Methylated Analogues of Methyl (*R*)-4-(3,4-Dichlorophenylacetyl)-3-(pyrrolidin-1-ylmethyl)piperazine-1-carboxylate (GR-89,696) as Highly Potent κ-Receptor Agonists: Stereoselective Synthesis, Opioid-Receptor Affinity, Receptor Selectivity, and Functional Studies

Stella Soukara,[†] Christoph A. Maier,[†] Ursula Predoiu,[†] Andreas Ehret,[‡] Rolf Jackisch,[‡] and Bernhard Wünsch*[,†]

Pharmazeutisches Institut der Universität Freiburg, Hermann-Herder-Strasse 9, 79104 Freiburg i. Br., Germany, and Institut für Experimentelle und Klinische Pharmakologie und Toxikologie II, Hirnforschung, Hansastrasse 9A, D-79104 Freiburg, Germany

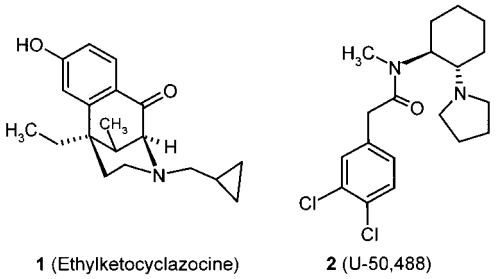
Received February 7, 2001

Analogues of the κ-receptor agonist methyl (*R*)-4-(3,4-dichlorophenylacetyl)-3-(pyrrolidin-1-ylmethyl)piperazine-1-carboxylate (GR-89,696, **6**) bearing an additional methyl substituent in the side chain are synthesized and evaluated for their κ-receptor affinity and selectivity. A key step in the synthesis is the stereoselective reductive amination of the ketones **9**, **18**, and **19** with pyrrolidine and $NaBH_3CN$, which succeeds only in the presence of the Lewis acid Ti(OiPr)$_4$. Whereas the BOC-substituted ketone **9** affords the unlike and like diastereomers of **10** in a ratio of 70:30, the diastereoselectivity during the reductive amination of the butyl and phenyl substituted ketones **18** and **19** is enhanced to 85:15 (butyl derivative) and >95:<5 (phenyl derivative) in favor of the unlike diastereomers. In receptor binding studies using the radioligand [^{3}H]U-69,593 the (*S*,*S*)-configured methyl carbamate (*S*,*S*)-**14** reveals the highest κ-receptor affinity (K_i = 0.31 nM) within this series, even exceeding the lead κ-agonist **6** (GR-89,696). A slightly reduced κ-receptor affinity is observed with the propionamide (*S*,*S*)-**13** (K_i = 0.67 nM). The κ-receptor affinity of piperazines with acyl or alkoxycarbonyl residues at both nitrogen atoms (**11**, **13**, **14**) decreases in the order (*S*,*S*) > (*R*,*R*) > (*S*,*R*) > (*R*,*S*). The methyl carbamate (*S*,*S*)-**14** discloses a unique activity profile also binding at μ-receptors in the subnanomolar range (K_i = 0.36 nM). In a functional assay, i.e., by measuring acetylcholine release in rabbit hippocampus slices, the agonistic effects of the methyl carbamate (*S*,*S*)-**14** and the propionamide (*S*,*S*)-**13** are demonstrated. Only weak κ- and μ-receptor affinities are found with the butyl- and phenyl-substituted piperazines **22** and **23**. However, considerable σ_1-receptor affinity is determined for the enantiomeric, unlike-configured butyl derivatives (*R*,*S*)-**22** and (*S*,*R*)-**22** with K_i-values of 40.2 nM and 81.0 nM, respectively.

Introduction

It is well-established that opioid analgesics mediate their effects through three opioid receptor subtypes named after their prototypical agonists, μ (OP$_3$), κ (OP$_2$) and δ (OP$_1$) receptors (nomenclature according to the IUPHAR nomenclature committee in parentheses).[1] Whereas agonists at each of these receptor subtypes represent strong analgesics, the side effect profiles associated with these subtypes differ distinctly. In view of their side effect profile, κ-agonists are of particular interest, because in contrast to μ-agonists, they cause minimal physical dependence, respiratory depression, and inhibition of gastrointestinal motility. In addition to their analgesic effects in in vivo models, κ-agonists have been shown to be potent neuroprotective and antihyperalgesic agents. However, sedation, dysphoria, and strong diuresis usually accompany κ-agonist applications.[2]

Chart 1



1 (Ethylketocyclazocine) **2** (U-50,488)

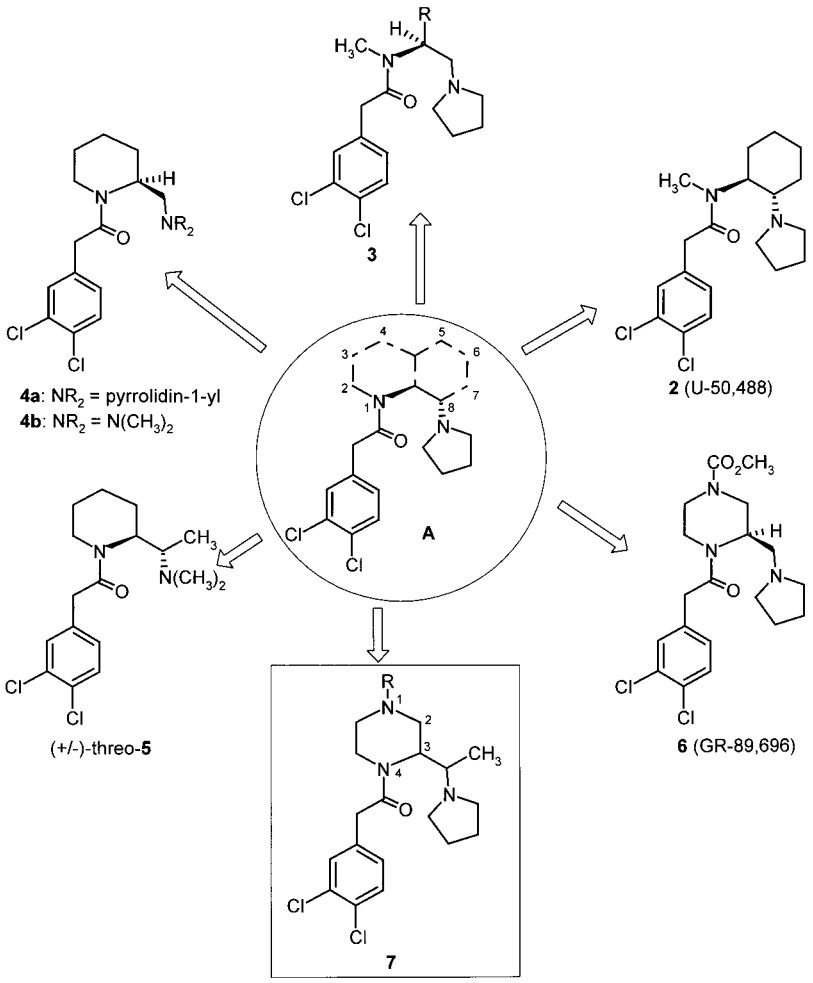
The first κ-selective agonists were found in the benzomorphan substance class, which contains the prototypical κ-ligand ethyl*k*etocyclazocine (**1**, Chart 1) giving name to κ-receptors. More potent and κ-selective are, however, ethylenediamines monoacylated with (3,4-dichlorophenyl)acetic acid. The cyclohexane derivative U-50,488 (**2**) represents the prototypical κ-ligand within this substance class, with its (1*S*,2*S*)-*trans*-configuration being crucial for high κ-receptor affinity and selectivity.[3]

During the past decade potent κ-ligands were derived from the lead structure **2** (U-50,488) including annulated compounds[4,5] and simplified ligands (e.g. **3**),[6,7] as

* To whom correspondence should be addressed. Phone: +49-(0)-761/203-6320. Fax: +49-(0)761/203-6321. E-mail: wuensch@ruf.uni-freiburg.de.
[†] Pharmazeutisches Institut der Universität Freiburg.
[‡] Institut für Experimentelle und Klinische Pharmakologie und Toxikologie II.

10.1021/jm0108395 CCC: $20.00
Published on Web 07/24/2001

Chart 2



well as heterocyclic analogues, e.g., 2-(aminomethyl)-piperidines **4** and **5**[8,9] and 2-(aminomethyl)piperazines **6**.[10−12] As observed with **2**, the κ-receptor affinity and selectivity of these monoacylated ethylenediamines is strongly dependent on the stereochemistry. In Chart 2 the most κ-active stereoisomers are shown, respectively.

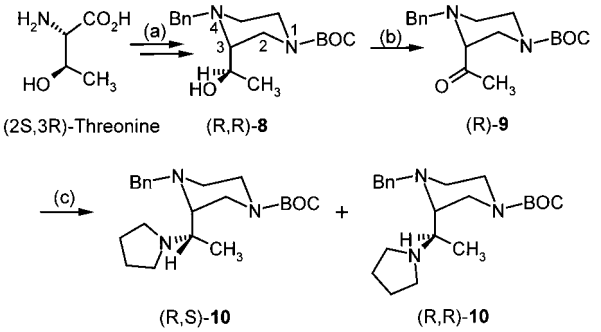
According to structure/κ-receptor affinity relationship studies, ligands with the basic amino functionality incorporated into a pyrrolidine substituent (cf. Scheme 2) display the best κ-receptor affinities. The same is true for the piperidine series, with the κ-receptor affinity of the racemic dimethylamine (±)-**4b** (K_i = 1.36 nM) being exceeded by that of the analogous pyrrolidine derivative (±)-**4a** (K_i = 0.53 nM). Introduction of an additional methyl substituent in the side chain of (±)-**4b**, however, dramatically changes the κ-receptor affinity: Whereas, an increased κ-receptor affinity was observed with the racemic *threo*-isomer **5** [(±)-*threo*-**5**: K_i = 0.60 nM] only low κ-receptor affinity was found for the corresponding *erythro*-isomer of **5** [(±)-*erythro*-**5**: K_i. 1000 nM].[8]

With the exception of the pyrrolidine derivative of **5**, which is mentioned in a patent without stereochemical assignment and pharmacological data,[9] methylated analogues of the most active pyrrolidine containing ligands are not yet described. Therefore, we decided to synthesize and evaluate pharmacologically methylated analogues **7** of the very potent piperazine κ-agonist methyl (*R*)-4-(3,4-dichlorophenylacetyl)-3-(pyrrolidin-1-ylmethyl)piperazine-1-carboxylate (**6**, GR-89,696, IC_{50} = 0.018 nM).[11] Herein, we report on the stereoselective synthesis of all four stereoisomers of the piperazines **7** with various substituents (R) in position 1. A comparison of the projected ligands **7** with **2** (U-50,488) reveals that in both ligands *two* carbon atoms of the model perhydroquinoline **A** are missing-in **2** the carbon atoms 3 and 4, in the piperazine derivative **7** the carbon atoms 5 and 6. The opioid receptor affinities of the piperazines **7** are determined in receptor binding assays and discussed in terms of receptor selectivity, stereochemistry, and N-1 substitution. In addition, we show in a functional model that two of the newly synthesized compounds are potent agonists at presynaptic κ-opioid receptors on cholinergic nerve terminals in the rabbit hippocampus.

Chemistry

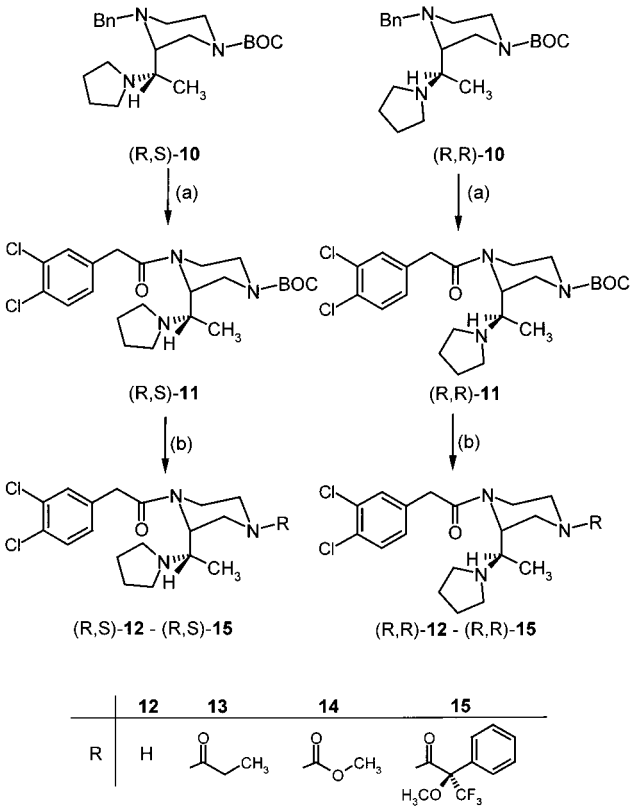
A chiral pool synthesis was employed for the preparation of the piperazines **7**. At first, the naturally occurring amino acid (2*S*,3*R*)-threonine was transformed into the orthogonally protected (piperazin-2-yl)ethanol (*R*,*R*)-**8**. (Scheme 1)[13] For the oxidation of the alcohol (*R*,*R*)-**8**, several oxidants (PCC, Ag_2CO_3 on Celite) were investigated. Among these oxidants, a catalytic amount of tetrapropylammonium perruthenate (TPAP) combined with an excess of the reoxidant *N*-methylmorpholine *N*-oxide (NMMO)[14] turned out to give the best yields of the ketone (*R*)-**9**.

Scheme 1[a]



[a] Reagents and reaction conditions: (a) see ref 13; (b) $(Pr_4N)RuO_4$, NMMO, molecular sieves, CH_2Cl_2, 16 h, rt, 85 %; (c) pyrrolidine, $Ti(OiPr)_4$, CH_2Cl_2, 90 min, rt, then methanol, $NaBH_3CN$, molecular sieves, 40 h, rt, 62 % (*R*,*S*)-**10**, 25 % (*R*,*R*)-**10**.

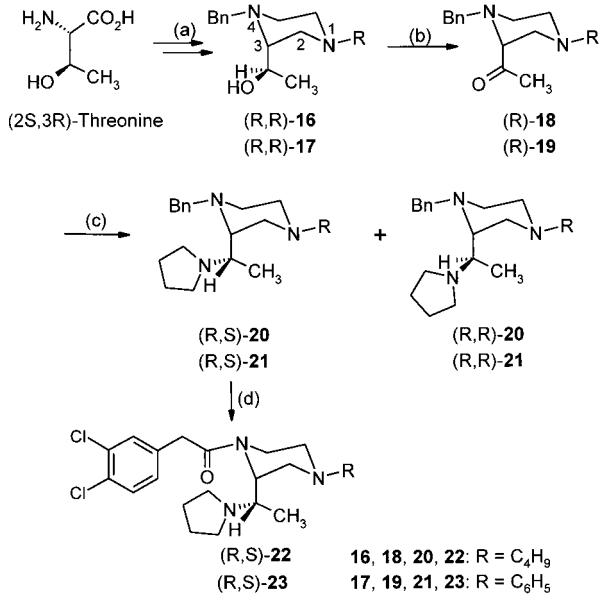
Scheme 2[a]



[a] Reagents and reaction conditions: (a) (i) H_2, 1.3 bar, Pd/C, methanol, 9 h, rt, (ii) CDI, (3,4-dichlorophenyl)acetic acid, CH_2Cl_2, 24 h, rt, 73 % (*R*,*S*)-**11**, 74 % (*R*,*R*)-**11**; (b) (i) CF_3CO_2H, CH_2Cl_2, 10 h, rt, (ii) NaOH, CH_3CH_2COCl or CH_2Cl_2, Et_3N, CH_3OCOCl, 40 h, rt, 73 % (*R*,*S*)-**13**, 75 % (*R*,*R*)-**13**, 64 % (*R*,*S*)-**14**, 67 % (*R*,*R*)-**14**.

Reductive amination of the ketone (*R*)-**9** with pyrrolidine (and other amines such as dimethylamine) in the presence of $NaBH_3CN$[15] failed to provide the amines **10**. This is probably due to the slow formation of an iminium ion from the sterically hindered ketone (*R*)-**9** and the cyclic secondary amine pyrrolidine. However, acceleration of iminium ion formation by addition of the Lewis acid tetraisopropyl orthotitanate $[Ti(OiPr)_4]$[16] afforded the diastereomeric pyrrolidines (*R*,*S*)-**10**[17] and (*R*,*R*)-**10**[17] in the ratio 70:30, which were separated by flash chromatography. Attempts to optimize the diastereoselectivity always led to diminished yields of the pyrrolidines **10**.

Scheme 3[a]



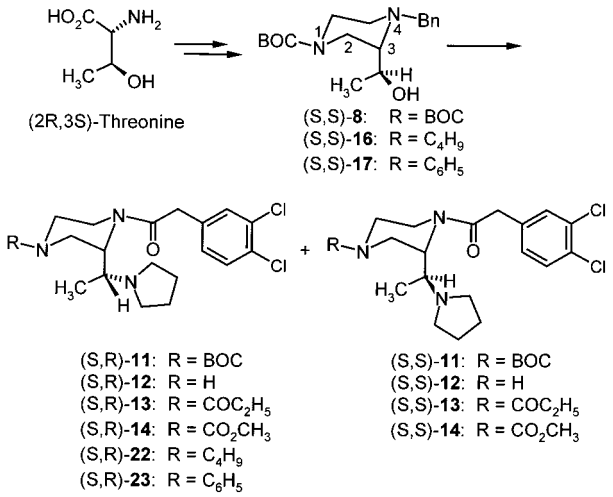
(a) See ref 13; (b) $(Pr_4N)RuO_4$, NMMO, molecular sieves, CH_2Cl_2, 16 h, rt, 71 % (*R*)-**18**, 75 % (*R*)-**19**; (c) pyrrolidine, $Ti(OiPr)_4$, CH_2Cl_2, 90 min, rt, then methanol, $NaBH_3CN$, molecular sieves, 40 h, rt, 70 % (*R*,*S*)-**20**, 15 % (*R*,*R*)-**20**, 83 % (*R*,*S*)-**21**, 1,7 % (*R*,*R*)-**21**; (d) (i) H_2, 1.3 bar, Pd/C, methanol, 9 h, rt, (ii) CDI, (3,4-dichlorophenyl)acetic acid, CH_2Cl_2, 24 h, rt, 74 % (*R*,*S*)-**22**, 65 % (*R*,*S*)-**23**.

Hydrogenolytic cleavage[18] of the benzyl protective group followed by acylation with (3,4-dichlorophenyl)-acetic acid in the presence of the coupling reagent 1,1′-carbonyldiimidazole (CDI) furnished the diastereomeric phenylacetamides (*R*,*S*)-**11** and (*R*,*R*)-**11**, respectively (Scheme 2). Trifluoroacetic acid hydrolysis[19] of the BOC derivatives **11** led to the secondary amines **12**, which were acylated with propionyl chloride or methyl chloroformate to yield the diastereomeric amides (*R*,*S*)-**13** and (*R*,*R*)-**13** and carbamates (*R*,*S*)-**14** and (*R*,*R*)-**14**, respectively.

The (piperazin-2-yl)ethanols with a butyl [(*R*,*R*)-**16**] and a phenyl [(*R*,*R*)-**17**] residue in position 4 were also prepared according to our method[13] starting with the amino acid (2*S*,3*R*)-threonine (Scheme 3). The alcohols (*R*,*R*)-**16** and (*R*,*R*)-**17** were oxidized with TPAP and NMMO[14] to provide the ketones (*R*)-**18** and (*R*)-**19** in good yields. As observed for the BOC-protected (piperazin-2-yl)ethanone (*R*)-**9**, the reductive amination of the alkylated and arylated ketones (*R*)-**18** and (*R*)-**19** succeeded only in the presence of the Lewis acid $Ti(OiPr)_4$.[16] Surprisingly, the diastereoselectivity was enhanced to 85:15 (butyl derivatives) and >95:<5 (phenyl derivatives) in favor of the *unlike*-diastereomers[20] (*R*,*S*)-**20** and (*R*,*S*)-**21**. This enhanced diastereoselectivity may be explained by an altered piperazine ring geometry of the alkylated and arylated derivatives (*R*)-**18** and (*R*)-**19** in comparison to the BOC-derivative (*R*)-**9**. Hydrogenolysis and acylation transformed the main diastereomers (*R*,*S*)-**20** and (*R*,*S*)-**21** into the phenylacetamides (*R*,*S*)-**22** and (*R*,*S*)-**23**, respectively.

The reductive amination of the ketones (*R*)-**18** and (*R*)-**19** yielded only small amounts of the minor diastereomers (*R*,*R*)-**20** and (*R*,*R*)-**21**, which were sufficient for identification and characterization. Since the unlike-configured main diastereomers (*R*,*S*)-**22** and (*R*,*S*)-**23** as

Scheme 4



well as their enantiomers (*S*,*R*)-**22** and (*S*,*R*)-**23** (vide infra) displayed only little κ-receptor affinities it was renounced to convert the like-configured minor diastereomers (*R*,*R*)-**20** and (*R*,*R*)-**21** into the corresponding phenylacetamides.

The enantiomeric piperazines with the (*S*)-configuration in position 3 of the piperazine ring system were prepared starting with the enantiomeric amino acid (2*R*,3*S*)-threonine (see Scheme 4). As described for (2*S*,3*R*)-threonine, the (piperazin-2-yl)ethanol (*S*,*S*)-**8** was generated first[21] and, subsequently, (*S*,*S*)-**8** was transformed into the unlike-configured phenylacetamides (*S*,*R*)-**11**, (*S*,*R*)-**12**, (*S*,*R*)-**13**, (*S*,*R*)-**14** and the like-configured phenylacetamides (*S*,*S*)-**11**, (*S*,*S*)-**12**, (*S*,*S*)-**13**, and (*S*,*S*)-**14**. The butyl- and phenyl-substituted derivatives (*S*,*R*)-**22** and (*S*,*R*)-**23** were obtained from the alcohols (*S*,*S*)-**16** and (*S*,*S*)-**17**, respectively.[21]

During reductive amination of the ketones (*R*)-**9**, (*R*)-**18**, and (*R*)-**19**, a new stereocenter is created in the side chain of the piperazines. Careful interpretation of the NMR spectra did not lead to an unequivocal determination of the relative configuration. Therefore, an X-ray structure analysis of the propionamide (*R*,*S*)-**13** was performed that revealed the unlike-configuration of the stereocenters.[22] The relative and absolute configurations of the stereoisomers and analogues of the propionamide (*R*,*S*)-**13** were deduced from this X-ray structure analysis in combination with analogies of spectroscopic data and chromatographic behaviors.

To determine the enantiomeric purity of the piperazines, the BOC-protected derivatives (*R*,*S*)-**11** and (*S*,*R*)-**11** were hydrolyzed with trifluoroacetic acid and, without purification, the resulting secondary amines (*R*,*S*)-**12** and (*S*,*R*)-**12** were acylated with (*R*)-Mosher's acid [(*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropionic acid] and CDI to afford the diastereomeric Mosher amides (*R*,*S*,*R*)-**15** and (*S*,*R*,*R*)-**15**, respectively. The ^{19}F NMR spectra of both Mosher amides (*R*,*S*,*R*)-**15** and (*S*,*R*,*R*)-**15** revealed about 8% of the diastereomeric amides. It is concluded from these results that the piperazines **12** as well as their acylated, alkoxycarbonylated, alkylated, and arylated derivatives contain about 8% of their enantiomers, respectively. Probably, partial racemization had occurred during reductive amination of the ketones **9**, **18**, and **19**.

At room temperature the mono- and diacylpiperazines **8**−**15**, **22**, and **23** exist as mixtures of rotational isomers, caused by restricted rotation around the amidic C−N bonds. The interpretation of the NMR spectra is especially complicated by the existence of an amide and a carbamate moiety or two amide moieties with different coalescence ranges. Therefore, ^{1}H and ^{13}C as well as homo- and heteronuclear correlated NMR spectra were recorded at various temperatures, employing as a rule [D_5]nitrobenzene as solvent. For example, the NMR spectra of the BOC derivative (*R*,*S*)-**11** at room temperature and at 80 °C are discussed. At 80 °C a single set of signals is seen in the ^{1}H and ^{13}C NMR spectra, which can be assigned with the help of the C,H-correlated NMR spectrum. However, signals for the 3-CH and the 5-CH_2 groups are missing at 80 °C, indicating coalescence around the phenylacetamide moiety. At room temperature double sets of signals for the 3-CH and 5-CH_2 groups emerge, which can be explained by rotational isomerism around the phenylacetamide moiety. Yet, signals for the 2-CH_2 and 6-CH_2 groups have disappeared, because of slow rotation (coalescence) around the carbamate C−N bond.

Receptor Binding Studies. To determine κ-, μ-, σ_1-, and NMDA-receptor affinities of the stereoisomeric phenylacetamides, receptor binding studies of **11**, **13**, **14**, **22**, and **23** with radioligands were performed.

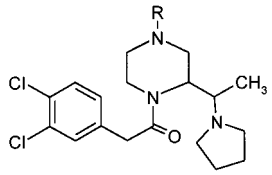
κ-Receptor Affinity. The κ-receptor binding of the test compounds was determined with homogenates of guinea pig brain (without cerebellum) membranes as receptor material using the κ-selective radioligand [^{3}H]U-69,593.[23−26] The nonspecific binding was determined with an excess of U-50,488 (**2**).

In Table 1 the κ-receptor affinities of the test compounds are summarized. In this series the highest κ-receptor affinity is found for the (*S*,*S*)-configured methyl carbamate (*S*,*S*)-**14** with a K_i value of 0.31 nM. In comparison with the methyl carbamate (*S*,*S*)-**14**, the κ-receptor affinity of the bioisosteric propionamide (*S*,*S*)-**13** (K_i = 0.67 nM) is reduced by the factor 2, whereas the binding of the sterically demanding *tert*-butyl carbamate (*S*,*S*)-**11** (K_i = 13.0 nM) is decreased by a factor of 40. The κ-receptor affinity of the diacylated piperazines **11**, **13**, and **14** is strongly dependent on the stereochemistry. In the methyl carbamate series **14** the κ-receptor affinity is decreasing in the order (*S*,*S*) > (*R*,*R*) > (*S*,*R*) > (*R*,*S*). The same rank is observed in the propionamide **13** and the *tert*-butyl carbamate **11** series.

Introduction of a phenyl moiety in position 1 of the piperazine ring system (**23**) almost completely abolishes the ability of the ligands to bind at κ-receptors. Also, ligands with a butyl residue in position 1 (**22**) show minimal κ-receptor affinities. Obviously, a 1-acyl moiety (compounds **11**, **13**, and **14**), which leads to a planar, nonbasic nitrogen atom in position 1, is necessary for high κ-receptor binding.

The κ-receptor affinity of the lead structure **6** (GR-89,696) was also determined in our assay (see Table 1). The data reveal that the K_i value of the most κ-active compound of this series [(*S*,*S*)-**14**, K_i = 0.31 nM] is lower than the K_i value of **6** (K_i = 0.45 nM). Thus, we conclude that a methyl substituent at the pyrrolidinylmethyl

Table 1. Affinities of the Phenylacetamides to κ-, μ-, σ_1-, and NMDA-Receptors[a]



		$K_i \pm$ SEM (n)			
compd	R	κ (U-69,593)	μ (DAMGO)	σ_1 (pentazocine)	NMDA (MK-801)
(*R*,*S*)-**11**	CO_2-*t*-Bu	>10 μM[a]	>10 μM	>1 μM	>10 μM
(*S*,*R*)-**11**	CO_2-*t*-Bu	500 nM (1)	>1 μM	>1 μM	>10 μM
(*R*,*R*)-**11**	CO_2-*t*-Bu	206 nM (1)	>10 μM	>1 μM	>10 μM
(*S*,*S*)-**11**	CO_2-*t*-Bu	13.0 ± 1.8 nM (3)	>1 μM	>1 μM	>10 μM
(*R*,*S*)-**13**	$COCH_2CH_3$	>10 μM	>10 μM	>1 μM	>10 μM
(*S*,*R*)-**13**	$COCH_2CH_3$	43.4 ± 6.1 nM (3)	>1 μM	>1 μM	>10 μM
(*R*,*R*)-**13**	$COCH_2CH_3$	4.20 ± 0.38 nM (3)	>1 μM	>1 μM	>10 μM
(*S*,*S*)-**13**	$COCH_2CH_3$	0.67 ± 0.17 nM (3)	19.1 ± 8.1 nM (2)	>1 μM	>10 μM
(*R*,*S*)-**14**	CO_2CH_3	34.9 ± 11.0 nM (3)	>10 μM	>1 μM	>10 μM
(*S*,*R*)-**14**	CO_2CH_3	8.8 ± 1.7 nM (3)	>1 μM	>1 μM	>10 μM
(*R*,*R*)-**14**	CO_2CH_3	2.40 ± 0.81 nM (3)	6.8 ± 1.2 nM (2)	>1 μM	>10 μM
(*S*,*S*)-**14**	CO_2CH_3	0.31 ± 0.04 nM (3)	0.36 ± 0.06 nM (3)	>1 μM	>10 μM
(*R*,*S*)-**22**	C_4H_9	237 nM (1)	>1 μM	40.2 nM (1)	>10 μM
(*S*,*R*)-**22**	C_4H_9	134 nM (1)	>1 μM	81.0 nM (1)	>10 μM
(*R*,*S*)-**23**	C_6H_5	>10 μM	>10 μM	>1 μM	>10 μM
(*S*,*R*)-**23**	C_6H_5	>1 μM	>1 μM	>1 μM	>10 μM
U-69,593		1.4 ± 0.67 nM (3)	nd[b]	nd	nd
U-50,488 (**2**)		0.49 ± 0.16 nM (3)	nd	nd	nd
GR-89,696 (**6**)		0.45 ± 0.065 nM (3)	nd	nd	nd
naloxone		3.2 nM (1)	0.68 ± 0.04 nM (2)	nd	nd
haloperidol		nd	nd	2.20 ± 0.31 nM (3)	nd
(+)-pentazocine		nd	nd	3.58 ± 0.20 nM (4)	nd
phencyclidine		nd	nd	nd	15.3 nM (1)

[a] >10 μM, >1 μM: In the screening with one test concentration a significant competition between the test compound and the radioligand was not observed. Therefore, the exact K_i value was not determined. The given value was estimated from the residual binding. [b] nd = not determined

residue with the correct configuration is able to increase κ-receptor affinity.

The excellent κ-receptor binding of the methoxycarbonyl-substituted derivative (*S*,*S*)-**14** is in accordance with observations in the (aminomethyl)piperazine series of κ-ligands with different substituents in position 1. In this series the (*R*)-configured methyl carbamate (compound **6** in Scheme 2) also displays the best κ-receptor affinity.[11] In contrast to the literature reports,[11] only little difference in the κ-receptor affinity (factor 2) is seen between the carbamate (*S*,*S*)-**14** and the propionamide (*S*,*S*)-**13**.

Among the acylated piperazines, stereoselective receptor binding is observed, with the (*S*,*S*)-configured derivatives showing the highest κ-receptor affinity, respectively. Obviously, the (*S*,*S*)-stereoisomers fit best into the binding site of the κ-receptor protein. The three-dimensional structure of these optimal fitting (*S*,*S*)-configured piperazines corresponds to the stereostructure of the known κ-ligands **2**−**6**. However, in some cases the stereodescriptors of corresponding stereocenters are opposite, because of alterations in the ligand priority according to the CIP rules.

***μ*-Receptor Affinity.** In addition to κ-receptor binding the μ-receptor affinities of the stereoisomeric piperazines were investigated. As described for the κ-assay, membrane preparations from whole guinea pig brains without cerebellum were used as receptor material. The radioligand [^{3}H]DAMGO was employed for labeling the μ-receptors, and nonspecific binding was determined in the presence of 1 μM naloxone.[23−26]

At first all test compounds were screened in a test concentration of 10 μM. At this concentration most of the test compounds did not compete significantly with the radioligand. Therefore, it is concluded that the IC_{50} values are at least greater than 10 μM.

A considerable competition, however, was observed with the propionamide (*S*,*S*)-**13** and the enantiomeric methyl carbamates (*R*,*R*)-**14** and (*S*,*S*)-**14**. Therefore, the complete competition curves were recorded for these compounds and the K_i values were calculated. In Table 1 the K_i values for μ-receptor affinity are summarized. Surprisingly, the most potent κ-ligand, the methyl carbamate (*S*,*S*)-**14**, interacted with the μ-receptors in the subnanomolar range (K_i = 0.36 nM). The enantiomeric methyl carbamate (*R*,*R*)-**14** and the bioisosteric propionamide (*S*,*S*)-**13** display lower μ-receptor affinities by a factor of 19 and 53, respectively.

These results lead to κ/μ-selectivity of about 1:1, 3:1, and 29:1 for (*S*,*S*)-**14**, (*R*,*R*)-**14**, and (*S*,*S*)-**13**, respectively. In contrast to the κ-selective lead structure GR-89,696[11] (**6**, Scheme 2), the methyl carbamates (*S*,*S*)-**14** and (*R*,*R*)-**14** as well as the propionamide (*S*,*S*)-**13** represent potent κ-ligands with reduced κ/μ-selectivity. A similar observation was made in the piperidine series of κ-ligands. Introduction of a methyl group with *threo*-configuration into the side chain provided the racemate (±)-*threo*-**5** with enhanced κ-receptor affinity but diminished κ/μ-selectivity.[8]

***σ*$_1$-Receptor Affinity.** Depending on the stereochemistry, ligands derived from U-50,488 can display high affinity for σ-receptors.[3] Therefore, we investigated the σ_1-receptor affinity of the test compounds with receptor preparations from guinea pig brains. σ_1-Receptors were labeled with the σ_1-selective radioligand [^{3}H]-(+)-pen-

tazocine; for the determination of the nonspecific binding, an excess of haloperidol was employed.[27]

As described for the determination of μ-receptor affinities, the σ_1-receptor affinities of the test compounds were screened at a test concentration of 1 μM. With the exception of the butylated derivatives (*R*,*S*)-**22** and (*S*,*R*)-**22**, the residual binding of the radioligand to the σ_1-receptor containing membrane preparations in the presence of 1 μM test compound was greater than 73%. Thus, the complete competition curves were recorded only for the enantiomeric butyl derivatives (*R*,*S*)-**22** and (*S*,*R*)-**22**. As depicted in Table 1, both enantiomers show considerable σ_1-receptor affinity, the enantiomer (*R*,*S*)-**22** (K_i = 40.2 nM) being more active by a factor of 2. Since 1-butyl-4-[2-(3,4-dichlorophenyl)ethyl]piperazine is reported to be a highly potent σ_1-receptor ligand (K_i = 0.55 nM),[28] the high affinity of the unlike-configured butyl derivatives (*R*,*S*)-**22** and (*S*,*R*)-**22** is not surprising.

NMDA-Receptor Affinity. The affinity for the phencyclidine binding site of the NMDA receptor was investigated with membrane preparations from pig brain cortex. [^{3}H]-(+)-MK-801 was used as radioligand, and the nonspecific binding was determined in the presence of an excess of nonradiolabeled (+)-MK-801.[29] In addition to the above-mentioned compounds, the synthetic intermediates (*R*,*S*)-**10**, (*S*,*R*)-**10**, (*R*,*S*)-**20**, and (*S*,*R*)-**20** were also included in the study.

All test compounds were screened in a test concentration of 100 μM. At this concentration the residual binding of the tritiated radioligand was always greater than 50%, pointing to IC_{50} values of greater than 50−100 μM. Therefore, the exact K_i values for NMDA-receptor binding were not determined. With respect to NMDA-receptor affinity, the described piperazines can be regarded as κ-selective ligands.

Functional Assay. Both in the peripheral and central nervous system inhibition of transmitter release via presynaptic opioid receptors is a well-known phenomenon.[30−33] Interestingly, not only species differences in the opioid receptor types modulating release of a given transmitter in a specific brain region have been observed but also regional differences within the same species. For instance, in hippocampus[34,35] and striatum[36] of the rat, the evoked release of acetylcholine is modulated via presynaptic μ- and δ-opioid receptors, respectively, whereas in both corresponding tissues of the guinea pig, κ-opioid receptors[35] seem to be involved in this response. In the rabbit hippocampus κ-opioid receptors inhibit the evoked release of acetylcholine,[34] whereas the cholinergic axon terminals of the caudate/putamen of this species seem to lack opioid receptors.[37] Finally, in human neocortex tissue, acetylcholine release is inhibited via both κ- and δ-opioid receptors.[38,39]

To find out whether the two most active κ-receptor ligands (*S*,*S*)-**13** and (*S*,*S*)-**14** of the present study exhibit agonistic or antagonistic properties, their presynaptic effects were studied in rabbit hippocampal slices, in which κ-opioid receptor agonists have been shown to strongly reduce the electrically evoked release of acetylcholine.[34]

Figure 1 shows that (*S*,*S*)-**13** and (*S*,*S*)-**14** as well as the parent compound U-50,488 strongly reduce the electrically evoked release of [^{3}H]acetylcholine in rabbit hippocampal slices. Moreover, their effect is antagonized by a low concentration (0.01 μM) of the selective κ-opioid receptor antagonist norbinaltorphimine.[40] Finally, the data from Figure 1 also suggest that (*S*,*S*)-**14** may be significantly more potent at κ-opioid receptors than the two other compounds: Even at a concentration of 0.01 μM its effect is not completely antagonized by norbinaltorphimine. Although the latter observation has to be confirmed by a more detailed analysis of concentration/response curves, these data support the conclusion that (*S*,*S*)-**13** and especially (*S*,*S*)-**14** are potent agonists at presynaptic κ-opioid receptors in the central nervous system.

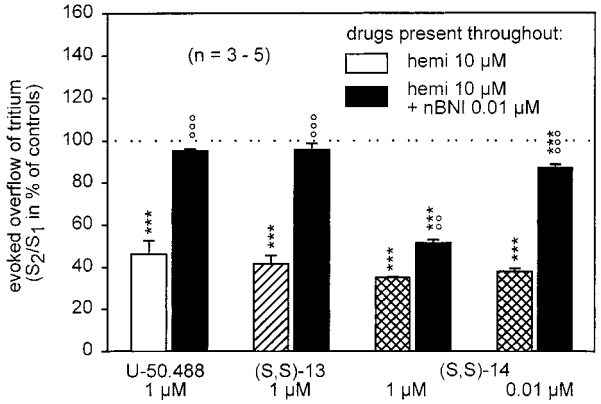


Figure 1. Effects of U-50,488, (*S*,*S*)-**13**, and (*S*,*S*)-**14** on the electrically evoked overflow of [^{3}H] from rabbit hippocampal slices preincubated with [^{3}H]-choline. Following preincubation, the slices were superfused with physiological medium containing either hemicholinium-3 alone (hemi; 10 μM; open columns) or, in addition, norbinaltorphimine (nBNI; 0.01 μM; filled columns). During superfusion they were stimulated electrically twice (360 pulses, 3 Hz), after 57 (S_1) and 89 min (S_2) of superfusion. The evoked overflow at S_1 (in % of tissue-[^{3}H]) amounted to 3.4 ± 0.1 in the absence and to 3.5 ± 0.2 in the presence of nBNI; it has been shown previously[34] that it represents action potential-induced exocytotic release of acetylcholine. Drugs were added to the superfusion medium as shown on the abscissa from 16 min before S_2 onward. Their effects are shown as the S_2/S_1-ratios (means ± SEM) expressed as % of the corresponding control ratios (no drug addition before S_2). Significance of differences: ***p < 0.001 vs untreated controls; °°p < 0.01 and °°°p < 0.001 vs same drug concentration in the absence of nBNI; *n*, number of single observations.

Conclusion

The present work demonstrates that introduction of an additional methyl substituent into the side chain of the κ-agonist **6** (GR-89,696) leads to potent κ-agonists, with the methyl carbamate (*S*,*S*)-**14** displaying the highest κ-receptor affinity. (*S*,*S*)-**14** even exceeds **6** in κ-receptor binding. The κ-agonistic properties of the carbamate (*S*,*S*)-**14** and the propionamide (*S*,*S*)-**13** were shown by reduction of the electrically evoked release of [^{3}H]acetylcholine in rabbit hippocampal slices. The κ-receptor affinity is strongly dependent on the configuration of both stereocenters. The stereostructure of the most potent (*S*,*S*)-configured stereoisomers corresponds to the three-dimensional structure of known κ-agonists. In contrast to **6** (GR-89,696) and analogous κ-agonists, the methyl carbamate (*S*,*S*)-**14** binds with high affinity to μ-receptors, giving this ligand a unique receptor binding profile. The importance of this observation has to be elucidated in further pharmacological studies.

Experimental Section

General. Unless otherwise noted, moisture-sensitive reactions were conducted under dry nitrogen. Thin layer chromatography (TLC) was performed with silica gel 60 F_{254} plates (Merck). In the flash chromatography (fc)[41] silica gel 60, 0.040−0.063 mm, (Merck) was used and the diameter of the column (cm), eluent, fraction size (mL), and R_f are included in parentheses. Melting points are uncorrected and were determined with a Dr. Tottoli apparatus (Büchi). Optical rotation was measured with a Polarimeter 241 (Perkin-Elmer) having a 1.0 dm tube; the concentration *c* is given in g/100 mL at 20 °C. Elemental analyses were determined with CHN elemental analyzer Rapid (Heraeus) and Elemental Analyzer 240 (Perkin-Elmer) instruments. MS was measured with 5989A (Hewlett-Packard), MAT 312, MAT 8200, MAT 4456, and TSQ 7000 (Finnigan) instruments in the EI (electron impact), CI (chemical ionization), or ESI (electron spray ionization) mode. IR spectra were generated with IR 1600 FT-IR, 2000 FT-IR, and 841-IR (Perkin-Elmer) spectrophotometers. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), and ^{19}F NMR spectra were recorded with a GSX FT NMR spectrometer (JEOL), and ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz)spectra were generated with a Unity 300 FT NMR spectrometer (Varian); tetramethylsilane was the internal standard, δ is in ppm, and coupling constants are given with 0.5 Hz resolution. The assignments of ^{13}C and ^{1}H NMR signals were supported by 2D NMR techniques (COSY, DEPT); in the case of rotational isomers, the signals of the minor rotamer are marked as ^{mr}.

(+)-*tert*-Butyl (*R*)-3-Acetyl-4-benzylpiperazine-1-carboxylate [(*R*)-9]. Molecular sieves (4 Å, 4 g), *N*-methylmorpholine *N*-oxide (NMMO, 1.10 g, 9.37 mmol), and tetrapropylammonium perruthenate (TPAP, 171 mg, 0.5 mmol) were successively added at 5 °C to a solution of (*R*,*R*)-**8**[13] (1.25 g, 3.89 mmol) in CH_2Cl_2 (100 mL). The reaction mixture was stirred for 16 h at room temperature. The suspension was filtered with Celite, the filtrate was concentrated in vacuo, and the black residue was purified by fc (4 cm, petroleum ether/ethyl acetate 2:1, 30 mL, R_f = 0.66): pale yellow oil, yield 1.05 g (85%); $[\alpha]^{22}_{589}$ = +28.2 (*c* = 0.68, CH_2Cl_2); ^{1}H NMR (d_6-DMSO, 60 °C) δ = 1.39 (s, 9 H, C(CH_3)$_3$), 2.17 (s, 3 H, CH_3), 2.15−2.24 (m, 1 H, 5-H), 2.88 (ddd, *J* = 11.7/6.6/3.7 Hz, 1 H, 5-H), 3.18 (dd, *J* = 6.1/4.6 Hz, 1 H, 3-H), 3.27 (ddd, *J* = 13.0/7.0/3.7 Hz, 1 H, 6-H), 3.36 (ddd, *J* = 13.1/6.7/3.9 Hz, 1 H, 6-H), 3.45 (d, *J* = 13.6 Hz, 1 H, CH_2Ph), 3.49 (dd, *J* = 13.2/4.4 Hz, 1 H, 2-H), 3.57 (dd, *J* = 13.1, 6.1 Hz, 1 H, 2-H), 3.73 (d, *J* = 13.7 Hz, 1 H, CH_2Ph), 7.22−7.29 (m, 1 H, arom), 7.29−7.37 (m, 4 H, arom). Anal. ($C_{18}H_{26}N_2O_3$) C, H, N.

(−)-*tert*-Butyl (*S*)-3-Acetyl-4-benzylpiperazine-1-carboxylate [(*S*)-9]. As described for (*R*)-**9**, the alcohol (*S*,*S*)-**8**[21] (330 mg, 1.03 mmol) was oxidized with NMMO (241 mg, 2.06 mmol), pulverized 4 Å molecular sieves (1 g), and TPAP (45 mg, 0.13 mmol) in CH_2Cl_2 (20 mL): pale yellow oil, yield 268 mg (82%); $[\alpha]^{22}_{589}$ = −32.1 (*c* = 1.30, CH_2Cl_2). Anal. ($C_{18}H_{26}N_2O_3$) C, H, N.

(+)-*tert*-Butyl (*R*)-4-Benzyl-3-[(*S*)-1-(pyrrolidin-1-yl)-ethyl]piperazine-1-carboxylate [(*R*,*S*)-10] and (+)-*tert*-Butyl (*R*)-4-Benzyl-3-[(*R*)-1-(pyrrolidin-1-yl)ethyl]piperazine-1-carboxylate [(*R*,*R*)-10]. Pyrrolidine (0.42 mL, 5.0 mmol) and Ti(OiPr)$_4$ (1.1 mL, 3.7 mmol) were slowly added to a cooled (0 °C) solution of (*R*)-**9** (790 mg, 2.49 mmol) in CH_2Cl_2 (2 mL), and the reaction mixture was stirred for 90 min at room temperature. Methanol (7 mL), 3 Å molecular sieves, and $NaBH_3CN$ (450 mg, 7.1 mmol) were successively added to the cooled (0 °C) solution, and the reaction mixture was stirred for 40 h at room temperature. Then, water (2 mL) and ethyl acetate (50 mL) were added, the suspension was filtered, and the filtrate was washed with saturated solutions of Na_2CO_3 (2 × 25 mL) and NaCl (25 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue (936 mg) was purified by fc (4 cm, petroleum ether/ethyl acetate/ethyldimethylamine 80:20:0.4; 30 mL).

(*R*,*S*)-10 (R_f = 0.78): colorless oil, yield 580 mg (62%); $[\alpha]^{22}_{589}$ = +44.3 (*c* = 0.57, CH_2Cl_2); $C_{22}H_{35}N_3O_2$ (373.5); ^{1}H NMR (d_6-DMSO, 60 °C) δ = 1.13 (d, *J* = 6.6 Hz, 3 H, CHCH_3), 1.44 (s, 9 H, C(CH_3)$_3$), 1.63−1.74 (m, 4 H, CH_2CH$_2$NCH$_2$CH_2), 2.12−2.22 (m, 1 H, 5-H), 2.43−2.56 (m, 3 H, 3-H, CH$_2$CH_2-NCH_2CH$_2$), 2.56−2.65 (m, 2 H, CH$_2$CH_2NCH_2CH$_2$), 2.65−2.69 (m, 1 H, CHCH$_3$), 2.70−2.74 (m, 1 H, 5-H), 3.03 (ddd, *J* = 12.6/9.0/3.5 Hz, 1 H, 6-H), 3.12−3.28 (m, 1 H, 2-H), 3.35 (d, *J* = 13.9 Hz, 1 H, CH_2Ph), 3.45−3.54 (m, 1 H, 6-H), 3.54−3.62 (m, 1 H, 2-H), 4.52 (d, *J* = 13.9 Hz, 1 H, CH_2Ph), 7.21−7.28 (m, 1 H, arom), 7.30−7.39 (m, 4 H, arom).

(*R*,*R*)-10 (R_f = 0.30): colorless oil, yield 233 mg (25%); $[\alpha]^{26}_{589}$ = +88.7 (*c* = 1.39, CH_2Cl_2); $C_{22}H_{35}N_3O_2$ (373.5); ^{1}H NMR (d_6-DMSO, 60 °C) δ = 1.11 (d, *J* = 6.6 Hz, 3 H, CHCH_3), 1.37 (s, 9 H, C(CH_3)$_3$), 1.68 (s broad, 4 H, CH_2CH$_2$NCH$_2$CH_2), 1.93 (td, *J* = 11.5/3.3 Hz, 1 H, 5-H), 2.31−2.39 (m, 1 H, 2-H), 2.48 (s broad, 2 H, CH$_2$CH_2NCH_2CH$_2$), 2.54 (s broad, 2 H, CH$_2$CH_2NCH_2CH$_2$), 2.62 (d broad, *J* = 11.9 Hz, 1 H, 5-H), 2.66−2.82 (m, 3 H, CHCH$_3$, 6-H and 2-H), 3.02 (d, *J* = 13.4 Hz, 1 H, CH_2Ph), 3.66 (d broad, *J* = 12.7 Hz, 1 H, 6-H), 3.98−4.07 (m, 1 H, 2-H), 4.05 (d, *J* = 13.4 Hz, 1 H, CH_2Ph), 7.17−7.26 (m, 1 H, arom), 7.27−7.32 (m, 4 H, arom).

(−)-*tert*-Butyl (*S*)-4-Benzyl-3-[(*R*)-1-(pyrrolidin-1-yl)-ethyl]piperazine-1-carboxylate [(*S*,*R*)-10] and (−)-*tert*-Butyl (*S*)-4-Benzyl-3-[(*S*)-1-(pyrrolidin-1-yl)ethyl]piperazine-1-carboxylate [(*S*,*S*)-10]. As described for the preparation of (*R*,*S*)-**10** and (*R*,*R*)-**10**, the ketone (S)-**9** (742 mg, 2.33 mmol) was reductively aminated with pyrrolidine (0.4 mL, 4.80 mmol), Ti(OiPr)$_4$ (1.4 mL, 4.78 mmol), CH_2Cl_2 (2 mL), methanol (15 mL), and $NaBH_3CN$ (413 mg, 6.56 mmol).

(*R*,*S*)-10: colorless oil, yield 466 mg (54%); $[\alpha]^{20}_{589}$ = −49.1 (*c* = 1.00, CH_2Cl_2).

(*S*,*S*)-10: colorless oil, yield 181 mg (21%); $[\alpha]^{20}_{589}$ = −96.0 (*c* = 1.16, CH_2Cl_2).

(+)-*tert*-Butyl (*R*)-4-[2-(3,4-Dichlorophenyl)acetyl]-3-[(*S*)-1-(pyrrolidin-1-yl)ethyl]piperazine-1-carboxylate [(*R*,*S*)-11]. A mixture of (*R*,*S*)-**10** (879 mg, 2.36 mmol), Pd/C (10%, 600 mg), and methanol (40 mL) was stirred under hydrogen (balloon) for 9 h at room temperature. The suspension was filtered with Celite and the filtrate was concentrated in vacuo to give *tert*-butyl (*R*)-3-[(*S*)-1-(pyrrolidin-1-yl)ethyl]-piperazine-1-carboxylate as colorless oil, yield 643 mg: IR (film) $\tilde{\nu}$ (cm^{-1}) = 3600−3150 (br, ν_{NH}), 2970 (s, ν_{CH}), 2795 (m, ν_{CH}), 1694 (s, $\nu_{C=O}$), 1420, 1170 (s).

At 0 °C, 1,1′-carbonyldiimidazole (CDI, 535 mg, 3.30 mmol) was added to a solution of (3,4-dichlorophenyl)acetic acid (580 mg, 2.83 mmol) in CH_2Cl_2 (10 mL). After stirring for 2 h at room temperature, a solution of *tert*-butyl (*R*)-3-[(*S*)-1-(pyrrolidin-1-yl)ethyl]piperazine-1-carboxylate in CH_2Cl_2 (10 mL) was added and the reaction mixture was stirred for 24 h at room temperature. Then, CH_2Cl_2 (50 mL) was added, the CH_2Cl_2 layer was washed with saturated solutions of Na_2CO_3 (2 × 20 mL) and NaCl (50 mL), dried ($MgSO_4$), and evaporated in vacuo. The residue (1.27 g) was purified by fc (4 cm, CH_2Cl_2/ethyl acetate/methanol 6:1:0.5, 15 mL, R_f = 0.48): pale yellow solid, mp 95 °C, yield 810 mg (73%); $[\alpha]^{21}_{589}$ = +30.4 (*c* = 1.05, CH_2Cl_2); ^{1}H NMR (d_5-nitrobenzene, 80 °C) δ = 0.88 (d, *J* = 6.6 Hz, 3 H, CHCH_3), 1.48−1.59 (m, 4 × 0.13 H, CH_2-CH$_2$NCH$_2$CH_2^{mr}), 1.54 (s, 9 H, C(CH_3)$_3$), 1.64−1.75 (m, 4 × 0.87 H, CH_2CH$_2$NCH$_2$CH_2), 2.11−2.31 (m, 4 × 0.13 H, CH$_2$CH_2-NCH_2CH$_2^{mr}$), 2.56−2.77 (m, 4 × 0.87 H, CH$_2$CH_2NCH_2CH$_2$), 2.79−2.94 (m, 1 H, 6-H), 2.84 (dd, *J* = 12.8/3.8 Hz, 1 H, 2-H), 3.07−3.36 (m broad, 2 H, CHCH$_3$ and 5-H), 3.86 (s, 2 H, COCH$_2$), 3.79−3.94 (m broad, 1 H, 5-H), 4.22 (d broad, *J* = 11.8 Hz, 1 H, 6-H), 4.59−4.84 (m broad, 1 H, 3-H), 4.73 (d broad, *J* = 12.8 Hz, 1 H, 2-H), 7.25 (d, *J* = 8.3 Hz, 1 H, arom), 7.36 (d, *J* = 8.2 Hz, 1 H, arom), 7.47 (s, 1 H, arom); ^{13}C NMR (d_5-nitrobenzene, 80 °C) δ = 7.0 (1 C, CHCH$_3$), 23.9 (2 C, CH$_2$-CH$_2$NCH$_2$$CH_2$), 28.0 (3 C, C($CH_3$)$_3$), 39.2 (1 C, CO$CH_2$), 42.3 (broad, 1 C, C-6), 43.2 (broad, 1 C, C-2), 47.3 (2 C, CH$_2$$CH_2$-N$CH_2CH_2$), 49.5 (1 C, CHCH$_3$), 79.1 (1 C, C(CH$_3$)$_3$), 129.1 (1 C, arom CH), 130.1 (1 C, arom CH), 130.3 (1 C, arom CCl), 131.3 (1 C, arom CH), 131.9 (1 C, arom CCl), 136.7 (1 C, arom C), 154.7 (1 C, NCO_2tBu), 168.7 (1 C, C=O); ^{1}H NMR (d_5-nitrobenzene, 27 °C) δ = 2.39−2.67 (m, 5 H, CH_2CH$_2$-NCH$_2$CH_2 and 5-H^{mr}), 2.71−2.93 (m broad, 1 H, 6-H), 2.81 (dd, *J* = 13.0/3.5 Hz, 1 H, 2-H), 2.95−3.10 (m broad, 1 H, 6-H),

3.14 (dq, J = 11.3/6.6 Hz, 1 H, C*H*CH$_3$), 3.26 (dq, J = 9.9/6.9 Hz, C*H*CH$_3$mr), 3.76−3.97 (m, 3-H^{mr} and 5-H), 3.87 (s, 2 H, COCH$_2$), 3.98 (s, COCH$_2^{mr}$), 3.87 (s, 2 H, COCH$_2$), 4.12−4.43 (m broad, 1 H, 6-H), 4.65−4.73 (d broad, 5-H^{mr}), 4.69−4.89 (m, 3-H and 2-H). Anal. ($C_{23}H_{33}Cl_2N_3O_3$) C, H, N.

(−)-*tert*-Butyl (*S*)-4-[2-(3,4-Dichlorophenyl)acetyl]-3-[(*R*)-1-(pyrrolidin-1-yl)ethyl]piperazine-1-carboxylate [(*S*,*R*)-11]. As described for the preparation of (*R*,*S*)-**11**, the piperazine (*S*,*R*)-**10** (158 mg, 0.42 mmol) was hydrogenated with the catalyst Pd/C (10%, 80 mg) in methanol (40 mL) and the resulting secondary amine (113 mg) was acylated with (3,4-dichlorophenyl)acetic acid (103 mg, 0.50 mmol) and CDI (92 mg, 0.57 mmol) in CH_2Cl_2 (15 mL): pale yellow oil, yield 147 mg (74%); $[\alpha]^{25}_{589} = -26.2$ (c = 1.22, CH_2Cl_2). Anal. ($C_{23}H_{33}Cl_2N_3O_3$) C, H, N.

(−)-*tert*-Butyl (*R*)-4-[2-(3,4-Dichlorophenyl)acetyl]-3-[(*R*)-1-(pyrrolidin-1-yl)ethyl]piperazine-1-carboxylate [(*R*,*R*)-11]. As described for the preparation of (*R*,*S*)-**11**, the piperazine (*R*,*R*)-**10** (257 mg, 0.69 mmol) was hydrogenated with the catalyst Pd/C (10%, 200 mg) in methanol (40 mL) and the resulting secondary amine (190 mg) was acylated with (3,4-dichlorophenyl)acetic acid (148 mg, 0.72 mmol) and CDI (185 mg, 1.1 mmol) in CH_2Cl_2 (15 mL). The crude product (356 mg) was purified by fc (3 cm, CH_2Cl_2/ethyl acetate/methanol 6:1:1, 10 mL, R_f = 0.41): colorless solid, mp 150−153 °C, yield 198 mg (61%); $[\alpha]^{21}_{589} = -3.8$ (c = 1.00, CH_2Cl_2); ^{1}H NMR (d_5-nitrobenzene, 80 °C) δ = 1.14 (d, J = 6.4 Hz, 3 H, CHC*H*$_3$), 1.54 (s, 9 H, C(CH$_3$)$_3$), 1.59−1.69 (m, 4 H, C*H*$_2$CH$_2$NCH$_2$C*H*$_2$), 2.11−2.36 (m broad, 4 × 0.26 H, CH$_2$C*H*$_2$NC*H*$_2$CH$_2^{mr}$), 2.48−2.64 (m, 2 × 0.74 H, CH$_2$C*H*$_2$NC*H*$_2$CH$_2$), 2.62−2.76 (m, 2 × 0.74 H, CH$_2$C*H*$_2$NC*H*$_2$CH$_2$), 2.96 (td, J = 12.7/3.9 Hz, 1 H, 6-H), 3.06 (dd, J = 13.9/4.0 Hz, 1 H, 2-H), 3.18−3.32 (m, 1 H, 5-H), 3.26 (dq, J = 9.7/6.5 Hz, 1 H, C*H*CH$_3$), 3.71−3.80 (m broad, 1 H, 5-H), 3.78 (d, J = 15.9 Hz, 1 H, COCH$_2$), 3.89 (d, J = 15.7 Hz, 1 H, COCH$_2$), 4.13−4.21 (d broad, 1 H, 6-H), 4.19−4.27 (d broad, 1 H, 2-H), 4.39−4.84 (m broad, 1 H, 3-H), 7.25 (dd, J = 8.2/2.0 Hz, 1 H, arom), 7.33 (d, J = 8.2 Hz, 1 H, arom), 7.43 (d, J = 1.8 Hz, 1 H, arom); ^{13}C NMR (d_5-nitrobenzene, 70 °C) δ = 9.7 (1 C, CH*C*H$_3$), 23.9 (2 C, *C*H$_2$CH$_2$NCH$_2$*C*H$_2$), 28.0 (3 C, C(*C*H$_3$)$_3$), 39.4 (1 C, CO*C*H$_2$), 43.8 (1 C, C-3), 44.4 (1 C, C-5), 48.3 (2 C, CH$_2$*C*H$_2$N*C*H$_2$CH$_2$), 51.5 (1 C, *C*HCH$_3$), 79.6 (1 C, *C*(CH$_3$)$_3$), 129.2 (1 C, arom CH), 130.1 (1 C, arom CH), 130.2 (1 C, arom CCl), 131.3 (1 C, arom CH), 131.9 (1 C, arom CCl), 137.2 (1 C, arom C), 154.6 (1 C, NCO$_2$tBu), 168.5 (1 C, C═O); ^{1}H NMR (d_5-nitrobenzene, 27 °C) δ = 2.80−3.12 (m broad, 6-H), 3.00−3.08 (d broad, J = 12.8 Hz, 2-H), 3.14−3.56 (m, 2 H, 5-H and C*H*CH$_3$), 3.65−3.76 (m, 1 H, 5-H), 3.65−3.75 (m, COCH$_2^{mr}$), 3.77 (d, J = 16.2 Hz, 1 H, COCH$_2$), 3.93 (d, J = 16.0 Hz, 1 H, COCH$_2$), 4.06−4.25 (m, 1 H, 2-H), 4.22−4.35 (m, 1 H, 6-H), 4.63−4.76 (d broad, J = 9.2 Hz, 1 H, 3-H). Anal. ($C_{23}H_{33}Cl_2N_3O_3$) C, H, N.

(+)-*tert*-Butyl (*S*)-4-[2-(3,4-Dichlorophenyl)acetyl]-3-[(*S*)-1-(pyrrolidin-1-yl)ethyl]piperazine-1-carboxylate [(*S*,*S*)-11]. As described for the preparation of (*R*,*R*)-**11**, the piperazine (*S*,*S*)-**10** (390 mg, 1.05 mmol) was hydrogenated with the catalyst Pd/C (10%, 250 mg) in methanol (25 mL), and the resulting secondary amine (279 mg) was acylated with (3,4-dichlorophenyl)acetic acid (254 mg, 1.24 mmol) and CDI (254 mg, 1.57 mmol) in CH_2Cl_2 (20 mL): colorless solid, mp 150−153 °C, yield 289 mg (59%); $[\alpha]^{25}_{589} = +2.9$ (c = 1.10, CH_2Cl_2). Anal. ($C_{23}H_{33}Cl_2N_3O_3$) C, H, N.

(+)-1-{(*R*)-4-[2-(3,4-Dichlorophenyl)acetyl]-3-[(*S*)-1-(pyrrolidin-1-yl)ethyl]piperazin-1-yl}propan-1-one [(*R*,*S*)-13]. Trifluoroacetic acid (0.5 mL, 6.53 mmol) was added to a cold solution of (*R*,*S*)-**11** (124 mg, 0.26 mmol) in CH_2Cl_2 (3 mL) and the reaction mixture was stirred for 9 h at room temperature. Then, 2 N NaOH (6 mL) and propionyl chloride (50 μL, 0.53 mmol) were added, and the reaction mixture was stirred for an additional 40 h at room temperature. After addition of CH_2Cl_2 (50 mL), the organic layer was washed with 2 N NaOH (20 mL) and saturated NaCl (25 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue (110 mg) was purified by fc (1.6 cm, CH_2Cl_2/ethyl acetate/methanol 8:1:0.5, 10 mL, R_f = 0.33): colorless needles (ethyl acetate), mp 127 °C, yield 82 mg (73%); $[\alpha]^{23}_{589} = +32.4$ (c = 1.03, CH_2Cl_2); MS (CI) m/z (%) = 431/429/427 (M + H, 3/15/23), 430/428/426 (M, 12/67/100), 98 ($C_6H_{12}N$, 87); IR (film) = $\tilde{\nu}$ (cm^{-1}) 2969 (m, ν_{CH}), 1642 (s, $\nu_{C=O}$), 1433 (s), 1212, 1032 (m); ^{1}H NMR (d_5-nitrobenzene, 80 °C) δ = 0.83 (d, J = 5.8 Hz, 3 H, CHC*H*$_3$), 1.15 (t, J = 7.4 Hz, 3 H, CH$_2$C*H*$_3$), 1.63 (s broad, 4 H, C*H*$_2$CH$_2$NCH$_2$C*H*$_2$), 2.13−2.24 (m broad, 2 H, CH$_2$C*H*$_2$NC*H*$_2$CH$_2$), 2.24−2.40 (m, 1 H, C*H*$_2$CH$_3$), 2.42−2.66 (m, 3 H, C*H*$_2$CH$_3$ and CH$_2$C*H*$_2$NC*H*$_2$CH$_2$), 2.60−2.80 (m broad, 1 H, 6-H), 2.96 (d broad, J = 11.2 Hz, 1 H, 2-H), 3.00−3.26 (m, 2 H, C*H*CH$_3$ and 5-H), 3.76−3.96 (m broad, 1 H, 5-H), 3.83 (s, 2 H, COCH$_2$), 4.30−4.90 (m broad, 3 H, 2-H, 3-H and 6-H), 7.21 (d, J = 8.1 Hz, 1 H, arom), 7.32 (d, J = 8.2 Hz, 1 H, arom), 7.43 (s, 1 H, arom); ^{13}C NMR (d_5-nitrobenzene, 80 °C) δ = 6.4 (1 C, CH*C*H$_3$), 9.0 (1 C, CH$_2$*C*H$_3$), 23.9 (2 C, *C*H$_2$CH$_2$NCH$_2$*C*H$_2$), 25.4 (1 C, *C*H$_2$CH$_3$), 39.2 (1 C, CO*C*H$_2$), 47.0 (2 C, CH$_2$*C*H$_2$NC*H*$_2$CH$_2$), 51.1 (1 C, *C*HCH$_3$), 129.2 (1 C, arom CH), 130.2 (1 C, arom CH), 130.4 (1 C, arom CCl), 131.4 (1 C, arom CH), 132.0 (1 C, arom CCl), 136.6 (1 C, arom *C*), 168.7 (1 C, C═O), 172.5 (1 C, C═O); ^{1}H NMR (d_5-nitrobenzene, 27 °C) δ = 2.50−2.60 (m, 5-H^{mr}), 2.60−2.80 (dd broad, 6-H), 2.94−3.10 (m, 2-H, 5-H and C*H*CH$_3$), 3.11−3.28 (m, C*H*CH$_3^{mr}$), 3.80−3.90 (m, 5-H), 3.85 (s, COCH$_2$), 3.87−3.95 (m, 3-H^{mr}), 4.39−4.50 (m, 2-H), 4.66−4.77 (m, 6-H and 5-H^{mr}), 4.75−4.85 (m, 3-H); ^{13}C NMR (d_5-nitrobenzene, 27 °C) δ = 37.3 (C-5^{mr}), 39.5 (CO*C*H$_2^{mr}$), 39.7 (CO*C*H$_2$), 41.2 (C-6^{mr}), 41.6 (C-6), 42.0 (C-5), 45.1 (C-2), 45.6 (C-2^{mr}), 47.1 (CH$_2$*C*H$_2$NCH$_2$CH$_2$), 47.3 (CH$_2$*C*H$_2$NCH$_2$CH$_2^{mr}$), 50.7 (*C*HCH$_3^{mr}$), 50.9 (*C*HCH$_3$), 52.4 (C-3), 57.3 (C-3^{mr}). Anal. ($C_{21}H_{29}Cl_2N_3O_2$) C, H, N.

(−)-1-{(*S*)-4-[2-(3,4-Dichlorophenyl)acetyl]-3-[(*R*)-1-(pyrrolidin-1-yl)ethyl]piperazin-1-yl}propan-1-one [(*S*,*R*)-13]. As described for the preparation of (*R*,*S*)-**13**, the BOC-protected piperazine (*S*,*R*)-**11** (152 mg, 0.32 mmol) was treated with trifluoroacetic acid (0.6 mL, 7.84 mmol) in CH_2Cl_2 (5 mL) and, subsequently, with propionyl chloride (57 μL, 0.65 mmol) and 2 N NaOH (7.5 mL): colorless needles (ethyl acetate), mp 127 °C, yield 93 mg (68%); $[\alpha]^{18}_{589} = -40.1$ (c = 0.97, CH_2Cl_2). Anal. ($C_{21}H_{29}Cl_2N_3O_2$) C, H, N.

(−)-1-{(*R*)-4-[2-(3,4-Dichlorophenyl)acetyl]-3-[(*R*)-1-(pyrrolidin-1-yl)ethyl]piperazin-1-yl}propan-1-one [(*R*,*R*)-13]. As described for the preparation of (*R*,*S*)-**13**, the BOC-protected piperazine (*R*,*R*)-**11** (169 mg, 0.36 mmol) was treated with trifluoroacetic acid (0.6 mL, 7.84 mmol) in CH_2Cl_2 (5 mL) and, subsequently, with propionyl chloride (63 μL, 0.72 mmol) and 2 N NaOH (7.5 mL). The residue (140 mg) was purified by fc (2 cm, CH_2Cl_2/ethyl acetate/methanol 8:1:0.5, 10 mL, R_f = 0.16): pale yellow oil, yield 115 mg (75%); $[\alpha]^{22}_{589} = -6.1$ (c = 1.36, CH_2Cl_2); MS (CI) m/z (%) = 431/429/427 (M + H, 2/10/17), 430/428/426 (M, 8/41/66), 98 ($C_6H_{12}N$, 100); IR (film) $\tilde{\nu}$ (cm^{-1}) = 2967 (m, ν_{CH}), 1641 (s, $\nu_{C=O}$), 1432 (s), 1216, 1031 (m); ^{1}H NMR (d_5-nitrobenzene, 80 °C) δ = 1.12 (d, J = 6.4 Hz, 3 H, CHC*H*$_3$), 1.20 (t, J = 7.4 Hz, 3 H, CH$_2$C*H*$_3$), 1.53−1.73 (m, 4 H, C*H*$_2$CH$_2$NCH$_2$C*H*$_2$), 2.25−2.52 (m, 2 H, C*H*$_2$CH$_3$), 2.49−2.62 (m, 2 H, CH$_2$C*H*$_2$NC*H*$_2$CH$_2$), 2.62−2.73 (m, 2 H, CH$_2$C*H*$_2$NC*H*$_2$CH$_2$), 3.05−3.26 (m, 3 H, 2-H, 6-H and C*H*CH$_3$), 3.24−3.44 (m, 1 H, 5-H), 3.82 (d, J = 16.0 Hz, 1 H, COCH$_2$), 3.85−4.27 (m broad, 2 H, 5-H and 6-H), 3.93 (d, J = 16.0 Hz, 1 H, COCH$_2$), 4.30−4.88 (m broad, 2 H, 2-H and 3-H), 7.27 (d, J = 8.2 Hz, 1 H, arom), 7.34 (d, J = 8.2 Hz, 1 H, arom), 7.46 (s, 1 H, arom); ^{13}C NMR (d_5-nitrobenzene, 80 °C) δ = 8.8 (1 C, CH$_2$*C*H$_3$), 10.0 (1 C, CH*C*H$_3$), 23.6 (2 C, *C*H$_2$CH$_2$NCH$_2$CH$_2$), 25.8 (1 C, *C*H$_2$CH$_3$), 39.0 (1 C, CO*C*H$_2$), 48.3 (2 C, CH$_2$*C*H$_2$NCH$_2$CH$_2$), 51.8 (1 C, *C*HCH$_3$), 122.6 (1 C, arom CCl), 128.9 (1 C, arom CH), 129.9 (1 C, arom CH), 131.0 (1 C, arom CH), 131.7 (arom CCl), 136.8 (1 C, arom C), 168.3 (1 C, C═O), 172.2 (1 C, C═O); ^{1}H NMR (d_5-nitrobenzene, 27 °C) δ = 2.81−3.09 (m, 2-H and 2-H^{mr}), 3.12−3.30 (m, C*H*CH$_3$), 3.21−3.50 (m, 5-H and 6-H), 3.73−4.07 (m, 5-H, 6-H and COCH$_2$), 4.54−4.65 (d broad, 2-H^{mr}), 4.70−4.88 (m, 2-H and 3-H); ^{13}C NMR (d_5-nitrobenzene, 27 °C) δ = 37.8 (C-5^{mr}), 39.9 (CO*C*H$_2^{mr}$), 40.1 (CO*C*H$_2$), 42.0 (C-2^{mr}), 42.2 (C-2), 42.4 (C-5), 45.1 (C-6^{mr}), 45.8 (C-6), 48.3 (CH$_2$*C*H$_2$NCH$_2$CH$_2$), 48.7 (CH$_2$*C*H$_2$NCH$_2$CH$_2^{mr}$), 51.1 (*C*HCH$_3$), 51.6 (*C*HCH$_3^{mr}$), 53.7 (C-3), 58.3 (C-3^{mr}). Anal. ($C_{21}H_{29}Cl_2N_3O_2$) C, H, N.

(+)-1-{(*S*)-4-[2-(3,4-Dichlorophenyl)acetyl]-3-[(*S*)-1-(pyrrolidin-1-yl)ethyl]piperazin-1-yl}propan-1-one [(*S,S*)-13]. As described for the preparation of (*R,R*)-**13**, the BOC-protected piperazine (*S,S*)-**11** (165 mg, 0.35 mmol) was treated with trifluoroacetic acid (0.6 mL, 7.84 mmol) in CH_2Cl_2 (5 mL) and, subsequently, with propionyl chloride (60 μL, 0.69 mmol) and 2 N NaOH (7.5 mL): pale yellow oil, yield 101 mg (68%); $[\alpha]^{21}_{589} = +8.4$ ($c = 0.74$, CH_2Cl_2). Anal. ($C_{21}H_{29}Cl_2N_3O_2$) C, H, N.

(+)-Methyl (*R*)-4-[2-(3,4-Dichlorophenyl)acetyl]-3-[(*S*)-1-(pyrrolidin-1-yl)ethyl]piperazine-1-carboxylate [(*R,S*)-14]. Trifluoroacetic acid (0.6 mL, 7.84 mmol) was added to a cold solution of (*R,S*)-**11** (155 mg, 0.33 mmol) in CH_2Cl_2 (5 mL) and the reaction mixture was stirred for 10 h at room temperature. Then, 2 N NaOH (25 mL) was added, the organic layer was separated, the aqueous layer was extracted with CH_2Cl_2 (2 × 75 mL), and the combined organic layers were dried ($MgSO_4$). Concentration in vacuo gave (*R,S*)-**12** as colorless oil: 138 mg; IR (film) $\tilde{\nu}$ (cm^{-1}) = 2961 (m, ν_{CH}), 1700 (s, $\nu_{C=O}$), 1645 (s, $\nu_{C=O}$), 1450 (s broad).

(*R,S*)-**12** was dissolved in CH_2Cl_2 (3 mL) and Et_3N (0.1 mL, 0.72 mmol). At 0 °C methyl chloroformate (40 μL, 0.52 mmol) was added and the reaction mixture was stirred at room temperature for 40 h. After addition of CH_2Cl_2 (50 mL) the mixture was washed with saturated solutions of $NaHCO_3$ (2 × 25 mL) and NaCl (25 mL), and the organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue (150 mg) was purified by fc (2 cm, CH_2Cl_2/ethyl acetate/methanol 8:1:0.5, 10 mL, $R_f = 0.41$); colorless oil, yield 90 mg (64%); $[\alpha]^{23}_{589} = +25.9$ ($c = 1.23$, CH_2Cl_2); MS (CI) *m/z* (%) = 433/431/429 (M + H, 0.5/3.0/5.0), 432/430/428 (M, 2.5/14/21), 98 ($C_6H_{12}N$, 100); IR (film) $\tilde{\nu}$ (cm^{-1}) = 2961 (m, ν_{CH}), 1700 (s, $\nu_{C=O}$), 1645 (s, $\nu_{C=O}$), 1450 (s broad); ^{1}H NMR (d_5-nitrobenzene, 80 °C) $\delta = 0.88$ (d, $J = 6.7$ Hz, 3 H, CHCH_3), 1.21−1.35 (m broad, 4 × 0.26 H, CH_2CH$_2$NCH$_2$CH_2^{mr}), 1.56−1.73 (m, 4 × 0.74 H, CH_2CH$_2$NCH$_2$CH_2), 2.26−2.45 (m broad, 4 × 0.26 H, CH$_2$CH_2NCH_2CH$_2^{mr}$), 2.56−2.68 (m, 4 × 0.74 H, CH$_2$CH_2NCH_2CH$_2$), 2.77−3.00 (m, 1 H, 6-H), 2.88 (dd, $J = 13.0/4.0$ Hz, 1 H, 2-H), 3.10−3.30 (m, 2 H, CHCH$_3$ and 5-H), 3.61−3.96 (m broad, 1 H, 5-H), 3.73 (s, 3 H, OCH_3), 3.88 (s, 2 H, $COCH_2$), 4.15 (d broad, $J = 11.4$ Hz, 1 H, 6-H), 4.50−4.82 (m broad, 1 H, 3-H), 4.71 (d, $J = 13.1$ Hz, 1 H, 2-H), 7.25 (dd, $J = 8.2/2.0$ Hz, 1 H, arom), 7.36 (d, $J = 8.2$ Hz, 1 H, arom), 7.47 (d, $J = 1.8$ Hz, 1 H, arom); ^{13}C NMR (d_5-nitrobenzene, 80 °C) $\delta = 7.2$ (1 C, CHCH$_3$), 23.9 (2 C, CH$_2$CH$_2$NCH$_2$$CH_2$), 39.2 (1 C, CO$CH_2$), 43.4 (broad, 1 C, C-6), 43.6 (broad, 1 C, C-2), 47.3 (2 C, CH$_2$$CH_2NCH_2CH_2$), 51.6 (1 C, CHCH$_3$), 52.0 (1 C, OCH_3), 129.2 (1 C, arom CH), 130.2 (1 C, arom CH), 130.4 (1 C, arom CCl), 131.3 (1 C, arom CH), 132.0 (1 C, arom CCl), 136.6 (1 C, arom C), 156.0 (1 C, NCO$_2$CH$_3$), 168.8 (1 C, CH$_2$$C$=O); ^{1}H NMR ($d_5$-nitrobenzene, 27 °C) $\delta = 2.50$−2.65 (m broad, 5-H^{mr}), 2.60−2.97 (m broad, 6-H), 2.83 (dt, $J = 12.9/4.0$ Hz, 1 H, 2-H), 2.89−3.15 (m broad, CHCH$_3$ and 5-H), 3.70 (s, OCH_3), 3.78−3.90 (m broad, 3-H^{mr} and 5-H), 3.86 (s, 2 H, $COCH_2$), 3.92−4.13 (m broad, 6-H^{mr}), 4.11−4.34 (m broad, 6-H), 4.55−4.89 (m broad, 3-H, 2-H, 2-H^{mr} and 5-H^{mr}); ^{13}C NMR (d_5-nitrobenzene, 27 °C) $\delta = 37.5$ (C-6^{mr}), 39.6 (COCH$_2$^{mr}), 39.7 (COCH$_2$), 42.2 (C-6), 43.3−44.4 (broad, C-3 and C-5), 47.6 (CH$_2$$CH_2NCH_2CH_2$), 51.3−51.6 (broad, CHCH$_3$), 51.8 (C-2), 52.6 (OCH_3), 56.7 (C-2^{mr}). Anal. ($C_{20}H_{27}Cl_2N_3O_3$) C, H, N.

(−)-Methyl (*S*)-4-[2-(3,4-Dichlorophenyl)acetyl]-3-[(*R*)-1-(pyrrolidin-1-yl)ethyl]piperazine-1-carboxylate [(*S,R*)-14]. As described for the preparation of (*R,S*)-**14**, the BOC group of (*S,R*)-**11** (155 mg, 0.33 mmol) was cleaved with trifluoroacetic acid (0.6 mL, 7.84 mmol) in CH_2Cl_2 (5 mL) and the resulting secondary amine (*S,R*)-**12** was acylated with methyl chloroformate (31 μL, 0.40 mmol) and Et_3N (0.11 mL, 0.80 mmol) in CH_2Cl_2 (5 mL); colorless oil, yield 101 mg (72%); $[\alpha]^{22}_{589} = -29.5$ ($c = 1.16$, CH_2Cl_2). Anal. ($C_{20}H_{27}Cl_2N_3O_3$) C, H, N.

(−)-Methyl (*R*)-4-[2-(3,4-Dichlorophenyl)acetyl]-3-[(*R*)-1-(pyrrolidin-1-yl)ethyl]piperazine-1-carboxylate [(*R,R*)-14]. As described for the preparation of (*R,S*)-**14**, the BOC group of (*R,R*)-**11** (157 mg, 0.33 mmol) was cleaved with trifluoroacetic acid (0.6 mL, 7.84 mmol) in CH_2Cl_2 (3 mL) and the resulting secondary amine (*R,R*)-**12** (124 mg) was acylated with methyl chloroformate (50 μL, 0.65 mmol) and Et_3N (0.10 mL, 0.72 mmol) in CH_2Cl_2 (3 mL). The residue was purified by fc (2 cm, CH_2Cl_2/ethyl acetate/methanol 8:1:0.5, 10 mL, $R_f = 0.19$); colorless oil, yield 95 mg (67%); $[\alpha]^{21}_{589} = -6.1$ ($c = 1.54$, CH_2Cl_2); MS (CI) *m/z* (%) = 433/431/429 (M + H, 3/11/18), 432/430/428 (M, 9/51/77), 98 ($C_6H_{12}N$, 100); IR (film) $\tilde{\nu}$ (cm^{-1}) = 2962 (m, ν_{CH}), 1702 (s, $\nu_{C=O}$), 1641 (s, $\nu_{C=O}$), 1446 (broad s), 1124, 1029 (m); ^{1}H NMR (d_5-nitrobenzene, 80 °C) $\delta = 1.09$ (d, $J = 6.6$ Hz, 3 H, CHCH_3), 1.55−1.69 (m, 4 H, CH_2-CH$_2$NCH$_2$CH_2), 2.24−2.47 (broad, CH_2CH$_2$NCH$_2$CH_2^{mr}), 2.48−2.60 (m, 2 H, CH$_2$CH_2NCH_2CH$_2$), 2.61−2.73 (m, 2 H, CH$_2$CH_2-NCH_2CH$_2$), 3.00 (td, $J = 12.6/4.0$ Hz, 1 H, 6-H), 3.10 (dd, $J = 13.7/4.0$ Hz, 1 H, 2-H), 3.18−3.30 (m, 2 H, 5-H and CHCH$_3$), 3.70−3.78 (broad, 1 H, 5-H), 3.73 (s, 3 H, OCH_3), 3.79 (d, $J = 15.9$ Hz, 1 H, $COCH_2$), 3.90 (d, $J = 15.9$ Hz, 1 H, $COCH_2$), 4.10 (d, $J = 13.0$ Hz, 1 H, 6-H), 4.21 (d, $J = 13.7$ Hz, 1 H, 2-H), 4.38−4.78 (broad, 1 H, 3-H), 7.26 (dd, $J = 8.3/1.8$ Hz, 1 H, arom), 7.33 (d, $J = 8.2$ Hz, 1 H, arom), 7.44 (d, $J = 1.8$ Hz, 1 H, arom); ^{13}C NMR (d_5-nitrobenzene, 80 °C) $\delta = 9.7$ (1 C, CHCH$_3$), 23.8 (2 C, CH$_2$CH$_2$NCH$_2$$CH_2$), 39.2 (1 C, CO$CH_2$), 43.9 (broad, 1 C, C-3 or C-5), 44.2 (broad, 1 C, C-5 or C-3), 48.3 (2 C, CH$_2$$CH_2NCH_2CH_2$), 51.6 (1 C, CHCH$_3$), 52.1 (1 C, OCH_3), 129.0 (1 C, arom CH), 130.0 (1 C, arom CH), 130.2 (1 C, arom CCl), 131.2 (1 C, arom CH), 131.9 (1 C, arom CCl), 137.0 (1 C, arom C), 155.8 (1 C, NCO$_2$CH$_3$), 168.5 (1 C, C=O); ^{1}H NMR (d_5-nitrobenzene, 27 °C) $\delta = 2.73$−2.97 (m broad, 5-H^{mr}), 2.92−3.17 (m broad, 2-H and 6-H), 3.14−3.38 (m broad, CHCH$_3$ and 5-H), 3.43 (quint, $J = 6.6$ Hz, CHCH$_3^{mr}$), 3.67−3.87 (m, 5-H), 3.74 (s, OCH_3), 3.78 (d, $J = 16.0$ Hz, $COCH_2$), 3.92−4.43 (m broad, 2-H and 6-H), 3.94 (d, $J = 16.0$ Hz, $COCH_2$), 4.66−4.78 (d broad, $J = 8.7$ Hz, 3-H); ^{13}C NMR (d_5-nitrobenzene, 27 °C) $\delta = 37.5$ (C-5^{mr}), 38.7 (COCH$_2$^{mr}), 40.0 (COCH$_2$), 41.9 (C-5), 43.6−45.0 (broad, C-2 and C-6), 48.2 (CH$_2$$CH_2NCH_2CH_2$), 49.2 (CH$_2$$CH_2NCH_2CH_2^{mr}$), 50.9 ($CHCH_3$), 52.8 ($OCH_3$), 53.3 (C-3), 58.2 (C-3^{mr}). Anal. ($C_{20}H_{27}Cl_2N_3O_3$) C, H, N.

(+)-Methyl (*S*)-4-[2-(3,4-Dichlorophenyl)acetyl]-3-[(*S*)-1-(pyrrolidin-1-yl)ethyl]piperazine-1-carboxylate [(*S,S*)-14]. As described for the preparation of (*R,R*)-**14**, the BOC group of (*S,S*)-**11** (157 mg, 0.33 mmol) was cleaved with trifluoroacetic acid (0.6 mL, 7.84 mmol) in CH_2Cl_2 (5 mL) and the resulting secondary amine (*S,S*)-**12** was acylated with methyl chloroformate (31 μL, 0.40 mmol) and Et_3N (0.11 mL, 0.80 mmol) in CH_2Cl_2 (5 mL): colorless oil, yield 95 mg (67%); $[\alpha]^{21}_{589} = +6.3$ ($c = 1.08$, CH_2Cl_2). Anal. ($C_{20}H_{27}Cl_2N_3O_3$) C; H, N calcd, H 6.35, N 9.81; found, H 5.86, N 9.34.

(+)-1-[(2*R*)-1-Benzyl-4-butylpiperazin-2-yl]ethan-1-one [(*R*)-18]. As described for (*R*)-**9** the alcohol (*R,R*)-**16**[13] (1.41 g, 5.11 mmol) was oxidized with NMMO (1.19 g, 10.22 mmol), pulverized 4 Å molecular sieves (6 g), and TPAP (240 mg, 0.68 mmol) in CH_2Cl_2 (150 mL) and purified by fc (4 cm, petroleum ether/ethyl acetate 1:1, 30 mL, $R_f = 0.56$): pale yellow oil, yield 987 mg (71%); $[\alpha]^{25}_{589} = +49.3$ ($c = 1.54$, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) $\delta = 0.82$ (t, $J = 7.3$ Hz, 3 H, CH$_2$CH$_2$CH$_2$CH_3), 1.22 (sext, $J = 7.2$ Hz, 2 H, CH$_2$CH$_2$CH_2CH$_3$), 1.30−1.43 (m, 2 H, CH$_2$CH_2CH$_2$CH$_3$), 2.07−2.29 (m, 3 H, 3-H, 5-H and 6-H), 2.17 (s, 3 H, $COCH_3$), 2.25 (t, $J = 7.5$ Hz, 2 H, CH_2CH$_2$CH$_2$CH$_3$), 2.54−2.63 (m, 1 H, 5-H), 2.67−2.75 (m, 1 H, 3-H), 2.76−2.83 (m, 1 H, 6-H), 3.10 (dd, $J = 9.4/3.3$ Hz, 1 H, 2-H), 3.19 (d, $J = 13.5$ Hz, 1 H, CH_2Ph), 3.66 (d, $J = 13.5$ Hz, 1 H, CH_2Ph), 7.12−7.22 (m, 1 H, arom), 7.22−7.28 (m, 4 H, arom). Anal. ($C_{17}H_{26}N_2O$) C, H, N.

(−)-1-[(2*S*)-1-Benzyl-4-butylpiperazin-2-yl]ethan-1-one [(*S*)-18]. As described for (*R*)-**18**, the alcohol (*S,S*)-**16**[21] (2.16 g, 7.83 mmol) was oxidized with NMMO (1.83 g, 15.7 mmol), pulverized 4 Å molecular sieves (9 g), and TPAP (368 mg, 1.05 mmol) in CH_2Cl_2 (150 mL): pale yellow oil, yield 1.69 g (79%); $[\alpha]^{21}_{589} = -35.5$ ($c = 0.45$, CH_2Cl_2). Anal. ($C_{17}H_{26}N_2O$) C, H, N.

(+)-1-[(2*R*)-1-Benzyl-4-phenylpiperazin-2-yl]ethan-1-one [(*R*)-19]. As described for (*R*)-**9**, the alcohol (*R,R*)-**17**[13] (1.60 g, 5.40 mmol) was oxidized with NMMO (1.27 g, 10.84

mmol), pulverized 4 Å molecular sieves (6 g), and TPAP (250 mg, 0.73 mmol) in CH_2Cl_2 (200 mL) and purified by fc (5 cm, petroleum ether/ethyl acetate 3:1, 35 mL, R_f = 0.46): pale yellow oil, yield 1.19 g (75%); $[\alpha]^{20}_{589}$ = +51.8 (c = 1.18, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ = 2.23 (s, 3 H, CH_3), 2.24−2.34 (m, 1 H, 5-H), 2.84−3.04 (m, 3 H, 3-H, 5-H, 6-H), 3.20 (dd, J = 9.2/3.4 Hz, 1 H, 2-H), 3.23−3.32 (m, 1 H, 6-H), 3.27 (d, J = 13.4 Hz, 1 H, CH_2Ph), 3.43 (ddd, J = 11.7/3.2/1.6 Hz, 1 H, 3-H), 3.73 (d, J = 13.6 Hz, 1 H, CH_2Ph), 6.76−6.87 (m, 3 H, arom), 7.14−7.32 (m, 7 H, arom). Anal. ($C_{19}H_{22}N_2O$) C, H, N.

(−)-1-[(2*S*)-1-Benzyl-4-phenylpiperazin-2-yl]ethan-1-one [(*S*)-19]. As described for (*R*)-**19**, the alcohol (*S*,*S*)-**17**[21] (652 mg, 2.20 mmol) was oxidized with NMMO (600 mg, 5.13 mmol), pulverized 4 Å molecular sieves (2.4 g), and TPAP (100 mg, 0.28 mmol) in CH_2Cl_2 (50 mL): pale yellow oil, yield 534 mg (82.6%) 0.1.69 g (79%); $[\alpha]^{25}_{589}$ = −60.8 (c = 1.25, CH_2Cl_2). Anal. ($C_{19}H_{22}N_2O$) C, H, N.

(*R*)-(+)-1-Benzyl-4-butyl-2-[(*S*)-1-(pyrrolidin-1-yl)ethyl]-piperazine [(*R*,*S*)-20] and (*R*)-(+)-1-Benzyl-4-butyl-2-[(*R*)-1-(pyrrolidin-1-yl)ethyl]piperazine [(*R*,*R*)-20]. To a cooled solution (0 °C) of (*R*)-**18** (706 mg, 2.57 mmol) in CH_2Cl_2 (2 mL) were successively added pyrrolidine (0.43 mL, 5.1 mmol) and Ti(OiPr)$_4$ (1.1 mL, 3.7 mmol), and the reaction mixture was stirred for 90 min at room temperature. Then, methanol (5 mL), 3 Å molecular sieves, and $NaBH_3CN$ (450 mg, 7.1 mmol) were added at 0 °C, and the reaction mixture was stirred at room temperature. After 40 h water (2 mL) and ethyl acetate (25 mL) were added, the suspension was filtered, and the filtrate was washed with saturated solutions of Na_2CO_3 (2 × 25 mL) and NaCl (50 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue (1.01 g) was purified by fc (4 cm, petroleum ether/ethyl acetate/ethyldimethylamine 75:25:0.5; 30 mL).

(*R*,*S*)-20 (R_f = 0.55): pale yellow oil, yield 590 mg (70%); $[\alpha]^{20}_{589}$ = +20.1 (c = 1.56, CH_2Cl_2); $C_{21}H_{35}N_3$ (329.5); ^{1}H NMR ($CDCl_3$) δ = 0.84 (t, J = 7.2 Hz, 3 H, $CH_2CH_2CH_2CH_3$), 1.17 (d, J = 6.7 Hz, 3 H, CHCH_3), 1.24 (sext, J = 7.1 Hz, 2 H, $CH_2CH_2CH_2CH_3$), 1.41 (quint, J = 7.5 Hz, 2 H, $CH_2CH_2CH_2CH_3$), 1.60−1.67 (m, 4 H, CH_2CH$_2$NCH$_2$CH_2), 1.89 (td, J = 10.9/2.3 Hz, 1 H, 3-H or 5-H or 6-H), 1.95−2.06 (m, 1 H, 3-H or 5-H or 6-H), 2.07 (dd, J = 11.4/2.6 Hz, 1 H, 3-H or 5-H or 6-H), 2.15−2.34 (m, 3 H, CHCH$_3$ and CH_2CH$_2$CH$_2$CH$_3$), 2.39−2.51 (m, 2 H, $CH_2CH_2NCH_2CH_2$), 2.52−2.62 (m, 2 H, $CH_2CH_2NCH_2CH_2$), 2.52−2.72 (m, 4 H, 2-H, 3-H, 5-H and 6-H), 2.90 (d, J = 14.0 Hz, 1 H, CH_2Ph), 4.94 (d, J = 14.2 Hz, 1 H, CH_2Ph), 7.09−7.17 (m, 1 H, arom), 7.18−7.31 (m, 4 H, arom).

(*R*,*R*)-20 (R_f = 0.29): pale yellow oil, yield 100 mg (15%); $[\alpha]^{20}_{589}$ = +30.0 (c = 1.01, CH_2Cl_2); $C_{21}H_{35}N_3$ (329.5); ^{1}H NMR ($CDCl_3$) δ = 0.83 (t, J = 7.2 Hz, 3 H, $CH_2CH_2CH_2CH_3$), 1.16 (d, J = 6.1 Hz, 3 H, CHCH_3), 1.22 (sext, J = 7.3 Hz, 2 H, $CH_2CH_2CH_2CH_3$), 1.32−1.49 (m, 2 H, $CH_2CH_2CH_2CH_3$), 1.63−1.75 (m, 4 H, CH_2CH$_2$NCH$_2$CH_2), 1.83−1.98 (m, 2 H, 3-H and 5-H), 2.08 (td, J = 11.3/2.4 Hz, 1 H, 6-H), 2.14−2.37 (m, 2 H, CH_2-CH$_2$CH$_2$CH$_3$), 2.38−2.53 (m, 2 H, $CH_2CH_2NCH_2CH_2$), 2.48−2.69 (m, 4 H, CHCH$_3$, 2-H, 5-H, 6-H), 2.85 (d, J = 13.1 Hz, 1 H, CH_2Ph), 2.97 (d broad, J = 11.3 Hz, 1 H, 3-H), 4.11 (d, J = 13.1 Hz, 1 H, CH_2Ph), 7.12−7.28 (m, 5 H, arom).

(*S*)-(−)-1-Benzyl-4-butyl-2-[(*R*)-1-(pyrrolidin-1-yl)ethyl]-piperazine [(*S*,*R*)-20] and (*S*)-(−)-1-Benzyl-4-butyl-2-[(*S*)-1-(pyrrolidin-1-yl)ethyl]piperazine [(*S*,*S*)-20]. As described for the preparation of (*R*,*S*)-**20** and (*R*,*R*)-**20**, the ketone (*S*)-**18** (1.01 g, 3.70 mmol) was reductively aminated with pyrrolidine (0.6 mL, 7.39 mmol), Ti(OiPr)$_4$ (1.6 mL, 5.55 mmol), CH_2Cl_2 (5 mL), methanol (20 mL), and $NaBH_3CN$ (675 mg, 10.71 mmol).

(*S*,*R*)-20: pale yellow oil, yield 895 mg (74%); $[\alpha]^{20}_{589}$ = −23.0 (c = 1.01, CH_2Cl_2).

(*S*,*S*)-20: pale yellow oil, yield 123 mg (10%); $[\alpha]^{20}_{589}$ = −35.3 (c = 0.49, CH_2Cl_2).

(*R*)-(+)-1-Benzyl-4-phenyl-2-[(*S*)-1-(pyrrolidin-1-yl)ethyl]piperazine [(*R*,*S*)-21] and (*R*)-(+)-1-Benzyl-4-phenyl-2-[(*R*)-1-(pyrrolidin-1-yl)ethyl]piperazine [(*R*,*R*)-21]. As described for the preparation of (*R*,*S*)-**20** and (*R*,*R*)-**20**, the ketone (*R*)-**19** (1.04 mg, 3.55 mmol) was reductively aminated with pyrrolidine (0.6 mL, 7.1 mmol), Ti(OiPr)$_4$ (1.6 mL, 5.3 mmol), CH_2Cl_2 (5 mL), methanol (10 mL), and $NaBH_3CN$ (600 mg, 9.52 mmol). The crude product (1.35 g) was purified by fc (5 cm, CH_2Cl_2/ethyl acetate/methanol 6:1:0.5, 25 mL).

(*R*,*S*)-21 (R_f = 0.35): pale yellow oil, yield 1.03 g (83%); $[\alpha]^{25}_{589}$ = +36.1 (c = 1.56, CH_2Cl_2); $C_{23}H_{31}N_3$ (349.5); ^{1}H NMR ($CDCl_3$) δ = 1.17 (d, J = 6.6 Hz, 3 H, CHCH_3), 1.63 (br s, 4 H, CH_2CH$_2$NCH$_2$CH_2), 2.22 (ddd, J = 11.9/10.1/3.1 Hz, 1 H, 5-H), 2.39−2.49 (m, 2 H, $CH_2CH_2NCH_2CH_2$), 2.50−2.61 (m, 3 H, $CH_2CH_2NCH_2CH_2$ and CHCH$_3$), 2.65−2.73 (m, 2 H, 6-H and 2-H), 2.82 (ddd, J = 12.1/4.2/3.3 Hz, 1 H, 5-H), 2.89 (dd, J = 11.7/9.2 Hz, 1 H, 3-H), 3.09 (d, J = 14.0 Hz, 1 H, CH_2Ph), 3.22 (d broad, 1 H, 6-H), 3.30 (d broad, 1 H, 3-H), 4.80 (d, J = 14.0 Hz, 1 H, CH_2Ph), 6.74 (t, J = 7.3 Hz, 1 H, arom), 6.85 (d, J = 8.2 Hz, 2 H, arom), 7.10−7.34 (m, 7 H, arom).

(*R*,*R*)-21 (R_f = 0.15): Pale yellow oil, yield 21 mg (1.7%); $[\alpha]^{25}_{589}$ = +36.1 (c = 1.56, CH_2Cl_2); $C_{23}H_{31}N_3$ (349.5); ^{1}H NMR ($CDCl_3$) δ = 1.19 (d, J = 6.4 Hz, 3 H, CHCH_3), 1.71 (s broad, 4 H, CH_2CH$_2$NCH$_2$CH_2), 2.16 (td, J = 11.5/3.0 Hz, 1 H, 5-H), 2.40−2.60 (m, 4 H, $CH_2CH_2NCH_2CH_2$), 2.58−2.72 (m, 3 H, 2-H, 3-H and CHCH$_3$), 2.67 (dd, J = 11.5/3.1 Hz, 1 H, 5-H), 2.78 (dt, J = 11.3/2.6 Hz, 1 H, 6-H), 2.89 (d, J = 12.9 Hz, 1 H, CH_2Ph), 3.32 (dd, J = 11.6/2.3 Hz, 1 H, 6-H), 3.74 (dd, J = 16.4/9.1 Hz, 1 H, 3-H), 4.18 (d, J = 13.2 Hz, 1 H, CH_2Ph), 6.73 (tt, J = 7.0/1.0 Hz, 1 H, arom), 6.85 (dd, J = 8.8/1.0 Hz, 2 H, arom), 7.16 (dd, J = 8.7/7.4 Hz, 2 H, arom), 7.18−7.32 (m, 5 H, arom).

(*S*)-(−)-1-Benzyl-4-phenyl-2-[(*R*)-1-(pyrrolidin-1-yl)ethyl]piperazine [(*S*,*R*)-21] and (*S*)-(−)-1-Benzyl-4-phenyl-2-[(*S*)-1-(pyrrolidin-1-yl)ethyl]piperazine [(*S*,*S*)-21]. As described for the preparation of (*R*,*S*)-**21** and (*R*,*R*)-**21**, the ketone (*R*)-**18** (0.95 g, 3.23 mmol) was reductively aminated with pyrrolidine (0.4 mL, 4.84 mmol), Ti(OiPr)$_4$ (1.9 mL, 6.46 mmol), CH_2Cl_2 (4 mL), methanol (10 mL), and $NaBH_3CN$ (514 mg, 8.16 mmol).

(*S*,*R*)-21: pale yellow oil, yield 740 mg (66%); $[\alpha]^{20}_{589}$ = −30.0 (c = 1.56, CH_2Cl_2).

(*S*,*S*)-21: pale yellow oil, yield 18 mg (1.6%).

(+)-1-{(*R*)-4-Butyl-2-[(*S*)-1-(pyrrolidin-1-yl)ethyl]piperazin-1-yl}-2-(3,4-dichlorophenyl)ethan-1-one [(*R*,*S*)-22]. As described for the preparation of (*R*,*S*)-**11**, the piperazine (*R*,*S*)-**20** (340 mg, 1.03 mmol) was hydrogenated with the catalyst Pd/C (10%, 370 mg) in methanol (20 mL) for 9 h, and the resulting secondary amine (227 mg) was acylated with (3,4-dichlorophenyl)acetic acid (222 mg, 1.09 mmol) and CDI (209 mg, 1.29 mmol) in CH_2Cl_2 (14 mL). The residue (577 mg) obtained after work up was purified by fc (3 cm, CH_2Cl_2/ethyl acetate/methanol 8:1:0.5, 15 mL, R_f = 0.42): pale yellow oil, yield 324 mg (74 %); $[\alpha]^{23}_{589}$ = +34.8 (c = 1.43, CH_2Cl_2); MS (CI) m/z (%) = 431/429/427 (M + H, 0.6/3.5/6.2), 430/428/426 (M, 3/14/23), 98 ($C_6H_{12}N$, 100); IR (film) $\tilde{\nu}$ (cm^{-1}) = 2959, 2802 (s, ν_{CH}), 1640 (s, $\nu_{C=O}$), 1440 (br s), 1147 (s), 1030 (m, ν_{CCl}); ^{1}H NMR (d_5-nitrobenzene, 80 °C) δ = 0.85 (d, J = 6.1 Hz, 3 H, CHCH_3), 0.85 (t, J = 6.8 Hz, 3 H, $CH_2CH_2CH_2CH_3$), 1.21−1.41 (m, 4 H, $CH_2CH_2CH_2CH_3$), 1.61−1.71 (m, 4 H, CH_2CH$_2$-NCH$_2$CH_2), 1.73 (dd, J = 10.9/3.2 Hz, 1 H, 3-H), 1.93 (td, J = 11.5/2.7 Hz, 1 H, 5-H), 2.10−2.18 (m, 2 H, CH_2CH$_2$CH$_2$CH$_3$), 2.53−2.70 (m, 4 H, $CH_2CH_2NCH_2CH_2$), 2.72 (d, J = 11.0 Hz, 1 H, 5-H), 3.03−3.30 (m, 1 H, 6-H), 3.41 (d, J = 10.8 Hz, 1 H, 3-H), 3.48−3.74 (m, 2 H, CHCH$_3$ and 6-H), 3.80 (s broad, 2 H, COCH$_2$), 4.39−4.85 (m, 1 H, 2-H), 7.23 (dd, J = 8.2/2.0 Hz, 1 H, arom), 7.33 (d, J = 8.2 Hz, 1 H, arom), 7.45 (d, J = 1.6 Hz, 1 H, arom); ^{13}C NMR (d_5-nitrobenzene, 80 °C) δ = 7.0 (1 C, CHCH_3), 13.4 (1 C, $CH_2CH_2CH_2CH_3$), 20.2 (1 C, $CH_2CH_2CH_2$-CH$_3$), 23.9 (2 C, CH_2CH$_2$NCH$_2$CH_2), 28.9 (1 C, $CH_2CH_2CH_2$-CH$_3$), 39.2 (1 C, COCH_2), 47.2 (2 C, $CH_2CH_2NCH_2CH_2$), 51.8 (1 C, CHCH$_3$), 52.7 (1 C, C-3), 53.6 (1 C, C-5), 57.5 (1 C, CH_2-CH$_2$CH$_2$CH$_3$), 129.2 (1 C, arom CH), 130.1 (1 C, arom CH), 130.2 (1 C, arom CCl), 131.4 (1 C, arom CH), 131.9 (1 C, arom CCl), 137.0 (1 C, arom C), 168.3 (1 C, C=O); ^{1}H NMR (d_5-nitrobenzene, 27 °C) δ = 1.66 (d broad, J = 10.8 Hz, 3-H), 1.80−1.99 (m, 5-H), 2.00−2.27 (m, CH_2CH$_2$CH$_2$CH$_3$), 2.43−2.63 (m, $CH_2CH_2NCH_2CH_2$), 2.62−2.73 (m, 6-H^{mr}), 2.69 (t, J = 9.5 Hz, 5-H), 3.13 (t, J = 12.7 Hz, 6-H), 3.40 (d, J = 10.7 Hz, 3-H), 3.52 (dq, J = 10.8/6.6 Hz, CHCH$_3$), 3.59−3.73 (m,

C*H*CH_3^{mr}), 3.73−3.86 (m, 2-H^{mr} and 6-H), 3.83 (s, $COCH_2$), 3.83 (s, $COCH_2$), 3.88 (s, $COCH_2^{mr}$), 4.60 (d broad, J = 12.4 Hz, 6-H^{mr}), 4.70 (d broad, J = 10.4 Hz, 2-H); ^{13}C NMR (d_5-nitrobenzene, 27 °C) δ = 38.2 (C-6^{mr}), 39.3 ($COCH_2^{mr}$), 39.5 ($COCH_2$), 42.8 (C-6), 47.2 ($CH_2CH_2NCH_2CH_2$), 51.6 (C*H*CH_3), 52.4 (C-2), 52.5 (C-3), 54.1 (C-5), 57.2 (C-2^{mr}), 57.5 (CH_2CH_2-CH_2CH_3). Anal. ($C_{22}H_{33}Cl_2N_3O$) C, H, N.

(−)-1-{(*S*)-4-Butyl-2-[(*R*)-1-(pyrrolidin-1-yl)ethyl]piperazin-1-yl}-2-(3,4-dichlorophenyl)ethan-1-one [(*S*,*R*)-22]. As described for the preparation of (*R*,*S*)-**22**, the piperazine (*S*,*R*)-**20** (440 mg, 1.34 mmol) was hydrogenated with the catalyst Pd/C (10%, 350 mg) in methanol (20 mL) for 9 h and the resulting secondary amine was acylated with (3,4-dichlorophenyl)acetic acid (310 mg, 1.51 mmol) and CDI (330 mg, 2.04 mmol) in CH_2Cl_2 (15 mL): pale yellow oil, yield 355 mg (62 %); $[\alpha]^{18}_{589}$ = −27.2 (c = 1.03, CH_2Cl_2). Anal. ($C_{22}H_{33}$-Cl_2N_3O) H, N; C calcd, 62.0; found, 61.5.

(+)-2-(3,4-Dichlorophenyl)-1-{(*R*)-4-phenyl-2-[(*S*)-1-(pyrrolidin-1-yl)ethyl]piperazin-1-yl}ethan-1-one [(*R*,*S*)-23]. As described for the preparation of (*R*,*S*)-**11**, the piperazine (*R*,*S*)-**21** (372 mg, 1.07 mmol) was hydrogenated with the catalyst Pd/C (10%, 400 mg) in methanol (20 mL) for 16 h, and the resulting secondary amine (235 mg) was acylated with (3,4-dichlorophenyl)acetic acid (262 mg, 1.28 mmol) and CDI (243 mg, 1.50 mmol) in CH_2Cl_2 (25 mL). The residue (551 mg) obtained after work up was purified by fc (3 cm, CH_2Cl_2/ethyl acetate/methanol 6:1:0.25, 15 mL, R_f = 0.39): pale yellow oil, yield 312 mg (66%); $[\alpha]^{18}_{589}$ = +3.4 (c = 0.96, CH_2Cl_2); Anal. ($C_{24}H_{29}Cl_2N_3O$) N; C, H calcd, C 64.6, H 6.55; found, C 64.1, H 6.02. MS (CI) m/z (%) = 451/449/447 (M + H, 2.5/16/30), 450/448/446 (M, 11/61/100), 99 ($C_6H_{13}N$, 39); IR (film) $\tilde{\nu}$ (cm^{-1}) = 2964, 2811 (m, ν_{CH}), 1642 (s, $\nu_{C=O}$), 1434 (m), 1148 (m), 1034 (m, ν_{CCl}); ^{1}H NMR (d_5-nitrobenzene, 80 °C) δ = 0.94 (d, J = 6.6 Hz, 3 H, CHC*H*$_3$), 1.65−1.77 (m, 4 H, C*H*$_2$CH$_2$NCH$_2$C*H*$_2$), 2.55−2.71 (m, 4 H, CH$_2$C*H*$_2$NC*H*$_2$CH$_2$), 2.69 (dd, J = 12.0/3.6 Hz, 1 H, 3-H), 2.71−2.85 (m, 1 H, 5-H), 3.30−3.50 (m broad, 1 H, 6-H), 3.48−3.67 (m, 2 H, 5-H and C*H*CH$_3$), 3.82−4.04 (m broad, 1 H, 6-H), 3.89−3.94 (m, 2 H, $COCH_2$), 4.34 (dd, J = 12.0/1.8 Hz, 1 H, 3-H), 4.60−5.03 (m broad, 1 H, 2-H), 6.83 (t broad, J = 7.3 Hz, 1 H, arom), 6.94 (d broad, J = 8.1 Hz, 2 H, arom), 7.23 (t broad, J = 7.9 Hz, 2 H, arom), 7.29 (dd, J = 8.2/2.0 Hz, 1 H, arom), 7.37 (d, J = 8.1 Hz, 1 H, arom), 7.51(d, J = 2.0 Hz, 1 H, arom); ^{13}C NMR (d_5-nitrobenzene, 80 °C) δ = 6.5 (1 C, CH*C*H$_3$), 23.8 (2 C, *C*H$_2$CH$_2$NCH$_2$*C*H$_2$), 39.0 (1 C, CO*C*H$_2$), 47.0 (2 C, CH$_2$*C*H$_2$N*C*H$_2$CH$_2$), 47.8 (broad, 1 C, C-5), 50.3 (broad, 1 C, C-3), 51.3 (1 C, *C*HCH$_3$), 115.8 (2 C, arom CH), 119.0 (1 C, arom CH), 128.8 (2 C, arom CH), 129.0 (1 C, arom CH), 129.9 (1 C, arom CH), 130.1 (1 C, arom CCl), 131.2 (1 C, arom CH), 131.7 (1 C, arom CCl), 136.6 (1 C, arom C), 151.5 (1 C, arom C), 168.3 (1 C, C=O); ^{1}H NMR (d_5-nitrobenzene, 27 °C) δ = 2.68 (dd, J = 12.0/3.2 Hz, 3-H), 2.79 (td, J = 11.8/3.0 Hz, 5-H), 2.90 (dd, J = 12.4/3.2 Hz, 6-H^{mr}), 3.36 (dd, J = 12.9/3.2 Hz, 6-H), 3.43−3.59 (m, C*H*CH$_3$ and 5-H), 3.60−3.70 (m, C*H*CH$_3^{mr}$), 3.83−4.05 (m, 2-H^{mr} and 6-H), 3.92 (s, $COCH_2$), 4.35 (d, J = 11.9 Hz, 3-H), 4.83 (d broad, J = 13.3 Hz, 6-H^{mr}), 4.90 (d broad, J = 11.0 Hz, 2-H); ^{13}C NMR (d_5-nitrobenzene, 27 °C) δ = 37.8 (C-6^{mr}), 39.4 ($COCH_2^{mr}$), 39.6 ($COCH_2$), 42.4 (C-6), 47.4 ($CH_2CH_2NCH_2CH_2$), 47.8 (CH_2CH_2-$NCH_2CH_2^{mr}$), 48.3 (C-5), 50.7 (C-3), 51.3 (C*H*CH$_3^{mr}$), 51.4 (C*H*CH$_3$), 52.7 (C-2), 57.6 (C-2^{mr}).

(−)-2-(3,4-Dichlorophenyl)-1-{(*S*)-4-phenyl-2-[(*R*)-1-(pyrrolidin-1-yl)ethyl]piperazin-1-yl}ethan-1-one [(*S*,*R*)-23]. As described for the preparation of (*R*,*S*)-**23**, the piperazine (*S*,*R*)-**21** (362 mg, 1.04 mmol) was hydrogenated with the catalyst Pd/C (10%, 150 mg) in methanol (10 mL), and the resulting secondary amine was acylated with (3,4-dichlorophenyl)acetic acid (233 mg, 1.14 mmol) and CDI (201 mg, 1.24 mmol) in CH_2Cl_2 (15 mL): pale yellow oil, yield 306 mg (66%); $[\alpha]^{24}_{589}$ = −4.4 (c = 0.50, CH_2Cl_2). Anal. ($C_{24}H_{29}Cl_2N_3O$) H; C, N calcd, C 64.6, N 9.41; found, C 64.1, N 8.73.

Receptor Binding Studies. General. The following equipment was used in the studies: homogenizer, Potter S (B. Braun Biotech International); Ultraturrax, Euroturrax T20 (Ika Labortechnik); centrifuge, high-speed refrigerating centrifuge model J2-HS (Beckman); filter, Whatman glass-fiber filters GF/B, presoaked in the medium described below before use; filtration was performed with a Brandel 24-well cell harvester; scintillation cocktail, Ultima Gold (Canberra Packard); liquid scintillation analyzer, TriCarb 2100 TR (Canberra Packard), counting efficiency 66%. All experiments were carried out in triplicate. IC_{50} values were determined from competition experiments with at least six concentrations of test compounds and were calculated with the program GraphPad Prism 3.0 (GraphPad Software) by nonlinear regression analysis. K_i values were calculated according to Cheng and Prusoff.[42] K_D values for the radioligands were taken from the literature. For compounds with high affinity (low K_i values) mean values ± SEM from three independent experiments are given.

Investigation of κ-Receptor Affinity.[23−26] [^{3}H]U-69,593 binding to guinea pig brain membrane preparations was determined according to standard radioligand binding assays,[23−25] which were slightly modified as described below.

Preparation of the Receptor Material. The cerebellum was removed from guinea pig brains (Dunkin Hartley, Harlan), and the brains were bisected. Three brain halves were homogenized in 50 mL of buffer (50 mM Tris HCl pH 7.4) with a homogenizer (800 rpm, 10 up-and-down strokes). The suspension was centrifuged at 49000 g for 10 min at 4 °C. The supernatant was removed and the pellet was resuspended in buffer (30 mL) with an Ultraturrax (8000 rpm). Subsequently, it was centrifuged at 49000*g* for 10 min at 4 °C. The pellet was resuspended in buffer, incubated for 45 min at 37 °C, and centrifuged (49000*g*, 10 min, 4 °C). Again the pellet was resuspended and centrifuged. Then, the pellet was resuspended in buffer (30 mL), the protein concentration was determined according to the method of Bradford[43] using bovine serum albumin as standard, and subsequently, the preparation was frozen (−83 °C) in 5 mL portions of about 3 mg protein/mL.

Performance. The test was performed with the radioligand [^{3}H]U-69,593 (1468.9 GBq/mmol; NEN Life Science Products). The thawed membrane preparation (about 900 μg of the protein) was incubated with various concentrations of test compounds, 1 nM [^{3}H]U-69,593, 5 mM $MgCl_2$, and buffer (50 mM Tris HCl, pH 7.5) in a total volume of 500 μL at 25 °C for 90 min. The incubation was terminated by rapid filtration through presoaked Whatman GF/B filters (0.25% polyethylenimine in 50 mM Tris HCl, pH 7.4 for 2 h at 4 °C) using a cell harvester. After washing four times with 2 mL of cold buffer, 3 mL of scintillation cocktail was added to the filters. After at least 8 h, bound radioactivity trapped on the filters was counted in a liquid scintillation analyzer. Nonspecific binding was determined with 1 μM U-50,488.

Investigation of the μ-Receptor-Affinity.[23−26] [^{3}H]-DAMGO binding to guinea pig brain membrane preparations was determined according to standard radioligand binding assays,[23−25] which were slightly modified as described below.

Preparation of the receptor material was as described under Investigation of κ-Receptor Affinity.

Performance. The test was performed with the radioligand [^{3}H]DAMGO (2016.5 GBq/mmol; NEN Life Science Products). The thawed membrane preparation (about 400 μg of the protein) was incubated with various concentrations of test compounds, 1 nM [^{3}H]DAMGO, 5 mM $MgCl_2$, 100 μM PMSF (phenylmethanesulfonyl fluoride), and buffer (50 mM Tris HCl, pH 7.4) in a total volume of 500 μL at 25 °C for 90 min. The incubation was terminated by rapid filtration through presoaked Whatman GF/B filters (50 mM Tris HCl, pH 7.4 for 2 h at 4 °C) using a cell harvester. After washing four times with 2 mL of cold buffer, 3 mL of scintillation cocktail was added to the filters. After at least 8 h, bound radioactivity trapped on the filters was counted in a liquid scintillation analyzer. Nonspecific binding was determined with 1 μM naloxone.

Investigation of the σ_1-Receptor Affinity.[27] [^{3}H]-(+)-Pentazocine binding to guinea pig brain membrane preparations was determined according to standard radioligand binding assays,[27] which were slightly modified as described below.

Preparation of the Receptor Material. Thawed guinea pig brains (Dunkin Hartley, Harlan) were homogenized (ultraturrax, 8000 rpm) in 10 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 900*g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 22000*g* for 20 min at 4 °C. The pellet was resuspended in 10 volumes of buffer (50 mM Tris HCl, pH 7.4) with an Ultraturrax (8000 rpm), incubated for 30 min at 25 °C, and centrifuged at 22000*g* (2 min, 4 °C). The pellet was resuspended in buffer, the protein concentration was determined according to the method of Bradford[43] using bovine serum albumin as standard, and subsequently, the preparation was frozen (−83 °C) in 5 mL portions of about 2 mg protein/mL.

Performance. The test was determined with the radioligand [^{3}H]-(+)-pentazocine (1036 GBq/mmol; NEN Life Science Products). The thawed membrane preparation (about 150 Fg of the protein) was incubated with various concentrations of test compounds, 3 nM [^{3}H]-(+)-pentazocine, and buffer (50 mM Tris HCl, pH 7.4) in a total volume of 500 μL for 150 min at 37 °C. The incubation was terminated by rapid filtration through presoaked Whatman GF/B filters (0.5% polyethylenimine in water for 2 h at 4 °C) using a cell harvester. After washing four times with 2 mL of cold buffer, 3 mL of scintillation cocktail was added to the filters. After at least 8 h, bound radioactivity trapped on the filters was counted in a liquid scintillation analyzer. Nonspecific binding was determined with 10 μM haloperidol.

Investigation of the Affinity for the Phencyclidine Binding Site of the NMDA Receptor.[29] [^{3}H]-(+)-MK-801 binding to pig cortex membrane preparations was determined according to standard radioligand binding assays,[29] which were slightly modified as described below.

Preparation of the Receptor Material. Fresh pig cortex was homogenized (500 rpm, 10 up-and-down strokes) in 10 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 10000*g* for 20 min at 4 °C. The pellet was resuspended in buffer (5 mM Tris-acetate with 1 mM EDTA, pH 7.5) with an Ultraturrax (8000 rpm) and centrifuged at 20000*g* (20 min, 4 °C). This procedure was repeated twice. The pellet was resuspended in buffer, the protein concentration was determined according to the method of Bradford[43] using bovine serum albumin as standard, and subsequently, the preparation was frozen (−83 °C) in 5 mL portions of about 1 mg protein/mL.

Performance. The test was determined with the radioligand [^{3}H]-(+)-MK-801 (832.5 GBq/mmol; NEN Life Science Products). The thawed membrane preparation (about 100 μg of the protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-MK-801, and buffer (5 mM Tris-acetate, 1 mM EDTA, pH 7.5) in a total volume of 500 μL for 90 min at 25 °C. The incubation was terminated by rapid filtration through presoaked Whatman GF/C filters (1% polyethylenimine in water for 3 h at 4 °C) using a cell harvester. After washing four times with 2 mL of cold buffer, 3 mL of scintillation cocktail was added to the filters. After at least 8 h, bound radioactivity trapped on the filters was counted in a liquid scintillation analyzer. Nonspecific binding was determined with 10 μM (+)-MK-801.

Electrically Evoked Release of Acetylcholine in Rabbit Hippocampal Slices. Slices (350 μM thick) of the rabbit hippocampus were prepared as described previously.[34] They were transferred to small Petri dishes containing 2 mL each of modified Krebs-Henseleit (KH) buffer with [^{3}H]choline (0.1 μM; Amersham-Pharmacia, Freiburg, Germany) and incubated for 30 min at 37 °C under carbogen (95% O_2, 5% CO_2). The KH buffer had the following composition (in mM): NaCl, 118; KCl, 4.8; $CaCl_2$, 1.3; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose, 11; ascorbic acid, 0.57; Na_2EDTA, 0.03; saturated with carbogen; pH adjusted to 7.4. After incubation, the slices were transferred to superfusion chambers and superfused with KH buffer (37 °C, gassed with carbogen) at a rate of 0.6 mL/min. The superfusion medium contained hemicholinium-3 (10 μM; Sigma-Aldrich, München, Germany) and, when indicated, norbinaltorphimine dihydrochloride (0.1 μM; Research Biochemicals International, Köln, Germany) throughout superfusion. Collection of 4-min fractions started after 49 min. Release of [^{3}H]acetylcholine was induced by electrical field stimulation (360 rectangular pulses, 3 Hz, 2 ms, 48−52 mA) after 57 (S_1) and 89 min (S_2) of superfusion. Drugs to be tested (i.e. the opioid receptor agonists) were added to the superfusion medium from 16 min before S_2 onward. At the end of the experiment (after 105 min of superfusion), the radioactivity of superfusate samples and slices (dissolved in 500 μL Soluene 350; Packard, Frankfurt, Germany) was determined by liquid scintillation counting. *The fractional rate of tritium outflow* (in percent of tissue tritium) was calculated as (picomoles of tritium outflow per 4 min) × 100/4 × (picomoles of tritium in the slices at the start of the respective 4-min period). *The stimulation-evoked overflow of tritium* was calculated by subtraction of the basal outflow; the latter was assumed to decline linearly from the 4 min *before* to the 4-min period, 12−16 min *after* the onset of the stimulation. The evoked overflow was expressed as percent of the tritium content of the slices at the onset of the respective stimulation period. Effects of drugs added before S_2 are expressed as the ratio of the overflow evoked by the corresponding stimulation periods (S_2/S_1) and shown as the percent of the appropriate control ratios (no drug addition before S_2). None of the drugs studied affected the basal outflow of [^{3}H]. Significance of differences was tested using ANOVA followed by Bonferroni's test. All data are shown as means ± SEM; n = total number slices in at least three independent superfusion experiments.

Acknowledgment. We wish to thank the Deutsche Forschungsgemeinsrchaft and the Fonds der Chemischen Industrie for financial support. Thanks are also due to the Degussa AG, Janssen Cilag GmbH,Upjohn Pharmacia, and GlaxoSmithKline for donation of chemicals and reference compounds.

Supporting Information Available: Spectral data for (*R*)-**9**, (*R*,*S*)-**10**, (*R*,*R*)-**10**, (*R*,*S*)-**11**, (*R*,*R*)-**11**, (*R*)-**18**, (*R*)-**19**, (*R*,*S*)-**20**, (*R*,*R*)-**20**, (*R*,*S*)-**21**, and (*R*,*R*)-**21**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dhawan, B. N.; Cesselin, F.; Raghubir, R.; Reisine, T.; Bradley, P. B.; Portoghese, P. S.; Hamon, M. International Union of Pharmacology XII. Classification of opioid receptors. *Pharmacol. Rev.* **1996**, *48*, 567−592

(2) Scopes, D. I. C. Recent developments in non-peptide kappa receptor agonists. *Drug Future* **1993**, *18*, 933−947.

(3) de Costa, B. R.; Bowen, W. D.; Hellewell, S. B.; George, C.; Rothman, R. B.; Reid, A. A.; Walker, J. M.; Jacobson, A. E.; Rice, K. C. Alterations in the Stereochemistry of the κ-Selective Opioid Agonist U50,488 Result in High-Affinity σ-Ligands. *J. Med. Chem.* **1989**, *32*, 1996−2002.

(4) Freeman, J. P.; Michalson, E. T.; D'Andrea, S. V.; Baczynskyj, L.; VonVoigtländer, P. F.; Lahti, R. A.; Smith, M. W.; Lawson, C. F.; Scahill, T. A.; Mizsak, S. A.; Szmuszkovicz, J. Naphtho and Benzo Analogues of the κ Opioid Agonist *trans*-(±)-3,4-Dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzene acetamide. *J. Med. Chem.* **1991**, *34*, 1891−1896.

(5) Vecchietti, V.; Clarke, G. D.; Colle, R.; Dondio, G.; Giardina, G.; Petrone, G.; Sbacchi, M. Substituted 1-(Aminomethyl)-2-(arylacetyl)-1,2,3,4-tetrahydroisoquinolines: A Novel Class of Very Potent Antinociceptive Agents with Varying Degrees of Selectivity for κ and μ Opioid Receptors. *J. Med. Chem.* **1992**, *35*, 2970−2978.

(6) Barlow, G. J.; Blackburn, T. P.; Costello, G. F.; James, R.; Le Count, D. J.; Main, B. G.; Pearce, R. J., Russell, K.; Shaw, J. S. Structure/Activity Studies Related to 2-(3,4-Dichlorophenyl)-*N*-methyl-N-[2-(1-pyrrolidinyl)-1-substituted-ethyl]acetamides: A Novel Series of Potent and Selective κ-Opioid Agonists. *J. Med. Chem.* **1991**, *34*, 3149−3158.

(7) Chang, A.-C.; Takemori, A. E.; Ojala, W. H.; Gleason, W. B.; Portoghese P. S. κ-Opioid Receptor Selective Affinity Labels: Electrophilic Benzeneacetamides. *J. Med. Chem.* **1994**, *37*, 4490−4498.

(8) Vecchietti, V.; Giordani, A.; Giardina, G.; Colle, R.; Clarke, G. D. (2S)-1-(Arylacetyl)-2-(aminomethyl)piperidine Derivatives: Novel, Highly Selective κ Opioid Analgesics. *J. Med. Chem.* **1991**, *34*, 397−403.

(9) Vecchietti, V.; Giordani, A. *Eur. Pat. Appl. EP-275, 696,* July 27, **1988**.
(10) Birch, P. J.; Rogers, H.; Hayes, A. G.; Hayward, N. J.; Tyers, M. B.; Scopes, D. I. C.; Naylor, A.; Judd, D. B. Neuroprotective actions of GR 89696, a highly potent and selective κ-opioid receptor agonist. *Br. J. Pharmacol.* **1991**, *103*, 1829−1823.
(11) Naylor, A.; Judd, D. B.; Lloyd, J. E.; Scopes, D. I. C.; Hayes, A. G.; Birch, P. A Potent New Class of κ-Receptor Agonist: 4-Substituted 1-(Arylacetyl)-2-[(dialkylamino)methyl]piperazines. *J. Med. Chem.* **1993**, *36*, 2075−2083.
(12) Naylor, A.; Judd, D. B.; Brown, D. S. Eur. Pat. Appl. EP-343,900, November 29, **1989**.
(13) Soukara, S.; Wünsch, B. A Facile Synthesis of Enantiomerically Pure 1-(Piperazin-2-yl)ethan-1-ol Derivatives from (2*S*,3*R*)-Threonine. *Synthesis* **1999**, 1739−1746.
(14) Ley, S. V.; Norman, J.; Griffith, W. P.; Marsden, S. P. Tetrapropylammonium Perruthenate, $Pr_4N^+ RuO_4^-$, TPAP: A Catalytic Oxidant for Organic Synthesis. *Synthesis* **1994**, 639−666.
(15) Borch, R. P.; Bernstein, M. D.; Durst, H. D. The Cyanohydridoborate Anion as a Selective Reducing Agent. *J. Am. Chem. Soc.* **1971**, *93*, 2897−2904.
(16) Mattson, R. J.; Pharm, K. M.; Leuck, D. J.; Cowen, K. A. An Improved Method for Reductive Alkylation of Amines Using Titanium(IV) Isopropoxide and Sodium Cyanoborohydride. *J. Org. Chem.* **1990**, *55*, 2552−2554.
(17) The first stereodescriptor characterizes the configuration in position 3 of the piperazine ring and the second stereodesriptor the configuration in the side chain.
(18) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 2nd ed.; John Wiley & Sons: New York, 1991, pp 364 f.
(19) Ref. 18 pp 327 f.
(20) The terms like and unlike are used as defined in the following: Eliel, E. L.; Wilen S. H. *Stereochemistry of Organic Compounds*; John Wiley & Sons: New York, 1994; pp 120 f and p 1210.
(21) The synthesis of the enantiomers of the alcohols (*S*,*S*)-**8**, (*S*,*S*)-**16**, and (*S*,*S*)-**17** is detailed in ref.[13]
(22) Röhr, C.; Soukara, S.; Wünsch, B. Synthesis and Stereoselective κ-Receptor Binding of Methylated Analogues of GR-89, 696. *Eur. J. Med. Chem.* **2001**, *36*, 211−214.
(23) Smith, J. A. M.; Hunter, J. C.; Hill, R. G.; Hughes, J. A Kinetic Analysis of κ-Opioid Agonist Binding Using the Selective Radioligand [^{3}H]-U-69, 593. *J. Neurochem.* **1989**, *53*, 27−36.
(24) Hunter, J. C.; Leighton, G. E.; Meecham, K. C.; Boyle, S. J.; Horwell, D. C.; Rees, D. C.; Hughes, J. Cl-977, a novel and selective agonist for the κ-opioid receptor. *Br. J. Pharmacol.* **1990**, *101*, 183−189.
(25) Meecham, K. G.; Boyle, S. J.; Hunter, J. C.; Hughes, J. An in vitro profile of activity for the (+) and (−) enantiomers of spiradoline and PD117302. *Eur. J. Pharmacol.* **1989**, *173*, 151−157.
(26) Wanner, K. Th.; Praschak, I.; Höfner, G.; Beer, H. Asymmetric Synthesis and Enantiospecificity of Binding of 2-(1,2,3,4-Tetrahydro-1-isoquinolinyl)-ethanol Derivatives to μ and κ Receptors. *Arch. Pharm. Phar. Med. Chem.* **1996**, *329*, 11−22.
(27) DeHaven-Hudkins, D. L.; Fleissner, L. C.; Ford-Rice, F. Y. Characterization of the binding of [^{3}H](+)-pentazocine to σ fecognition sites in guinea pig brain. *Eur. J. Pharmacol.* **1992**, *227*, 371−378.
(28) de Costa, B. R.; He, X.; Linders, J. T. M.; Dominguez, C.; Gu, Z. Q.; Williams, W.; Bowen, W. D. Synthesis and Evaluation of Conformationally Restricted N-[2-(3,4-Dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamines at σ-Receptors. 2. Piperazines, Bicyclic Amines, Bridged Bicyclic Amines, and Miscellaneous Compounds. *J. Med. Chem.* **1993**, *36*, 2311−2320.
(29) McKernan, R. M.; Castro, S.; Poat, J. A.; Wong, E. H. F. Solubilization of the *N*-Methyl-D-Aspartate Receptor Channel Complex from Rat and Porcine Brain. *J. Neurochem.* **1989**, *52*, 777−785.
(30) Illes, P. Modulation of transmitter and hormone release by multiple neuronal opioid receptors. *Rev. Physiol. Biochem. Pharmacol.* **1989**, *112*, 139−233.
(31) Illes, P.; Jackisch, R. Modulation of catecholamine release in the central nervous system by multiple opioid receptors. In *Neurobiology of opioids;* Almeida, O. F. X., Shippenberg, T. S., Eds.; Springer: New York, 1991; pp 213−225.
(32) Jackisch, R. Regulation of neurotransmitter release by opiates and opioid peptides in the central nervous system. In *Presynaptic regulation of transmitter release;* Feigenbaum, J., Hanani, M., Eds.; Freund Publishing House: Tel Aviv, 1991; pp 551−592.
(33) Mulder, A. H.; Schoffelmeer, A. N. M. Multiple opioid receptors and presynaptic modulation of neurotransmitter release in the brain. In *Handbook Exptl Pharmacol;* Herz, A., Ed.; Springer-Verlag: Berlin, 1993; 104/1, pp 125−144.
(34) Jackisch, R.; Geppert, M.; Brenner, A. S.; Illes, P. Presynaptic opioid receptors modulating acetylcholine release in the hippocampus of the rabbit. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1986**, *332*, 156−162
(35) Lapchak, P. A.; Araujo, D. M.; Collier, B. Regulation of endogenous acetylcholine release from mammalian brain slices by opiate receptors: hippocampus, striatum and cerebral cortex of guinea-pig and rat. *Neurosci.* **1989**, *31*, 313−325.
(36) Mulder, A. H.; Wardeh, G.; Hogenboom, F.; Frankhuyzen, A. L. κ- and δ-opioid receptor agonists differentially inhibit striatal dopamine and acetylcholine release. *Nature* **1984**, *308*, 278−280.
(37) Jackisch, R.; Hotz, H.; Hertting, G. No evidence for presynaptic opioid receptors on cholinergic, but presence of κ-receptors on dopaminergic neurons in the rabbit caudate nucleus. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1993**, *348*, 234−241.
(38) Feuerstein, T. J.; Gleichauf, O.; Peckys, D.; Landwehrmeyer, G. B.; Scheremet, R.; Jackisch, R. Opioid receptor-mediated control of acetylcholine release in human neocortex tissue. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1996**, *354*, 586−592.
(39) Feuerstein, T. J.; Albrecht, C.; Wessler, I.; Zentner, J.; Jackisch, R. δ_1-Opioid receptor-mediated control of acetylcholine (ACh) release in human neocortex slices. *Int. J. Devl. Neurosci.* **1998**, *16*, 795−802.
(40) Portoghese, P. S.; Lipkowski, A. W.; Takemori, A. E. Binaltorphimine and nor-binaltorphimine, potent and selective κ-opioid receptor antagonists. *Life Sci.* **1987**, *40*, 1287−1292.
(41) Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923−2925.
(42) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition of constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(43) Bradford, M., M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein−Dye Binding. *Anal. Biochem.* **1976**, *72*, 248−254.

JM0108395