Dancing of the Second Aromatic Residue around the 6,8-Diazabicyclo[3.2.2]nonane Framework: Influence on σ Receptor Affinity and Cytotoxicity

Ralph Holl,[†] Dirk Schepmann,[†] Roland Fröhlich,[‖,‡] Renate Grünert,[§] Patrick J. Bednarski,[§] and Bernhard Wünsch*[,†]

Institut für Pharmazeutische und Medizinische Chemie der Westfälischen Wilhelms-Universität Münster, Hittorfstrasse 58-62, D-48149 Münster, Germany, Organisch-chemisches Institut der Westfälischen Wilhelms-Universität Münster, Correnstrasse 40, D-48149 Münster, Germany, Institut für Pharmazie der Ernst-Moritz-Arndt-Universität Greifswald, Friedrich-Ludwig-Jahn-Strasse 17, D-17489 Greifswald, Germany

Received December 3, 2008

A series of 6,8-diazabicyclo[3.2.2]nonane derivatives bearing two aromatic moieties was prepared, the affinity toward σ_1 and σ_2 receptors was investigated, and the growth inhibition of six human tumor cell lines was determined. The enantiopure bicyclic ketones **5a** ((+)-(1*S*,5*S*)-6-allyl-8-(4-methoxybenzyl)-6,8-diazabicyclo-[3.2.2]nonane-2,7,9-trione) and **5b** ((+)-(1*S*,5*S*)-6-allyl-8-(2,4-dimethoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-2,7,9-trione) as well as their enantiomers ent-**5a** and ent-**5b** served as chiral building blocks, which were derived from (*S*)- and (*R*)-glutamate, respectively. Structure−affinity relationships revealed that **11a** (K_i = 154 nM), ent-**11a** (K_i = 91 nM), and ent-**17a** (K_i = 104 nM) are the most potent σ_1 ligands. High σ_2 affinity was achieved with **17b** (K_i = 159 nM) and **8b** (K_i = 400 nM). The bicyclic σ ligands showed a selective growth inhibition of the small cell lung cancer cell line A-427 with the benzyl ethers **11** and the benzylidene derivatives **17** being the most potent compounds. **11a** has a cytotoxic potency (IC_{50} = 0.92 μM), which exceeds the activity of cisplatin and interacts considerably with both σ_1 and σ_2 receptors.

Introduction

When the σ receptor was described first more than 30 years ago, it was assumed to be an opioid receptor subtype.[1] However, today it is accepted to be a distinct pharmacological entity, with the class of σ receptors being subdivided into σ_1 and σ_2 receptors.[2]

The σ_1 receptor has been cloned.[3] The amino acid sequence of the receptor shows no similarity to any other known mammalian protein but shows about 30% homology to the yeast enzyme sterol Δ^8/Δ^7-isomerase. The cloned receptor is postulated to possess two transmembrane domains with the amino and carboxy termini on the intracellular side of the membrane.[4] Presently, neurosteroids such as progesterone are discussed to be the endogenous ligands for σ_1 receptors. Cloning of the σ_2 receptor has not yet been reported.

Although the biochemical role as well as the mechanism of signal transduction of σ receptors are not completely understood, so far they have been implicated in a multitude of biological and pathophysiological processes such as psychosis,[5] depression,[6] and uncontrolled cell proliferation.[7] Therefore, high affinity σ receptor ligands could be developed as atypical antipsychotics, antidepressants, and antitumor agents.

The ethylenediamine substructure substituted with different residues at the nitrogen atoms represents a crucial pharmacophoric element of several σ_1 receptor ligands. Incorporation of the ethylenediamine substructure into a piperazine ring leads to very potent σ_1 receptor ligands, e.g., **1** (K_i = 0.47 nM)[8] and **2** (K_i = 12 nM).[9] We recently reported on the synthesis and σ receptor affinity of a series of 6,8-diazabicyclo[3.2.2]nonane derivatives **3** and **4** (Figure 1).[10−12] In these bridged piperazine derivatives, the conformational flexibility of the ethylenediamine substructure is considerably reduced. This class of bridged piperazines also contains potent σ_1 receptor ligands like **3a** (K_i = 6.5 nM)[10] and **4a** (K_i = 53 nM).[11] However, when the N-6 benzyl group was replaced by an allyl group, which represents a smaller π-system, a dramatic loss of σ_1 receptor affinity was observed (e.g., **3b**: K_i = 2240 nM,[12] **4b**: K_i = 1600 nM[11]). This result was surprising, because in the benzomorphan compound class, the *N*-allyl (SKF-10,047) and *N*-dimethylally substituents (pentazocine) lead to high affinity σ_1 ligands. Moreover, replacement of the N-6-benzyl group of **4a** by a benzoyl moiety led to almost complete loss of σ_1 and σ_2 receptor affinity.[12] These results suggest a basic N-atom in position 6 and a second aromatic moiety are important for high σ_1 receptor affinity.

The question arose whether N-6 is the only position for the second aromatic residue or whether it could be attached to other positions of the bicyclic framework. This work is devoted to find the optimal position for the second aromatic residue with

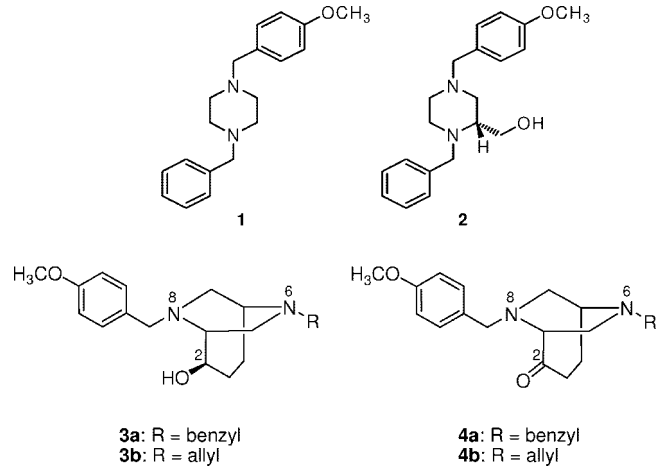


Figure 1. Lead structures: monocyclic (**1**, **2**) and bridged (**3**, **4**) piperazine derivatives with high σ receptor affinity.

* To whom correspondence should be addressed. Phone: +49-251-8333311. Fax: +49-251-8332144. E-mail: wuensch@uni-muenster.de.

[†] Institut für Pharmazeutische and Medizinische Chemie der Westfälischen Wilhelms-Universität Münster.

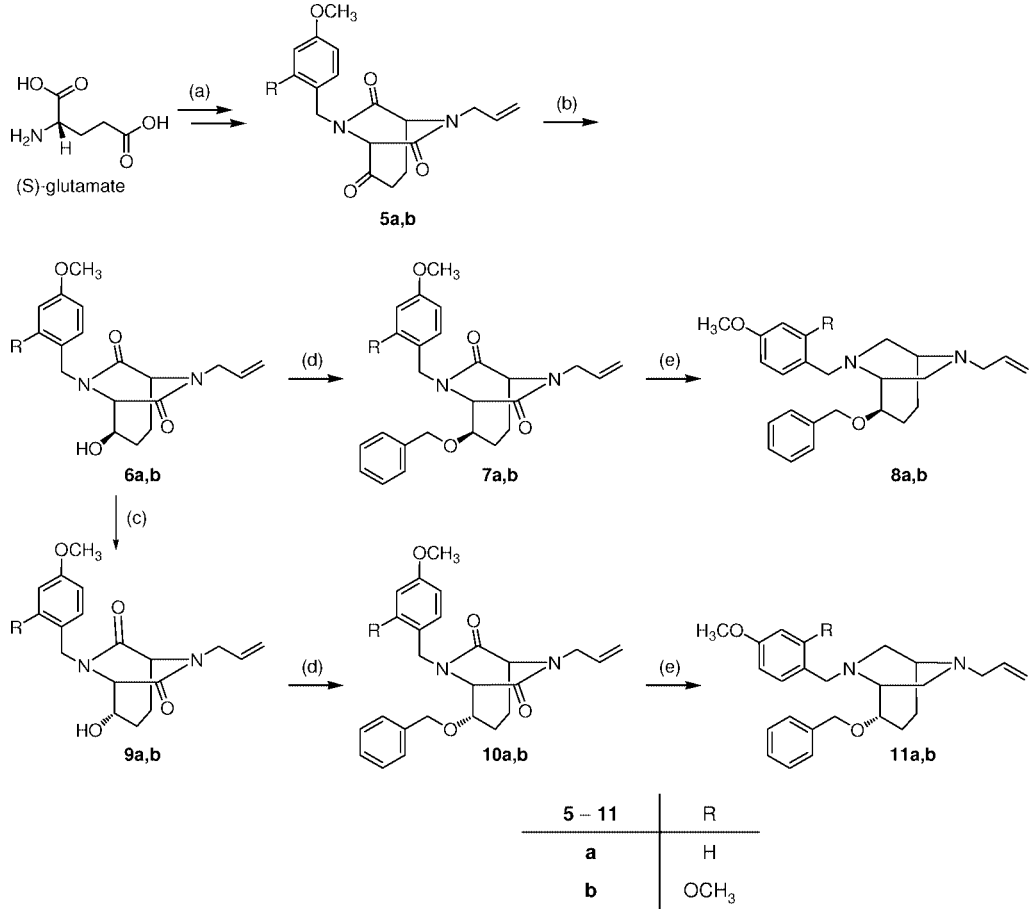
[‡] Organisch-chemisches Institut der Westfälischen Wilhelms-Universität Münster.

[§] Institut für Pharmazie der Ernst-Moritz-Arndt-Universität Greifswald.

[‖] X-ray structure analysis.

10.1021/jm801522j CCC: $40.75
Published on Web 02/25/2009

Scheme 1[a]



[a] (a) Ref 13. (b) $LiBH_4$, THF, −78 °C, 3 h, 92% (**5a**), 92% (**5b**).[11] (c) (1) DIAD, PPh_3, *p*-nitrobenzoic acid, THF, rt, 6 h; (2) MeOH, K_2CO_3, rt, 16 h, 78% (**6a**), 72% (**6b**).[11] (d) NaH, benzyl bromide, THF, rt, 16 h, 94% (**7a**), 84% (**7b**), 86% (**10a**), 82% (**10b**). (e) $LiAlH_4$, THF, reflux, 16 h, 60% (**8a**), 49% (**8b**), 58% (**11a**), 52% (**11b**).

respect to σ_1 receptor affinity. For this purpose, the relatively small allyl moiety with only minor contribution to σ_1 receptor binding was retained in position 6.

In addition to the σ_1 receptor affinity, we have shown that bicyclic compounds of type **3** and **4** are able to inhibit the growth of human tumor cell lines. In particular, the methyl ether of **3a** inhibited selectively the growth of the small cell lung cancer cell line A-427 with an IC_{50} value of 1.23 μM. This cytotoxicity was correlated with the σ_1 affinity (K_i = 26 nM) and stereochemistry of the ligands.[10] Therefore the influence of the aryl ring position and the stereochemistry of the ligands on the tumor cell growth inhibition are of particular interest.

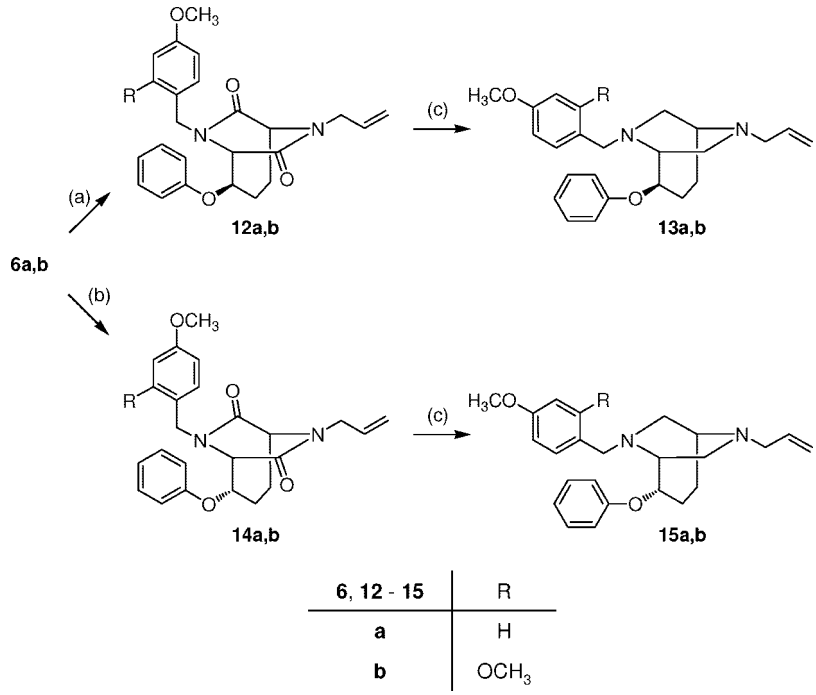
In this paper, we report on the synthesis, σ receptor affinity, and growth inhibition of human tumor cell lines of a series of 6,8-diazabicyclo[3.2.2]nonane derivatives bearing a second aromatic moiety in the propano bridge. The aromatic residue has been attached to different positions of the propano bridge, resulting in various orientations in relation to the first aromatic residue. Moreover the stereochemistry and the conformational flexibility of the attached phenyl moiety have been carefully considered.

Chemistry. The chiral-pool synthesis of the central bicyclic building block **5** starting with the proteinogenic amino acid (*S*)-glutamate has recently been described by us.[13] The enantiomerically pure bicyclic ketones **5a** and **5b** were diastereoselectively reduced with $LiBH_4$ in THF at −78 °C to give the (2*R*)-configured alcohols **6a** and **6b**.[12] To obtain the (2*S*)-configured alcohols **9a** and **9b**, the (2*R*)-configured alcohols **6a** and **6b** were inverted by a Mitsunobu reaction with DIAD, PPh_3, and *p*-nitrobenzoic acid, followed by methanolytic cleavage of the resulting *p*-nitrobenzoates.[12] The diastereomeric alcohols **6a**,**b** and **9a**,**b** were transformed into the benzyl ethers **7a**,**b** and **10a**,**b** with benzyl bromide after deprotonation with NaH. Treatment of **7a**,**b** and **10a**,**b** with $LiAlH_4$ afforded the basic piperazine derivatives **8a**,**b** and **11a**,**b** (Scheme 1).

The phenyl ethers **13** and **15** were synthesized in order to reduce the distance between the propano bridge and the aromatic moiety. For the 4-methoxybenzyl as well as for the 2,4-dimethoxybenzyl derivatives, both possible diastereomeric phenyl ethers were obtained from the (2*R*)-configured alcohols **6a** and **6b**. An Ullmann reaction of **6a**,**b** with iodobenzene and CuI[14] yielded the (2*R*)-configured ethers **12a**,**b** and a Mitsunobu reaction[15] with phenol, DIAD, and PPh_3 led to the (2*S*)-configured phenyl ethers **14a**,**b** under inversion of configuration in position 2. Reduction of the ethers **12a**,**b** and **14a**,**b** with $LiAlH_4$ provided the bridged piperazines **13a**,**b** and **15a**,**b** (Scheme 2).

To reduce the conformational flexibility of the second aromatic moiety, the position of the aromatic moiety should be fixed relative to the bicyclic system. The benzylidene derivatives **17a**,**b** were envisaged for this purpose. A Wittig reaction[16] of **5a**,**b** with benzyltriphenylphosphonium bromide and potassium *tert*-butoxide gave the benzylidene derivatives **16a**,**b**. The (*Z*)-configuration of the double bond of **16a**,**b** was determined by NOE experiments. Irradiation at 6.4 ppm (PhC<u>H</u>= of **16a**) led to an increased signal at 2.3 ppm (3-CH_2). In the case of the

Scheme 2[a]



[a] (a) CuI, 1,10-phenanthroline, Cs_2CO_3, iodobenzene, 110 °C, 36 h, 48% (**12a**), 47% (**12b**); (b) phenol, DIAD, PPh_3, toluene, reflux, 4 h, 31% (**14a**), 35% (**14b**); (c) $LiAlH_4$, THF, reflux, 16 h, 24% (**13a**), 20% (**13b**), 27% (**15a**), 44% (**15b**).

4-methoxybenzyl substituent at N-8, only the (*Z*)-configured benzylidene derivative **16a** was obtained. However, when the 2,4-dimethoxybenzyl residue was attached to N-8, the Wittig reaction led to both diastereomers (*Z*)-**16b** and (E)-**16b** in the ratio of 2:1. The (*Z*)-configured benzylidene derivatives (*Z*)-**16a** and (*Z*)-**16b** were subsequently reduced with $LiAlH_4$ to yield the basic piperazine derivatives **17a**,**b**.

A Fischer indole synthesis[17] of **5a**,**b** with phenylhydrazine and *p*-methoxyphenylhydrazine gave the indole annulated bicyclic compounds **18a**,**b** and **19a**,**b**, respectively. The quinoline annulated compounds **22a**,**b** were obtained by a Friedländer quinoline synthesis[18] of the ketones **5a**,**b** with *o*-aminoacetophenone. Reduction of **18a**,**b**, **19a**,**b**, and **22a**,**b** with $LiAlH_4$ yielded the indole and quinoline annulated bridged piperazine derivatives **20a**,**b**, **21a**,**b**, and **23a**,**b**, respectively (Scheme 3).

Figure 2 shows the X-ray crystal structure analysis of the enantiomer of **20a** (ent-**20a**), which was prepared analogously starting with (*R*)-glutamate. Recrystallization of ent-**20a** from diisopropyl ether gave crystals which were suitable for X-ray crystal structure analysis. Solution of the structure revealed the twist-boat conformation of the piperazine ring and the endo orientation of the *p*-methoxybenzyl and allyl substituents (equatorial position relative to the piperazine ring plane). It is well-known that the structure of a compound in the solid state (X-ray crystal structure) is not necessarily identical with the bioactive conformation. However, the structure in the solid state represents a stable conformation. In case of ent-**20a**, the X-ray crystal structure reveals an endo orientation of both N-substituents, which indicates this structure as a possible bioactive conformation. We assume that this is one of the most preferred conformations of all bicyclic compounds, in particular concerning the orientation of the residues at the N-atoms.

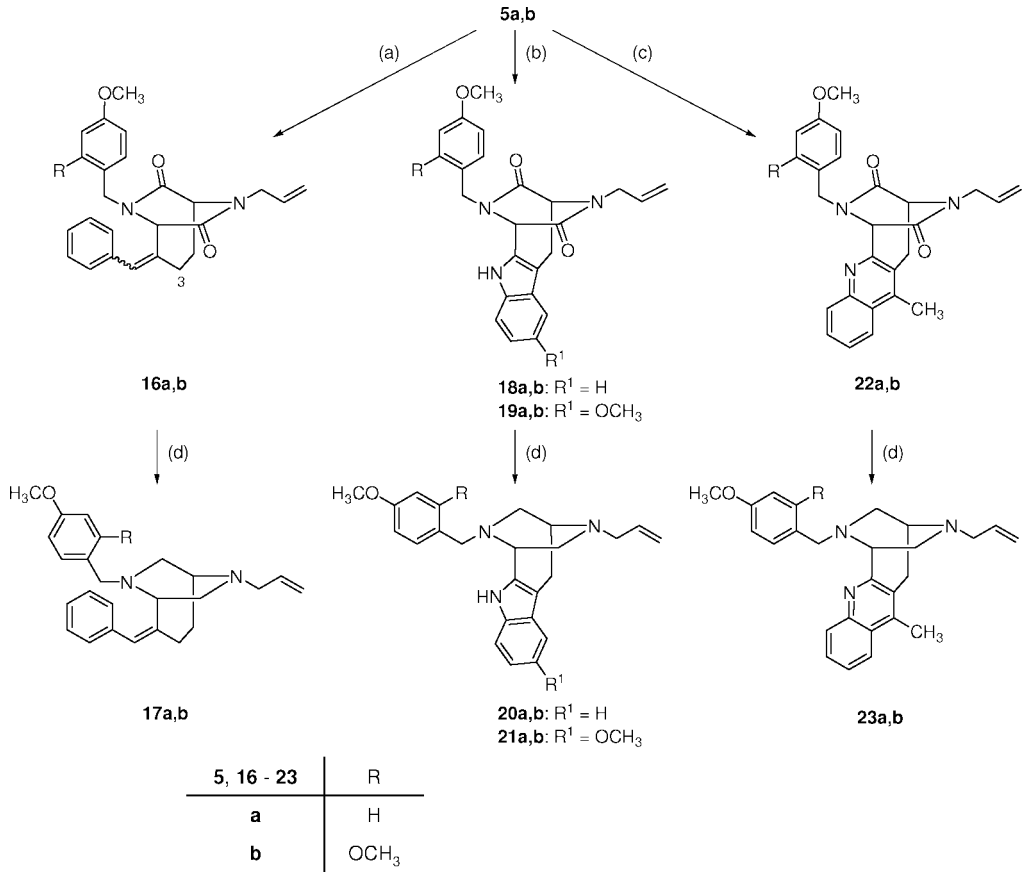
The reaction of the bicyclic ketone **5a** with 1-bromo-2-(2-bromoethyl)benzene[19] and 1 equiv of *n*-butyllithium (Parham reaction)[20] led to the spirocyclic system **24**, which was also reduced with $LiAlH_4$ to give **25**. As reported for the $LiBH_4$ reduction[11] and the Grignard reaction[13] of the bicyclic ketone **5a**, the nucleophilic attack during the Parham reaction occurred stereoselectively from the *Si* face of the ketone, giving the spirocyclic compound **24** with (2*S*)-configuration of the spiro-C-atom (Scheme 4).

To introduce substituents in position 4 of the bicyclic system, the basic piperazine derivatives **26** and **27** were used because the corresponding bislactams did not lead to the α,β-unsaturated ketones. The synthesis of **26** and **27** has recently been described by us.[11] The N-6 benzyl derivative **26** was transformed into the α,β-unsaturated ketone **28** with benzeneselenic anhydride.[21] 1,4-Addition of Ph_2CuLi in THF at −50 °C[22] afforded the diastereomeric 4-phenyl substituted bicyclic compounds **29**. The (4*R*)- and (4*S*)-configured compounds **29a** and **29b** were separated by flash column chromatography. The configuration at position 4 was established by NOE experiments. In case of the (4*R*)-configured compound **29a** (phenyl up), irradiation at 3.1 ppm (9-H) led to an increase of the signal at 3.3 ppm (4-H). The 4-substituted N-6 propyl derivative **31** was synthesized in the same manner starting from **27**. However, in the case of the propyl derivative **31**, only the (4*R*)-configured diastereomer was isolated (Scheme 5).

The enantiomers of the 6,8-diazabicyclo[3.2.2]nonane derivatives **8a**,**b**, **11a**,**b**, **13a**,**b**, **15a**,**b**, **17a**,**b**, **20a**,**b**, **21a**,**b**, **23a**,**b**, **25**, **29a**,**b**, and **31** were prepared in the same manner starting with (*R*)-glutamate. Therefore, all possible stereoisomers of the described compounds were available for pharmacological evaluation. The purity of all tested compounds was determined by RP-HPLC and found to be >95%.

Pharmacological Evaluation. Receptor Binding Studies. The σ receptor affinities of the synthesized compounds were determined in competition experiments with radioligands. Homogenates of guinea pig brains were used as receptor material in the σ_1 assay and the σ_1 selective ligand [^{3}H]-(+)-pentazocine was employed as a radioligand. The nonspecific binding was determined in the presence of a large excess of nontritiated (+)-pentazocine. In the σ_2 assay, homogenates of rat liver served as source for σ_2 receptors. The nonselective radioligand [^{3}H]-

Scheme 3[a]



[a] (a) Benzyltriphenylphosphonium bromide, KO*t*Bu, THF, rt, 16 h, 89% ((*Z*)-**16a**), 17% ((*Z*)-**16b**), 9% ((*E*)-**16b**); (b) phenylhydrazine or *p*-methoxyphenylhydrazine, HCl(g)-sat. EtOH, reflux, 16 h, 42% (**18a**), 59% (**18b**), 49% (**19a**), 61% (**19b**); (c) *o*-aminoacetophenone, AcOH, reflux, 16 h, 65% (**22a**), 63% (**22b**); (d) $LiAlH_4$, THF, reflux, 16 h, 46% (**17a**), 41% (**17b**), 41% (**20a**), 61% (**20b**), 45% (**21a**), 57% (**21b**), 25% (**23a**), 39% (**23b**).

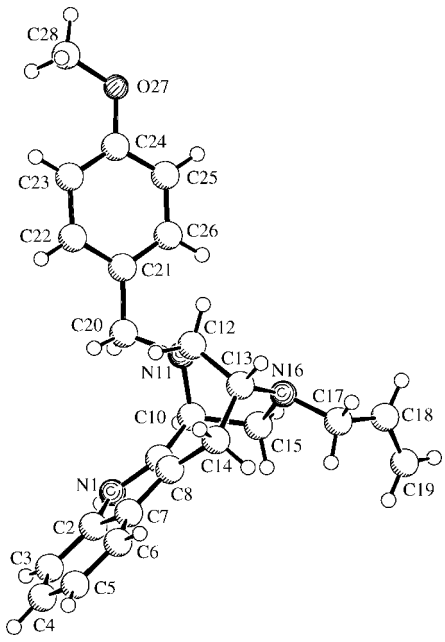
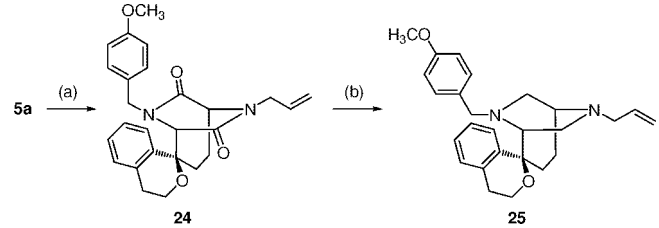


Figure 2. X-ray structure analysis of ent-**20a**.

1,3-di(*o*-tolyl)guanidine was employed in the presence of an excess of nontritiated (+)-pentazocine for selective occupation of σ_1 receptors. The nonspecific binding of the radioligand was determined by performing the σ_2 assay in the presence of an excess of nontritiated 1,3-di(*o*-tolyl)guanidine.[23,26]

Scheme 4[a]

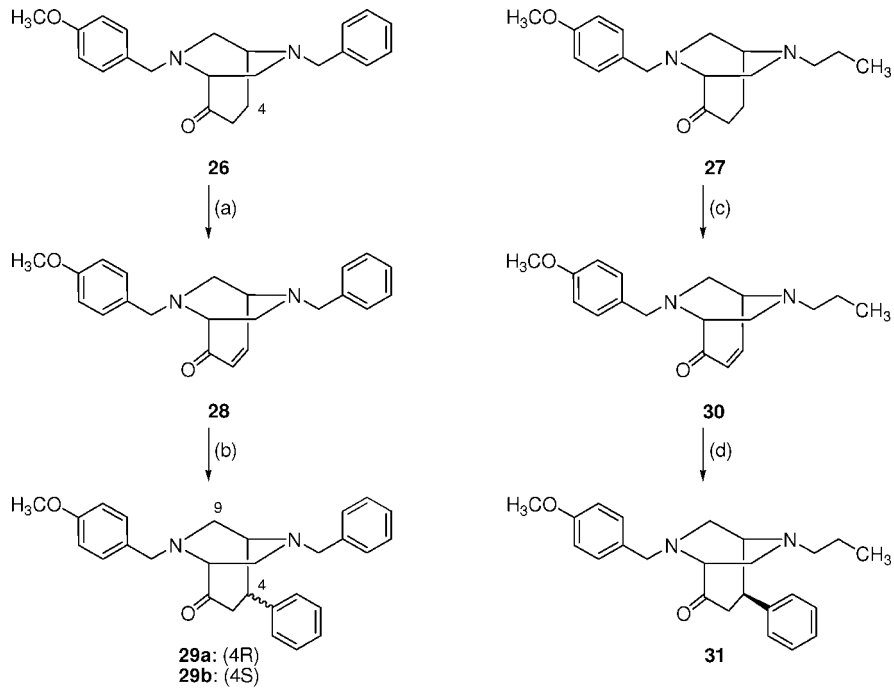


[a] (a) (1) 1-Bromo-2-(2-bromoethyl)benzene, *n*-BuLi, THF, −90 °C, 10 min; (2) **5a**, −90 °C, 30 min, then rt, 2 h, 12%. (b) $LiAlH_4$, THF, reflux, 16 h, 27%.

Inhibition of Cell Growth of Human Tumor Cell Lines. In literature, the overexpression of σ_1 and σ_2 receptors in human tumor cell lines has been reported.[7] Furthermore, some σ_2 agonists and σ_1 antagonists (e.g., haloperidol) have shown antiproliferative and cytotoxic effects in some tumor cell lines.[24] Therefore, the antiproliferative effects of the synthesized compounds were investigated in a panel of six human tumor cell lines, including the cell lines 5637 (bladder cancer), RT-4 (bladder cancer), A-427 (small cell lung cancer), LCLC-103H (large cell lung cancer), DAN-G (pancreas cancer), and MCF-7 (breast cancer).

In the primary screening, the tumor cells were incubated with a 20 μM solution of the test compound at 37 °C for 96 h. Compounds that reduced cell growth by greater than 50% compared to untreated controls, as measured by staining cells with crystal violet,[25] were considered active. The IC_{50} values of all active compounds were determined by subjecting the cells

Scheme 5[a]



[a] (a) Benzeneselenic anhydride, chlorobenzene, 95 °C, 16 h, 20%. (b) (1) $CuBr \cdot S(CH_3)_2$, PhLi, THF, −50 °C, 1 h; (2) **28**, −50 °C, 15 min, then rt 16 h, 14% (**29a**), 29% (**29b**). (c) Benzeneselenic anhydride, chlorobenzene, 95 °C, 16 h. (d) Ph_2CuLi, THF, −50 °C, 15 min, then rt 16 h, 4%.

to 5 serial dilutions of test compounds for 96 h and measuring the remaining cell density by crystal violet staining followed by comparison with untreated controls.

Biological Results and Discussion

Table 1 shows the σ_1 and σ_2 receptor affinities of the synthesized compounds. The benzyl ethers **11a** and **11b** with (2*S*)-configuration as well as their enantiomers ent-**11a** and ent-**11b** display greater σ_1 receptor affinity than their diastereomers **8a**,**b** and ent-**8a**,**b**. ent-**11a**, the compound with the highest σ_1 receptor affinity of this series of compounds ($K_i = 91$ nM), has a 70-fold selectivity against the σ_2 receptor. However, especially the benzyl ethers **8a**,**b** and **11a**,**b** derived from (*S*)-glutamate show considerable affinity toward the σ_2 receptor. The most potent σ_2 ligand with a 2-benzyloxy moiety is **8b** ($K_i = 400$ nM), showing at least a 5-fold selectivity over the σ_1 receptor.

In the series of phenyl ethers (**13**, **15**), the σ_1 receptor affinity is rather low, all K_i values are greater than 1000 nM. This might be due to the higher electron density of the phenyl moiety or the reduced distance between the propano bridge and the aromatic moiety. As described for the benzyl ethers **8a**,**b** and **11a**,**b**, the (*S*)-glutamate derived phenyl ethers **13a** ($K_i = 479$ nM) and **13b** ($K_i = 469$ nM) display moderate σ_2 affinity and considerable selectivity against the σ_1 receptor. The diastereomers **15a** ($K_i = 8390$ nM) and **15b** ($K_i = 2090$ nM) show a much lower affinity toward the σ_2 receptor. These results demonstrate that the stereochemistry, in particular at position 2, is crucial for the interaction with σ_2 receptors.

Compared to the benzyl and phenyl ethers, the conformational flexibility of the second aromatic moiety is considerably reduced in case of the benzylidene derivatives **17**, indoles **20** and **21**, quinolines **23**, and spirocyclic compounds **25**.

For the σ_1 and σ_2 affinities of the benzylidene derivatives, the stereochemistry plays a crucial role. Whereas ent-**17a** and ent-**17b**, which were derived from (*R*)-glutamate, are preferably bound at σ_1 receptors, their enantiomers **17a** and **17b** show a higher affinity toward the σ_2 receptor. ent-**17a** ($K_i = 104$ nM) reveals a σ_1 affinity comparable to that of the benzyl ether ent-**11a**, indicating a similar orientation of the aromatic moieties of these two compounds.

Neither the indole (**20**, **21**) nor the quinoline (**23**) annulated bicyclic compounds show σ_1 or σ_2 receptor affinity in the submicromolar range. Also the spirocyclic compounds **25** and ent-**25** do not interact significantly with σ_1 or σ_2 receptors.

Introduction of a phenyl substituent in position 4 (**29**, **31**) does not lead to high σ_1 or σ_2 affinity. In fact, a phenyl moiety at position 4 of the propano bridge rather decreases the σ_1 receptor affinity compared to the 4-unsubstituted analogue **4b** (σ_1: $K_i = 1600$ nM).

Taken together, these findings indicate that the second aromatic moiety is best located within the residue at the N-atom in position 6[12] with respect to high σ_1 receptor affinity. Nevertheless, the benzyl ethers **11a** (σ_1: $K_i = 154$ nM) and ent-**11a** (σ_1: $K_i = 91$ nM) as well as the benzylidene derivatives ent-**17a** (σ_1: $K_i = 104$ nM) and ent-**17b** (σ_1: $K_i = 316$ nM) show promising σ_1 receptor affinity. We assume that the N-6-benzyl derivatives, the benzyl ethers, and the benzylidene compounds are able to present the aromatic moieties in a similar manner to the σ_1 receptor protein although these groups are attached to different positions of the bicyclic scaffold.

Comparing the K_i values of the enantiomers of the most potent σ_1 ligands (**11a**/ent-**11a**, **17a**/ent-**17a**) clearly indicates that the enantiomers derived from (*R*)-glutamate are more potent than the corresponding enantiomers synthesized from (*S*)-glutamate. The eudismic ratios are 1.5 and 12, respectively.

The affinity of the bridged piperazines toward the phencyclidine binding site of the NMDA receptor (pig brain cortex membrane preparations, [^{3}H]-MK801[26]) was also determined because it is known that some σ receptor ligands show considerable affinity toward the NMDA receptor.[1] However, the synthesized 6,8-diazabicyclo[3.2.2]nonanes showed only

Table 1. σ_1 and σ_2 Receptor Affinity of the 6,8-Diazabicyclo[3.2.2]nonane Derivatives and Some Reference Compounds

			$K_i \pm$ SEM [nM]	
compound	configuration	structure	σ_1 affinity	σ_2 affinity
8a	(*R*,*R*,*S*)	benzyl ether	366 ± 23	802
ent-**8a**	(*S*,*S*,*R*)	benzyl ether	1040 ± 200	4750
8b	(*R*,*R*,*S*)	benzyl ether	20%[a]	400 ± 121
ent-**8b**	(*S*,*S*,*R*)	benzyl ether	13%[a]	2810
11a	(*R*,*S*,*S*)	benzyl ether	154 ± 33	578
ent-**11a**	(*S*,*R*,*R*)	benzyl ether	91 ± 16	6460
11b	(*R*,*S*,*S*)	benzyl ether	385 ± 61	588
ent-**11b**	(*S*,*R*,*R*)	benzyl ether	1270	22700
13a	(*R*,*R*,*S*)	phenyl ether	2120	479 ± 102
ent-**13a**	(*S*,*S*,*R*)	phenyl ether	29%[a]	862
13b	(*R*,*R*,*S*)	phenyl ether	4050	469 ± 49
ent-**13b**	(*S*,*S*,*R*)	phenyl ether	0%[a]	5880
15a	(*R*,*S*,*S*)	phenyl ether	5460	8390
ent-**15a**	(*S*,*R*,*R*)	phenyl ether	1620	688
15b	(*R*,*S*,*S*)	phenyl ether	1580	2090
ent-**15b**	(*S*,*R*,*R*)	phenyl ether	2840	907
17a	(*S*,*S*,*Z*)	benzylidene	1200	576 ± 85
ent-**17a**	(*R*,*R*,*Z*)	benzylidene	104 ± 23	566
17b	(*S*,*S*,*Z*)	benzylidene	465 ± 109	159 ± 24
ent-**17b**	(*R*,*R*,*Z*)	benzylidene	316 ± 57	679 ± 80
20a	(*R*,*S*)	indole	44%[a]	23600
ent-**20a**	(*S*,*R*)	indole	7%[a]	0%[a]
20b	(*R*,*S*)	indole	13%[a]	3350
ent-**20b**	(*S*,*R*)	indole	11%[a]	3210
21a	(*R*,*S*)	indole	10500	0%[a]
ent-**21a**	(*S*,*R*)	indole	37%[a]	44%[a]
21b	(*R*,*S*)	indole	6350	9330
ent-**21b**	(*S*,*R*)	indole	0%[a]	10200
23a	(*R*,*S*)	quinoline	1630	5610
ent-**23a**	(*S*,*R*)	quinoline	18%[a]	57200
23b	(*R*,*S*)	quinoline	5460	8390
ent-**23b**	(*S*,*R*)	quinoline	1630	5610
25	(*R*,*S*,*S*)	spirocycle	36%[a]	58%[a]
ent-**25**	(*S*,*R*,*R*)	spirocycle	24%[a]	0%[a]
29a	(*R*,*R*,*S*)	4-phenyl	8%[a]	0%[a]
ent-**29a**	(*S*,*S*,*R*)	4-phenyl	25%[a]	0%[a]
29b	(*R*,*S*,*S*)	4-phenyl	17%[a]	0%[a]
ent-**29b**	(*S*,*R*,*R*)	4-phenyl	14%[a]	0%[a]
31	(*R*,*R*,*S*)	4-phenyl	3%[a]	10%[a]
ent-**31**	(*S*,*S*,*R*)	4-phenyl	40%[a]	11%[a]
(+)-pentazocine	(*S*,*S*,*S*)		4.2 ± 1.1	
ditolylguanidine			61 ± 18	42 ± 17
haloperidol			3.9 ± 1.5	78 ± 2.3

[a] Inhibition of radioligand binding at a concentration of the test compound of 1 μM.

negligible affinity toward the phencyclidine binding site of the NMDA receptor.

Table 2 reports the IC_{50} values for cell growth inhibiting properties of the synthesized compounds. Active compounds were those that showed IC_{50} values below 20 μM. In general, the A-427 cell line was the most sensitive to this series of compounds; only in the cases of the indoles **20a** and **21a** other cell lines were more sensitive than the A-427 cell line. The next most sensitive cell lines were the MCF-7 and DAN-G cell lines. Interestingly, another lung cancer cell line, LCLC-103H, appeared to be the least sensitive to the antiproliferative effects of these compounds. The selectivity of this new series of compounds toward the A-427 line was not as great as with the series of 6,8-diazabicyclo[3.2.2]nonanes we reported earlier,[10] indicating that the new compounds have a broader spectrum of activity.

The benzyl ethers **8** and **11** as well as the benzylidene derivatives **17** showed the strongest growth inhibition in the A-427 cell line. The most active compounds, **11a** and **11b**, had activities comparable to cisplatin (IC_{50} = 1.96 μM) in that cell line. Most of the compounds were also more active than haloperidol in the A-427 cell line, a known σ_1 antagonist with cell growth inhibitory activity in some cancer cell lines (Table 2). Compounds **11a** and **11b** appeared to be the most selective compounds of this series for the A-427 cell line. The most active compound in this work, **11a** (IC_{50} = 0.92 μM), which is also among the most potent σ_1 ligands of this series, is almost as active as the most potent compound in our previous publication,[10] which gave an IC_{50} value of 0.51 ± 0.21 μM in the A-427 cell line.

With exception of the most potent compounds **11a** and **11b**, the stereochemistry does not appear to affect the activity of the ligands. However, for **11a** and **11b**, 5- and 3-fold lower IC_{50} values were recorded in the A-427 cell line compared to their enantiomers ent-**11a** and ent-**11b**, respectively. Such differences in potency were not as apparent as for these two pairs of enantiomers in the other five cell lines. The reduced stereoselectivity in the cytotoxicity assay is due to the reduced activity in these cell lines. In the first series of 6,8-diazabicyclo-[3.2.2]nonanes with a hydroxy or methoxy group in position 2, a more pronounced difference in cytotoxicity between enantiomers was observed for the most potent σ ligands.[10]

The selective growth inhibition of the A-427 cell line suggests a specific mechanism of action. Moreover, the benzyl ethers **11** and the benzylidene derivatives **17**, which display high σ_1 and σ_2 receptor affinities, show relatively low IC_{50} values, indicating high cytotoxicity. In the case of these compounds, the combination of σ_1 and σ_2 receptor affinity may be responsible for the overall cytotoxic activity.

Conclusion

A comparison of the σ receptor affinities of the synthesized 6,8-diazabicyclo[3.2.2]nonanes bearing a second aromatic moiety in different positions of the propano bridge shows that the 4-phenyl substituted compounds **29** and **31**, the spirocyclic compounds **25**, as well as the indole and quinoline annulated bicyclic compounds **20**, **21**, and **23** do not exhibit considerable σ_1 or σ_2 receptor affinity.

High σ_1 receptor affinity was observed for the benzyl ethers **11** and the benzylidene derivatives **17**, with **11a** (K_i = 154 nM), ent-**11a** (K_i = 91 nM), and ent-**17a** (K_i = 104 nM) showing the highest σ_1 affinity in this series of compounds. While ent-**11a** revealed a 70-fold selectivity against the σ_2 receptor, in the case of ent-**17a**, only a 5-fold selectivity was observed. The slightly increased cytotoxicity of ent-**17a** (IC_{50} = 3.8 μM) compared with ent-**11a** (IC_{50} = 4.6 μM) may be caused by a σ_2 contribution.

For the benzyl ether **8b** (K_i = 400 nM) and the phenyl ethers **13a** (K_i = 479 nM) and **13b** (K_i = 469 nM), a relatively high σ_2 receptor affinity was observed, showing at least a 5-fold selectivity over the σ_1 receptor. Furthermore, both (*S*)-glutamate derived benzylidene derivatives **17a** and **17b** displayed a preference for the σ_2 receptor, with **17a** having the highest σ_2 receptor affinity (K_i = 159 nM) in this series of compounds.

In the series of benzylidene derivatives **17**, all compounds show considerable cell growth inhibiting properties against the cell line A-427, with IC_{50} values comparable to that of the cytostatic drug cisplatin. Because the benzylidene derivatives **17** also show considerable affinity toward σ_1 and σ_2 receptors, there might be a correlation between these properties and their cell growth inhibiting activity. However, further investigations are required to find the definite mechanism of antiproliferative activity of these interesting compounds.

All these findings indicate that the stereochemistry of the bicyclic scaffold (e.g., **17a**: σ_1, K_i = 1200 nM and ent-**17a**: σ_1,

Table 2. Cell Growth Inhibitory Activity (IC_{50} Values)[a] of the 6,8-Diazabicyclo[3.2.2]nonane Derivatives in Six Human Cancer Cell Lines Following a Continuous 96 h Exposure to the Test Compounds

compd	5637[b]	RT-4[c]	A-427[d]	LCLC-103H[e]	DAN-G[f]	MCF-7[g]
8a	6.53	11.1	4.71	21.8	7.34	8.66
ent-**8a**	12.7 ± 1.74	13.3 ± 3.40	4.45 ± 0.68	16.7 ± 6.25	8.97 ± 1.28	6.51 ± 0.66
8b	4.73	8.57	3.55	11.1	4.58	5.81
ent-**8b**	7.66 ± 1.49	5.87 ± 2.68	3.53 ± 0.75	8.67 ± 4.44	5.89 ± 1.77	5.85 ± 1.38
11a	6.22 ± 4.98	10.6 ± 0.21	0.92 ± 1.43	20.3 ± 8.78	7.45 ± 1.81	10.1 ± 2.01
ent-**11a**	11.2 ± 2.30	14.3 ± 3.58	4.61 ± 1.11	19.9 ± 3.77	12.7 ± 1.01	9.13 ± 1.53
11b	5.25 ± 3.32	4.26 ± 0.94.	1.25 ± 0.92	8.30 ± 3.81	6.36 ± 3.22	5.22 ± 2.84
ent-**11b**	5.12 ± 1.18	6.28 ± 3.17	3.67 ± 0.99	8.04 ± 3.14	7.16 ± 2.86	5.41 ± 1.30
13a	14.0 ± 7.14	19.1 ± 7.52	5.95 ± 0.39	>20	15.8 ± 6.46	12.7 ± 3.87
ent-**13a**	9.79 ± 1.35	17.9 ± 6.50	6.29 ± 0.24	>20	10.6 ± 3.60	9.23 ± 0.53
13b	11.7 ± 0.65	15.3 ± 4.39	7.09 ± 1.29	>20	12.5 ± 3.82	9.11 ± 0.44
ent-**13b**	15.2 ± 3.48	16.5 ± 2.27	6.28 ± 1.02	>20	10.9 ± 3.57	9.09 ± 1.26
15a	15.7 ± 6.34	20.1 ± 11.1	5.62 ± 1.71	>20	11.6 ± 4.25	11.9 ± 1.80
ent-**15a**	14.1	>20	6.44	>20	10.2	9.40
15b	14.0 ± 5.14	>20	9.01 ± 0.50	>20	13.0 ± 6.31	11.4 ± 1.68
ent-**15b**	16.3 ± 4.26	23.7 ± 9.41	7.00 ± 1.24	>20	15.0 ± 3.06	10.4 ± 4.18
17a	9.92 ± 1.00	10.3 ± 3.83	3.53 ± 0.77	16.8 ± 6.92	7.80 ± 2.49	8.75 ± 1.01
ent-**17a**	10.9 ± 4.38	14.1 ± 2.39	3.93 ± 1.37	17.8 ± 7.17	9.82 ± 2.32	7.25 ± 0.82
17b	5.13 ± 1.09	8.90 ± 3.44	4.28 ± 0.85	10.9 ± 4.00	7.20 ± 1.56	6.15 ± 0.92
ent-**17b**	6.22 ± 3.88	8.05 ± 2.44	3.07 ± 0.37	12.6 ± 3.24	8.90 ± 3.94	7.20 ± 0.46
20a	11.2	11.2	12.3	12.9	7.70	22.5
ent-**20a**	nd	>20	nd	>20	nd	nd
20b	nd	>20	nd	>20	nd	nd
ent-**20b**	12.5	7.97	6.41	8.62	5.99	5.04
21a	10.8 ± 4.92	>20	13.2 ± 6.03	>20	>20	25.5 ± 5.99
ent-**21a**	16.1 ± 2.25	>20	15.4 ± 5.05	>20	>20	>20
21b	11.7 ± 3.44	19.0 ± 6.18	8.29 ± 1.49	15.3 ± 2.49	9.21 ± 2.07	13.8 ± 2.44
ent-**21b**	16.6 ± 5.61	11.1 ± 3.00	6.28 ± 2.60	8.57 ± 2.49	7.12 ± 2.50	11.4 ± 3.98
23a	13.8	>20	15.7	>20	21.5	18.5
ent-**23b**	14.0	10.6	9.30	13.2	6.62	14.7
29a	nd	nd	nd	>20	>20	nd
ent-**29a**	nd	nd	nd	>20	>20	nd
29b	18.3	20.5	10.3	>20	16.1	19.4
ent-**29b**	10.4	15.7	7.88	19.8	10.1	19.0
31	nd	>20	nd	>20	>20	nd
ent-**31**	>20	>20	>20	>20	>20	>20
haloperidol	>20	>20	10.0 ± 1.71	>20	nd	>20
cisplatin[a]	0.35 ± 0.10	1.61 ± 0.16	1.96 ± 0.54	0.90 ± 0.19	0.73 ± 0.34	1.38 ± 0.29
methotrexate[a]	0.016 ± 0.009	0.04 ± 0.02	5.52 ± 3.55	0.025 ± 0.012	0.077 ± 0.005	0.05 ± 0.02

[a] IC_{50} values (μM): values with standard deviations (SD) are averages of 3 or more independent determinations. Values without SD are from 1 to 2 determinations; nd: not determined. [b] Bladder cancer. [c] Bladder cancer. [d] Small cell lung cancer. [e] Large cell lung cancer. [f] Pancreas cancer. [g] Breast cancer.

K_i = 104 nM, or **17b**: σ_2, K_i = 159 nM, and ent-**17b**: σ_2, K_i = 679 nM) as well as the configuration in position 2 (e.g., ent-**8a**: σ_1, K_i = 1040 nM and ent-**11a**: σ_1, K_i = 91 nM) strongly influence σ receptor affinity and subtype selectivity of the 6,8-diazabicyclo[3.2.2]nonane derivatives.

Finally, it should be noted that the N-8-dimethoxybenzyl substituted derivatives ent-**8b** (IC_{50} = 3.53 μM) and ent-**11b** (IC_{50} = 3.67 μM) display high cytotoxic activity against the A-427 tumor cell line but show only low σ_1 and σ_2 receptor affinity. This result may indicate an additional target for these compounds, which is distinct from σ receptors. However, the penetration of the compounds into the tumor cells as well as transformations of the compounds by tumor cell enzymes should be considered.

Experimental Section

Chemistry. General. Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. Flash chromatography (fc): Silica gel 60, 40−64 μm (Merck); parentheses include: diameter of the column, eluent, fraction size, R_f value. Optical rotation: Polarimeter 341 (Perkin-Elmer); 1.0 dm tube; concentration c [g/100 mL], the unit of [α] [grad·mL·dm^{-1}·g^{-1}]. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz): Unity Mercury Plus 400 spectrometer (Varian); δ in ppm related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution. The purity of all test compounds is greater than 95%, which was determined with two independent HPLC methods.

(+)-(1*S*,2*R*,5*S*)-6-Allyl-2-benzyloxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (7a). Under N_2 atmosphere and ice-cooling NaH (153 mg, 6.36 mmol, prepared from 254 mg 60% NaH suspension in paraffin oil), was suspended in THF (70 mL) and **6a**[11] (350 mg, 1.06 mmol) and tetrabutylammonium iodide (78 mg, 0.21 mmol) were added. After 20 min, benzyl bromide (0.38 mL, 544 mg, 3.18 mmol) was added dropwise and the mixture was stirred at room temperature for 16 h. Then the solvent was removed in vacuo, the residue was dissolved in CH_2Cl_2, and the organic layer was washed with water (2×), 0.5 M HCl (1×), and 0.5 M NaOH (1×). The organic layer was dried (Na_2SO_4), filtered, and the solvent was removed in vacuo. The residue was purified by flash column chromatography (Ø = 3 cm, h = 15 cm, petroleum ether/ethyl acetate = 3/7, V = 20 mL, R_f = 0.35) to give **7a** as a colorless solid, mp 95 °C, yield 418 mg (94%). $[\alpha]_D^{20}$ = +144 (c = 0.50; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.60−1.72 (m, 1H, 4-H), 1.78−1.88 (m, 2H, 3-H (1H), 4-H (1H)), 1.92−2.02 (m, 1H, 3-H), 3.07−3.12 (m, 1H, 2-H), 3.79 (s, 3H, ArOCH_3), 3.92 (dd, J = 5.5/2.3 Hz, 1H, 5-H), 3.98−4.12 (m, 2H, NCH_2CH=CH_2), 4.04 (d, J = 3.9 Hz, 1H, 1-H), 4.20 (d, J = 12.5 Hz, 1H, OCH_2Ar), 4.26 (d, J = 14.1 Hz, 1H, NCH_2Ar), 4.50 (d, J = 12.5 Hz, 1H, OCH_2Ar), 4.66 (d, J = 14.1 Hz, 1H, NCH_2Ar), 5.21−5.28 (m, 2H, NCH$_2$CH=CH_2), 5.78 (ddt, J = 17.2/10.2/6.3 Hz, 1H, NCH$_2$CH=CH$_2$), 6.84 (d, J = 8.6 Hz, 2H, 3′-H$_{4\text{-methoxybenzyl}}$, 5′-H$_{4\text{-methoxybenzyl}}$), 7.17 (d, J = 8.6 Hz, 2H, 2′-H$_{4\text{-methoxybenzyl}}$, 6′-H$_{4\text{-methoxybenzyl}}$), 7.19−7.34 (m, 5H, OCH$_2C_6H_5$).

(+)-(1*R*,2*R*,5*S*)-6-Allyl-2-benzyloxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane (8a). Under N_2 atmosphere, $LiAlH_4$ (173 mg, 4.6 mmol) was added to an ice-cooled solution of **7a** (383 mg, 0.91 mmol) in THF (40 mL). The mixture was stirred at 0 °C for 10 min and then heated to reflux for 16 h. Then water was added under ice-cooling until H_2 formation was finished. The mixture was stirred at 0 °C for 10 min and then heated to reflux for 30 min. After cooling down, the mixture was filtered, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (Ø = 3 cm, h = 15 cm, CH_2Cl_2/methanol = 50/1, V = 20 mL, R_f = 0.07) to give **8a** as a yellow oil, yield 215 mg (60%). $[\alpha]_D^{20}$ = +4.8 (c = 0.55; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.51−1.61 (m, 1H, 3-H), 1.84−1.95 (m, 3H, 3-H (1H), 4-H (2H)), 2.65 (dd, J = 10.2/2.4 Hz, 1H, 7-H), 2.71−2.81 (m, 2H, 9-H (1H), 1-H), 2.86 (dd, J = 10.2/1.8 Hz, 1H, 7-H), 2.92−2.98 (m, 1H, 5-H), 3.12−3.19 (m, 3H, 9-H (1H), NC*H*$_2$CH=CH$_2$), 3.42−3.48 (m, 1H, 2-H), 3.61 (d, J = 12.6 Hz, 1H, NC*H*$_2$Ar), 3.69 (d, J = 12.6 Hz, 1H, NC*H*$_2$Ar), 3.81 (s, 3H, ArOC*H*$_3$), 4.19 (d, J = 12.6 Hz, 1H, OC*H*$_2$Ph), 4.26 (d, J = 12.6 Hz, 1H, OC*H*$_2$Ph), 5.08 (d, J = 10.2 Hz, 1H, NCH$_2$CH=C*H*$_2$), 5.18 (dd, J = 16.9/1.8 Hz, 1H, NCH$_2$CH=C*H*$_2$), 5.86 (ddt, J = 16.9/10.2/6.0 Hz, 1H, NCH$_2$C*H*=CH$_2$), 6.84 (d, J = 8.4 Hz, 2H, 3′-H$_{4\text{-methoxybenzyl}}$, 5′-H$_{4\text{-methoxybenzyl}}$), 7.17 (d, J = 8.4 Hz, 2H, 2′-H$_{benzyloxy}$, 6′-H$_{benzyloxy}$), 7.25 (d, J = 8.4 Hz, 2H, 2′-H$_{4\text{-methoxybenzyl}}$, 6′-H$_{4\text{-methoxybenzyl}}$), 7.22−7.32 (m, 3H, OCH$_2C_6$*H*$_5$).

(+)-(1*S*,2*S*,5*S*)-6-Allyl-2-benzyloxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (10a). Under N_2 atmosphere and ice-cooling, NaH (180 mg, 7.52 mmol, prepared from 301 mg 60% NaH suspension in paraffin oil), was suspended in THF (70 mL) and **9a**[11] (414 mg, 1.25 mmol) and tetrabutylammonium iodide (93 mg, 0.25 mmol) were added. After 20 min, benzyl bromide (0.45 mL, 643 mg, 3.76 mmol) was added dropwise and the mixture was stirred at room temperature for 16 h. Then the solvent was removed in vacuo, the residue was dissolved in CH_2Cl_2, and the organic layer was washed with water (2×), 0.5 M HCl (1×), and 0.5 M NaOH (1×). The organic layer was dried (Na_2SO_4), filtered, and the solvent was removed in vacuo. The residue was purified by flash column chromatography (Ø = 3 cm, h = 15 cm, cyclohexane/ethyl acetate = 2/1, V = 20 mL, R_f = 0.16) to give **10a** as a colorless solid, mp 83 °C, yield 452 mg (86%). $[\alpha]_D^{20}$ = +14.0 (c = 0.32; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.55−1.64 (m, 1H, C*H*$_2$CH$_2$), 1.74−1.86 (m, 1H, C*H*$_2$CH$_2$), 2.02−2.19 (m, 2H, CH$_2$C*H*$_2$), 3.69−3.80 (m, 2H, 2-H, NC*H*$_2$CH=CH$_2$ (1H)), 3.78 (s, 3H, ArOC*H*$_3$), 3.89 (dd, J = 5.5/1.6 Hz, 1H, 5-H), 4.02 (d, J = 14.9 Hz, 1H, NC*H*$_2$Ar), 4.09 (d, J = 0.8 Hz, 1H, 1-H), 4.16 (ddt, J = 14.9/6.3/1.6 Hz, 1H, NC*H*$_2$CH=CH$_2$), 4.47 (d, J = 11.7 Hz, 1H, OC*H*$_2$Ar), 4.56 (d, J = 11.7 Hz, 1H, OC*H*$_2$Ar), 5.18−5.26 (m, 2H, NCH$_2$CH=C*H*$_2$), 5.28 (d, J = 14.9 Hz, 1H, NC*H*$_2$Ar), 5.72 (ddt, J = 17.2/10.2/6.3 Hz, 1H, NCH$_2$C*H*=CH$_2$), 6.83 (d, J = 8.6 Hz, 2H, 3′-H$_{4\text{-methoxybenzyl}}$, 5′-H$_{4\text{-methoxybenzyl}}$), 7.10 (d, J = 8.6 Hz, 2H, 2′-H$_{4\text{-methoxybenzyl}}$, 6′-H$_{4\text{-methoxybenzyl}}$), 7.28−7.39 (m, 5H, OCH$_2C_6$*H*$_5$).

(−)-(1*R*,2*S*,5*S*)-6-Allyl-2-benzyloxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane (11a). Under N_2 atmosphere, $LiAlH_4$ (47 mg, 1.25 mmol) was added to an ice-cooled solution of **10a** (105 mg, 0.25 mmol) in THF (30 mL). The mixture was stirred at 0 °C for 10 min and then heated to reflux for 16 h. Then water was added under ice-cooling until H_2 formation was finished. The mixture was stirred at 0 °C for 10 min and then heated to reflux for 30 min. After cooling down, the mixture was filtered, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, CH_2Cl_2/methanol = 50/1, V = 10 mL, R_f = 0.06) to give **11a** as a yellow oil, yield 57 mg (58%). $[\alpha]_D^{20}$ = −30.2 (c = 0.35; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.57−1.67 (m, 1H, C*H*$_2$CH$_2$), 1.75−1.83 (m, 1H, C*H*$_2$CH$_2$), 1.85−1.92 (m, 1H, C*H*$_2$CH$_2$), 2.12−2.23 (m, 1H, C*H*$_2$CH$_2$), 2.69−2.84 (m, 4H, piperazine-H), 2.91 (dd, J = 10.2/1.6 Hz, 1H, piperazine-H), 3.05−3.08 (m, 1H, piperazine-H), 3.09−3.21 (m, 2H, NC*H*$_2$CH=CH$_2$), 3.68 (s, 2H, NC*H*$_2$Ar), 3.74 (dd, J = 11.0/4.7 Hz, 1H, 2-H), 3.77 (s, 3H, ArOC*H*$_3$), 4.30 (d, J = 12.5 Hz, 1H, OC*H*$_2$Ph), 4.34 (d, J = 12.5 Hz, 1H, OC*H*$_2$Ph), 5.10 (d, J = 10.2 Hz, 1H, NCH$_2$CH=C*H*$_2$), 5.16 (dd, J = 17.2/1.6 Hz, 1H, NCH$_2$CH=C*H*$_2$), 5.85 (ddt, J = 17.2/10.2/6.3 Hz, 1H, NCH$_2$C*H*=CH$_2$), 6.82 (d, J = 8.6 Hz, 2H, 3′-H$_{4\text{-methoxybenzyl}}$, 5′-H$_{4\text{-methoxybenzyl}}$), 7.17−7.31 (m, 5H, OCH$_2C_6$*H*$_5$), 7.33 (d, J = 8.6 Hz, 2H, 2′-H$_{4\text{-methoxybenzyl}}$, 6′-H$_{4\text{-methoxybenzyl}}$).

(+)-(1*S*,2*R*,5*S*)-6-Allyl-8-(4-methoxybenzyl)-2-phenoxy-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (12a). Under N_2 atmosphere, **6a** (161 mg, 0.49 mmol), CuI (9.3 mg, 0.05 mmol), 1,10-phenanthroline (18 mg, 0.10 mmol), and Cs_2CO_3 (238 mg, 0.73 mmol) were suspended in iodobenzene (5.5 mL, 9.9 g, 49 mmol). The mixture was heated to 110 °C for 36 h. After the mixture was cooled to room temperature, water was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (Ø = 3 cm, h = 15 cm, cyclohexane/ethyl acetate = 2/1, V = 20 mL, R_f = 0.15) to give **12a** as a colorless solid, mp 116 °C, yield 95 mg (48%). $[\alpha]_D^{20}$ = +147 (c = 0.34; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.75−1.97 (m, 2H, 3-H (1H), 4-H (1H)), 2.04−2.14 (m, 2H, 3-H (1H), 4-H (1H)), 3.78 (s, 3H, ArOC*H*$_3$), 3.87−3.92 (m, 1H, 2-H), 3.97−4.01 (m, 1H, 5-H), 4.04−4.10 (m, 3H, 1-H, NC*H*$_2$CH=CH$_2$ (2H)), 4.27 (d, J = 14.5 Hz, 1H, NC*H*$_2$Ar), 4.71 (d, J = 14.5 Hz, 1H, NC*H*$_2$Ar), 5.24−5.31 (m, 2H, NCH$_2$CH=C*H*$_2$), 5.81 (ddt, J = 17.2/10.2/6.3 Hz, 1H, NCH$_2$C*H*=CH$_2$), 6.65 (d, J = 7.8 Hz, 2H, 2′-H$_{phenoxy}$, 6′-H$_{phenoxy}$), 6.81 (d, J = 8.6 Hz, 2H, 3′-H$_{4\text{-methoxybenzyl}}$, 5′-H$_{4\text{-methoxybenzyl}}$), 6.98 (t, J = 7.8 Hz, 1H, 4′-H$_{phenoxy}$), 7.09 (d, J = 8.6 Hz, 2H, 2′-H$_{4\text{-methoxybenzyl}}$, 6′-H$_{4\text{-methoxybenzyl}}$), 7.21 (t, J = 7.8 Hz, 2H, 3′-H$_{phenoxy}$, 5′-H$_{phenoxy}$).

(+)-(1*R*,2*R*,5*S*)-6-Allyl-8-(4-methoxybenzyl)-2-phenoxy-6,8-diazabicyclo[3.2.2]nonane (13a). Under N_2 atmosphere, $LiAlH_4$ (43 mg, 1.12 mmol) was added to an ice-cooled solution of **12a** (91 mg, 0.22 mmol) in THF (20 mL). The mixture was stirred at 0 °C for 10 min and then heated to reflux for 16 h. Then water was added under ice-cooling until H_2 formation was finished. The mixture was stirred at 0 °C for 10 min and then heated to reflux for 30 min. After cooling down, the mixture was filtered, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (Ø = 1 cm, h = 15 cm, CH_2Cl_2/methanol = 50/1, V = 5 mL, R_f = 0.13) to give **13a** as a yellow oil, yield 20 mg (24%). $[\alpha]_D^{20}$ = +9.1 (c = 0.44; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.71 (td, J = 13.3/3.9 Hz, 1H, C*H*$_2$CH$_2$), 1.90−2.03 (m, 2H, C*H*$_2$CH$_2$), 2.06−2.17 (m, 1H, C*H*$_2$CH$_2$), 2.70 (dd, J = 11.7/3.1 Hz, 1H, piperazine-H), 2.76 (dd, J = 10.2/2.3 Hz, 1H, piperazine-H), 2.83 (s br, 1H, piperazine-H), 2.87 (dd, J = 10.2/1.6 Hz, 1H, piperazine-H), 2.96−3.01 (m, 1H, piperazine-H), 3.14−3.20 (m, 3H, NC*H*$_2$CH=CH$_2$, piperazine-H), 3.57 (d, J = 12.5 Hz, 1H, NC*H*$_2$Ar), 3.70 (d, J = 12.5 Hz, 1H, NC*H*$_2$Ar), 3.85 (s, 3H, ArOC*H*$_3$), 4.18−4.24 (m, 1H, 2-H), 5.09 (dd, J = 10.2/1.6 Hz, 1H, NCH$_2$CH=C*H*$_2$), 5.20 (dd, J = 17.2/1.6 Hz, 1H, NCH$_2$CH=C*H*$_2$), 5.87 (ddt, J = 17.2/10.2/6.3 Hz, 1H, NCH$_2$C*H*=CH$_2$), 6.37 (d, J = 8.6 Hz, 2H, 3′-H$_{4\text{-methoxybenzyl}}$, 5′-H$_{4\text{-methoxybenzyl}}$), 6.79 (t, J = 7.8 Hz, 1H, 4′- H$_{phenoxy}$), 6.89 (d, J = 7.8 Hz, 2H, 2′-H$_{phenoxy}$, 6′-H$_{phenoxy}$), 7.03 (t, J = 7.8 Hz, 2H, 3′-H$_{phenoxy}$, 5′-H$_{phenoxy}$), 7.29 (d, J = 8.6 Hz, 2H, 2′-H$_{4\text{-methoxybenzyl}}$, 6′-H$_{4\text{-methoxybenzyl}}$).

(+)-(1*S*,2*S*,5*S*)-6-Allyl-8-(4-methoxybenzyl)-2-phenoxy-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (14a). Under N_2 atmosphere, **6a** (177 mg, 0.54 mmol) and triphenylphosphine (351 mg, 1.34 mmol) were dissolved in toluene (30 mL) and heated to reflux. A solution of phenol (101 mg, 1.07 mmol) and diisopropyl azodicarboxylate (0.53 mL, 542 mg, 2.68 mmol) in toluene (10 mL) was added dropwise and the mixture was refluxed for 4 h. Then the solvent was removed in vacuo and the residue was purified by flash column chromatography (Ø = 3 cm, h = 15 cm, cyclohexane/ethyl acetate = 2/1, 20 mL, R_f = 0.15) to give **14a** as a colorless solid, mp 106 °C, yield 67 mg (31%). $[\alpha]_D^{20}$ = +28.2 (c = 0.11; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.66−1.75 (m, 1H, 4-H), 1.90−2.01 (m, 1H, 3-H), 2.11−2.27 (m, 2H, 3-H (1H), 4-H (1H)), 3.75 (s, 3H, ArOC*H*$_3$), 3.78−3.86 (m, 1H, NC*H*$_2$CH=CH$_2$), 3.93−3.97 (m, 1H, 5-H), 4.03 (d, J = 14.1 Hz, 1H, NC*H*$_2$Ar), 4.15−4.22 (m, 2H, 1-H, NC*H*$_2$CH=CH$_2$ (1H)), 4.58 (dd, J = 8.6/

5.5 Hz, 1H, 2-H), 5.13 (d, J = 14.1 Hz, 1H, NCH_2Ar), 5.20−5.29 (m, 2H, $NCH_2CH{=}CH_2$), 5.75 (ddt, J = 17.2/10.2/6.3 Hz, 1H, $NCH_2CH{=}CH_2$), 6.74 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 6.90 (d, J = 7.8 Hz, 2H, 2′-$H_{phenoxy}$, 6′-$H_{phenoxy}$), 6.96−7.03 (m, 3H, 4′-$H_{phenoxy}$, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$), 7.31 (t, J = 7.8 Hz, 2H, 3′-$H_{phenoxy}$, 5′-$H_{phenoxy}$).

(−)-(1*R*,2*S*,5*S*)-6-Allyl-8-(4-methoxybenzyl)-2-phenoxy-6,8-diazabicyclo[3.2.2]nonane (15a). Under N_2 atmosphere, $LiAlH_4$ (56 mg, 1.48 mmol) was added to an ice-cooled solution of **14a** (120 mg, 0.30 mmol) in THF (30 mL). The mixture was stirred at 0 °C for 10 min and then heated to reflux for 16 h. Then water was added under ice-cooling until H_2 formation was finished. The mixture was stirred at 0 °C for 10 min and then heated to reflux for 30 min. After cooling down, the mixture was filtered, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, CH_2Cl_2/methanol = 50/1, V = 10 mL, R_f = 0.11) to give **15a** as a yellow oil, yield 30 mg (27%). $[\alpha]_D^{20}$ = −56.3 (c = 2.9; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.64−1.76 (m, 1H, CH_2CH_2), 1.81−1.89 (m, 1H, CH_2CH_2), 1.92−2.01 (m, 1H, CH_2CH_2), 2.28−2.41 (m, 1H, CH_2CH_2), 2.72−2.77 (m, 1H, piperazine-H), 2.79−2.85 (m, 2H, piperazine-H), 2.87−2.95 (m, 2H, piperazine-H), 3.05−3.08 (m, 1H, piperazine-H), 3.14−3.26 (m, 2H, NCH_2CH=CH_2), 3.59−3.67 (s, 2H, NCH_2Ar), 3.79 (s, 3H, ArOCH_3), 4.65 (dd, J = 10.2/4.7 Hz, 1H, 2-H), 5.13 (d, J = 10.2 Hz, 1H, $NCH_2CH{=}CH_2$), 5.20 (d, J = 17.2 Hz, 1H, $NCH_2CH{=}CH_2$), 5.89 (ddt, J = 17.2/10.2/6.3 Hz, 1H, $NCH_2CH{=}CH_2$), 6.72 (d, J = 7.8 Hz, 2H, 2′-$H_{phenoxy}$, 6′-$H_{phenoxy}$), 6.81 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 6.85 (t, J = 7.8 Hz, 1H, 4′-$H_{phenoxy}$), 7.19 (t, J = 7.8 Hz, 2H, 3′-$H_{phenoxy}$, 5′-$H_{phenoxy}$), 7.29 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$).

(+)-(1*S*,5*S*,*Z*)-6-Allyl-2-benzylidene-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (16a). Under N_2 atmosphere, benzyltriphenylphosphonium bromide (317 mg, 0.73 mmol) was suspended in THF (50 mL) and the mixture was cooled to −10 °C. Then potassium *tert*-butoxide (103 mg, 0.91 mmol) was added in portions. Subsequently, a solution of **5a** (200 mg, 0.61 mmol) in THF (20 mL) was added dropwise and the mixture was stirred at room temperature for 16 h. Then water was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), filtered, and the solvent was removed in vacuo. The residue was purified by flash column chromatography (Ø = 3 cm, h = 15 cm, cyclohexane/ethyl acetate = 2/1, 20 mL, R_f = 0.18) to give **16a** as a colorless solid, mp 156 °C, yield 219 mg (89%). $C_{25}H_{26}N_2O_3$ (402.5). $[\alpha]_D^{20}$ = +267 (c = 0.29; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.79−1.89 (m, 1H, 4-H), 2.15−2.23 (m, 1H, 4-H), 2.34−2.50 (m, 2H, 3-H), 3.75 (s, 3H, ArOCH_3), 3.85 (dd, J = 14.9/6.3 Hz, 1H, NCH_2CH=CH_2), 3.99 (dd, J = 5.5/2.3 Hz, 1H, 5-H), 4.21 (dd, J = 14.9/6.3 Hz, 1H, NCH_2CH=CH_2), 4.27 (d, J = 14.1 Hz, 1H, NCH_2Ar), 4.33 (d, J = 14.1 Hz, 1H, NCH_2Ar), 4.82 (s, 1H, 1-H), 5.22−5.28 (m, 2H, $NCH_2CH{=}CH_2$), 5.78 (ddt, J = 16.4/10.2/6.3 Hz, 1H, $NCH_2CH{=}CH_2$), 6.48 (s, 1H, C=CHPh), 6.61 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 6.77 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$), 7.31 (t, J = 7.0 Hz, 1H, 4′-$H_{benzylidene}$), 7.38 (t, J = 7.0 Hz, 2H, 3′-$H_{benzylidene}$, 5′-$H_{benzylidene}$), 7.50 (d, J = 7.0 Hz, 2H, 2′-$H_{benzylidene}$, 6′-$H_{benzyidene}$). NOE: irradiation at 4.82 ppm (1-H): δ [ppm] = 4.27 (NCH_2Ar), 4.33 (NCH_2Ar), 6.77 (2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$), 7.50 (2′-$H_{benzylidene}$, 6′-$H_{benzyidene}$); irradiation at 6.48 ppm (C=CHPh): δ [ppm] = 2.34−2.50 (3-H), 7.50 (2′-$H_{benzylidene}$, 6′-$H_{benzyidene}$).

(−)-(1*S*,5*S*,*Z*)-6-Allyl-2-benzylidene-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane (17a). Under N_2 atmosphere, $LiAlH_4$ (51 mg, 1.33 mmol) was added to an ice-cooled solution of **16a** (107 mg, 0.27 mmol) in THF (30 mL). The mixture was stirred at 0 °C for 10 min and then heated to reflux for 16 h. Then water was added under ice-cooling until H_2 formation was finished. The mixture was stirred at 0 °C for 10 min and then heated to reflux for 30 min. After cooling down, the mixture was filtered, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, CH_2Cl_2/methanol = 50/1, V = 10 mL, R_f = 0.07) to give **17a** as a yellow oil, yield 46 mg (46%). $[\alpha]_D^{20}$ = −82.9 (c = 0.73; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.76−1.95 (m, 2H, 4-H), 2.35 (ddd, J = 14.8/6.5/2.8 Hz, 1H, 3-H), 2.69 (dd, J = 10.6/1.6 Hz, 1H, 7-H), 2.80−2.90 (m, 2H, 3-H (1H), 9-H (1H)), 2.96−3.03 (m, 2H, 7-H (1H), 9-H (1H)), 3.04−3.08 (m, 1H, 5-H), 3.15−3.26 (m, 2H, NCH_2CH=CH_2), 3.55 (s, 2H, NCH_2Ar), 3.78 (s, 3H, ArOCH_3), 3.85 (d br, J = 2.8 Hz, 1H, 1-H), 5.10 (d, J = 10.2 Hz, 1H, $NCH_2CH{=}CH_2$), 5.18 (d, J = 16.6 Hz, 1H, $NCH_2CH{=}CH_2$), 5.91 (ddt, J = 16.6/10.2/6.5 Hz, 1H, $NCH_2CH{=}CH_2$), 6.31 (s, 1H, C=CHPh), 6.77 (d, J = 8.3 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 6.94 (d, J = 7.4 Hz, 2H, 2′-$H_{benzylidene}$, 6′-$H_{benzylidene}$), 7.09−7.21 (m, 5H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$, 4′-$H_{benzylidene}$, 3′-$H_{benzylidene}$, 5′-$H_{benzylidene}$).

(+)-(1*S*,4*S*)-11-Allyl-2-(4-methoxybenzyl)-1,2,5,10-tetrahydro-4,1-iminomethanoazepino[3,4-*b*]indole-3,12(4H)-dione (18a). A solution of **5a** (130 mg, 0.40 mmol) and phenylhydrazine (107 mg, 0.99 mmol) in HCl(g)-saturated ethanol (50 mL) was heated to reflux under N_2 for 16 h. Then the solvent was evaporated and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, cyclohexane/ethyl acetate = 2/1, 10 mL, R_f = 0.13) to give **18a** as a colorless solid, mp 253 °C, yield 67 mg (42%). $[\alpha]_D^{20}$ = +232 (c = 0.21; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 3.23 (dd, J = 17.2/3.9 Hz, 1H, 5-H), 3.32 (dd, J = 17.2/2.3 Hz, 1H, 5-H), 3.70 (s, 3H, ArOCH_3), 3.94−4.01 (m, 1H, NCH_2CH=CH_2), 4.18−4.25 (m, 1H, NCH_2CH=CH_2), 4.40 (d, J = 14.9 Hz, 1H, NCH_2Ar), 4.47 (dd, J = 3.9/2.3 Hz, 1H, 4-H), 4.56 (s, 1H, 1-H), 4.71 (d, J = 14.9 Hz, 1H, NCH_2Ar), 5.25−5.31 (m, 2H, $NCH_2CH{=}CH_2$), 5.81 (ddt, J = 17.2/10.2/6.3 Hz, 1H, $NCH_2CH{=}CH_2$), 6.68 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.01 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-Methoxybenzyl}}$), 7.06−7.12 (m, 1H, H_{indole}), 7.14−7.17 (m, 2H, H_{indole}), 7.42 (d, J = 7.8 Hz, 1H, H_{indole}), 7.81 (s br, 1H, NH).

(+)-(1*S*,4*S*)-11-Allyl-7-methoxy-2-(4-methoxybenzyl)-1,2,5,10-tetrahydro-4,1-iminomethanoazepino[3,4-*b*]indole-3,12(4*H*)-dione (19a). A solution of **5a** (141 mg, 0.43 mmol) and *p*-methoxyphenylhydrazine hydrochloride (187 mg, 1.07 mmol) in HCl(g)-saturated ethanol (70 mL) was heated to reflux under N_2 for 16 h. Then the solvent was evaporated and the residue was purified by flash column chromatography (Ø = 3 cm, h = 15 cm, cyclohexane/ethyl acetate = 2/1, 20 mL, R_f = 0.10) to give **19a** as a colorless solid, mp 263 °C, yield 90 mg (49%). $[\alpha]_D^{20}$ = +214 (c = 0.29; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 3.18 (dd, J = 17.2/3.9 Hz, 1H, 5-H), 3.27 (dd, J = 17.2/3.1 Hz, 1H, 5-H), 3.71 (s, 3H, ArOCH_3), 3.83 (s, 3H, ArOCH_3), 3.97 (dd, J = 15.7/6.3 Hz, 1H, NCH_2CH=CH_2), 4.20 (dd, J = 15.7/6.3 Hz, 1H, NCH_2CH=CH_2), 4.40 (d, J = 14.9 Hz, 1H, NCH_2Ar), 4.46 (dd, J = 3.9/3.1 Hz, 1H, 4-H), 4.50 (s, 1H, 1-H), 4.72 (d, J = 14.9 Hz, 1H, NCH_2Ar), 5.25−5.31 (m, 2H, $NCH_2CH{=}CH_2$), 5.80 (ddt, J = 17.2/10.2/6.3 Hz, 1H, $NCH_2CH{=}CH_2$), 6.68 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 6.80−6.84 (m, 2H, 6-H, 8-H), 7.00 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$), 7.04 (d, J = 9.4 Hz, 1H, 9-H), 7.57 (s br, 1H, NH).

(+)-(1*R*,4*S*)-11-Allyl-2-(4-methoxybenzyl)-1,2,3,4,5,10-hexahydro-4,1-iminomethanoazepino[3,4-*b*]indole (20a). Under N_2 atmosphere, $LiAlH_4$ (52 mg, 1.37 mmol) was added to an ice-cooled solution of **18a** (110 mg, 0.27 mmol) in THF (30 mL). The mixture was stirred at 0 °C for 10 min and then heated to reflux for 16 h. Then water was added under ice-cooling until H_2 formation was finished. The mixture was stirred at 0 °C for 10 min and then heated to reflux for 30 min. After cooling down, the mixture was filtered, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, CH_2Cl_2/methanol = 50/1, V = 10 mL, R_f = 0.02) to give **20a** as a pale-yellow solid, mp 161 °C, yield 42 mg (41%). $[\alpha]_D^{20}$ = +97.4 (c = 0.15; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.40−2.46 (m, 1H, 3-H), 2.72−2.82 (m, 2H, 5-H (1H), 12-H (1H)), 3.24−3.42 (m, 4H, NCH_2CH=CH_2 (2H), 5-H (1H), NCH_2Ar (1H)), 3.55−3.60 (m, 4H, 4-H, 3-H (1H), 12-H (1H), NCH_2Ar (1H)), 3.67−3.71 (m, 1H, 1-H), 3.82 (s, 3H, ArOCH_3), 5.11−5.16 (m, 1H, $NCH_2CH{=}CH_2$), 5.19−5.26 (m, 1H, $NCH_2CH{=}CH_2$), 5.94−6.06 (m, 1H, $NCH_2CH{=}CH_2$), 6.88 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$,

5′-$H_{4\text{-methoxybenzyl}}$), 7.11−7.21 (m, 2H, 7-H, 8-H), 7.30 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$), 7.35 (d, J = 7.7 Hz, 1H, 9-H), 7.50 (d, J = 7.7 Hz, 1H, 6-H), 7.73 (s br, 1H, N*H*).

(−)-(1*S*,4*R*)-11-Allyl-2-(4-methoxybenzyl)-1,2,3,4,5,10-hexahydro-4,1-iminomethanoazepino[3,4-*b*]indole (ent-20a). As described for the preparation of **20a**, the enantiomer ent-**18a** (111 mg, 0.28 mmol) was reacted with $LiAlH_4$ (53 mg, 1.38 mmol) in THF (30 mL) to give ent-**20a** as a pale-yellow solid, mp 160 °C, yield 41 mg (40%). $[\alpha]_D^{20}$ = −94.1 (c = 0.19; CH_2Cl_2). Recrystallization of ent-**20a** from diisopropyl ether gave crystals which were suitable for X-ray crystal structure analysis. Details on the X-ray crystal structure analysis are given in the Supporting Information. CCDC 693445 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*R*,4*S*)-11-Allyl-7-methoxy-2-(4-methoxybenzyl)-1,2,3,4,5,10-hexahydro-4,1-iminomethanoazepino[3,4-*b*]indole (21a). Under N_2 atmosphere, $LiAlH_4$ (29 mg, 0.76 mmol) was added to an ice-cooled solution of **19a** (66 mg, 0.15 mmol) in THF (20 mL). The mixture was stirred at 0 °C for 10 min and then heated to reflux for 16 h. Then water was added under ice-cooling until H_2 formation was finished. The mixture was stirred at 0 °C for 10 min and then heated to reflux for 30 min. After cooling down, the mixture was filtered, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (Ø = 1 cm, h = 15 cm, CH_2Cl_2/methanol = 50/1, V = 5 mL, R_f = 0.03) to give **21a** as a pale-yellow solid, mp 197 °C, yield 28 mg (45%). $[\alpha]_D^{20}$ = +87.8 (c = 0.68; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.47 (d, J = 11.4 Hz, 1H, 3-H), 2.75 (dd, J = 16.9/4.2 Hz, 1H, 5-H), 2.83 (d, J = 10.2 Hz, 1H, 12-H), 3.26 (d, J = 16.9 Hz, 1H, 5-H), 3.31−3.44 (m, 2H, NCH_2CH=CH_2), 3.49−3.63 (m, 5H, NCH_2Ar (2H), 3-H (1H), 4-H, 12-H (1H)), 3.79 (s, 3H, ArOCH_3), 3.86 (s, 3H, ArOCH_3), 3.91 (s, 1H, 1-H), 5.16 (d, J = 10.2 Hz, 1H, NCH$_2$CH=CH_2), 5.23 (d, J = 16.9 Hz, 1H, NCH$_2$CH=CH_2), 5.96 (ddt, J = 16.9/10.2/6.6 Hz, 1H, NCH$_2$CH=CH$_2$), 6.81−6.87 (m, 3H, 8-H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 6.92 (s, 1H, 6-H), 7.23−7.29 (m, 3H, 9-H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$), 8.41 (s br, 1H, N*H*).

(+)-(1*S*,4*S*)-12-Allyl-2-(4-methoxybenzyl)-6-methyl-1,2,4,5-tetrahydro-4,1-iminomethanoazepino[3,4-*b*]quinoline-3,13-dione (22a). A solution of **5a** (187 mg, 0.57 mmol) and *o*-aminoacetophenone (192 mg, 1.42 mmol) in glacial acetic acid (50 mL) was heated to reflux under N_2 for 16 h. Then the solvent was evaporated in vacuo and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, cyclohexane/ethyl acetate = 2/1, 10 mL, R_f = 0.07) to give **22a** as a colorless solid, mp 177 °C, yield 159 mg (65%). $[\alpha]_D^{20}$ = +105 (c = 0.29; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.54 (s, 3H, ArCH_3), 3.28 (dd, J = 18.0/4.7 Hz, 1H, 5-H), 3.41 (dd, J = 18.0/2.3 Hz, 1H, 5-H), 3.67 (s, 3H, ArOCH_3), 3.99 (dd, J = 15.7/6.3 Hz, 1H, NCH_2CH=CH$_2$), 4.19−4.28 (m, 2H, NCH_2CH=CH$_2$ (1H), NCH_2Ar (1H)), 4.33 (dd, J = 3.9/2.3 Hz, 1H, 4-H), 4.85 (d, J = 14.9 Hz, 1H, NCH_2Ar), 5.10 (s, 1H, 1-H), 5.25−5.31 (m, 2H, NCH$_2$CH=CH_2), 5.77 (ddt, J = 17.2/10.2/6.3 Hz, 1H, NCH$_2$CH=CH$_2$), 6.71 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.15 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$), 7.55 (t, J = 7.8 Hz, 1H, 8-H), 7.66 (t, J = 7.8 Hz, 1H, 9-H), 7.95−8.00 (m, 2H, 7-H, 10-H).

(−)-(1*R*,4*S*)-12-Allyl-2-(4-methoxybenzyl)-6-methyl-2,3,4,5-tetrahydro-4,1-iminomethano-1*H*-azepino[3,4-*b*]quinoline (23a). Under N_2 atmosphere, $LiAlH_4$ (59 mg, 1.56 mmol) was added to an ice-cooled solution of **22a** (133 mg, 0.31 mmol) in THF (30 mL). The mixture was stirred at 0 °C for 10 min and then heated to reflux for 16 h. Then water was added under ice-cooling until H_2 formation was finished. The mixture was stirred at 0 °C for 10 min and then heated to reflux for 30 min. After cooling down, the mixture was filtered, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, CH_2Cl_2/methanol = 50/1, V = 10 mL, R_f = 0.05) to give **23a** as a yellow oil, yield 31 mg (25%). $[\alpha]_D^{20}$ = −42.4 (c = 0.39 mg; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.60 (s, 3H, ArCH_3), 2.65 (d, J = 11.0 Hz, 1H, 3-H), 2.99−3.06 (m, 1H, 13-H), 3.17−3.43 (m, 7H, 3-H (1H), 4-H, 5-H (2H), 13-H (1H), NCH_2CH=CH$_2$), 3.66 (d, J = 12.5 Hz, 1H, NCH_2Ar), 3.74 (d, J = 12.5 Hz, 1H, NCH_2Ar), 3.78 (s, 3H, ArOCH_3), 4.20−4.23 (m, 1H, 1-H), 5.15 (d, J = 10.2 Hz, 1H, NCH$_2$CH=CH_2), 5.21 (d, J = 17.2 Hz, 1H, NCH$_2$CH=CH_2), 5.85−5.96 (m, 1H, NCH$_2$CH=CH$_2$), 6.80 (d, J = 8.6 Hz, 2H, 3′- $H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.21 (d, J = 8.6 Hz, 2H, 2′- $H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$), 7.50 (t, J = 7.0 Hz, 1H, 8-H), 7.61 (t, J = 7.0 Hz, 1H, 9-H), 7.94−8.03 (m, 2H, 7-H, 10-H).

(+)-(1′*S*,2′*S*,5′*S*)-6′-Allyl-8′-(4-methoxybenzyl)-3,4-dihydrospiro[2-benzopyran-1,2′-6′,8′-diazabicyclo[3.2.2]nonane]-7′,9′-dione (24). Under N_2 atmosphere, 1-bromo-2-(2-bromoethyl) benzene (235 mg, 0.89 mmol) was dissolved in THF (30 mL) and the solution was cooled to −90 °C. Then a 1.6 M solution of *n*-butyllithium in hexane (0.56 mL, 0.89 mmol) was slowly added. The reaction mixture was stirred for 10 min at −90 °C. Then a solution of **5a** (292 mg, 0.89 mmol) in THF (10 mL) was added dropwise. After stirring at −90 °C for 30 min, the mixture was allowed to warm to room temperature. After 2 h, water was added and the resulting mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), filtered, and the solvent was removed in vacuo. The residue was purified by flash column chromatography (Ø = 3 cm, h = 15 cm, cyclohexane/ethyl acetate = 2/1, V = 20 mL, R_f = 0.16) to give **24** as a colorless oil, yield 46 mg (12%). $[\alpha]_D^{20}$ = +18.7 (c = 0.12; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.97−2.13 (m, 3H, 3′-H (1H), 4′-H (2H)), 2.41−2.51 (m, 1H, 3′-H), 2.77 (dt, J = 16.4/3.9 Hz, 1H, PhCH_2CH$_2$O), 2.93 (ddd, J = 16.4/9.4/5.5 Hz, 1H, PhCH_2CH$_2$O), 3.67 (d, J = 14.9 Hz, 1H, NCH_2Ar), 3.74 (s, 3H, ArOCH_3), 3.78 − 3.89 (m, 2H, PhCH$_2$CH_2O (1H), NCH_2CH=CH$_2$ (1H)), 3.98 (dd, J = 5.5/1.6 Hz, 1H, 5′-H), 4.11−4.21 (m, 2H, PhCH$_2$CH_2O (1H), NCH_2CH=CH$_2$ (1H)), 4.15 (s, 1H, 1′-H), 5.12−5.21 (m, 2H, NCH$_2$CH=CH_2), 5.36 (d, J = 14.9 Hz, 1H, NCH_2Ar), 5.73 (ddt, J = 16.4/10.2/6.3 Hz, 1H, NCH$_2$CH=CH$_2$), 6.75 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 6.94 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$), 7.15−7.27 (m, 4H, H_{arom}). NOE: irradiation at 3.67 ppm (NCH_2Ar): δ [ppm] = 4.15 (1′-H), 5.36 (NCH_2Ar), 6.94 (2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$), 7.15−7.27 ($H_{arom}$); irradiation at 5.36 ppm (NCH_2Ar): δ [ppm] = 3.67 (NCH_2Ar), 6.94 (2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$); irradiation at 7.15−7.27 ppm (H_{arom}): δ [ppm] = 2.41−2.51 (3′-H), 3.67 (NCH_2Ar).

(−)-(1′*R*,2′*S*,5′*S*)-6′-Allyl-8′-(4-methoxybenzyl)-3,4-dihydrospiro[2-benzopyran-1,2′-6′,8′-diazabicyclo[3.2.2]nonane] (25). Under N_2 atmosphere, $LiAlH_4$ (22.8 mg, 0.60 mmol) was added to an ice-cooled solution of **24** (52 mg, 0.12 mmol) in THF (20 mL). The mixture was stirred at 0 °C for 10 min and then heated to reflux for 16 h. Then water was added under ice-cooling until H_2 formation was finished. The mixture was stirred at 0 °C for 10 min and then heated to reflux for 30 min. After cooling down, the mixture was filtered, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (Ø = 1 cm, h = 15 cm, CH_2Cl_2/methanol = 9.5/0.5, V = 5 mL, R_f = 0.12) to give **25** as a yellow oil, yield 12.9 mg (27%). $[\alpha]_D^{20}$ = −38.2 (c = 0.05; CH_2Cl_2). ^{1}H NMR (CDCl3): δ [ppm] = 1.62−1.71 (m, 1H, 4′-H), 1.87 (ddd, J = 14.1/5.5/3.1 Hz, 1H, 3′-H), 2.00−2.14 (m, 1H, 4′-H), 2.42 (ddd, J = 14.1/11.7/6.3 Hz, 1H, 3′-H), 2.66−2.90 (m, 4H, 9′-H (1H), 7′-H (1H), PhCH_2CH$_2$O), 2.92 (dd, J = 11.0/2.3 Hz, 1H, 9′-H), 2.99−3.05 (m, 1H, 5′-H), 3.08−3.11 (m, 1H, 1′-H), 3.21−3.31 (m, 3H, 7′-H (1H), NCH_2CH=CH$_2$), 3.63 (d, J = 17.2 Hz, 1H, NCH_2Ar), 3.66 (d, J = 17.2 Hz, 1H, NCH_2Ar), 3.81 (s, 3H, ArOCH_3), 3.83−3.89 (m, 1H, PhCH$_2$CH_2O), 3.92−3.99 (m, 1H, PhCH$_2$CH_2O), 5.09−5.14 (m, 1H, NCH$_2$CH=CH_2), 5.19−5.25 (m, 1H, NCH$_2$CH=CH_2), 5.92−6.03 (m, 1H, NCH$_2$CH=CH$_2$), 6.88 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.05 (dd, J = 7.8/1.6 Hz, 1H, 5-H), 7.14 (td, J = 7.8/1.6 Hz, 1H, 7-H), 7.20 (td, J = 7.8/1.6 Hz, 1H, 6-H), 7.29 (d,

J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$), 8.04 (dd, J = 7.8/1.6 Hz, 1H, 8-H).

(+)-(1*R*,5*S*)-6-Benzyl-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]non-3-en-2-one (28). The ketone **26** (160 mg, 0.46 mmol) was dissolved in dry chlorobenzene (15 mL). Then benzeneseleninic anhydride (181 mg, 0.50 mmol) was added and the mixture was heated to 95 °C for 16 h. Then the solvent was removed in vacuo and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, CH_2Cl_2/methanol = 100/1, V = 10 mL, R_f = 0.28) to give **28** as a yellow oil, yield 32 mg (20%). $[\alpha]_D^{20}$ = +71.4 (c = 0.19; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.46−2.54 (m, 2H, 7-H (1H), 9-H (1H)), 3.45−3.58 (m, 5H, 1-H, 5-H, 7-H (1H), 9-H (1H), NCH_2Ar (1H)), 3.60 (s, 2H, NCH_2Ar), 3.74 (d, J = 13.3 Hz, 1H, NCH_2Ar), 3.79 (s, 3H, ArOCH_3), 6.48 (d, J = 11.0 Hz, 1H, 3-H), 6.84 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.08 (dd, J = 11.0/7.8 Hz, 1H, 4-H), 7.23 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$), 7.25−7.29 (m, 1H, 4′-$H_{\text{benzyl}}$), 7.29−7.32 (m, 4H, 2′-$H_{\text{benzyl}}$, 3′-$H_{\text{benzyl}}$, 5′-$H_{\text{benzyl}}$, 6′-$H_{\text{benzyl}}$).

(−)-(1*R*,4*R*,5*S*)-6-Benzyl-8-(4-methoxybenzyl)-4-phenyl-6,8-diazabicyclo[3.2.2]nonan-2-one (29a) and (−)-(1*R*,4*S*,5*S*)-6-Benzyl-8-(4-methoxybenzyl)-4-phenyl-6,8-diazabicyclo[3.2.2]nonan-2-one (29b). Under N_2 atmosphere copper(I) bromide−dimethyl sulfide complex (27 mg, 0.13 mmol) was suspended in THF (10 mL). The mixture was cooled to −50 °C, and a 2 M solution of phenyllithium in dibutyl ether (0.13 mL, 0.26 mmol) was added. The mixture was stirred at −50 °C for 1 h followed by 10 min at 0 °C. Then at −50 °C, **28** (42 mg, 0.12 mmol) in THF (5 mL) was added dropwise. The mixture was stirred at −50 °C for 15 min and at room temperature for 16 h. Then a 2 M NaOH solution was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (Ø = 1 cm, h = 30 cm, CH_2Cl_2/methanol = 100/1, V = 5 mL) to give **29a** (R_f = 0.56) and **29b** (R_f = 0.42). **29a**: yellow oil, yield 7 mg (14%). $C_{28}H_{30}N_2O_2$ (426.6). $[\alpha]_D^{20}$ = −23.2 (c = 0.05; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.63 (dd, J = 11.0/2.3 Hz, 1H, 7-H), 2.67−2.75 (m, 2H, 3-H (1H), 7-H (1H)), 2.92 (dd, J = 11.0/3.1 Hz, 1H, 9-H), 3.11 (dd, J = 11.0/1.6 Hz, 1H, 9-H), 3.20−3.22 (m, 1H, 5-H), 3.23−3.26 (m, 1H, 1-H), 3.26 (d, J = 12.5 Hz, 1H, NCH_2Ar), 3.30−3.34 (m, 1H, 4-H), 3.35 (d, J = 12.5 Hz, 1H, NCH_2Ar), 3.63 (dd, J = 14.1/9.4 Hz, 1H, 3-H), 3.64 (d, J = 12.5 Hz, 1H, NCH_2Ar), 3.69 (d, J = 12.5 Hz, 1H, NCH_2Ar), 3.81 (s, 3H, ArOCH_3), 6.85 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.01 (dd, J = 7.8/1.6 Hz, 2H, H_{arom}), 7.17−7.23 (m, 6H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$, H_{arom} (4H)), 7.27−7.30 (m, 4H, H_{arom}). NOE: irradiation at 2.92 ppm (9-H): δ [ppm] = 2.63 (7-H), 3.11 (9-H), 3.20−3.22 (5-H); irradiation at 3.11 ppm (9-H): δ [ppm] = 2.92 (9-H), 3.20−3.22 (5-H), 3.30−3.34 (4-H). **29b**: yellow oil, yield 15 mg (29%). $[\alpha]_D^{20}$ = −24.7 (c = 0.16; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.49 (dd, J = 12.5/7.8 Hz, 1H, 3-H), 2.63 (dd, J = 11.7/2.3 Hz, 1H, 9-H), 2.79−2.83 (m, 1H, 5-H), 2.90−3.01 (m, 3H, 9-H (1H), 7-H (1H), 4-H), 3.02 (dd, J = 9.4/3.9 Hz, 1H, 7-H), 3.29−3.32 (m, 1H, 1-H), 3.61 (d, J = 13.3 Hz, 1H, NCH_2Ph), 3.66 (s, 2H, NCH_2Ar), 3.78 (dd, J = 12.5/10.2 Hz, 1H, 3-H), 3.79 (d, J = 13.3 Hz, 1H, NCH_2Ph), 3.80 (s, 3H, ArOCH_3), 6.86 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 6.95 (dd, J = 8.6/1.6 Hz, 2H, H_{arom}), 7.13−7.21 (m, 3H, H_{arom}), 7.25 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$), 7.28−7.35 (m, 5H, H_{arom}). NOE: irradiation at 3.31 ppm (1-H): δ [ppm] = 2.90−2.96 (7-H), 3.02 (7-H), 3.66 (NCH_2Ar); irradiation at 2.63 ppm (9-H): δ [ppm] = 2.79−2.83 (5-H), 2.90−2.96 (9-H), 3.66 (NCH_2Ar); irradiation at 2.79−2.83 (5-H): δ [ppm] = 2.63 (9-H), 2.90−3.01 (9-H, 4-H), 3.61 (NCH_2Ph).

(−)-(1*R*,4*R*,5*S*)-8-(4-Methoxybenzyl)-4-phenyl-6-propyl-6,8-diazabicyclo[3.2.2]nonan-2-one (31). The ketone **27** (200 mg, 0.66 mmol) was dissolved in dry chlorobenzene (20 mL). Then benzeneseleninic anhydride (262 mg, 0.73 mmol) was added and the mixture was heated to 95 °C for 16 h. Then the solvent was removed in vacuo and the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, CH_2Cl_2/methanol = 100/1, V = 10 mL, R_f = 0.11) to give a mixture of the α,β-unsaturated ketone **30** and the saturated ketone **27** (101 mg, ratio about 1/3), which was dissolved in THF (10 mL) and employed for the subsequent 1,4-addition reaction. Under N_2 atmosphere, copper(I) bromide−dimethyl sulfide complex (69 mg, 0.34 mmol) was suspended in THF (20 mL). The mixture was cooled to −50 °C, and a 2 M solution of phenyllithium in dibutyl ether (0.34 mL, 0.67 mmol) was added. The mixture was stirred at −50 °C for 1 h followed by 10 min at 0 °C. Then at −50 °C, the previously prepared solution of the α,β-unsaturated ketone **30** was added dropwise. The mixture was stirred at −50 °C for 15 min and at room temperature for 16 h. Then a 2 M NaOH solution was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (Ø = 1 cm, h = 30 cm, CH_2Cl_2/methanol = 100/1, V = 5 mL, R_f = 0.45) to give **31** as a yellow oil, yield 10 mg (4% over 2 steps). $[\alpha]_D^{20}$ = −38.3 (c = 0.20; CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ [ppm] = 0.60 (t, J = 7.0 Hz, 3H, $NCH_2CH_2CH_3$), 1.00−1.15 (m, 2H, $NCH_2CH_2CH_3$), 1.93 (ddd, J = 11.7/9.4/7.0 Hz, 1H, NC$H_2$$CH_2CH_3$), 2.27 (ddd, J = 11.7/8.6/4.7 Hz, 1H, NC$H_2$$CH_2CH_3$), 2.63 (dd, J = 14.1/3.1 Hz, 1H, 3-H), 2.73 (dd, J = 11.0/2.3 Hz, 1H, 7-H), 2.89−2.96 (m, 3H, 5-H, 7-H, 9-H), 3.03−3.08 (m, 1H, 9-H), 3.23−3.27 (m, 1H, 4-H), 3.28−3.31 (m, 1H, 1-H), 3.59 (dd, J = 14.1/9.4 Hz, 1H, 3-H), 3.67 (d, J = 13.3 Hz, 1H, NCH_2Ar), 3.71 (d, J = 13.3 Hz, 1H, NCH_2Ar), 3.81 (s, 3H, ArOCH_3), 6.86 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.15−7.25 (m, 7H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$, H_{phenyl} (5H)). NOE: irradiation at 1.93 ppm (NC$H_2$$CH_2CH_3$): δ [ppm] = 2.27 (NC$H_2$$CH_2CH_3$), 2.89−2.96 (5-H); irradiation at 2.27 ppm (NC$H_2$$CH_2CH_3$): δ [ppm] = 1.93 (NC$H_2$$CH_2CH_3$), 2.73 (7-H), 2.89−2.96 (5-H); irradiation at 2.73 ppm (7-H): δ [ppm] = 2.63 (3-H), 2.89−2.96 (7-H), 3.28−3.31 (1-H); irradiation at 3.03−3.08 ppm (9-H): δ [ppm] = 2.89−2.96 (9-H), 3.23−3.27 (4-H); irradiation at 3.59 ppm (3-H): δ [ppm] = 2.63 (3-H), 3.23−3.27 (4-H).

Receptor Binding Studies. Materials and General Procedures. The guinea pig brains and rat livers were commercially available (Harlan-Winkelmann, Germany). The protein concentration was determined according to the method of Bradford[27] using bovine serum albumin as standard. 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%.

Performing of the σ_1 Assay (Modified According to Refs 23, 26). 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.[28]

Performing of the σ_2 Assay (Modified According to Refs 23, 26). 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.[29]

NMDA Assay. The preparation of the receptor material and the assay were performed according to a literature procedure.[26]

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.[30] The K_i values are given as the mean value ± SEM from three independent experiments.

Cytotoxicity Assay.[25] 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.[25] To determine the IC_{50} values, five serially diluted stock solutions of test substance in DMSO 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.

Acknowledgment. This work was performed within the International Research Training Group "Complex Functional Systems in Chemistry: Design, Synthesis and Applications" in collaboration with the University of Nagoya. Financial support of this IRTG by the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

Supporting Information Available: Physical and spectroscopic data of all new compounds. Purity data of all test compounds. General chemistry methods. Details of the pharmacological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Walker, J. M.; Bowen, W. D.; Walker, F. O.; Matsumoto, R. R.; De Costa, B.; Rice, K. C. Sigma receptors: biology and function. *Pharmacol. Rev.* **1990**, *42*, 355–402.
(2) Quirion, R.; Bowen, W. D.; Itzhak, Y.; Junien, J. L.; Musacchio, J. M.; Rothman, R. B.; Su, T. P.; Tam, S. W.; Taylor, D. P. A proposal for the classification of sigma binding sites. *Trends Pharmacol. Sci.* **1992**, *13*, 85–86.
(3) Hanner, M.; Moebius, F. F.; Flandorfer, A.; Knaus, H.; Striessnig, J.; Kempner, E.; Glossman, H. Purification, molecular cloning, and expression of the mammalian sigma1-binding site. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8072–8077.
(4) Aydar, E.; Palmer, C. P.; Klyachko, V. A.; Jackson, M. B. The sigma receptor as a ligand-regulated auxiliary potassium channel subunit. *Neuron* **2002**, *34*, 399–410.
(5) Guitart, X.; Codony, X.; Monroy, X. Sigma receptors: biology and therapeutic potential. *Psychopharmacology (Berlin, Germany)* **2004**, *174*, 301–319.
(6) Skuza, G. Potential antidepressant activity of sigma ligands. *Pol. J. Pharmacol.* **2003**, *55*, 923–934.
(7) Aydar, E.; Palmer, C. P.; Djamgoz, M. B. A. Sigma Receptors and Cancer: Possible Involvement of Ion Channels. *Cancer Res.* **2004**, *64*, 5029–5035.
(8) Foster, A.; Wu, H.; Chen, W.; Williams, W.; Bowen, W. D.; Matsumoto, R. R.; Coop, A. 1,4-Dibenzylpiperazines possess anticocaine activity. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 749–751.
(9) Bedürftig, S.; Wünsch, B. Chiral, nonracemic (piperazin-2-yl)methanol derivatives with σ-receptor affinity. *Bioorg. Med. Chem.* **2004**, *12*, 3299–3311.
(10) Geiger, C.; Zelenka, C.; Weigl, M.; Fröhlich, R.; Wibbeling, B.; Lehmkuhl, K.; Schepmann, D.; Grünert, R.; Bednarski, P. J.; Wünsch, B. Synthesis of Bicyclic σ Receptor Ligands with Cytotoxic Activity. *J. Med. Chem.* **2007**, *50*, 6144–6153.
(11) Holl, R.; Schepmann, D.; Grünert, R.; Bednarski, P. J.; Wünsch, B. Relationships between the Structure of 6-Allyl-6,8-diazabicyclo[3.2.2]nonane Derivatives and their σ Receptor Affinity and Cytotoxic Activity. *Bioorg. Med. Chem.* **2009**, in press.
(12) Holl, R.; Schepmann, D.; Bednarski, P. J.; Grünert, R.; Wünsch, B. Relationships between the Structure of 6-Substituted 6,8-diazabicyclo[3.2.2]nonan-2-ones and their σ Receptor Affinity and Cytotoxic Activity. *Bioorg. Med. Chem.*, **2009**, in press.
(13) Jung, B.; Englberger, W.; Wünsch, B. Molecular modeling directed synthesis of a bicyclic analogue of the δ opioid receptor agonist SNC 80. *Arch. Pharm. Pharm. Med. Chem. (Weinheim, Germany)* **2005**, *338*, 281–290.
(14) Wolter, M.; Nordmann, G.; Job, G. E.; Buchwald, S. L. Copper-Catalyzed Coupling of Aryl Iodides with Aliphatic Alcohols. *Org. Lett.* **2002**, *4*, 973–976.
(15) Shi, Y.; Hughes, D. L.; McNamara, J. M. Stereospecific synthesis of chiral tertiary alkyl aryl ethers via Mitsunobu reaction with complete inversion of configuration. *Tetrahedron Lett.* **2003**, *44*, 3609–3611.
(16) Wünsch, B. A new method for the preparation of 3-alkoxy- and 3-hydroxy-3,4-dihydro-1*H*-2-benzopyrans. *Arch. Pharm. (Weinheim, Germany)* **1990**, *323*, 493–499.
(17) Robinson, B. Fischer indole synthesis. *Chem. Rev.* **1963**, 373–401.
(18) Cheng, C. C.; Yan, S. J. The Friedlander synthesis of quinolines. *Org. React. (New York)* **1982**, *28*, 37–201.
(19) Blanckaert, P.; Vandcapelle, M.; Staelens, L.; Burvenich, I.; Dierckx, R. A.; Slegers, G. Synthesis, radiosynthesis and preliminary in vivo evaluation of [^{123}I]-(4-fluorophenyl) {1-[2-(2-iodophenyl)ethyl]piperidin-4-yl}methanone, a potential 5-HT2A-antagonist for SPECT brain imaging. *J. Lab. Comp. Radiopharm.* **2004**, *47*, 591–598.
(20) Parham, W. E.; Jones, L. D.; Sayed, Y. A. Selective halogen-lithium exchange in bromophenylalkyl halides. *J. Org. Chem.* **1976**, *41*, 1184–1186.
(21) Barton, D. H. R.; Lester, D. J.; Ley, S. V. Dehydrogenation of steroidal and triterpenoid ketones using benzeneseleninic anhydride. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2209–2212.
(22) Bertz, S. H.; Gibson, C. P.; Dabbagh, G. New copper chemistry. 12. Preparation of lithium organocuprates from various Cu(I) salts. *Tetrahedron Lett.* **1987**, *28*, 4251–4254.
(23) Maier, C. A.; Wünsch, B. Novel Spiropiperidines as Highly Potent and Subtype Selective s-Receptor Ligands. Part 1. *J. Med. Chem.* **2002**, *45*, 438–448.
(24) Colabufo, N. A.; Berardi, F.; Contino, M.; Niso, M.; Abate, C.; Perrone, R.; Tortorella, V. Antiproliferative and cytotoxic effects of some σ2 agonists and σ1 antagonists in tumour cell lines. *Naunyn-Schmiedebergs Arch. Pharmacol.* **2004**, *370*, 106–113.
(25) Bracht, K.; Boubakari; Grunert, 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 National Cancer Institute data. *Anticancer Drugs* **2006**, *17*, 41–51.
(26) Wirt, U.; Schepmann, D.; Wünsch, B. Asymmetric synthesis of 1-substituted tetrahydro-3-benzazepines as NMDA receptor antagonists. *Eur. J. Org. Chem.* **2007**, *46*, 2–475.
(27) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein−dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.
(28) DeHaven-Hudkins, D. L.; Fleissner, L. C.; Ford-Rice, F. Y. Characterization of the binding of [3H](+)-pentazocine to s recognition sites in guinea pig brain. *Eur. J. Pharmacol.* **1992**, *227*, 371–378.
(29) Mach, R. H.; Smith, C. R.; Childers, S. R. Ibogaine possesses a selective affinity for σ2 receptors. *Life Sci.* **1995**, *57*, PL57−PL62.
(30) Cheng, Y. C.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

JM801522J