Synthesis and biological evaluation of conformationally restricted σ_1 receptor ligands with 7,9-diazabicyclo[4.2.2]decane scaffold†

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The key step in the synthesis of the 7,9-diazabicyclo[4.2.2]decane system was a modified Dieckmann condensation of piperazinebutyrate **11**, which makes use of trapping the first cyclized intermediate with TMS-Cl. Reduction of the bicyclic ketone **14** with $LiBH_4$ at –90 °C provided diastereoselectively (>99 : 1) the *syn*-configured alcohol **15a**, which was converted into the final alcohol and ethers **16a–g**. The configuration at the 2-position was established by X-ray structure analysis of methyl and ethyl ethers **15b** and **15c**. In contrast to bicyclic systems with a three-carbon bridge, inversion of the configuration at the 2-position of the alcohol **15a** failed to give the inverted alcohol **19a**. However, an unselective reduction of the ketone **24** with L-Selectride led to the diastereomeric alcohols **16a** and **25a** in the ratio 36 : 64. $LiAlH_4$ reduction of the tosylate **20** and the alkene **18** yielded the diazabicyclo-decane **26** and -decene **27** without further substituents at the four-carbon bridge. The σ_1 and σ_2 receptor affinities were investigated in receptor binding studies with radioligands. All test compounds showed a lower σ_1 affinity than the corresponding bicyclic derivatives with a three-membered bridge. The reduced σ_1 receptor affinity is attributed to the larger four-membered bridge. This hypothesis is supported by the alkene **27**, which represents the most potent σ_1 ligand of this series (K_i = 7.5 nM). In the alkene **27** the size and flexibility of the bridge is considerably reduced by the double bond. The methyl ether **25b** and the unsubstituted derivatives **26** and **27** revealed moderate inhibition of the growth of the human tumor cell lines A-427, 5637 and MCF-7. Again, these compounds are less potent than the analogues with a three-membered bridge. The IC_{50}-value of the most potent σ_1 ligand **27** against the small cell lung cancer cell line A-427 (IC_{50} = 10 μM) should be emphasized, since this cell line is particularly sensitive to homologues with a three-carbon bridge.

1. Introduction

σ Receptors, which were initially misclassified as a subtype of opioid receptors[1] are now generally accepted as a separate class of receptors including two subtypes termed σ_1 and σ_2 receptors.[2] Cloning of the σ_1 receptor subtype has proved that this receptor does not have any structural similarity with any other known mammalian protein. However 30% homology to the yeast enzyme Δ^8/Δ^7-isomerase was found.[3] Aydar *et al.* have postulated a σ_1 receptor model with two transmembrane domains and both the amino and carboxy termini located intracellularly.[4] Site directed mutagenesis experiments have unraveled that the acidic amino acids aspartate 126 and glutamate 172 in the carboxy terminus,[5] as well as serine 99 and tyrosine 103, which are located in or close to the second transmembrane helix (first steroid binding domain) are crucial for (+)-pentazocine binding.[6]

Although the intracellular signal transduction pathway is not yet elucidated, σ_1 receptors are involved in the modulation of various systems including the glutamatergic,[7] dopaminergic[8] and cholinergic[9] neurotransmission. Additionally, the influence on the activity of a variety of ion channels including K^+-channels[10,11] and Ca^{2+}-channels[12] is an important feature of σ_1 receptors.

Both σ receptor subtypes are involved in neuromodulatory processes and, in particular the σ_1 receptor can be exploited as target for the development of novel drugs for the treatment of different neurological disorders, *e.g.* schizophrenia,[13] depression,[14] dementia[15] and cocaine induced locomotor activity and toxicity.[16] In addition to the relevance of σ receptors in neurological disorders, overexpression of σ_1 and σ_2 receptors in various human tumor cell lines, including breast, lung and prostate cancer cell lines, has been found.[17,18] Since antiproliferative and cytotoxic activity of σ_1 antagonists and σ_2 agonists has been shown, both σ receptor subtypes represent interesting targets for the development of novel antitumor drugs.[9]

The class of σ_1 receptor ligands comprises potent but structurally very diverse ligands, including aryl alkyl amines (*e.g.* **1**),[19] guanidine derivatives (*e.g.* di-*o*-tolylguanidine **2**),[20] dextrorotatory benzomorphans (*e.g.* (+)-pentazocine **3**),[21] and spirocyclic compounds (*e.g.* **4**) (Fig. 1).[22–27]

Based on structure affinity relationships of a series of *N*,*N*-dialkylamine derivatives Glennon has established a pharmacophore model for σ_1 receptor ligands. According to this model a potent σ_1 receptor ligand should contain a basic amine, which is substituted with two hydrophobic substituents at distances of

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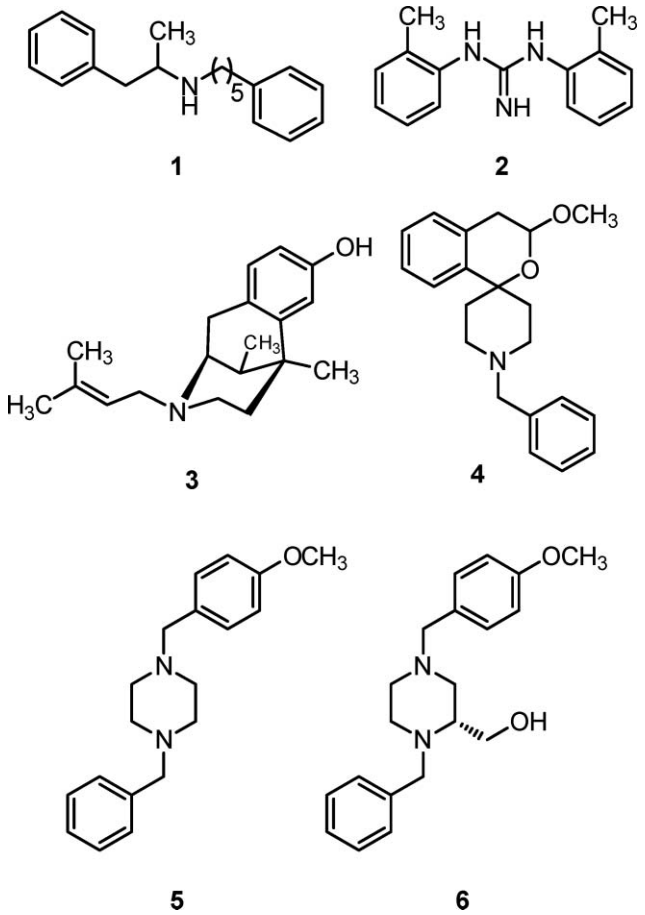


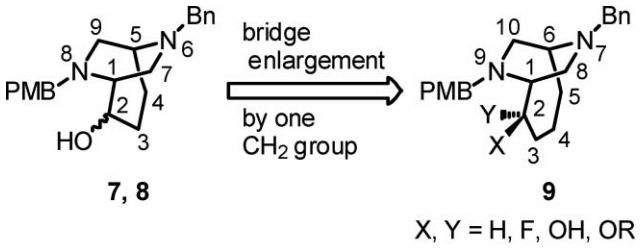
Fig. 1 Structurally diverse σ_1 receptor ligands.

2.5–3.9 Å and 6–10 Å.[19] However, the two-dimensional model does not reflect the three dimensional orientation of the particular pharmacophoric elements. For the development of a more detailed three dimensional model, novel σ_1 receptor ligands with restricted conformational flexibility and defined stereochemistry are required.

Several σ_1 receptor ligands contain the ethylenediamine substructure as a pivotal pharmacophoric element. The piperazines **5** (σ_1: K_i = 0.47 nM)[28] and **6** (σ_1: K_i = 12.4 nM)[29] are further examples for potent σ_1 ligands with the ethylenediamine substructure.

Recently we have reported on the synthesis and pharmacological evaluation of 6,8-diazabicyclo[3.2.2]nonanes **7** and **8** representing conformationally restricted piperazine derivatives with a bridge consisting of three carbon atoms.[30] The enantiomeric pairs **7**/*ent*-**7** and **8**/*ent*-**8** have almost the same σ_1 affinity, whereas the σ_1 affinities of the diastereomers **7**/**8** and *ent*-**7**/*ent*-**8** differ considerably. Obviously the relative orientation of the OH moiety in the bicyclic framework is responsible for high σ_1 receptor affinity (Fig. 2). In order to learn more about the influence of the bridge size, its type of substituents and the relative configuration on the σ_1 affinity, bicyclic ligands **9** with a four-carbon bridge were envisaged. In addition to the increased size of the bridge in **9** the orientation of the substituents at the 2-position is changed when compared with compounds **7** and **8** with a three-carbon bridge.

Herein, we report on the conformational analysis, synthesis and pharmacological evaluation of 7,9-diazabicyclo[4.2.2]decanes



Compd.	configuration	σ_1: K_i (nM)
7	(1*R*,2*R*,5*S*)	6.5
ent-**7**	(1*S*,2*S*,5*R*)	7.5
8	(1*R*,2*S*,5*S*)	125
ent-**8**	(1*S*,2*R*,5*R*)	118

Fig. 2 Comparison of the lead compounds **7** and **8** with the planned σ_1 ligands **9**.

9 with various substituents and different stereochemistry at the 2-position.

2. Conformational analysis

At first the conformational flexibility of the largest bridge of 6,8-diazabicyclo[3.2.2]nonanes (three-carbon bridge) and 7,9-diazabicyclo[4.2.2]decanes (four-carbon bridge) as well as the orientation of the substituent at the 2-position were considered theoretically. For this purpose a stochastic conformational search (molecular modeling program MOE[31]) was performed with model compounds (1*R*,2*R*,5*S*)-**A** (model for **7**), (1*R*,2*S*,5*S*)-**B** (model for **8**), (1*R*,2*R*,6*S*)-**C** (model for **16a**) and (1*R*,2*S*,6*S*)-**D** (model for **25a**), which are substituted with small methyl groups at both N-atoms (Fig. 3). Subsequent geometry optimization resulted in 4–10 conformations (cutoff energy 7 kcal mol^{-1}). (see Tables 6–9 in the Supporting Information†).

For all types of compounds two different conformations of the bridge were found with considerably lower energy than the residual conformations. The relative energies and the dihedral angles N$^{8/9}$–C^1–C^2–OH of these conformations are summarized in Table 1. As examples the two energetically most favored conformations of the model compounds (1*R*,2*R*,5*S*)-**A** and (1*R*,2*R*,6*S*)-**C** are depicted in Fig. 3. It can be seen clearly that the bridges of both bicyclic systems can adopt two generally different conformations, which result in considerably different dihedral angles of 157° and −158° for the three-membered bridge of (1*R*,2*R*,5*S*)-**A** and −165° and 164° for the four-membered bridge of (1*R*,2*R*,6*S*)-**C**.

Although being rather similar the dihedral angles of the bicyclic systems (1*R*,2*R*,5*S*)-**A** and (1*R*,2*S*,5*S*)-**B** with a three-membered bridge differ by 2–16° from the dihedral angles of (1*R*,2*R*,6*S*)-**C** and (1*R*,2*S*,6*S*)-**D** with a four-membered bridge indicating a fine tuning of the orientation of the OH-moiety attached at the 2-position. Moreover the additional methylene moiety within the bridge of the bicyclic systems **C** and **D** leads to an increased occupied space by the larger bridge.

Table 1 Relative energies and dihedral angles of the energetically most favored conformations of model compounds **A–D**

Model for compd.	Compd.	Conformation	ΔE/kcal mol^{-1}	Dihedral angle N$^{8/9}$–C^{1}–C^{2}–OH (°)
7	(1*R*,2*R*,5*S*)-**A**	1	0.00	157
		2	0.69	−158
8	(1*R*,2*S*,5*S*)-**B**	1	0.00	−83
		2	0.57	−35
16a	(1*R*,2*R*,6*S*)-**C**	1	0.00	−165
		2	2.90	164
25a	(1*R*,2*S*,6*S*)-**D**	1	0.00	−51
		2	3.44	−81

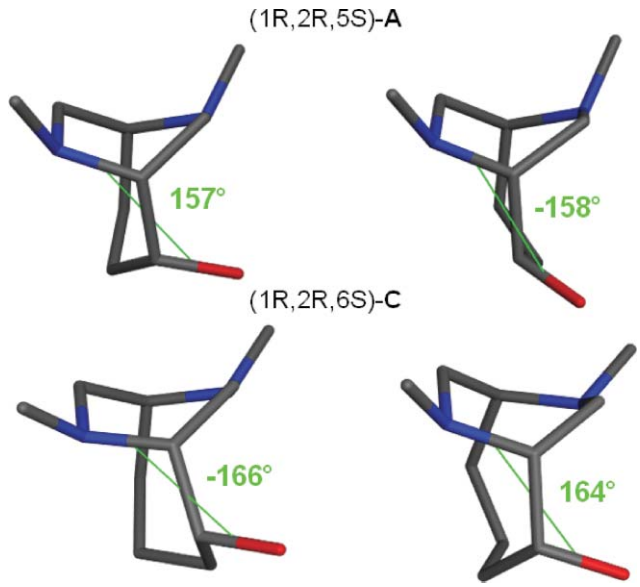


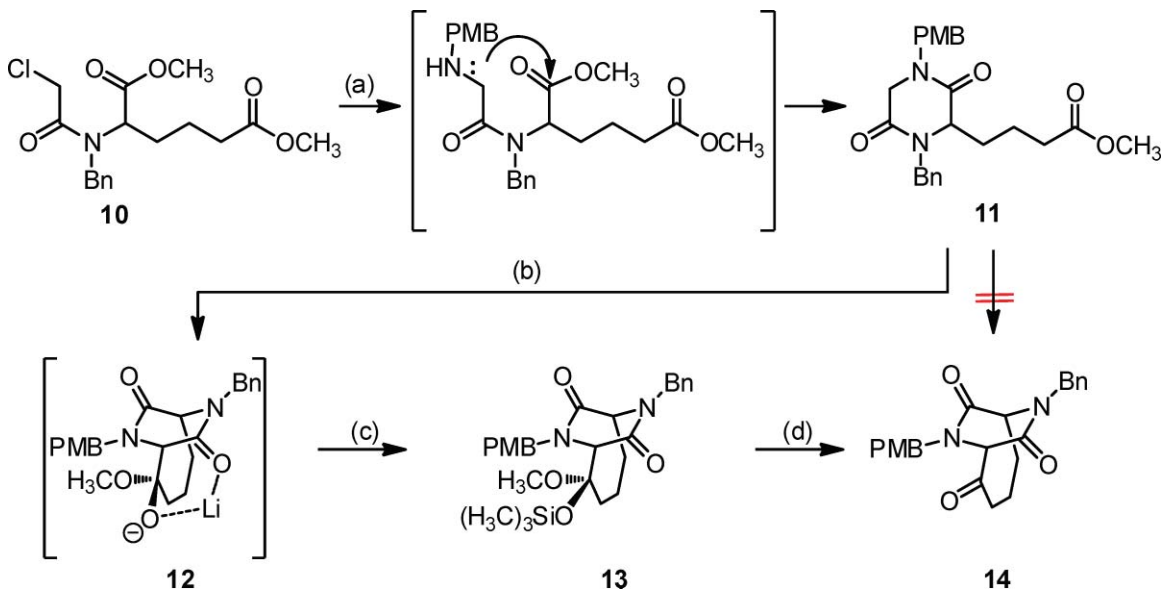
Fig. 3 Energetically most favored conformations of model compound (1*R*,2*R*,5*S*)-**A** (model for **7**, top) compared with those of model compound (1*R*,2*R*,6*S*)-**C** (model for **9**, bottom).

3. Chemistry

Recently we have reported on the synthesis of racemic chloroacetamide **10**, which started from racemic 2-aminoadipic acid.[32] Treatment of the chloroacetamide **10** with *p*-methoxybenzylamine (PMB-NH_2) afforded in a one-pot procedure the dioxopiperazine **11** in 96% yield. In the first step a S_N2 reaction of **10** with *p*-methoxybenzylamine led to a secondary amine, which reacted directly with the ester in an intramolecular aminolysis to give the dioxopiperazine **11** (Scheme 1).

The classical Dieckmann condensation of the dioxopiperazine **11** (*e.g.* with KHMDS), did not provide the bicyclic ketone **14** due to reduced stabilization of the anion of the resulting β-ketoamide **14**. Usually, the driving force in ester condensations under equilibrium conditions is the formation of the enolate of the corresponding β-ketoester. Due to the negative charge on the bridgehead position, the enolate of **14** is rather unstable (compare Bredt's rule[33]), which explains the failure of the direct Dieckmann cyclization to obtain **14**. (Scheme 1).

In order to establish the four-carbon bridge, a Dieckmann analogous cyclization[34] was followed, which involves trapping of the first cyclized intermediate **12** with trimethylsilyl chloride to afford diastereoselectively the mixed methyl trimethylsilyl ketal



Scheme 1 Synthesis of dioxopiperazine **11** and its Dieckmann analogous cyclization. Reagents and reaction conditions: (a) 4-methoxybenzylamine, NEt_3, CH_3CN, rt, 18 h, 96%. (b) LHMDS, THF, 0.5 h, −78 °C then (c) Me_3SiCl, 2 h, −78 °C, 0.5 h, rt, 89%. (d) *p*-toluenesulfonic acid, THF–H_2O, rt, 16 h, 99%.

13. Hydrolysis of the mixed ketal **13** produced the bicyclic ketone **14** in 99% yield (Scheme 1). In analogy to the corresponding mixed ketal of the *N*-methyl analogue[32] and the mixed ketals of bicyclic systems with shorter bridges[34–38] we assume that the TMSO group is oriented towards the carbonyl moiety in position 8, since the corresponding Li^+-alcoholate **12** will be stabilized by forming a chelate with this carbonyl moiety.

The ketone **14** was the central building block for the synthesis of σ_1 receptor ligands with a 7,9-diazabicyclo[4.2.2]decane scaffold. At first **14** was reduced chemo- and diastereoselectively with $LiBH_4$ at –90 °C to provide the alcohol **15a** in 87% yield. The unequivocal assignment of the configuration of the newly formed center of chirality at the 2-position was not possible by different NMR spectroscopic techniques. Since the alcohol **15a** appeared to be a spongy solid, performing of an X-ray crystal structure analysis was not possible. However, the methyl ether **15b**, which was prepared by a Williamson ether synthesis of the alcohol **15a** with CH_3I and NaH (Scheme 2) gave nice crystals, which upon recrystallization from ethyl acetate–*n*-hexane mixture were suitable for X-ray crystal structure analysis. The X-ray crystal structure analysis revealed the relative configuration as (1*RS*,2*SR*,6*RS*) for the methyl ether **15b** (Fig. 4).

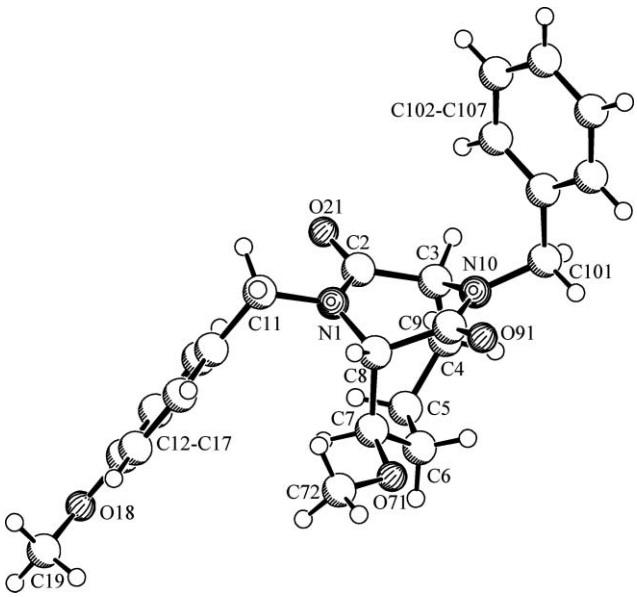
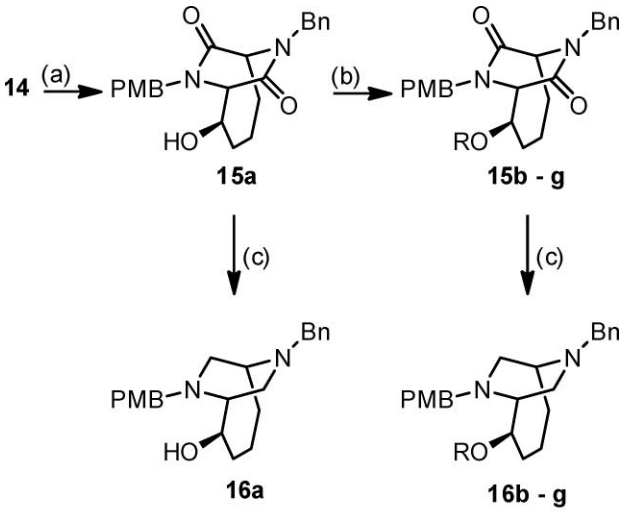


Fig. 4 X-Ray crystal structure of methyl ether **15b**.

The corresponding ethers **15c–g** were prepared using the same conditions (NaH, R-X) (Scheme 2). A second X-ray crystal structure analysis of the ethyl ether **15c** was also recorded (Fig. 5). A comparison of the dihedral angles and angles between the planes of different atoms of the crystal structures of methyl ether **15b** and ethyl ether **15c** showed that the orientation of the bridge in both compounds is very similar. We assume that the orientation of the bridge in the test compounds **16** with different 2-alkoxy groups and with the same configuration is very similar to the orientation of the bridge in the dilactam systems **15b** and **15c**. This assumption is supported by comparing the orientation of the four-membered bridge of the energetically most favored conformation of model compound (1*R*,2*R*,6*S*)-**C** (see Fig. 3, conformation of the left side)



15, 16	R
a	H
b	CH_3
c	CH_2CH_3
d	$CH_2CH_2CH_3$
e	$CH_2CH_2CH_2CH_3$
f	$CH_2CH_2CH(CH_3)_2$
g	$CH_2C_6H_5$

Scheme 2 Synthesis of bicyclic piperazines **15a–g** and **16a–g**. Reagents and reaction conditions: (a) $LiBH_4$, THF, –90 °C, 2.5 h, 87%. (b) NaH, RX, *n*-$Bu_4N^+I^-$, THF, 1.5–24 h, 62–99%. (c) $LiAlH_4$, THF, reflux, 12–24 h, 23–96%.

with the conformation of the bridge in the energetically favored solid state of the dilactams **15b** and **15c**.

Finally, the two lactam groups of the bridged piperazinediones **15a–g** were reduced with $LiAlH_4$ in boiling THF[39] to produce the tertiary amines **16a–g** (Scheme 2).

For the evaluation of the pharmacological activity of the 7,9-diazabicyclo[4.2.2]decane compound class the diastereomers of **16** with opposite configuration in position 2 were to be synthesized. At first different reducing agents for the reduction of the ketone **14** were investigated with the aim of finding a method for the stereoselective synthesis of the diastereomeric alcohol **19a** (Table 2).

The results in Table 2 reveal that all reducing agents led preferentially to the alcohol **15a**. $LiBH_4$ at –90 °C gave exclusively the alcohol **15a**. The lowest diastereoselectivity was observed for the reduction of **14** with $NaBH_4$ at rt. However, the alcohol **19a** was still formed as minor product (21%). Moreover, the separation of the two diastereomeric alcohols **15a** and **19a** was not possible by flash chromatography due to their similar interactions with silica gel.

Next it was planned to invert the configuration of the alcohol **15a** by a Mitsunobu reaction.[40] Unfortunately, Mitsunobu reaction of the alcohol **15a** with *p*-nitrobenzoic acid, diisopropyl

Table 2 Reduction of ketone **14** with various reducing agents

Reducing agent	Temp.	Time	Ratio[a] **15a**:**19a**
$LiBH_4$	−90 °C	3 h	>99 : 1
$NaBH_4$	rt	5 h	79 : 21
BH_3·THF	rt	1 h	84 : 16
KS-Selectride	−80 °C	2 h	93 : 7
BH_3·THF	−78 °C	1 h	93 : 7
L-Selectride	−80 °C	3 h	97.5 : 2.5
DIBAL	68 °C	1.5 h	98.0 : 2.0
$LiAlH_4$	−78 °C	15 min	99.0 : 1.0

[a] The ratio of diastereomeric alcohols **15a** and **19a** was determined by ^{1}H NMR spectroscopy.

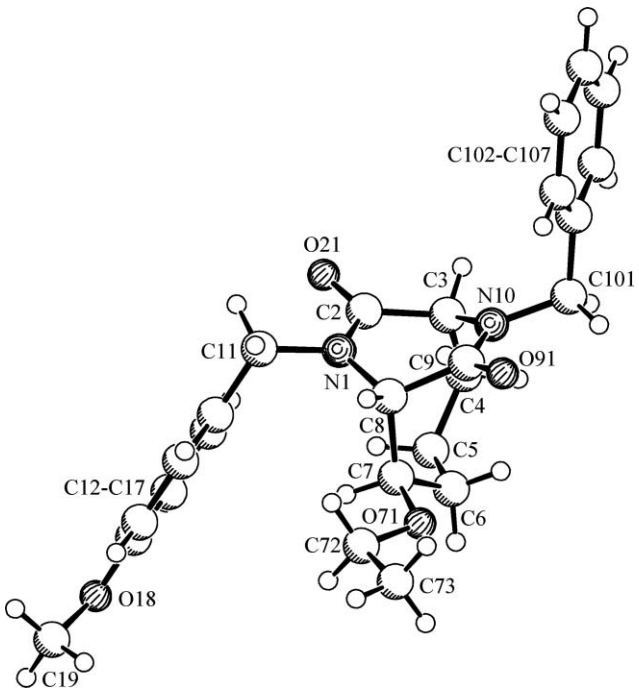


Fig. 5 X-Ray crystal structure of ethyl ether **15c**.

azodicarboxylate (DIAD) and triphenylphosphine (PPh_3) gave an inseparable mixture of inverted *p*-nitrobenzoate **17** and elimination product **18**. The mixture was treated with CH_3OH and K_2CO_3 to transform the ester **17** into the inverted alcohol **19a** in 6% yield, whereas the elimination product **18** was isolated in 50% yield (Scheme 3).

In the ^{1}H NMR spectrum of **19a**, the coupling constants of 1-H (singlet at 4.20 ppm) differ considerably from those of the starting alcohol **15a** (doublet at 4.06 ppm, ^3J = 4.9 Hz) confirming the inversion of configuration at position 2.

The low yield (6%) of the inverted alcohol **19a** encouraged us to modify the Mitsunobu reaction of the alcohol **15a**. In particular more reactive azodicarboxylates (azodicarbonyldipiperidine (ADDP), diethyl azodicarboxylate (DEAD)), modified phosphines (PBu_3) and different acids (acetic acid, *p*-nitrobenzoic acid, *p*-methoxybenzoic acid) were employed.[40] However, all these variations did not improve the yield of inverted alcohol **19a**.

Moreover, the three step inversion of the configuration by activation of the alcohol **15a** as tosylate **20** followed by substitution with CH_3CO_2Na and *p*-$NO_2C_6H_4CO_2K$ failed (Scheme 3). Additionally, the activation of alcohol **15a** as mesylate, trichloromesylate, nosylate and triflate followed by S_N2 reaction with different nucleophiles (CH_3CO_2Na, *p*-$NO_2C_6H_4CO_2K$, KNO_2 and KNO_3) in different solvents (DMF, acetone, acetonitrile) also failed to give the inverted alcohol **19a**. Generally inseparable mixtures of retention and inversion products together with elimination product **18** were formed.

Since all attempts to invert the configuration of the conformationally restricted alcohol **15a** had failed, the Mitsunobu inversion was tried with the more flexible reduced bicyclic alcohol **16a**. The transformation of **16a** with DIAD–PPh_3 and *p*-nitrobenzoic acid proceeded smoothly providing the *p*-nitrobenzoate **21** in 69% yield. Subsequently, the ester **21** was cleaved with CH_3OH–K_2CO_3 (Scheme 3). Surprisingly, the ^{1}H NMR spectra of the hydrolysis product and the starting alcohol **16a** were completely identical. The X-ray crystal structure analysis of the *p*-nitrobenzoate **21** (Fig. 6) confirmed that the Mitsunobu reaction of **16a** had taken place with retention of configuration at the 2-position. We assume that the retention of configuration is due to the anchimeric assistance of the tertiary amine (*N*-9) of the piperazine alcohol **16a** initiating a double inversion.

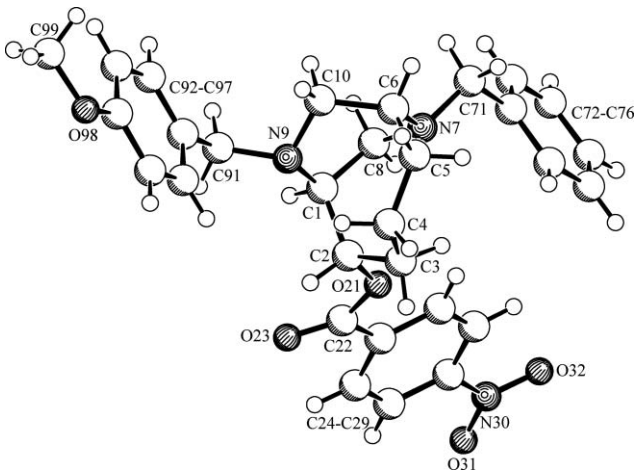
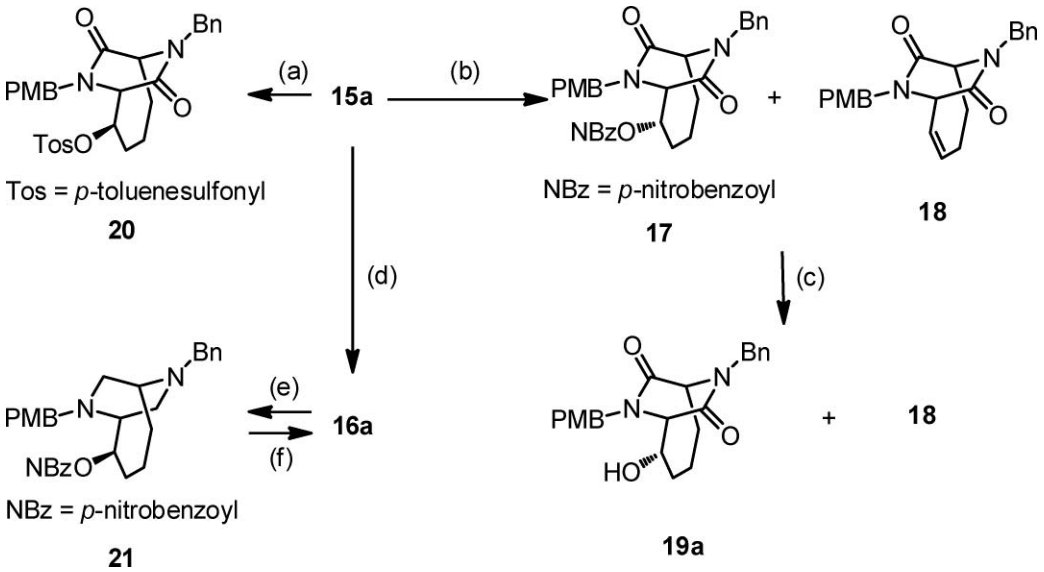


Fig. 6 X-Ray crystal structure of *p*-nitrobenzoate **21**.

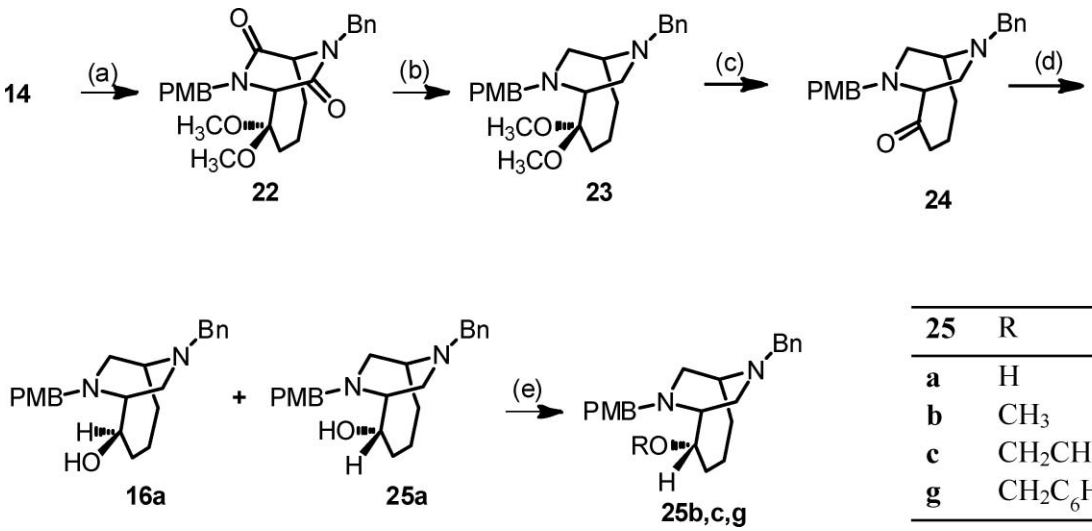
Finally, reduction of the ketone in position 2 of the bridged piperazine **24** was envisaged instead of reduction of the ketone in the bicyclic dilactam **14**. For the preparation of **24**, the ketone **14** was protected as dimethyl ketal **22**. Subsequent reduction of **22** with $LiAlH_4$ in boiling THF afforded the ketal **23**, which was hydrolyzed with diluted HCl to yield the ketone **24**.

Reduction of **24** with L-Selectride at rt provided the diastereomeric alcohols **16a** and **25a** in the ratio of 36 : 64, which were separated by flash chromatography. The desired diastereomer **25a** was isolated in 27% yield (Scheme 4). The alkylation of the alcohol **25a** was performed using the same reaction conditions, *i.e.* NaH, THF, RX, as for the alkylation of the alcohol **15a** containing lactam moieties in the bicyclic system. Surprisingly these conditions did not lead to the ethers **25b** and **25c**. However, after changing the solvent from THF to DMF the ethers **25b**, **25c**, and **25g** were obtained in 55–72% yield (Scheme 4).

In order to include bicyclic compounds without substituents in position 2 into the structure–affinity study the tosylate **20**, which had been synthesized for purpose of substitution reactions, was



Scheme 3 Inversion of the alcohols **15a** and **16a**. Reagents and reaction conditions: (a) *p*-toluenesulfonyl chloride, powdered KOH, CH_2Cl_2, 0 °C, 2.5 h, 94%. (b) PPh_3, DIAD, *p*-nitrobenzoic acid, THF, reflux, 18 h. (c) CH_3OH, K_2CO_3, rt, 16 h, 6% (**19a**, calculated over 2 steps), 50% (**18** calculated over 2 steps). (d) $LiAlH_4$, THF, reflux, 15 h, 95%. (e) PPh_3, DIAD, *p*-nitrobenzoic acid, THF, rt, 16 h, 69%. (f) CH_3OH–CH_2Cl_2 (5 : 1), K_2CO_3, rt, 16 h, 97%.



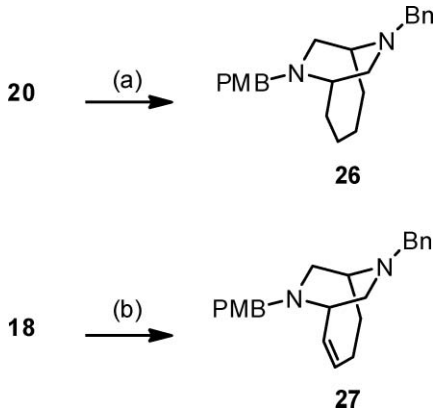
25	R
a	H
b	CH_3
c	CH_2CH_3
g	$CH_2C_6H_5$

Scheme 4 Synthesis of bicyclic piperazines **25a–c**, **g** with inverted configuration at the 2-position. Reagents and reaction conditions: (a) $HC(OCH_3)_3$, MeOH, *p*-toluenesulfonic acid, reflux, 15 h, 99.9%. (b) $LiAlH_4$, THF, reflux, 12 h, 80%. (c) 1 M HCl, THF–H_2O (1 : 1), rt, 10 h, 77%. (d) L-Selectride, THF, rt, 12 h, **25a** (28%), **16a** (24%) (e) NaH, DMF, rt, 3 h, **25b** (70%), **25c** (72%), **25g** (55%).

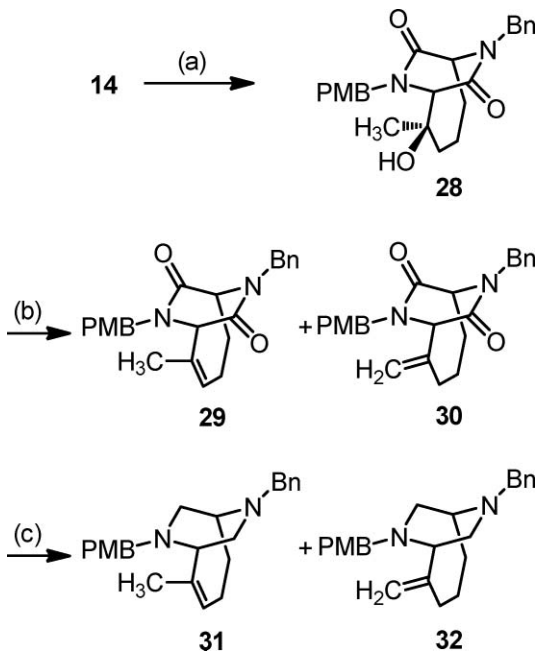
treated with $LiAlH_4$ to afford the unsubstituted compound **26** in 65% yield. Analogously the unsaturated dilactam **18**, which had been obtained as side product in the Mitsunobu reactions, was reduced with $LiAlH_4$ in boiling THF to give the bicyclic alkene **27** in 71% yield (Scheme 5).

The high σ_1 affinity and selectivity of the unsubstituted alkene **27** (K_i (σ_1) = 7.5 nM) stimulated the synthesis of the 2-methyl substituted alkene **31**. For this purpose, the ketone **14** was reacted with CH_3MgBr in THF to obtain the tertiary alcohol **28** as a single diastereomer in 48% yield. The tertiary alcohol was dehydrated with P_4O_{10} in toluene to provide two regioisomeric alkenes **29** and **30** in the ratio 2 : 3, according to the ^1H NMR spectrum of the crude sample (Scheme 6). Since separation of the regioisomers **29** and **30** was not possible by flash chromatography, the mixture of **29** and **30** was reduced with $LiAlH_4$ to obtain the piperazines **31** and **32**. After flash chromatography, the methylene derivative **32** was isolated in 54% yield whereas the methylated alkene **31** was isolated in very low amounts (<1 mg, 5%). (Scheme 6)

The introduction of fluorine atoms in position 2 as bioisosteric replacements of O-substituents was envisaged. Thus, the ketone **14** was treated with DAST[41] at −78 °C in CH_2Cl_2, which resulted in an inseparable 3 : 1 mixture of difluoro compound **33** and fluoroalkene **34** (Scheme 7). This mixture was reduced with $LiAlH_4$ in THF under reflux conditions.[39] Surprisingly, these reaction conditions converted both compounds **33** and **34** into fluoroalkene **35**, which was isolated in 54% yield.



Scheme 5 Synthesis of bicyclic compounds **26** and **27** without substituents in position 2. Reagents and reaction conditions: (a) $LiAlH_4$, THF, rt, 12 h then reflux, 12 h, 65%. (b) $LiAlH_4$, THF, reflux, 12 h, 71%.

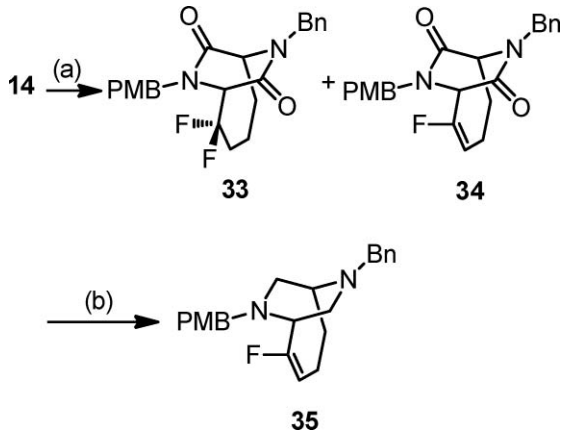


Scheme 6 Synthesis of methylene substituted bicyclic compound **32**. Reagents and reaction conditions: (a) CH_3MgBr, THF, −78 °C, 10 h then rt, 24 h, 48%. (b) P_4O_{10}, toluene, 90 °C, 12 h, 32% (**29** + **30**). (c) $LiAlH_4$, THF, reflux, 12 h, **32** (54%).

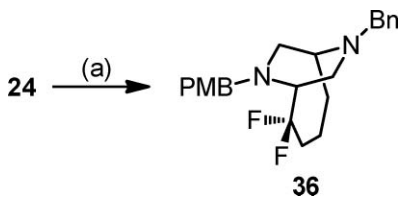
The synthesis of difluoro substituted compound **36** was achieved by treatment of piperazine ketone **24** with DAST at 0 °C in CH_2Cl_2 in 95% yield (Scheme 8).

4. Receptor affinity

The σ_1 and σ_2 receptor affinities of the bridged piperazines were determined in competition experiments with radioligands. In the σ_1 assay membrane preparations of guinea pig brains were used as receptor material and [^{3}H]-(+)-pentazocine as radioligand. The non-specific binding was determined in the presence of a large excess of non-tritiated (+)-pentazocine. Homogenates of rat liver served as source for σ_2 receptors in the σ_2 assay. Since a σ_2 selective radioligand is not commercially available, the non-selective radioligand [^{3}H]-di-*o*-tolylguanidine was employed in the



Scheme 7 Synthesis of fluoroalkene **35**. Reagents and reaction conditions: (a) DAST, CH_2Cl_2, −78 °C, 12 h, 81% (**33** +,**34**). (b) $LiAlH_4$, THF, reflux, 12 h, 54%.



Scheme 8 Synthesis of difluoride **36**. Reagents and reaction conditions: (a) DAST, CH_2Cl_2, 0 °C, 12 h, 95%.

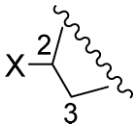
presence of an excess of non-radiolabeled (+)-pentazocine for selective masking of σ_1 receptors. An excess of non-tritiated di-*o*-tolylguanidine was used for determination of the non-specific binding.[22,26,42,43]

In the (1*RS*,2*RS*,6*SR*) series, the alcohol **16a** has moderate σ_1 affinity (K_i = 298 nM) and transformation of the alcohol **16a** into the methyl ether **16b** led to decreased σ_1 affinity (K_i > 1 μM) (Table 3). Enlargement of the alkyl residue to an ethyl group **16c** (K_i = 552 nM) or propyl group **16d** (K_i = 503 nM) retained the σ_1 affinity, whereas further extension of the O-residue to a butyl residue **16e** led to almost complete loss of σ_1 affinity (K_i > 1 μM). Interestingly, introduction of a branched residue, *e.g.* an isopentyl moiety, resulted in the most potent σ_1 ligand **16f** (K_i = 123 nM) of the ether series. In analogy to the butyl ether **16e** the benzyl ether **16g** reveals only very low σ_1 affinity (K_i > 1 μM).

The diastereomeric alcohol **25a** and ethers **25b**, **25c** and **25g** ((1*RS*,2*SR*,6*SR*)-configuration) show only very low σ_1 affinity. The σ_1 receptor affinity of the 2,2-dimethoxy derivative **23** is also very low. Since the smaller homologous alcohols **7**, *ent*-**7**, **8**, *ent*-**8** (compare Fig. 2) and the corresponding methyl ethers represent potent σ_1 receptor ligands,[30] it can be concluded that expansion of the bridge from three to four methylene moieties is unfavorable for high σ_1 receptor affinity. Either the changed orientation of the 2-OR moiety on the four-membered bridge or the increased spatial demand of the larger bridge itself may be the reason for the reduced σ_1 affinity. However, in both bicyclic systems with a three- and four-membered bridge the same effect of the stereochemistry is observed, because **7**/*ent*-**7** and **16a** show higher σ_1 receptor affinities than **8**/*ent*-**8** and **25a**, respectively.

Some derivatives without a substituent at the 2-position have been synthesized in order to check the tolerance of the σ_1 receptor

Table 3 σ_1 and σ_2 receptor affinities of 7,9-diazabicyclo[4.2.2]decanes



Compd.		$K_i \pm$ SEM[a]/nM		
		σ_1	σ_2	σ_1/σ_2 selectivity
16a	OH	298 ± 28	4.8 μM	—
16b	OCH_3	>1 μM	>1 μM	—
16c	OCH_2CH_3	552	>1 μM	—
16d	$OCH_2CH_2CH_3$	503	>1 μM	—
16e	$OCH_2CH_2CH_2CH_3$	>1 μM	>1 μM	—
16f	$OCH_2CH_2CH(CH_3)_2$	123 ± 19	>1 μM	—
16g	$OCH_2C_6H_5$	17 μM	>1 μM	—
25a	OH	>1 μM	640 nM	—
25b	OCH_3	600 nM	>1 μM	—
25c	OCH_2CH_3	>1 μM	>1 μM	—
25g	$OCH_2C_6H_5$	>1 μM	>1 μM	—
23	2-C: $C(OCH_3)_2$	>1 μM	>1 μM	—
26	H	253 ± 34	674	2.7
27	2/3-CH=CH-	7.5 ± 1.57	184	25
32	2-C: $C=CH_2$	1.0 μM	>1 μM	—
35	2/3-CF=CH	361	387	≈ 1
36	2-C: CF_2	1.0 μM	619	0.62
(+)-Pentazocine		5.6 ± 2.2	—	—
Haloperidol		6.3 ± 1.6	78 ± 2.3	12
Ditolylguanidine		89 ± 29	57 ± 18	0.64

[a] SEM values are determined only for high affinity (K_i < 300 nM) compounds.

with respect to 2-substitution. The unsubstituted derivative **26** has almost the same σ_1 affinity as the alcohol **16a**. This result indicates that the changed orientation of the 2-OH or 2-OCH_3 moiety is not the reason for the reduced σ_1 affinity but the increased size of the four-carbon bridge itself. Moreover, the analogous bicyclic compound with an unsubstituted three-carbon bridge represents a very potent σ_1 ligand ((*S*,*S*): K_i = 0.91 nM; (*R*,*R*): K_i = 4.4 nM).[44] This hypothesis is further supported by introduction of a double bond into the four-carbon bridge (**27**), which results in reduced bridge size and high σ_1 affinity of the bicyclic alkene **27** (K_i = 7.5 nM). The corresponding bicyclic compounds with an unsaturated three-membered bridge bearing only a small allyl substituent at the 6-position are also very potent σ_1 ligands.[44]

An additional fluorine atom in position 2 of the alkene substructure (**35**) again leads to reduced σ_1 affinity in the range of the σ_1 affinity of the alcohol **16a** and the alkane **26**. An *exo*-methylene moiety (**32**) and two fluorine atoms (**36**) are too big to be tolerated by the σ_1 receptor protein. Altogether it can be concluded that a reduced size of the bridge is favoring σ_1 affinity.

The most potent σ_1 ligands of this series **27**, **16a**, **16f** and **26** interact with higher affinity with σ_1 receptors than with σ_2 receptors and the most potent compound **27** is the most selective one (σ_1 : σ_2 = 25). On the contrary a slight preference for the σ_2 subtype is observed for the inverted alcohol **25a** and the difluoride **36**. However the corresponding K_i-values (*ca.* 650 nM) are rather high, so that both compounds cannot be considered as potent σ_2 ligands.

5. Inhibition of growth of human tumor cell lines

6,8-Diazabicycyclo[3.2.2]nonane derivatives with a methoxy moiety in position 2 are able to inhibit the growth of some human tumor cell lines. The human small cell lung cancer cell line A-427 is particularly sensitive to these bicyclic σ_1 ligands.[30] Therefore, the antiproliferative effects of some of the synthesized compounds were investigated in a panel of six human tumor cell lines, including the cell lines A-427 (small cell lung cancer), 5637 (bladder cancer), RT-4 (bladder cancer), LCLC-103H (large cell lung cancer), MCF-7 (breast cancer) and DAN-G (pancreas cancer).

In the primary screening the tumor cells were incubated with a 20 μM solution of the test compound 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. The IC_{50} values of all active compounds were determined by subjecting the cells 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.[45]

In the primary screening the compounds **26** and **27** without further substituents at the bridge showed considerable inhibition of the growth of tumor cells. (Table 4) The growth of the tumor cell lines A-427, 5637 and MCF-7 was moderately inhibited while little or no activity for any of the compounds was seen in the DAN-G, LCLC-103H and RT-4 cell lines. For compounds that inhibited cell growth by > 50% at 20 μM, IC_{50}-values were determined.

Table 5 shows that the bladder cancer cell line 5637 is particularly sensitive towards the methyl ether **25b**, and the unsubstituted compounds **26** and **27**, which gave IC_{50} values of 6.8, 8.8 and 13 μM, respectively. In addition to the growth inhibition of the tumor cell line 5637, the growth of the small cell lung cancer cell line A-427 and the breast cancer cell line MCF-7 are also inhibited.

The bicyclic derivative **27**, with an unsaturated bridge, represents the most potent σ_1 ligand (K_i = 7.5 nM) of this novel compound class. In addition to its high σ_1 receptor affinity **27** inhibits the growth of the cell line A-427 with an IC_{50}-value of 10 μM, which represents the highest activity within this compound class against this cell line. Although a clear correlation between

Table 4 Cell growth inhibitory activity (% of untreated control) of 7,9-diazabicyclo[4.2.2]decane derivatives in six human tumor cell lines[a]

	A-427[b]	5637[c]	MCF-7[d]	DAN-G[e]	LCLC-103H[f]	RT-4[g]
16a	86 ± 18	78 ± 15	63 ± 29	92 ± 11	125 ±30	72 ± 1
16b	53 ± 23	45 ± 18	68 ± 22	76 ±11	122 ± 45	62 ±13
25a	85 ± 23	71 ±19	63 ± 17	80 ± 6	147 ± 40	82 ± 14
25b	25 ± 9	28 ±13	37 ± 17	70 ± 3	114 ± 12	53 ± 16
26	−10 ± 17	11 ± 6	9 ± 7	18 ± 23	37 ± 34	37 ± 12
27	−6 ± 14	29 ± 11	44 ± 24	50 ± 6	99 ± 8	52 ± 14

[a] Relative cell growth [%] in relation to untreated control of the tumor cell lines after 96 h exposure to test compound at a dose of 20 μM. Values are averages of three independent experiments. [b] Small cell lung cancer. [c] Bladder cancer. [d] Breast cancer. [e] Pancreas cancer. [f] Large cell lung cancer. [g] Bladder cancer.

Table 5 IC_{50} values (μM) of growth inhibition of tumor cell lines following a continuous 96 h exposure to the respective compounds. Unless otherwise noted, all values are the averages of three independent determinations

	A-427[a]	5637[b]	MCF-7[c]	DAN-G[d]	LCLC-103H[e]	RT-4[f]
16a	>20	>20	>20	>20	>20	>20
16b	>20	19 ± 2	>20.	>20	>20	>20
25a	>20	n.d.	n.d.	>20	>20	>20
25b	17 ± 2	6.8 ± 5.3	13 ± 3	>20	>20	>20
26	15 ± 2	8.8 ± 7.2	16[h]	17 ±4	>20	>20
27	10 ± 1	13 ± 2	18 ± 3	18 ± 5	>20	>20
Haloperidol	10 ± 2	27 ± 9	25 ± 10	29 ± 7	23 ± 5	16 ± 5
Methotrexate[g]	5.5 ± 3.6	0.016 ± 0.009	0.05 ± 0.02	0.077 ± 0.005	0.025 ± 0.012	0.04 ± 0.02

[a] Small cell lung cancer. [b] Bladder cancer. [c] Breast cancer. [d] Pancreas cancer. [e] Large cell lung cancer. [f] Bladder cancer; average of two determinations. [g] IC_{50} values are from ref. 45. [h] n.d. not determined.

the σ_1 (or σ_2) receptor affinity and the cell growth inhibition in any of the cell lines cannot be derived from these data, the growth inhibition of the A-427 cell line may to some extent be associated with interaction of the compounds with σ_1 receptors. This cell line is selectively sensitive to haloperidol, a known σ_1 receptor antagonist, which indicates that **27** could also be a σ_1 receptor antagonist. (Table 5) While **27** shows growth inhibitory activity in the A-427 line, it also shows activity in the 5637 cell line, which is relatively insensitive to haloperidol. Presently, studies are under way to clarify whether receptor binding is a cause for cytotoxicity.

6. Conclusion

In this manuscript, the conformational analysis, synthesis, σ_1 and σ_2 receptor affinities as well as growth inhibition of some human tumor cell lines of 7,9-diazabicyclo[4.2.2]decanes with a benzyl group at N-7 and a *p*-methoxybenzyl group at N-9 are reported. Starting from the chloroacetamide **10**, the bicyclic framework was established by a Dieckmann analogous cyclization which involves trapping of the first cyclized intermediate with trimethylsilyl chloride. The yield of the resulting mixed methyl silyl ketal **13** was even higher than the yields of the bicyclic analogues with a three-carbon bridge. This observation is in good agreement with our calculations showing that the diazabicyclo[4.2.2]decane system is slightly more stable than the diazabicyclo[3.2.2]nonane system.[36] The bicyclic ketone **14** was used as central building block for the synthesis of diastereoisomerically pure bicyclic alcohols, ethers and fluorine derivatives. Whereas the ketone **14** was reduced with high diastereoselectivity (dr > 99 : 1) to produce the alcohol **15a**, the diastereomeric alcohol **25a** was only available by an unselective reduction of the ketone **24**.

Generally, the 7,9-diazabicyclo[4.2.2]decane system showed considerably different chemical and pharmacological behavior compared with the corresponding 6,8-diazabicyclo[3.2.2]nonane derivatives: (1) A nucleophilic substitution at the 2-position either *via* a Mitsunobu inversion or after activation of the alcohol as sulfonate did not take place. It is assumed that this failure is due to higher shielding of this position by the unfavorable orientation of the larger bridge. (2) The σ_1 and σ_2 receptor affinities of all alcohols and ethers with a four-membered bridge are considerably lower than the σ affinities of the corresponding analogues with a three-carbon bridge. This may be attributed to the larger bridge itself. This hypothesis is supported by introduction of a double bond into the bridge (compound **27**), which reduced the size and flexibility of the bridge, and led to the most potent σ_1 ligand of this study (K_i = 7.5 nM). (3) The activity against human tumor cell lines is also reduced considerably. Again the larger, more flexible four-carbon bridge might be responsible for the reduced cell growth inhibition compared to the three-carbon bridge homologues.

7. Experimental part

7.1. Conformational analysis

3D-Structures were generated with MOE (Molecular Operating Environment), Version 2009.10, Chemical computing group AG (CCG, Montreal, Canada). Stochastic conformational search was carried out at standard conditions. Method: stochastic, rejection limit: 100, iteration limit 10000, RMS gradient: 0.005, MM iteration limit: 500, RMSD limit: 0.25, strain cutoff: 7 kcal mol^{-1}, conformation limit: 10000). Tables 6–9 in the Electronic Supplementary Information show the relative calculated energies and dihedral angles of all conformations.†

7.2. Chemistry

7.2.1. General. Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. THF was dried with sodium/benzophenone and was freshly distilled before use. Thin layer chromatography (tlc): Silica gel 60 F_{254} plates (Merck). Flash chromatography (fc): Silica gel 60, 40–64 µm (Merck); parentheses include: eluent, diameter of the column, height of the column packed, fraction size, R_f value. Melting point: Melting point apparatus SMP 3 (Stuart Scientific), uncorrected. MS: MAT GCQ (Thermo–Finnigan), EI (electron impact); Thermo Finnigan LCQ® ion trap mass spectrometer with an ESI (electrospray ionization) interface, exact mass (ESI): MicroTof (Bruker Daltonics) Finnigan MAT 4200s. IR: IR spectrophotometer 480Plus FT-ATR-IR (Jasco). ^{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 ^{1}H NMR and ^{13}C NMR were supported by COSY and GHSQC two dimensional NMR techniques. HPLC: Merck Hitachi Equipment; UV detector: L-7400; autosampler: L-7200; pump: L-7100; degasser: L-7614; Method A: column: LiChrospher® 60 RP-select B (5 µm), 250-4 mm; flow rate: 1.00 mL min^{-1}; injection volume: 5.0 µL; detection at λ = 210 nm; solvents: A: water with 0.05% (v/v) trifluoroacetic acid; B: acetonitrile with 0.05% (v/v) trifluoroacetic acid: gradient elution: (A%): 0–4 min: 90%, 4–29 min: 90% to 0%, 29–31 min: 0%, 31–31.5 min: 0% to 90%, 31.5–40 min: 90%. Method B: column: LiChrospher® 60 RP-select B (5 µm), 250-4 mm; flow rate: 1.00 mL min^{-1}; injection volume: 5.0 µL; detection at λ = 210 nm; solvents: A: water with 0.05% (v/v) trifluoroacetic acid, B: methanol with 0.05% (v/v) trifluoroacetic acid: gradient elution: (A%): 0–1 min: 80%, 1–22 min: 80% to 0%, 22–30 min: 0%, 30–31.5 min: 0% to 80%, 31.5–40 min: 80%.

7.2.2. Methyl 4-[(*RS*)-1-benzyl-4-(4-methoxybenzyl)-3,6-dioxopiperazin-2-yl]butanoate (11). Under ice-cooling NEt_3 (2.4 mL, 17.5 mmol) and 4-methoxybenzylamine (2.4 mL, 18.6 mmol) were added slowly to a solution of **10** (4.1 g, 11.6 mmol) in CH_3CN (60 mL) over a period of 30 min. The mixture was warmed to rt and stirred for 18 h. Then the solvent was evaporated under vacuum, Et_2O (150 mL) was added to the residue, the mixture was filtered and the filtrate was concentrated under vacuum. The residue was purified by fc (cyclohexane/ethyl acetate = 1/1, 5 cm, 15 cm, 30 mL, R_f 0.27) to yield a colorless oil, yield 1.25 g (96%). $C_{24}H_{28}N_2O_5$ (424.5). Purity (HPLC, method A): 94.6%, t_R = 19.3 min. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.39–1.51 (m, 1H, $CH_2CH_2CH_2CO_2CH_3$), 1.52–1.63 (m, 1H, $CH_2CH_2CH_2CO_2CH_3$), 1.69–1.78 (m, 1H, $CH_2CH_2CH_2CO_2CH_3$), 1.80–1.88 (m, 1H, $CH_2CH_2CH_2CO_2CH_3$), 2.07–2.26 (m, 2H, $CH_2CH_2CH_2CO_2CH_3$), 3.58 (s, 3H, $CH_2CH_2CH_2CO_2CH_3$), 3.73 (s, 3H, $C_6H_4OCH_3$), 3.77 (d, J = 17.6 Hz, 1H, NCH_2CO), 3.84–3.89 (m, 2H, NCH_2CO, $NCHCO$), 3.94 (d, J = 14.9 Hz, 1H, $NCH_2C_6H_4OCH_3$), 4.22 (d, J = 14.3 Hz, 1H, $NCH_2C_6H_5$), 4.67 (d, J = 14.3 Hz, 1H, $NCH_2C_6H_5$), 5.17 (d, J = 14.9 Hz, 1H, $NCH_2C_6H_4OCH_3$), 6.79 (d, J = 8.6 Hz, 2H, 3-H, 5-H$_{methoxybenzyl}$), 7.11 (d, J = 8.6 Hz, 2H, 2-H, 6-H$_{methoxybenzyl}$), 7.15–7.28 (m, 5H, 2-H, 3-H, 4-H, 5-H, 6-H$_{benzyl}$). ^{13}C NMR ($CDCl_3$): δ (ppm) = 18.6 (1C, C-3), 30.0 (1C, C-4), 32.1 (1C, C-2), 46.1 (1C, $NCH_2C_6H_4OCH_3$), 47.8 (1C, $NCH_2C_6H_5$), 47.9 (1C, C-8), 50.6 (1C, $C_6H_4OCH_3$), 54.3 (1C, CO_2CH_3), 57.9 (1C, C-5), 113.3 (2C, C-3, C-5$_{methoxybenzyl}$), 126.1 (1C, C-4$_{benzyl}$), 127.0 (1C, C-1$_{methoxybenzyl}$), 127.2 (2C, C-3, C-5$_{benzyl}$), 127.9 (2C, C-2, C-6$_{benzyl}$), 128.8 (2C, C-2, C-6$_{methoxybenzyl}$), 134.4 (1C, C-1$_{benzyl}$), 158.4 (1C, C-4$_{methoxybenzyl}$), 163.1 (1C, amide carbonyl), 164.8 (1C, amide carbonyl), 172.0 (1C, CO_2CH_3). MS (EI): m/z (%) = 424 [(M)$^+$, 59], 303 [(M-methoxybenzyl)$^+$, 37], 121 [methoxybenzyl$^+$, 100], 91 [benzyl$^+$, 46]. IR (neat): ν/cm^{-1} = 1732 (C═O), 1654 (C═O), 1243 (C–O), 1171 (C–O).

7.2.3. (1*RS*,2*SR*,6*RS*)-7-Benzyl-2-methoxy-9-(4-methoxybenzyl)-2-(trimethylsilyloxy)-7,9-diazabicyclo[4.2.2]decane-8,10-dione (13). Lithium hexamethyldisilazide (LHMDS), freshly prepared from *n*-BuLi (1.6 M in *n*-hexane, 11.5 mL, 18.4 mmol) and hexamethyldisilazane (3.8 mL, 18.4 mmol) in dry THF (25 mL) at 0 °C, was added slowly to a solution of **11** (4.7 g, 11.2 mmol) in dry THF (70 mL) at −78 °C. The mixture was stirred for 0.5 h at −78 °C, chlorotrimethylsilane $(CH_3)_3SiCl$ (4.2 mL, 33.5 mmol) was slowly added to this solution over a period of 10 min and the mixture was stirred at −78 °C for 2 h, then warmed to rt and stirred for additional 0.5 h. The solvent was evaporated to half of the original volume and ethyl acetate (50 mL) was added. The mixture was washed with H_2O (50 mL) and the aqueous layer was extracted with CH_2Cl_2 (5 × 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, concentrated under vacuum and the residue was purified by fc (cyclohexane/ethyl acetate = 7/3, 8 cm, 14 cm, 50 mL, R_f 0.25) to obtain a colorless viscous oil, yield 4.93 g (89%). $C_{27}H_{36}N_2O_5Si$ (496.6). Purity (HPLC, method A): 98.2%, t_R = 23.2 min. Elemental analysis: calcd. C 65.29 H 7.31 N 5.64 found C 65.39 H 7.65 N 5.06. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.13 (s, 9H, $(CH_3)_3Si$), 1.34–1.53 (m, 3H, 3-H, 4-H), 1.74-1.92 (m, 3H, 3-H, 5-H), 3.29 (s, 3H, $OCH_{3\,ketal}$), 3.73 (s, 3H, $C_6H_4OCH_3$), 3.88 (d, J = 14.6 Hz, 1H, $NCH_2C_6H_4OCH_3$), 3.96 (d, J = 1.0 Hz, 1H, 1-H), 4.0 (dd, J = 6.4/1.8 Hz, 1H, 6-H), 4.18 (d, J = 14.7 Hz, 1H, $NCH_2C_6H_5$), 4.72 (d, J = 14.7 Hz, 1H, $NCH_2C_6H_5$), 5.29 (d, J = 14.6 Hz, 1H, $NCH_2C_6H_4OCH_3$), 6.78 (d, J = 8.7 Hz, 2H, 3-H, 5-H$_{methoxybenzyl}$), 7.02 (d, J = 8.5 Hz, 2H, 2-H, 6-H$_{methoxybenzyl}$), 7.14 (dd, J = 7.5/1.9 Hz, 2H, 2-H, 6-H$_{benzyl}$), 7.20– 7.26 (m, 3H, 3-H, 4-H, 5-H$_{benzyl}$). ^{13}C NMR ($CDCl_3$): δ (ppm) = 0.0 (3C, $(CH_3)_3Si$), 15.6 (1C, C-4), 32.0 (1C, C-5), 32.1 (1C, C-3), 45.9 (1C, $CH_2C_6H_4OCH_3$), 46.2 (1C, $CH_2C_6H_5$), 47.3 (1C, $OCH_{3\,ketal}$), 53.5 (1C, $C_6H_4OCH_3$), 57.9 (1C, C-6), 62.1 (1C, C-1), 103.8 (1C, C-2), 112.4 (2C, C-3, C-5$_{methoxybenzyl}$), 126.1 (1C, C-4$_{benzyl}$), 126.2 (1C, C-1$_{methoxybenzyl}$), 126.4 (2C, C-3, C-5$_{benzyl}$), 127.0 (2C, C-2, C-6$_{benzyl}$), 127.7 (2C, C-2, C-6$_{methoxybenzyl}$), 134.6 (1C, C-1$_{benzyl}$), 157.4 (1C, C-4$_{methoxybenzyl}$), 162.7 (1C, carbonyl), 167.6 (1C, carbonyl). MS (EI): m/z (%) = 496 (M$^+$, 89), 375 [(M-methoxybenzyl)$^+$, 42], 121 [(methoxybenzyl)$^+$, 100], 91 [(benzyl)$^+$, 85]. IR (neat): ν/cm^{-1} = 1666 (C═O), 1451 (C–N), 1243 (C–O), 839 (Si–O).

7.2.4. (1*RS*,6*RS*)-7-Benzyl-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2]decane-2,8,10-trione (14). The mixed methyl silyl ketal **13** (0.15 g, 0.3 mmol) was dissolved in a mixture of THF (2.5 mL) and H_2O (1 drop, ≈ 0.05 mL). *p*-Toluenesulfonic acid (30 mg, 0.18 mmol) was added and the mixture was stirred for 16 h at rt. The mixture was concentrated to half of its original volume, then it was washed with saturated $NaHCO_3$ solution (5 mL) and the aqueous layer was extracted with CH_2Cl_2 (5 × 10 mL). The combined organic layers were washed with brine,

dried over Na_2SO_4 and the solvent was evaporated under vacuum to obtain a colorless solid. Purification by recrystallization with *n*-hexane–ethyl acetate (4/1) mixture yielded colorless crystals, mp 190–195 °C, yield 0.12 g (99%). $C_{23}H_{24}N_2O_4$ (392.4). Purity (HPLC, method A): 99.9%, t_R = 18.9 min. Elemental analysis: calcd. C 70.39 H 6.16 N 7.14 found C 69.77 H 6.24 N 6.91. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.11–1.22 (m, 1H, 4-H), 1.67–1.75 (m, 1H, 4-H), 1.95–1.99 (m, 2H, 5-H), 2.01–2.07 (m, 1H, 3-H), 2.19–2.25 (m, 1H, 3-H), 3.78 (s, 3H, $C_6H_4OCH_3$), 4.26–4.28 (m, 1.5H, 6-H, $NCH_2C_6H_4OCH_3$), 4.31 (s, 0.5H, $NCH_2C_6H_4OCH_3$), 4.35 (d, J = 14.2 Hz, 1H, $NCH_2C_6H_5$), 4.56 (d, J = 0.7 Hz, 1H, 1-H), 4.64 (d, J = 14.2 Hz, 1H, $NCH_2C_6H_5$), 4.82 (d, J = 14.6 Hz, 1H, $NCH_2C_6H_4OCH_3$), 6.82 (d, J = 8.7 Hz, 2H, 3-H, 5-H$_{methoxybenzyl}$), 7.18 (d, J = 8.7 Hz, 2H, 2-H, 6-H$_{methoxybenzyl}$), 7.24–7.27 (m, 2H, 2-H, 6-H$_{benzyl}$), 7.30–7.33 (m, 3H, 3-H, 4-H, 5-H$_{benzyl}$). ^{13}C NMR ($CDCl_3$): δ (ppm) = 19.4 (1C, C-4), 31.3 (1C, C-5), 38.9(1C, C-3), 48.8 (1C, $CH_2C_6H_4OCH_3$), 49.8 (1C, $CH_2C_6H_5$), 55.5 (1C, $C_6H_4OCH_3$), 59.6 (1C, C-6), 71.6 (1C, C-1), 114.6 (2C, C-3, C-5$_{methoxybenzyl}$), 125.8, (1C, C-4$_{benzyl}$), 128.6, (1C, C-1$_{methoxybenzyl}$), 128.7, (2C, C-3, C-5$_{benzyl}$), 129.2, (2C, C-2, C-6$_{benzyl}$), 131.1, (2C, C-2, C-6$_{methoxybenzyl}$), 135.3, (1C, C-1$_{benzyl}$), 160.0, (1C, C-4$_{methoxybenzyl}$), 161.4 (1C carbonyl), 166.8, 1C, carbonyl), 203.5 (1C, C-2$_{carbonyl}$). MS (EI): m/z (%) = 392.0 [M$^+$, 22], 301.0 [(M-benzyl)$^+$, 6], 121.1 [(methoxybenzyl)$^+$, 100], 91.2 [(benzyl)$^+$, 17]. IR (neat): ν/cm^{-1} = 1716 (C═O), 1668 (C═O), 1449 (C–N), 1240 (C–O).

7.2.5. (1*RS*,2*SR*,6*RS*)-7-Benzyl-2-hydroxy-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2] decane-8,10-dione (15a). Under N_2 $LiBH_4$ (4.3 mL, 8.56 mmol, 2 M solution in THF) was slowly added to a solution of **14** (2.23 g, 5.7 mmol) in dry THF (120 mL) at –90 °C and the mixture was stirred for 2.5 h at –90 °C. The excess $LiBH_4$ was destroyed with 1 M HCl (50 mL), then 2 M NaOH (20 mL) was added until the solution attained a pH of 9 and the mixture was stirred for 0.5 h at rt. The aqueous layer was extracted with CH_2Cl_2 (5 × 30 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and the solvent was evaporated under vacuum to obtain a colorless viscous oil. The crude oil was purified by fc (cyclohexane/ethyl acetate = 3/7, 3.5 cm, 15 cm, 30 mL, R_f 0.1) to obtain a spongy colorless solid. Yield 1.95 g (87%). $C_{23}H_{26}N_2O_4$ (394.4). Purity (HPLC, method A): 97.2%, t_R = 17.4 min. Diastereomeric purity: >99% (from the ^{1}H NMR spectrum of the crude sample). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.26–1.35 (m, 1H, 4-H), 1.35–1.58 (m, 2H, 3-H, 4-H), 1.63–1.69 (m, 1H, 3-H), 1.74–1.81 (m, 1H, 5-H), 1.91–1.98 (m, 1H, 5-H), 2.99 (d, J = 7.5 Hz, 0.8H, CHO*H*), 3.73 (s, 3H, OCH_3), 3.81–3.87 (m, 1H, 2-H), 3.99 (dd, J = 4.7/3.2 Hz, 1H, 6-H), 4.05 (d, J = 14.7 Hz, 1H, $NCH_2C_6H_4OCH_3$), 4.06 (d, J = 4.9 Hz, 1H, 1-H), 4.18 (d, J = 14.6 Hz, 1H, $NCH_2C_6H_5$), 4.76 (d, J = 14.6 Hz, 1H, $NCH_2C_6H_5$), 4.96 (d, J = 14.7 Hz, 1H, $NCH_2C_6H_4OCH_3$), 6.79 (d, J = 8.7 Hz, 2H, 3-H, 5-H$_{methoxybenzyl}$), 7.13 (d, J = 8.7 Hz, 2H, 2-H, 6-H$_{methoxybenzyl}$), 7.16–6.18 (m, 2H, 2-H, 6-H$_{benzyl}$), 7.23–7.29 18 (m, 3H, 3-H, 4-H, 5-H$_{benzyl}$). ^{13}C NMR ($CDCl_3$): δ (ppm) = 17.6 (1C, C-4), 32.2 (1C, C-3), 32.3 (1C, C-5), 47.7 (1C, $CH_2C_6H_4OCH_3$), 48.0 (1C, $CH_2C_6H_5$), 55.5 (1C, $C_6H_4OCH_3$), 59.5 (1C, C-6), 63.1 (1C, C-1), 72.0 (1C, C-2), 114.6 (2C, C-3, C-5$_{methoxybenzyl}$), 127.7 (1C, C-4$_{benzyl}$), 128.5 (1C, C-1$_{methoxybenzyl}$), 128.6 (2C, C-3, C-5$_{benzyl}$), 129.2 (2C, C-2, C-6$_{benzyl}$), 130.1 (2C, C-2, C-6$_{methoxybenzyl}$), 135.4 (1C, C-1$_{benzyl}$), 159.7 (1C, C-4$_{methoxybenzyl}$), 167.3 (1C, carbonyl), 167.7 (1C, carbonyl). MS (EI): m/z (%) = 394.0 [(M)$^+$, 32], 121.0 [(methoxybenzyl)$^+$, 100], 91.1 [(benzyl)$^+$, 42]. IR (neat): ν/cm^{-1} = 3398 (O–H), 1657 (C═O), 1451 (C–N), 1244 (C–O), 1173 (C–O).

7.2.6. (1*RS*,2*SR*,6*RS*)-7-Benzyl-2-methoxy-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2] decane-8,10-dione (15b). Under N_2 atmosphere, **15a** (0.1 g, 0.25 mmol) and CH_3I (0.05 mL, 0.76 mmol) were added to a solution of NaH (0.1 g, 2.5 mmol, 60% in paraffin oil) in dry THF (12 mL) under ice-cooling. The mixture was warmed to rt and stirred for 3 h. Excess NaH was destroyed with H_2O (3 mL) under ice-cooling. The reaction mixture was washed with 2 M NaOH (15 mL) solution. The aqueous layer was extracted with CH_2Cl_2 (5 × 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent was evaporated under vacuum. The crude solid was purified by fc (cyclohexane/ethyl acetate = 1/1, 2 cm, 13 cm, 7 mL, R_f 0.21). to obtain colorless crystals, mp 136–140 °C, yield 0.105 g (99%). $C_{24}H_{28}N_2O_4$ (408.2) Purity (HPLC, method A): 99.7%, t_R = 17.0 min. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.32–1.41 (m, 1H, 4-H), 1.58–1.78 (m, 3H, 4-H, 3-H), 1.81–1.90 (m, 1H, 5-H), 1.96-2.04 (m, 1H, 5-H), 3.36 (s, 4H, $CHOCH_3$), 3.81 (s, 3H, $C_6H_4OCH_3$), 4.05 (dd, J = 5.1/2.9 Hz, 1H, 6-H), 4.11 (d, J = 14.8 Hz, 1H, $NCH_2C_6H_4OCH_3$), 4.26 (d, J = 5.0 Hz, 1H, 1-H), 4.31 (d, J = 14.6 Hz, 1H, $NCH_2C_6H_5$), 4.76 (d, J = 14.6 Hz, 1H, $NCH_2C_6H_5$), 5.04 (d, J = 14.8 Hz, 1H, $NCH_2C_6H_4OCH_3$), 6.87 (d, J = 8.7 Hz, 2H, 3-H, 5-H$_{methoxybenzyl}$), 7.20 (d, J = 8.7 Hz, 2H, 2-H, 6-H$_{methoxybenzyl}$), 7.22-7.24 (m, 2H, 3-H, 5-H$_{benzyl}$), 7.28-7.34 (m, 3H, 2-H, 4-H, 6-H$_{benzyl}$). ^{13}C NMR ($CDCl_3$): δ (ppm) = 17.8 (1C, C-4), 28.1 (1C, C-3), 32.1 (1C, C-5), 47.4 (1C, $CH_2C_6H_4OCH_3$), 48.4 (1C, $CH_2C_6H_5$), 55.4 (1C, $C_6H_4OCH_3$), 57.7 (1C, $CHOCH_3$), 59.4 (1C, C-6), 60.7 (1C, C-1), 81.63 (1C, C-2), 114.4 (2C, C-3, C-5$_{methoxybenzyl}$), 127.6 (1C, C-4$_{benzyl}$), 128.3 (1C, C-1$_{methoxybenzyl}$), 128.5 (2C, C-3, C-5$_{benzyl}$), 129.0 (2C, C-2, C-6$_{benzyl}$), 130.0 (2C, C-2, C-6$_{methoxybenzyl}$), 135.3 (1C, C-1$_{benzyl}$), 159.5 (1C, C-4$_{methoxybenzyl}$), 167.1 (1C, carbonyl), 167.6 (1C, carbonyl). MS (EI): m/z (%) = 406.6 [(M)$^+$, 2], 120.1[(methoxybenzyl)$^+$, 100], 91.1 [(benzyl)$^+$, 25]. IR (neat): ν/cm^{-1} = 1653 (C═O), 1509 (C═C), 1244 (C–O).

7.2.7. (1*RS*,2*RS*,6*SR*)-7-Benzyl-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2]decan-2-ol (16a).

*Method 1 from **15a**.* Under N_2 $LiAlH_4$ (13.6 mL, 13.6 mmol, 1 M solution in THF) was added dropwise under ice cooling to a solution of **15a** (0.89 g, 2.27 mmol) in dry THF (73 mL). The mixture was warmed to rt and stirred under reflux for 15 h. The excess $LiAlH_4$ was destroyed with H_2O (3 mL) under ice-cooling. The mixture was again stirred under reflux for 1 h, cooled to rt and filtered. The precipitate was washed with CH_2Cl_2 (50 mL) and the solvent was evaporated under vacuum. The crude viscous oil was purified by fc (petroleum ether/ethyl acetate = 95/5 + 0.2% *N*,*N*-dimethylethylamine, 3 cm, 13 cm, 12 mL, R_f 0.27). Colorless oil, yield 0.79 g (95%).

*Method 2 from **21**.* To a suspension of **21** (1.2 g, 2.3 mmol) in CH_3OH–CH_2Cl_2 (5/1, 50 mL), K_2CO_3 (0.96 g, 6.9 mmol) was added and the mixture was stirred at rt for 16 h. The solvent was evaporated under vacuum and H_2O (20 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (5 × 20 mL) and the solvent was evaporated under vacuum to obtain **16a** as colorless viscous oil. Purification by fc (petroleum ether/ethyl acetate = 95/5 + 0.2% N,N-dimethylethylamine, 4 cm, 15 cm, 20 mL, R_f 0.27). Colorless oil, yield 0.82 g (97%).

$C_{23}H_{30}N_2O_2$ (366.5). Purity (HPLC, method A): 98.0%, t_R = 18.7 min. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 1.21-1.31 (m, 1H, 5-H), 1.48-1.53 (m, 1H, 5-H), 1.61-1.69 (m, 1H, 4-H), 1.95-1.99 (m, 1H, 3-H), 2.22 (ddd, J = 17.5/13.8/4.9 Hz, 1H, 4-H), 2.37 (q, J = 10.7 Hz, 1H, 3-H), 2.56 (dd, J = 11.1/1.3 H, 1H, 10-H), 2.79-2.81 (m, 2H, 2-H, 8-H), 2.85 (s(b), 1H, 6-H), 2.95 (dd, J = 12.5/3.1 Hz, 1H, 8-H), 3.01 (ddd, J = 10.9/4.1/1.5 Hz, 1H, 10-H), 3.52-3.65 (m, 5H, 1-H, $NCH_2C_6H_4OCH_3$, $NCH_2C_6H_5$), 3.77 (s, 3H, OCH_3), 6.81 (d, J = 8.6 Hz, 2H, 3-H, 5-$H_{methoxybenzyl}$), 7.21-7.24 (m, 3H, 2-H, 6-$H_{methoxybenzyl}$,4-H_{benzyl}), 7.28-7.31 (m, 2H, 2-H, 6-H_{benzyl}), 7.34-7.36 (m, 2H, 3-H, 5-H_{benzyl}). ^{13}C NMR ($CDCl_3$): δ (ppm) = 21.7 (1C, C-4), 33.9 (1C, C-3), 36.2 (1C, C-5), 44.6 (1C, C-10), 50.5 (1C, C-8), 55.4 (1C, $C_6H_4OCH_3$), 57.9 (1C, C-6), 62.9 (1C, $CH_2C_6H_5$), 63.4 (1C, C-1), 63.6 (1C, $CH_2C_6H_4OCH_3$), 74.2 (1C, C-2), 113.7 (2C, C-3, C-5$_{methoxybenzyl}$), 127.1 (1C, C-4$_{benzyl}$), 128.3 (1C, C-1$_{methoxybenzyl}$), 129.1 (2C, C-3, C-5$_{benzyl}$), 130.3 (2C, C-2, C-6$_{benzyl}$), 131.8 (2C, C-2, C-6$_{methoxybenzyl}$), 140.0 (1C, C-1$_{benzyl}$), 158.8 (1C, C-4$_{methoxybenzyl}$). MS (EI): m/z (%) = 366.2 [(M)$^+$, 64], 245.3 [(M-methoxybenzyl)$^+$, 56], 121.0 [(methoxybenzyl)$^+$, 100], 91.1 [(benzyl)$^+$, 38]. IR (neat): ν/cm^{-1} = 3405 (O–H), 1509 (C=C aromatic), 1442 (C–N), 1244 (C–O).

7.2.8. (1*RS*,2*RS*,6*SR*)-7-Benzyl-2-methoxy-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2] decane (16b). Under N_2 $LiAlH_4$ (0.76 mL, 0.76 mmol, 1 M solution in THF) was slowly added to a solution of **15b** (0.10 g, 0.25 mmol) in dry THF (25 mL) under ice-cooling. The mixture was warmed to rt and stirred under reflux for 14 h. Excess $LiAlH_4$ was destroyed with H_2O (1 mL) under ice-cooling. The mixture was again stirred under reflux for 1 h. The solution was cooled to rt and filtered through a sintering funnel. The precipitate was washed with CH_2Cl_2 (50 mL). The solvent was evaporated under vacuum. The crude oil was purified by fc (ethyl acetate/petroleum ether = 5/95, 2.5 cm, 13 cm, 10 mL, R_f 0.45 (ethyl acetate/cyclohexane = 3/7)). Colorless oil, yield 0.092 g (91%). $C_{24}H_{32}N_2O_2$ (380.2). Purity (HPLC, method A): 98.6%, t_R = 19.1 min. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.22-1.29 (m, 1H, 5-H), 1.35-1.45 (m, 1H, 5-H), 1.56-1.64 (m, 1H, 4-H), 1.87-1.94 (m, 1H, 3-H), 2.12-2.18 (m, 1H, 4-H), 2.25-2.34 (m, 1H, 3-H),2.55 (dd, J = 10.9/1.8 Hz, 1H, 10-H), 2.75-2.80 (m, 2H, 6-H, 8-H), 2.84-2.91 (m, 3H, 1-H, 2-H, 8-H), 2.98 (s, 4H, $CHOCH_3$, 10-H), 3.45 (d, J = 12.4 Hz, 2H, CH_2Ph, $CH_2C_6H_4OCH_3$), 3.52 (d, J = 12.5 Hz, 1H, $CH_2C_6H_5$), 3.59 (d, J = 13.5 Hz, 1H, $CH_2C_6H_4OCH_3$), 3.72 (s, 3H, $C_6H_4OCH_3$), 6.79 (d, J = 8.7 Hz, 2H, 3-H, 5-$H_{methoxybenzyl}$), 7.13-7.24 (m, 5H, 2-H, 6-$H_{methoxybenzyl}$, 2-H, 4-H, 6-H_{benzyl}), 7.26-7.29 (m, 2H, 3-H, 5-H_{benzyl}). ^{13}C NMR ($CDCl_3$): δ (ppm) = 22.0 (1C, C-4), 30.1 (1C, C-3), 36.3 (1C, C-5), 45.4 (1C, C-8), 50.7 (1C, C-10), 55.5 (1C, $C_6H_4OCH_3$), 56.9 (1C, $CHOCH_3$), 57.6 (1C, C-6), 59.5 (1C, C-1), 62.8 (1C, $CH_2C_6H_5$), 63.3 (1C, $CH_2C_6H_4OCH_3$), 83.9 (1C, C-2), 113.6 (2C, C-3, C-5$_{methoxybenzyl}$), 126.9 (1C, C-4$_{benzyl}$), 128.2 (1C, C-1$_{methoxybenzyl}$), 129.3 (2C, C-3, C-5$_{benzyl}$), 130.6 (2C, C-2, C-6$_{benzyl}$), 131.8 (2C, C-2,C-6$_{methoxybenzyl}$), 140.0 (1C, C-1$_{benzyl}$), 158.8 (1C, C-4$_{methoxybenzyl}$). MS (EI): m/z (%) = 380.1 [(M)$^+$, 66], 289.0 [(M-benzyl)$^+$, 12], 259.0 [(M-methoxybenzyl)$^+$, 55], 121.0 [(methoxybenzyl)$^+$, 100], 91.1 [(benzyl)$^+$, 33]. IR (neat): ν/cm^{-1} = 1510 (C=C aromatic), 1244 (C–O), 1088 (C–O).

7.2.9. (1*RS*,6*RS*)-7-Benzyl-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2]dec-2-ene-8,10-dione (18) and (1*RS*,2*RS*,6*RS*)-7-benzyl-2-hydroxy-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2]decane-8,10-dione (19a). Under N_2 triphenylphosphine (0.10 g, 0.38 mmol), *p*-nitrobenzoic acid (0.040 g, 0.25 mmol) were added to a solution of **15a** (50 mg, 0.13 mmol) in THF (15 mL). Diisopropyl azodicarboxylate (0.07 mL, 0.38 mmol) was added dropwise to this mixture under ice-cooling. The mixture was heated to reflux and stirred for 18 h. The solvent was evaporated under vacuum. Without purification, the residue (mixture of **17** and **18**) was dissolved in CH_3OH–CH_2Cl_2 (14 mL (1/1)), K_2CO_3 was added and the mixture was stirred at rt for 30 h. The solvent was evaporated under vacuum. H_2O (20 mL) was added to the crude residue and extracted with CH_2Cl_2 (5 × 20 mL). The combined organic layers were dried (Na_2SO_4) and the solvent was evaporated under vacuum. The crude residue was separated by fc (ethyl acetate/cyclohexane = 1/4–ethyl acetate/cyclohexane = 7/3, 2.5 cm, 16 cm, 10 mL) to obtain alkene **18** as a colorless solid and alcohol **19a** as a colorless viscous oil.

18. R_f 0.40 (ethyl acetate/cyclohexane = 1/1). Colorless solid, mp 143–147 °C, yield 24 mg (52%). $C_{23}H_{24}N_2O_3$ (376.4). Purity (HPLC, method A): 97.6%, t_R = 18.8 min. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.73-1.82 (m, 1H, 5-H), 1.97-2.09 (m, 2H, 4-H, 5-H), 2.15-2.24 (m, 1H, 4-H), 3.73 (s, 3H, OCH_3), 4.05 (t, J = 4.4 Hz, 1H, 6-H),4.11 (d, J = 14.8 Hz, 1H, $NCH_2C_6H_4OCH_3$), 4.12 (d, J = 14.5 Hz, 1H, $NCH_2C_6H_5$), 4.27 (d, J = 6.8 Hz, 1H, 1-H), 4.76 (d, J = 14.5 Hz, 1H, $NCH_2C_6H_5$), 4.85 (d, J = 14.8 Hz, 1H, $NCH_2C_6H_4OCH_3$), 5.68-5.78 (m, 2H, 2-H, 3-H), 6. 78 (d, J = 8.5 Hz, 2H, 3-H, 5-$H_{methoxybenzyl}$), 7.11 (d, J = 8.6 Hz, 2H, 2-H, 6-$H_{methoxybenzyl}$), 7.15-7.17 (m, 2H, 2-H, 6-H_{benzyl}), 7.22-7.29 (m, 3H, 3-H, 4-H, 5-H_{benzyl}). ^{13}C NMR ($CDCl_3$): δ (ppm) = 22.1 (1C, C-5), 30.1 (1C, C-4), 46.6 (1C, $NCH_2C_6H_4OCH_3$), 47.2 (1C, $NCH_2C_6H_5$), 54.2 (1C, OCH_3), 57.9 (1C, C-6), 58.2 (1C, C-1), 113.2 (2C, C-3, C-5$_{methoxybenzyl}$), 126.5 (1C, C-3), 127.0 (1C, C-3), 127.3 (1C, C-4$_{benzyl}$), 127.6 (1C, C-1$_{methoxybenzyl}$), 127.9 (2C, C-3, C-5$_{benzyl}$), 128.9 (2C, C-2, C-6$_{benzyl}$), 132.1 (2C, C-2, C-6$_{methoxybenzyl}$), 134.5 (1C, C-1$_{benzyl}$), 159.6 (1C, C-4$_{methoxybenzyl}$), 165.0 (d, J = 14.8 Hz, 1C, carbonyl), 169.0 (1C, carbonyl). MS (EI): m/z (%) = 375 [(M-H)$^+$, 43], 255 [(M-methoxybenzyl)$^+$, 32], 91 [(benzyl)$^+$, 100]. IR (neat): ν/cm^{-1} = 1658 (C=O), 1511 (C=C aromatic), 1243 (C–O), 699 (C–H).

19a. R_f 0.10 (cyclohexane/ethyl acetate = 3/7). Colorless oil, yield (3 mg, 6%, calculated over two steps from **15a**). $C_{23}H_{26}N_2O_4$ (394.4). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.26-1.35 (m, 1H, 4-H), 1.35-1.58 (m, 2H, 3-H, 4-H), 1.63-1.69 (m, 1H, 3-H), 1.74-1.81 (m, 1H, 5-H), 1.91-1.98 (m, 1H, 5-H), 3.73 (s, 3H, OCH_3), 3.99 (d, J = 14.5 Hz, 1H, $NCH_2C_6H_4OCH_3$), 4.05 (d, J = 3.7 Hz, 1H, 6-H), 4.07 (s, 1H, 2-H), 4.11 (d, J = 14.6 Hz, 1H, $NCH_2C_6H_5$), 4.20 (s, 1H, 1-H), 4.86 (d, J = 14.7 Hz, 1H, $NCH_2C_6H_5$), 5.27 (d, J = 14.5 Hz, 1H, $NCH_2C_6H_4OCH_3$), 6.79 (d, J = 8.7 Hz, 2H, 3-H, 5-$H_{methoxybenzyl}$), 7.13 (d, J = 8.7 Hz, 2H, 2-H, 6-$H_{methoxybenzyl}$), 7.16-7.18 (m, 2H, 2-H, 6-H_{benzyl}), 7.23-7.29 18 (m, 3H, 3-H, 4-H, 5-H_{benzyl}). MS (EI): m/z (%) = 394.0 [(M)$^+$, 32], 121.0 [(methoxybenzyl)$^+$, 100], 91.1 [(benzyl)$^+$, 42]. IR (neat): ν/cm^{-1} = 3398 (O–H), 1657 (C=O), 1451 (C–N), 1244 (C–O), 1173 (C–O).

7.2.10. [(1*RS*,2*SR*,6*RS*)-7-Benzyl-9-(4-methoxybenzyl)-8,10-dioxo-7,9-diazabicyclo[4.2.2]dec-2-yl] *p*-toluenesulfonate (20). The alcohol **15a** (0.10 g, 0.25 mmol) was dissolved in CH_2Cl_2 (5 mL). *p*-Toluenesulfonyl chloride (0.070 g, 0.38 mmol) and powdered KOH (15 mg, 0.28 mmol) were added and the mixture was stirred at 0 °C for 2.5 h. The reaction mixture was acidified with 1 M HCl and sat. $NaHCO_3$ solution was added (pH 7.5).

The aqueous layer was extracted with CH_2Cl_2 (5 × 10 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and evaporated under vacuum to obtain a colorless viscous oil. Purification by fc (ethyl acetate/petroleum ether = 1/1, 3 cm, 13 cm, 10 mL, R_f 0.26) gave a colorless oil, yield 0.13 g (94%). $C_{30}H_{32}N_2O_6S$ (548.6). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.24-1.37 (m, 1H, 4-H), 1.56-1.67 (m, 2H, 4-H, 5-H) 1.69-1.78 (m, 2H, 3-H, 5-H), 1.90-1.96 (m, 1H, 3-H), 2.39 (s, 3H, $CH_{3\,tosyl}$), 3.76 (s, 3H, OCH_3), 3.98 (dd, J = 4.6/3.2 Hz, 1H, 6-H), 4.06 (d, J = 14.7 Hz, 1H, $NCH_2C_6H_4OCH_3$), 4.15 (d, J = 4. 8 Hz, 1H, 1-H), 4.18 (d, J = 14.5 Hz, 1H, $NCH_2C_6H_5$), 4.45-4.48 (m, 1H, 2-H), 4.62 (d, J = 14.4 Hz, 1H, $NCH_2C_6H_5$), 4.92 (d, J = 14.7 Hz, 1H, $NCH_2C_6H_4OCH_3$), 6. 76 (d, J = 8.5 Hz, 2H, 3-H, 5-$H_{methoxybenzyl}$), 7.15-7.25 (m, 5H, 2-H, 6-$H_{methoxybenzyl}$,2-H, 4-H, 6-H_{benzyl}), 7.28 (d, J = 7.3 Hz, 2H, 3-H, 5-H_{benzyl}), 7.30 (d, J = 8.0 Hz, 2H, 3-H, 5-H_{tosyl}), 7.76 (d, J = 8.2 Hz, 2H, 4-H, 6-H_{tosyl}). Exact mass (ESI): m/z = calculated for $C_{30}H_{32}N_2O_6SNa^+$ 571.1878, found 571.1874.

7.2.11. (1*RS*,6*RS*)-7-Benzyl-2,2-dimethoxy-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2] decane-8,10-dione (22). *p*-Toluenesulfonic acid (1.21 g, 6.37 mmol) and trimethyl orthoformate (2.0 mL, 17.85 mmol) were slowly added to a solution of ketone **14** (1.0 g, 2.55 mmol) in CH_3OH (70 mL) under ice-cooling. The solution was warmed to rt and stirred under reflux for 15 h. The mixture was cooled to rt, the solvent was evaporated under vacuum and CH_2Cl_2 (10 mL) was added to residue. The mixture was washed with sat. $NaHCO_3$ and the aqueous layer was extracted with CH_2Cl_2 (5 × 25 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent was evaporated under vacuum. The crude viscous oil was purified by fc (cyclohexane/ethyl acetate = 2/3, 3.5 cm, 15 cm, 30 mL, R_f 0.26) to obtain a colorless viscous oil, yield 1.12 g (99.9%). $C_{25}H_{30}N_2O_5$ (438.2). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.43-1.64 (m, 3H, 4-H, 5-H), 1.88-2.44 (m, 3H, 3-H, 5-H), 3.21 (s, 3H, $OCH_{3\,ketal}$), 3.33 (s, 3H, $OCH_{3\,ketal}$), 3.79 (s, 3H, $C_6H_4OCH_3$), 3.93 (d, J = 14.8 Hz, 1H, $CH_2C_6H_4OCH_3$), 4.03 (d, J = 14.8 Hz, 1H, $CH_2C_6H_5$), 4.07 (dd, J = 5.7/2.5 Hz, 1H, 6-H), 4.17 (d, J = 0.6 Hz, 1H, 1-H), 5.04 (d, J = 14.8 Hz, 1H, $CH_2C_6H_5$), 5.38 (d, J = 14.8 Hz, 1H, $CH_2C_6H_4OCH_3$), 6.84 (d, J = 8.7 Hz, 2H, 3-H, 5-$H_{methoxybenzyl}$), 7.08 (d, J = 8.7 Hz, 2H, 2-H, 6-$H_{methoxybenzyl}$), 7.19 (dd, J = 7.6 Hz, 1.6 Hz, 2H, 3-H, 5-H_{benzyl}), 7.26-7.33 (m, 3H, 2-H, 4-H, 6-H_{benzyl}). Exact mass (ESI): m/z = calculated for $C_{25}H_{30}N_2O_5Na^+$ 461.2052, found 461.2051. I.R. (neat): ν/cm^{-1} = 1663 (C═O), 1511 (C═C), 1244 (C–O), 1091 (C–O), 730 (C═C).

7.2.12. (1*RS*,6*SR*)-7-Benzyl-2,2-dimethoxy-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2] decane (23). Under N_2 $LiAlH_4$ (10.8 mL, 0.10.8 mmol, 1 M solution in THF) was slowly added to a solution of **22** (0.95 g, 2.15 mmol) in dry THF (43 mL) under ice-cooling. The mixture was warmed to rt and stirred under reflux for 12 h. Excess $LiAlH_4$ was destroyed with H_2O (3 mL) under ice-cooling and the mixture was again stirred under reflux for 1 h. The solution was cooled to rt and filtered through a sintering funnel. The precipitate was washed with CH_2Cl_2 (100 mL) and the solvent was evaporated under vacuum. The crude oil was purified by fc (petroleum ether/ethyl acetate = 95/5, 4.5 cm, 16 cm, 30 mL, R_f 0.42 (cyclohexane/ethyl acetate = 4/1)) to obtain a colorless viscous oil, yield 0.71 g (80%). $C_{25}H_{34}N_2O_3$ (410.2). Purity (HPLC, method B): 97.2%, t_R = 16.33 min. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.11 (dd, J = 13.5/3.2 Hz, 1H, 5-H), 1.32–1.40 (m, 1H, 5-H), 1.54 (s(b), 1H, 4-H), 2.02 (t, J = 11.1 Hz, 1H, 3-H), 2.31 (d, J = 10.6 Hz, 2H, 4-H, 10-H), 2.42 (s(b), 1H, 3-H), 2.71 (dd, J = 11.2/4.0 Hz, 2H, 6-H, 8-H), 2.77 (ddd, J = 10.7/3.7/1.3 Hz, 1H, 10-H), 2.88 (d, J = 11.1 Hz, 1H, 8-H), 2.97 (s(b), 1H, 1-H), 3.09 (s, 3H, $OCH_{3\,ketal}$), 3.16 (s, 3H, $OCH_{3\,ketal}$),3.37 (d, J = 12.7 Hz, 1H, $CH_2C_6H_4OCH_3$), 3.47 (d, J = 13.2 Hz, 1H, $CH_2C_6H_5$), 3.58 (d, J = 13.2 Hz, 1H, $CH_2C_6H_5$),3.71 (s, 3H, $C_6H_4OCH_3$), 3.85 (d, J = 12.7 Hz, 1H, $CH_2C_6H_4OCH_3$), 6.76 (d, J = 8.6 Hz, 2H, 3-H, 5-$H_{methoxybenzyl}$), 7.11-7.27 (m, 7H, 2-H, 6-$H_{methoxybenzyl}$, 2-H, 3-H, 4-H, 5-H, 6-H_{benzyl}). ^{13}C NMR ($CDCl_3$): δ (ppm) = 19.8 (1C, C-4), 31.9 (1C, C-3), 36.0 (1C, C-5), 46.9 (1C, C-10), 48.1 (1C, $OCH_{3\,ketal}$), 48.2 (1C, $OCH_{3\,ketal}$), 49.1 (1C, C-8), 55.4 (1C, $C_6H_4OCH_3$), 57.6 (1C, C-6), 61.9 (1C, 1C, C-1), 63.1 (1C, $CH_2C_6H_5$), 63.6 (1C, $CH_2C_6H_4OCH_3$), 103.5 (1C, C-2), 113.6 (2C, C-3, C-5$_{methoxybenzyl}$), 126.9 (1C, C-4$_{benzyl}$), 128.2 (1C, C-1$_{methoxybenzyl}$), 129.1 (2C, C-3, C-5$_{benzyl}$), 130.1 (2C, C-2, C-6$_{benzyl}$), 132.8 (2C, C-2, C-6$_{methoxybenzyl}$), 140.1 (1C, C-1$_{benzyl}$), 158.6 (1C, C-4$_{methoxybenzyl}$). Exact mass (ESI): m/z = calculated for $C_{25}H_{35}N_2O_3H^+$ 411.2648, found 411.2642. I.R. (neat): ν/cm^{-1} = 2909 (C–H), 1509 (C═C), 1245 (C–O), 1108 (C–O).

7.2.13. (1*RS*,6*SR*)-7-Benzyl-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2]decan-2-one (24). 1M HCl (1.6 mL, 1.6 mmol) was added to a solution of **23** (0.66 g, 1.6 mmol) in THF–H_2O (1/1, 30 mL) and the mixture was stirred for 10 h at rt. Saturated $NaHCO_3$ solution was added until the mixture attained a pH of 7.5. The aqueous layer was extracted with Et_2O (5 × 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent was evaporated under vacuum. The crude viscous oil was purified by fc (ethyl acetate/petroleum ether = 5/95+0.05% *N*,*N*-dimethylethylamine, 4 cm, 10 cm, 30 mL, R_f 0.4 (ethyl acetate/cyclohexane = 1/4)) to obtain a colorless viscous oil, yield 0.45 g (77%). $C_{23}H_{28}N_2O_2$ (364.2). Purity (HPLC, method A): 96.0%, t_R = 17.5 min. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.36 (dt, J = 11.4/4.3 Hz, 1H, 5-H), 1.54-1.61 (m, 1H, 5-H), 1.70–1.85 (m, 2H, 4-H), 2.10(dt, J = 11.4/3.2 Hz, 1H, 3-H), 2.54 (d, J = 10.6 Hz, 1H, 10-H), 2.92 (dd, J = 10.6/2.4 Hz, 1H, 10-H), 2.96-3-00 (m, 2H, 6-H, 8-H), 3.02 (t, J = 3.4 Hz, 1H, 8-H), 3.08 (s(b), 1H, 1-H), 3.31 (dt, J = 11.4/3.2 Hz, 1H, 3-H), 3.47 (d, J = 13.0 Hz, 1H, $CH_2C_6H_4OCH_3$), 3.56 (t, J = 12.5 Hz, 2H, $CH_2C_6H_5$), 3.71 (s, 3H, OCH_3), 6.75 (d, J = 8.6 Hz, 2H, 3-H, 5-$H_{methoxybenzyl}$), 7.11-7.22 (m, 7H, 2-H, 6-$H_{methoxybenzyl}$, 2-H, 3-H, 4-H, 5-H, 6-H_{benzyl}). ^{13}C NMR ($CDCl_3$): δ (ppm) = 22.4 (1C, C-4), 35.3 (1C, C-5), 40.7 (1C, C-3), 50.9 (1C, C-10), 52.33 (1C, C-8), 55.4 (1C, OCH_3), 55.8 (1C, C-6), 61.8 (1C, $CH_2C_6H_5$), 62.5 (1C, $CH_2C_6H_4OCH_3$), 69.0 (1C, C-1), 114.0 (2C, C-3, C-5$_{methoxybenzyl}$), 127.2 (1C, C-4$_{benzyl}$), 128.4 (1C, C-1$_{methoxybenzyl}$), 129.1 (2C, C-2, C-6$_{benzyl}$), 130.1 (2C, C-3, C-5$_{benzyl}$), 130.7 (2C, C-2, C-6$_{methoxybenzyl}$), 139.1 (1C, C-1$_{benzyl}$), 158.9 (1C, C-4$_{methoxybenzyl}$), 222.6 (1C, C-2). MS (EI): m/z (%) = 365.1 [M+H)$^+$, 3] 121.1 [(methoxybenzyl)$^+$, 100], 91.1 [(benzyl)$^+$, 48]. IR (neat) ν/cm^{-1} = 1698 (C═O), 1510 (C═C aromatic), 1244 (C–O).

7.2.14. (1*RS*,2*SR*,6*SR*)-7-Benzyl-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2]decan-2-ol (25a) and (1*RS*,2*RS*,6*SR*)-7-benzyl-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2]decan-2-ol (16a). Under N_2 L-Selectride (Lithium tri-*sec*-butylborohydride, 1M in THF, 4.15 mL, 4.15 mmol) was slowly added to a solution of **24** (0.76 g, 2.07 mmol) in dry THF (40 mL) under ice-cooling and the mixture was stirred for 10 min at low temperature and for 12 h at rt. The excess L-Selectride was destroyed by addition of 1 M HCl

(5 mL) under ice-cooling. Saturated $NaHCO_3$ solution was added until the solution attained a pH of 7.5, then the aqueous layer was extracted with ethyl acetate (5 × 30 mL). The combined organic layers were washed with brine, dried over K_2CO_3 and the solvent was evaporated under vacuum to obtain the diastereomeric alcohols **16a** and **25a**. The ^{1}H NMR spectrum of the crude sample showed a ratio of **16a** : **25a** = 36 : 64. The diastereomers were separated by fc (started from ethyl acetate/petroleum ether = 5/95+0.05% *N*,*N*-dimethylethylamine to ethyl acetate/petroleum ether = 7/93+0.05% *N*,*N*-dimethylethylamine, 1.5 cm, 13 cm, 10 mL).

25a (R_f 0.27, ethyl acetate/cyclohexane = 3/7): Colorless viscous oil, yield 0.21 g (28%). $C_{23}H_{30}N_2O_2$ (366.2). Purity (HPLC, method B): 96.0%, t_R = 14.9 min. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.18–1.27 (m, 1H, 5-H), 1.39-1.51 (m, 2H, 4-H, 5-H), 1.76-1.83 (m, 1H, 3-H), 1.95-2.07 (m, 1H, 4-H), 2.36 (ddd, *J* = 14.0/11.1/2.9 Hz, 1H, 3-H), 2.51 (s, 1H, 8-H), 2.54-2.55 (m, 1H, 10-H), 2.77 (d (b), *J* = 4.5 Hz, 0.5H, 8-H), 2.80 (s(b), 1.5H, 8-H, 10-H), 2.86 (dd, *J* = 11.6/4.2 Hz, 1H, 6-H), 2.98 (s(b), 1H, 2-H), 3.15 (s(b), 0.6H, O*H*), 3.46 (d, *J* = 12.7 Hz, 2H, 1-H, C$H_2C_6H_5$), 3.53 (d, *J* = 12.9 Hz, 1H, C$H_2C_6H_4OCH_3$), 3.58 (d, *J* = 12.9 Hz, 1H, C$H_2C_6H_4OCH_3$), 3.66 (d, *J* = 12.7 Hz, 1H, C$H_2C_6H_5$), 3.72 (s, 3H, OCH_3), 6.79 (d, *J* = 8.4 Hz, 2H, 3-H, 5-$H_{methoxybenzyl}$), 7.16 (d, *J* = 8.5 Hz, 2H, 2-H, 6-$H_{methoxybenzyl}$), 7.22-7.24 (m, 3H, 3-H, 4-H, 5-H_{benzyl}). ^{13}C NMR ($CDCl_3$): δ (ppm) = 18.9 (1C, C-4), 33.6 (1C, C-3), 35.9 (1C, C-5), 47.5 (1C, C-10), 51.0 (1C, C-8), 55.4 (1C, $C_6H_4OCH_3$), 57.9 (1C, C-6), 63.2 (1C, CH_2Ph), 63.7 (1C, C-1), 64.9 (1C, C$H_2C_6H_4OCH_3$), 71.4 (1C, C-2), 114.1 (2C, C-3, C-5$_{methoxybenzyl}$), 127.1 (1C, C-4$_{benzyl}$), 128.3 (1C, C-1$_{methoxybenzyl}$), 129.1 (2C, C-2, C-6$_{benzyl}$), 130.0 (2C, C-3, C-5$_{benzyl}$), 131.3 (2C, C-2, C-6$_{methoxybenzyl}$), 139.8 (1C, C-1$_{benzyl}$), 159.0 (1C, C-4$_{methoxybenzyl}$). Exact mass (ESI): *m*/*z* = calculated for $C_{23}H_{31}N_2O_2H^+$ 367.2386, found 367.2380 $^+$. IR (neat): ν/cm^{-1} = 3401 (O–H), 1510 (C═C), 1442 (C–N), 1247 (C–O).

16a (R_f 0.20, ethyl acetate/cyclohexane = 3/7): Colorless viscous oil, yield 0.18 g (24%).

7.2.15. (1*RS*,2*SR*,6*SR*)-7-Benzyl-2-methoxy-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2] decane (25b). Under N_2 NaH (10 mg, 0.22 mmol, 60% in paraffin oil) was slowly added to a solution of **25a** (55 mg, 0.15 mmol) and CH_3I (0.02 mL, 0.3 mmol) in dry DMF (2 mL) under ice-cooling. The reaction mixture was stirred for 3 h at rt. The excess NaH was destroyed with H_2O (5 mL) under ice-cooling. The aqueous layer was extracted with ethyl acetate (7 × 10 mL). The combined organic layers were washed with brine, dried over K_2CO_3 and the solvent was evaporated under vacuum. The crude viscous oil was purified by fc (ethyl acetate/petroleum ether = 5/95+0.05% *N*,*N*-dimethylethylamine, 1.5 cm, 13 cm, 10 mL, R_f 0.36 (ethyl acetate/cyclohexane = 3/7)) to obtain a colorless viscous oil, yield 40 mg (70%). $C_{24}H_{32}N_2O_2$ (380.2). Purity (HPLC, method B): 97.2%, t_R = 16.3 min. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.09–1.14 (m, 1H, 5-H), 1.46 (dt, *J* = 12.9/5.6 Hz, 1H, 5-H), 1.56-1.64 (m, 1H, 4-H), 1.92-2.13 (m, 3H, 3-H, 4-H), 2.28 (d, *J* = 10.3 Hz, 1H, 10-H), 2.50 (d, *J* = 11.1 Hz, 1H, 8-H), 2.78 (dd, *J* = 10.3/6.4 Hz, 2H, 6-H, 10-H), 2.86 (dd, *J* = 11.1/4.4 Hz, 1H, 8-H), 2.94-2.97 (m, 1H, 2-H), 3.11 (s(b), 1H, 1-H), 3.21 (s, 3H, CHOCH_3), 3.46 (d, *J* = 12.7 Hz, 1H, C$H_2C_6H_4OCH_3$), 3.53 (s, 2H, C$H_2C_6H_5$), 3.72 (s, 3H, $C_6H_4OCH_3$), 3.76 (d, *J* = 12.7 Hz, 1H, C$H_2C_6H_4OCH_3$), 6.79 (d, *J* = 8.7 Hz, 2H, 3-H, 5-$H_{methoxybenzyl}$), 7.13-7.24 (m, 5H, 2-H, 6-$H_{methoxybenzyl}$, 2-H, 4-H, 6-H_{benzyl}), 7.26-7.29 (m, 2H, 3-H, 5-H_{benzyl}). ^{13}C NMR ($CDCl_3$): δ (ppm) = 19.2 (1C, C-4), 29.2 (1C, C-3), 34.4 (1C, C-5), 48.8 (1C, C-10), 48.9 (1C, C-8), 54.2 (1C, $C_6H_4OCH_3$), 55.2 (1C, CHOCH_3), 56.3 (1C, C-6), 59.9 (1C, C-1), 62.0 (1C, $CH_2C_6H_5$), 62.5 (1C, $CH_2C_6H_4OCH_3$), 85.7 (1C, C-2), 112.3 (2C, C-3, C-5$_{methoxybenzyl}$), 125.7 (1C, C-4$_{benzyl}$), 127.0 (1C, C-1$_{methoxybenzyl}$), 127.8 (2C, C-2, C-6$_{benzyl}$), 129.0 (2C, C-3, C-5$_{benzyl}$), 131.3 (2C, C-2, C-6$_{methoxybenzyl}$), 138.9 (1C, C-1$_{benzyl}$), 157.3 (1C, C-4$_{methoxybenzyl}$). Exact mass (ESI): *m*/*z* = calculated for $C_{24}H_{33}N_2O_2H^+$ 381.2542, found 381.2537. I.R. (neat): ν/cm^{-1} = 2905 (C–H), 1509 (C–O), 1244 (C–O), 698 (C═C).

7.2.16. (1*RS*,6*RS*)-7-Benzyl-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2]decane (26). Under N_2 $LiAlH_4$ (0.03 g, 0.87 mmol) was added to a solution of **20** (0.12 g, 0.22 mmol) in THF (10 mL) under ice-cooling. The mixture was stirred at rt for 12 h and under reflux for 3 h. The excess $LiAlH_4$ was destroyed with H_2O (1 mL) under ice-cooling and the mixture was refluxed for an additional hour. The mixture was cooled to rt and filtered. The filtrate was concentrated under vacuum to obtain a colorless viscous oil. Purification by fc (*t*-butyl methyl ether/petroleum ether = 7.5/92.5, 1.5 cm, 18 cm, 2 mL, R_f 0.26 ethyl acetate/cyclohexane = 1/9)) gave a colorless oil, yield 0.05 g (65%). $C_{23}H_{30}N_2O$ (350.5). Purity (HPLC, method A): 96.5%, t_R = 19.4 min. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.26 (d, *J* = 12.8 Hz, 2H, 2-H, 5-H), 1.47 (s (broad), 2H, 2-H, 5-H), 1.69 (d, *J* = 9.9 Hz, 2H, 3-H, 4-H), 2.09 (t, *J* = 10.2 Hz, 2H, 3-H, 4-H), 2.47 (d, *J* = 10.2 Hz, 2H, 8-H, 10-H), 2.80 (s, 2H, 1-H, 6-H), 2.88 (dd, *J* = 10.8/2.7 Hz, 2H, 8-H, 10-H), 3.48 (s, 2H, NC$H_2C_6H_5$), 3.55 (s, 2H, NC$H_2C_6H_4OCH_3$), 3.72 (s, 3H, OCH_3), 6. 76 (d, *J* = 8.5 Hz, 2H, 3-H, 5-$H_{methoxybenzyl}$), 7.13-7.24 (m, 5H, 2-H, 6-$H_{methoxybenzyl}$, 2-H, 4-H, 6-H_{benzyl}), 7.28 (d, *J* = 7.3 Hz, 2H, 3-H, 5-H_{benzyl}). ^{13}C NMR ($CDCl_3$): δ (ppm) = 25.5 (2C, C-2, C-5), 36.3 (2C, C-3, C-4), 50.9 (2C, C-8, C-10), 55.4 (1C, OCH_3), 58.1 (1C, C-6), 58.3 (1C, C-1), 62.8 (1C, NC$H_2C_6H_5$), 63.6 (1C, NC$H_2C_6H_4OCH_3$), 113.6 (2C, C-3, C-5$_{methoxybenzyl}$), 126.9 (1C, C-4$_{benzyl}$), 128.3 (1C, C-1$_{methoxybenzyl}$), 129.2 (2C, C-3, C-5$_{benzyl}$), 130.2 (2C, C-2, C-6$_{benzyl}$), 132.5 (2C, C-2, C-6$_{methoxybenzyl}$), 140.5 (1C, C-1$_{benzyl}$), 160.2 (1C, C-4$_{methoxybenzyl}$). Exact mass (ESI): *m*/*z* = calculated for $C_{23}H_{30}N_2OH^+$ 351.2436, found 351.2435. IR (neat): ν/cm^{-1} = 1611, 1511 (C═C), 1242 (C–O), 699 (C═C).

7.2.17. (1*RS*,6*RS*)-7-Benzyl-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2]dec-2-ene (27). Under N_2 $LiAlH_4$ (0.01 g, 0.26 mmol) was added to a solution of **18** (50 mg, 0.13 mmol) under ice-cooling. The mixture was heated to reflux for a period of 12 h. H_2O (1 mL) was added to the mixture under ice-cooling and the mixture was heated to reflux for an additional hour. The solution was cooled to rt and filtered through a sintering funnel. The precipitate was washed with CH_2Cl_2 (20 mL) and the solvent was evaporated under vacuum. The crude oil was purified by fc (petroleum ether/ethyl acetate = 95/5, 1.5 cm, 22 cm, 5 mL, R_f 0.23 (ethyl acetate/cyclohexane = 2/8)) to obtain **27** as a colorless viscous oil, yield 32 mg (71%). $C_{23}H_{28}N_2O$ (348.4). Purity (HPLC, method A): 99.1%, t_R = 18.2 min. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.43 (dq, *J* = 13.9/4.7 Hz, 1H, 5-H), 1.64 (s (broad), 1H, 5-H), 2.02 (s, 1H, 4-H), 2.58 (d, *J* = 8.6 Hz, 1H, 8-H), 2.65 (d, *J* = 10.4 Hz, 1H, 10-H), 2.87 (d, *J* = 9.2 Hz, 1H, 8-H), 2.93-3.03 (m, 3H; 4-H, 6-H, 10-H), 3.24 (s, 1H, 1-H), 3.58 (s, 2H, NC$H_2C_6H_5$), 3.66 (d, *J* = 13.8 Hz, 1H, NC$H_2C_6H_4OCH_3$), 3.72 (s, 3H, OCH_3), 3.76 (d, *J* = 13.1 Hz, 1H, NC$H_2C_6H_4OCH_3$), 5.37 (dd, *J* = 10.6/5.5 Hz,

1H, 2-H),5.90 (dt, J = 11.3/7.6 Hz, 1H, 3-H), 6. 76 (d, J = 8.5 Hz, 2H, 3-H, 5-$H_{methoxybenzyl}$), 7.15-7.25 (m, 5H, 2-H, 6-$H_{methoxybenzyl}$,2-H, 4-H, 6-H_{benzyl}), 7.28 (d, J = 7.3 Hz, 2H, 3-H, 5-H_{benzyl}). ^{13}C NMR ($CDCl_3$): δ (ppm) = 24.2 (1C, C-4), 35.9 (1C, C-5), 49.6 (1C, C-8), 50.7 (1C, C-10), 55.4 (1C, OCH_3), 56.2 (1C, C-6), 58.8 (1C, C-1), 61.3 (1C, N$CH_2C_6H_4OCH_3$), 61.9 (1C, N$CH_2C_6H_5$), 113.7 (2C, C-3, C-5$_{methoxybenzyl}$), 127.0 (1C, C-4$_{benzyl}$), 128.3 (3C, C-1$_{methoxybenzyl}$, C-3, C-5$_{benzyl}$), 129.2 (2C, C-2, C-6$_{benzyl}$), 129.8 (3C, C-2, C-6$_{methoxybenzyl}$, C-1$_{benzyl}$), 130.3 (1C, C-3), 130.9 (1C, C-2), 158.7 (1C, C-4$_{methoxybenzyl}$). MS (EI): m/z (%) = 347 [M$^+$, 3], 121 [methoxybenzyl$^+$, 100], 91 [(benzyl)$^+$, 56]. IR (neat): ν/cm^{-1} = 1611 (C═C), 1511 (C═C), 1244 (C–O), 699 (C–H).

7.2.18. (1*RS*,6*SR*)-7-Benzyl-2,2-difluoro-9-(4-methoxybenzyl)-7,9-diazabicyclo[4.2.2] decane (36). DAST (0.016 mL, 0.12 mmol) was added to an ice-cold solution of ketone **24** (22 mg, 0.06 mmol) in CH_2Cl_2 (2 mL) and the mixture was stirred at 0 °C for 12 h. The reaction mixture was washed with sat. $NaHCO_3$ solution (10 mL) and the aqueous layer was extracted with CH_2Cl_2 (5 × 10 mL). The combined organic layers were dried (K_2CO_3) and the solvent was evaporated under vacuum to obtain a colorless viscous oil. Purification by fc (ethyl acetate/petroleum ether = 3/97, 1.5 cm, 15 cm, 5 mL, R_f 0.52 (ethyl acetate/cyclohexane = 1/4)) gave a colorless viscous oil, yield 0.22 g (95%). $C_{23}H_{28}F_2N_2O$ (386.2). Purity (HPLC, method A): 96.4%, t_R = 19.7 min. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.28-1.32 (m, 1H, 5-H), 1.43-1.52 (m, 1H, 5-H), 1.67 (ddd, J = 14.2/8.7/4.1 Hz, 1H, 4-H), 2.20 (ddd, J = 25.1/12.5/10.6 Hz, 1H, 3-H), 2.39 (ddd, J = 15.4/13.1/4.1 Hz, 1H, 4-H), 2.45 (d, J = 9.2 Hz, 1H, 10-H), 2.64-2.81 (m, 1H, 3-H), 2.84-2.92 (m, 4H, 6-H, 8-H, 10-H), 3.16 (t, J = 9.1 Hz, 1H, 1-H), 3.53 (d, J = 12.7 Hz, 1H, N$CH_2C_6H_4OCH_3$), 3.61 (d, J = 12.9 Hz, 1H, N$CH_2C_6H_5$), 3.65 (d, J = 12.9 Hz, 1H, N$CH_2C_6H_5$), 3.80 (s, 3H, OCH_3), 3.88 (d, J = 12.9 Hz, 1H, N$CH_2C_6H_5$), 6.84 (d, J = 8.8 Hz, 2H, 3-H, 5-$H_{methoxybenzyl}$), 7.24 (d, J = 8.6 Hz, 3H, 2-H, 6-$H_{methoxybenzyl}$, 4-H_{benzyl}), 7.30-7.36 (m, 4H, 2-H, 3-H, 5-H, 6-H_{benzyl}). ^{13}C NMR ($CDCl_3$): δ (ppm) = 19.8 (t, J = 5.6 Hz, 1C, C-4), 35.1 (t, J = 25.6 Hz, 1C, C-3), 35.4 (1C, C-5), 45.5 (t, J = 4.9 Hz, 1C, C-8), 48.6 (1C, 10-C), 55.4 (1C, OCH_3), 57.7 (1C, C-6), 62.8 (1C, N$CH_2C_6H_4OCH_3$), 63.2 (1C, N$CH_2C_6H_5$), 64.7 (t, J = 29.2 Hz, 1C, C-1), 113.7 (2C, C-3, C-5$_{methoxybenzyl}$), 127.2 (1C, C-4$_{benzyl}$), 124.8 (t, J = 246.2 Hz, 1C, C-2), 128.4 (1C, C-1$_{methoxybenzyl}$), 129.2 (2C, C-3, C-5$_{benzyl}$), 130.2 (2C, C-2, C-6$_{benzyl}$), 131.5 (2C, C-2, C-6$_{methoxybenzyl}$), 139.4 (1C, C-1$_{benzyl}$), 158.8 (1C, C-4$_{methoxybenzyl}$). Exact mass (ESI): m/z = calculated for $C_{23}H_{28}F_2N_2OH^+$ 387.2247, found 387.2242. IR (neat): ν/cm^{-1} = 1511 (C═C), 1245 (C–F), 698 (C═C).

7.3. Receptor binding studies

7.3.1. Materials and general procedures. The guinea pig brains and rat livers were commercially available (Harlan–Winkelmann, Borchen, Germany). The pig brains were a donation of the local slaughterhouse (Coesfeld, Germany). Homogenizer: Elvehjem Potter (B. Braun Biotech International, Melsungen, Germany). Centrifuge: High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Fisher Scientific, Langenselbold, Germany). Filter: Printed Filtermat Typ A and B (Perkin Elmer LAS, Rodgau-Jügesheim, Germany), presoaked in 0.5% aqueous polyethylenimine for 2 h at rt before use. The filtration was carried out with a MicroBeta FilterMate-96 Harvester (Perkin Elmer). The scintillation analysis was performed using Meltilex (Typ A or B) solid scintillator (Perkin Elmer). The solid scintillator was melted on the filter mat at a temperature of 95 °C for 5 min. After solidifying of the scintillator at rt, the scintillation was measured using a MicroBeta Trilux scintillation analyzer (Perkin Elmer). The counting efficiency was 40%. All experiments were carried out in triplicates using standard 96-well-multiplates (Diagonal, Muenster, Germany). The IC_{50}-values were determined in competition experiments with at least six concentrations of the test compounds and were calculated with the program GraphPad Prism® 3.0 (GraphPad Software, San Diego, CA, USA) by non-linear regression analysis. The K_i-values were calculated according to the formula of Cheng and Prusoff.[46] The K_i-values are given as mean value ± SEM from three independent experiments.

7.3.2. Membrane preparation for the σ_1 assay. 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[47] 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^{-1}.

7.3.3. Performing the σ_1 assay. The test was performed with the radioligand [^{3}H]-(+)-pentazocine (32,2 Ci/mmol; Perkin Elmer LAS). 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 150 min at 37 °C. The incubation was terminated by rapid filtration through the presoaked filter mats using a cell harvester. After washing each well five times with 300 µl of water, the filter mats were dried at 95 °C. Subsequently, the solid scintillator was placed on the filter mat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at rt. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The non-specific binding was determined with 10 µM unlabeled (+)-pentazocine. The K_d-value of (+)-pentazocine is 2.9 nM.[42]

7.3.4. Membrane preparation for the σ_2 assay. Two rat livers were cut into smaller pieces and 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 31000 × g for 20 min at 4 °C. The pellet was resuspended in 5–6 volumes of buffer (50 mM TRIS, pH 8.0) and incubated at rt 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 5–6 volumes of buffer, the protein concentration was determined according to the method of Bradford[47] 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^{-1}.

7.3.5. Performing the σ_2 assay. The test was performed with the radioligand [^{3}H]-ditolylguanidine (50 Ci/mmol; ARC, St.

Louis, MO, USA). The thawed membrane preparation (about 100 µg of the protein) was incubated with various concentrations of test compounds, 3 nM [^{3}H]-ditolylguanidine, and buffer containing (+)-pentazocine (2 µM (+)-pentazocine in 50 mM TRIS, pH 8.0) in a total volume of 200 µL for 150 min at rt. The incubation was terminated by rapid filtration through the presoaked filter mats using a cell harvester. After washing each well five times with 300 µL of water, the filter mats were dried at 95 °C. Subsequently, the solid scintillator was placed on the filter mat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at rt. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The non-specific binding was determined with 10 µM unlabeled ditolylguanidine. The K_d-value of ditolylguanidine is 17.9 nM.[43]

7.4. Cancer cell growth inhibition assay

A well established microtiter assay based on the staining of cell components with crystal violet was used to measure the inhibition of cell growth, as described in detail in ref. 45

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