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Conformationally Constrained κ Receptor Agonists: Stereoselective Synthesis and Pharmacological Evaluation of 6,8-Diazabicyclo[3.2.2]nonane Derivatives†

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Three sets of stereoisomeric bicyclic κ agonists with defined orientation of the pharmacophoric elements pyrrolidine and dichlorophenylacetamide were stereoselectively prepared and pharmacologically evaluated. Stereoselective reduction, reductive amination, and Mitsunobu inversions were the key steps for the establishment of the desired stereochemistry. The κ affinity decreased in the following order depending on the N-substituent: CO_2CH_3 > benzyl > $COCH_2CH_3$. Bicyclic derivatives with (1*S*,2*R*,5*R*)-configuration showed the highest κ receptor affinity, which led to dihedral angles of 97° and 45° for the *N*(pyrrolidine)−C−C−*N*(phenylacetamide) structural element. The most potent κ agonist of this series was (+)-methyl (1*S*,2*R*,5*R*)-8-[2-(3,4-dichlorophenyl)acetyl]-2-(pyrrolidin-1-yl)-6,8-diazabicyclo[3.2.2]nonane-6-carboxylate (*ent*-**23**, WMS-0121) with an K_i value of 1.0 nM. *ent*-**23** revealed high selectivity against the other classical opioid receptors and related receptor systems. In the [^{35}S]GTPγS-binding assay at human κ-opioid receptors, *ent*-**23** was proved to be a full agonist with the same EC_{50} value (87 nM) as the prototypical full agonist U-69,593 (EC_{50} = 80 nM).

Introduction

Agonists of the three classical opioid receptors, μ, κ, and δ receptors, can be used for the treatment of strong pain, caused e.g. by surgery, accident, or cancer. The clinically used opioid analgesics are more or less selective μ receptor agonists. Therefore, their application is accompanied by severe side effects like euphoria, respiratory depression, constipation, tolerance, and dependence. The side effect profile of κ receptor agonists differs considerably from that of μ agonists. In contrast to μ agonists, they cause only minimal physical dependence, respiratory depression, and inhibition of gastrointestinal motility. However, sedation, dysphoria, and strong diuresis are the most severe side effects associated with the application of κ agonists. In addition to the strong analgesic effect, κ agonists have been shown to be potent neuroprotective and antihyperalgesic agents in in vivo.[1,2] Moreover, growing interest is observed in peripherally acting κ agonists for the selective treatment of inflammatory visceral and neuropathic pain as well as pruritus in the periphery, which are devoid of centrally mediated side effects.[3,4]

Although all three opioid receptor subtypes have already been cloned,[5,6] X-ray crystal structures of these G-protein coupled membrane bound receptors showing the exact three-dimensional construction of the ligand binding sites are not yet available. In this project, compounds with high κ receptor affinity were selected as lead compounds for the development of novel potent and selective κ agonists.

Most of the reported κ agonists belong to four compound classes:[1,2,7] peptides (e.g., the physiological agonist dynorphin A[1,7]), benzomorphans (e.g., ethylketocyclazocine[1,7]), arylacetamides (e.g., the prototypical κ agonist 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1*S*,2*S*)-2-pyrrolidinocyclohexyl]acetamide (U-50,488)[8]), and the nonbasic natural product salvinorin A.[9,10]

The prototypical κ agonist U-50,488 from the arylacetamide class has served as lead compound for the development of various potent κ agonists. In particular, annulated compounds,[11,12] simplified ligands,[13,14] as well as heterocyclic analogues like 2-(aminomethyl)piperidines[15,16] and 2-(aminomethyl)piperazines[17−19] have been developed. The (2*R*)-configured 2-(aminomethyl)piperazine **1** (GR-89,696[18]) belongs to the most potent κ agonists with an IC_{50} value of 0.018 nM in the rabbit vas deferens model[17−19] and a K_i value of 0.36 nM in receptor binding studies.[20] (Figure 1) Introduction of an additional methyl moiety into the pyrrolidinylmethyl side chain of **1** led to the pyrrolidinylethyl derivative **2** with slightly increased κ receptor affinity (K_i = 0.31 nM).[20,21] In both compounds **1** and **2**, the stereochemistry is crucial for high κ receptor affinity. In Figure 1, the stereoisomers with the highest κ affinity are shown, respectively.

Because of allylic strain, the pyrrolidinylalkyl side chain in position 2 of both 1,4-diacylpiperazines **1** and **2** is forced to adopt an axial orientation.[21] In both compounds, an almost free rotation around the indicated axial bond is possible.[22] To investigate the bioactive conformation of **1** and **2**, the conformational flexibility of the side chain should be reduced by connection of the terminal methyl moiety to the piperazine scaffold.

In the first series of ligands, the methyl moiety of **2** was connected with the N-atom in position 4 of the piperazine system, which resulted in the bicyclic κ agonists **3**. The diastereomeric compounds *exo*-**3** (K_i > 1000 nM) and *endo*-**3** (K_i = 73 nM) showed negligible and low κ receptor affinity,

†Dedicated to Prof. Dr. h.c. F. Eiden, Munich, on occasion of his 85th birthday.

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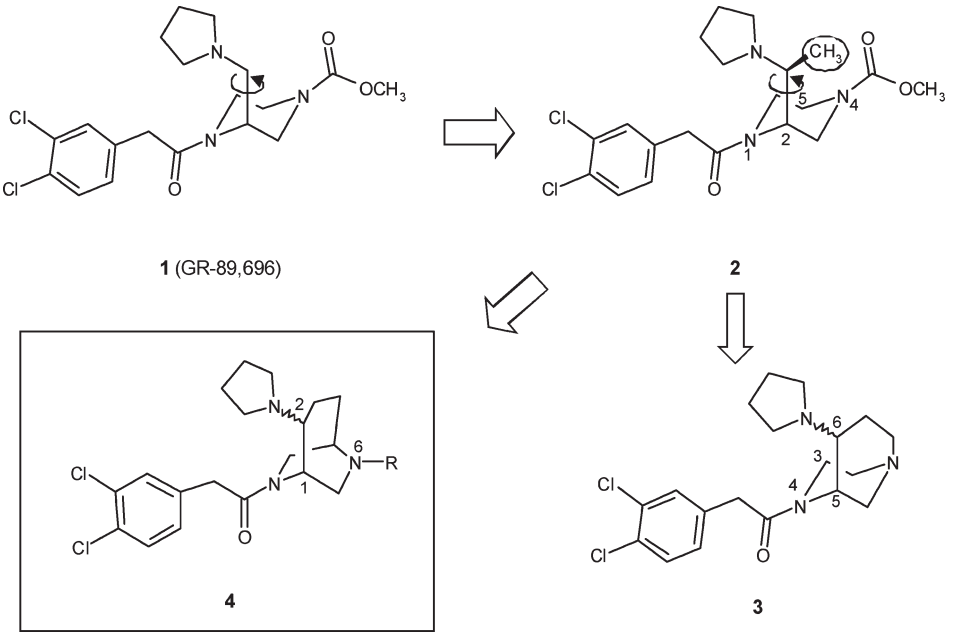
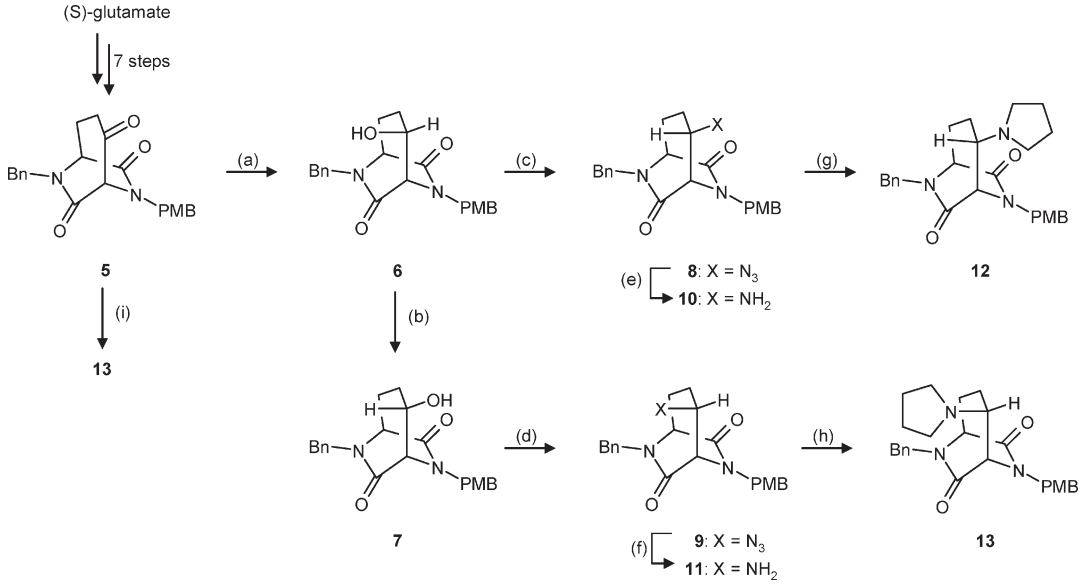


Figure 1. Structural development of bicyclic κ receptor agonists.

Scheme 1[a]



[a] Reagents and reaction conditions: (a) $LiBH_4$, THF, −90 °C, 84%, ref 26; (b) (1) DIAD, PPh_3, 4-nitrobenzoic acid, THF, ref 26, (2) CH_3OH, K_2CO_3, rt, 74% from 6, ref 26; (c) HN_3, PPh_3, DIAD, benzene, THF, rt, 98%; (d) $Zn(N_3)_2 \cdot (pyridine)_2$, PPh_3, DIAD, toluene, rt, 72%; (e) H_2, Pd/C, 1 bar, rt, 99%; (f) H_2, Pd/C, 1 bar, rt, 98%; (g) $I(CH_2)_4I$, CH_3CN, $NaHCO_3$, 18 h, reflux, 82% (from 8); (h) $I(CH_2)_4I$, CH_3CN, $NaHCO_3$, 16 h, reflux, 60% (from 9); (i) pyrrolidine, $NaBH(OAc)_3$, 1,2-dichloroethane, 0−12 °C, 76%, de 94%.

respectively, indicating that a dihedral angle (*N*(pyrrolidine)−C−C−*N*(phenylacetamide)) of 180° (*exo*-**3**) is not tolerated and a dihedral angle of 58° (*endo*-**3**) is better accepted by the κ receptor protein.[22,23]

In a new series of conformationally constrained κ agonists, the methyl moiety of **2** was connected with the C-atom in position 5 of the piperazine ring. This connection leads to 6,8-diazabicyclo[3.2.2]nonanes of type **4**, which allows further modifications by introduction of various residues R at the N-atom in position 6. Herein, we report on the stereoselective synthesis and pharmacological evaluation of all possible stereoisomers of the κ receptor agonists **4** with a 6,8-diazabicyclo[3.2.2]nonane framework.

Chemistry

Synthesis of the designed κ agonists of type **4** started with bicyclic ketone **5**, which was derived from the proteinogenic amino acid (*S*)-glutamate (Scheme 1).[24−26] At first the ketone in position 2 should be stereoselectively converted into the desired pyrrolidine moiety. Then the orthogonal protective groups at the N-atoms will allow successive cleavage and introduction of different acyl residues. Because the transformations have been carefully optimized with (*S*)-glutamate derived bicyclic ketone **5**, the syntheses in the reaction schemes are shown with the corresponding enantiomers.

$LiBH_4$ reduction of **5** at −90 °C provided diastereoselectively alcohol **6** (**6**:**7** = 99:1), which was transformed into the

diastereomeric alcohol **7** by a Mitsunobu inversion using *p*-nitrobenzoic acid (Scheme 1).[26] According to our first idea, the alcohols **6** and **7** were activated as mesylates.[27] But when the mesylates were subsequently reacted with pyrrolidine, starting material with few elimination products was obtained instead of the desired substitution products **12** and **13**.

Therefore, a stepwise setup of the pyrrolidine ring was envisaged (Scheme 1). For this purpose, alcohol **6** should be converted into azide **8**, which should be reduced and alkylated to form the pyrrolidine ring. In the literature, several methods for the direct conversion of alcohols into azides by a Mitsunobu reaction[28] have been reported. Reaction of alcohol **6** with diphenyl phosphoryl azide ($(PhO)_2PON_3$), triphenylphosphane (PPh_3), and diisopropyl azodicarboxylate (DIAD[a])[29] led to bicyclic azide **8** in 78% yield. Using the complex $Zn(N_3)_2 \cdot (pyridine)_2$[30] increased the yield of azide **8** to 91%. However, the best yield of azide **8** (98%) was obtained using a solution of HN_3 in benzene[31] as azide source. Nucleophilic substitution of mesylate of **6** with NaN_3 also gave the bicyclic azide **8** in 70% yield, but additionally considerable amounts of the elimination product were produced. Because of the excellent yields, HN_3 and $Zn(N_3)_2 \cdot (pyridine)_2$ complex were the standard reagents in this project. The diastereomeric azide **9** was synthesized from diastereomeric alcohol **7** in a Mitsunobu reaction with $Zn(N_3)_2 \cdot (pyridine)_2$ complex. Under the Mitsunobu conditions, all reactions took place with complete inversion of configuration at position 2 and elimination products were not formed.

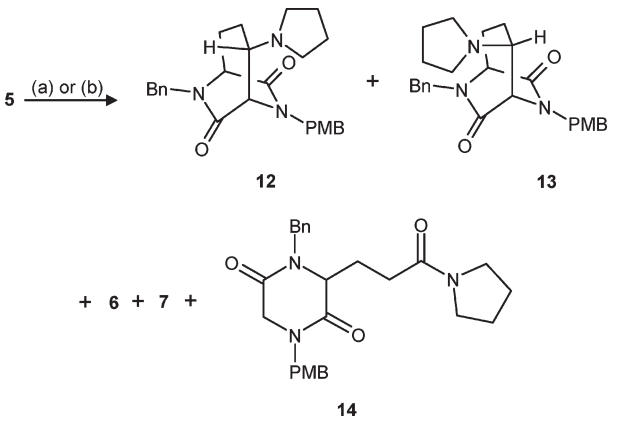
The diastereomeric azides **8** and **9** were reduced with H_2 in the presence of Pd/C to give the primary amines **10** and **11** in 99% and 98% yield, respectively. To develop an one-pot consecutive Mitsunobu−Staudinger reaction azide, **8** was treated with PPh_3 in THF to provide the primary amine **10**. A quantitative transformation of azide **8** into primary amine **10** was observed by thin layer chromatography, however, the isolated yield of the primary amine **10** was only 37% due to problems during separation of Ph_3PO.

Reaction of the primary amines **10** and **11** with 1,4-diiodobutane in boiling acetonitrile led to the pyrrolidine derivatives **12** and **13**. High yields of **12** (82%) and **13** (60%) were only achieved by use of freshly distilled 1,4-diiodobutane.

Altogether, pyrrolidine **12** was stereoselectively prepared in four reaction steps starting from ketone **5**. The overall yield of 67% over four steps was obtained only, when the primary amine **10** was directly transformed into the pyrrolidine **12**. The stereoselective synthesis of diastereomeric pyrrolidine **13** required six reaction steps starting with ketone **5** because an additional Mitsunobu inversion (**6** → **7**) was necessary. The overall yield was 26% over six steps.

To shorten the synthesis of the diastereomeric pyrrolidines **12** and **13**, the direct reductive amination of ketone **5** with pyrrolidine was investigated (Scheme 2). Reaction of ketone **5** with pyrrolidine and $NaBH_3CN$[32] at pH 6 led to a mixture of the diastereomeric pyrrolidines **12** and **13** (yield 49%, ratio 40: 60) and the diastereomeric alcohols **6** and **7** (yield 20%, ratio 75: 25). Addition of the Lewis acid $Ti(OiPr)_4$[33] did not improve the yield of pyrrolidines **12**/**13** (27−52%) but led to considerable amounts of amide **14** as an additional side product (27−44%). The formation of amide **14** does obviously occur by a pyrrolidine induced ester cleavage, which is similar to the ring-opening observed after treatment of ketone **5** with methanolate.[34] The reaction of ketone **5** with $NaBH(OAc)_3$[35] in dichloroethane at 0−12 °C without $Ti(OiPr)_4$ provided the pyrrolidines **12** and **13** in 76% yield with an excellent diastereoselectivity of 3:97. Only traces of the amide **14** were formed under these reaction conditions. The reductive amination of ketone **5** represents a considerable improvement of the six-step synthesis of pyrrolidine **13** that includes two Mitsunobu inversion reactions. Removal of small amounts (3%) of diastereomer **12** succeeded only after reduction of **13** to piperazine derivative **16**.

Scheme 2[a]



[a] Reagents and reaction conditions: (a) pyrrolidine, $NaBH_3CN$, $Ti(OiPr)_4$, CH_2Cl_2, CH_3OH, rt, 27% of **12**/**13** (34:66), 44% of **14**; (b) pyrrolidine, $NaBH(OAc)_3$, 1,2-dichloroethane, 0−12 °C, 76% of **13**, **12**:**13** = 3:97, traces of **14**.

Next, bicyclic dilactams **12** and **13** were reduced with $LiAlH_4$ to give piperazine derivatives **15** and **16** with orthogonal protective groups at the N-atoms (Scheme 3). The *p*-methoxybenzyl protective group at N-8 was selectively removed with trifluoroacetic acid, and the resulting secondary amines **17** and **18** were acylated with (3,4-dichlorophenyl)-acetyl chloride (DCPA-Cl) to afford acetamides **19** and **20**, respectively. The benzyl protective group at N-6 was removed hydrogenolytically. However, dechlorinated product **27** was formed exclusively when the hydrogenolytic cleavage was performed with ammonium formate and Pd/C in boiling methanol (Scheme 4). Selective *N*-debenzylation without dechlorination was achieved using H_2 (balloon) and Pd/C in aqueous THF. The benzylamino moiety was activated by addition of a few drops of concentrated HCl, the reaction time was limited to 30 min, and the reaction mixture was stirred at room temperature. These reaction conditions led to the (dichlorophenyl)acetamides **21** and **22** in 69% and 83% yield, respectively. Finally, the secondary amines **21** and **22** were acylated with methyl chloroformate or propionyl chloride to provide the methoxycarbonyl and propionyl derivatives **23**−**26** in more than 90% yields, respectively.

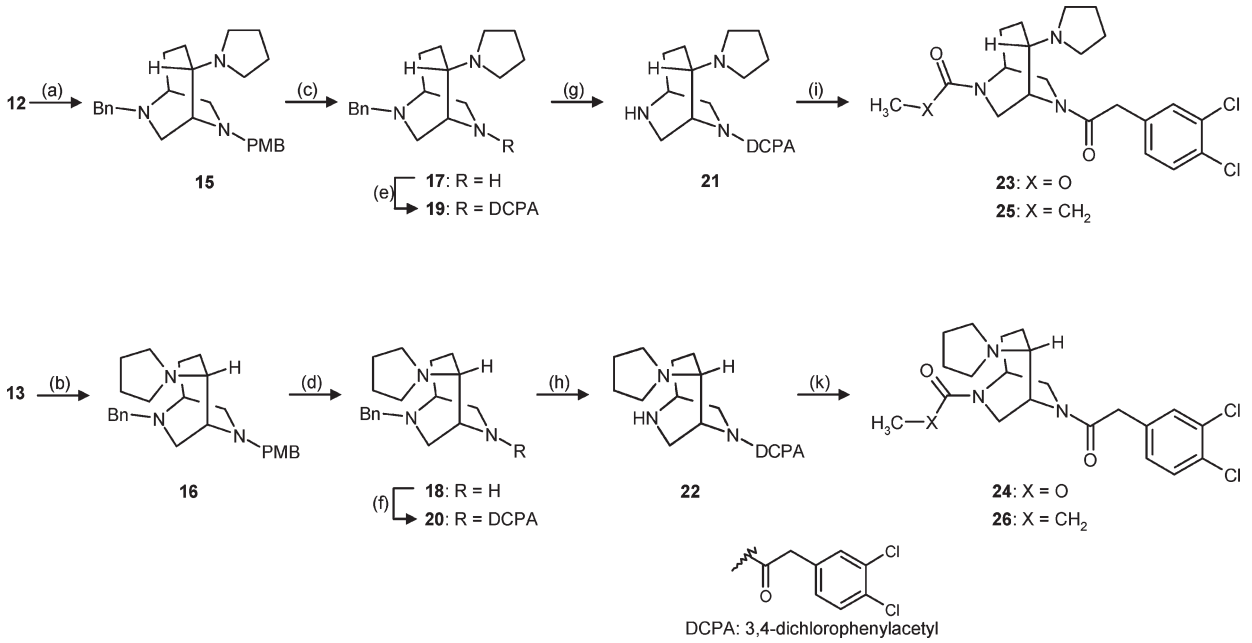
The corresponding enantiomers of the κ agonists **19**/**20**, **23**/**24**, and **25**/**26**, which are termed using the prefix *ent* (e.g., *ent*-**19**, *ent*-**26**), were prepared in the same manner.

κ Receptor Affinity

The κ receptor affinities of the bicyclic amines were determined in competition experiments with the radioligand [^{3}H]-U-69,593.[20,22] Membrane homogenates prepared from guinea pig brains were used as receptor material. Nonspecific binding

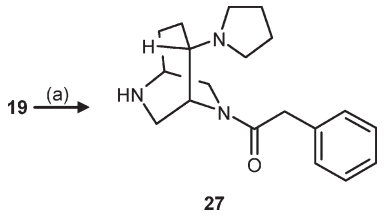
[a] Abbreviations: DIAD, diisopropyl azodicarboxylate; DCPA-Cl, (dichlorophenyl)acetyl chloride; MOE, molecular operating system; *ent*, enantiomeric; PCP, phencyclidine [(1-phenylcyclohexyl)piperidine]; NMDA, *N*-methyl-D-aspartate.

Scheme 3[a]



[a] Reagents and reaction conditions: (a) $LiAlH_4$, THF, 66 °C, 19 h; (b) $LiAlH_4$, THF, 66 °C, 16 h; (c) CF_3CO_2H, CH_2Cl_2, 40 °C, 24 h, 60% (from **12**); (d) CF_3CO_2H, CH_2Cl_2, 40 °C, 16 h; (e) (3,4-dichlorophenyl)acetyl chloride (DCPA-Cl), CH_2Cl_2, rt, 3 h, 95%; (f) DCPA-Cl, CH_2Cl_2, rt, 16 h, 49% (from **13**); (g) H_2,Pd/C, THF, H_2O, HCl conc, rt, 30 min, 69%; (h) H_2, Pd/C, THF, H_2O, HCl conc, rt, 30 min, 83%; (i) $H_3COCOCl$, NEt_3, CH_2Cl_2, rt, 16 h, 92%; (k) $H_3COCOCl$, NEt_3, CH_2Cl_2, rt, 1 h, 91%; (l) H_3CCH_2COCl, NEt_3, CH_2Cl_2, rt, 16 h, 92%; (m) H_3CCH_2COCl, NEt_3, CH_2Cl_2, rt, 16 h, 92%.

Scheme 4[a]



[a] Reagents and reaction conditions: (a) NH_4HCO_2, Pd/C, CH_3OH, 65 °C, 3.5 h, 74%.

was determined in the presence of a large excess of nontritiated U-69,593 (10 μM).[20,22]

The κ receptor affinities of the bicyclic dichlorophenylacetamides are summarized in Table 1. A strong dependence of the κ receptor affinity from the stereochemistry and the N-substituent is demonstrated. Generally, the compounds with (1*S*,2*R*,5*R*)-configuration *ent*-**19**, *ent*-**23**, and *ent*-**25** show the highest κ receptor affinities with excellent eudismic ratios. The corresponding diastereomers are considerably less potent κ ligands.

The most potent κ agonist of this series is the (1*S*,2*R*,5*R*)-configured methyl carbamate *ent*-**23** (WMS-0121) with a K_i value of 1.0 nM. The structural element of the methoxycarbonyl moiety of *ent*-**23** has been derived from the lead compounds **1** and **2**. The bioisosteric replacement of the oxygen atom of the methoxycarbonyl group by a methylene moiety led to the propionyl derivative *ent*-**25** (WMS-0122) with a >10-fold reduced κ receptor affinity. Surprisingly, the benzylamine *ent*-**19** (WMS-0114) displayed a κ affinity of 8.7 nM. Although this κ affinity is considerably lower than the κ affinity of *ent*-**23**, it demonstrates that the benzyl moiety of *ent*-**19** is better tolerated by the κ receptor than the propionyl residue of *ent*-**25**. Moreover, *ent*-**19** represents the first potent κ agonist with an additional basic structural element.

Table 1. Affinities of Bicyclic (3,4-Dichlorophenyl)acetamides towards κ-Opioid Receptors

compd	R	configuration	$K_i \pm$ SEM (nM), $n = 3$ κ ([^{3}H]-U-69,593)	eudismic ratio
2[20]	C(=O)OCH_3	(2*S*,1′*S*)	0.31 ± 0.04	8
ent-**2**[20]		(2*R*,1′*R*)	2.40 ± 0.81	
19	CH_2Ph	(1*R*,2*S*,5*S*)	>1000 (25%[a])	>110
ent-**19**		(1*S*,2*R*,5*R*)	8.7 ± 1.3	
20		(1*R*,2*R*,5*S*)	152 ± 27	2.8
ent-**20**		(1*S*,2*S*,5*R*)	421 ± 23	
23	C(=O)OCH_3	(1*R*,2*S*,5*S*)	150 ± 4	150
ent-**23**		(1*S*,2*R*,5*R*)	1.0 ± 0.09	
24		(1*R*,2*R*,5*S*)	515 ± 80	6
ent-**24**		(1*S*,2*S*,5*R*)	80 ± 3.7	
25	C(=O)CH_2CH_3	(1*R*,2*S*,5*S*)	464 ± 98	35
ent-**25**		(1*S*,2*R*,5*R*)	13 ± 1.6	
26		(1*R*,2*R*,5*S*)	>1000 (6%[a])	
ent-**26**		(1*S*,2*S*,5*R*)	>1000 (35%[a])	
U-50,488		(1*S*,2*S*)	0.31 ± 0.1	
U-69,593		(1*S*,2*S*,4*R*)	0.97 ± 0.4	
naloxone			7.3 ± 0.4	

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

This additional N-atom allows further fine-tuning of the pharmacological and pharmacokinetic properties of this compound class.

Discussion of the Dihedral Angle N−C−C−N

To achieve high κ affinity, the relative orientation of the pharmacophoric structural elements is of particular relevance. Therefore, the dihedral angles *N*(pyrrolidine)−C−C−*N*-(phenylacetamide) defining the relative orientation of the

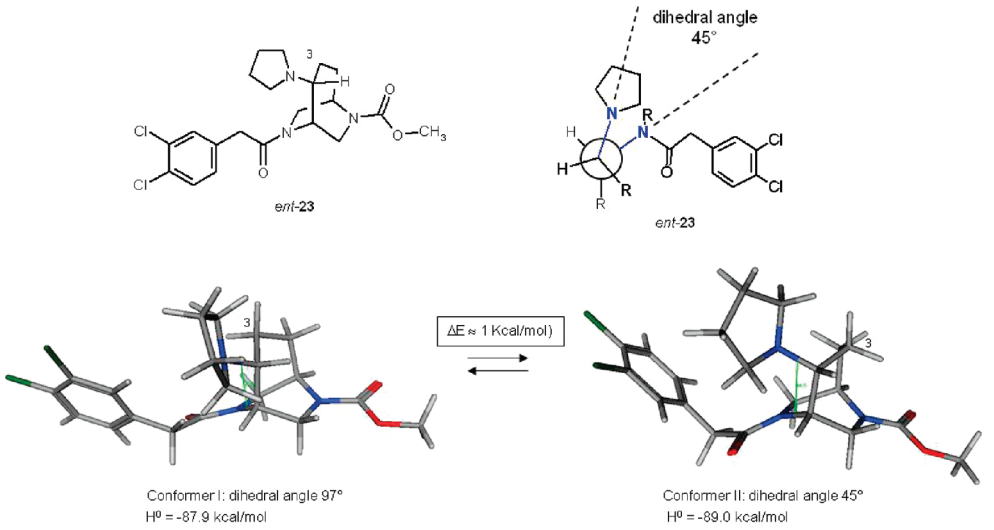


Figure 2. Correlation of the dihedral angle *N*(pyrrolidine)−C−C−*N*(phenylacetamide) of the bicyclic compounds with their κ affinity.

pharmacophoric pyrrolidine and dichlorophenylacetyl moieties were calculated and correlated with the κ affinities of the corresponding compounds. The detection of all favorable orientations of the three-carbon bridge was of particular interest.

The calculations were performed with the force field MMFF94x of the Molecular Modeling Program MOE (molecular operating environment). In Figure 2, the dihedral angle of interest is illustrated for *ent*-**23**, the most potent κ agonist of this series. In general, the three-carbon bridge of the bicyclic compounds can adopt two energetically favored conformations: in conformer I, the methylene moiety in the middle of the bridge (3-CH_2) is pointing to the "left side" (to the dichlorophenylacetyl moiety) and in conformer II 3-CH_2 is directed to the "right side" (to the methoxycarbonyl moiety). According to AM1 calculations, the energy of the two conformers is rather similar, with a little preference for conformer II in the case of **23**/*ent*-**23** and for conformer I in the case of **24**/*ent*-**24** (Table 2). However, the small difference of 1−2 kcal/mol does not allow the unequivocal definition of the bioactive conformation of the bicyclic κ agonists **23**/*ent*-**23** and **24**/*ent*-**24**.

Recently, we have reported on a systematic conformational analysis of the flexible κ agonist **2**, showing an energy minimum at a dihedral angle *N*(pyrrolidine)−C−C−*N*(phenylacetamide) of 70°.[22] The calculated dihedral angles of 97° and 45° for *ent*-**23** are rather close to this energy minimum. The difference of 25−27° might contribute to the reduced κ affinity of *ent*-**23** compared with **2**.

The calculated dihedral angles of the stereoisomers of *ent*-**23** are summarized in Table 2. It is obvious that the dihedral angles of **23**, **24**, and *ent*-**24** differ considerably from the calculated optimal dihedral angle of 70° for the very potent κ agonist **2**. The modified orientation of the pharmacophoric elements in the different stereoisomers might be responsible for their various κ receptor affinities.

Table 2. Correlation of the Dihedral Angle *N*(Pyrrolidine)−C−C−*N*-(Phenylacetamide) of the Bicyclic Methyl Carbamates **23**/**24** with Their κ Receptor Affinity

compd	κ: K_i (nM)	conformer	dihedral angle[a]	ΔH^0 (kcal/mol)[b]
23	150	I	−96	−0.8
		II	−45	
ent-**23**	1.0	I	97	−1.2
		II	45	
24	515	I	−149	+2.4
		II	170	
ent-**24**	80	I	150	+2.3
		II	−170	

[a] *N*(Pyrrolidine)−C−C−*N*(Phenylacetamide). [b] ΔH^0 = H^0(conformer II) − H^0(conformer I); H^0 = heat of formation calculated with AM1.

Selectivity against Opioid and Other Related Receptors

In collaboration with Grünenthal GmbH, the κ, μ, and δ receptor affinities of the bridged piperazine derivatives **19**, **20**, **23**−**26**, and their corresponding enantiomers with the prefix *ent* were investigated. In these assays, transfected HEK-293 cell lines expressing the human κ-opioid receptor and CHO-K_1 cell lines expressing the human μ- and δ-opioid receptors served as receptor material. The following radioligands were used: [^{3}H]-Cl-977 in the κ assay, [^{3}H]-naloxone in the μ assay, and [^{3}H]-deltorphine II in the δ assay.[36,37]

The results of the receptor binding studies are summarized in Table 3. Generally, the κ assay using transfected HEK-293 cell lines and the radioligand [^{3}H]-Cl-977 led to 6−10-fold higher K_i values (lower κ affinities) compared with the assay employing guinea pig brains and [^{3}H]-U-69,593. However, the relative order of κ affinities in both assays is the same. The methyl carbamate *ent*-**23** displays the highest κ affinity (K_i = 6.0 nM), followed by the benzylamine *ent*-**19** (K_i = 70 nM) and the propionyl derivative *ent*-**25** (K_i = 120 nM).

The μ receptor affinity of almost all bicyclic compounds is rather low. Thus, the most potent κ-agonists *ent*-**19**, *ent*-**23**, and *ent*-**25** show high selectivity against the μ-opioid receptor with κ/μ selectivity factors of 6.7, 23, and 5.4, respectively. The most potent κ-agonist *ent*-**23** reveals the highest selectivity (23fold) over the μ-opioid receptor.

It should be noted that changing of the configuration in position 2 of the potent κ agonist *ent*-**19** led to *ent*-**20**, showing a considerable preference for the μ-opioid receptor.

The bicyclic dichlorophenylacetamides showed only negligible affinity toward the δ-opioid receptor, indicating high selectivity of the κ agonists over the δ receptor.

Table 3. Affinities of Bicyclic (3,4-Dichlorophenyl)acetamides towards κ-, μ-, and δ-Opioid Receptors

	$K_i \pm$ SEM (nM)			
compd	κ [^{3}H]-Cl-977	μ [^{3}H]-naloxone	δ [^{3}H]-deltorphine II	κ/μ selectivity
19	> 1 μM[a]	> 1 μM[a]	> 1 μM[a]	
ent-**19**	70	470	> 1 μM (42%[b])	6.7
20	920	1150	> 1 μM[a]	1.3
ent-**20**	1510	180	> 1 μM[a]	0.12
23	> 1 μM[a]	> 1 μM (57%[b])	> 1 μM[a]	
ent-**23**	6.0	140	> 1 μM[a]	23
24	> 1 μM[a]	> 1 μM[a]	> 1 μM[a]	
ent-**24**	850	6120	> 1 μM[a]	7.2
25	> 1 μM[a]	> 1 μM[a]	> 1 μM[a]	
ent-**25**	120	650	> 1 μM[a]	5.4
26	> 1 μM[a]	> 1 μM[a]	> 1 μM[a]	
ent-**26**	> 1 μM[a]	> 1 μM[a]	> 1 μM[a]	
norbinaltorphimine	0.34 ± 0.23			
U-50,488	1.5 ± 0.9			
naloxone	14.7 ± 9.3	2.3 ± 1.1	103	

[a] The inhibition of the radioligand binding at a test compound concentration of 1 μM was lower than 38%. [b] Inhibition of the radioligand binding at a test compound concentration of 1 μM.

Table 4. Affinities of Bicyclic (3,4-Dichlorophenyl)acetamides towards σ_1 and σ_2 Receptors and the PCP Binding Site of the NMDA Receptor

	$K_i \pm$ SEM (nM), $n = 3$		
compd	σ_1 [^{3}H]-(+)-pentazocine	σ_2 [^{3}H]-di-*o*-tolylguanidine	NMDA (PCP) [^{3}H]-MK-801
19	433 ± 39	880	> 1 μM[a]
ent-**19**	> 1 μM[b]	3200	> 1 μM[a]
20	198 ± 37	1700	> 1 μM[a]
ent-**20**	3700	5100	> 1 μM[a]
23	> 1 μM[a]	> 1 μM[a]	> 1 μM[a]
ent-**23**	> 1 μM[a]	> 1 μM[a]	> 1 μM[a]
24	> 1 μM[a]	624	> 1 μM[a]
ent-**24**	> 1 μM[a]	1500	> 1 μM[a]
25	> 1 μM[a]	> 1 μM[a]	> 1 μM[a]
ent-**25**	> 1 μM[a]	> 1 μM[a]	> 1 μM[a]
26	> 1 μM[a]	1100	> 1 μM[a]
ent-**26**	> 1 μM[a]	2100	> 1 μM[a]
(+)-pentazocine	4.2 ± 1.1		
haloperidol	3.9 ± 1.5	78 ± 2.3	
di-*o*-tolylguanidine	61 ± 18	42 ± 17	
MK-801			2.9 ± 0.6

[a] The inhibition of the radioligand binding at a test compound concentration of 10 μM was lower than 10%. [b] The inhibition of the radioligand binding at a test compound concentration of 90 μM was 50%.

It has been reported that variation of the stereochemistry or reduction of the amide moiety of prototypical κ agonists led to potent σ receptor ligands.[8,38] Therefore, the σ_1 and σ_2 receptor affinities of the bicyclic κ agonists were also recorded in receptor binding studies using the radioligands [^{3}H]-(+)-pentazocine and [^{3}H]-di-*o*-tolylguanidine, respectively.[39−41] Table 4 summarizes the results.

The bicyclic piperazines **23**−**26** with two acyl moieties at the N-atoms showed only negligible σ_1 and σ_2 receptor affinities, indicating high selectivities of the most potent κ agonists *ent*-**23** and *ent*-**25** against these receptors. Considerable σ_1 and σ_2 receptor affinities were found for the *N*-benzyl derivative **19**, which even exceeded its κ affinity. This result reveals that the stereochemistry strongly influences the receptor selectivity in this compound class, as one enantiomer (*ent*-**19**) is a selective κ agonist, whereas its enantiomer **19** interacts preferably with σ_1 and σ_2 receptors.

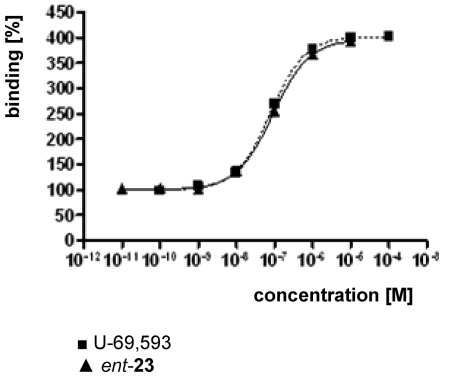


Figure 3. Intrinsic activity of the methyl carbamate *ent*-**23** compared with the full agonist U-69,593 in the [^{35}S]GTPγS-assay. ■ U-69,593; ▲ *ent*-**23**.

Table 5. Intrinsic Activity and EC_{50} Values of the Most Potent κ Agonists in the [^{35}S]GTPγS Assay

compound	EC_{50} (nM)	intrinsic activity (%)[a]
ent-**19**	820	90
ent-**23**	87	99
ent-**25**	1800	100
U-69,593	80	100

[a] Intrinsic activity relative to the full κ agonist U-69,593 (= 100%).

In the class of benzomorphans, the stereochemistry and the N-substituent determine whether a ligand interacts with κ, σ, or NMDA receptors.[42,43] Therefore, the affinity at NMDA receptors was also included in this study. Even at a test compound concentration of 10 μM in the assay, the prepared dichlorophenylacetamides did not compete with the radioligand [^{3}H]-MK-801.[41]

In summary, the most potent κ agonists *ent*-**19**, *ent*-**23**, and *ent*-**25** showed high selectivity for the κ receptor related to μ-opioid, δ-opioid, σ_1, and σ_2 receptors as well as the phencyclidine binding site of the NMDA receptor.

Functional Activity

To demonstrate the agonist activity of the most potent κ ligands *ent*-**19**, *ent*-**23**, and *ent*-**25**, a [^{35}S]GTPγS-binding assay at human κ-opioid receptors was performed. In Figure 3, the binding curve of *ent*-**23** is compared with the binding curve of the prototypical full agonist U-69,593.

In this assay, the bicyclic piperazines *ent*-**19**, *ent*-**23**, and *ent*-**25** behaved as full κ agonists with 90−100% relative potency compared with U-69,593 (see Table 5). The EC_{50} value of the most potent κ agonist *ent*-**23** (87 nM) is comparable with the EC_{50} value of U-69,593 (80 nM), indicating a comparable activation of the κ receptor. The less potent κ ligands *ent*-**19** and *ent*-**25** show reduced EC_{50} values, which correlate well with their reduced κ receptor affinities. Altogether, the 8−12-fold reduced κ receptor affinity of *ent*-**19** and *ent*-**25** found in the receptor binding studies is confirmed in the [^{35}S]GTPγS-assay.

Conclusion

In conclusion, a series of bicyclic κ receptor agonists with restricted conformational flexibility was synthesized. Compounds with (1*S*,2*R*,5*R*)-configuration showed the highest κ receptor affinity. The methyl carbamate *ent*-**23** was the most

potent κ agonist, followed by the benzyl derivative *ent*-**19** and the propionyl derivative *ent*-**25**. The reduction of the conformational flexibility of the κ agonists by introduction of the pharmacophoric elements into a bicyclic framework allowed the determination of their relative orientation by definition of the dihedral angle *N*(pyrrolidine)−C−C−*N*(phenylacetamide). For the most potent stereoisomer *ent*-**23**, dihedral angles of 97° (conformer I) and 45° (conformer II) were determined, which are close to the optimal dihedral angle of 70°. The novel κ agonists were selective against μ-opioid, δ-opioid, σ_1, and σ_2 receptors and the PCP binding site of the NMDA receptor. In the [^{35}S]GTPγS-assay the κ ligands showed full agonism and *ent*-**23** (EC_{50} = 87 nM) was equipotent with the prototypical κ agonist U-69,593 (EC_{50} = 80 nM).

Experimental Section

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, height of silica gel, eluent, R_f value. Melting point: melting point apparatus SMP 3 (Stuart Scientific), uncorrected. Optical rotation: Polarimeter 341 (Perkin-Elmer); 1.0 dm tube; concentration *c* in g/100 mL; *T* = 20 °C; wavelength 589 nm (D-line of Na light); unit of [α] is grad·mL·dm^{-1}·g^{-1}. ^{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. Elemental analyses: Vario EL (Elementaranalysesysteme GmbH). The purity of all test compounds was proved by elemental analysis; all values are within ±0.4%. The syntheses of (3,4-dichlorophenyl)acetyl chloride (DCPA-Cl), $Zn(N_3)_2$·(pyridine)$_2$ complex, HN_3 solution in benzene, and 1,4-diiodobutane are detailed in the Supporting Information.

(+)-(1*S*,2*S*,5*S*)-2-Azido-6-benzyl-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (8). Method A. Under N_2, triphenylphosphane (6.74 g, 25.7 mmol) and a solution of HN_3 in benzene (16.0 mL, 1.4 M, 22.4 mmol) were added to a solution of **6**[26] (2.00 g, 5.26 mmol) in THF (100 mL). At 0 °C, diisopropyl azodicarboxylate (DIAD, 5.0 mL, 25.7 mmol) was added and the mixture was stirred for 10 h at rt. Then CH_2Cl_2 (100 mL) was added and the mixture was extracted with 0.5 M NaOH (100 mL). Subsequently, the organic layer was dried (K_2CO_3), the solvent was removed in vacuo, and the residue was purified by fc (8 cm, h = 15 cm, elution with a gradient: petroleum ether/ethyl acetate 70/30 to petroleum ether/ethyl acetate 60/40, R_f = 0.31 (petroleum ether/ethyl acetate 60/40)). Colorless solid, mp 124 °C, yield 2.09 g (98%).

Method B. A solution of **6** (250 mg, 0.66 mmol) and triphenylphosphane (0.80 g, 3.05 mmol) in CH_2Cl_2 (4 mL) was diluted with toluene (38 mL). Subsequently, $Zn(N_3)_2$·(pyridine)$_2$ (0.45 g, 1.46 mmol) and diisopropyl azodicarboxylate (0.6 mL, 3.08 mmol) were added under ice cooling. The reaction mixture was stirred for 72 h at rt. Then the solvent was removed in vacuo. The residue was purified by fc (3 cm, h = 15 cm, petroleum ether/ethyl acetate 60/40, R_f = 0.31). Colorless solid, mp 124 °C, yield 243 mg (91%). [α] = +17.8 (*c* = 0.965, CH_2Cl_2). Anal. ($C_{22}H_{23}N_5O_3$) C, H, N.

(−)-(1*R*,2*R*,5*R*)-2-Azido-6-benzyl-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (*ent*-8). Under N_2, triphenylphosphane (1.68 g, 6.42 mmol) and a solution of HN_3 in benzene (4.0 mL, 1.4 M, 5.60 mmol) were added to a solution of *ent*-**6**[26] (0.50 g, 1.31 mmol) in THF (25 mL). At 0 °C, diisopropyl azodicarboxylate (1.25 mL, 6.43 mmol) was added and the mixture was stirred for 2 h at rt. Then NaOH (25 mL, 2 M) was added and the mixture was extracted with CH_2Cl_2 (3×). The organic layer was washed with water, dried (K_2CO_3), the solvent was removed in vacuo and the residue was purified by fc (6 cm, h = 15 cm, elution with a gradient: petroleum ether/ethyl acetate 7/3 to petroleum ether/ethyl acetate 60/40, R_f = 0.31 (petroleum ether/ethyl acetate 6/4)). Colorless solid, mp 123 °C, yield 0.485 g (91%). [α] = −17.7 (*c* = 0.815, CH_2Cl_2). Anal. ($C_{22}H_{23}N_5O_3$) C, H; N: calcd N 17.27; found N 16.75.

Spectroscopic Data of 8. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.33−1.43 (m, 1 H, 3-H or 4-H), 1.68−1.80 (m, 1 H, 3-H or 4-H), 1.95−2.07 (m, 2 H, 3-H and/or 4-H), 3.81 (s, 3 H, PhOCH_3), 3.89 (td, *J* = 4.5/2.0 Hz, 1 H, 2-H), 3.92 (dd, *J* = 5.1/2.7 Hz, 1 H, 5-H), 3.98 (d, *J* = 2.0 Hz, 1 H, 1-H), 4.00 (d, *J* = 14.7 Hz, 1 H, NCH_2Ar), 4.48 (d, *J* = 14.7 Hz, 1 H, NCH_2Ar), 4.58 (d, *J* = 14.7 Hz, 1 H, NCH_2Ar), 5.23 (d, *J* = 14.7 Hz, 1 H, NCH_2Ar), 6.88 (d broad, *J* = 8.6 Hz, 2 H, aromat 3-H, 5-$H_{methoxybenzyl}$), 7.16 (d, *J* = 8.6 Hz, 2 H, aromat 2-H, 6-$H_{methoxybenzyl}$), 7.21 (dd, *J* = 7.6/1.8 Hz, 2 H, aromat 2-H, 6-H_{benzyl}), 7.29−7.37 (m, 3 H, aromat H).

(−)-(1*S*,2*S*,5*S*)-6-Benzyl-8-(4-methoxybenzyl)-2-(pyrrolidin-1-yl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (12). A mixture of Pd/C (10%, 520 mg), **8** (2.1 g, 5.17 mmol), and methanol (200 mL) was stirred under a H_2 atmosphere (balloon) for 1.5 h at rt. The catalyst was removed upon filtration over celite, and the solvent was removed in vacuo. The residue (primary amine **10**) was dissolved in acetonitrile (200 mL). Then 1,4-diiodobutane (6.37 g, 20.55 mmol) and $NaHCO_3$ (2.93 g, 34.88 mmol) were added. The mixture was refluxed for 18 h. After cooling down to rt, the mixture was concentrated in vacuo (*T* < 40 °C) to a volume of approximately 45 mL. Then 2 M HCl (400 mL) was added, the mixture was washed with Et_2O, and the aqueous layer was made alkaline by addition of 2 M NaOH (pH 13−14) and extracted with Et_2O (5×). The combined Et_2O layers were dried (K_2CO_3), filtered, and evaporated in vacuo. The residue was purified by fc (5 cm, h = 15 cm, ethyl acetate, R_f = 0.30). Colorless oil, yield 1.83 g (82%). [α] = −12.9 (*c* = 0.325, $CHCl_3$). Anal. ($C_{26}H_{31}N_3O_3$) C, H, N.

(+)-(1*R*,2*R*,5*R*)-6-Benzyl-8-(4-methoxybenzyl)-2-(pyrrolidin-1-yl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (*ent*-12). Method A. A mixture of Pd/C (10%, 120 mg), *ent*-**8** (489 mg, 1.21 mmol), ammonium formate (400 mg, 6.35 mmol), and methanol (20 mL) was heated to reflux for 2 h. It was filtered, and the solvent was removed in vacuo. The residue was dissolved in CH_2Cl_2, the solution was washed with a half-saturated solution of NaCl (1×), dried (Na_2SO_4) and concentrated in vacuo. The residue (primary amine *ent*-**10**) was dissolved in acetonitrile (40 mL), and 1,4-diiodobutane (1.48 g, 4.79 mmol) and $NaHCO_3$ (0.74 g, 8.81 mmol) were added and the mixture was heated to reflux for 16 h. Workup and purification were performed as described for the enantiomer **12**. Colorless oil, yield 320 mg (61%).

Method B. Under cooling with ice a solution of *ent*-**6** (750 mg, 1.97 mmol) in dry CH_2Cl_2 (30 mL) was treated with NEt_3 (0.81 mL, 5.84 mmol) and methanesulfonyl chloride (0.26 mL, 3.29 mmol). The mixture was stirred at rt for 1.5 h. The mixture was washed with 0.5 M NaOH (1×) and 0.5 M HCl (1×), dried (Na_2SO_4), filtered, and concentrated in vacuo. The pale-yellow residue (mesylate of ent-**6**) was dissolved in DMF (20 mL), NaN_3 (950 mg, 14.6 mmol) was added, and the mixture was stirred at 130 °C for 18 h. Then water (200 mL) was added, the colorless precipitate (azide *ent*-**8**) was separated by filtration, and the filtrate was extracted with Et_2O (5×). The Et_2O layer was dried (Na_2SO_4), concentrated in vacuo, and the residue was combined with the first precipitate. The azide *ent*-**8** was dissolved in methanol (40 mL), Pd/C (10%, 200 mg), and ammonium formate (660 mg, 10.5 mmol) were added, and the mixture was heated to reflux for 1 h. After filtration, the solvent was removed in vacuo and the residue was purified by fc (3 cm, h = 10 cm, petroleum ether/EtOH 50/10, R_f = 0.16). The pale-yellow product (primary amine *ent*-**10**, 499 mg) was dissolved in acetonitrile (50 mL), 1,4-diiodobutane (0.70 mL, 5.31 mmol) and $NaHCO_3$ (750 mg, 8.93 mmol) were added, and the mixture was heated to reflux for 16 h. Workup and purification were performed as described for the enantiomer **12**. Colorless oil, yield 463 mg (54% calculated from the alcohol **6** over 4 reaction

steps). [α] = +20.5 (*c* = 0.17, CH_2Cl_2); [α] = +13.0 (*c* = 0.485, $CHCl_3$). Anal. ($C_{26}H_{31}N_3O_3$) H, N; C: calcd C 72.03; found C 71.59.

Spectroscopic Data of 12. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.22−1.36 (m, 1 H, 4-H), 1.56−1.69 (m, 1 H, 3-H), 1.70−1.83 (m, 4 H, $N(CH_2CH_2)_2$), 1.90−2.05 (m, 2 H, 3-H, 4-H), 2.45−2.58 (m, 5 H, $N(CH_2CH_2)_2$, 2-H), 3.80 (s, 3 H, $PhOCH_3$), 3.85 (d, *J* = 5.5 Hz, 1 H, 5-H), 4.02 (d, *J* = 14.9 Hz, 1 H, NCH_2Ar), 4.09 (s broad, 1 H, 1-H), 4.38 (d, *J* = 14.5 Hz, 1 H, NCH_2Ar), 4.64 (d, *J* = 14.9 Hz, 1 H, NCH_2Ar), 5.33 (d, *J* = 14.5 Hz, 1 H, NCH_2Ar), 6.86 (d broad, *J* = 8.6 Hz, 2 H, aromat 3-H, 5-$H_{methoxybenzyl}$), 7.13 (d, *J* = 8.6 Hz, 2 H, aromat 2-H, 6-$H_{methoxybenzyl}$), 7.21 (d broad, *J* = 7.8 Hz, 2 H, aromat H), 7.25−7.33 (m, 3 H, aromat H).

(+)-(1*R*,2*S*,5*S*)-6-Benzyl-2-(pyrrolidin-1-yl)-6,8-diazabicyclo[3.2.2]nonane (17). $LiAlH_4$ (725 mg, 19.1 mmol) was carefully added to an ice cold solution of **12** (1.66 g, 3.82 mmol) in THF (220 mL). The reaction mixture was heated to reflux for 19 h. A small amount of water was carefully added, it was filtered, and the solvent was removed in vacuo. The oily residue (**15**) was dissolved in CH_2Cl_2 (50 mL), trifluoroacetic acid (50 mL) was added, and the mixture was heated to reflux for 24 h. After cooling down to rt, the mixture was poured into cold water (300 mL) and made alkaline by addition of solid NaOH (pH 13−14). The aqueous layer was extracted with CH_2Cl_2 (5×), and the organic layer was dried (K_2CO_3) and concentrated in vacuo. The residue was dissolved in 2 M HCl, washed with Et_2O (2×), made alkaline with solid NaOH, and extracted with CH_2Cl_2. The combined organic layers were dried (K_2CO_3), concentrated in vacuo, and the residue was purified by fc (4.5 cm, h = 16 cm, ethyl acetate/methanol/ethyldimethylamine 50/50/1, R_f = 0.12). Pale-yellow oil, yield 0.652 g (60%). [α] = +46 (*c* = 0.15, $CHCl_3$). $C_{18}H_{27}N_3$ (285.4).

(−)-(1*S*,2*R*,5*R*)-6-Benzyl-2-(pyrrolidin-1-yl)-6,8-diazabicyclo[3.2.2]nonane (*ent*-17). As described for the synthesis of **17**, the enantiomeric dilactam *ent*-**12** (720 mg, 1.66 mmol) was reduced with $LiAlH_4$ (315 mg, 8.31 mmol) in THF (95 mL) and the residue (*ent*-**15**) was treated with trifluoroacetic acid (40 mL) and CH_2Cl_2 (40 mL). Workup and fc purification gave a pale-yellow oil, yield 309 mg (65%). [α] = −55.7 (*c* = 0.37, $CHCl_3$). $C_{23}H_{26}N_2O_4$ (285.4).

Spectroscopic Data of 17. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.55−1.65 (m, 1 H, 4-H), 1.70−1.88 (m, 6 H, 3-H, 4-H, $N(CH_2CH_2)_2$), 2.06−2.15 (m, 1 H, 3-H), 2.21 (s breit, 1 H, N−H), 2.46−2.49 (m, 1 H, 2-H), 2.50−2.58 (m, 2 H, $N(CH_2CH_2)_2$), 2.58−2.67 (m, 2 H, $N(CH_2CH_2)_2$), 2.75 (d breit, *J* = 12.1 Hz, 1 H, 9-H), 2.82−2.88 (m, 1 H, 5-H), 2.94 (d, *J* = 3.1 Hz, 2 H, 7-H), 3.26−3.35 (m, 2 H, 1-H, 9-H), 3.68 (d, *J* = 13.3 Hz, 1 H, NCH_2Ar), 3.73 (d, *J* = 13.7 Hz, 1 H, NCH_2Ar), 7.20−7.40 (m, 5 H, aromat H).

(−)-1-[(1*R*,2*S*,5*S*)-6-Benzyl-2-(pyrrolidin-1-yl)-6,8-diazabicyclo[3.2.2]nonan-8-yl]-2-(3,4-dichlorophenyl)ethanone (19). (3,4-Dichlorophenyl)acetyl chloride (DCPA-Cl, 648 mg, 2.90 mmol) was added to a solution of **17** (552 mg, 1.93 mmol) in CH_2Cl_2 (20 mL), and the mixture was stirred for 3 h at rt. Then 2 M NaOH (20 mL) was added, the mixture was stirred for an additional hour at rt, and the organic layer was separated and washed with saturated NaCl. The CH_2Cl_2 layer was dried (K_2CO_3), filtered, concentrated in vacuo, and the residue was purified by fc (3.5 cm, h = 17 cm, CH_2Cl_2/ethyl acetate/ethyldimethylamine 90/10/1, R_f = 0.31). Pale-yellow oil, yield 869 mg (95%). [α] = −63.5 (*c* = 0.23, $CHCl_3$). Anal. ($C_{26}H_{31}Cl_2N_3O$) C, H, N.

(+)-1-[(1*S*,2*R*,5*R*)-6-Benzyl-2-(pyrrolidin-1-yl)-6,8-diazabicyclo[3.2.2]nonan-8-yl]-2-(3,4-dichlorophenyl)ethanone (*ent*-19). As described for the synthesis of **19** the enantiomeric secondary amine *ent*-**17** (309 mg, 1.08 mmol) was acylated with DCPA-Cl (360 mg, 1.61 mmol) in CH_2Cl_2 (11 mL). Pale-yellow oil, yield 475 mg (93%). [α] = +60.0 (*c* = 0.25, $CHCl_3$). Anal. ($C_{26}H_{31}Cl_2N_3O$) C, H, N.

Spectroscopic Data of 19. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.41−1.92 (m, 8 H, 2× 3-H, 2× 4-H, $N(CH_2CH_2)_2$), 2.47−2.65 (m, 3× 0.3 H and 4× 0.7 H, 2-H^{NR}, $N(CH_2CH_2)_2{}^{NR}$, $N(CH_2CH_2)_2{}^{HR}$), 2.65−2.78 (m, 2× 0.3 H and 2× 0.7 H, $N(CH_2CH_2)_2{}^{NR}$, 2-H^{HR}, 7-H^{HR}), 2.82 (dd, *J* = 11.0/2.0 Hz, 0.3 H, 7-H^{NR}), 2.96 (dd, *J* = 10.9/2.7 Hz, 0.7 H, 7-H^{HR}), 2.98−3.06 (m, 2× 0.3 H and 1× 0.7 H, 5-H^{HR}, 5-H^{NR}, 7-H^{NR}), 3.44 (d, *J* = 10.2 Hz, 0.3 H, 9-H^{NR}), 3.56 (dd, *J* = 13.7/3.3 Hz, 0.7 H, 9-H^{HR}), 3.64−3.72 (m, 5× 0.3 H, 9-H^{NR}, NCH_2Ar^{NR}, $COCH_2Ar^{NR}$), 3.73 (s, 2× 0.7 H, NCH_2Ar^{HR}), 3.75 (dd, *J* = 14.1/1.6 Hz, 0.7 H, 9-H^{HR}), 3.83 (d, *J* = 15.3 Hz, 0.7 H, $COCH_2Ar^{HR}$), 4.01 (d, *J* = 15.3 Hz, 0.7 H, $COCH_2Ar^{HR}$), 4.11 (t, *J* = 2.7 Hz, 0.7 H, 1-H^{HR}), 5.08 (d, *J* = 5.5 Hz, 0.3 H, 1-H^{NR}), 7.10 (dd, *J* = 8.2/1.9 Hz, 0.7 H, aromat 6-$H^{HR}{}_{dichlorophenyl}$), 7.15 (dd, *J* = 8.2/2.3 Hz, 0.3 H, aromat 6-$H^{NR}{}_{dichlorophenyl}$), 7.22−7.40 (m, 6.7 H, aromat H), 7.44 (d, *J* = 1.9 Hz, 0.3 H, aromat 2-$H^{NR}{}_{dichlorophenyl}$). Ratio of rotamers 70(HR):30(NR).

(−)-1-[(1*R*,2*S*,5*S*)-2-(Pyrrolidin-1-yl)-6,8-diazabicyclo[3.2.2]nonan-8-yl]-2-(3,4-dichlorophenyl)ethanone (21). Pd/C (10%, 75 mg) was added to a solution of **19** (202 mg, 0.427 mmol) in THF/H_2O (1:1, 6.2 mL) and conc HCl (0.62 mL), and the mixture was stirred under a H_2 atmosphere (balloon) for 30 min at rt. The catalyst was filtered off, and the organic solvent was removed in vacuo. The remaining aqueous solution was treated with solid NaOH (pH 13−14) and subsequently extracted with CH_2Cl_2 (2×) and $CHCl_3$/ethanol (2:1, 3×). The combined organic layers were dried (K_2CO_3), filtered, and concentrated in vacuo and the residue was purified by fc (2 cm, h = 19 cm, gradient CH_2Cl_2/MeOH/NH_3 250/8/3 to CH_2Cl_2/MeOH/NH_3 200/8/3, R_f = 0.20 (CH_2Cl_2/MeOH/NH_3 200/8/3)). Colorless resin, yield 113 mg (69%). [α] = −56.5 (*c* = 0.115, $CHCl_3$). $C_{19}H_{25}Cl_2N_3O$ (382.3). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.44−2.10 (m, 8 H, 2 × 3-H, 2× 4-H, $N(CH_2CH_2)_2$), 2.48−2.57 (m, 3 × 0.7 H, 2-H^{HR}, $N(CH_2CH_2)_2{}^{HR}$), 2.58−2.70 (m, 3× 0.3 H and 2 × 0.7 H, $N(CH_2CH_2)_2{}^{HR}$, 2-H^{NR}, $N(CH_2CH_2)_2{}^{NR}$), 2.76−2.84 (m, 2 × 0.3 H, $N(CH_2CH_2)_2{}^{NR}$), 3.01 (dd, *J* = 12.3/2.1 Hz, 0.7 H, 7-H^{HR}), 3.06 (d broad, *J* = 12.1 Hz, 0.3 H, 7-H^{NR}), 3.19 (dd, *J* = 12.1/3.5 Hz, 0.7 H, 7-H^{HR}), 3.28 (dd, 12.1/5.7 Hz, 0.3 H, 7-H^{NR}), 3.37 (s broad, 1 H, 5-H^{HR}, 5-H^{NR}), 3.47 (dd, *J* = 13.5/2.1 Hz, 0.7 H, 9-H^{HR}), 3.55−3.74 (m, 4× 0.3 H, 2× 9-H^{NR}, $COCH_2Ar^{NR}$), 3.81 (d, *J* = 14.9 Hz, 2× 0.7 H, 9-H^{HR}, $COCH_2Ar^{HR}$), 4.01 (d, *J* = 15.3 Hz, 0.7 H, $COCH_2Ar^{HR}$), 4.09 (s broad, 0.7 H, 1-H^{HR}), 5.04 (d, *J* = 5.5 Hz, 0.3 H, 1-H^{NR}), 7.09 (dd, *J* = 8.2/1.6 Hz, 0.7 H, aromat 6-$H^{H}{}_{dichlorophenyl}$), 7.17 (dd, *J* = 8.2/2.0 Hz, 0.3 H, aromat 6-$H^{NR}{}_{dichlorophenyl}$), 7.34−7.40 (m, 2× 0.7 H and 1× 0.3 H, aromat 2-H^{HR}, 5-H^{HR}, 5-$H^{NR}{}_{dichlorophenyl}$), 7.42 (d, *J* = 1.6 Hz, 0.3 H, aromat 2-$H^{NR}{}_{dichlorophenyl}$). Ratio of rotamers 70(HR):30(NR).

(−)-Methyl (1*R*,2*S*,5*S*)-8-[2-(3,4-Dichlorophenyl)acetyl]-2-(pyrrolidin-1-yl)-6,8-diazabicyclo[3.2.2]nonane-6-carboxylate (23). Under cooling with ice, NEt_3 (18 μL, 0.13 mmol) and methyl chloroformate (15 μL, 0.195 mmol) were added to a solution of **21** (50 mg, 0.13 mmol) in CH_2Cl_2 (1 mL). The reaction mixture was stirred for 16 h at rt. Then CH_2Cl_2 was added, the organic layer was washed with 0.5 M NaOH, dried (K_2CO_3), and concentrated in vacuo, and the residue was purified by fc (2 cm, h = 10 cm, CH_2Cl_2/ethyl acetate/ethyldimethylamine 90/10/1, R_f = 0.13). Pale-yellow resin, yield 53 mg (92%). [α] = −59.7 (*c* = 0.37, $CHCl_3$). Anal. ($C_{21}H_{27}Cl_2N_3O_3$) C, H, N.

(+)-Methyl (1*S*,2*R*,5*R*)-8-[2-(3,4-Dichlorophenyl)acetyl]-2-(pyrrolidin-1-yl)-6,8-diazabicyclo[3.2.2]nonane-6-carboxylate (*ent*-23). As described for the synthesis of **21**, the benzyl derivative *ent*-**19** (150 mg, 0.32 mmol) was dissolved in THF (2.35 mL). After addition of water (2.35 mL), conc HCl (0.47 mL), and Pd/C (10%, 56 mg), the mixture was hydrogenated (balloon) for 28 min at rt. After workup and fc purification the residue (*ent*-**21**, 32 mg) was acylated with methyl chloroformate (10 μL, 0.13 mmol), NEt_3 (12 μL, 0.09 mmol), and CH_2Cl_2 (1 mL) for 16 h at rt as described for the synthesis of **23**. Pale-yellow resin, yield 36 mg (26% from *ent*-**19**). [α] = +57.1 (*c* = 0.34, $CHCl_3$). Anal.

($C_{21}H_{27}Cl_2N_3O_3$) H; C, N: calcd C 57.28, N 9.54; found C 56.84, N 9.11.

Spectroscopic Data of 23. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.46−2.01 (m, 8 H, $N(CH_2CH_2)_2$, 2× 3-H, 2× 4-H), 2.41−2.49 (m, 2× 0.4 H, 2-$H^{HR(1)+HR(2)}$), 2.49−2.66 (m, 8× 0.4 H, $N(CH_2CH_2)_2^{HR(1)+HR(2)}$), 2.66−2.86 (m, 10× 0.1 H, 2-$H^{NR(1)+NR(2)}$, $N(CH_2CH_2)_2^{NR(1)+NR(2)}$), 3.32−4.40 (m, 2× 0.4 H, 7-$H^{HR(1)+HR(2)}$), 3.42−3.52 (m, 2× 0.4 H and 4× 0.1 H, 9-$H^{HR(1)+HR(2)}$, 2× 7-$H^{NR(1)+NR(2)}$), 3.54−3.62 (m, 2× 0.4 H, 7-$H^{HR(1)+HR(2)}$), 3.62−3.76 (m, 3 H and 8× 0.1 H, OCH_3, $COCH_2Ar^{NR(1)+NR(2)}$, 2× 9-$H^{NR(1)+NR(2)}$), 3.78−3.94 (m, 4× 0.4 H, $COCH_2Ar^{HR(1)+HR(2)}$, 9-$H^{HR(1)+HR(2)}$), 3.99−4.10 (m, 2× 0.4 H, $COCH_2Ar^{HR(1)+HR(2)}$), 4.20−4.24 (broad signal, 0.4 H, 1-$H^{HR(1)}$), 4.27−4.32 (broad signal, 2× 0.4 H, 5-$H^{HR(1)}$, 1-$H^{HR(2)}$), 4.33−4.37 (broad signal, 0.1 H, 5-$H^{NR(1)}$), 4.40−4.45 (broad signal, 0.4 H, 5-$H^{HR(2)}$), 4.47−4.53 (broad signal, 0.1 H, 5-$H^{NR(2)}$), 5.16−5.20 (broad signal, 0.1 H, 1-$H^{NR(1)}$), 5.20−5.24 (broad signal, 0.1 H, 1-$H^{NR(2)}$), 7.09 (d broad, J = 7.8 Hz, 0.8 H, aromat 6-$H^{HR(1)+HR(2)}$), 7.13 (d broad, J = 8.2 Hz, 0.2 H, aromat 6-$H^{NR(1)+NR(2)}$), 7.34−7.41 (m, 2 H, aromat 2-H, 5-H). Ratio of rotamers: 40(HR1):40(HR2):10(NR1):10(NR2).

(−)-1-{(1*R*,2*S*,5*S*)-8-[2-(3,4-Dichlorophenyl)acetyl]-2-(pyrrolidin-1-yl)-6,8-diazabicyclo[3.2.2]nonan-8-yl}propan-1-one (25). Propanoyl chloride (25 μL, 0.286 mmol) was added under ice cooling to a solution of **21** (55 mg, 0.144 mmol) in CH_2Cl_2 (1 mL) and NEt_3 (20 μL, 0.144 mmol), and the mixture was stirred for 16 h at rt. Then CH_2Cl_2 was added, the mixture was extracted with 2 M NaOH, and the organic layer was dried (K_2CO_3), concentrated in vacuo, and the residue was purified by fc (2 cm, h = 15 cm, CH_2Cl_2/ethyl acetate/ethyldimethylamine 90/10/1, R_f = 0.36). Colorless resin, yield 58 mg (92%). [α] = −60.1 (c = 0.506, $CHCl_3$). Anal. ($C_{22}H_{29}Cl_2N_3O_2$) C, H, N.

(+)-1-{(1*S*,2*R*,5*R*)-8-[2-(3,4-Dichlorophenyl)acetyl]-2-(pyrrolidin-1-yl)-6,8-diazabicyclo[3.2.2]nonan-8-yl}propan-1-one (ent-25). The benzyl derivative *ent*-**19** (50 mg, 0.106 mmol) was dissolved in THF (0.8 mL). Water (0.8 mL), conc HCl (0.16 mL), and Pd/C (10%, 18.7 mg) were added and the mixture was stirred under H_2 (balloon) for 30 min at rt. This procedure was repeated with *ent*-**19** (47 mg, 0.10 mmol). After combining the reaction mixtures, it was filtered and concentrated in vacuo and the residue was purified by fc (2 cm, h = 15 cm, gradient CH_2Cl_2/MeOH/NH_3 300/8/3 to CH_2Cl_2/MeOH/NH_3 175/8/3, R_f = 0.20 (CH_2Cl_2/MeOH/NH_3 200/8/3)]. The resulting colorless oil (*ent*-**21**, 38 mg, 50%) was dissolved in CH_2Cl_2 (1 mL), NEt_3 (16 μL, 0.115 mmol), and propanoyl chloride (19 μL, 0.217 mmol) were added, and the mixture was stirred for 16 h at rt. Then CH_2Cl_2 was added, the mixture was extracted with 2 M NaOH, and the organic layer was dried (K_2CO_3), concentrated in vacuo, and the residue was purified by fc (2 cm, h = 15 cm, CH_2Cl_2/ethyl acetate/ethyldimethylamine 90/10/1, R_f = 0.36). Colorless resin, yield 38 mg (42% from *ent*-**19**). [α] = +58.1 (c = 0.26, $CHCl_3$). Anal. ($C_{22}H_{29}Cl_2N_3O_2$) C, H; N: calcd N 9.59; found N 10.10.

Spectroscopic Data of 25. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.07−1.18 (m, 3 H, CH_2CH_3), 1.46−2.01 (m, 7 and 0.35 H and 0.2 H, $N(CH_2CH_2)_2$, 2× 3-H, 1× 4-H, 4-$H^{HR(1)+NR(1)}$), 2.03−2.12 (m, 0.35 and 0.1 H, 4-$H^{HR(2)+NR(2)}$), 2.16−2.44 (m, 2 H and 2× 0.35 H, CH_2CH_3, 2-$H^{HR(1)+HR(2)}$), 2.46−2.68 (m, 4 H, $N(CH_2CH_2)_2$, 2.69−2.75 (m, 1× 0.2 H and 1× 0.1 H, 2-$H^{NR(1)+NR(2)}$), 3.29 (dd, J = 13.0/3.7 Hz, 0.35 H, 7-$H^{HR(2)}$), 3.36−3.74 [In this m, a broad d (J = 13.7 Hz) at 3.40 ppm for 9-$H^{HR(1)}$ is seen and at 3.47 ppm appears the broad d (J = 13.7 Hz) for 9-$H^{HR(2)}$.] (m, 4× 0.35 H and 6× 0.2 H and 6× 0.1 H, 2× 7-$H^{HR(1)+NR(1)+NR(2)}$, 9-$H^{HR(1)+HR(2)}$, 2× 9-$H^{NR(1)+NR(2)}$, 2× $COCH_2Ar^{NR(1)+NR(2)}$), 3.79 (d, J = 15.3 Hz, 0.35 H, $COCH_2Ar^{HR(2)}$), 3.82−3.94** [In this m, a d (J = 15.3 Hz) at 3.85 ppm for $COCH_2Ar^{HR(1)}$ is seen.] (m, 4× 0.35 H, 9-$H^{HR(1)+HR(2)}$, 7-$H^{HR(2)}$, $COCH_2Ar^{HR(1)}$), 4.00 (d, J = 15.3 Hz, 0.35 H, $COCH_2Ar^{HR(1)}$), 4.05 (d, J = 15.3 Hz, 0.35 H, $COCH_2Ar^{HR(2)}$), 4.11−4.18 (broad signal, 0.35 and 0.1 H, 5-$H^{HR(2)+NR(2)}$), 4.22−4.27 (broad signal, 0.35 H, 1-$H^{HR(1)}$), 4.29−4.33 (broad signal, 0.35 H, 1-$H^{HR(2)}$), 4.73−4.79 (broad signal, 0.35 H, 5-$H^{HR(1)}$), 4.83−4.89 (broad signal, 0.2 H, 5-$H^{NR(1)}$), 5.18−5.25 (broad signal, 0.2 and 0.1 H, 1-$H^{NR(1)+NR(2)}$), 7.03−7.12 (m, 1 H, aromat 6-H), 7.31−7.39 (m, 2 H, aromat 2-H, 5-H). Ratio of rotamers 35(HR1):35(HR2):20(NR1):10(NR2).

Receptor Binding Studies. κ Receptor Affinity Using Guinea Pig Brain Membrane Preparations. Membrane Preparation for the κ Assay (Modified According to refs 20,22). 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[44] using bovine serum albumin as standard, and subsequently the preparation was frozen (−80 °C) in 1.5 mL portions containing about 1.5 mg protein/mL.

Performing of the κ Assay (Modified According to refs 20,22). The test was performed with the radioligand [^{3}H]-U-69,593 (55 Ci/mmol, Amersham, Little Chalfont, UK). The thawed membrane preparation (about 75 μg of the protein) was incubated with various concentrations of test compounds, 1 nM [^{3}H]-U-69,593, and TRIS-$MgCl_2$ buffer (50 mM, 8 mM $MgCl_2$, 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 filtermats using a 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 scintillator was allowed to solidify at rt. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The nonspecific binding was determined with 10 μM unlabeled U-69,593. The K_d value of U-69,593 is 0.69 nM.

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

κ, μ, and δ Receptor Affinity Using Cell Lines Expressing Human Receptors. The affinity toward κ, μ, and δ receptors was determined as described in refs 36,37

σ_1 and σ_2 Receptor Affinity. The affinity toward σ_1 and σ_2 receptors was determined as described in refs 39−41

Affinity toward the Phencyclidine Binding Site of The NMDA Receptor. The affinity toward the phencyclidine binding site of the NMDA was determined as described in ref 41.

[^{35}S]GTPγS Binding Assay. The [^{35}S]-guanosine-5′-3-*O*-(thio)-triphosphate (GTPγS) assay was carried out as an homogeneous scintillation proximity assay using 1.5 mg of WGA-coated SPA-beads (Amersham, Cardiff, UK) in microtiter luminescence plates (Costar, Cambridge MA). To test the agonist activity of test compounds on human recombinant κ-opioid receptors, cell membranes from HEK-293-cells expressing human κ-opioid receptors (PerkinElmer Life Sciences), 10 μg membrane proteins per assay, were incubated with 0.4 nmol/L [^{35}S]GTPγS and series concentrations of agonists in buffer containing 20 mmol/L HEPES pH 7.4, 100 mmol/L NaCl, 10 mmol/L $MgCl_2$, 1 mmol/L EDTA, 1 mmol/L dithiothreitol, and 10 μmol/L GDP for 45 min at rt. The microtiter plates were thereafter centrifuged for 10 min at 2100 rpm in a GS6 microtiter plate centrifuge (Beckman Coulter, Krefeld, Germany) to sediment the SPA beads. The bound radioactivity was determined after a delay of 15 min by means of a 1450 Microbeta Trilux (Wallac, Freiburg,

Germany). The enhancement of [^{35}S]GTPγS binding above the basal activity was used to determine the potency (EC_{50}) and the relative efficacy (in % of maximal efficacy) of test compounds versus the reference compound U-69,593, which was set as 100%. EC_{50} values were calculated by means of nonlinear regression analysis with the software "GraphPad Prism 4 for Windows" (version 4.03, GrapPad Software, San Diego, CA).

Molecular Modeling. The conformational analysis was performed with force field MMFF94x of the Molecular Modeling Program MOE (molecular operating environment), version 2008.10 (Chemical Computing Group AG).

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Supporting Information Available: Purity data of all new compounds; physical and spectroscopic data of all new compounds; general chemistry methods; preparation of DCPA-Cl, $Zn(N_3)_2 \cdot (pyridine)_2$ complex, HN_3 solution in benzene, 1,4-diiodobutane; materials and general procedures of the receptor binding studies; details of pharmacological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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