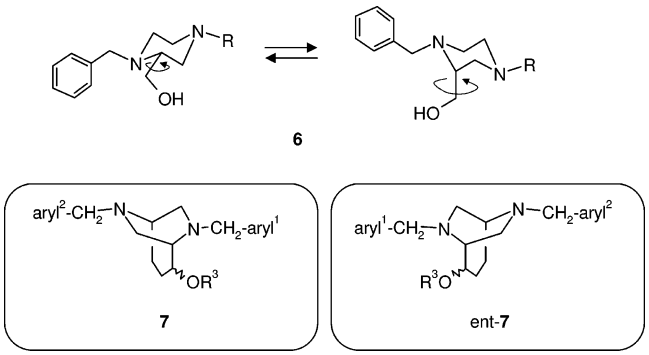


Figure 3. Structural development of novel σ receptor ligands.

Obviously, the pharmacophoric elements of **6**, which are responsible for the interactions with σ_1 receptors, are not in a defined orientation to each other. Therefore, we planned to synthesize bridged piperazines **7** with a 2-(hydroxymethyl)piperazine substructure. In the bicyclic compounds **7** the conformational flexibility is considerably reduced, which leads to a defined three-dimensional orientation of the pharmacophoric nitrogen atoms (together with their residues) and the hydroxy moiety. The stereoselective receptor binding of the bridged piperazines should be investigated after synthesizing all possible stereoisomers of **7**. Moreover, the cytotoxic activity of the synthesized bridged piperazines should be determined in a panel of human tumor cell lines and correlated with the σ_1 and/or σ_2 receptor affinity.

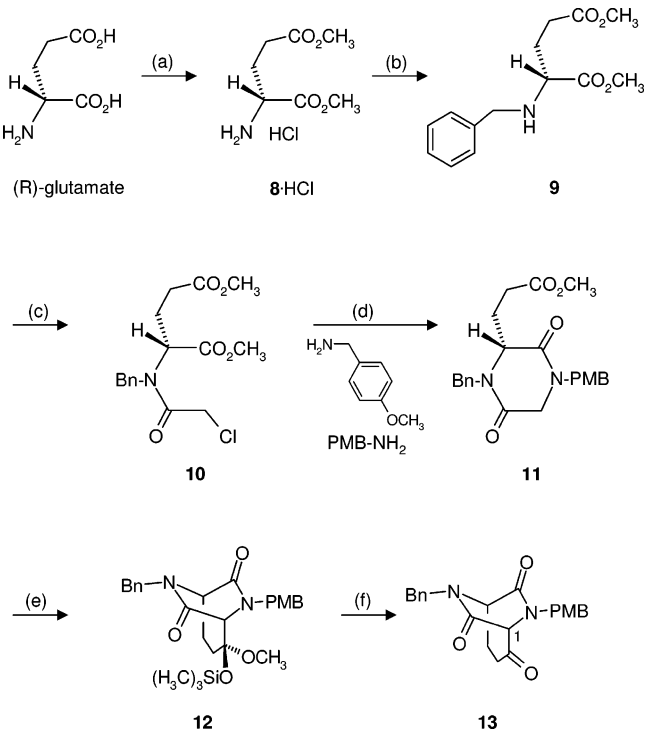
Chemistry. The synthesis was started by esterification of (*R*)-glutamate with methanol and trimethylsilyl chloride (Me_3SiCl) to give the hydrochloride of dimethyl glutamate **8**·HCl. Condensation of **8** with benzaldehyde and subsequent reduction with $NaBH_4$ yielded the benzylamine **9**, which was acylated with chloroacetyl chloride to form the chloroacetamide **10**. Since the intermediate imine tends to racemize even during storage at 4 °C and the benzylamine **9** gives rise to form a γ-lactam, the synthesis of the chloroacetamide **10** has to be performed without storing the intermediates. Reaction of the chloroacetamide **10** with *p*-methoxybenzylamine led to the nucleophilic substitution followed by intramolecular aminolysis, affording the piperazinedione **11** in 82% yield (Scheme 1).[19]

Since according to Bredt's rule[20] deprotonation at the bridgehead position of **13** (position 1) during Dieckmann cyclization is not allowed, the Dieckmann cyclization protocol recently developed by us was applied.[21−23] Trapping of the first reaction product with Me_3SiCl led diastereoselectively to the mixed methyl silyl acetal **12** in excellent yields (96%), which was carefully hydrolyzed (room temperature, low concentration of acid) with *p*-toluenesulfonic acid in THF/H_2O to provide the bicyclic ketone **13** (Scheme 1).

The reduction of the ketone **13** with $NaBH_4$ in aqueous THF provided a mixture of the diastereomeric alcohols **14** and **19** in the ratio 75:25 (Scheme 2). In order to improve the diastereoselectivity of this reduction several reducing agents and conditions were investigated. It was found that $LiBH_4$ in THF at −90 °C led to the diastereomeric alcohols **14** and **19** in the ratio of 99:1. Recrystallization of the crude reaction product from ethyl acetate/methanol (9:1) provided 82% of the alcohol **14** in diastereomerically and enantiomerically pure form (HPLC analysis).

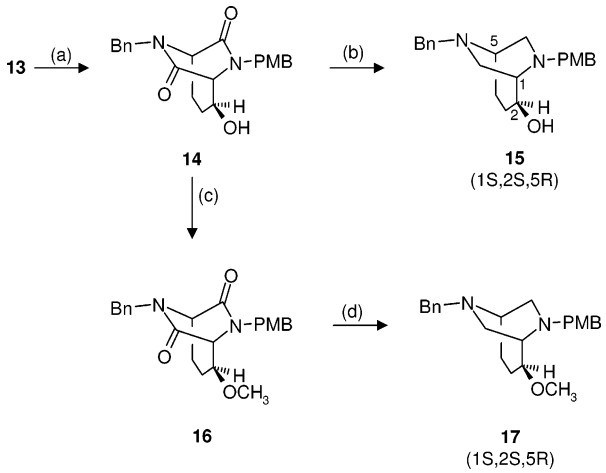
Because of the flexibility of the three-carbon bridge of the bicyclic alcohol **14**, it was not possible to unequivocally prove the configuration of the new center of chirality in position 2 by NMR experiments. Therefore an X-ray crystal structure analysis of the enantiomer of **14** (*ent*-**14**) was recorded, which was

Scheme 1[a]



[a] (a) Me_3SiCl, MeOH. (b) Benzaldehyde, $MgSO_4$, NEt_3, CH_2Cl_2 and then $NaBH_4$, MeOH. (c) $ClCH_2COCl$, NEt_3, CH_2Cl_2. (d) *p*-Methoxybenzylamine (PMB-NH_2), NEt_3, CH_3CN. (e) LiHMDS, THF, −78 °C, 30 min and then Me_3SiCl. (f) *p*-Toluenesulfonic acid, THF, H_2O.

Scheme 2[a]



[a] (a) $LiBlH_4$, THF, −90 °C. (b) $LiAlH_4$, THF, 66 °C. (c) NaH, MeI, THF. (d) $LiAlH_4$, THF, 66 °C.

analogously prepared starting with the proteinogenic amino acid (*S*)-glutamate. Recrystallization of *ent*-**14** from ethyl acetate gave colorless crystals, which were suitable for X-ray crystal structure analysis. Solution of the structure revealed the relative configuration of the alcohol *ent*-**14**. Since the absolute (*S*)-configuration of the center of chirality in position 5 is given from the synthesis educt (*S*)-glutamate, the absolute configuration of the alcohol *ent*-**14** is (1*S*,2*R*,5*S*) (Figure 4).

Methylation of the alcohol **14** with CH_3I and NaH gave the methyl ether **16** in 96% yield. Heating of the alcohol **14** and the methyl ether **16** with $LiAlH_4$ in THF led to the bridged piperazines **15** and **17** with a hydroxy and a methoxy group in position 2, respectively. The configuration of the alcohol **15** and the methyl ether **17** is (1*S*,2*S*,5*R*) (Scheme 2).

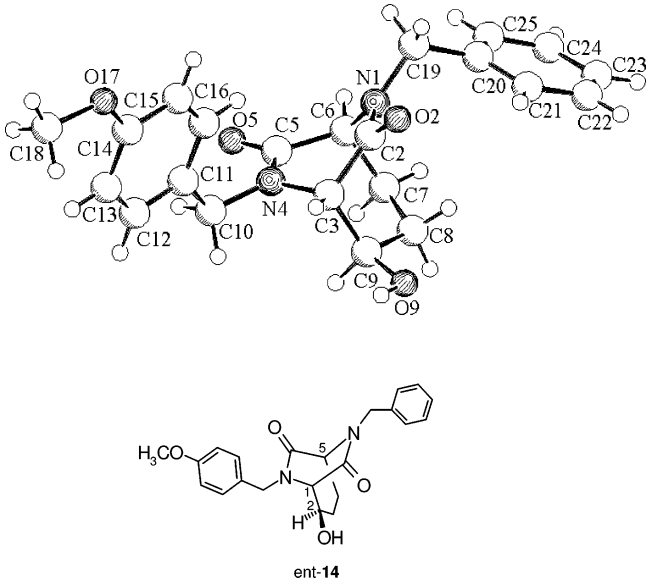
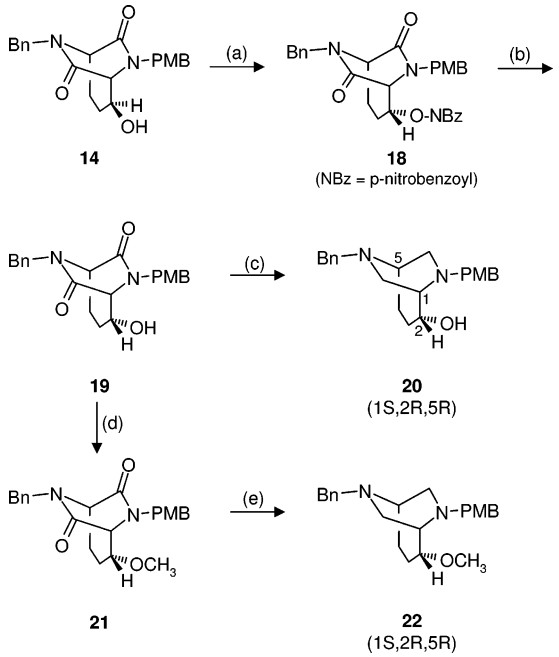


Figure 4. X-ray crystal structure analysis of *ent*-**14**.

Scheme 3[a]



[a] (a) DIAD, PPh_3, *p*-nitrobenzoic acid (NBzOH), THF. (b) MeOH, K_2CO_3. (c) $LiAlH_4$, THF, 66 °C. (d) NaH, MeI, THF. (e) $LiAlH_4$, THF, 66 °C.

In order to synthesize the diastereomeric alcohol **20** and methyl ether **22** with (*R*)-configuration in position 2 the configuration of the (2*S*)-configured alcohol **14** was inverted by a Mitsunobu reaction.[24] Thus, **14** reacted with 4-nitrobenzoic acid, diisopropyl azodicarboxylate (DIAD), and PPh_3 to provide the 4-nitrobenzoate **18** with inverted C-2-configuration. The inverted alcohol **19** was obtained by cleavage of the 4-nitrobenzoate **18** with CH_3OH and K_2CO_3 (Scheme 3).

The methyl ether **21** was prepared by reaction of the alcohol **19** with CH_3I and NaH. $LiAlH_4$ reduction of the alcohol **19** and the methyl ether **21** resulted in the basic bicyclic piperazines bearing a hydroxy (**20**) and a methoxy group (**22**) in position 2, respectively (Scheme 3).

The enantiomeric alcohols *ent*-**15** and *ent*-**20** as well as the enantiomeric methyl ethers *ent*-**17** and *ent*-**22** were prepared in the same manner, starting the synthesis with the enantiomeric amino acid (*S*)-glutamate. Thus, the four possible stereoisomeric alcohols **15**, *ent*-**15**, **20**, and *ent*-**20** as well as methyl ethers **17**, *ent*-**17**, **22**, and *ent*-**22** were available for pharmacological evaluation.

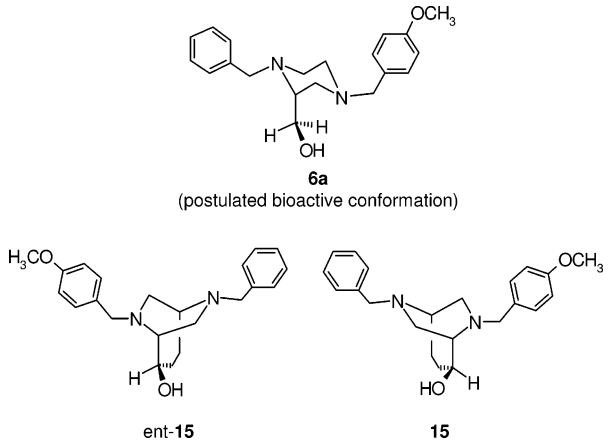


Figure 5. Structural comparison of the enantiomeric alcohols *ent*-**15** and **15** with the lead compound **6a**.

Receptor Binding Studies. The σ_1 and σ_2 receptor affinity of the stereoisomeric bicyclic alcohols **15**, *ent*-**15**, **20**, and *ent*-**20** and methyl ethers **17**, *ent*-**17**, **22**, and *ent*-**22** was determined in competition experiments with radioligands. In the σ_1 assay, guinea pig brain membrane preparations were used as receptor material, and [^{3}H]-(+)-pentazocine was the radioligand. The nonspecific binding was determined in the presence of a large excess of nontritiated (+)-pentazocine. The σ_2 assay was performed with rat liver membrane preparations and the nonselective radioligand [^{3}H]-ditolylguanidine. In order to gain σ_2 selectivity, an excess of the σ_1 selective ligand (+)-pentazocine was added to occupy the σ_1 receptors. A concentration of 10 μM of nontritiated ditolylguanidine was used for the determination of nonspecific binding. Since structural relationships between σ receptor ligands and NMDA receptor antagonists exist,[25,26] the affinity of the bridged piperazines toward the phencyclidine binding site of the NMDA receptor (pig brain cortex membrane preparations, [^{3}H]-MK801) was also included in this study.[27−29]

In Table 1 the σ_1 and σ_2 receptor affinity of the stereoisomeric bicyclic alcohols and methyl ethers is summarized. In the alcohol series, the σ_1 receptor affinity is strongly dependent on the configuration in position 2. Whereas **15** with (1*S*,2*S*,5*R*)-configuration interacts in the low nanomolar range (K_i = 7.5 nM) with σ_1 receptors, its (2*R*)-diastereomer **20** shows a considerably lower affinity (K_i = 118 nM). The same trend is observed for the enantiomeric alcohols *ent*-**15** and *ent*-**20**. It is striking that in this series the enantiomer pairs **15**/*ent*-**15** and **20**/*ent*-**20** display the same σ_1 receptor affinity, respectively.

In Figure 5, the structures of the flexible σ_1 receptor ligand **6a** (K_i = 12.4 nM)[18] and the conformationally constrained bicyclic σ_1 receptor ligands *ent*-**15** and **15** are compared. We assume that the hydrophobic pockets of the σ_1 receptor protein accept both the phenyl and the 4-methoxyphenyl moiety of the ligands in the same manner. Therefore, the phenyl and the 4-methoxyphenyl moiety are considered to be equivalent for the receptor.

Formally, the σ_1 ligands **6a** and *ent*-**15**, which are derived from the (*S*)-configured amino acids (*S*)-serine and (*S*)-glutamate, respectively, are related to each other. Therefore, we postulate that the bioactive conformation of the rather flexible 2-(hydroxymethyl)piperazine **6a**, which is shown in Figure 5, is similar to the structure of the bicyclic alcohol *ent*-**15**.

Table 1. σ_1 and σ_2 Receptor Affinity of the Stereoisomeric Alcohols and Methyl Ethers

			$K_i \pm$ SEM [nM] ($n = 3$)		
compound	configuration	C-2 substituent	σ_1 affinity ([^{3}H](+)-pentazocine)	σ_2 affinity ([^{3}H]-ditolylguanidine)	σ_1/σ_2 selectivity
15	(1*S*,2*S*,5*R*)	OH	7.5 ± 2.1	1700	227
ent-**15**	(1*R*,2*R*,5*S*)	OH	6.5 ± 0.67	806	124
20	(1*S*,2*R*,5*R*)	OH	118 ± 5.0	441	4
ent-**20**	(1*R*,2*S*,5*S*)	OH	125 ± 18	705	6
17	(1*S*,2*S*,5*R*)	OCH_3	258 ± 11	2430	9
ent-**17**	(1*R*,2*R*,5*S*)	OCH_3	26 ± 8.8	573	22
22	(1*S*,2*R*,5*R*)	OCH_3	126 ± 26	1440	11
ent-**22**	(1*R*,2*S*,5*S*)	OCH_3	25 ± 3.0	325 ± 20	13
(+)-pentazocine	(2*S*,6*S*,11*S*)	-	2.2 ± 1.2	-	-
ditolylguanidine	-	-	177 ± 6.6	20 ± 2.3	0.1
haloperidol	-	-	1.9 ± 0.4	78 ± 2.4	41

Table 2. σ_1 Receptor Affinities (K_i Values) and Cell Growth Inhibitory Activity (% of Untreated Control) of the Stereoisomeric Alcohols and Methyl Ethers in Five Human Cancer Cell Lines[a])

compound	configuration	σ_1 K_i [nM]	5637[b]	RT-4[c]	A-427[d]	LCLC-103H[e]	MCF-7[f]
15	(1*S*,2*S*,5*R*)	7.5	72 ± 32.6	72 ± 1.2	46 ± 8.6	80 ± 3.3	76 ± 1.9
ent-**15**	(1*R*,2*R*,5*S*)	6.5	88 ± 10.7	93 ± 10.3	46 ± 12.0	94 ± 6.9	91 ± 9.4
20	(1*S*,2*R*,5*R*)	118	88 ± 4.5	67 ± 4.2	33 ± 7.8	77 ± 18.0	73 ± 9.4
ent-**20**	(1*R*,2*S*,5*S*)	125	103 ± 7.7	95 ± 13.6	46 ± 11.1	96 ± 11.4	96 ± 14.1
17	(1*S*)	258	27 ± 13.6	29 ± 21.4	-10 ± 7.5	19 ± 4.4	−22 ± 20.2
ent-**17**	(1*R*,2*R*,5*S*)	26	68 ± 10.4	61 ± 21.7	-8 ± 3.9	72 ± 7.1	21 ± 6.0
22	(1*S*,2*R*,5*R*)	126	77 ± 8.5	65 ± 18.5	-7 ± 3.6	71 ± 4.4	30 ± 7.0
ent-**22**	(1*R*,2*S*,5*S*)	25	79 ± 7.5	75 ± 20.0	-5 ± 3.0	86 ± 4.1	56 ± 14.9
haloperidol		1.9 ± 0.4	63 ± 16.2	55 ± 5.9	5 ± 1.5	63 ± 12.1	60 ± 6.6
(+)-pentazocine		2.2 ± 1.2	96 ± 10.1	98 ± 1.0	76 ± 7.0	95 ± 6.2	94 ± 6.6

[a] Relative cell growth (%) in relation to an untreated control of the tumor cell lines after a 96 h exposure to substance at 20 μM. Results are averages ± standard deviation of three or more independent determinations. [b] Bladder cancer. [c] Bladder cancer. [d] Small cell lung cancer. [e] Large cell lung cancer. [f] Breast cancer.

However, the structure of the enantiomeric bicyclic alcohol **15** is also very similar to the postulated bioactive conformation of **6a**: The three-dimensional orientation of both the *p*-methoxybenzyl and benzyl residues of **15** and **6a** are quite similar. Moreover, the hydroxy moiety of **15** points in a similar direction as the hydroxy moiety of **6a** (and also *ent*-**15**) does. Therefore, a properly positioned H-bond acceptor group in the σ_1 receptor protein would be able to form H-bonds with the OH moieties of all three potent σ_1 ligands **6a**, **15**, and *ent*-**15**.

The methyl ethers **17**, *ent*-**17**, **22**, and *ent*-**22** display lower σ_1 receptor affinities than the more active alcohols **15** and *ent*-**15**. This result supports the idea that an H-bond contributes to the overall interaction of the ligands with σ_1 receptors, since the methoxy moiety cannot function as H-bond donor group. In the methyl ether series the diastereomers *ent*-**17** and *ent*-**22**, which are derived from (*S*)-glutamate, display the highest σ_1 receptor affinity with K_i-values of 26 and 25 nM, respectively.

Generally, the σ_2 receptor affinity of the investigated stereoisomeric alcohols and methyl ethers is very low, indicating high selectivity for σ_1 receptors over σ_2 receptors. The best σ_1/σ_2 selectivity was observed for the most potent σ_1 receptor ligands **15**, *ent*-**15**, *ent*-**17**, and *ent*-**22**.

In the NMDA assay the stereoisomeric alcohols and ethers did not interact with the phencyclidine binding site of the NMDA receptor. At a concentration of 10 μM the test compounds were not able to compete with the radioligand (100% radioligand binding) for the binding sites. Thus the bicyclic alcohols and ethers display high preference for σ_1 receptors compared with NMDA receptors.

Cytotoxicity Assay. The overexpression of σ_1 and σ_2 receptors in human tumor cell lines and the cytotoxic activity of some σ ligands have been documented in the literature (see Introduction). Therefore, we investigated the cytotoxic effects of the stereoisomeric alcohols and methyl ethers in a panel of five human tumor cell lines. This panel includes the cell lines 5637 (bladder cancer), RT-4 (bladder cancer), A-427 (small cell lung cancer), LCLC 103H (large cell lung cancer), and MCF-7 (breast cancer).

Table 3. IC_{50} Values of Growth Inhibition of the Cancer Cell Lines A-427 and MCF-7 Following a Continuous 96 h Exposure to the Various Compounds

		IC_{50} [μM][a]	
compound	configuration	A-427	MCF-7
17	(1*S*,2*S*,5*R*)	9.5 ± 2.32	13.4 ± 0.75
ent-**17**	(1*R*,2*R*,5*S*)	1.23 ± 0.46	12.2 ± 2.05
22	(1*S*,2*R*,5*R*)	8.00 ± 1.61	15.3 ± 3.34
ent-**22**	(1*R*,2*S*,5*S*)	0.51 ± 0.21	13.5 ± 2.34
haloperidol		10.0 ± 1.71	24.9 ± 9.77
cisplatin[b]		1.96 ± 0.54	1.38 ± 0.29
oxaliplatin[b]		0.76 ± 0.09	0.32 ± 0.04
methotrexate[b]		5.52 ± 3.55	0.05 ± 0.02

[a] Results are averages ± standard deviation of three or more independent determinations. [b] IC_{50} values are from ref 30.

In the primary screening the tumor cells were incubated with a 20 μM solution of the test compounds at 37 °C. After 96 h the medium was removed and the density of adherent cells (living cells) was measured by staining with crystal violet.[30] In Table 2 the results are given as part of living cells (in %) in relation to a control without test compound.

The data in Table 2 clearly indicate the tendency of the methyl ethers **17**, *ent*-**17**, **22**, and *ent*-**22** to reduce the cell growth of the tumor cells to a greater extent than the corresponding alcohols **15**, *ent*-**15**, **20**, and *ent*-**20**. It is possible that the higher lipophilicity of the methyl ethers is responsible for their stronger activity.

The cell growth inhibition of the small cell lung cancer cell line A-427 is striking. Whereas the stereoisomeric alcohols caused about 40% cell growth inhibition, the values of the stereoisomeric methyl ethers are negative, indicating not only

Table 4. Expression of σ_1 and σ_2 Receptors in the Five Human Tumor Cell Lines

cell line	SB[a] (%)	$\sigma_1 N^b$ (10^9)	$N_p{}^c$ (10^7) (N/μg)	$c_p{}^d$ (pmol/mg)	SB[a] (%)	$\sigma_2 N^b$ (10^9)	$N_p{}^c$ (10^7) (N/μg)	$c_p{}^d$ (pmol/mg)
5637	23	0.99	1.1	0.018	52	3.61	4.0	0.066
RT-4	92	18.5	21	0.35	97	110	122	2.03
A-427	73	5.97	6.6	0.11	43	2.33	2.6	0.043
LCLC-103H	87	8.36	9.3	0.15	88	14.9	16.6	0.28
MCF-7	74	10.4	11.5	0.19	89	12.6	14.1	0.23

[a] SB: specific binding in relation to the total binding in %. [b] *N*: Number of binding sites in the assay. [c] N_p: Number of bindings sites per μg protein. [d] c_p: molar amount of binding sites (pmol) per mg protein.

inhibition of cell growth but also death of existing cells. It is very likely that the growth inhibition of the A-427 cell line is not simply unspecific toxicity of the test compounds because the growth inhibition of the other four tumor cell lines is considerably lower. Obviously a definite target, which is selectively produced by A-427 cells and to a lesser extent in the MCF-7 cells, is interacting with the test compounds, possibly the σ_1 receptor. It is notable that at a concentration of 20 μM haloperidol, a known σ_1 receptor antagonist, also strongly and selectively inhibits the growth of the A-427 cell line while (+)-pentazocine, a known σ_1 receptor agonist, does not. Similar cytotoxic behavior of haloperidol and a lack of cytotoxic behavior of (+)-pentazocine in other cell lines expressing σ receptors have been documented.[42]

The IC_{50}-values of the diastereomeric methyl ethers *ent*-**17** and *ent*-**22**, which combine high σ_1 receptor affinity and good growth inhibition against both A-427 and MCF-7 cells, and the IC_{50}-values of their enantiomers **17** and **22** were determined. In Table 3 the IC_{50}-values of the test compounds are compared with the IC_{50}-values of three standard anticancer drugs. The cytotoxic effect of the (*R*,*R*,*S*)-configured methyl ether *ent*-**17** (IC_{50} = 1.23 μM) is comparable with the activity of cisplatin (IC_{50} = 1.27 μM). Moreover, the diastereomeric methyl ether *ent*-**22** (IC_{50} = 0.51 μM) is equipotent with the more active drug oxaliplatin (IC_{50} = 0.68 μM). The A-427 cell line is at least 10 times more sensitive to the antiproliferative effects of *ent*-**17** and *ent*-**22** than the MCF-7 cell lines. Comparing the IC_{50} values in the A-427 and MCF-7 cell lines, there is much less selectivity for haloperidol and the enantiomeric ethers **17** and **22** (Table 3) than for *ent*-**17** and *ent*-**22**.

***σ*$_1$ and *σ*$_2$ Receptor Expression in Tumor Cell Lines.** In order to investigate the σ_1 and σ_2 receptor expression of the tumor cell lines under consideration cell membrane preparations of these tumor cells were produced by homogenization and centrifugation. For a better comparison of the data, the protein amount was standardized with the Bradford method.[31] The specific σ_1 receptor binding (SB) was determined by incubation of the membrane preparations (1.8 mg protein/mL) with the σ_1 selective radioligand [^{3}H]-(+)-pentazocine in the absence (total binding, TB) and the presence (nonspecific binding, NSB) of the non-radiolabeled competitor (+)-pentazocine. The σ_2 receptor expression of the tumor cell lines was determined in the same manner using the radioligand [^{3}H]ditolylguanidine in the absence (TB) and the presence (NSB) of the non-radiolabeled competitor ditolylguanidine. In order to selectively label σ_2 receptors (ditolylguanidine is an unselective σ ligand) these experiments were performed in the presence of an excess of (+)-pentazocine, which selectively blocks σ_1 receptors.

With only few exceptions the five tumor cell preparations show a specific σ_1 and σ_2 receptor binding greater than 50% (Table 4). A particular high σ_1 (92% SB) and σ_2 (97% SB) receptor expression was found for the bladder cancer cell line RT-4. The high σ_1 and σ_2 receptor density of this cell line will be exploited to establish new σ_1 and σ_2 receptor assays on the basis of RT-4 cell membrane preparations. We were able to confirm the relatively high density of both σ_1 and σ_2 receptors in the MCF-7 cell line, which was reported previously by John and co-workers.[43] The specific σ_1 receptor binding in the small cell lung cancer cell line A-427 is also relatively high (73%). It is possible that the strong cytotoxic effects of the methyl ethers *ent*-**17** and *ent*-**22**, which interact in the nanomolar range with σ_1 receptors, are mediated by these receptors.

It should be noted that strong correlations between σ receptor affinities, tumor cell growth inhibition, and σ receptor expression are not apparent in our data. However, relationships between these three parameters are complicated and direct correlations may not be apparent. For example, the cell growth inhibition assay uses whole cells while the receptor binding assays use cell membranes isolated from cell homogenates. Thus, additional parameters influencing cell membrane penetration (e.g., lipophilicity) may be important for the compounds to access the receptors and act on the cells. The intrinsic function of the receptors may also be different in each cell line. In confirmation of this, Spruce and co-workers found no correlation between the expression levels of the σ_1 receptor and the susceptibility of tumor cells to the antiproliferative effects of σ antagonists.[42] Finally, the intrinsic activities of the new σ ligands (agonist, partial agonist, antagonist) have not yet been established, and this property will be a deciding factor as to whether a compound has antiproliferative activity (e.g., compare the σ_1 antagoinst haloperidol with the σ_1 agonist (+)-pentazocine in Table 2).

Conclusion

The stereoisomeric alcohols **15**, *ent*-**15**, **20**, and *ent*-**20** and methyl ethers **17**, *ent*-**17**, **22**, and *ent*-**22** with the bridged piperazine framework represent a novel class of potent σ_1 receptor ligands. The σ_1 receptor affinity appears to be correlated with cytotoxic effects against the A-427 cell line; in particular the diastereoisomeric methyl ethers *ent*-**17** and *ent*-**22** show promising σ_1 receptor affinity (K_i = 26 nM and 25 nM) and growth inhibition of the A-427 cell line (IC_{50} = 1.23 μM and 0.51 μM) while enantiomeric compounds **17** and **22** show weaker σ_1 receptor binding and also weaker antiproliferative activity. In the A-427 cell line, both σ_1 receptors with 73% specific binding and σ_2 receptors with only 43% specific binding were found. This lends support to the hypothesis that *ent*-**17** and *ent*-**22** act as antiproliferative agents through their interactions with the σ_1 receptor, possibly as antagonists. Ongoing competition experiments with known agonists and antagonists of the σ receptors are aiming to elucidate the mechanism of cell growth inhibition by these interesting compounds.

Experimental

Chemistry. General. Flash chromatography (fc):[32] silica gel 60, 40−64 μm (Merck; parentheses include: diameter of the column, eluent, Rf-value). Optical rotation: Polarimeter 341 (Perkin-Elmer); 1.0 dm tube; concentration *c* [g/100 mL]. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz): Mercury-400BB 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.

(−)-Dimethyl (*R*)-Glutamate Hydrochloride (8·HCl). As described for the preparation of *ent*-**8·**HCl,[19] (*R*)-glutamate (25.1 g, 171 mmol) was reacted with dry MeOH (350 mL) and Me_3SiCl (75 mL, 594 mmol). After workup, the residual viscous oil was dried in vacuo at 40 °C to obtain **8·**HCl as colorless crystals, yield 33 g (91%), mp 91 °C. $[\alpha]^{20}_{589} = -25.4$ (*c* 5.0, H_2O).

(+)-Dimethyl (*S*)-Glutamate Hydrochloride (*ent*-8·HCl).[19] *ent*-**8·**HCl was prepared as described in ref 19 from (*S*)-glutamate (15 g, 102 mmol). Colorless solid, yield 21.3 g (99%), mp 83 °C. $[\alpha]^{20}_{589} = +25.4$ (*c* 5.05, H_2O).

(+)-Dimethyl (*R*)-2-[*N*-Benzyl-*N*-(2-chloroacetyl)amino]pentanedioate (10). As described for the preparation of *ent*-**10**,[19] the hydrochloride **8·**HCl (10.0 g, 47.2 mmol) was treated with benzaldehyde (4.72 mL, 46.7 mmol), $MgSO_4$ (8 g), and triethylamine (6.5 mL, 46.9 mmol) in dry CH_2Cl_2 (75 mL). The formed imine was reduced with $NaBH_4$ (3.05 g, 80.9 mmol) in dry MeOH (120 mL), and the resulting secondary amine **9** (ca. 12 g) was acylated with chloroacetyl chloride (6.1 mL, 76.5 mmol) and triethylamine (5.5 mL, 39.6 mmol) in dry CH_2Cl_2 (65 mL). After workup and fc, the chloroacetamide **10** was isolated as colorless oil, $R_f = 0.27$ (clohexane/EtOAc 7:3), yield 11.33 g (70%). $[\alpha]^{20}_{589} = +63.0$ (*c* 1.925, $CHCl_3$). Anal. ($C_{16}H_{20}ClNO_5$) C, H, N.

(−)-Dimethyl (*S*)-2-[*N*-Benzyl-*N*-(2-chloroacetyl)amino]pentanedioate (*ent*-10).[19] *ent*-**10** was prepared as described in ref 19 from *ent*-**8·**HCl (10 g, 47.2 mmol). Colorless oil, yield 9.15 g (56%). $[\alpha]^{20}_{589} = -63.0$ (*c* 1.455, $CHCl_3$). Purity by HPLC analysis.

(+)-Methyl (*R*)-3-[1-Benzyl-4-(4-methoxybenzyl)-3,6-dioxopiperazin-2-yl]propanoate (11). As described for the preparation of *ent*-**11**,[19] a solution of the chloroacetamide **10** (11.3 g, 33.1 mmol) in acetonitrile (160 mL) was reacted with 4-methoxybenzylamine (6.2 mL, 47.9 mmol) and triethylamine (6.5 mL, 46.9 mmol). After workup and fc, the piperazinedione **11** was isolated as colorless solid, $R_f = 0.33$ (cyclohexane/EtOAc 1:1), yield 11.2 g (82%), mp 63 °C (Et_2O). $[\alpha]^{20}_{589} = +8.2$ (*c* 1.10, CH_2Cl_2). Anal. ($C_{23}H_{26}N_2O_5$) C, H, N.

(−)-Methyl (*S*)-3-[1-Benzyl-4-(4-methoxybenzyl)-3,6-dioxopiperazin-2-yl]propanoate (*ent*-11).[19] *ent*-**11** was prepared as described in ref 19 from *ent*-**10** (9.15 g, 26.8 mmol) and 4-methoxybenzylamine (4.97 mL, 38.4mmol). Pale yellow oil, yield 8.5 g (77%). $[\alpha]^{20}_{589} = -7.8$ (*c* 0.712, CH_2Cl_2). Purity by HPLC analysis.

(+)-(1*R*,2*S*,5*R*)-6-Benzyl-2-methoxy-8-(4-methoxybenzyl)-2-(trimethylsiloxy)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (12).[23] As described in ref 23, a solution of **11** (3.20 g, 7.79 mmol) in THF (70 mL) was treated with lithium hexamethyldisilazane (LiHMDS, 1 M in THF, 8.9 mL, 8.9 mmol) and Me_3SiCl (3.6 mL, 28.36 mmol) to yield **12**. Colorless solid (methanol/water (1:1)), mp 95 °C. $[\alpha]_{589} = +8.7$, (*c* 1.72, CH_2Cl_2).

(−)-(1*S*,2*R*,5*S*)-6-Benzyl-2-methoxy-8-(4-methoxybenzyl)-2-(trimethylsiloxy)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (*ent*-12). As described for the synthesis of **12**, the enantiomer *ent*-**11** (233 mg, 0.57 mmol) was reacted with LiHMDS (1 M in THF, 0.95 mL, 0.95 mmol) and Me_3SiCl (0.25 mL, 1.97 mmol) in THF (7.5 mL). After workup and purification, *ent*-**12** was obtained as colorless solid, yield 214 mg (78%), mp 96 °C (petroleum ether/ethyl acetate). $[\alpha]_{589} = -8.7$ (*c* 1.72, CH_2Cl_2). Anal. ($C_{26}H_{34}N_2O_5Si$) C, H, N.

(−)-(1*R*,5*R*)-6-Benzyl-8-(4-methoxybenzyl)-6,8-diazabicyclo-[3.2.2]nonane-2,7,9-trione (13). Under N_2 at −78 °C a solution of **11** (4.6 g, 11.2 mmol) in dry THF (40 mL) was added dropwise during 10 min to a solution of LiHMDS (1 M in THF, 18.5 mL, 18.5 mmol) in dry THF (100 mL). After the mixture was stirred for 30 min at −78 °C, Me_3SiCl (4.9 mL, 38.6 mmol) was added slowly and the reaction mixture was stirred for 30 min at −78 °C and for 3 h at rt. Then, the solvent was removed in vacuo, the residue was dissolved in EtOAc (75 mL), and the organic layer was washed with 0.5 M HCl (30 mL), 0.5 M NaOH (30 mL), and brine (30 mL), dried (Na_2SO_4), and concentrated in vacuo. Since further purification was not necessary, the resulting residue (methyl silyl acetal **12**) was dissolved in THF (70 mL), H_2O (7 mL) and *p*-TosOH (1.38 g, 7.25 mmol) were added, and the reaction mixture was stirred for 16 h at rt. Then, the mixture was concentrated in vacuo to half of the original volume, and after dilution with CH_2Cl_2 (150 mL), the organic layer was washed with a half-saturated solution of $NaHCO_3$ (50 mL), H_2O (50 mL), and brine (50 mL), dried (Na_2SO_4), and then concentrated in vacuo. The residue was washed with Et_2O to afford **13** as colorless solid, yield 3.6 g (85%), mp 160 °C. $[\alpha]^{20}_{589} = -72.6$ (*c* 0.113, CH_2Cl_2). Anal. ($C_{22}H_{22}N_2O_4$) C, H, N. ^{1}H NMR ($CDCl_3$): δ 1.73−1.84 (m, 1H, 4-H), 2.22−2.37 (m, 2H, 4-H, 3-H), 2.40−2.50 (m, 1H, 3-H), 3.79 (s, 3H, PhOCH_3), 4.05 (dd, J = 4.7/2.3 Hz, 1H, 5-H), 4.30 (s, 1H, 1-H), 4.39 (d, J = 14.3 Hz, 1H, NCH_2Ph), 4.56 (d, J = 14.7 Hz, 1H, NCH_2Ph), 4.62 (d, J = 14.7 Hz, 1H, NCH_2Ph), 4.76 (d, J = 14.5 Hz, 1H, NCH_2Ph), 6.84 (d, J = 8.6 Hz, 2H, arom 3-H, 5-$H_{methoxybenzyl}$), 7.16−7.38 (m, 7H, arom H).

(+)-(1*S*,5*S*)-6-Benzyl-8-(4-methoxybenzyl)-6,8-diazabicyclo-[3.2.2]nonane-2,7,9-trione (*ent*-13). As described for the preparation of **13**, the enantiomer *ent*-**11** (8.5 g, 20.7 mmol) was treated with LiHMDS (1 M in THF, 37.5 mL, 37.5 mmol) and Me_3SiCl (9.85 mL, 77.6 mmol) in dry THF (210 mL), and the resulting methyl silyl acetal *ent*-**12** was hydrolyzed in a mixture of THF (150 mL), H_2O (15 mL) and *p*-TosOH (2.76 g, 14.4 mmol). After purification by fc (8 cm, cyclohexane/EtOAc 1:1, $R_f = 0.25$), *ent*-**13** was obtained as colorless solid, yield 6.6 g (84%), mp 163 °C. $[\alpha]^{20}_{589} = +72.4$ (*c* 0.132, CH_2Cl_2). Anal. ($C_{22}H_{22}N_2O_4$) C, H, N. Purity by HPLC analysis.

(−)-(1*R*,2*S*,5*R*)-6-Benzyl-2-hydroxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3. 2.2]nonane-7,9-dione (14). Under N_2 at −90 °C a solution of $LiBH_4$ (2 M in THF, 25.3 mL, 50.6 mmol) was slowly added to a solution of **13** (4.8 g, 12.68 mmol) in dry THF (250 mL), and the reaction mixture was stirred for 3.5 h at low temperature. The reaction mixture was cautiously hydrolyzed at −90 °C with 2 M aq HCl (250 mL). The mixture was warmed to rt and was stirred for an additional 0.5 h. The resulting solution was neutralized under ice cooling with 2 M NaOH, and the pH was brought to 8−9. The mixture was extracted three times with CH_2Cl_2, and the combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. The residual solid was recrystallized (EtOAc/MeOH 9:1) to afford **14** as colorless crystals, yield 3.98 g (82%), mp 183 °C (EtOAc). $[\alpha]^{20}_{589} = -91.0$ (*c* 1.25, CH_2Cl_2). Anal. ($C_{22}H_{24}N_2O_4$) C, H, N. ^{1}H NMR ($CDCl_3$): δ 1.39−1.51 (m, 1H, 3-H or 4-H), 1.54−1.66 (m, 1H, 3-H or 4-H), 1.67−1.77 (m, 1H, 3-H or 4-H), 1.78−1.89 (m, 1H, 3-H or 4-H), 3.41−3.51 (m, 1H, 2-H), 3.79 (s, 3H, PhOCH_3), 3.93 (dd, J = 5.6/2.6 Hz, 1H, 5-H), 4.00 (d, J = 3.7 Hz, 1H, 1-H), 4.43 (d, J = 14.5 Hz, 1H, NCH_2-aryl), 4.51 (d, J = 14.5 Hz, 1H, NCH_2aryl), 4.59 (d, J = 14.5 Hz, 1H, NCH_2aryl), 4.70 (d, J = 14.5 Hz, 1H, NCH_2aryl), 6.86 (d, J = 8.6 Hz, 2H, arom 3-H, 5-$H_{methoxybenzyl}$), 7.20 (d, J = 8.6 Hz, 2H, arom 2-H, 6-$H_{methoxybenzyl}$), 7.25−7.37 (m, 5H, arom H). HPLC: Column: Merck Superspher 100 (4 μm) LiChroCART 250−4 mm; eluent CH_3OH/H_2O 40:60; 0.5 mL/min; retention times: **14**: 90 min; **19**: 102 min.

(+)-(1*S*,2*R*,5*S*)-6-Benzyl-2-hydroxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (*ent*-14). As described for the preparation of **14**, ketone *ent*-**13** (5.0 g, 13.21 mmol) was reduced with $LiBH_4$ (2 M in THF, 26.4 mL, 52.8 mmol) in dry THF (250 mL). The crude product was recrystallized (EtOAc) to afford *ent*-**14** as colorless solid, yield 4.22 g (84%), mp 182−183 °C (EtOAc). $[\alpha]^{20}_{589} = +91.5$ (*c* 1.40, CH_2Cl_2). Anal. ($C_{22}H_{24}N_2O_4$) C, H, N. Further recrystallization from ethyl acetate gave colorless crystals, which were suitable for X-ray crystal structure analysis.

X-ray Crystal Structure Analysis of *ent*-14. Formula $C_{22}H_{24}N_2O_4$, $M = 380.43$, $T = 223$ K, colorless crystal, crystal size 0.35 × 0.10 × 0.03 mm, $a = 9.973(1)$, $b = 7.210(1)$, $c = 13.836(1)$ Å, $\beta = 105.35(1)°$, $V = 959.4(2)$ Å^3, $\rho_{calcd} = 1.317$ g cm^{-3}, $\mu = 0.741$ mm^{-1}, empirical absorption correction ($0.781 \le T \le 0.978$), $Z = 2$, monoclinic, space group $P2_1$ (no. 4), $\lambda = 1.54178$ Å, ω and φ scans, 4392 reflections collected ($\pm h$, $\pm k$, $\pm l$), $[(\sin\theta)/\lambda_{max}] = 0.59$ Å^{-1}, 2457 independent ($R_{int} = 0.034$) and 2024 observed reflections [$I \ge 2\,\sigma I$)], 255 refined parameters, $R = 0.038$, $wR^2 = 0.099$, max. residual electron density 0.14 (−0.13) e Å^{-3}, Flack parameter 0.2-(3), hydrogens calculated and refined as riding atoms. The data set

was collected with a Nonius KappaCCD diffractometer. Programs used: data collection COLLECT,[33] data reduction Denzo-SMN,[34] absorption correction Denzo,[35] structure solution SHELXS-97,[36] structure refinement SHELXL-97,[37] graphics SCHAKAL.[38]

CCDC-635641 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (internat.) +44(1223)336−033, E-mail: deposit@ccdc.cam.ac.uk].

(−)-(1*S*,2*S*,5*R*)-6-Benzyl-8-(4-methoxybenzyl)-6,8-diazabicyclo-[3.2.2]nonan-2-ol (15). Under N_2, $LiAlH_4$ (75 mg, 1.98 mmol) was added in small portions to an ice-cooled solution of **14** (151 mg, 0.40 mmol) in dry THF (13 mL). The reaction mixture was heated to reflux for 16 h. Then, H_2O was added under ice cooling until H_2 formation was finished. After filtration the filtrate was evaporated in vacuo. The resulting crude product was purified by fc (2 cm, petroleum ether/EtOAc 8:2 + 1% *N*,*N*-dimethylethanamine, $R_f = 0.28$) to afford **15** as colorless viscous oil, yield 118 mg (84%). $[\alpha]^{20}_{589} = -12.7$ (*c* 0.845, CH_2Cl_2). Anal. ($C_{22}H_{28}N_2O_2$) H. C, N: calcd, C 74.97, N 7.95; found, C 74.52, N 7.53. ^{1}H NMR ($CDCl_3$): $\delta = 1.59-1.69$ (m, 1H, 4-H), 1.74−1.87 (m, 2H, 3-H, 4-H), 2.13−2.24 (m, 1H, 3-H), 2.67−2.74 (m, 2H, 7-H, 9-H), 2.76−2.87 (m, 3H, 1-H, 5-H, 9-H), 3.10 (dd, $J = 11.2/2.4$ Hz, 1H, 7-H), 3.57−3.74 (m, 4H, 2 × NC*H*$_2$aryl), 3.79 (s, 3H, PhOC*H*$_3$), 3.97 (q, $J = 5.7$ Hz, 1H, 2-H), 6.84 (d, $J = 8.6$ Hz, 2H, arom 3-H, 5-H$_{methoxybenzyl}$), 7.12−7.26 (m, 3H, arom H), 7.27−7.35 (m, 4H, arom H). ^{13}C NMR ($CDCl_3$): $\delta = 29.3$ (1C, C-3), 30.5 (1C, C-4), 46.7 (1C, C-7), 51.0 (1C, C-9), 54.5 (1C, C-5), 55.2 (1C, PhO*C*H$_3$), 60.2 (1C, C-1), 60.4 (1C, N*C*H$_2$atyl), 60.9 (1C, N*C*H$_2$aryl), 75.1 (1C, C-2), 113.6 (2C, arom CH$_{C-3,C-5\ methoxybenzyl}$), 126.9 (1C, arom CH), 128.2 (2C, arom CH), 128.5 (2C, arom CH), 129.6 (2C, arom CH), 131.5 (1C, arom C$_{C-1\ methoxybenzyl}$), 139.4 (1C, arom C $_{C-1\ benzyl}$), 158.5 (1C, arom C$_{C-4\ methoxybenzyl}$).

(+)-(1*R*,2*R*,5*S*)-6-Benzyl-8-(4-methoxybenzyl)-6,8-diazabicyclo-[3.2.2]nonan-2-ol (*ent*-15). As described for the preparation of **15**, the enantiomer *ent*-**14** (600 mg, 1.58 mmol) was reduced with $LiAlH_4$ (300 mg, 7.91 mmol) in dry THF (50 mL) to give *ent*-**15** as a colorless viscous oil, yield 480 mg (86%). $[\alpha]^{20}_{589} = +11.4$ (*c* 0.775, CH_2Cl_2). Anal. ($C_{22}H_{28}N_2O_2$) H, N. C: calcd, C 74.97; found, C 74.47.

(−)-(1*R*,2*S*,5*R*)-6-Benzyl-2-methoxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (16). The alcohol **14** (150 mg, 0.39 mmol) and MeI (0.12 mL, 1.88 mmol) were consecutively added to a suspension of NaH (135 mg obtained from 225 mg 60% dispersion in oil, 5.63 mmol) in dry THF (20 mL). The reaction mixture was stirred at rt for 3 h. After hydrolysis with H_2O (2 mL) under ice cooling, CH_2Cl_2 (50 mL) was added and the mixture was extracted twice with 0.5 M NaOH. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by fc (2 cm, petroleum ether/EtOAc 3:7, $R_f = 0.38$) to give **16** as colorless viscous oil, which solidified slowly. Colorless solid, yield 150 mg (96%), mp 102 °C. $[\alpha]^{20}_{589} = -119.4$ (*c* 0.535, CH_2Cl_2). Anal. ($C_{23}H_{26}N_2O_4$) C, H, N. ^{1}H NMR ($CDCl_3$): $\delta = 1.42-1.60$ (m, 2H, 3-H and/or 4-H), 1.63−1.80 (m, 2H, 3-H and/or 4-H), 2.86−2.92 (m, 1H, 2-H), 3.18 (s, 3H, OC*H*$_3$), 3.81 (s, 3H, PhOC*H*$_3$), 3.91 (dd, $J = 5.7/2.4$ Hz, 1H, 5-H), 4.05 (d, $J = 3.3$ Hz, 1H, 1-H), 4.33 (d, $J = 14.5$ Hz, 1H, NC*H*$_2$aryl), 4.45 (d, $J = 14.6$ Hz, 1H, NC*H*$_2$aryl), 4.72 (d, $J = 14.5$ Hz, 1H, NC*H*$_2$aryl), 4.75 (d, $J = 14.5$ Hz, 1H, NC*H*$_2$aryl), 6.88 (d, $J = 8.6$ Hz, 2H, arom 3-H, 5-H$_{methoxybenzyl}$), 7.22 (d, $J = 8.6$ Hz, 2H, arom 2-H, 6-H$_{methoxybenzyl}$), 7.26−7.35 (m, 5H, arom H).

(+)-(1*S*,2*R*,5*S*)-6-Benzyl-2-methoxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (*ent*-16). As described for the preparation of **16**, the enantiomer *ent*-**14** (530 mg, 1.39 mmol) was allowed to react with NaH (474 mg obtained from 790 mg 60% dispersion in oil, 19.8 mmol) and MeI (0.44 mL, 6.9 mmol) in dry THF (30 mL) to yield *ent*-**16** as a colorless solid, yield 491 mg (89%), mp 98 °C. $[\alpha]^{20}_{589} = +102.4$ (*c* 0.505, CH_2Cl_2). Anal. ($C_{23}H_{26}N_2O_4$) C, H, N.

(−)-(1*S*,2*S*,5*R*)-6-Benzyl-2-methoxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane (17). As described for the reduction of the alcohol **14**, the methyl ether **16** (139 mg, 0.35 mmol) dissolved in dry THF (15 mL) was reduced with $LiAlH_4$ (66 mg, 1.74 mmol). After purification by fc (3 cm, petroleum ether/EtOAc 9:1, $R_f = 0.11$), **17** was obtained as colorless viscous oil, yield 70 mg (54%). $[\alpha]^{20}_{589} = -12.0$ (*c* 0.70, CH_2Cl_2). Anal. ($C_{23}H_{30}N_2O_2$) C, H. N: calcd, N 7.64; found, N 7.11. ^{1}H NMR ($CDCl_3$): $\delta = 1.50-1.60$ (m, 1H, 4-H), 1.78−1.87 (m, 1H, 4-H), 1.88−1.96 (m, 2H, 3-H), 2.63 (dd, $J = 10.7/3.7$ Hz, 1H, 9-H), 2.68−2.76 (m, 2H,1-H, 7-H), 2.82−2.88 (m, 2H, 5-H, 9-H), 3.05 (s, 3H, OC*H*$_3$), 3.05−3.10 (m, 1H, 7-H), 3.24 (t, $J = 8.2$ Hz, 1H, 2-H), 3.64 (d, $J = 12.9$ Hz, 1H, NC*H*$_2$aryl), 3.67 (s, 2H, NC*H*$_2$aryl), 3.73 (d, $J = 12.9$ Hz, 1H, NC*H*$_2$aryl), 3.78 (s, 3H, PhOC*H*$_3$), 6.84 (d, $J = 8.6$ Hz, 2H, arom 3-H, 5-H$_{methoxybenzyl}$), 7.17−7.35 (m, 7H, arom H). ^{13}C NMR ($CDCl_3$): $\delta = 26.7$ (1C, C-3), 28.4 (1C, C-4), 47.8 (1C, C-7), 51.8 (1C, C-9), 53.4 (1C, C-5), 55.2 (1C, PhO*C*H$_3$), 56.2 (2C, C-1, O*C*H$_3$), 60.1 (1C, N*C*H$_2$aryl), 60.6 (1C, N*C*H$_2$aryl), 85.3 (1C, C-2), 113.4 (2C, arom CH$_{C-3,C-5\ methoxybenzyl}$), 126.6 (1C, arom CH), 128.1 (2C, arom CH), 128.3 (2C, arom CH), 130.0 (2C, arom CH), 131.6 (1C, arom C$_{C-1\ methoxybenzyl}$), 140.1 (1C, arom C$_{C-1\ benzyl}$), 158.6 (1C, arom C$_{C-4\ methoxybenzyl}$).

(+)-(1*R*,2*R*,5*S*)-6-Benzyl-2-methoxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane (*ent*-17). As described for the preparation of **17**, the enantiomer *ent*-**16** (630 mg, 1.60 mmol) was reduced with $LiAlH_4$ (312 mg, 8.22 mmol) in dry THF (50 mL) to give *ent*-**17** as a colorless viscous oil, yield 413 mg (70%). $[\alpha]^{20}_{589} = +10.5$ (*c* 1.005, CH_2Cl_2). Anal. ($C_{23}H_{30}N_2O_2$) H, N. C: calcd, C 75.37; found, C 74.86.

(+)-[(1*S*,2*S*,5*S*)-6-Benzyl-8-(4-methoxybenzyl)-7,9-dioxo-6,8-diazabicyclo[3.2.2]nonan-2-yl] 4-Nitrobenzoate (*ent*-18). Under N_2, diisopropyl azodicarboxylate (DIAD, 1.0 mL, 5.14 mmol) was added dropwise to an ice-cooled solution of *ent*-**14** (500 mg, 1.31 mmol), PPh_3 (1.69 g, 6.44 mmol), and *p*-nitrobenzoic acid (960 mg, 5.74 mmol) in dry THF (25 mL). The reaction temperature was allowed to rise to rt. After 6 h, the solvent was removed in vacuo and the residue was purified by fc (6 cm, petroleum ether/EtOAc 1:1, $R_f = 0.42$) to obtain *ent*-**18** as pale yellow solid, yield 555 mg (80%), mp 143 °C. $[\alpha]^{20}_{589} = +51.1$ (*c* 0.19, CH_2Cl_2). Anal. ($C_{29}H_{27}N_3O_7$) C, H, N: calcd. C 65.78; found C 65.36. ^{1}H NMR ($CDCl_3$): $\delta = 1.42-1.54$ (m, 1H, 3-H or 4-H), 1.82−1.94 (m, 1H, 3-H or 4-H), 2.05−2.16 (m, 2H, 3-H or 4-H), 3.75 (s, 3H, PhOC*H*$_3$), 4.00 (dd, $J = 4.9/2.5$ Hz, 1H, 5-H), 4.27 (d, $J = 2.0$ Hz, 1H, 1-H), 4.37 (d, $J = 14.9$ Hz, 1H, NC*H*$_2$aryl), 4.59 (s, 2H, NC*H*$_2$-aryl), 4.87 (d, $J = 14.9$ Hz, 1H, NC*H*$_2$aryl), 5.34 (ddd, $J = 9.3/5.0/1.9$ Hz, 1H, 2-H), 6.79 (d, $J = 9.0$ Hz, 2H, arom 3-H, 5-H$_{methoxybenzyl}$), 7.12 (d, $J = 8.6$ Hz, 2H, arom 2-H, 6-H$_{methoxybenzyl}$), 7.22−7.39 (m, 5H, arom$_{benzyl}$), 7.94 (d, $J = 9.0$ Hz, 2H, arom 2-H, 6-H$_{nitrophenyl}$), 8.28 (d, $J = 9.0$ Hz, 2H, arom 3-H, 5-H$_{nitrophenyl}$).

(+)-(1*R*,2*R*,5*R*)-6-Benzyl-2-hydroxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (19). Under N_2, DIAD (1.0 mL, 5.14 mmol) was added dropwise to an ice-cooled solution of **14** (500 mg, 1.31 mmol), PPh_3 (1.69 g, 6.44 mmol), and *p*-nitrobenzoic acid (960 mg, 5.74 mmol) in dry THF (25 mL). The reaction temperature was allowed to rise to rt. After 16 h, the solvent was removed in vacuo and the residue was purified by fc (3 cm, petroleum ether/EtOAc 1:1, $R_f = 0.42$). The resulting pale yellow solid (*p*-nitrobenzoate **18**) was immediately dissolved in MeOH (30 mL), K_2CO_3 (300 mg, 2.17 mmol) was added, and the reaction mixture was stirred for 16 h at rt. Then, H_2O (120 mL) was added, the aqueous solution was extracted with CH_2Cl_2, and the combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. The residual solid was purified by fc (3 cm, EtOAc, $R_f = 0.40$) to obtain **19** as a colorless solid, yield 301 mg (60%), mp 173−174 °C. $[\alpha]^{20}_{589} = +23.0$ (*c* 1.045, CH_2Cl_2). Anal. ($C_{22}H_{24}N_2O_4$) C, H, N. ^{1}H NMR ($CDCl_3$): δ 1.29−1.41 (m, 1H, 4-H), 1.50−1.64 (m, 1H, 3-H), 1.86−1.94 (m, 1H, 3-H), 1.94−2.01 (m, 1H, 4-H), 3.80 (s, 3H, PhOC*H*$_3$), 3.89 (dd, $J = 5.4/2.3$ Hz, 1H, 5-H), 3.99−4.06 (m, 2H, 1-H, 2-H), 4.42 (d, $J = 14.5$ Hz, 1H, NC*H*$_2$Ph), 4.44 (d, $J = 14.7$ Hz, 1H, NC*H*$_2$Ph), 4.60 (d, $J = 14.7$ Hz, 1H, NC*H*$_2$Ph), 4.88 (d, $J = 14.5$ Hz, 1H, NC*H*$_2$Ph), 6.87 (d, $J = 8.6$ Hz, 2H, arom

3-H, 5-$H_{methoxybenzyl}$), 7.20 (d, $J = 8.6$ Hz, 4H, arom H), 7.27−7.36 (m, 3H, arom H).

(−)-(1*S*,2*S*,5*S*)-6-Benzyl-2-hydroxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (*ent*-19). K_2CO_3 (300 mg, 2.17 mmol) was added to a solution of *ent*-**18** (514 mg, 0.97 mmol) in MeOH (35 mL), and the reaction mixture was stirred for 16 h at rt. Then, H_2O (140 mL) was added, the aqueous solution was extracted with CH_2Cl_2, and the combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. The residual solid was purified by fc (2 cm, EtOAc, $R_f = 0.40$) to obtain *ent*-**19** as a colorless solid, yield 340 mg (92%), mp 174 °C (EtOAc). $[\alpha]^{20}_{589} = -22.6$ (*c* 1.485, CH_2Cl_2). Anal. ($C_{22}H_{24}N_2O_4$) C, H, N.

(−)-(1*S*,2*R*,5*R*)-6-Benzyl-8-(4-methoxybenzyl)-6,8-diazabicyclo-[3.2.2]nonan-2-ol (20). As described for the reduction of **14**, the diastereomeric alcohol **19** (124 mg, 0.33 mmol) in dry THF (11 mL) was reduced with $LiAlH_4$ (62 mg, 1.64 mmol). After workup and fc purification (2 cm, petroleum ether/EtOAc 8:2 + 1% *N*,*N*-dimethylethanamine, $R_f = 0.33$), **20** was obtained as colorless viscous oil, yield 76 mg (66%). $[\alpha]^{20}_{589} = -24.0$ (*c* 0.325, CH_2Cl_2). Anal. ($C_{22}H_{28}N_2O_2$) C, H, N. ^{1}H NMR ($CDCl_3$): $\delta = 1.53-1.63$ (m, 2H, 3-H, 4-H), 1.70−1.79 (m, 1H, 4-H), 2.07−2.18 (m, 1H, 3-H), 2.57 (dd, $J = 10.8/2.9$ Hz, 1H, 9-H), 2.70 (dd, $J = 10.6/2.4$ Hz, 1H, 9-H), 2.79−2.86 (m, 2H, 5-H, 7-H), 2.88−2.96 (m, 2H, 1-H, 7-H), 3.67−3.73 (m, 4H, 2 × NC*H*$_2$aryl), 3.80 (s, 4H, PhOC*H*$_3$, 2-H), 6.87 (d, $J = 8.6$ Hz, 2H, arom 3-H, 5-$H_{methoxybenzyl}$), 7.20−7.34 (m, 7H, arom H). ^{13}C NMR ($CDCl_3$): $\delta = 27.8$ (1C, C-4), 30.0 (1C, C-3), 48.9 (1C, C-7), 51.6 (1C, C-9), 53.7 (1C, C-5), 55.2 (1C, PhO*C*H$_3$), 60.0 (1C, C-1), 60.5 (1C, N*C*H$_2$aryl), 61.3 (1C, N*C*H$_2$aryl), 70.3 (1C, C-2), 113.8 (2C, arom $CH_{C-3,C-5\ methoxybenzyl}$), 126.8 (1C, arom CH), 128.2 (4C, arom CH), 130.2 (2C, arom CH), 130.4 (1C, arom $C_{C-1\ methoxybenzyl}$), 139.8 (1C, arom $C_{C-1,benzyl}$), 158.9 (1C, arom $C_{C-4,methoxybenzyl}$).

(+)-(1*R*,2*S*,5*S*)-6-Benzyl-8-(4-methoxybenzyl)-6,8-diazabicyclo-[3.2.2]nonan-2-ol (*ent*-20). As described for the preparation of **20**, the enantiomer *ent*-**19** (600 mg, 1.58 mmol) was reduced with $LiAlH_4$ (300 mg, 7.91 mmol) in dry THF (50 mL) to give *ent*-**20** as a pale yellow viscous oil, yield 432 mg (77%). $[\alpha]^{20}_{589} = +20.4$ (*c* 0.790, CH_2Cl_2). Anal. ($C_{22}H_{28}N_2O_2$) C, H, N.

(+)-(1*R*,2*R*,5*R*)-6-Benzyl-2-methoxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (21). As described for the synthesis of the methyl ether **16**, the alcohol **19** (300 mg, 0.79 mmol) was reacted with MeI (0.12 mL, 1.88 mmol) and NaH (270 mg obtained from 450 mg 60% dispersion in oil, 11.25 mmol) in dry THF (40 mL). Workup and fc purification (3 cm, petroleum ether/EtOAc 3:7, $R_f = 0.38$) gave **21** as colorless viscous oil, yield 300 mg (96%). $[\alpha]^{20}_{589} = +70.7$ (*c* 0.42, CH_2Cl_2). Anal. ($C_{23}H_{26}N_2O_4$) C, H, N. H NMR ($CDCl_3$): $\delta = 1.28-1.38$ (m, 1H, 4-H), 1.58−1.71 (m, 1H, 3-H or 4-H), 1.94−2.06 (m, 2H, 3-H and/or 4-H), 3.33 (s, 3H, OC*H*$_3$), 3.51 (ddd, $J = 9.2/4.5/1.3$ Hz, 1H, 2-H), 3.81 (s, 3H, PhOC*H*$_3$), 3.88 (dd, $J = 5.5/2.0$ Hz, 1H, 5-H), 3.95 (d, $J = 14.5$ Hz, 1H, NC*H*$_2$aryl), 4.07 (d, $J = 1.0$ Hz,1H, 1-H), 4.42 (d, $J = 14.8$ Hz, 1H, NC*H*$_2$aryl), 4.63 (d, $J = 14.5$ Hz, 1H, NC*H*$_2$aryl), 5.27 (d, $J = 14.8$ Hz, 1H, NC*H*$_2$aryl), 6.88 (d, $J = 8.6$ Hz, 2H, arom 3-H, 5-$H_{methoxybenzyl}$), 7.15 (d, $J = 8.6$ Hz, 2H, arom 2-H, 6-$H_{methoxybenzyl}$), 7.21 (dd, $J = 7.4/1.6$ Hz, 2H, arom H), 7.27−7.36 (m, 3H, arom H).

(−)-(1*S*,2*S*,5*S*)-6-Benzyl-2-methoxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (*ent*-21). As described for the preparation of **21**, the enantiomeric alcohol *ent*-**19** (433 mg, 1.14 mmol) was allowed to react with NaH (390 mg obtained from 650 mg 60% dispersion in oil, 16.3 mmol) and MeI (0.36 mL, 5.66 mmol) in dry THF (25 mL) to give *ent*-**21** as a colorless solid, yield 367 mg (82%), mp 153 °C (petroleum ether/EtOAc). $[\alpha]^{20}_{589} = -67.1$ (*c* 0.505, CH_2Cl_2). Anal. ($C_{23}H_{26}N_2O_4$) C, H, N.

(+)-(1*S*,2*R*,5*R*)-6-Benzyl-2-methoxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane (22). As described for the reduction of **14** the methyl ether **21** (197 mg, 0.50 mmol) in dry THF (20 mL) was reduced with $LiAlH_4$ (94 mg, 2.48 mmol). Workup and fc purification (3 cm, petroleum ether/EtOAc 8:2, $R_f = 0.24$) led to **22** as colorless viscous oil, yield 105 mg (57%). $[\alpha]^{20}_{589} = +8.9$ (*c* 0.765, CH_2Cl_2). Anal. ($C_{23}H_{30}N_2O_2$) C, H, N. ^{1}H NMR ($CDCl_3$): $\delta = 1.59-1.75$ (m, 2H, 4-H), 1.82−1.90 (m, 1H, 3-H), 2.00−2.12 (m, 1H, 3-H), 2.67−2.77 (m, 3H, 5-H, 7-H, 9-H), 2.78−2.87 (m, 2H, 7-H, 9-H), 3.00 (t, $J = 2.7$ Hz, 1H, 1-H), 3.14 (s, 3H, OC*H*$_3$), 3.53 (dd, $J = 10.2/4.7$ Hz, 1H, 2-H), 3.63−3.74 (m, 4H, 2 × NC*H*$_2$aryl), 3.79 (s, 3H, PhOC*H*$_3$), 6.84 (d, $J = 8.6$ Hz, 2H, arom 3-H, 5-$H_{methoxybenzyl}$), 7.20−7.36 (m, 7H, arom H). ^{13}C NMR ($CDCl_3$): $\delta = 26.4$ (1C, C-3), 31.7 (1C, C-4), 50.0 (1C, C-7), 51.2 (1C, C-9), 54.6 (1C, C-5), 55.2 (1C, PhO*C*H$_3$), 55.7 (1C, O*C*H$_3$), 59.0 (1C, C-1), 61.0 (2C, N*C*H$_2$aryl), 86.0 (1C, C-2), 113.3 (2C, arom $CH_{C-3,C-5\ methoxybenzyl}$), 126.8 (1C, arom CH), 128.1 (2C, arom CH), 128.6 (2C, arom CH), 129.8 (2C, arom CH), 131.8 (1C, arom $C_{C-1\ methoxybenzyl}$), 139.7 (1C, arom $C_{C-1\ benzyl}$), 158.4 (1C, arom $C_{C-4\ methoxybenzyl}$).

(−)-(1*R*,2*S*,5*S*)-6-Benzyl-2-methoxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane (*ent*-22). As described for the preparation of **22**, the enantiomer *ent*-**21** (545 mg, 1.38 mmol) was reduced with $LiAlH_4$ (260 mg, 6.86 mmol) in dry THF (50 mL) to give *ent*-**22** as a colorless viscous oil, yield 415 mg (82%). $[\alpha]^{20}_{589} = -7.8$ (*c* 0.80, CH_2Cl_2). Anal. ($C_{23}H_{30}N_2O_2$) C, H, N.

Receptor Binding Studies. Materials and General Procedures. The guinea pig brains and rat livers were commercially available (Harlan-Winkelmann, Germany). Homogenizer: Elvehjem Potter (B. Braun Biotech International). Centrifuge: High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Finnigan). Filter: Printed Filtermat Typ B (Perkin-Elmer), presoaked in 0.5% aqueous polyethylenimine for 2 h at room temperature before use. The filtration was carried out with a MicroBeta FilterMate-96 Harvester (Perkin-Elmer). The scintillation analysis was performed using Meltilex (Typ A) solid scintillator (Perkin-Elmer). The solid scintillator was melted on the filtermat at a temperature of 95 °C for 5 min. After solidification of the scintillator at room temperature, the scintillation was measured using a MicroBeta Trilux scintillation analyzer (Perkin-Elmer). The counting efficiency was 20%.

Membrane Preparation for the σ_1 Assay (modified according to refs 27, 29). Five guinea pig brains were homogenized with the potter (500−800 rpm, 10 up-and-down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1200*g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 23500*g* for 20 min at 4 °C. The pellet was resuspended in 5−6 volumes of buffer (50 mM Tris, pH 7.4) and centrifuged again at 23500*g* (20 min, 4 °C). This procedure was repeated twice. The final pellet was resuspended in 5−6 volumes of buffer, the protein concentration was determined according to the method of Bradford[31] using bovine serum albumin as standard, and subsequently the preparation was frozen (−80 °C) in 1.5 mL portions containing about 1.5 mg protein/mL.

Performing of the σ_1 Assay (modified according to refs 27, 29). The test was performed with the radioligand [^{3}H]-(+) pentazocine (42.5 Ci/mmol; Perkin-Elmer). The thawed membrane preparation (about 75 μg of the protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-pentazocine, and buffer (50 mM Tris, pH 7.4) in a total volume of 200 μL for 180 min at 37 °C. The incubation was terminated by rapid filtration through the presoaked filtermats by using the cell harvester. After washing each well five times with 300 μL of water, the filtermats were dried at 95 °C. Subsequently, the solid scintillator was placed on the filtermat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at room temperature. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The nonspecific binding was determined with 10 μM unlabeled (+) pentazocine. The K_d-value of the radioligand [^{3}H]-(+)-pentazocine is 2.9 nM.[39]

Membrane Preparation for the σ_2 Assay (modified according to refs 27, 29). Two rat livers were cut into smaller pieces and homogenized with a potter (500−800 rpm, 10 up-and-down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1200*g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 31000*g* for 20 min at 4 °C. The pellet was resuspended in buffer (50 mM Tris, pH 8.0) and incubated at room temperature for 30 min. After the incubation, the suspension was centrifuged again at 31000*g* for 20 min at 4 °C. The final pellet

was resuspended in buffer, the protein concentration was determined according to the method of Bradford[31] using bovine serum albumin as standard, and subsequently the preparation was frozen (−80 °C) in 1.5 mL portions containing about 2 mg protein/mL.

Performing of the σ_2-Assay (modified according to refs 27, 29). The test was performed with the radioligand [^{3}H]ditolylguanidine (50 Ci/mmol; ARC). The thawed membrane preparation (about 100 μg of the protein) was incubated with various concentrations of test compounds, 3 nM [^{3}H]-ditolylguanidine, 500 nM (+)-pentazocine, and buffer (50 mM Tris, pH 8.0) in a total volume of 200 μL for 180 min at room temperature. The incubation was terminated by rapid filtration through the presoaked filtermats using a cell harvester. After each well was washed five times with 300 μL of water, the filtermats were dried at 95 °C. Subsequently, the solid scintillator was placed on the filtermat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at room temperature. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The nonspecific binding was determined with 10 μM unlabeled ditolylguanidine. The K_d-value of the radioligand [^{3}H]-ditolylguanidine is 17.9 nM.[40]

NMDA Assay. The preparation of the receptor material and the assay were performed according to ref 29.

Data Analysis. All experiments were carried out in triplicates using standard 96-well multiplates (Diagonal). The IC_{50}-values were determined in competition experiments with six concentrations of the test compounds and were calculated with the program GraphPad Prism 3.0 (GraphPad Software) by nonlinear regression analysis. The K_i-values were calculated according to Cheng and Prusoff.[41] The K_i-values are given as the mean value + SEM from three independent experiments.

Cytotoxicity Assay.[30] All cell lines were obtained from the German Collection of Microbiology and Cell Culture (DSZK, Braunschweig, FRG). Cytotoxicity testing was done by using a microtiter assay based on staining cells with crystal violet as described in detail elsewhere.[30] To determine the IC_{50} values, five serially diluted stock solutions of test substance in DMF were used in the studies; concentrations giving T/C values between 10 and 90% were used to estimate the IC_{50} values, which were calculated by least-squares analysis of the dose−response curves.

σ_1 and σ_2 Expression in Tumor Cell Lines. A homogenized and standardized membrane preparation (1.8 mg protein/mL) of the corresponding tumor cell line was prepared as previously described[15] and incubated with the corresponding radioligand in the presence or absence of an excess of an inhibitor ((+)-pentazocine in the σ_1 assay, ditolylguanidine in the σ_2 assay). The experimental details are given above for the σ_1 and σ_2 assays. In the assay 90 μg protein/well were employed. For each tumor cell line preparation, the specific binding of the radioligand was calculated. Additionally the number of binding sites (receptors) in the assay was calculated according to the following equation:

$$N = \mathrm{Cpm} \times L/60 \times EA$$

where N is the number of binding sites (receptors) in the assay, Cpm is the counts per minute, L is Avogadro's number (6.022×10^{23} molecules/mol), E is the efficiency of the counter (20%), and A is the specific activity of the radioligand in Bq/mol. Furthermore, N_p, the number of binding sites (receptors) per μg protein as well as the molar amount of binding sites (pmol) per mg protein were calculated.

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Supporting Information Available: Purity data of the prepared compounds (elemental analysis, HPLC analysis), some experimental details, and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Brasili, L. Enigmatic receptors. *Pharm. Acta Helv.* **2000**, *74*, 201−203.
(2) Hanner, M.; Moebius, F. F.; Flandorfer, A.; Knaus, H.-G.; Striessnig, J.; Kempner, E.; Glossmann, H. Purification, molecular cloning, and expression of the mammalian sigma$_1$-binding site. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8072−8077.
(3) Kekuda, R.; Prasad, P. D.; Fei, Y.-J.; Leibach, F. H.; Ganapathy, V. Cloning and Functional Expression of the Human Type 1 Sigma Receptor (hSigmaR1). *Biochem. Biophys. Res. Commun.* **1996**, *229*, 553−558.
(4) Seth, P.; Fei, Y.-J.; Li, H. W.; Huang, W.; Leibach, F. H.; Ganapathy, V. Cloning and Functional Characterization of a σ Receptor from Rat Brain. *J. Neurochem.* **1998**, *70*, 922−931.
(5) Prasad, P. D.; Li, H. W.; Fei, Y.-J.; Ganapathy, M. E.; Fujita, T.; Plumley, L. H.; Yang-Feng, T. L.; Leibach, F. H.; Ganapathy, V. Exon-Intron structure, Analysis of Promoter Region, and Chromosomal Localization of the Human Type 1 σ Receptor Gene. *J. Neurochem.* **1998**, *70*, 443−451.
(6) Aydar, E.; Palmer, C. P.; Djamgoz, M. B. A. Sigma receptors and Cancer: Possible Involvement of Ion Channels. *Cancer Res.* **2004**, *64*, 5029−5035.
(7) Monnet, F. P.; Morin-Surun, P.; Leger, J.; Comettes, L. Protein Kinase C-Dependent Potentiation of Intracellular Calcium Influx by σ_1 Receptor Agonists in Rat Hippocampal Neurons. *J. Pharmacol. Exp. Ther.* **2003**, *307*, 705−712.
(8) Aydar, E.; Palmer, C. P.; Klyachko, V. A.; Meyer, B. J. The Sigma Receptor as a Ligand-Regulated Auxiliary Potassium Channel Subunit. *Neuron* **2002**, *34*, 399−410.
(9) Bowen, W. D. Sigma receptors: recent advances and new clinical potentials. *Pharm. Acta Helv.* **2000**, *74*, 211−218.
(10) Hayashi, T.; Su, T.-P. σ_1 Receptor Ligands: Potential in the Treatment of Neuropsychiatric Disorders. *CNS Drugs* **2004**, *18*, 269 − 284.
(11) Kassiou, M.; Dannals, R. F.; Liu, X.; Wong, D. F.; Ravert, H. T.; Scheffel, U. A. Synthesis and in vivo evaluation of a new PET radioligand for Studying sigma-2 receptors. *Biorg. Med. Chem.* **2005**, *13*, 3623−3626.
(12) John, C. S.; Lim, B. B.; Viler, B. J.; Geyer, B.; C.; Bowen, W. D. Substituted Halogenated Arylsulfonamides: A New Class of σ Receptor Binding Tumor Imaging Agents. *J. Med. Chem.* **1998**, *41*, 2445−2450.
(13) John, C. S.; Bowen, W. D.; Varma, V. M.; McAfee, J. G.; Moody, T. W. Sigma Receptors are Expressed in Human Non-Small Cell Lung Carcinoma. *Life Sci.***1995**, *56*, 2385−2392.
(14) John, C. S.; Vilner, B. J.; Geyer, B. C.; Moody, T.; Bowen W. D. Targeting Sigma Receptor-binding Benzamides as *in Vivo* Diagnostic and Therapeutic Agents for Human Prostate Tumors. *Cancer Res.* **1999**, *59*, 4578−4583.
(15) Vilner, B. J.; de Costa, B. R.; Bowen, W. D. Cytotoxic Effects of Sigma Ligands: Sigma Receptor-mediated Alterations in Cellular Morphology and Viability. *J. Neurosci.* **1995**, *15*, 117−134.
(16) Colabufo, N. A.; Berardi, F.; Contino, M.; Niso, M.; Abate, C.; Perrone, R.; Tortorella, V. Antiproliferative and cytotoxic effects of some σ_2 agonists and σ_1 antagonists in tumour cell lines. *Arch. Pharmacol.* **2004**, *370*, 106−113.
(17) Nordenberg, J.; Perlmutter, I.; Lavie, G.; Beery, E.; Uziel, O.; Morgenstern, C.; Fenig, E.; Weizman, A. Anti-proliferative activity of haloperidol in B16 mouse and human SK-MEL-28 melanoma cell lines. *Int. J. Oncol.* **2005**, *27*, 1097−1103.
(18) Bedürftig, S.; Wünsch, B. Chiral, nonracemic (piperazin-2-yl)-methanol derivatives with σ-receptor affinity. *Biorg. Med. Chem.* **2004**, *12*, 3299−3311.
(19) Weigl, M.; Wünsch, B. The synthesis of the enantiomer *ent*-**11** was described in literature: Synthesis of chiral non-racemic 3-(dioxopiperazin-2-yl)propionic acid derivatives. *Tetrahedron* **2002**, *58*, 1173−1183.
(20) Warner, P. M. Strained Bridgehead Double Bonds. *Chem. Rev.* **1989**, *89*, 1067 1093.
(21) Weigl, M.; Wünsch, B. Synthesis of 6,8-Diazabicyclo[3.2.2]nonanes: Conformationally Restricted Piperazine Derivatives. *Org. Lett.* **2000**, *2*, 1177−1179.
(22) Weigl, M.; Bedürftig, S.; Maier, C. A.; Wünsch, B. Conformationally Constrained Ethylenediamines: Synthesis and Receptor Binding of 6,8-Diazabicyclo[3.2.2]nonanes. *Biorg. Med. Chem.* **2002**, *10*, 2245−2257.
(23) Geiger, C.; Zelenka, C.; Fröhlich, R.; Wibbeling, B.; Wünsch, B. Stereoselectivity during a Dieckmann Analogous Cyclization of (Piperazin-2-yl)propionic Acid Esters. *Z. Naturforsch.* **2005**, *60b*, 1068−1070.
(24) Mitsunobu, O. The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products. *Synthesis* **1981**, 1−28.

(25) Carroll, F. I.; Abraham, P.; Parham, K.; Bai, X.; Zhang, X.; Brine, G. A.; Mascarella, S. W.; Martin, B. R.; May, E. L.; Sauss, C.; Di, Paolo, L.; Wallace, P.; Walker, J. M.; Bowen, W. D. Enantiomeric N-substituted N-Normetazocines: A Comparative Study of Affinities at *σ*, PCP and *μ* Opioid Receptors. *J. Med. Chem*. **1992**, *35*, 2812 -2818.
(26) May, E. L.; Aceto, M. D.; Bowman, E. R.; Bentley, C.; Martin, B. R.; Harris, L. S.; Medzihradsky, F.; Mattson, M. V.; Jacobson, A. E. Antipodal α-N-(Methyl through Decyl)-*N*-normetacozines (5,9a-Dimethyl-2′-hydroxy-6,7-benzomorphans): In Vitro and in Vivo Properties. *J. Med. Chem*. **1994**, *37*, 3408−3418.
(27) Maier, C. A.; Wünsch, B. Novel Spiropiperidines as Highly Potent and Subtype Selective Sigma Receptor Ligands. Part 1. *J. Med. Chem.* **2002**, *45*, 438 − 448.
(28) Maier, C. A.; Wünsch, B. Novel *σ* Receptor Ligands. Part 2. SAR of Spiro[[2]benzopyran-1,4′piperidines] and Spiro[[2]benzofuran-1,4′piperidines] with Carbon Substituents in Position 3. *J. Med. Chem.* **2002**, *45*, 4923−4930.
(29) Wirt, U.; Schepmann, D.; Wünsch, B. Asymmetric Synthesis of 1-Substituted 3-Benzazepines as NMDA Receptor Antagonists. *Eur. J. Org. Chem.* **2007**, 462 − 475.
(30) Bracht, K.; Boubakari, Grünert, R.; Bednarski, P. J. Correlations between the Activities of 19 Anti-tumor Agents and the Intracellular Glutathione Concentrations in a Panel of 14 Human Cancer Cell Lines: Comparisons with the NCI Data. *Anti-Cancer Drugs* **2006**, *17*, 41−51.
(31) Bradford, M. M. A Rapid and Sensitive Method fort he Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem*. **1976**, *72*, 248 − 254.
(32) Still, W. S.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923 − 2925.
(33) "Collect" data collection software. Nonius, B.V. Delft, The Netherlands 1998.
(34) Otwinowski, Z.; Minor, W. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* **1997**, *276*, 307−326.
(35) Otwinowski, Z.; Borek, D.; Majewski, W.; Minor, W. Multiparametric scaling of diffraction data. *Acta Crystallogr*. **2003**, *A59*, 228−234.
(36) Sheldrick, G. M. Phase annealing in SHELX-90: direct methods for larger structures. *Acta Crystallogr*. **1990**, *A46*, 467−473.
(37) Sheldrick, G. M. SHELXL-97 − a program for the refinement of crystal structures from diffraction data. Universität Göttingen 1997.
(38) Keller, E. SCHAKAL − a computer program for the graphic representation of molecular and crystallographic models. Universität Freiburg 1997.
(39) De-Haven-Hudkins, D. L.; Fleissner, L. C.; Ford-Rice, F. Y. Characterization of the binding of [^{3}H](+)-pentazocine to *σ* recognition sites in guinea pig brain. *Eur. J. Pharmacol. Mol. Pharmacol. Sect.* **1992**, *227*, 371−378.
(40) Mach, R. H.; Smith, C. R.; Childers, S. R. Ibogaine possesses a selective affinity for σ_2 receptors. *Life Sci.* **1995**, *57*, 57−62.
(41) Cheng, Y.; Prusoff, W. H. Relationship between the Inhibition constant (K_i) and the Concentration of Inhibitor which causes 50 per Cent Inhibition (I_{50}) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099 − 3108.
(42) Spruce, B. A.; Campbell, L. A.; McTavish, N.; Cooper, M. A.; Appleyard, M. V. L.; O'neill, M.; Howie, J.; Samson, J.; Watt, S.; Murray, K.; McLean, D.; Leslie, N. R.; Safrany, S. T.; Ferguson, M. J.; Peters, J. A.; Prescott, A. R.; Box, G.; Hayes, A.; Nutley, B.; Raynaud, F.; Downes, C. P.; Lambert, J. J.; Thompson, A. M.; Eccles, S. Small Molecule Antagonists of the *σ*-1 Receptor Cause Selective Release of the Death Program in Tumor and Self-Reliant Cells and Inhibit Tumor Growth in Vitro and in Vivo. *Cancer Res.* **2004**, *64*, 4875−4886.
(43) John, C. S.; Vilner, B. J.; Gulden, M. E.; Efange, S. M. N.; Langason, R. B.; Moody, T. W.; Bowen, W. D. Synthesis and Pharmacological Characterization of 4-[^{125}I]-*N*-(-*N*-Benzylpiperidin-4-yl)-4-iodobenzamide: A High Affinity *σ* Receptor Ligand for Potential Imaging of Breast Cancer. *Cancer Res.* **1995**, *55*, 3022−3027.

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