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Original article

Synthesis and receptor binding studies of 3-substituted piperazine derivatives

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Abstract

In order to find novel receptor ligands various substituents were introduced into the side chain in position 3 of the piperazine **5**. During nucleophilic substitution of the hydroxy group of **5** aziridinium ions were formed, resulting in rearranged 1,4-diazepanes and piperazines as side products. 1,2-anellated piperazines **15**, **18** and **19** were prepared by hydrogenation of the α,β-unsaturated ester **13** and by condensation of the primary amine **16b** with formaldehyde, respectively. Receptor binding studies with radioligands revealed that the phenylacetamide **17b** interacts with moderate affinity (K_i = 181 nM) and considerable selectivity with σ_1 receptors.

Keywords: 3-Substituted piperazines; 1,2-Anellated piperazines; Aziridinium ions; σ Receptor ligands

1. Introduction

The piperazine ring represents the core structure of several pharmacologically active compounds. In particular piperazine derivatives bearing substituents in position 3 can strongly interact with various receptors within the central nervous system. In Fig. 1 some examples of piperazine derivatives with high receptor affinity are given.

The (pyrrolidinylalkyl) substituted piperazines **1** are among the most active and selective κ-opioid receptor agonists [1,2]. The competitive NMDA receptor antagonist **2** is derived from the lead compound (R)-2-aminoadipic acid [3]. Several 1,4-disubstituted piperazine derivatives display high affinity to σ receptors, e.g. the 1-butyl-4-(3,4-dichlorophenethyl)piperazine (**3**) binds with high affinity at σ_1 receptors (K_i = 0.55 nM) [4]. Recently we have described the synthesis and σ receptor affinity of 2-(hydroxymethyl) substituted piperazine derivatives. We found that an additional aromatic element in the N-4 residue is favorable for high σ_1 receptor affinity. As an example the phenethyl derivative **4** reveals a K_i–value of 36.8 nM [5].

In this article we report on the synthesis and receptor affinities (NMDA, κ-opioid, μ-opioid, σ_1, σ_2,) of piperazine derivatives with various substituents in position 3. Modification of the hydroxymethyl substituent of the piperazine ring is investigated and special aspects of the transformation are discussed. With respect to receptor interactions introduction of nitrogen containing substituents is of particular interest. Pharmacophoric elements, which are crucial for high σ and κ receptor affinity (compare compounds **1**, **3**, and **4** of Scheme 1), are especially considered.

2. Chemistry

Starting from the proteinogenic amino acid (S)-serine the ester **5** was prepared in seven steps including chloroacetylation of serine methyl ester, N/O-acetal formation with benzaldehyde dimethyl acetal, piperazine ring formation via an azide, and reductive cleavage of the oxazolidine ring with $LiAlH_4$ [5]. In order to introduce further substituents in the side chain the OH-group of **5** was reacted with methanesulfonyl chloride and triethylamine to obtain the sulfonate **6**, an activated intermediate for following nucleophilic substitutions. After stirring the reaction mixture for 15 min at 0 °C thin layer chromatography (TLC) showed complete transformation. However, after work up with water only the chloride **7** could be isolated. In TLC the

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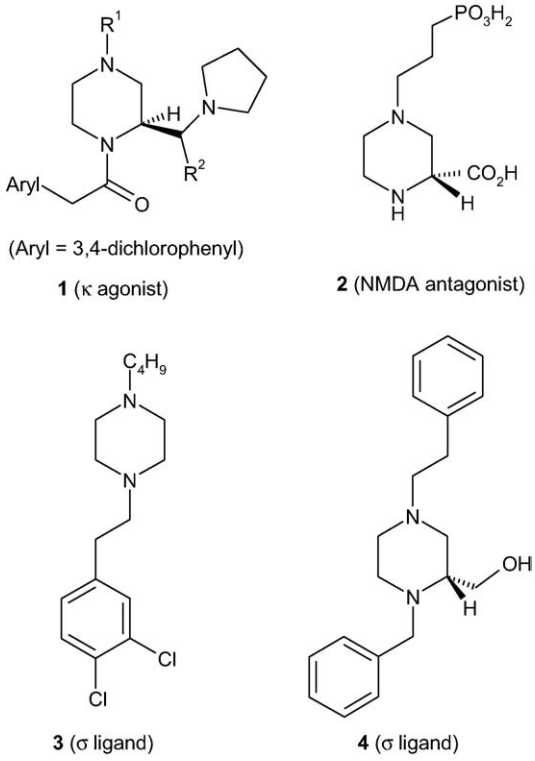
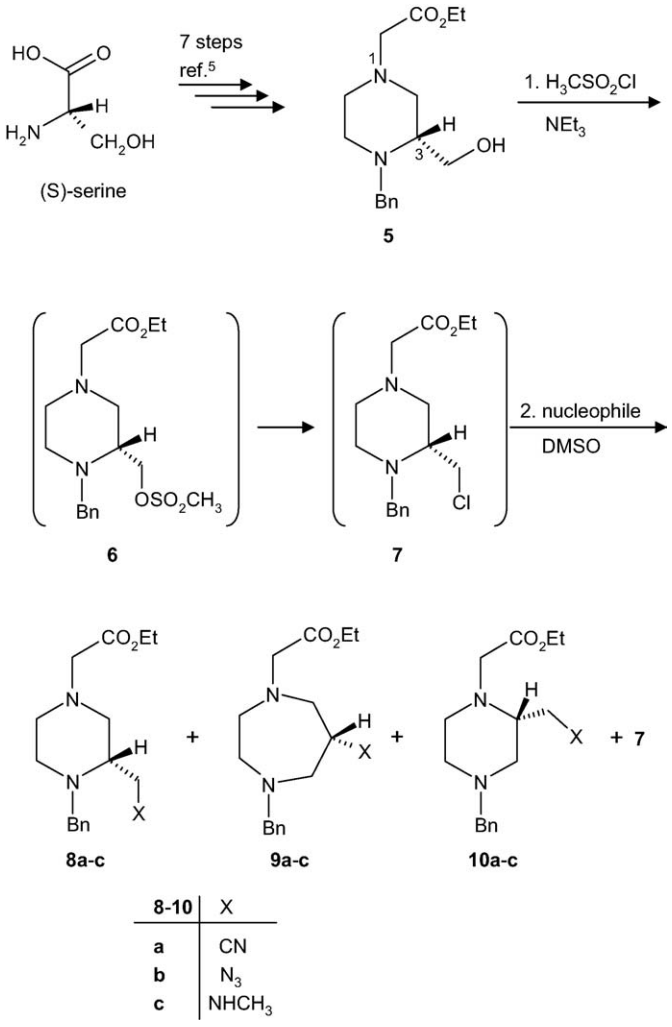


Fig. 1.

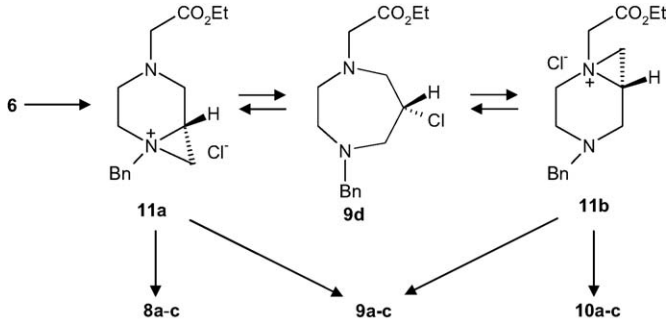
Rf values of the originally formed compound (**6**) and the isolated product (**7**) were not identical. We assume that the originally formed mesylate **6** reacted further with chloride via intermediate aziridinium ion **11a** (see Scheme 2) to yield the chloride **7**. The existence of similar aziridinium ions has been described in literature [6].

In order to avoid the formation of chloride **7** nucleophilic substitutions were performed immediately after preparation of the mesylate **6**. Reaction of the alcohol **5** with methanesulfonyl chloride and subsequently with KCN in DMSO at room temperature provided the cyanomethyl derivative **8a** in 54% yield. However, isolation of the rearranged 1,4-diazepane derivative **9a** (11% yield) demonstrated that the formation of the aziridinium intermediate **11a** had not been completely suppressed. In addition to these main products careful chromatographic separation provided the isomeric nitrile **10a** (0.4%) and the chloride **7** (1.0%). The structures of the isomeric nitriles **8a**, **9a**, and **10a** and the chloride **7** were unequivocally proven by NMR spectroscopy.

The formation of the isomeric nitriles **8a–10a** is explained with intermediate aziridinium ions. Nucleophilic attack of cyanide at the aziridinium corners of **11a** results in the nitriles **8a** and **9a**. A preceding isomerization of the aziridinium ion **11a** into the regioisomeric aziridinium ion **11b** and subsequent cyanide attack is responsible for the generation of the nitriles **9a** and **10a**. We assume that the isomerization of **11a** into **11b** could occur via nucleophilic attack of chloride to form the seven-membered chloro derivative **9d**. Subsequent intramolecular S_N2 reaction should provide the isomeric aziridinium ions **11a** and **11b**. Ring opening of the aziridinium ion **11a** to give a



Scheme 1.



Scheme 2.

seven-membered carbenium ion is unlikely since the isolated products **8a**, **9a**, and **10a** show optical activity.

The analogous reaction of the alcohol **5** with methanesulfonyl chloride and subsequently with NaN_3 also resulted in regioisomeric products. Purification of the reaction mixture by flash chromatography (FC) led to the expected (azidomethyl) piperazine **8b** (65%) and the 6-azidodiazepane **9b** (11%). In spite of intensive search the third regioisomeric azide **10b** was not detected.

In order to study the temperature effect on the nucleophilic substitution reactions of the mesylate **6** with cyanide and azide transformations were performed at 20 and 50 °C and the composition of the crude product mixtures was analyzed by ^{1}H NMR spectroscopy. These experiments demonstrated that the amounts of rearranged products were enhanced with increasing temperature: Performing the substitution reactions with cyanide or azide at 20 °C resulted in product ratios of **8a**/**9a**/**10a** = 84.5:15.0:0.5 and **8b**/**9b** = 77:23, respectively. At 50 °C the product ratios amounted **8a**/**9a**/**10a** = 67.6:22.7:9.7 and **8b**/**9b** = 70:30. These results lead to the conclusion that lower temperatures might suppress rearrangement and increase the yields of the desired substitution products **8a** and **8b**. However, the high melting point of the solvent DMSO (18.5 °C) prevents further reduction of the temperature. Therefore a solvent mixture THF/DMSO 3:1 was used for the 0 °C substitution reactions. After a reaction time of 44 h at 0 °C the ^{1}H NMR spectra revealed large amounts of starting mesylate **6** and chloride **7** which impede the exact interpretation of the spectra. Obviously, a reaction temperature of 20 °C represents the best compromise between selectivity and reactivity.

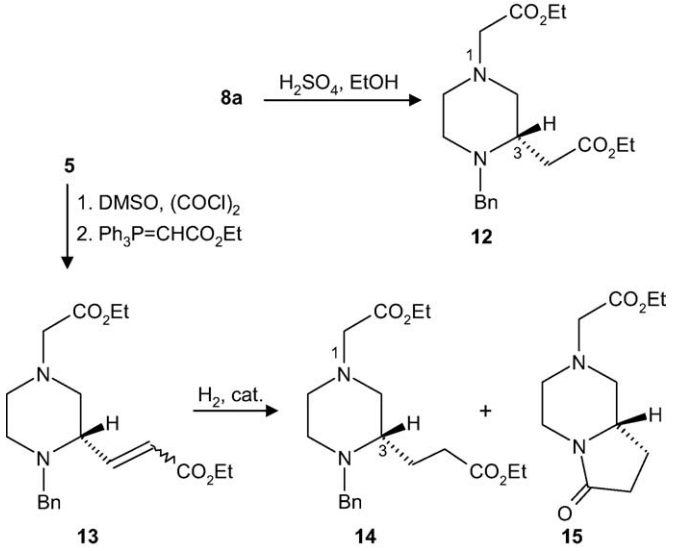
The nucleophilic substitution of the alcohol **5** with methylamine was performed according to the same procedure affording the methylamine **8c** in 52% yield. TLC showed additional spots which might represent rearranged products **9c** and/or **10c**. Because of strong tailing it was not possible to separate and analyze the corresponding compounds (Scheme 1).

The nitrile **8a** was transformed into the diester **12** with ethanol and H_2SO_4 [7]. The synthesis of the homologous diester **14** started with the alcohol **5**. Combination of a Swern oxidation [8] (oxalyl chloride, DMSO) with a subsequent Wittig reaction using the stabilized phosphor ylide $Ph_3P{=}CH{-}CO_2Et$ provided the α,β-unsaturated ester **13** as a mixture of (E) and (Z) isomers (90:10). The hydrogenation of the α,β-unsaturated ester **13** was controlled by the catalyst: Hydrogenation using Raney Nickel and 1 bar H_2 predominantly furnished the propionic acid ester **14** (64%) and only small amounts of the γ-lactam **15**; whereas hydrogenation of **13** with the catalyst Pd/C at elevated H_2 pressure (4 bar) led to saturation of the double bond and simultaneously hydrogenolysis of the benzyl group providing a γ-aminoester which spontaneously cyclized to yield the γ-lactam **15**. Analogous transformations have recently been described in [9,10] (Scheme 3).

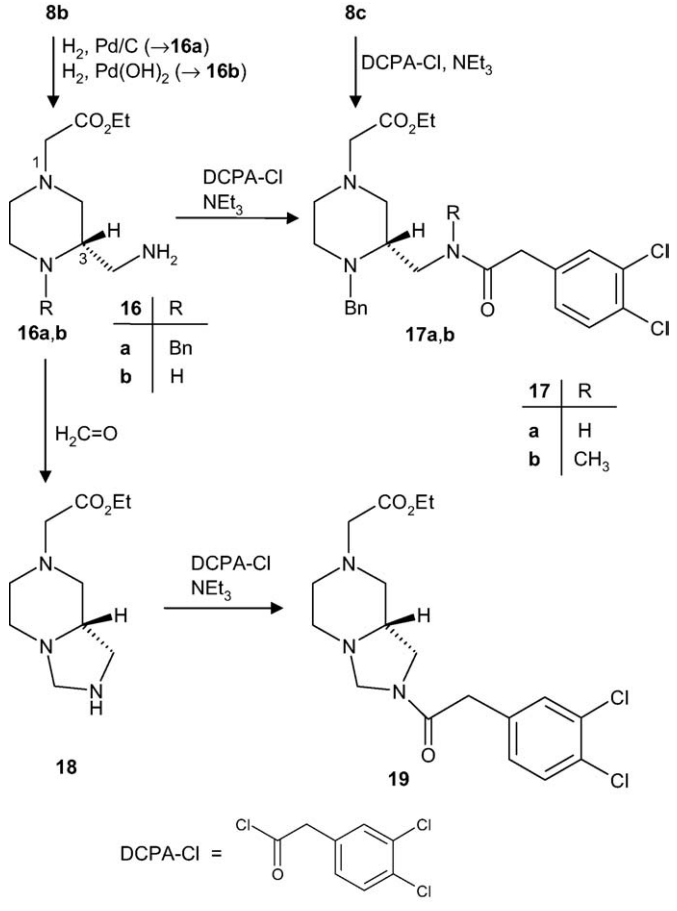
Depending on the reaction conditions hydrogenation of the azide **8b** yielded the benzylated primary amine **16a** (H_2, Pd/C, 1 bar, 0.5 h, 20 °C) or the debenzylated primary amine **16b** (H_2, $Pd(OH)_2$/C, 4 bar, 8 h, 20 °C) in 96% and 97% yield, respectively (Scheme 4).

Great efforts were undertaken to synthesize 1,3-bridged piperazine derivatives. However, all attempts failed to obtain bicyclic lactones or lactams by cyclization of the hydroxyester **5** or the aminoesters **8c**, **16a** or **16b**. Moreover, the projected Dieckmann cyclization of the diesters **12** and **14** failed to give bicyclic derivatives.

The secondary amine **8c** and the benzylated primary amine **16a** were used for the introduction of the κ- [11] and σ-pharmacophoric [12] dichlorophenylacetyl residue. Acylation with (dichlorophenyl)acetyl chloride (DCPA-Cl) provided the secondary and tertiary amides **17a** and **17b**, respectively. The debenzylated primary amine **16b** reacted with formaldehyde to afford the aminal **18**, which was acylated with DCPA-Cl to yield the amide **19**, which is regarded as conformationally constrained analogue of the methylated amide **17b**.



Scheme 3.



Scheme 4.

3. Receptor binding studies

The affinities of the 3-substituted and 1,2-anellated piperazine derivatives **15**, **17a**, **17b**, **18** and **19** towards NMDA, κ-opioid, μ-opioid, σ_1 and σ_2 receptors were determined in receptor binding studies using radioligands with high affinity and selectivity to the corresponding receptors. The affinity to the phencyclidine binding site of the NMDA receptor was measured with tritium labeled (+)-MK-801. [^{3}H]-U-69593 and [^{3}H]-DAMGO were employed for the determination of κ-opioid and μ-opioid receptor affinity. The σ receptors were labeled with [^{3}H]-(+)-pentazocine (σ_1) and [^{3}H]-ditolylguanidine in the presence of non-tritiated (+)-pentazocine (σ_2). Membrane preparations from pig brain cortex were employed in the NMDA assay. In the κ-opioid, μ-opioid and σ_1 assay homogenates of guinea pig brains were used as receptor material. Homogenates of rat liver served as source for σ_2 receptors in the σ_2 assay [5,13].

At first the receptor interaction of the test compounds was screened with 1 and 10 μM test compound concentrations. Only when considerable inhibition of the radioligand binding was observed at a concentration of 10 μM the exact K_i-values were determined.

4. Results and discussion

In Table 1 the results of the receptor binding studies are summarized.

It is shown that the test compounds do not interact significantly with the phencyclidine binding site of the NMDA receptor and with κ- and μ-opioid receptors. However, considerable σ_1 receptor affinity was found for the ligands **17a**, **17b** and **19** containing the σ_1- and κ-pharmacophoric (dichlorophenyl)acetamide substructure. The K_i value of the secondary amide **17a** is in the low micromolar range (K_i = 1341 nM). Methylation of the secondary amide led to a 10-fold increase of σ_1 receptor affinity (**17b**: K_i = 181 nM). However, cyclization of the N-methyl derivative **17b** to the imidazolidine derivative **19** reduced the interaction with σ_1 receptors. The loss of the benzyl moiety (second aromatic system) might be responsible for the diminished σ_1 receptor affinity of **19**. At a concentration of 10 μM all test compounds were inactive at σ_2 receptors. Obviously, the most σ_1 active compound **17b** displays considerable selectivity versus σ_2 receptors.

5. Conclusion

Within this series of compounds the phenylacetamide **17b** reveals considerable affinity towards σ_1 receptors, which is in the range of the σ_1 receptor affinity of ditolylguanidine (K_i = 164 nM). Despite the fact that **17b** contains the σ_1- and κ-pharmacophoric (dichlorophenyl)acetamide substructure affinity towards κ receptors was not found indicating high σ_1 receptor selectivity with regard to the investigated receptor systems. **17b** represents a monoacylated 1,2-diamine. It is known, that the stereochemistry of analogously substituted 1,2-diamines (compare compounds **1**, **3**, and **4** in Scheme 1) determines, whether a compound reacts with σ_1 or κ receptors [12]. Therefore, it might be possible that the (R)-configuration contributes to the σ_1 receptor selectivity of **17b**. Further investigations will be directed to improve the σ_1 receptor affinity of **17b** and to keep its selectivity versus σ_2, κ and other receptors.

6. Experimental

6.1. Chemistry, general

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. – THF was distilled from sodium/benzophenone ketyl prior to use. Petroleum ether used refers to the fraction boiling at 40–60 °C. – TLC: silica gel 60 F_{254} plates (Merck). – FC [14]: silica gel 60, 0.040–0.063 mm (Merck); parentheses include: diameter of the column (cm), eluent, fraction size (ml), R_f. – Optical rotation: Polarimeter 241 (Perkin–Elmer); 1.0 dm tube; concentration *c* (g/100 ml); temperature 20 °C. – Elemental analyses: Vario EL (Elementaranalysesysteme GmbH). – MS: MAT 312, MAT 8200, MAT 44, and TSQ 7000 (Finnigan); EI = electron impact, CI = chemical ionization. – High resolution MS (HRMS): MAT 8200 (Finnigan). – IR: IR spectrophotometer 1605 FT-IR (Perkin–Elmer). (br = broad, m = medium, s = strong). – ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz): Unity 300 FT NMR spectrometer (Varian), δ in ppm related to tetramethylsilane, coupling constants are given with 0.5 Hz resolution; the assignments of ^{13}C and of ^{1}H NMR signals were supported by 2D NMR techniques.

Table 1
Inhibition of radioligand binding at a test compound concentration of 10 μM

Compound	NMDA [(+)-MK-801]	κ (U-69593)	μ (DAMGO)	σ_1 [(+)-Pentazocine]	σ_2 Ditolylguanidine
15	15%	0%	4%	24%	14%
17a	15%	0%	22%	K_i = 1341 nM ± 241 nM (n = 3)	14%
17b	14%	27%	38%	K_i = 181 nM ± 12 nM (n = 3)	53%
18	n.d.	n.d.	n.d.	46%	21%
19	19%	2%	8%	K_i = 907 nM ± 93 nM (n = 3)	41%
Haloperidol	–	–	–	K_i = 2.20 nM ± 0.31 nM (n = 3) [13]	K_i = 34.2 nM ± 2.3 nM (n = 3) [13]
Ditolylguanidine	–	–	–	K_i = 164 nM ± 47 nM (n = 3) [13]	K_i = 63.9 nM ± 10.8 nM (n = 3) [13]
BMY 14802	–	–	–	K_i = 265 nM ± 32 nM (n = 3) [13]	K_i = 391 nM ± 63 nM (n = 3) [13]

*6.2. (–)-Ethyl 2-[(3R)-4-benzyl-3-(chloromethyl)piperazin-1-yl] acetate (**7**), (–)-Ethyl 2-[(3S)-4-benzyl-3-(cyanomethyl) piperazin-1-yl]acetate (**8a**), (+)-Ethyl 2-[(6R)-4-benzyl-6-cyano-1,4-diazepan-1-yl]acetate (**9a**) and (+)-Ethyl 2-[(2R)-4-benzyl-2-(cyanomethyl)piperazin-1-yl]acetate (**10a**)*

Triethylamine (0.35 ml, 2.5 mmol) and methanesulfonyl chloride (0.15 ml, 1.5 mmol) were added to a cooled (0 °C) solution of **5** [5] (0.367 g, 1.25 mmol) in CH_2Cl_2 (30 ml). The mixture was stirred at 0 °C for 15 min. The organic layer was washed with water (2 × 10 ml), dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in DMSO (5 ml) and potassium cyanide (0.817 g, 12.5 mmol) was added. The mixture was stirred for 24 h at room temperature. Then a saturated solution of $NaHCO_3$ (5 ml), water (10 ml) and diethyl ether (20 ml) were added and the organic layer was separated. The DMSO/water-layer was extracted with diethyl ether (5 × 10 ml). The organic layers were combined, washed with brine (30 ml), dried (Na_2SO_4) and evaporated in vacuo. Purification of the residue by FC (3 cm, petroleum ether/ethyl acetate 1:1, 5 ml) afforded **7**, **8a**, **9a** and **10a**, as well as a mixture of **8a/10a** (yield 41 mg, 11%).

7 (R_f = 0.41): Colorless oil, yield 4 mg (1.0%), $[\alpha]_{589}$ = –12.9 (c = 0.605, CH_2Cl_2).- $C_{16}H_{23}ClN_2O_2$ (310.82) calcd. C 61.83 H 7.46 N 9.01 found C 61.14 H 7.86 N 9.37.- MS (EI): *m/z* (%) = 312/310 (M, 1/4), 275 (M – Cl, 10), 239/237 (M –CO_2Et, 4/12), 91 (benzyl, 100).- MS (CI): *m/z* (%) = 313/311 (MH^+, 37/100), 275 (M – Cl, 21).- IR (film): ν (cm^{-1}) = 2939 (m, C–H), 2817 (m, C–H), 1748 (s, C=O), 834 (m, C–Cl), 736 and 699 (m, aryl-C–H).- ^{1}H NMR ($CDCl_3$): δ = 1.27 (t, J = 7.2 Hz, 3 H, OCH_2CH_3), 2.37–2.50 (m, 2 H, 5-H and 6-H), 2.61–2.76 (m, 3 H, 2-H, 5-H and 6-H), 2.78–2.89 (m, 2 H, 2-H and 3-H), 3.17 (d, J = 16.4 Hz, 1 H, NCH_2CO_2Et), 3.23 (d, J = 16.5 Hz, 1 H, NCH_2CO_2Et), 3.45 (d, J = 13.4 Hz, 1 H, $PhCH_2N$), 3.70 (dd, J = 11.0/2.8 Hz, 1 H, CH_2Cl), 3.87 (dd, J = 11.3/7.7 Hz, 1 H, CH_2Cl), 3.93 (d, J = 13.7 Hz, 1 H, $PhCH_2N$), 4.18 (q, J = 7.1 Hz, 2 H, OCH_2CH_3), 7.21–7.34 (m, 5 H, arom).- ^{13}C NMR ($CDCl_3$): δ = 14.2 (1 C, OCH_2CH_3), 42.5 (1 C, CH_2Cl), 49.2 (1 C, C-5 or C-6), 52.6 (1 C, C-6 or C-5), 55.1 (1 C, C-2), 58.0 (1 C, $PhCH_2N$), 59.3 (1 C, NCH_2CO_2Et), 60.2 (1 C, C-3), 60.6 (1 C, OCH_2CH_3), 127.1 (1 C, C-4 arom), 128.3 (2 C, C-3 and C-5 arom), 128.8 (2 C, C-2 and C-6 arom), 138.2 (1 C, C-1 arom), 170.2 (1 C, CO_2Et).

8a (R_f = 0.24): Pale yellow oil, yield 0.206 g (54 %), $[\alpha]_{589}$ = –5.8 (c = 0.595, CH_2Cl_2).- $C_{17}H_{23}N_3O_2$ (301.39) calcd. C 67.75 H 7.69 N 13.94 found C 68.41 H 7.25 N 13.73.- HRMS: Calcd. 301.1790, found 301.1798 (+ 0.8 ppm).- MS (EI): *m/z* (%) = 301 (M, 15), 261 (M – CH_2CN, 56), 228 (M –CO_2Et, 54), 170 (M – benzyl. – CH_2CN, 90), 91 (benzyl, 100).- IR (film): ν (cm^{-1}) = 2938 (m, C–H), 2818 (m, C–H), 2245 (m, C≡N), 1741 (s, C=O), 739 and 699 (m, aryl-C–H).- ^{1}H NMR ($CDCl_3$): δ = 1.29 (t, J = 7.2 Hz, 3 H, OCH_2CH_3), 2.45 (ddd, J = 11.0/5.3/3.2 Hz, 1 H, 5-H or 6-H), 2.54–2.71 (m, 5 H, 2-H, 5-H, 6-H and CH_2CN), 2.80 (dd, J = 11.0/3.1 Hz, 1 H, 2-H), 2.91 (dd, J = 16.7/8.1 Hz, 1 H, CH_2CN), 3.01–3.07 (m, 1 H, 3-H), 3.19 (d, J = 17.1 Hz, 1 H, NCH_2CO_2Et), 3.24 (d, J = 17.1 Hz, 1 H, NCH_2CO_2Et), 3.46 (d, J = 13.4 Hz, 1 H, $PhCH_2N$), 3.80 (d, J = 13.2 Hz, 1 H, $PhCH_2N$), 4.19 (q, J = 7.1 Hz, 2 H, OCH_2CH_3), 7.26–7.36 (m, 5 H, arom).- ^{13}C NMR ($CDCl_3$): δ = 14.2 (1 C, OCH_2CH_3), 16.2 (1 C, CH_2CN), 48.2 (1 C, C-5 or C-6), 52.4 (1 C, C-6 or C-5), 55.6 (1 C, C-3), 56.3 (1 C, C-2), 58.3 (1 C, $PhCH_2N$), 59.1 (1 C, NCH_2CO_2Et), 60.7 (1 C, OCH_2CH_3), 118.5 (1 C, CN), 127.3 (1 C, C-4 arom), 128.4 (2 C, C-3 and C-5 arom), 128.7 (2 C, C-2 and C-6 arom), 137.8 (1 C, C-1 arom), 170.1 (1 C, CO_2Et).

9a (R_f = 0.37): Pale yellow oil, yield 43 mg (11 %), $[\alpha]_{589}$ = +5.5 (c = 0.145, CH_2Cl_2).- $C_{17}H_{23}N_3O_2$ (301.39).- MS (EI): *m/z* (%) = 301 (M, 26), 228 (M –CO_2Et, 45), 210 (M – benzyl, 13), 91 (benzyl, 100).- IR (film): ν (cm^{-1}) = 2924 (m, C–H), 2844 (m, C–H), 2268 (m, C≡N), 1732 (s, C=O), 740 and 700 (m, aryl-C–H).- ^{1}H NMR ($CDCl_3$): δ = 1.28 (t, J = 7.2 Hz, 3 H, OCH_2CH_3), 2.69–2.79 (m, 2 H, 2-H and 3-H), 2.81–2.96 (m, 3 H, 2-H, 3-H, and 6-H), 2.98–3.09 (m, 2 H, 5-H or 7-H), 3.18 (dd, J = 14.0/6.6 Hz, 1 H, 5-H or 7-H), 3.27 (dd, J = 14.0/5.2 Hz, 1 H, 5-H or 7-H), 3.48 (d, J = 17.5 Hz, 1 H, NCH_2CO_2Et), 3.55 (d, J = 17.3 Hz, 1 H, NCH_2CO_2Et), 3.76 (s, 2 H, $PhCH_2N$), 4.18 (q, J = 7.1 Hz, 2 H, OCH_2CH_3), 7.23–7.36 (m, 5 H, arom).

10a (R_f = 0.26): Pale yellow oil, yield 2 mg (0.4%), $[\alpha]_{589}$ = +3.2 (c = 0.335, CH_2Cl_2).- $C_{17}H_{23}N_3O_2$ (301.39).- MS (EI): *m/z* (%) = 301 (M, 36), 261 (M – CH_2CN, 33), 228 (M –CO_2Et, 67), 210 (M – benzyl, 5), 170 (M – benzyl. – CH_2CN, 59), 91 (benzyl, 100).- IR (film): ν (cm^{-1}) = 2936 (m, C–H), 2819 (m, C–H), 2246 (m, C≡N), 1732 (s, C=O), 742 and 700 (m, aryl-C–H).- ^{1}H NMR ($CDCl_3$): δ = 1.28 (t, J = 7.0 Hz, 3 H, OCH_2CH_3), 2.41–2.48 (m, 2 H, 3-H and 5-H or 6-H), 2.51–2.56 (m, 2 H, CH_2CN and 5-H or 6-H), 2.68 (dd, J = 11.3/2.5 Hz, 1 H, 3-H), 2.73–2.82 (m, 3 H, CH_2CN, 5-H and 6-H), 3.15–3.22 (m, 1 H, 2-H), 3.34 (d, J = 16.5 Hz, 1 H, NCH_2CO_2Et), 3.44 (d, J = 16.4 Hz, 1 H, NCH_2CO_2Et), 3.52 (s, 2 H, $PhCH_2N$), 4.18 (q, J = 7.2 Hz, 2 H, OCH_2CH_3), 7.25–7.35 (m, 5 H, arom).- ^{13}C NMR ($CDCl_3$): δ = 14.2 (1 C, OCH_2CH_3), 17.4 (1 C, CH_2CN), 49.7 (1 C, C-5 or C-6), 52.5 (1 C, C-6 or C-5), 54.9 (1 C, C-2), 55.3 (1 C, NCH_2CO_2Et), 56.9 (1 C, C-3), 60.8 (1 C, OCH_2CH_3), 62.6 (1 C, $PhCH_2N$), 118.1 (1 C, CN), 127.2 (1 C, C-4 arom), 128.3 (2 C, C-3 and C-5 arom), 128.9 (2 C, C-2 and C-6 arom), 137.7 (1 C, C-1 arom), 170.0 (1 C, CO_2Et).

*6.3. (–)-Ethyl 2-[(3R)-3-(azidomethyl)-4-benzylpiperazin-1-yl] acetate (8b) and (+)-ethyl 2-[(6R)-6-azido-4-benzyl-1,4-diazepan-1-yl]acetate (**9b**)*

Triethylamine (1.03 ml, 7.42 mmol) and methanesulfonyl chloride (0.43 ml, 4.45 mmol) were added to a cooled (0 °C) solution of **5** (1.085 g, 3.71 mmol) in CH_2Cl_2 (100 ml). The mixture was stirred at 0 °C for 15 min. The organic layer was washed with water (2 × 30 ml), dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in DMSO (30 ml) and sodium azide (1.207 g, 18.55 mmol) was added. The suspension was stirred for 22 h at room temperature. Then water (50 ml) and diethyl ether (70 ml) were added and the organic

layer was separated. The DMSO/water-layer was extracted with diethyl ether (5 × 30 ml). The organic layers were combined, washed with brine (100 ml), dried (Na_2SO_4) and evaporated in vacuo. Purification of the residue by FC (4 cm, petroleum ether/ethyl acetate 3:1, 20 ml) afforded **8b** and **9b**.

8b (R_f = 0.14): Colorless oil, yield 0.767 g (65%), $[\alpha]_{589}$ = –3.2 (c = 0.445, CH_2Cl_2).- $C_{16}H_{23}N_5O_2$ (317.39) calcd. C 60.55 H 7.30 N 22.07 found C 60.63 H 7.33 N 21.76.- MS (EI): *m/z* (%) = 275 (M – N_3, 3), 261 (M – CH_2N_3, 81), 170 (M – benzyl – CH_2N_3, 100).- MS (CI): *m/z* (%) = 318 (MH^+, 100).- IR (film): ν (cm^{-1}) = 2940 (m, C–H), 2814 (m, C–H), 2097 (s, N_3), 1745 (s, C=O), 738 and 699 (m, aryl-C–H).- ^{1}H NMR ($CDCl_3$): δ = 1.27 (t, J = 7.0 Hz, 3 H, OCH_2CH_3), 2.35–2.52 (m, 3 H, 2-H, 5-H and 6-H), 2.60–2.68 (m, 1 H, 5-H or 6-H), 2.72–2.83 (m, 3 H, 2-H, 3-H and 6-H or 5-H), 3.16 (d, J = 16.4 Hz, 1 H, NCH_2CO_2Et), 3.22 (d, J = 16.5 Hz, 1 H, NCH_2CO_2Et), 3.42 (d, J = 13.5 Hz, 1 H, $PhCH_2N$), 3.52 (dd, J = 12.8/4.1 Hz, 1 H, CH_2N_3), 3.62 (dd, J = 12.8/6.5 Hz, 1 H, CH_2N_3), 3.96 (d, J = 13.4 Hz, 1 H, $PhCH_2N$), 4.18 (q, J = 7.1 Hz, 2 H, OCH_2CH_3), 7.21–7.34 (m, 5 H, arom).- ^{13}C NMR ($CDCl_3$): δ = 14.2 (1 C, OCH_2CH_3), 49.6 (1 C, C-5 or C-6), 50.3 (1 C, CH_2N_3), 52.4 (1 C, C-6 or C-5), 55.6 (1 C, C-2), 58.2 (1 C, $PhCH_2N$), 58.8 (1 C, C-3), 59.3 (1 C, NCH_2CO_2Et), 60.6 (1 C, OCH_2CH_3), 127.1 (1 C, C-4 arom), 128.3 (2 C, C-3 and C-5 arom), 128.7 (2 C, C-2 and C-6 arom), 138.3 (1 C, C-1 arom), 170.2 (1 C, CO_2Et).

9b (R_f = 0.29): Colorless oil, yield 0.127 g (11 %), $[\alpha]_{589}$ = +4.5 (c = 1.025, CH_2Cl_2).- $C_{16}H_{23}N_5O_2$ (317.39) calcd. C 60.55 H 7.30 N 22.07 found C 60.58 H 7.35 N 21.96.- MS (EI): *m/z* (%) = 275 (M – N_3, 100), 244 (M – CO_2Et, 13), 189 (M– N_3 – CH_2CO_2Et, 36), 91 (benzyl, 100).- MS (CI): *m/z* (%) = 318 (MH^+, 100).- IR (film): ν (cm^{-1}) = 2938 (m, C–H), 2095 (s, N_3), 1735 (s, C=O), 738 and 698 (m, aryl-C–H).- ^{1}H NMR ($CDCl_3$): δ = 1.27 (t, J = 7.1 Hz, 3 H, OCH_2CH_3), 2.64–2.70 (m, 2 H, 2-H and 3-H), 2.79–2.90 (m, 3 H, 5-H or 7-H, 2-H and 3-H), 2.96–3.03 (m, 2 H, 5-H and 7-H), 3.23 (dd, J = 14.1/4.6 Hz, 1 H, 5-H or 7-H), 3.41–3.49 (m, 1 H, 6-H), 3.43 (d, J = 17.0 Hz, 1 H, NCH_2CO_2Et), 3.50 (d, J = 17.3 Hz, 1 H, NCH_2CO_2Et), 3.67 (d, J = 13.4 Hz, 1 H, $PhCH_2N$), 3.74 (d, J = 13.4 Hz, 1 H, $PhCH_2N$), 4.17 (q, J = 7.2 Hz, 2 H, OCH_2CH_3), 7.21–7.36 (m, 5 H, arom).- ^{13}C NMR ($CDCl_3$): δ = 14.2 (1 C, OCH_2CH_3), 56.3 (1 C, C-2 or C-3), 56.6 (1 C, C-3 or C-2), 58.2 (1 C, C-5 or C-7), 59.2 (1 C, C-7 or C-5), 59.6 (1 C, NCH_2CO_2Et), 60.0 (1 C, C-6), 60.4 (1 C, OCH_2CH_3), 62.8 (1 C, $PhCH_2N$), 127.1 (1 C, C-4 arom), 128.3 (2 C, C-3 and C-5 arom), 128.7 (2 C, C-2 and C-6 arom), 139.1 (1 C, C-1 arom), 171.3 (1 C, CO_2Et).

*6.4. (–)-Ethyl 2-[(3S)-4-benzyl-3-(methylaminomethyl) piperazin-1-yl]acetate (**8c**)*

Triethylamine (0.09 ml, 0.66 mmol) and methanesulfonyl chloride (39 µl, 0.396 mmol) were added to a cooled (0 °C) solution of **5** (98 mg, 0.33 mmol) in CH_2Cl_2 (10 ml). The mixture was stirred at 0 °C for 15 min. The organic layer was washed with water (8 ml), dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in DMSO (3 ml) and a solution of methylamine in ethanol (8 M, 0.41 ml, 3.3 mmol) was added dropwise. The reaction mixture was stirred for 48 h at room temperature. Then water (10 ml) and diethyl ether (8 ml) were added and the organic layer was separated. The DMSO/water layer was extracted with diethyl ether (5 × 5 ml). The organic layers were combined, washed with brine (10 ml), dried (Na_2SO_4) and evaporated in vacuo. Purification of the residue by FC (2 cm, at first ethanol [130 ml], then ethyl acetate/acetone 8:2 + 2% ethyldimethylamine, 2 ml, R_f = 0.10 (EtOH), R_f = 0.21 (ethyl acetate/acetone 8:2 + 2% ethyldimethylamine)) afforded **8c** as a colorless, viscous oil. Yield 53 mg (52%), $[\alpha]_{589}$ = –29.2 (c = 0.55, CH_2Cl_2).- $C_{17}H_{27}N_3O_2$ (305.42) calcd. C 66.85 H 8.91 N 13.76 found C 66.04 H 8.92 N 13.46.- MS (EI): *m/z* (%) = 261 (M – CH_2NHCH_3, 74), 232 (M – CO_2Et, 5), 170 (261 – benzyl, 100).- MS (CI): *m/z* (%) = 306 (MH^+, 100).- IR (film): ν (cm^{-1}) = 2935 (m, C–H), 2807 (s, C–H), 1747 (s, C=O), 736 and 699 (m, aryl-C–H).- ^{1}H NMR ($CDCl_3$): δ = 1.24 (t, J = 7.2 Hz, 3 H, OCH_2CH_3), 1.46 (s broad, 1 H, N*H*), 2.25–2.39 (m, 2 H, 5-H and 6-H), 2.35 (s, 3 H, $NHCH_3$), 2.48 (dd, J = 10.7/8.5 Hz, 1 H, 2-H), 2.61–2.80 (m, 4 H, 2-H, 3-H, 5-H and 6-H), 2.84 (dd broad, J = 11.9/5.4 Hz, 2 H, CH_2NHCH_3), 3.11 (d, J = 16.5 Hz, 1 H, NCH_2CO_2Et), 3.19 (d, J = 16.5 Hz, 1 H, NCH_2CO_2Et), 3.27 (d, J = 13.6 Hz, 1 H, $PhCH_2N$), 3.99 (d, J = 13.8 Hz, 1 H, $PhCH_2N$), 4.15 (q, J = 7.2 Hz, 2 H, OCH_2CH_3), 7.19–7.30 (m, 5 H, arom).- ^{13}C NMR ($CDCl_3$): δ = 14.2 (1 C, OCH_2CH_3), 36.9 (1 C, $NHCH_3$), 50.7 (1 C, C-5 or C-6), 51.9 (1 C, CH_2NHCH_3), 52.8 (1 C, C-6 or C-5), 56.6 (1 C, C-2), 57.9 (1 C, $PhCH_2N$), 59.4 (1 C, C-3), 59.5 (1 C, NCH_2CO_2Et), 60.6 (1 C, OCH_2CH_3), 126.9 (1 C, C-4 arom), 128.3 (2 C, C-3 and C-5 arom), 128.7 (2 C, C-2 and C-6 arom), 139.0 (1 C, C-1 arom), 170.4 (1 C, CO_2Et).

*6.5. (–)-Ethyl 2-[(2S)-1-benzyl-4-(ethoxycarbonylmethyl) piperazin-2-yl]acetate (**12**)*

H_2SO_4 conc. (4.5 ml) was added slowly to a solution of **8a** (0.110 g, 0.367 mmol) in ethanol (13.5 ml). The mixture was heated to reflux for 3 h and, subsequently, concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (15 ml), extracted with water (2 × 10 ml), the pH of the combined aqueous layers was adjusted to 10 with NaOH and then the aqueous layer was extracted with CH_2Cl_2 (5 × 15 ml). The organic layer was washed with a saturated solution of $NaHCO_3$ (2 × 40 ml), dried (Na_2SO_4), concentrated in vacuo and the residue was purified by FC (2 cm, petroleum ether/ethyl acetate 1:1, 5 ml, R_f = 0.36). Pale yellow oil, yield 60 mg, 47%, $[\alpha]_{589}$ = –5.9 (c = 0.535, CH_2Cl_2).- $C_{19}H_{28}N_2O_4$ (348.44) calcd. C 65.49 H 8.10 N 8.04 found C 64.74 H 8.09 N 7.85.- MS (EI): *m/z* (%) = 348 (M, 46), 275 (M – CO_2Et, 67), 261 (M – CH_2CO_2Et, 100), 170 (261 – Benzyl, 97).- IR: $\tilde{\nu}$ [cm^{-1}] = 2938 (m, C–H), 2811 (m, C–H), 1732 (s, C=O), 1167 (s, C-O), 738 and 699 (m, aryl-C–H).- ^{1}H NMR ($CDCl_3$): δ = 1.22 (t, J = 7.1 Hz, 3 H, OCH_2CH_3), 1.25 (t, J = 7.2 Hz, 3 H, OCH_2CH_3), 1.92–2.02 (m, 2 H, $CH_2CH_2CO_2Et$), 2.24–2.37 (m, 4 H, 3-H, 5-H, 6-H and $CH_2CH_2CO_2Et$), 2.45 ("dt", J = 15.8/8.1 Hz, 1 H, $CH_2CH_2CO_2Et$), 2.51–2.60 (m, 1 H, 2-H), 2.62–2.76 (m, 3

H, 3-H, 5-H and 6-H), 3.11 (d, J = 16.4 Hz, 1 H, NC$H_2$$CO_2$Et), 3.18 (d, J = 16.4 Hz, 1 H, NC$H_2$$CO_2$Et), 3.21 (d, J = 13.4 Hz, 1 H, PhCH_2N), 4.01 (d, J = 13.7 Hz, 1 H, PhCH_2N), 4.09 (q, J = 7.1 Hz, 2 H, OC$H_2$$CH_3$), 4.16 (q, J = 7.1 Hz, 2 H, OC $H_2$$CH_3$), 7.21–7.34 (m, 5 H, arom).- ^{13}C NMR ($CDCl_3$): δ = 14.2 (2 C, 2 × OCH$_2$*C*H$_3$), 24.6 (1 C, *C*H$_2$CH$_2$CO$_2$Et), 30.2 (1 C, CH$_2$*C*H$_2$CO$_2$Et), 50.2 (1 C, C-5 or C-6), 52.6 (1 C, C-6 or C-5), 56.8 (1 C, C-3), 57.4 (1 C, PhCH$_2$N), 58.5 (1 C, C-2), 59.6 (1 C, NCH$_2$CO$_2$Et), 60.4 (1 C, OCH$_2$CH$_3$), 60.6 (1 C, OCH$_2$CH$_3$), 126.9 (1 C, C-4′), 128.2 (2 C, C-3′ and C-5′), 128.8 (2 C, C-2′ and C-6′), 138.9 (1 C, C-1′), 170.2 (1 C, *C*=O), 173.6 (1 C, *C*=O).

*6.6. (+)-Ethyl 2-[(6S)-9-oxo-1,4-diazabicyclo[4.3.0]nonan-4-yl]acetate (**15**)*

Pd/C (10%, 47 mg) was added to a solution of **13** (50 mg, 0.14 mmol) in ethanol (10 ml). The suspension was stirred under an H_2 atmosphere (4 bar) at room temperature for 8 h. The mixture was filtered through a pad of Celite®AFA and the filtrate was stirred at room temperature for additional 40 h. Removal of the solvent in vacuo and purification by FC (1 cm, ethanol, 1 ml, R_f = 0.26) gave **15** as a pale yellow oil. Yield 10 mg (30%), $[\alpha]_{589}$ = +17.7 (c = 0.81, CH_2Cl_2).- $C_{11}H_{18}N_2O_3$ (226.27).- HRMS: Calcd. 226.1317, found 226.1317 (±0 ppm).- MS (EI): *m/z* (%) = 226 (M, 9), 153 (M – CO_2Et, 100), 138 (M – CH_2CO_2Et, 11).- IR (film): ν (cm^{-1}) = 2934 (m, C–H), 1736 (s, C=O ester), 1682 (s, C=O amide), 1034 (m, C-O).- ^{1}H NMR ($CDCl_3$): δ = 1.26 (t, J = 7.3 Hz, 3 H, OCH$_2$C*H$_3$*), 1.50–1.63 (m, 1 H, 7-H), 2.00 (t, J = 10.7 Hz, 1 H, 5-H), 2.09–2.20 (m, 1 H, 7-H), 2.22 (td, J = 11.5/3.7 Hz, 1 H, 3-H), 2.34–2.41 (m, 2 H, 8-H), 2.83–2.88 (m, 1 H, 3-H), 2.94 (td, J = 12.4/3.4 Hz, 1 H, 2-H), 3.02 (ddd, J = 10.9/3.7/1.6 Hz, 1 H, 5-H), 3.22 (d, J = 16.6 Hz, 1 H, NC$H_2$$CO_2$Et), 3.28 (d, J = 16.7 Hz, 1 H, NC$H_2$$CO_2$Et), 3.69 (dtd, J = 10.6/7.1/3.6 Hz, 1 H, 6-H), 3.99 (ddd, J = v12.9/3.6/1.4 Hz, 1 H, 2-H), 4.17 (q, J = 7.2 Hz, 2 H, OC$H_2$$CH_3$).- ^{13}C NMR ($CDCl_3$): δ = 14.2 (1 C, OCH$_2$*C*H$_3$), 22.0 (1 C, C-7), 30.1 (1 C, C-8), 39.4 (1 C, C-2), 51.5 (1 C, C-3), 55.4 (1 C, C-6), 59. 0 (1 C, NCH$_2$CO$_2$Et), 59.5 (1 C, C-5), 60.7 (1 C, OCH$_2$CH$_3$), 170.0 (1 C, C=O), 173.3 (1 C, C-9).

*6.7. (–)-Ethyl 2-[(3S)-3-(aminomethyl)-4-benzylpiperazin-1-yl] acetate (**16a**)*

Pd/C (10%, 45 mg) was added to a solution of **8b** (100 mg, 0.315 mmol) in ethanol (10 ml). The suspension was stirred under an H_2 atmosphere (balloon) at room temperature for 30 min. The mixture was filtered through a pad of Celite®AFA and the filtrate was concentrated in vacuo. The residue was characterized without further purification and then acylated to afford **17a**. Pale yellow, viscous oil, yield 88 mg (96%), $[\alpha]_{589}$ = –8.2 (c = 1.15, CH_2Cl_2).- $C_{16}H_{25}N_3O_2$ (291.39).- MS (EI): *m/z* (%) = 261 (M – CH_2NH_2, 72), 179 (M – CH_2NH_2 – benzyl, 100), 91 (benzyl, 46).- MS (ESI): *m/z* (%) = 292 (MH^+, 100).- IR (film): ν (cm^{-1}) = 3397 (broad, N–H), 2935 (m, C–H), 2812 (m, C–H), 1733 (s, C=O), 731 and 698 (m, aryl-C–H).- ^{1}H NMR ($CDCl_3$): δ = 1.26 (t, J = 7.2 Hz, 3 H, OCH$_2$C*H$_3$*), 2.29–2.43 (m, 2 H, 5-H and 6-H), 2.47–2.60 (m, 2 H, 2-H and 3-H), 2.65–2.73 (m, 1 H, 5-H or 6-H), 2.76–2.85 (m, 3 H, 2-H and 6-H or 5-H and C $H_2$$NH_2$), 3.11 (dd, J = 13.2/5.7 Hz, 1 H, C$H_2$$NH_2$), 3.14 (d, J = 16.5 Hz, 1 H, NC$H_2$$CO_2$Et), 3.21 (d, J = 16.5 Hz, 1 H, NC $H_2$$CO_2$Et), 3.29 (d, J = 13.5 Hz, 1 H, PhCH_2N), 4.02 (d, J = 13.4 Hz, 1 H, PhCH_2N), 4.17 (q, J = 7.0 Hz, 2 H, OC $H_2$$CH_3$), 7.21–7.33 (m, 5 H, arom). The signals for the protons of the NH_2 group could not be detected.- ^{13}C NMR ($CDCl_3$): δ = 14.2 (1 C, OCH$_2$*C*H$_3$), 41.1 (1 C, *C*H$_2$NH$_2$), 50.3 (1 C, C-5 or C-6), 52.7 (1 C, C-6 or C-5), 55.5 (1 C, C-2), 57.7 (1 C, Ph *C*H$_2$N), 59.5 (1 C, NCH$_2$CO$_2$Et), 60.6 (2 C, C-3 and O *C*H$_2$CH$_3$), 126.9 (1 C, C-4 arom), 128.3 (2 C, C-3 and C-5 arom), 128.8 (2 C, C-2 and C-6 arom), 138.8 (1 C, C-1 arom), 170.3 (1 C, *C*O$_2$Et).

*6.8. (–)-Ethyl 2-[(3S)-3-(aminomethyl)piperazin-1-yl]acetate (**16b**)*

A suspension of **8b** (0.423 g, 1.33 mmol), $Pd(OH)_2$/C (Pearlman catalyst, 0.237 g) and ethanol (10 ml) was stirred under an H_2 atmosphere (4 bar) at room temperature for 8 h. The mixture was filtered through a pad of Celite®AFA and the filtrate was concentrated in vacuo. The residue was characterized without further purification and then reacted with formaldehyde to give **18**. Pale yellow, viscous oil, yield 0.260 g (97%), $[\alpha]_{589}$ = −0.6 (c = 0.86, CH_2Cl_2).- $C_9H_{19}N_3O_2$ (201.27).- MS (EI): *m/z* (%) = 201 (M, 3), 171 (M – CH_2NH_2, 100), 128 (M – CO_2Et, 17).- MS (CI): *m/z* (%) = 202 (MH^+, 100).- IR (film): ν (cm^{-1}) = 3262 (br, N–H), 2934 (m, C–H), 2814 (s, C–H), 1739 (s, C=O).- ^{1}H NMR ($CDCl_3$): δ = 1.25 (t, J = 7.1 Hz, 3 H, OCH$_2$C*H$_3$*), 1.66 (s broad, 3 H, N*H* and N*H*$_2$), 1.94 (t, J = 9.6 Hz, 1 H, 2-H), 2.23 (ddd, J = 15.3/9.8/4.4 Hz, 1 H, 5-H or 6-H), 2.58 (dd, J = 12.4/7.2 Hz, 1 H, 6-H or 5-H), 2.70–2.86 (m, 4 H, 2-H, 3-H, 5-H and 6-H), 2.94–2.98 (m, 2 H, C$H_2$$NH_2$), 3.18 (s, 2 H, NC $H_2$$CO_2$Et), 4.16 (q, J = 7.2 Hz, 2 H, OC$H_2$$CH_3$).- ^{13}C NMR ($CDCl_3$): δ = 14.2 (1 C, OCH$_2$*C*H$_3$), 45.3, (2 C, C-5 or C-6 and *C*H$_2$NH$_2$), 53.7 (1 C, C-6 or C-5), 57.0 (1 C, C-3), 57.2 (1 C, C-2), 59.8 (1 C, NCH$_2$CO$_2$Et), 60.5 (1 C, OCH$_2$CH$_3$), 170.2 (1 C, C=O).

*6.9. (–)-Ethyl 2-[(3S)-4-benzyl-3-{N-[2-(3,4-dichlorophenyl) acetyl]aminomethyl}-piperazin-1-yl]acetate (**17a**)*

To a stirred and cooled (0 °C) solution of 3,4-dichlorophenylacetic acid (0.137 g, 0.67 mmol) in CH_2Cl_2 (10 ml), 1,1′-carbonyldiimidazole (CDI) (0.127 g, 0.78 mmol) was added under N_2 atmosphere. It was allowed to warm to room temperature and the reaction mixture was stirred for 2 h. Under N_2 atmosphere and cooling (ice bath) a cooled solution of **16a** (0.163 g, 0.56 mmol) in CH_2Cl_2 (10 ml) was added dropwise. The reaction mixture was stirred for 24 h at room temperature. The organic layer was washed with a saturated solution of Na_2CO_3 (2 × 10 ml) and brine (10 ml), dried with Na_2SO_4 and concentrated in vacuo. FC purification of the re-

sidue (3 cm, ethyl acetate, 7 ml, $R_f = 0.24$) afforded **17a** as a pale yellow, viscous oil; yield 0.233 g (87%), $[\alpha]_{589} = -6.7$ (c = 0.385, CH_2Cl_2).- $C_{24}H_{29}Cl_2N_3O_3$ (478.42) calcd. C 60.25 H 6.11 N 8.78 found C 59.96 H 6.40 N 8.05.- MS (EI): *m/z* (%) = 478 (M, 1), 261 (M – $CH_2NHCOCH_2PhCl_2$, 100), 170 (261 – benzyl, 76).- MS (CI): *m/z* (%) = 482/480/478 (MH^+, 14/67/100).- IR (film): ν (cm^{-1}) = 3299 (broad, N–H), 2938 (m, C–H), 2815 (m, C–H), 1737 (s, C=O ester), 1648 (s, C=O amide), 1470 (s, N–H amide II), 1031 (s, C–Cl), 826, 738 and 699 (m, aryl-C–H).- ^{1}H NMR ($CDCl_3$): δ = 1.28 (t, J = 7.2 Hz, 3 H, OCH_2CH_3), 2.32–2.42 (m, 2 H, 5-H and 6-H), 2.47–2.62 (m, 3 H, 2-H and 5-H or 6-H), 2.67–2.73 (m, 1 H, 6-H or 5-H), 2.75–2.80 (m, 1 H, 3-H), 3.06 (d, J = 17.1 Hz, 1 H, NCH_2CO_2Et), 3.17 (d, J = 16.7 Hz, 1 H, NCH_2CO_2Et), 3.42 (d, J = 13.4 Hz, 1 H, $PhCH_2N$), 3.43 (dt, J = 14.0/4.6 Hz, 1 H, CH_2NHCO), 3.51 (s, 2 H, $COCH_2PhCl_2$), 3.62 (dt, J = 14.0/4.7 Hz, 1 H, CH_2NHCO), 3.78 (d, J = 13.5 Hz, 1 H, $PhCH_2N$), 4.13–4.22 (m, 2 H, OCH_2CH_3), 6.92 (s broad, 1 H, NH), 7.13–7.17 (m, 3 H, arom), 7.23–7.32 (m, 3 H, arom), 7.39 (d, J = 6.5 Hz, 1 H, 6-H $PhCl_2$), 7.41 (s, 1 H, 2-H $PhCl_2$).- ^{13}C NMR ($CDCl_3$): δ = 14.2 (1 C, OCH_2CH_3), 38.4 (1 C, CH_2NHCO), 42.9 (1 C, $COCH_2PhCl_2$), 49.0 (1 C, C-5 or C-6), 52.8 (1 C, C-6 or C-5), 55.6 (1 C, C-2), 56.1 (1 C, C-3), 57.8 (1 C, $PhCH_2N$), 58.9 (1 C, NCH_2CO_2Et), 60.8 (1 C, OCH_2CH_3), 127.1 (1 C, C-4 benzyl), 128.4 (2 C, C-3 and C-5 benzyl), 128.5 (2 C, C-2 and C-6 benzyl), 128.9, 130.6, 131.2, 131.4, 132.6 and 135.5 (6 C, $PhCl_2$), 138.3 (1 C, C-1 benzyl), 170.5 (1 C, CO_2Et).

*6.10. (–)-Ethyl 2-[(3R)-4-benzyl-3-{N-[2-(3,4-dichlorophenyl) acetyl]-N-methylamino-methyl}-piperazin-1-yl]acetate (**17b**)*

To a stirred and cooled (0 °C) solution of 3,4-dichlorophenylacetic acid (43 mg, 0.21 mmol) in CH_2Cl_2 (5 ml), 1,1′-CDI (40 mg, 0.245 mmol) was added under N_2 atmosphere. It was allowed to warm to room temperature and the reaction mixture was stirred for 2 h. Under N_2 atmosphere and cooling (ice bath) a cooled solution of **8c** (53 mg, 0.175 mmol) in CH_2Cl_2 (10 ml) was added dropwise. The reaction mixture was stirred for 24 h at room temperature. The organic layer was washed with a saturated solution of Na_2CO_3 (2 × 7 ml) and brine (7 ml), dried (Na_2SO_4) and concentrated in vacuo. FC of the residue (2 cm, ethyl acetate, 3 ml, $R_f = 0.32$) yielded **17b** as a pale yellow, viscous oil. Yield 62 mg (72%), $[\alpha]_{589} = -5.6$ (c = 0.51, CH_2Cl_2).- $C_{25}H_{31}Cl_2N_3O_3$ (492.44) calcd. C 60.98 H 6.34 N 8.53 found C 60.42 H 6.28 N 8.22.- MS (EI): *m/z* (%) = 418 (M – H – CO_2Et, 3), 261 (M – $CH_2N(CH_3)COCH_2PhCl_2$, 100), 170 (261 – benzyl, 75).- MS (CI): *m/z* (%) = 496/494/492 (MH^+, 12/69/100).- IR (film): ν (cm^{-1}) = 2937 (m, C–H), 2816 (m, C–H), 1743 (s, C=O ester), 1643 (s, C=O amide), 1031 (s, C–Cl), 832, 738 and 699 (m, aryl-C–H).- ^{1}H NMR ($CDCl_3$): δ = 1.25 (t, J = 7.1 Hz, 3 × 0.5 H, OCH_2CH_3 rotamer 1), 1.26 (t, J = 7.2 Hz, 3 × 0.5 H, OCH_2CH_3 rotamer 2), 2.43–2.51 (m, 2 H, 2-H and 5-H or 6-H), 2.52–2.60 (m, 2 H, 5-H and 6-H), 2.67–2.71 (m, 2 H, 2-H and 5-H or 6-H), 2.81 (s, 3 × 0.5 H, NCH_3 rotamer 1), 2.89–2.93 (m, 0.5 H, 3-H rotamer 1), 2.93- 2.99 (m, 0.5 H, 3-H rotamer 2), 3.07 (s, 3 × 0.5 H, NCH_3 rotamer 2), 3.14–3.24 (m, 2 H, NCH_2CO_2Et), 3.31 (dd broad, J = 14.4/4.3 Hz, 0. 5 H, $COCH_2PhCl_2$ rotamer 1), 3.50–3.69 (m, 2 H, C $H_2N(CH_3)CO$ and 3 × 0.5 H, $COCH_2PhCl_2$ rotamer 2 and PhC H_2N rotamer 1 and 2), 3.79–3.97 (m, 4 × 0.5 H, $COCH_2PhCl_2$ rotamer 1 and 2 and $PhCH_2N$ rotamer 1 and 2), 4.16 (q, J = 7.1 Hz, 2 H, OCH_2CH_3), 7.06–7.12 (m, 1 H, arom), 7.25–7.35 (m, 7 H, arom).- ^{1}H NMR (DMSO, 27 °C): δ = 1.12 (t, J = 7.0 Hz, 3 × 0.5 H, OCH_2CH_3 rotamer 1), 1.16 (t, J = 7.2 Hz, 3 × 0.5 H, OCH_2CH_3 rotamer 2), 2.20–2.59 (m, 6 H, 2-H, 5-H and 6-H), 2.72 (s, 3 × 0.5 H, NCH_3 rotamer 1), 3.01 (s, 3 × 0.5 H, NCH_3 rotamer 2), 3.07–3.14 (m, 2 H, 3-H and $CH_2N(CH_3)CO$ or NCH_2CO_2Et), 3.19–3.45 (m, 3 H, C $H_2N(CH_3)CO$ and/or NCH_2CO_2Et), 3.64–3.88 (m, 4 H, PhC H_2N and $COCH_2PhCl_2$), 4.03 (q, J = 6.8 Hz, 2 × 0.5 H, OC H_2CH_3 rotamer 1), 4.05 (q, J = 6.9 Hz, 2 × 0.5 H, OCH_2CH_3 rotamer 2), 7.31–7.41 (m, 6 H, arom), 7.41 (s broad, 0.5 H, 2-H $PhCl_2$ rotamer 1), 7.44 (s broad, 0.5 H, 2-H $PhCl_2$ rotamer 2), 7.51 (d, J = 8.3 Hz, 1 H, 5-H $PhCl_2$).- ^{1}H NMR (DMSO, 120 °C): δ = 1.19 (t, J = 7.1 Hz, 3 H, OCH_2CH_3), 2.40–2.52 (m, 5 H, 2-H, 5-H and 6-H), 2.62 (dd, J = 11.3/3.4 Hz, 1 H, 2-H), 2.76–2.96 (m, 4 H, 3-H and NCH_3), 3.15 (s, 2 H, NC H_2CO_2Et), 3.59 (d, J = 14.7 Hz, 1 H, $PhCH_2N$), 3.70 (s broad, 4 H, $CH_2N(CH_3)CO$ and $COCH_2PhCl_2$), 3.85 (d, J = 14.2 Hz, 1 H, $PhCH_2N$), 4.09 (q, J = 7.1 Hz, 2 H, OCH_2CH_3), 7.15–7.21 (m, 2 H, arom), 7.28 (m, 4 H, arom), 7.42 (s broad, 1 H, 2-H $PhCl_2$), 7.46 (d, J = 8.2 Hz, 1 H, 5-H $PhCl_2$).- ^{13}C NMR ($CDCl_3$): δ = 14.5 (1 C, OCH_2CH_3), 34.2 (0.5 C, N CH_3 rotamer 1), 37.7 (0.5 C, NCH_3 rotamer 2), 40.2 1 C, $CH_2N(CH_3)CO$), 46.5 (0.5 C, $COCH_2PhCl_2$ rotamer 1), 47.7 (0.5 C, $COCH_2PhCl_2$ rotamer 2), 51.9 (1 C, C-5 or C-6), 52.9 (1 C, C-6 or C-5), 55.4 (1 C, C-2 and 0.5 C, C-3 rotamer 1), 58.1 (2 × 0.5 C, C-3 rotamer 2 and $PhCH_2N$ rotamer 1), 58.4 (0.5 C, $PhCH_2N$ rotamer 2), 59.7 (1 C, NCH_2CO_2Et), 60.8 (0.5 C, OCH_2CH_3 rotamer 1), 60.9 (0.5 C, OCH_2CH_3 rotamer 2), 127.2 and 127.6 (both 0.5 C, C-4 benzyl rotamer 1 and 2), 128.69 and 128.72 (both 2 × 0.5 C, C-3 and C-5 benzyl rotamer 1 and 2), 128.8 and 128.9 (both 2 × 0.5 C, C-2 and C-6 benzyl rotamer 1 and 2), 130.6, 130.7, 131.2, 131.3, 132.6 and 135.3 (6 C, $PhCl_2$), 136.2 (1 C, C-1 benzyl), 170.2, 170.4, 170.5 and 171.0 (all 0.5 C, 2 × CO_2Et rotamer 1 and 2).

*6.11. (+)-Ethyl 2-[(6S)-1,4,8-triazabicyclo[4.3.0]nonan-4-yl] acetate (**18**)*

An aqueous solution of formaldehyde (37%, 24 µl, 0.3 mmol) was added to a solution of **16b** (50 mg, 0.25 mmol) in ethanol (5 ml). The mixture was heated to reflux for 15 min, then stirred at 35 °C for 30 h. After removal of the solvent the residue was characterized without further purification. Pale yellow, viscous oil, yield 53 mg (99%), $[\alpha]_{589} = +11.9$ (c = 0.12, CH_2Cl_2).- $C_{10}H_{19}N_3O_2$ (213.28).- MS (EI): *m/z* (%) = 213 (M, 29), 170 (piperazinylethylacetate, 26), 140 (M – CO_2Et, 64), 97 (170 – CH_2CO_2Et, 100).- MS (CI): *m/z* (%) = 214 (MH^+, 100).- IR (film): ν (cm^{-1}) = 3387 (br, N–H), 2934 (m, C–H), 2814 (s, C–H), 1736 (s, C=O).- ^{1}H NMR ($CDCl_3$): δ = 1.26 (t, J = 7.2 Hz, 3 H, OCH_2CH_3), 2.18–2.25 (m, 1 H, 2-H, 3-H, 5-H

or 7-H), 2.41–2.59 (m, 4 H, 2-H, 3-H, 5-H, 6-H and/or 7-H), 2.82–2.97 (m, 4 H, 2-H, 3-H, 5-H, 6-H and/or 7-H), 3.22 (d, J = 5.2 Hz, 1 H, 9-H), 3.25 (s, 2 H, NC$H_2$$CO_2$Et), 3.93 (d, J = 5.4 Hz, 1 H, 9-H), 4.17 (q, J = 7.1 Hz, 2 H, OC$H_2$$CH_3$). The signal for the proton of the NH group could not be detected.

*6.12. (+)-Ethyl 2-{(6R)-8-[2-(3,4-dichlorophenyl)acetyl]-1,4,8-triazabicyclo[4.3.0]nonan-4-yl}-acetate (**19**)*

To a stirred and cooled (0 °C) solution of 3,4-dichlorophenylacetic acid (0.111 g, 0.54 mmol) in CH_2Cl_2 (5 ml), 1,1′-CDI (0.103 g, 0.63 mmol) was added under N_2 atmosphere. It was allowed to warm to room temperature and the reaction mixture was stirred for 2 h. Under N_2 atmosphere and cooling (ice bath) a cooled solution of **18** (0.096 g, 0.45 mmol) in CH_2Cl_2 (5 ml) was added slowly. The reaction mixture was stirred for 24 h at room temperature. The organic layer was washed with a saturated solution of $NaHCO_3$ (2 × 7 ml) and brine (7 ml), dried (Na_2SO_4) and concentrated in vacuo. FC purification of the residue (2 cm, ethyl acetate/ethanol, 1:1, 3 ml, R_f = 0.43) afforded **19** as a viscous oil. Yield 77 mg (43%), $[\alpha]_{589}$ = +12.1 (c = 0.195, CH_2Cl_2).- $C_{18}H_{23}Cl_2N_3O_3$ (400.30) calcd. C 54.01 H 5.79 N 10.50 found C 53.81 H 5.99 N 11.10.- MS (EI): *m/z* (%) = 403/401/399 (M, 4/24/36), 240 (M – dichlorobenzyl, 15), 212 (M – $COCH_2PhCl_2$, 27), 163/161/159 (dichlorobenzyl, 11/53/82), 140 (212 – CO_2Et, 100).- IR (film): ν (cm^{-1}) = 2937 (m, C–H), 2817 (m, C–H), 1739 (s, C=O ester), 1643 (s, C=O amide), 1030 (s, C–Cl), 821 (m, aryl-C–H).- ^{1}H NMR ($CDCl_3$): δ = 1.26 (t, J = 7.1 Hz, 3 × 0.45 H, OCH_2CH_3 rotamer 1), 1.27 (t, J = 7.2 Hz, 3 × 0.55 H, OCH_2CH_3 rotamer 2), 2.33 (dd, J = 10.8/8.9 Hz, 0.45 H, 5-H rotamer 1), 2,47 (dd, J = 11.0/8.0 Hz, 0.55 H, 5-H rotamer 2), 2.54–2.64 (m, 2 H, 2-H and 3-H both rotamers), 2.71–2.78 (m, 0.45 H, 6-H rotamer 1, and 0.55 H, 2-H rotamer 2), 2.84–3.03 (m, 0.55 H, 6-H rotamer 2, and 0.45 H, 2-H rotamer 1, and 2 H, 3-H and 5-H both rotamers), 3.17 (t, J = 10.7 Hz, 0.45 H, 7-H rotamer 1), 3.26 (s, 2 × 0.55 H, NC$H_2$$CO_2$Et rotamer 2), 3.27 (s, 2 × 0.45 H, NC$H_2$$CO_2$Et rotamer 1), 3.36 (t, J = 9.2 Hz, 0.55 H, 7-H rotamer 2), 3.49 (dd, J = 8.6/6.4 Hz, 0.55 H, 7-H rotamer 2), 3.52 (s, 2 × 0.45 H, $COCH_2PhCl_2$ rotamer 1), 3.56 (s, 2 × 0.55 H, $COCH_2PhCl_2$ rotamer 2), 3.67 (dd, J = 10.7/6.4 Hz, 0.45 H, 7-H rotamer 1), 3.82 (d, J = 7.1 Hz, 0.55 H, 7-H rotamer 2), 3.83 (d, J = 4.7 Hz, 0.45 H, 9-H rotamer 1), 4.18 (q, J = 7.1 Hz, 2 H, OC$H_2$$CH_3$), 4.46 (d, J = 4.9 Hz, 0.45 H, 9-H rotamer 1), 4.62 (d, J = 7.7 Hz, 0.55 H, 9-H rotamer 2), 7.10 (dd, J = 8.2/2.1 Hz, 1 H, 6-H arom), 7.37 (d, J = 8.3 Hz, 1 H, 5-H arom), 7.36 (s broad, 1 H, 2-H arom).- ^{1}H NMR (DMSO, 27 °C): δ = 1.17 (t broad, J = 6.3 Hz, 3 H, OCH_2CH_3), 2.30–2.56 (m, 3 H, 2-H, 3-H and 5-H), 2.61–2.68 (m, 1 H, 2-H and 6-H rotamers), 2.72–2.84 (m, 1 H, 2-H and 6-H rotamers, and 2 H, 3-H and 5-H), 2.96 (t, J = 10.6 Hz, 0.4 H, 7-H rotamer 1), 3.27 (s, 2 H, NC$H_2$$CO_2$Et), 3.40–3.70 (m, 0.6 H, 7-H rotamer 2, and 1 H, 7-H), 3.60 (s, 2 × 0.4 H, $COCH_2PhCl_2$ rotamer 1), 3.65 (s, 2 × 0.6 H, $COCH_2PhCl_2$ rotamer 2), 3.83 (d broad, J = 5.2 Hz, 1 H, 9-H both rotamers), 4.07 (q, broad, J = 6.6 Hz, 2 H, OC$H_2$$CH_3$), 4.38 (d, J = 7.3 Hz, 0.6 H, 9-H rotamer 2), 4.53 (d, J = 4.7 Hz, 0.4 H, 9-H rotamer 1), 7.21 (d, J = 7.6 Hz, 1 H, 6-H arom), 7.49 (s, 1 H, 2-H arom), 7.53 (d, J = 7.9 Hz, 1 H, 5-H arom).- ^{1}H NMR (DMSO, 160 °C): δ = 1.22 (t, J = 7.0 Hz, 3 H, OCH_2CH_3), 2.42–2.52 (m, 2 H, 2-H, 3-H and/or 5-H), 2.56–2.71 (m, 2 H, 2-H, 3-H, 5-H and/or 6-H), 2.80–2.90 (m, 3 H, 2-H, 3-H, 5-H and/or 6-H), 3.19 (d broad, J = 11.6 Hz, 1 H, 7-H), 3.25 (s, 2 H, NC$H_2$$CO_2$Et), 3.46 (d broad, J = 11.5 Hz, 1 H, 7-H), 3.62 (s, 2 H, $COCH_2PhCl_2$), 3.78–3.82 (m, 1 H, 9-H), 4.13 (q, J = 7.1 Hz, 2 H, OC$H_2$$CH_3$), 4.47 (d, J = 6.7 Hz, 1 H, 9-H), 7.23 (d broad, J = 8.2 Hz, 1 H, 6-H arom), 7.45 (s, 1 H, 2-H arom), 7.47 (d broad, J = 7.6 Hz, 1 H, 5-H arom).- ^{13}C NMR ($CDCl_3$): δ = 14.2 (1 C, OCH_2CH_3), 40.6 (0.55 C, $COCH_2PhCl_2$ rotamer 2), 40.7 (0.45 C, $COCH_2PhCl_2$ rotamer 1), 47.4 (1 C, C-7), 47.9 (1 C, C-3), 51.3 (0.45 C, C-2 rotamer 1), 51.6 (0.55 C, C-2 rotamer 2), 53.2 (0.55 C, C-5 rotamer 2), 54.0 (0.45 C, C-5 rotamer 1), 58.9 (1 C, NC$H_2$$CO_2$Et), 59.3 (0.45 C, C-6 rotamer 1), 60.2 (0.55 C, C-6 rotamer 2), 60.7 (1 C, OC$H_2$$CH_3$), 68.1 (0.55 C, C-9 rotamer 2), 68.3 (0.45 C, C-9 rotamer 1), 128.5 (0.45 C, C $PhCl_2$ rotamer 1), 128.6 (0.55 C, C $PhCl_2$ rotamer 2), 130.4 (1 C, C $PhCl_2$), 131.0 (0.45 C, C $PhCl_2$ rotamer 1), 131.1 (0.55 C, C $PhCl_2$ rotamer 2), 132.5, 134.4 and 134.5 (3 C, C $PhCl_2$), 167.1 (0.45 C, *C*=O amide rotamer 1), 167.9 (0.55 C, *C*=O amide rotamer 2), 170.1 (1 C, *C*=O ester).

7. Receptor binding studies

7.1. General information

Homogenizer: Potter®S (B. Braun Biotech International). – Ultraturrax: Euroturrax® T20 (Ika Labortechnik). – Centrifuge: High speed cooling centrifuge model J2-HS (Beckman). - Filter: Whatman glass fiber filters GF/B, presoaked in 0.5% polyethylenimine in water for 2 h at 4 °C before use. - Filtration was performed with a Brandel 24-well cell harvester. - Scintillation cocktail: Rotiscint Eco Plus (Roth). - Liquid scintillation analyzer: TriCarb 2100 TR (Canberra Packard), counting efficiency 66%. – All experiments were carried out in triplicates. - 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 [15]. – The K_i-values are given as mean value ± S.E.M. from three independent experiments.

7.2. Performance of the σ_1-assay

For the σ_1-assay guinea pig brain membranes were prepared as described in Ref. [13]. The test was performed with the radioligand [^{3}H]-pentazocine (1036 GBq $mmol^{-1}$; NEN™ Life Science Products). The thawed membrane preparation (about 150 μg of protein) was incubated with various concentrations of the test compound, 3 nM [^{3}H]-pentazocine, and buffer (50 mM Tris HCl, pH 7.4) in a total volume of 500 μl for 120 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 were 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.

7.3. NMDA, κ-opioid, μ-opioid, and σ_2 receptor assays

NMDA, κ-opioid, μ-opioid, and σ_2 receptor assays were performed as described in reference [5].

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