Eur. J. Med. Chem. 36 (2001) 211–214

PII: S0223-5234(00)01208-3/SCO

**Short communication**

# Synthesis and stereoselective κ-receptor binding of methylated analogues of GR-89.696

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Received 28 August 2000; revised 30 November 2000; accepted 1 December 2000

Dedicated to Prof. Dr. August W. Frahm on the occasion of his 65th birthday

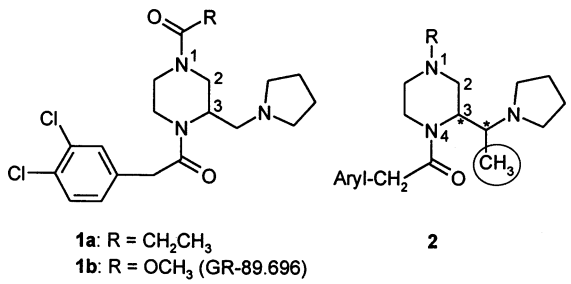
**Abstract** – Stereoselective synthesis of all four stereoisomers of methylated analogues **8** of the κ-receptor agonist GR-89.696 is presented. Starting with orthogonally protected piperazine derivatives (R,R)-**4** and (S,S)-**4**, the reaction sequence involves oxidation, reductive amination and modification of the piperazine nitrogen protective groups. The configuration of the stereocentre in α-position to the pyrrolidine moiety is determined by X-ray structure analysis of (R,S)-**8**. In receptor-binding studies with the radioligand U-69.593, the stereoisomer with (S)-configuration at both stereogenic centres (S,S)-**8** displayed the highest κ-receptor affinity with a $K_i$-value of 0.67 nM. © 2001 Éditions scientifiques et médicales Elsevier SAS

**κ-receptor agonists / 2-[1-(pyrrolidin-1-yl)ethyl]-piperazines / diastereoselective synthesis / X-ray crystal structure / stereoselective κ-receptor binding**

## 1. Introduction

In the brain and peripheral tissues, three distinct opioid receptor subtypes, μ, κ and δ, have been identified. It is well established that activation of each of these receptor subtypes can produce analgesic effects. Among ligands for these opioid receptor subtypes, κ-agonists are of particular interest, since only minimal physical dependence, respiratory depression and obstipation are associated with a strong analgesic profile. Apart from their analgesic effects, κ-agonists may play a role as neuroprotectants in certain animal models of cerebral ischaemia. However, application of κ-agonists may be limited by adverse side effects including sedation, dysphoria and strong diuresis [1, 2].

The 3-(pyrrolidin-1-ylmethyl) substituted 1,4-diacylpiperazines **1** belong to the most active and selective κ-receptor agonists. In the diamide series, the N1-propionyl-substituted piperazine **1a** revealed the highest κ-receptor affinity in the rabbit vas deferens functional in vitro assay ($IC_{50} = 6.9$ nM). A dramatic enhancement of the κ-receptor affinity by a factor of 170 was achieved with the bioisosteric carbamate **1b** (GR-89.696; $IC_{50} = 0.041$ nM), the κ-receptor affinity of which resides almost exclusively in the (R)-enantiomer [(R)-**1b**: $IC_{50} = 0.018$ nM] [3, 4] (*figure 1*).




**1a**: R = $CH_2CH_3$
**1b**: R = $OCH_3$ (GR-89.696)

**2**

**Figure 1.**

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In the 2-(aminomethyl)piperidine substance class of κ-agonists an additional methyl group in α-position to the dimethylamino group is, depending on the stereochemistry, able to enhance κ-receptor affinity [5]. Accordingly, we envisioned that yet unknown methylated (pyrrolidinylmethyl)piperidines or -piperazines of general structure **2** should be potent κ-receptor ligands. This prompted us to undertake the synthesis and in vitro testing of those compounds. One of the major issues of this study was to investigate the influence of the stereochemistry of the second chiral centre on κ-receptor affinity.

In this communication, we report on the stereoselective synthesis of all four stereoisomeric methylated (pyrrolinin-1-ylmethyl)piperazines **8**, the elucidation of their stereochemistry as well as on their κ-receptor affinities.

## 2. Chemistry

The synthesis of the piperazines **8** with (R)-configuration in position 3 starts from the proteinogenic amino acid (2S,3R)-threonine, which was transformed via the intermediate bicyclic piperazinedione (S,R)-**3** into the orthogonally protected (R,R)-configured 3-(1-hydroxyethyl)piperazine (R,R)-**4** according to literature [6]. During $LiAlH_4$ reduction of the bicyclic piperazinedione (S,R)-**3** the stereodescriptor denoting the stereocentre in position 3 is changed from (S) in **3** to (R) in the piperazines **4**–**8** since the priority of the substituents according to the CIP rules is changed (*figure 2*) [6].

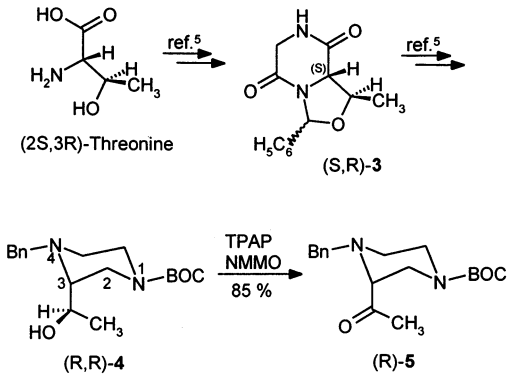


**Figure 2.**

The oxidation of the secondary alcohol (R,R)-**4** succeeded with a catalytic amount of tetrapropylammonium perruthenate ($Pr_4N^+$ $RuO_4^-$, TPAP) and an excess of the reoxidant *N*-methylmorpholine-*N*-oxide (NMMO) [7, 8] to provide the 3-acetylpiperazine (R)-**5** [9] in 85% yield.

Attempts to perform the reductive amination of the ketone (R)-**5** with pyrrolidine and $NaBH_3CN$ according to the procedure of Borch and coworkers [10] failed. However, a variation using the Lewis acid $Ti(OiPr)_4$ to accelerate iminium ion formation [11] led to the diastereomeric pyrrolidines (R,S)-**6** and (R,R)-**6** in the ratio 7:3. Flash chromatographic separation afforded the diastereomers (R,S)-**6** and (R,R)-**6** in 62 and 25% yield, respectively.

The last steps in the reaction sequence represent modifications of the piperazine nitrogen protective groups. At first, the benzyl protective group (Bn) of **6** was hydrogenolytically ($H_2$, Pd/C) [12] removed and, subsequently, the secondary amines were acylated with (3,4-dichlorophenyl)acetic acid (DCPAA) in the presence of 1,1'-carbonyldiimidazole (CDI) to yield the diastereomeric phenylacetamides (R,S)-**7** and (R,R)-**7**, respectively. Finally, the *tert*-butoxycarbonyl protective group (BOC) of **7** was cleaved with trifluoroacetic acid/$CH_2Cl_2$ [13]. The resulting crude secondary amines were acylated with propionyl chloride and NaOH in a biphasic system to afford the diacyl piperazines (R,S)-**8** and (R,R)-**8**, respectively (*figure 3*).

The respective (3S)-configured enantiomers (S,R)-**8** and (S,S)-**8** were prepared in an analogous manner starting with the enantiomeric amino acid (2R,3S)-threonine.

## 3. X-ray analysis

The absolute configuration of the new stereocentre generated by reductive amination of the ketone (R)-**5**, could not be determined unequivocally by means of NMR spectroscopy. However, X-ray suitable crystals of the major diastereomer (R,S)-**8** could be obtained by means of purification by flash chromatography and repeated recrystallisation from ethyl acetate. According to the X-ray structure analysis (*figure 4*), unlike configurations [14] could be established undoubtedly for the two adjacent stereocentres. Since the absolute configuration of the stereocentre in posi-

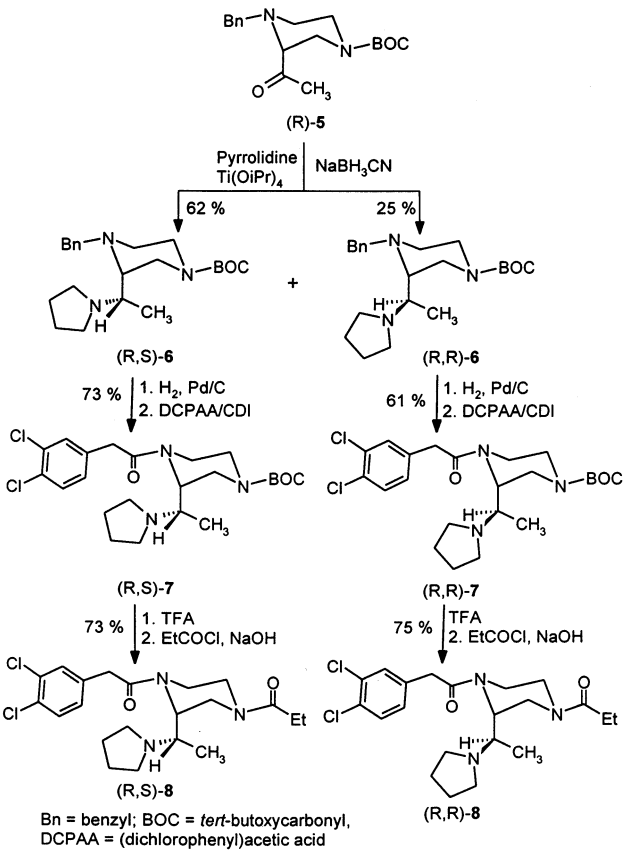


**Figure 3.**

tion 3 of the piperazine ring is known from the synthetic pathway to be (R), the newly generated side chain stereocentre has to be (S)-configured. Conclusively, and since the C-3 stereocentre remains unaffected in the reaction sequence from **6** to **8**, the stereochemistry of the intermediates [(R,S)-**7** and (R,S)-**6**)] and diastereomers [(R,R)-**6**, (R,R)-**7**, (R,R)-**8**] is established beyond any doubt.

In addition to elucidation of the configuration, the X-ray analysis indicates that the 1-(pyrrolidin-1-yl)ethyl residue in position 3 of the piperazine moiety adopts an axial orientation. In solution, the C-3 substituent is also most likely to be forced into the axial position because of the allylic 1,3-strain ($A^{1,3}$ strain) of the amide moiety [16]. This result is in accordance with the X-ray structure of piperazine and piperidine analogues with (aminomethyl) substituents [3, 17].

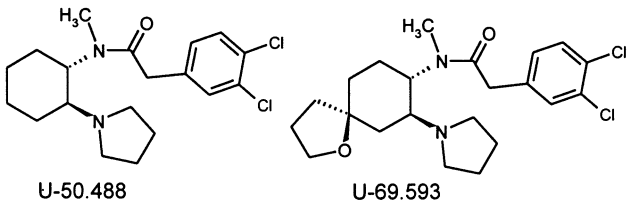


**Figure 5.**

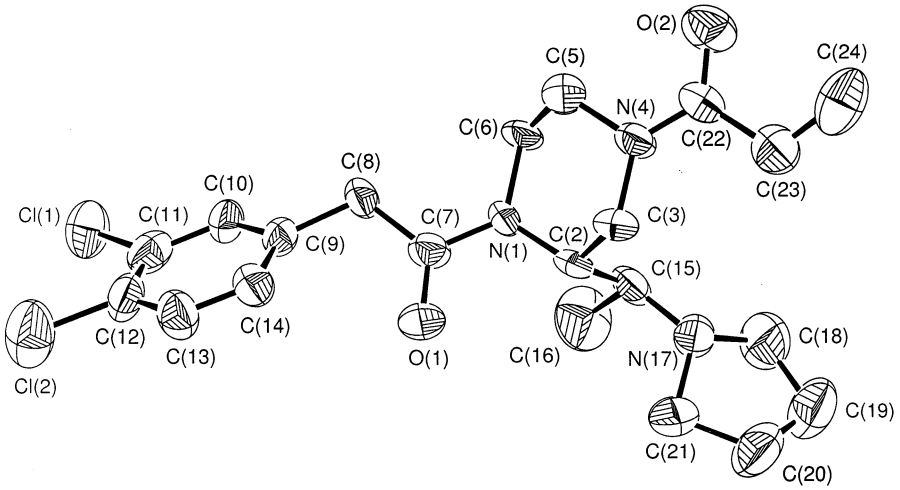


**Figure 4.** ORTEP drawing of the X-ray structure of (R,S)-**8** [15].

**Table I.** κ-Receptor affinities of the stereoisomeric piperazines **8**.

| Compound | $K_i$ (nM) ± S.E.M. |
|---|---|
| (R,S)-**8** | > 10 000 |
| (S,R)-**8** | 43 ± 6.1 |
| (R,R)-**8** | 4.2 ± 0.38 |
| (S,S)-**8** | 0.67 ± 0.17 |
| U-50.488 | 0.49 ± 0.16 |

## 4. Receptor-binding studies

In order to determine the κ-receptor affinities of the stereoisomeric piperazines **8**, in vitro receptor-binding assays were performed using guinea-pig brain membrane preparations as receptor material [18]. κ-Receptors were labelled with the radioligand [$^3$H]-U-69.593 and non-specific binding was defined with an excess of U-50.488 (*figure 5*) [19, 20].

The $K_i$-values of the stereoisomeric piperazines **8**, which are summarised in *table I*, reveal that in this substance class the κ-receptor affinity is strongly dependent on the stereochemistry.

Thus, the *like*-configured [14] isomers (R,R)-**8** and (S,S)-**8** show much higher affinities when compared to the *unlike* stereoisomers (R,S)-**8** and (S,R)-**8**. In both diastereomeric pairs, the enantiomers with (3S)-configuration display higher κ-receptor affinities than their (3R)-enantiomers. This observation is in accordance with the reported higher κ-receptor affinity of (R)-**1b** [3, 4]. [Note, that the stereochemistry in position 3 of (S,S)-**8** and (S,R)-**8** is equivalent to the stereochemistry of (R)-**1b**, since the priority of the substituents according to the CIP rules is changed.] Comparison of the κ-receptor affinities of the diastereomers (S,S)-**8** ($K_i = 0.67$ nM) and (S,R)-**8** ($K_i = 43$ nM) indicates that (S)-configuration in the side chain increases κ-receptor affinity. Thus, combination of (S)-configuration in the piperazine ring system with (S)-configuration in the side chain provides (S,S)-**8** with the highest κ-receptor affinity.

## Acknowledgements

We gratefully acknowledge the financial support of the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. Thanks are also due to the Degussa AG for donation of chemicals.

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