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# Stereoselective synthesis of conformationally restricted KOR agonists based on the 2,5-diazabicyclo[2.2.2]octane scaffold

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## Abstract

It has been postulated that the KOR affinity depends on the dihedral angle of the ethylenediamine pharmacophore. Herein, 2,5-diazabicyclooctanes bearing a pyrrolidino moiety in 7-position were envisaged to study KOR agonists with a conformationally rigid ethylenediamine pharmacophore and thus a defined N(pyrrolidine)-C7-C1-N2 dihedral angle. The first approach with an intramolecular addition at the chiral sulfinylimines **9** failed to give bicyclic products. The key step in the second approach was a Dieckmann analogous cyclization providing mixed methyl silyl ketals **11a**-**e** as key intermediates. The highest KOR affinity was found for the 2,5-dibenzyl substituted derivatives (*S*,*R*,*S*)-

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**16a** ($K_i$ = 31 nM) and (*R*,*S*,*R*)-**16a** ($K_i$ = 74 nM) with the pyrrolidine ring oriented towards N-5. The high KOR affinity of (*S*,*R*,*S*)-**16a** is unexpected, since the KOR pharmacophoric ethylenediamine system adopts a dihedral angle of about 160°, which is quite different from the angle of the energetically most favored conformer of the flexible and potent KOR agonist **2**. (*S*,*R*,*S*)-**16a** represents a KOR agonist with moderate selectivity over MOR (8-fold) and DOR (5-fold), but high selectivity over both σ receptor subtypes. In the [$^{35}$S]GTPγS assay (*S*,*R*,*S*)-**16a** reacted as full KOR agonist with an $EC_{50}$ value of 240 nM.

**Keywords:** KOR agonists; 2,5-diazabicyclo[2.2.2]octanes; chiral pool synthesis; conformational restriction; ethano bridge; structure affinity relationships; receptor selectivity; dihedral angle; [$^{35}$S]GTPγS assay

## 1. Introduction

Behavioral studies with chronic spinal dogs in 1976 resulted in differentiation of the opioid receptor into three subtypes, which were termed μ-opioid receptor (MOR, agonist morphine), σ-opioid receptor (agonist SKF-10,047) and κ-opioid receptor (KOR, agonist ketocyclazocine).[1] Later, the σ receptor was removed from the class of opioid receptors and the δ-opioid receptor (DOR, vas deferens) and the opioid receptor-like 1 (ORL1) receptor (NOR) were added to the class of opioid receptors.[2] In the beginning of the 1990ies, the four opioid receptors were cloned. The homology of the four opioid receptors is higher than 60 % on the level of amino acid sequences.[3] The opioid receptors belong to the γ subfamily of class A (rhodopsin-like) G-protein coupled receptors (GPCRs) and are coupled to $G_i$-proteins.[4] The KOR,[4] MOR,[5] DOR,[6] and NOR receptors[7] were crystallized with various ligands leading to interesting information about the three dimensional structure of the receptors and their binding sites.

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The molecular structure of the T4-lysozyme construct of the human KOR in complex with the antagonist JDTic ((*R*)-7-hydroxy-N-{(*S*)-2-[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethylpiperidin-1-yl]-1-isopropylethyl}-1,2,3,4-tetrahydroisoquinoline-3-carboxamide) was determined as homodimer with a resolution of 2.9 Å. It allows a precise insight into the binding pocket occupied by the KOR antagonist JDTic. The salt bridge between two protonated amino moieties of JDTic and the carboxy moiety of Asp138 represents the key interaction stabilizing binding of the ligand to the binding pocket. Whereas Asp138 is conserved in all opioid receptors ("message" structure),[8] lipophilic interactions of the ligand with lipophilic side chains of the amino acids Val108, Val118, Ile294 and Tyr312 exclusively found in the KOR are responsible for the subtype selectivity ("address" features).[4,8]

The strong analgesia mediated by activation of KOR, MOR and DOR opioid receptors is accompanied by specific receptor subtype side effects. Activation of MOR can cause respiratory depression, physical dependence and reduced gastrointestinal motility. Although these very problematic side effects are not observed after activation of KOR, undesirable centrally mediated side effects such as dysphoria, sedation and diuresis are mediated by KOR agonists.[9]

The known KOR agonists belong to four compound classes: peptides derived from the endogenous KOR agonist dynorphin A (1-17),[10] morphinoids with ketocyclazocine, nalbuphine and buprenorphine as typical representatives,[11] the natural product salvinorin A isolated from the leaves of S*alvia divinorum*[12] and ethylenediamines with U-50,488 as the first synthetic KOR agonist.[13] (Figure 1) With exception of salvinorin A

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and its derivatives, KOR agonists contain a basic amino group which forms the crucial ionic interaction with Asp138.[4]

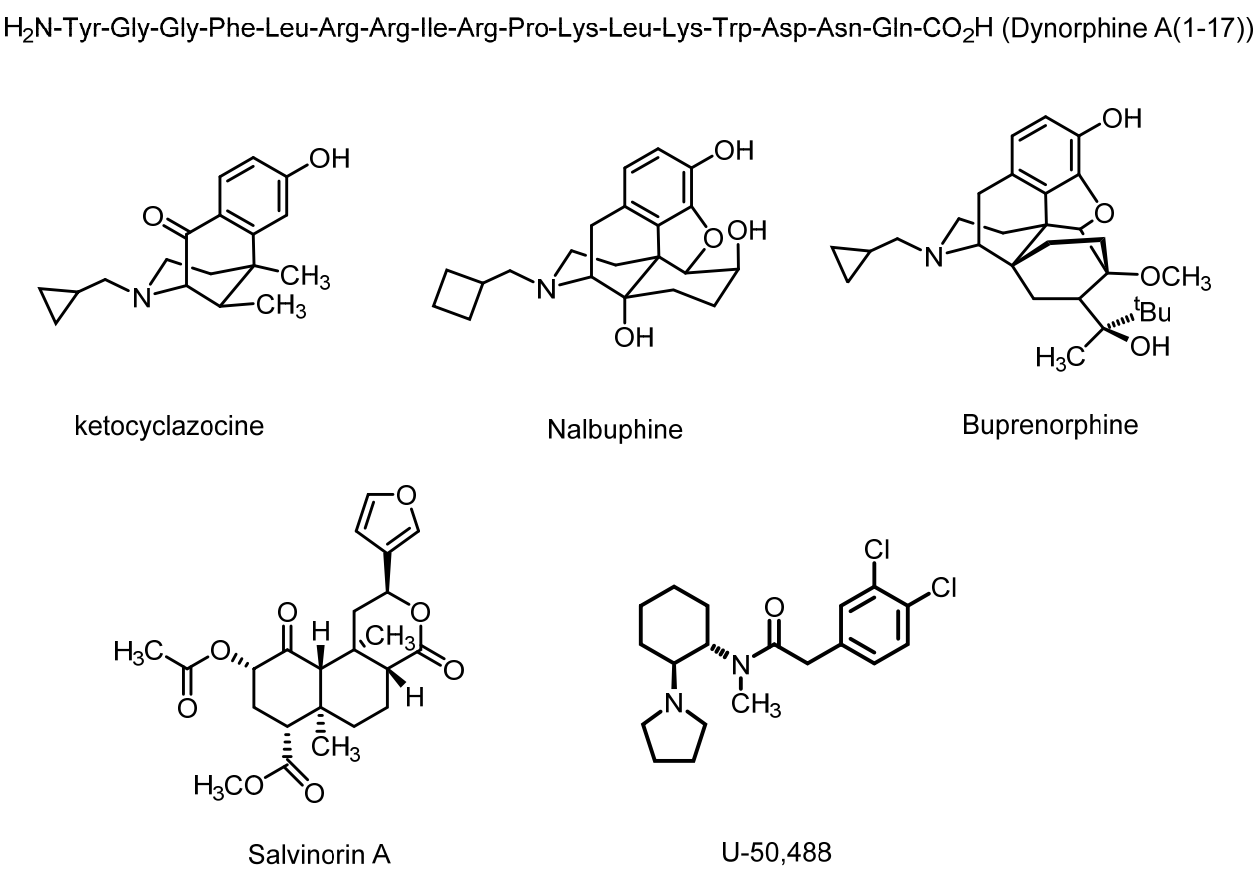


Figure 1: Prominent KOR agonists.

In case of ethylenediamine KOR agonists the pyrrolidine ring at one end of the ethylene moiety is featuring the basic functional group. Usually the second N-atom of the central ethylenediamine unit is bearing the (3,4-dichloropheyl)acetyl moiety representing the second pharmacophoric substructure of this type of KOR agonists. In addition to U-50,488, the piperazine derivative GR-89,696 (**1**) follows this principle.[14] The piperazine **1** with the axially oriented pyrrolidinomethyl moiety in 2-position shows very high KOR affinity ($K_i$ = 0.45 nM).[15] Introduction of an additional methyl group into the axially

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oriented substituent led to the pyrrolidinoethyl derivative **2** with even higher KOR affinity ($K_i$ = 0.31 nM).[15] (Figure 2)

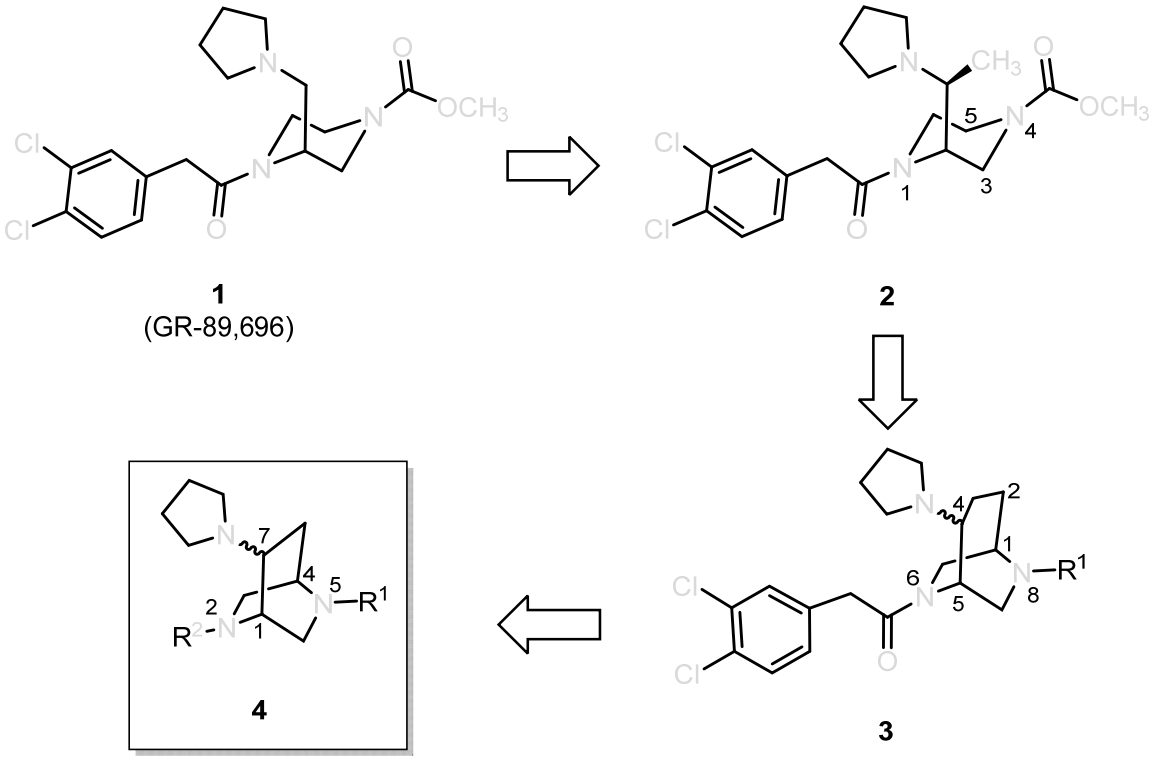


Figure 2: Design of conformationally restricted KOR agonists **4**.

Due to free rotation around the axially oriented C-C-bond, the pyrrolidine ring of piperazines **1** and **2** can adopt several orientations relative to the (dichlorophenyl)acetamido moiety. Calculations of the energy depending on the N(pyrrolidine)-C-C-N(acyl) dihedral angle resulted in an energy minimum at around 70°.[16] In order to learn more about the bioactive conformation of the rather flexible KOR agonists **1** and **2**, we decided to synthesize and pharmacologically evaluate KOR agonists with reduced conformational flexibility around the axial C-C-bond by connecting the methyl moiety of **2** with the 5-position of the piperazine ring (2,5-bridged piperazines). Recently, we have reported on the synthesis of piperazines **3** with a three-carbon bridge across the piperazine ring. The (1*R*,4*R*,5*S*)-configured stereoisomer (*R*,*R*,*S*)-**3a** ($R^1$ = $CO_2CH_3$) reveals high KOR affinity with a $K_i$ value of 1.0 nM. The three-

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carbon bridge of (*R*,*R*,*S*)-**3** can adopt two energetically favored conformations resulting in dihedral angles of 45° and 97° of the ethylenediamine pharmacophore.[17]

## 2. Results and discussion

### 2.1. Conformational analysis

Herein, we report the synthesis and pharmacological evaluation of piperazines **4** with a two-carbon bridge instead of the three-carbon bridge of **3**. Theoretical calculations led to N(pyrrolidine)-C-C-NR dihedral angles of 69° and 159° for the diastereomers with (*R*)- and (*S*)-configuration at 7-position, respectively. (Figure 3) In particular the dihedral angle of (1*S*,4*R*,7*R*)-**4a** is between the dihedral angles calculated for the conformers of (*R*,*R*,*S*)-**3**[17] and is close to the dihedral angle of the energetically most favored conformer of **2** with (*S*,*S*)-configuration at both centers of chirality.[16]

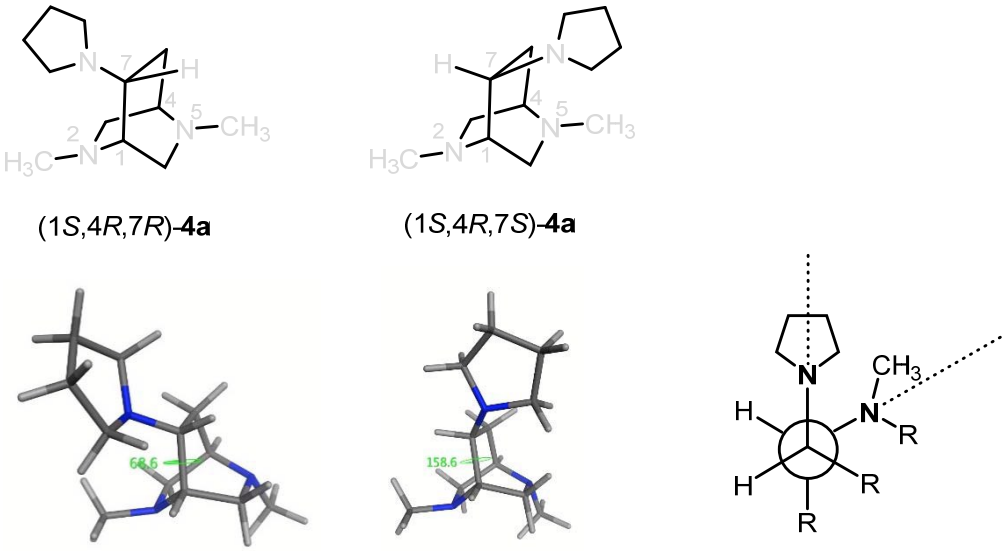


Figure 3: Calculated dihedral angles of the KOR pharmacophoric substructure N(pyrrolidine)-C-C-N($CH_3$) of diastereomeric model compounds. (1*S*,4*R*,7*R*)-**4a**: 69°; (1*S*,4*R*,7*S*)-**4a**: 159°. Right: Definition of the dihedral angle.

### 2.2. Synthesis plan

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Two strategies were pursued for the synthesis of the appropriately substituted bicyclic compounds **4**. (Figure 4) In a chiral-pool synthesis (*R*)-aspartate should be converted into piperazinediones **6** with an acetate side chain. After transformation of the ester **6** into the sulfinylimine **9**, intramolecular addition of an enolate at the activated imino moiety should give diazabicyclo[2.2.2]octanes **10**. The last steps comprise establishment of the pyrrolidine moiety, reduction of the lactam groups and modification of the substituents at the N-atoms.

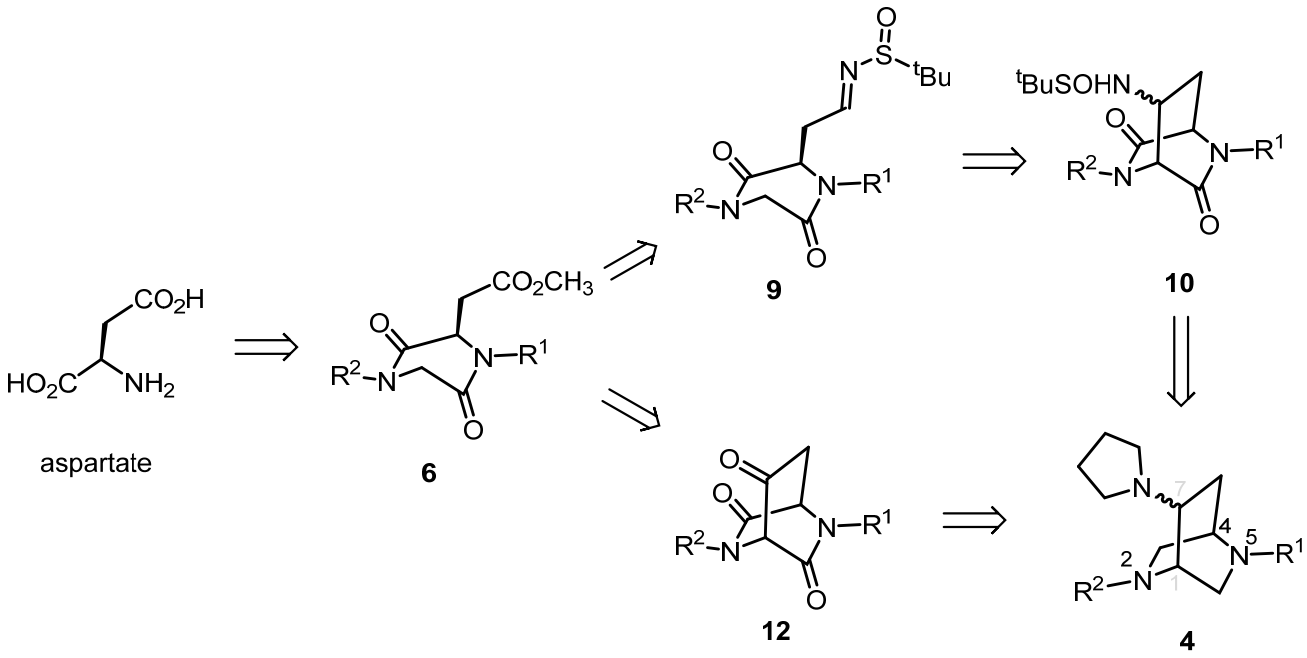
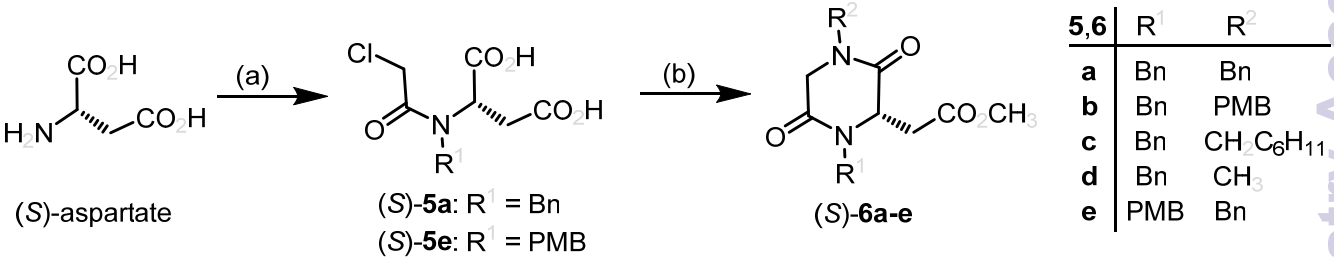



Figure 4: Plans for the synthesis of conformationally constrained KOR agonists **4** depicted with (*R*)-aspartate as starting material.

According to an alternative plan, the ester **6** will be used in a Dieckmann cyclization resulting in the tricarbonyl compound **12**. The keto group in 7-position of **12** should be converted into the pyrrolidine ring and the lactam groups of the piperazinedione system should be reduced to end-up with **4**.

## 2.3. Synthesis

In Scheme 1 the synthesis of piperazinediones (*S*)-**6a**-**e** with an acetate side chain is shown starting with the proteinogenic amino acid (*S*)-aspartate. The chloroacetamides (*S*)-**5a** and (*S*)-**5e** were obtained in four reaction steps comprising esterification, imine formation, $NaBH_4$ reduction and acylation.[18,19] Various primary amines reacted with the chloroacetamides (*S*)-**5a** and (*S*)-**5e** in a domino reaction starting with a nucleophilic substitution followed by intramolecular aminolysis to provide the piperazinediones (*S*)-**6a**-**e** in 61-83 % yield.[18] (Scheme 1)




| **5**,**6** | $R^1$ | $R^2$ |
|---|---|---|
| **a** | Bn | Bn |
| **b** | Bn | PMB |
| **c** | Bn | $CH_2C_6H_{11}$ |
| **d** | Bn | $CH_3$ |
| **e** | PMB | Bn |

Scheme 1: Synthesis of (dioxopiperazinyl)acetates **6** with diverse substituents at the N-atoms. Reagents and reaction conditions: (a) 4 steps: 1. $CH_3OH$, TMS-Cl; 2. PhCH=O or 4-$CH_3O(C_6H_4)CH$=O; 3. $NaBH_4$; 4. $ClCH_2COCl$. (b) $R^2$-$NH_2$, $NEt_3$, $CH_3CN$, reflux, 61 – 83 %. (*S*)-**6c** and (*S*)-**6d** have already been reported in ref.[18] The enantiomer (*R*)-**6a** was prepared in the same manner starting from (*R*)-aspartate.

The sulfinylimines **9** represent the key intermediates in the first strategy. Ester (*S*)-**6e** was converted into the *N*,*O*-dimethylhydroxamic acid (*S*)-**7** upon treatment with *N*,*O*-dimethylhydroxylamine and $Al(CH_3)_3$. Reduction of (*S*)-**7** with $LiAlH_4$ led to the aldehyde (*S*)-**8**, which was condensed with *Ellman's* chiral sulfinamide ((*R*)-2-methylpropane-2-sulfinamide)[20,21] and $Ti(OEt)_4$ to give the sulfinylimine (*S*,*R*)-**9**. (Scheme 2).

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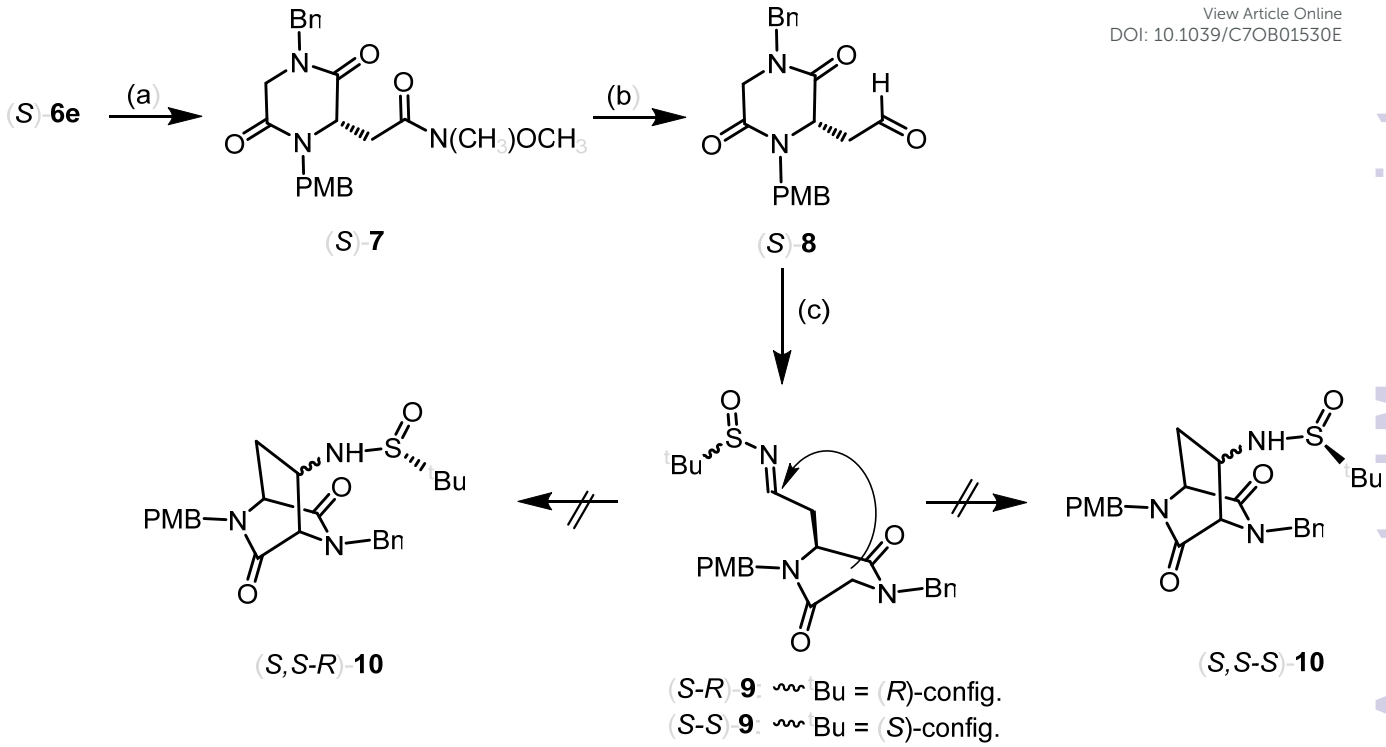


Scheme 2: Synthesis of sulfinylimines **9** and attempts to obtain bicyclic systems **10**. Reagents and reaction conditions: (a) $HN(CH_3)OCH_3 \cdot HCl$, $Al(CH_3)_3$, $CH_2Cl_2$, rt, 4 h, 97 %. (b) $LiAlH_4$, THF, -78 °C, 16 h, 49 %. (c) (*R*)-$^t$BuS(=O)$NH_2$ or (*S*)-$^t$BuS(=O)$NH_2$, $Ti(OEt)_4$, THF, rt, 3.5 h, 43 % ((*S*-*R*)-**9**); 64 % ((*S*-*S*)-**9**).

Several reaction conditions were investigated to induce the intramolecular addition at the sulfinylimine. Various bases (e.g. LiHMDS, NaHMDS, KO$^t$Bu, 1-6 equivalents) at various temperatures (-78 °C to 25 °C) as well as various additives (e.g. $BF_3$, $Ti(OEt)_4$,$ClSiMe_3$) were tried. However, the bicyclic compound **10** was not formed. In order to exclude that (*S*,*R*)-**9** represents the mismatched configured diastereomer for this conversion, the diastereomer (*S*,*S*)-**9** was prepared by condensation of the aldehyde (*S*)-**8**, with (*S*)-configured *Ellman's* chiral sulfinamide ((*S*)-2-methylpropane-2-sulfinamide). However, as observed for (*S*,*R*)-**9** the intramolecular nucleophilic addition at the sulfinylimine (*S*,*S*)-**9** to form 2,5-diazabicyclo[2.2.2]octanes **10** could not be observed. (Scheme 2)

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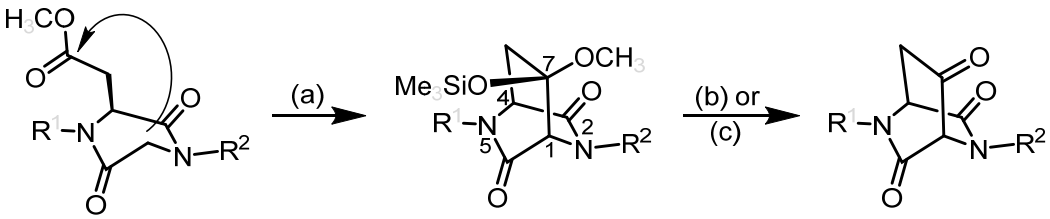


(*S*)-**6a**-**e** (*S*,*S*,*R*)-**11a**-**e** (*S*,*S*)-**12a**-**c**

| **11** | $R^1$ | $R^2$ | yield |
|---|---|---|---|
| **a** | Bn | Bn | 39 % |
| **b** | Bn | PMB | 12 % |
| **c** | Bn | $CH_2C_6H_{11}$ | 15 % |
| **d** | Bn | $CH_3$ | 34 %[19] |
| **e** | PMB | Bn | 0.3 % |

Scheme 3: Synthesis of 2,5-diazabicyclo[2.2.2]octane-3,6,7-triones **12** by Dieckmann analogous cyclization of **6**. Reagents and reaction conditions: (a) NaHMDS, THF, -78 °C, 45 min, then TMS-Cl, -78 °C, 1 h, rt, 2 h, yields are given in Scheme. (b) *p*TosOH, THF, $H_2O$, rt, 16 h, 70 % ((*S*,*S*)-**12a**), 95 % ((*S*,*S*)-**12b**). (c) HCl, THF, rt, 16 h, 74 % ((*S*,*S*)-**12c**). The enantiomers (*R*,*R*,*S*)-**11a** and (*R*,*R*)-**12a** were prepared in the same manner starting from (*R*)-configured piperazinedione (*R*)-**6a**.

According to the second strategy, a *Dieckmann* cyclization of the piperazinediones (*S*)-**6** should give the bicyclic compounds (*S*,*S*)-**12**. (Scheme 3) However, reaction of the piperazinediones (*S*)-**6** with various bases did not lead to bicyclic tricarbonyl compounds (*S*,*S*)-**12**, since the cyclization product could not be stabilized by deprotonation. Therefore, the piperazinediones (*S*)-**6** were treated with NaHMDS at -78 °C and the primary cyclization products, the anion of hemiketals, was trapped with chlorotrimethylsilane to yield the mixed methyl silyl ketals (*S*,*S*,*R*)-**11**. The yields of this transformation were strongly dependent on the substitution pattern of the piperazinediones (*S*)-**6**. As reported previously, (*S*,*S*,*R*)-**11d** with a methyl moiety at N-2 was obtained in 34 % yield.[19] The dibenzyl derivative (*S*,*S*,*R*)-**11a** was isolated in slightly higher yield (39 %) after careful optimization, but a cyclohexylmethyl moiety at

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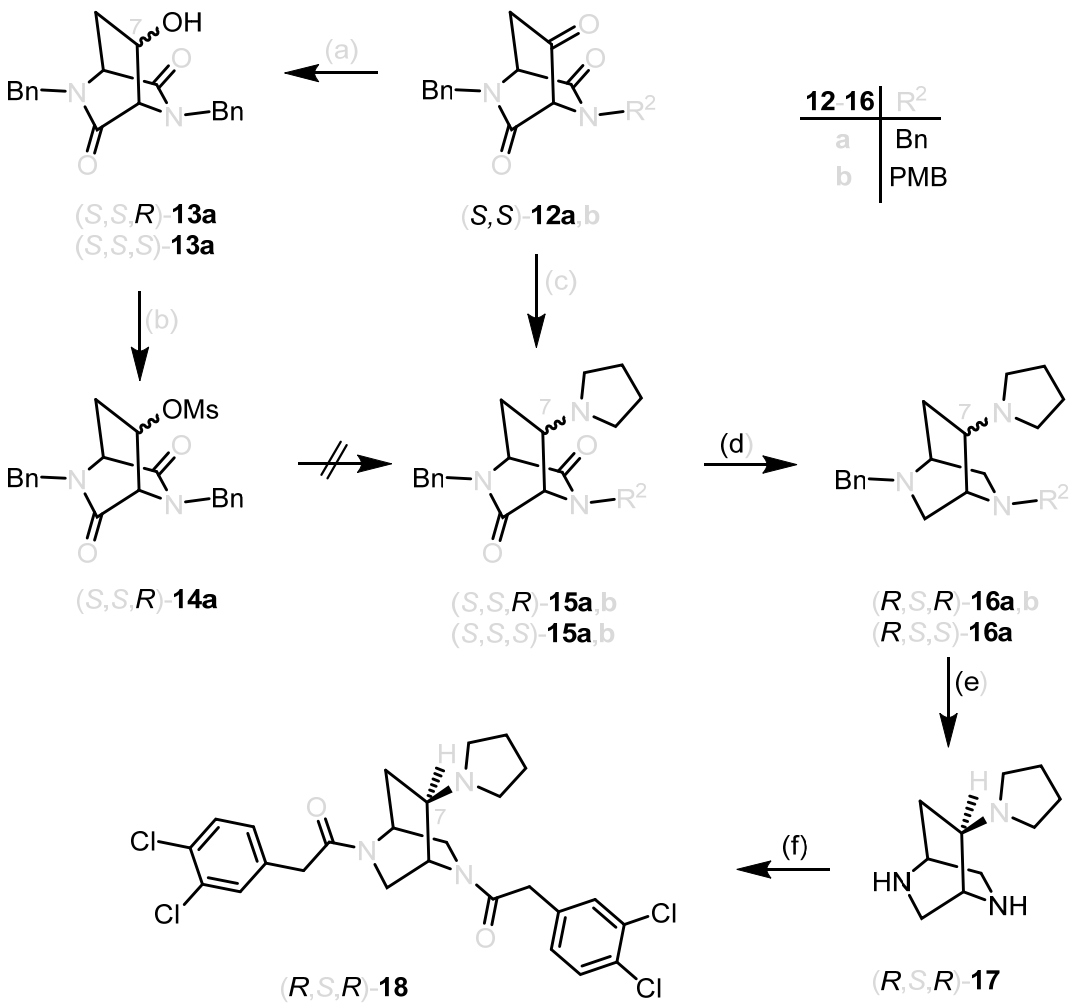
N-2 led to considerably reduced yields ((*S*,*S*,*R*)-**11c**, 15 % yield). The piperazinediones (*S*)-**6b** and (*S*)-**6e** with one *p*-methoxybenzyl moiety were prepared in order to have orthogonal protective groups at the N-atoms. Despite intensive optimization, the mixed methyl silyl ketals (*S*,*S*,*R*)-**11b** and (*S*,*S*,*R*)-**11e** were obtained in only 12 % and 0.3 % yield, respectively. It was concluded that the *p*-methoxybenzyl moiety in particular at N-5 is detrimental for this cyclization reaction. Hydrolysis of the mixed methyl silyl ketals (*S*,*S*,*R*)-**11a**-**c** provided the ketones (*S*,*S*)-**12a**-**c** on 70-95 % yields.




Scheme 4: Synthesis of 7-pyrrolidino substituted 2,5-diazabicyclo[2.2.2]octanes containing KOR-pharmacophoric structural elements. Reagents and reaction conditions: (a) $NaBH_4$, $CH_3OH$, 5-10 °C, 30 min, 84 %. (b) $CH_3SO_2Cl$, $CH_2Cl_2$, $NEt_3$, DMAP, rt, 16 h, 78 %. (c) Pyrrolidine, $NaBH(OAc)_3$, THF, AcOH, rt, 3 d or 16 h, fc

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separation, 56 % (*S*,*S*,*R*)-**15a** and 6 % (*S*,*S*,*S*)-**15a**; 79 % (*S*,*S*,*R*)-**15b** and 8 % (*S*,*S*,*S*)-**15b**. (d) $LiAlH_4$, THF, reflux, 16 h, 30-67 %. (e) $H_2$, balloon, Pd/C, THF, rt, 16 h, 74 %. (f) 2-(3,4-dichlorophenyl)acetyl chloride, $CH_2Cl_2$, rt, 16 h, 45 %. The enantiomers (*S*,*R*,*S*)-**16a**, (*S*,*R*,*R*)-**16a** and (*S*,*R*,*S*)-**18** were prepared in the same manner starting from (*R,R*)-configured trione (*R,R*)-**12a**.

$NaBH_4$ reduction of the ketone (*S*,*S*)-**12a** with two benzyl moieties led to a mixture of diastereomeric alcohols (*S*,*S*,*R*)-**13a** and (*S*,*S*,*S*)-**13a** (ratio 80:20), which was reacted with methanesulfonyl chloride. (Scheme 4) After chromatographic purification, only the major diastereomer (*S*,*S*,*R*)-**14a** was isolated in 78 % yield. However, all attempts failed to convert the mesylate (*S*,*S*,*R*)-**14a** into pyrrolidine derivatives **15a**.

Next, the direct reductive amination of the ketone (*S*,*S*)-**12a** with pyrrolidine and $NaBH(OAc)_3$[22] was investigated. This transformation was successful only after addition of an acid. The yields and the ratio of diastereomers obtained with the additives HOAc ((*S*,*S*,*R*)-**15a**: 56 %; (*S*,*S*,*S*)-**15a**: 6 %) and $Ti(OiPr)_4$ ((*S*,*S*,*R*)-**15a**: 55 %; (*S*,*S*,*S*)-**15a**: 9 %) were very similar. Therefore, HOAc, which could be handled and removed easily, was used preferably for the synthesis of the pyrrolidines (*S*,*S*,*R*)-**15a** and (*S*,*S*,*S*)-**15a** in larger amounts, for the synthesis of the enantiomeric pyrrolidines (*R*,*R*,*S*)-**15a** and (*R*,*R*,*R*)-**15a** and the *p*-methoxybenzyl substituted analogs (*S*,*S*,*R*)-**15b** and (*S*,*S*,*S*)-**15b**. (Scheme 4)

Reduction of the dilactams (*S*,*S*,*R*)-**15a**,**b** and (*S*,*S*,*S*)-**15a** with $LiAlH_4$ led to the diazabicyclooctanes (*R*,*S*,*R*)-**16a**,**b** and (*R*,*S*,*S*)-**16a** in 30-67 % yields. Hydrogenolytic removal of the benzyl moieties of the major diastereomer (*R*,*S*,*R*)-**16a** resulted in the unsubstituted bicyclic compound (*R*,*S*,*R*)-**17**, which was acylated with

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(dichlorophenyl)acetyl chloride to afford the bis[(dichlorophenyl)acetamide] (*R*,*S*,*R*)-**18** (Scheme 4)

The enantiomers (*S*,*R*,*S*)-**16a**, (*S*,*R*,*R*)-**16a**, and (*S*,*R*,*S*)-**18** were prepared in the same manner starting from the enantiomeric amino acid (*R*)-aspartate.

### 2.4. Structure determination

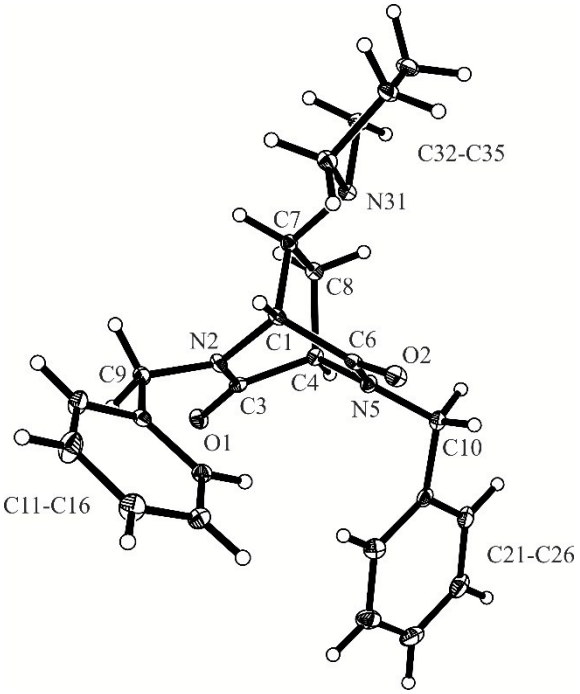


Figure 5: Molecular structure of (*R*,*R*,*S*)-**15a**, the main diastereomer after reductive amination of ketone (*R*,*R*)-**12a** with pyrrolidine and $NaBH(OAc)_3$. Thermal ellipsoids are shown at 30 % probability. P43 chiral space group with Flack parameter 0.01(5). Dihedral angles: N31-C7-C1-N2: 179,5(1)°; H7-C7-C1-N2: 59.0°.

The configuration of the major diastereomer obtained by reductive amination of the ketone (*R*,*R*)-**12a** was assigned unequivocally by recording an X-ray crystal structure, which is shown in Figure 5. The crystal structure displays the pyrrolidine ring in anti-periplanar orientation to N-2 of the bicyclic framework. This orientation leads to (*S*)-configuration of the newly formed center of chirality in 7-position. (*S*)-configuration in 7-

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position originates from *Re*-face attack of the reducing agent at the intermediate iminium ion. This result fits nicely to the hypothesis that the large benzyl moiety at 5-position inhibits the attack of the nucleophile along the Bürgi-Dunitz trajectory of 107° from the *SI*-face.[17] The dihedral angle of the ethylenediamine pharmacophore in the solid state is 179.5°.

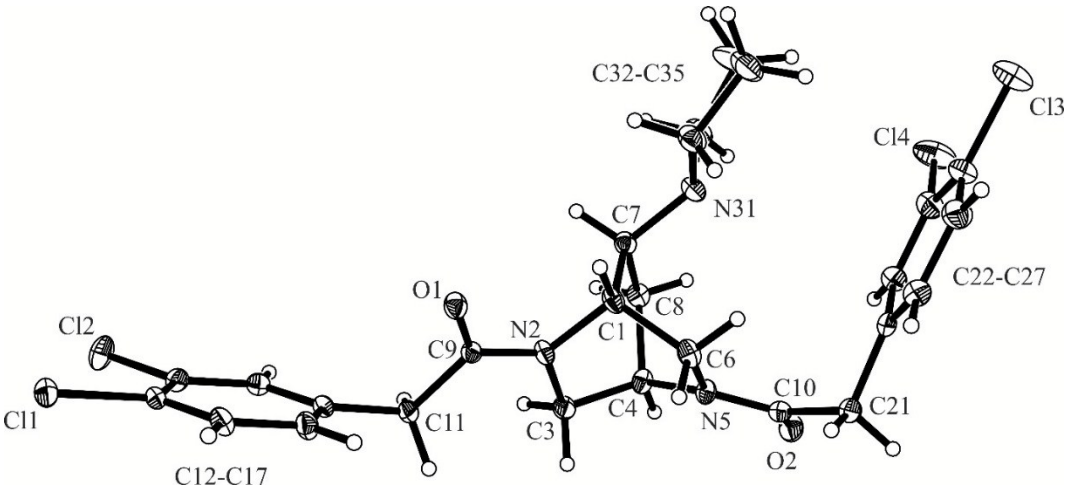


Figure 6: Molecular structure of racemic **18**. Thermal ellipsoids are shown at 30 % probability. The crystal contains both enantiomers in the ratio 1:1. Centrosymmetric space group P2/c. Dihedral angles: N31-C7-C1-N2: 178.4(3)°; H7-C7-C1-N2: - 61.0°.

Analysis of the structure of diamides (*S*,*R*,*S*)-**18** and (*R*,*S*,*R*)-**18** by $^{1}$H NMR spectroscopy was difficult due to broad signals caused by rotational isomerism along the N-C(=O) bonds. Therefore, an X-ray crystal structure was recorded. Due to partial racemization of diamide (*S*,*R*,*S*)-**18** during the synthesis, a racemic mixture (*S*,*R*,*S*)-**18**/(*R*,*S*,*R*)-**18** was crystallized; the elemental unit contained the enantiomers in a 1:1 ratio. The molecular structure of **18** shows two acyl moieties at the ring N-atoms confirming the constitution of the final product. (Figure 6) Moreover, the orientation of the pyrrolidine ring towards N-5 of the bicyclic framework was proved by the crystal structure. The amide moieties together with the adjacent substituents form an almost

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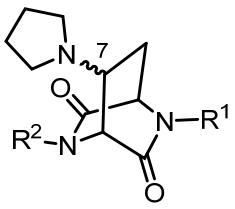
planar structure. According to the molecular structure, the ethylenediamine pharmacophoric system adopts a dihedral angle of 178.4°.

## 2.5. Pharmacological evaluation

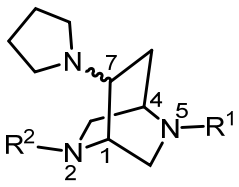
*KOR affinity*

Competitive radioligand receptor binding studies were used to determine the KOR affinity of 2,5-diazabicyclocotanes **15**-**18**. [$^3$H]U-69,593 served as radioligand and guinea pig brain membrane preparations as receptor material. A large excess of U-69,593 (10 µM) was used to determine the non-specific binding of the radioligand in the assay.[16,17] Table 1 summarizes the KOR affinity recorded for the test compounds **15**-**18** and the reference compounds **2**, U-69,593, naloxone and morphine.

Table 1: Affinities of 2,5-diazabicyco[2.2.2]octanes with pyrrolidine moiety towards KOR and related receptors.



**15**



**16**, **18**

| Compd. | $R^1$ | $R^2$ | $K_i$ ± SEM (nM)[a,b] | | | | |
|---|---|---|---|---|---|---|---|
| | | | KOR[b)] | MOR[c)] | DOR[c)] | $\sigma_1$ | $\sigma_2$ |
| | | | [$^3$H]U-69,593 | [$^3$H]DAMGO | [$^3$H]DPDPE | [$^3$H]-(+)-pentazocine | [$^3$H]DTG |
| (*S*,*S*,*R*)-**15a** | Bn | Bn | 0 % | 24 % | 8 % | 0 % | 0 % |
| (*S*,*S*,*S*)-**15a** | Bn | Bn | 0 % | 0 % | 4 % | 0 % | 0 % |
| (*R*,*R*,*S*)-**15a** | Bn | Bn | 0 % | 0 % | 0 % | 0 % | 0 % |
| (*R*,*R*,*R*)-**15a** | Bn | Bn | 4 % | 0 % | 16 % | 0 % | 5 % |
| (*S*,*S*,*R*)-**15b** | Bn | PMB | 5 % | 38 % | 12 % | 0 % | 4 % |
| (*S*,*S*,*S*)-**15b** | Bn | PMB | 0 % | 0 % | 3 % | 0 % | 3 % |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| (*R*,*S*,*R*)-**16a** | Bn | Bn | 74 ± 14 | >1 µM | 318 | 755 | >1 µM |
| (*R*,*S*,*S*)-**16a** | Bn | Bn | 0 % | 25 % | >1 µM | 13 % | >1 µM |
| (*S*,*R*,*S*)-**16a** | Bn | Bn | 31 ± 9 | 241 | 146 | 882 | >1 µM |
| (*S*,*R*,*R*)-**16a** | Bn | Bn | 0 % | 27 % | >1 µM | >1 µM | >1 µM |
| (*R*,*S*,*R*)-**16b** | Bn | PMB | 155 | 23 ± 9 | 30 ± 13 | 343 | >1 µM |
| (*R*,*S*,*R*)-**18** | DCPA[c] | DCPA[c] | 92 ± 26 | 270 | >1 µM | 0 % | 3 % |
| (*S*,*R*,*S*)-**18** | DCPA[c] | DCPA[c] | 253 | 260 | 10 % | 5 % | 0 % |
| **2** | | | 0.31 ± 0.04 | - | - | - | - |
| U-69,593 | | | 0.88 ± 0.10 | - | - | - | - |
| naloxone | | | 6.9 ± 0.50 | 2.1 ± 0.50 | 2.4 ± 0.50 | - | - |
| morphine | | | 35 ± 6.0 | 3.9 ± 2.1 | 2.0 ± 0.30 | - | - |
| (+)-pentazocine | | | - | - | - | 5.4 ± 0.5 | - |
| haloperidol | | | - | - | - | 6.6 ± 0.90 | 78 2.3 |

[a] In case of high affinity ($K_i$ < 100 nM) the experiments were repeated twice (n = 3) and SEM values are given in Table 1.

[b] Values in % reflect the inhibition of the radioligand binding at a test compound concentration of 1 µM.

[c] DCPA = 2-(3,4-dichlorophenyl)acetyl

The data in Table 1 indicate very low KOR affinity for compounds **15** with two carbonyl moieties within the bicyclic framework. After reduction, pyrrolidine derivatives **16** display promising KOR affinity, which is strongly dependent on the configuration: Only compounds (*S*,*R*,*S*)-**16a** and (*R*,*S*,*R*)-**16a** with the pyrrolidine ring oriented towards N-5 show high KOR affinity. (*S*,*R*,*S*)-**16a** represents the eutomer with a $K_i$ value of 31 nM. Taking into account that partial racemization had occurred, the eudismic ratio is expected to be higher than 2. The dihedral angle of the ethylenediamine pharmacophore of (*S*,*R*,*S*)-**16a** is in the range 159-180° (compare Figures 3, 5, and 7). This result is unexpected, as this arrangement is quite different from the energetically favored conformation (70°) of the flexible KOR agonist **2**. A possible explanation is a revers

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orientation of (*S*,*R*,*S*)-**16a** in the KOR binding pocket with N-5 together with the pyrrolidine ring representing the KOR-pharmacophoric structural elements. Although the dihedral angle of the diastereomer (*S*,*R*,*R*)-**16a** (69°, see Figure 3) is close to the energetically favored conformation (70°), its KOR affinity is negligible.

Replacement of the benzyl groups of the KOR agonists (*S*,*R*,*S*)-**16a** and (*R*,*S*,*R*)-**16a** by (dichlorophenyl)acetyl moieties resulted in reduced KOR affinity. Although (*S*,*R*,*S*)-**18** contains both pharmacophoric elements, an 8-fold reduction of KOR affinity was detected. It is postulated that the large second (dichlorophenyl)acetyl moiety at N-5 might inhibit the interaction with KOR.

*Receptor selectivity*

The MOR and DOR affinity was recorded in receptor binding studies using the radioligands [$^3$H]DAMGO and [$^3$H]DPDPE, respectively.[23] The bislactams **15** as well as the dibenzyl derivatives (*R*,*S*,*S*)-**16a** and (*S*,*R*,*R*)-**16a** without KOR affinity did not display any MOR and DOR affinity. However, the KOR agonists (*S*,*R*,*S*)-**16a** and (*R*,*S*,*R*)-**16a** showed moderate MOR and DOR affinity leading for the most potent KOR agonist (*S*,*R*,*S*)-**16a** to KOR/MOR and KOR/DOR selectivity factors of 8-fold and 5-fold respectively.

Introduction of a methoxy moiety into the N-2 benzyl group of (*R*,*S*,*R*)-**16a** led to (*R*,*S*,*R*)-**16b** with lightly reduced KOR affinity, but considerably increased MOR and DOR affinity. Altogether, (*R*,*S*,*R*)-**16b** represents a ligand with 5- to 6-fold preference for MOR and DOR compared with KOR. An increased MOR affinity was also observed for the (dichlorophenyl)acetyl derivatives (*R*,*S*,*R*)-**18** and (*S*,*R*,*S*)-**18** leading to unselective ligands.

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Originally, σ receptors were regarded as opioid receptor subtype.[1] Moreover, the ligand binding profile of opioid receptors, in particular KORs and σ receptors is often very similar.[24-27] Therefore, determination of $\sigma_1$ and $\sigma_2$ receptor affinity was included into this study. [$^3$H](+)-pentazocine and [$^3$H]di-*o*-tolylguanidine were employed as radioligands in the receptor binding studies.[28-30] As described for MOR and DOR affinity the bislactams **15** and the dibenzyl derivatives (*R*,*S*,*S*)-**16a** and (*S*,*R*,*R*)-**16a** without KOR affinity did not interact with both σ receptor subtypes. Weak $\sigma_1$ receptor affinity was detected for (*R*,*S*,*R*)-**16a** and (*S*,*R*,*S*)-**16a** resulting in 10- and 25-fold KOR/$\sigma_1$ receptor selectivity, respectively. The (dichlorophenyl)acetamides (*R*,*S*,*R*)-**18** and (*S*,*R*,*S*)-**18** did not show any $\sigma_1$ or $\sigma_2$ receptor affinity.

*Intrinsic activity*

The intrinsic activity of the most promising KOR ligands (*S*,*R*,*S*)-**16a** and (*R*,*S*,*R*)-**16a** was investigated with the [$^{35}$S]GTPγS ([$^{35}$S]-guanosine-5´-3-O-(thio)triphosphate) binding assay.[31,32]

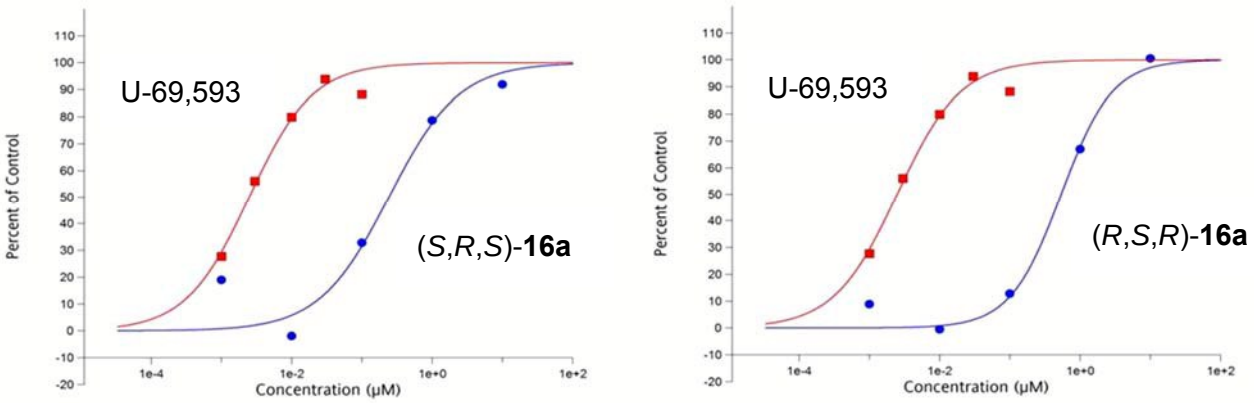


Figure 7: KOR agonistic activity of (*S*,*R*,*S*)-**16a** and of (*R*,*S*,*R*)-**16a** in the [$^{35}$S]GTPγS assay. Left: Comparison of [$^{35}$S]GTPγS binding after activation of KORs by U-69,593 (red curve) and by (*S*,*R*,*S*)-**16a** (blue curve, $EC_{50}$ = 240 nM); Right: Comparison of

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[$^{35}$S]GTPγS binding after activation of KORs by U-69,593 (red curve) and by (*R*,*S*,*R*)-**16a** (blue curve, $EC_{50}$ = 540 nM).

In this assay both KOR ligands (*S*,*R*,*S*)-**16a** and (*R*,*S*,*R*)-**16a** behaved as full agonists with the same intrinsic activity as the reference compound U-69,593. (*S*,*R*,*S*)-**16a**, the ligand with higher KOR affinity, led to an $EC_{50}$ value of 240 nM, whereas the lower KOR affinity enantiomer (*R*,*S*,*R*)-**16a** provided an $EC_{50}$ value of 540 nM. (Figure 7) Although these data show a nice correlation between KOR affinity and activation of KORs, the $EC_{50}$ values are considerably higher than the $EC_{50}$ value of U-69-593 ($EC_{50}$ = 2.5.nM). However, it is demonstrated that ethylenediamine ligands without the dichlorophenylacetyl KOR pharmacophoric group are able to activate KORs.

## Conclusion

The synthesis of ethano bridged piperazine derivatives is rather difficult, due to high conformational constraints. Trapping of intermediate hemiketal anions resulted in very low to moderate yields of mixed methyl silyl ketals **11**. Although both pharmacophoric elements (pyrrolidine ring, (dichlorophenyl)acetamide) are present in (*R*,*S*,*R*)-**18** and (*S*,*R*,*S*)-**18**, only moderate to low KOR affinity was found. It is postulated that either an unfavorable dihedral angle of 178° (Figure 6) or the large substituent at N-5 is responsible for the low KOR affinity. The most promising KOR agonists are the 2,5-dibenzyl substituted derivatives (*R*,*S*,*R*)-**16a** and (*S*,*R*,*S*)-**16a** with $K_i$ values of 74 nM and 31 nM, respectively. The nanomolar KOR affinity of (*S*,*R*,*S*)-**16a** was unexpected, since the (dichlorophenyl)acetyl moiety is missing and the dihedral angle of the ethylenediamine KOR pharmacophore (159-180°) differs considerably from the expected value. (*S*,*R*,*S*)-**16a** requires higher concentrations ($EC_{50}$ = 240 nM) than U-69,593 ($EC_{50}$ = 2.5 nM) to reach the same full KOR agonistic effects as U-69,593, which is in accordance to the 30-fold lower KOR affinity of (*S*,*R*,*S*)-**16a**.

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## 5. Experimental Chemistry

### 5.1. General Methods

Unless otherwise noted, reactions were conducted under dry nitrogen in absolute solvents. THF was dried with sodium/benzophenone and was freshly distilled before use.

### 5.2. Instruments

Thin layer chromatography (tlc): Silica gel 60 $F_{254}$ plates (Merck). Flash chromatography (fc): Silica gel 60, 40–64 µm (Merck); parentheses include: diameter of the column, eluent, fraction size, $R_f$ value. Melting point: Melting point apparatus SMP 3 (Stuart Scientific), uncorrected. MS: MAT GCQ (Thermo-Finnigan); IR: IR spectrophotometer 480Plus FT-ATR-IR (Jasco). $^1$H NMR (400 MHz): Unity Mercury Plus 400 spectrometer (Varian); $\delta$ in ppm related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution. Optical rotation: Polarimeter 341 (Perkin Elmer); 1.0 dm tube; concentration *c* in g/100 mL; T = 20 °C; wavelength 589 nm (D-line of Na light); the unit of the specific rotation ($[\alpha]_D^T$ deg·mL·dm$^{-1}$·g$^{-1}$) is omitted for clarity.

### 5.3. HPLC method for the determination of the purity

Merck Hitachi Equipment; UV detector: L-7400; autosampler:L-7200; pump: L-7100; degasser: L-7614; column: LiChrospher® 60 RP-select B (5 µm); LiCroCART® 250-4 mm cartridge; flow rate: 1.00 mL/min; injection volume: 5.0 µL; detection at λ = 210 nm; solvents: A: water with 0.05% (v/v) trifluoroacetic acid; B: acetonitrile with 0.05% (v/v) trifluoroacetic acid: gradient elution: 0.0 min: 90.0 % of A, 10.0 % of B; 4.0 min: 90.0 % of A, 10.0 % of B; 29.0 min: 0.0 % of A, 100.0 % of B; 31.0 min: 0.0 % of A, 100.0 % of

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B; 31.5 min: 90.0 % of A, 10.0 % of B; 40.0 min: 90.0 % of A, 10.0 % of B. According to this HPLC method the purity of all test compounds was greater than 95 %.

### 5.4. X-ray crystallography

Data sets for compounds (*R*,*R*,*S*)-**15a**, and **18** were collected with a D8 Venture Dual Source 100 CMOS diffractometer. Programs used: data collection: APEX2 V2014.5-0 (Bruker AXS Inc., **2014**);[33] cell refinement: SAINT V8.34A (Bruker AXS Inc., **2013**);[33] data reduction: SAINT V8.34A (Bruker AXS Inc., **2013**);[33] absorption correction, SADABS V2014/2 (Bruker AXS Inc., **2014**);[33] structure solution SHELXT-2014 (Sheldrick, **2014**);[34] structure refinement SHELXL-2014 (Sheldrick, **2014**)[34] and graphics, XP (Bruker AXS Inc., **2014**).[35] *R*-values are given for observed reflections, and *w*$R^2$ values are given for all reflections. Thermals ellipsoids are shown with 30 % probability.

### 5.6. Synthetic Procedures

**Dimethyl (*S*)-2-[N-(2-chloroacetyl)-N-(4-methoxybenzyl)amino]butanedioate ((*S*)-5e)**

Dimethyl (*S*)-aspartate·HCl (6.9 g, 32.6 mmol) was dissolved in $CH_2Cl_2$ (75 mL). Triethylamine (4.5 mL, 32.6 mmol) and freshly distilled *p*-methoxybenzaldehyde (4.0 mL, 32.6 mmol) were added successively. Afterwards, $Na_2SO_4$ was added and the mixture was stirred for 16 h at room temperature. Then, the suspension was filtered and the filtrate was concentrated in vacuo. The resulting residue was suspended in $Et_2O$ and the mixture was filtered again. Removing of the solvent of the filtrate in vacuo gave the imine (8.4 g, 30.1 mmol) as a colorless oil. The imine was dissolved in $CH_3OH$ (50 mL). $NaBH_4$ (1.9 g, 51.2 mmol) was added over 45 min under ice cooling. Afterwards, the mixture was stirred at room temperature for further 5 min, before the solvent was

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removed in vacuo. For work-up, $H_2O$ (60 mL) was added to the residue and the aqueous layer was extracted with $Et_2O$ (6 x 30 mL). The combined organic layers were dried ($K_2CO_3$), filtered and the solvent was removed under reduced pressure to give the secondary amine (8.1 g, 28.8 mmol) as a colorless solid, which was dissolved in $CH_2Cl_2$ (75 mL). Under $N_2$-atmosphere and ice cooling, chloroacetyl chloride (3.7 mL, 46.1 mmol) and triethylamine (3.2 mL, 23.0 mmol) were added slowly and the mixture was then stirred for 2.5 h at room temperature. Afterwards the solvent was removed in vacuo and diethyl ether was added to the resulting residue. This suspension was filtered and the filtrate was concentrated in vacuo. The orange oily residue was purified by fc (Ø 6 cm, h = 19 cm, v = 65 mL, cyclohexane : EtOAc = 2:1, $R_f$ = 0.25). Orange oil, yield 6.3 g (55 % over 3 steps). $C_{16}H_{20}ClNO_6$ (357.8). $[\alpha]_D^{20}$ = -83.7 (c = 0.41; $CH_3OH$). HPLC: Purity 90.9 %, $t_R$ = 17.5 min. $^1$H NMR (600 MHz, $CDCl_3$): δ (ppm) = 2.69 (dd, *J* = 17.1 / 6.6 Hz, 0.85H, CHC*H*$_2$CO$_2$CH$_3^{HR}$ ), 2.86 (dd, *J* = 17.3 / 4.6 Hz, 0.15H, CHC*H*$_2$CO$_2$CH$_3^{NR}$), 3.08 (dd, *J* = 17.3 / 4.4 Hz, 0.15H, CHC*H*$_2$CO$_2$CH$_3^{NR}$), 3.28 (dd, *J* = 17.1, 6.6 Hz, 0.85H, CHC*H*$_2$CO$_2$CH$_3^{HR}$ ), 3.58 (s, 3 x 0.15H, OC*H*$_3^{NR}$), 3.59 (s, 3 x 0.15H, OC*H*$_3^{NR}$), 3.65 (s, 3 x 0.85H, OC*H*$_3^{HR}$), 3.67 (s, 3 x 0.85H, OC*H*$_3^{HR}$), 3.77 (s, 3 x 0.15H, OCH$_3^{NR}$), 3.81 (s, 3 x 0.85H, OC*H*$_3^{HR}$), 4.07 (d, *J* = 12.7 Hz, 0.85H, O=CC*H*$_2$Cl$^{HR}$), 4.14 (d, *J* = 12.6 Hz, 0.85H, O=CC*H*$_2$Cl$^{HR}$), 4.23 (d, *J* = 15.5 Hz, 0.15H, NC*H*$_2$Ar$^{NR}$) ,4.42 (s, broad, 2 x 0.15H, O=CC*H*$_2$Cl$^{NR}$), 4.45 (t, *J* = 6.6 Hz, 0.85H, C*H*CH$_2$CO$_2$CH$_3^{HR}$ ), 4.60 (d, *J* = 16.2 Hz, 0.85H, NC*H*$_2$Ar$^{HR}$ ), 4.66 (d, *J* = 16.2 Hz, 0.85H, NC*H*$_2$Ar$^{HR}$), 4.77 (d, *J* = 15.4 Hz, 0.15H, NC*H*$_2$Ar$^{NR}$ ), 5.00 (t, *J* = 7.5 Hz, 0.15H, C*H*CH$_2$CO$_2$CH$_3^{NR}$), 6.80 (d, *J* = 8.4 Hz, 2 x 0.15H, Ar-*H*$^{NR}$), 6.90 (d, *J* = 8.6 Hz, 2 x 0.85H, Ar-*H*$^{HR}$), 7.12 (d, *J* = 8.3 Hz, 2 x 0.15H, Ar-*H*$^{NR}$), 7.28 (d, *J* = 8.6 Hz, 2 x 0.85H, Ar-*H*$^{HR}$). The ratio of rotamers is 85:15. HR = major rotamer, NR = minor rotamer. $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) = 34.4 (1C, CHCH$_2$CO$_2$CH$_3^{HR}$), 34.6 (1C, CHCH$_2$CO$_2$CH$_3^{NR}$), 41.5 (1C, O=CCH$_2$Cl$^{HR}$), 42.0 (1C, O=CCH$_2$Cl$^{NR}$), 47.0 (1C, NCH$_2$Ar$^{NR}$), 52.1 (1C, OCH$_3^{HR}$), 52.3 (1C, OCH$_3^{NR}$),

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52.7 (1C, O$CH_3^{HR}$), 53.1 (2C, N$CH_2Ar^{HR+NR}$), 55.4 (1C, O$CH_3^{HR}$), 55.5 (1C, O$CH_3^{NR}$), 56.9 (1C, $CHCH_2CO_2CH_3^{NR}$), 57.1 (1C, $CHCH_2CO_2CH_3^{HR}$), 114.0 (1C, Ar-$C^{NR}$), 114.4 (2C, Ar-$C^{HR}$), 127.0 (1C, Ar-$C_q^{HR}$), 129.1 (2C, Ar-$C^{HR}$), 129.2 (1C, Ar-$C^{NR}$), 159.7 (1C, Ar-$C^{HR}$), 167.0 (1C, O=$CCH_2Cl^{HR}$), 167.8 (1C, O=$CCH_2Cl^{NR}$), 169.8 (1C, $CO_2CH_3^{HR}$), 169.9 (1C, $CO_2CH_3^{NR}$), 170.6 (1C, $CO_2CH_3^{NR}$), 171.8 (1C, $CO_2CH_3^{HR}$). HR = major rotamer, NR = minor rotamer. Exact mass (APCI): m/z = 358.1057 (calcd. 358.1052 for $C_{16}H_{21}Cl^{35}NO_6^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2951 (w, C-$H_{aliph.}$), 1732 (s, C=$O_{ester}$), 1654 (s, C=$O_{amide}$), 1512 (m, C=C $_{arom.}$), 1435 (m, C-$H_{arom.}$), 1246, 1173 (s, C-$O_{ether}$), 818 (m, C-$H_{arom.\ disubst.}$) 798 (w, C-Cl).

**Methyl (*S*)-2-(1,4-dibenzyl)-3,6-dioxopiperazin-2-yl)acetate ((*S*)-6a)**

Under $N_2$, (*S*)-**5a** (9.6 g, 29.1 mmol) was dissolved in dry acetonitrile (molecular sieves 3 Å, 200 mL). Benzylamine (4.8 mL, 43.7 mmol) and triethylamine (2.6 mL, 34.9 mmol) were added dropwise. The mixture was heated to 70 °C for 1 h and then stirred at room temperature for further 16 h. The solvent was removed in vacuo and the residue was dissolved in EtOAc (150 mL). The organic layer was washed with 0.5 M HCl (2 x 30 mL), 0.5 M NaOH (30 mL) and brine (30 mL). After drying ($Na_2SO_4$), it was filtered and concentrated under reduced pressure. The residue was purified by fc (Ø 8cm, h = 12 cm, v = 65 mL, cyclohexane : EtOAc = 3 : 2 → 2 : 3 → 0 : 1, $R_f$ = 0.22 (cyclohexane : EtOAc = 3:2)). Yellow solid, mp 97-98 °C, yield 8.9 g (83 %). $C_{21}H_{22}N_2O_4$ (366.4). $[\alpha]_D^{20}$ = +34.8 (c = 0.75; $CH_2Cl_2$). Purity by HPLC: 94.2 %, $t_R$ = 19.5 min.

**Methyl (*R*)-2-(1,4-dibenzyl-3,6-dioxopiperazin-2-yl)acetate ((*R*)-6a)**

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Under $N_2$, (*R*)-**5a** (18.6 g, 56.7 mmol) was dissolved in dry acetonitrile (molecular sieves 3 Å, 220 mL). Benzylamine (9.3 mL, 85.1 mmol) and triethylamine (9.4 mL, 68.04 mmol) were added dropwise. The mixture was heated to 70 °C for 1 h and then stirred at room temperature for further 16 h. The solvent has then been removed in vacuo and the residue was dissolved in EtOAc (150 mL). The organic layer was washed with 0.5 M HCl (2 x 30 mL), 0.5 M NaOH (30 mL) and brine (30 mL). After drying ($Na_2SO_4$), it was filtered and concentrated under reduced pressure. The residue was purified by fc (Ø 8 cm, h = 20 cm, v = 65 mL, cyclohexane:EtOAc = 3:2 → 2:3 → 0:1, $R_f$ = 0.22 (cyclohexane : EtOAc = 3 : 2)). Pale yellow solid, mp 99-100 °C, yield 18.2 g (88 %). $C_{21}H_{22}N_2O_4$ (366.4). $[\alpha]_D^{20}$ = -25.9 (c = 1.58; $CH_3OH$). Purity by HPLC: 94.0 %, $t_R$ = 19.1 min.

Spectroscopic data of (*S*)-**6a** and (*S*+*R*)-**6a**

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 2.82 (dd, *J* = 17.1 / 5.0 Hz, 1H, CHC*H*$_2$CO$_2$CH$_3$), 3.08 (dd, *J* = 17.1 / 3.3 Hz, 1H, CHC*H*$_2$CO$_2$CH$_3$), 3.54 (s, 3H, OC*H*$_3$), 3.87 (d, *J* = 17.3 Hz, 1H, O=CC*H*$_2$N ), 4.15 (d, *J* = 16.6 Hz, 1H, O=CC*H*$_2$N), 4.16 (t, *J* = 4.34 Hz, 1H, C*H*CH$_2$CO$_2$CH$_3$), 4.21 (d, *J* = 15.1 Hz, 1H, NC*H*$_2$Ph), 4.51 (d, *J* = 14.5 Hz, 1H, NC*H*$_2$Ph), 4.71 (d, *J* = 14.5 Hz, 1H, NC*H*$_2$Ph), 5.07 (d, *J* = 15.1 Hz, 1H, NC*H*$_2$Ph), 7.21 – 7.38 (m, 10H, Ar-*H*). $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) = 35.3 (1C, CH*C*H$_2$CO$_2$CH$_3$), 47.3 (1C, NCH$_2$Ph), 49.5 (1C, O=C*C*H$_2$N), 49.8 (1C, N*C*H$_2$Ph), 52.2 (1C, O*C*H$_3$), 56.2 (1C, *C*HCH$_2$CO$_2$CH$_3$), 128.19 (2C, Ar-*C*), 128.20 (1C, Ar-C), 128.3 (1C, Ar-C), 128.7 (2C, Ar-*C*), 129.0 (2C, Ar-*C*), 129.1 (2C, Ar-*C*), 135.1 (1C, *C*-1$_{(benzyl)}$), 135.6 (1C, *C*-1$_{(benzyl)}$), 164.5 (1C, *C*=O), 165.5 (1C, *C*=O), 170.2 (1C, *C*O$_2$CH$_3$). Exact mass (APCI): m/z = 367.1660 (calcd. 367.1652 for $C_{21}H_{23}N_2O_4^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2947 (w, C-H$_{aliph.}$), 1732 (s, C=O$_{ester)}$, 1655 (s, C=O$_{amide}$), 1204, 1180 (s, C-O$_{ester}$), 752, 702 (m, C-H$_{arom. monosubst.}$).

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**Methyl (*S*)-2-[1-benzyl-4-(4-methoxybenzyl)-3,6-dioxopiperazin-2-yl]acetate ((*S*)-6b)**

Under $N_2$, (*S*)-**5a** (18.4 g, 56.2 mmol) was dissolved in dry acetonitrile (molecular sieves 3 Å, 230 mL). *p*-Methoxybenzylamine (11.0 mL, 84.3 mmol) and triethylamine (9.4 mL, 67.5 mmol) were added dropwise. The mixture was heated to 70 °C for 1 h and then stirred at room temperature for further 16 h. The solvent was removed in vacuo and the residue was dissolved in EtOAc (150 mL). The organic layer was washed with 0.5 M HCl (2 x 30 mL), 0.5 M NaOH (30 mL) and brine (30 mL). After drying ($Na_2SO_4$), it was filtered and concentrated under reduced pressure. The residue was purified by fc (Ø 8 cm, h = 15 cm, v = 65 mL, cyclohexane/EtOAc = 2:1 → 1:1 → 1:2, $R_f$ = 0.16 (cyclohexane : EtOAc = 1 : 1)). Colorless solid, mp 99-101 °C, yield 17.5 g (79 %). $C_{22}H_{24}N_2O_5$ (396.4). $[\alpha]_D^{20}$ = +69.8 (c = 1.2; $CH_3OH$). Purity by HPLC: 92.9 %, $t_R$ = 19.9 min. $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 2.80 (dd, *J* = 17.1 / 5.1 Hz, 1H, CHC*H*$_2$CO$_2$CH$_3$ ), 3.06 (dd, *J* = 17.1 / 3.3 Hz, 1H, CHC*H*$_2$CO$_2$CH$_3$ ), 3.54 (s, 3H, CO$_2$C*H*$_3$), 3.80 (s, 3H, Ar-OC*H*$_3$), 3.85 (d, *J* = 17.2 Hz, 1H, O=CC*H*$_2$N), 4.11 (d, *J* = 17.5 Hz, 1H, O=CC*H*$_2$N), 4.14 (t, *J* = 4.1 Hz, 1H, C*H*CH$_2$CO$_2$CH$_3$), 4.20 (d, *J* = 15.1 Hz, 1H, NC*H*$_2$Ph), 4.43 (d, *J* = 14.4 Hz, 1H, NC*H*$_2$PMB), 4.65 (d, *J* = 14.4 Hz, 1H, CH$_2$NC*H*$_2$PMB), 5.07 (d, *J* = 15.1 Hz, 1H, NC*H*$_2$Ph), 6.87 (d, *J* = 8.4 Hz, 2H, 3-*H*, 5-*H* $_{(p\text{-methoxybenzyl})}$), 7.20 (d, *J* = 8.2 Hz, 2H, 2-*H*, 6-*H* $_{(p\text{-methoxybenzyl})}$), 7.22 – 7.37 (m, 5H, Ar-*H*). $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) = 35.3 (1C, CH*C*H$_2$CO$_2$CH$_3$), 47.3 (1C, CHN*C*H$_2$Ar), 49.2 (1C, CH$_2$N*C*H$_2$Ar), 49.3 (1C, O=C*C*H$_2$N), 52.1 (1C, O*C*H$_3$), 55.4 (1C, CO$_2$*C*H$_3$), 56.2 (1C, *C*HCH$_2$CO$_2$CH$_3$), 114.3 (2C, *C*-3$_{(p\text{-methoxybenzyl})}$, *C*-5$_{(p\text{-methoxybenzyl})}$), 127.1 (1C, *C*-1$_{(p\text{-methoxybenzyl})}$), 128.18 (2C, *C*-2, *C*-6$_{(benzyl)}$), 128.20 (1C, *C*-4$_{(benzyl)}$), 129.1 (2C, *C*-3, *C*-5$_{(benzyl)}$), 130.1 (2C, *C*-2, *C*-6$_{(p\text{-methoxybenzyl})}$), 135.6 (1C, *C*-1$_{(benzyl)}$), 159.6 (1C, *C*-4$_{(p\text{-methoxybenzyl})}$),164.5 (1C, *C*=O), 165.4 (1C, *C*=O), 170.2 (1C, *C*O$_2$CH$_3$). Exact mass

(APCI): m/z = 397.1810 (calcd. 397.1758 for $C_{22}H_{25}N_2O_5$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2947 (w, C-$H_{aliph.}$), 1732 (s, C=$O_{ester}$), 1651 (s, C=$O_{amide}$), 1474 (m, C-$H_{arom.}$), 1246, 1173 (s, C-$O_{ester}$), 818 (m, C-$H_{arom.\ disubst.}$), 729, 702 (m, C-$H_{arom.\ monosubst.}$).

**Methyl (*S*)-2-[1-benzyl-4-(cyclohexylmethyl)-3,6-dioxopiperazin-2-yl]acetate ((*S*)-6c)[18]**

Under $N_2$, (*S*)-**5a** (5.5 g, 16.8 mmol) was dissolved in dry acetonitrile (molecular sieves 3 Å, 200 mL). Cyclohexylmethylamine (2.8 mL, 21.8 mmol) and triethylamine (2.8 mL, 20.1 mmol) were added dropwise and the mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo and the residue was dissolved in EtOAc (150 mL). The organic layer was washed with 0.5 M HCl (2 x 30 mL), 0.5M NaOH (30 mL) and brine (30 mL). After drying ($Na_2SO_4$), it was filtered and concentrated under reduced pressure. The residue was purified by fc (Ø 5.5 cm, h = 17 cm, v = 65 mL, cyclohexane : EtOAc = 1 : 1, $R_f$ = 0.40 (cyclohexane : EtOAc = 1 : 1)). Colorless solid, mp 93 – 94 °C, yield 4.6 g (73 %). $C_{21}H_{28}N_2O_4$ (372.5). $[\alpha]_D^{20}$ = +57.3 (c = 1.12; $CH_3OH$). Purity by HPLC: 97.5 %, $t_R$ = 20.5 min. $^1$H NMR (600 MHz, $CDCl_3$): δ (ppm) = 0.89 – 1.01 (m, 2H, $NCH_2C_6H_{11}$), 1.10 – 1.23 (m, 3H, $NCH_2C_6H_{11}$), 1.62 – 1.74 (m, 6H, $NCH_2C_6H_{11}$), 2.77 (dd, *J* = 17.1 / 5.1 Hz, 1H, $CHCH_2CO_2CH_3$), 3.00 (dd, *J* = 17.1 / 3.5 Hz, 1H, $CHCH_2CO_2CH_3$), 3.19 (dd, *J* = 13.5 / 6.7 Hz, 1H, $NCH_2C_6H_{11}$), 3.26 (dd, *J* = 13.5 / 7.6 Hz, 1H, $NCH_2C_6H_{11}$), 3.59 (s, 3H, $OCH_3$), 3.92 (d, *J* = 17.2 Hz, 1H, O=$CCH_2$N), 4.07 – 4.10 (m, 1H, $CHCHCO_2CH_3$), 4.23 (d, *J* = 15.1 Hz, 1H, $NCH_2$Ph), 4.33 (d, *J* = 17.2 Hz, 1H, O=$CCH_2$N), 5.05 (d, *J* = 15.1 Hz, 1H, $NCH_2$Ph), 7.20 – 7.34 (m, 5H, Ar-*H*). $^{13}$C NMR (151 MHz, $CDCl_3$): δ (ppm) = 25.80 (1C, $NCH_2C_6H_{11}$), 25.83 (1C, $NCH_2C_6H_{11}$), 26.4 (1C, $NCH_2C_6H_{11}$), 30.6 (1C, $NCH_2C_6H_{11}$), 30.8 (1C, $NCH_2C_6H_{11}$), 35.2 (1C, $CHCHCO_2CH_3$), 35.5 (1C, $NCH_2C_6H_{11}$), 47.4 (1C, $NCH_2$Ph), 50.9 (1C, O=$CCH_2$N), 52.1 (1C, $OCH_3$), 52.8 (1C, $NCH_2C_6H_{11}$), 56.3 (1C, $CHCHCO_2CH_3$), 128.1 (2C, Ar-*C*), 128.2 (1C, Ar-*C*), 129.0

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(2C, Ar-*C*), 135.7 (1C, Ar-$C_q$), 164.7 (1C, *C*=O), 165.5 (1C, *C*=O), 170.3 (1C, $CO_2CH_3$). Exact mass (APCI): m/z = 373.2159 (calcd. 373.2122 for $C_{21}H_{29}N_2O_4^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2920, 2851 (w, C-$H_{aliph.}$), 1732 (s, C=$O_{ester}$), 1647 (s, C=$O_{amide}$), 1200 (m, C-$O_{ester}$), 725, 698 (m, C-$H_{arom.\ monosubst.}$).

**Methyl (*S*)-2-(1-benzyl-4-methyl-3,6-dioxopiperazin-2-yl)acetate ((*S*)-6d)[18]**

Under $N_2$, (*S*)-**5a** (3.6 g, 11.0 mmol) was dissolved in dry acetonitrile (molecular sieves 3 Å, 200 mL). A solution of methylamine in THF (2 M, 7.2 mL, 14.4 mmol) and triethylamine (1.8 mL, 13.2 mmol) were added dropwise and the reaction was stirred for 3 d at room temperature. The solvent was removed in vacuo and the residue was dissolved in EtOAc (150 mL). The organic layer was washed with 0.5 M HCl (2 x 30 mL), 0.5 M NaOH (30 mL) and brine (30 mL). After drying ($Na_2SO_4$), it was filtered and concentrated under reduced pressure. The residue was purified by fc (Ø 4 cm, h = 18 cm, v = 30 mL, EtOAc : acetone = 4 : 1, $R_f$ = 0.44). Colorless solid, mp 99-100°C, yield 2.0 g (61 %). $C_{15}H_{18}N_2O_4$ (290.3). $[\alpha]_D^{20}$ = +92.7 (c = 1.86; $CH_3OH$). Purity by HPLC: 97.0 %, $t_R$ = 14.5 min. $^1$H NMR (600 MHz, $CDCl_3$): δ (ppm) = 2.80 (dd, *J* = 17.4 / 4.9 Hz, 1H, CHC*H*$_2$$CO_2CH_3$), 2.97 (s, 3H, NC*H*$_3$), 3.04 (dd, *J* = 17.4 / 3.3 Hz, 1H, CHC*H*$_2$$CO_2CH_3$), 3.60 (s, 3H, OC*H*$_3$), 3.95 (d, *J* = 17.1 Hz, 1H, O=CC*H*$_2$N), 4.04 (t, *J* = 3.9 Hz, 1H, C*H*$CH_2CO_2CH_3$ ), 4.15 (d, *J* = 15.1 Hz, 1H, NC*H*$_2$Ar), 4.33 (d, *J* = 17.1 Hz, 1H, O=CC*H*$_2$N), 5.12 (d, *J* = 15.1 Hz, 1H, NC*H*$_2$Ar), 7.20 – 7.35 (m, 5H, Ar-*H*). $^{13}$C NMR (151 MHz, $CDCl_3$): δ(ppm) = 33.7 (1C, N*C*$H_3$), 34.9 (1C, CH*C*$H_2CO_2CH_3$), 47.1 (1C, NC$H_2$Ar), 52.1 (1C, O=C*C*$H_2$N), 52.2 (1C, O*C*$H_3$), 55.7 (1C, *C*H$CH_2CO_2CH_3$), 128.2 (3C, Ar-*C*), 129.1 (2C, Ar-*C*), 135.5 (1C, Ar-$C_q$), 164.3 (1C, *C*=O), 165.6 (1C, *C*=O), 170.4 (1C, *C*$O_2CH_2$). Exact mass (APCI): m/z = 291.1344 (calcd. 291.1339 for $C_{15}H_{19}N_2O_4^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2951, 2920 (w, C-$H_{aliph.}$), 1728 (s, C=$O_{ester}$), 1643 (s, C=$O_{amide}$), 1196 (m, C-$O_{ester}$), 725, 698 (m, C-$H_{arom.\ monosubst.}$).

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**Methyl (*S*)-2-[4-benzyl-1-(4-methoxybenzyl)-3,6-dioxopiperazin-2-yl]acetate ((*S*)-6e)**

Under $N_2$, (*S*)-**5e** (7.9 g, 22 mmol) was dissolved in dry acetonitrile (molecular sieves 3 Å, 200 mL). Freshly distilled benzylamine (3.4 mL, 30.7 mmol) and triethylamine (4.3 mL, 30.7 mmol) were added dropwise. The reaction mixture was heated to reflux for 16 h. After cooling to room temperature, the solvent was removed in vacuo and the residue was dissolved in EtOAc (100 mL). The organic layer was washed with 0.5 M HCl (2 x 30 mL), 0.5 M NaOH (1 x 30 mL) and brine (1 x 30 mL). After drying ($Na_2SO_4$), it was filtered and concentrated under reduced pressure. The residue was purified by fc (Ø 6 cm, h = 17 cm, v = 65 mL, cyclohexane/EtOAc = 1:1 → 1:2, $R_f$ = 0.44 (cyclohexane:EtOAc = 1:2)). Colorless solid, mp 97-98 °C, yield 6.7 g (77 %). $C_{22}H_{24}N_2O_5$ (396.4). $[\alpha]_D^{20}$ = +59.0 (c = 0.24; $CH_3OH$). Purity by HPLC: 94.3 %, $t_R$ = 19.5 min. $^1$H NMR (600 MHz, $CDCl_3$): δ (ppm) = 2.83 (dd, *J* = 17.1 / 5.1 Hz, 1H, CHC*H*$_2$CO$_2$CH$_3$), 3.07 (dd, *J* = 17.0 / 3.3 Hz, 1H, CHC*H*$_2$CO$_2$CH$_3$), 3.54 (s, 3H, CO$_2$C*H*$_3$), 3.79 (s, 3H, Ar-OC*H*$_3$), 3.85 (d, 1H, *J* = 17.2 Hz, 1H, O=CC*H*$_2$N ), 4.10 (d, 1H, *J* = 15.0 Hz, NC*H*$_2$ArOCH$_3$), 4.12 (d, *J* = 17.9 Hz, 1H, O=CC*H*$_2$N), 4.15 (m, 1H, C*H*CH$_2$CO$_2$CH$_3$), 4.48 (d, *J* = 14.5 Hz, 1H, CHNC*H*$_2$Ph), 4.71 (d, *J* = 14.6 Hz, 1H, CHNC*H*$_2$Ph), 5.05 (d, 1H, *J* = 15.0 Hz, NC*H*$_2$ArOCH$_3$), 6.85 (d, *J* = 8.6 Hz, 2H, 3-*H*, 5-*H* $_{(p\text{-methoxybenzyl})}$), 7.16 (d, *J* = 8.4 Hz, 2H, 2-*H*, 6-*H* $_{(p\text{-methoxybenzyl})}$), 7.24 – 7.37 (m, 5H, Ar-*H*). $^{13}$C NMR (151 MHz, $CDCl_3$): δ (ppm) = 35.2 (1C, CH*C*H$_2$CO$_2$CH$_3$ ), 46.6 (1C, CH$_2$N*C*H$_2$ArOCH$_3$), 49.5 (1C, O=C*C*H$_2$N), 49.8 (1C, CHN*C*H$_2$Ar), 52.1 (1C, CO$_2$*C*H$_3$), 55.4 (1C, Ar-O*C*H$_3$), 55.8 (1C, *C*HCH$_2$CO$_2$CH$_3$), 114.5 (2C, *C*-3$_{(p\text{-methoxybenzyl})}$, *C*-5$_{(p\text{-methoxybenzyl})}$), 127.4 (1C, *C*-1$_{(p\text{-methoxybenzyl})}$), 128.2 (1C, *C*-4$_{(benzyl)}$), 128.6 (2C, *C*-2, *C*-6$_{(benzyl)}$), 128.9 (2C, *C*-3, *C*-5$_{(benzyl)}$), 129.6 (2C, *C*-2, *C*-6$_{(p\text{-methoxybenzyl})}$), 135.0 (1C, *C*-1$_{(benzyl)}$), 159.6 (1C, *C*-4$_{(p\text{-methoxybenzyl})}$), 164.4 (1C, *C*=O), 165.6 (1C, *C*=O), 170.2 (1C, *C*O$_2$CH$_3$). Exact mass (APCI): m/z =

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397.1780 (calcd. 397.1758 for $C_{22}H_{25}N_2O_5^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2978 (w, C-$H_{aliph.}$), 1736 (s, C=$O_{ester}$), 1651 (s, C=$O_{amide}$), 1450 (m, C-$H_{arom.}$), 1234 (s, C-$O_{ether}$), 1180, 1165 (s, C-$O_{ester}$), 826 (w, C-$H_{arom.\ disubst.}$), 745, 710 (w, C-$H_{arom.\ monosubst.}$).

**(*S*)-2-[4-Benzyl-1-(4-methoxybenzyl)-3,6-dioxopiperazin-2-yl]-N-methoxy-N-methylacetamide ((*S*)-7)**

Under $N_2$ and ice cooling, $Al(CH_3)_3$ solution (2 M in toluene, 16.0 mL, 32 mmol) was added slowly to a solution of $H_3CNHOCH_3{\cdot}HCl$ (3.2 g, 32.7 mmol) in dry $CH_2Cl_2$ (60 mL). After stirring for 30 min at room temperature, a solution of (*S*)-**6e** (4.3 g, 10.9 mmol) in dry $CH_2Cl_2$ (60 mL) was added and the mixture was stirred for 4 h at room temperature. An aqueous sodium potassium tartrate solution (10 %, 50 mL) was added and the resulting suspension was stirred for 1 h. Then the mixture was filtered through Celite® using $CH_2Cl_2$ as eluent. The filtrate was concentrated under reduced pressure and the residue was purified by fc (Ø 6 cm, h = 18 cm, v = 65 mL, cyclohexane:EtOAc = 2:9, $R_f$ = 0.20). Colorless solid, mp 89-90 °C, yield 4.5 g (97 %). $C_{23}H_{27}N_3O_5$ (425.5). $[\alpha]_D^{20}$ = +38.4 (c = 0.17; $CH_3CN$). Purity by HPLC: 99.2 %, $t_R$ = 19.3 min. $^1H$ NMR (400 MHz, $CDCl_3$): δ (ppm) = 2.96 (dd, *J* = 17.3 / 3.8 Hz, 1H, CHC*H*$_2$CON($OCH_3$)$CH_3$), 3.03 (s, 3H, NC*H*$_3$), 3.04 (dd, *J* = 16.7 / 3.9 Hz, 1H, CHC*H*$_2$CON($OCH_3$)$CH_3$), 3.47 (s, 3H, NOC*H*$_3$), 3.73 (s, 3H, Ar-OC*H*$_3$), 3.77 (d, *J* = 17.0 Hz, 1H, O=CC*H*$_2$N), 4.15 (t, *J* =3.8 Hz, 1H, C*H*$CH_2$CON($OCH_3$)$CH_3$), 4.17 (d, *J* = 12.7 Hz, 1H, NC*H*$_2$Ar), 4.20 (d, *J* = 16.9 Hz, 1H, O=CC*H*$_2$N), 4.40 (d, *J* = 14.7 Hz, 1H, NC*H*$_2$Ph), 4.68 (d, *J* = 14.7 Hz, 1H, NC*H*$_2$Ph), 4.87 (d, *J* = 14.9 Hz, 1H, NC*H*$_2$Ar), 6.79 (d, *J* = 8.6 Hz, 2H, 3-*H*, 5-*H* $_{(p\text{-methoxybenzyl})}$), 7.11 (d, *J* = 8.6 Hz, 2H, 2-*H*, 6-*H* $_{(p\text{-methoxybenzyl})}$), 7.20 – 7.30 (m, 5H, Ar-*H*). $^{13}C$ NMR (101 MHz, $CDCl_3$): δ (ppm) = 32.1 (1C, N*C*$H_3$), 32.9 (1C, CH*C*$H_2$CON($OCH_3$)$CH_3$), 46.9 (1C, NC$H_2$Ar), 49.7 (1C, NC$H_2$Ph), 49.8 (1C, O=C*C*$H_2$N), 55.5 (1C, Ar-O*C*$H_3$), 56.1 (1C, *C*HC$H_2$CON($OCH_3$)$CH_3$), 61.3 (1C, NO*C*$H_3$), 114.4 (2C, *C*-3, *C*-5$_{(p\text{-methoxybenzyl})}$), 128.0

(2C, *C*-4$_{(benzyl)}$, *C*-1$_{(p\text{-}methoxybenzyl)}$), 128.5 (2C, *C*-2, *C*-6$_{(benzyl)}$), 128.9 (2C, *C*-3, *C*-5$_{(benzyl)}$), 129.5 (2C, *C*-2, *C*-6$_{(p\text{-}methoxybenzyl)}$), 135.3 (1C, *C*-1$_{(benzyl)}$), 159.4 (1C, *C*-4$_{(p\text{-}methoxybenzyl)}$), 164.9 (1C, *C*=O), 166.5 (1C, *C*=O), 170.1 (1C, O=*C*N(OCH$_3$)CH$_3$). Exact mass (APCI): m/z = 426.2023 (calcd. 426.2065 for $C_{23}H_{28}N_3O_5^+$ [MH]$^+$). IR (neat): ũ [cm$^{-1}$] = 2936 (w, C-H$_{aliph.}$), 1651 (s, C=O$_{amide}$), 1242 (s, C-O$_{ether}$), 1177 (m, C-N$_{weinreb\text{-}amide}$), 818 (w, C-H$_{arom.\ disubst.}$), 733, 702 (w, C-H$_{arom.\ monosubst.}$).

**(*S*)-2-[4-Benzyl-1-(4-methoxybenzyl)-3,6-dioxopiperazin-2-yl]acetaldehyde ((*S*)-8)**

Under $N_2$, *Weinreb* amide (*S*)-**7** (452 mg, 1.1 mmol) was dissolved in THF abs. (30 mL) and the mixture was cooled to -78 °C. Then, 0.9 equiv. of $LiAlH_4$ solution (1 M in THF, 0.95 mL, 0.95 mmol) were added slowly and the mixture was stirred for 16 h at -78 °C. The reaction mixture was treated with HCl (1 M, 8 mL) and warmed to room temperature. After extraction of the aqueous layer with diethyl ether (5 x 10 mL), the combined organic layers were dried ($Na_2SO_4$), filtered and the solvent was removed in vacuo. The resulting residue was purified by fc (Ø 4 cm, h = 18 cm, v = 30 mL, cyclohexane:EtOAc = 2:9, $R_f$ = 0.33). Orange-brown oil, yield 190 mg (49 %). $C_{21}H_{22}N_2O_4$ (366.4). $[\alpha]_D^{20}$ = +50.6 (c = 0.38; $CH_3CN$). Purity by HPLC: 86.1 %, $t_R$ = 17.0 min. $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 2.97 (dd, *J* = 18.6 / 5.0 Hz, 1H, CHC*H*$_2$CHO ), 3.12 (dd, *J* = 18.6 / 3.8 Hz, 1H, CHC*H*$_2$CHO), 3.79 (s, 3H, OC*H*$_3$), 3.88 (d, *J* = 17.2 Hz, 1H, O=CC*H*$_2$N), 4.19 (t, *J* = 4.3 Hz, 1H, C*H*CH$_2$CHO), 4.23 (d, *J* = 18.1 Hz, 1H, O=CC*H*$_2$N), 4.27 (d, 1H, *J* = 15.4 Hz, NC*H*$_2$Ar), 4.49 (d, *J* = 14.5 Hz, 1H, NC*H*$_2$Ph), 4.69 (d, *J* = 14.5 Hz, 1H, NC*H*$_2$Ph), 4.82 (d, *J* = 15.0 Hz, 1H, NC*H*$_2$Ar), 6.85 (d, *J* = 8.5 Hz, 2H, 3-*H*, 5-*H* $_{(p\text{-}methoxybenzyl)}$), 7.14 (d, *J* = 8.5 Hz, 2H, 2-*H*, 6-*H* $_{(p\text{-}methoxybenzyl)}$), 7.23 – 7.37 (m, 5H, Ar-*H*), 9.53 (s, 1H, C*H*O). $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) = 44.7 (1C, CH*C*H$_2$CHO), 47.3 (1C, NCH$_2$Ar ), 49.5 (1C, O=C*C*H$_2$N), 49.8 (1C, NCH$_2$Ph), 54.9 (1C, *C*HCH$_2$CHO), 55.5 (1C, OCH$_3$), 114.6 (2C, *C*-3, *C*-5$_{(p\text{-}methoxybenzyl)}$), 127.7 (1C, *C*-1$_{(p\text{-}methoxybenzyl)}$), 128.3 (1C, *C*-4$_{(benzyl)}$), 128.6

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(2C, *C*-2, *C*-6$_{(benzyl)}$), 129.0 (2C, *C*-3, *C*-5$_{(benzyl)}$), 129.6 (2C, *C*-2, *C*-6$_{(p\text{-}methoxybenzyl)}$), 135.0 (1C, *C*-1$_{(benzyl)}$), 159.6 (1C, *C*-4$_{(p\text{-}methoxybenzyl)}$), 164.4 (1C, *C*=O), 165.7 (1C, *C*=O), 198.0 (1C, *C*H=O). Exact mass (APCI): m/z = 367.1652 (calcd. 367.1618 for $C_{21}H_{23}N_2O_4^+$ [MH]$^+$). IR (neat): ũ [cm$^{-1}$] = 2932 (w, C-H $_{aliph.}$), 2835 (w, C-H$_{aldehyde.}$), 1732 (w, C=O$_{aldehyde}$), 1655 (s, C=O$_{amide}$),1512 (m, C=C$_{aromat.}$), 1242 (s, C-O$_{ether}$), 821 (w, C-H$_{arom.\ disubst.}$), 737, 702 (w, C-H$_{arom.\ monosubst.}$).

**(*R*,*E*)-N-{2-[(*S*)-4-Benzyl-1-(4-methoxybenzyl)-3,6-dioxopiperazin-2-yl]ethylidene}-2-methylpropane-2-sulfinamide ((*S*,*R*)-9)**

(*R*)-2-Methylpropane-2-sulfinamide (162 mg, 1.34 mmol) and Ti(OEt)$_4$ (0.5 mL, 2.23 mmol) were added to a solution of aldehyde (*S*)-**8** (326 mg, 0.89 mmol) in THF abs. (20 mL) and the reaction mixture was stirred for 3.5 h at room temperature. For work up, a 1:1 mixture of $CH_2Cl_2$ (15 mL) and saturated $NaHCO_3$-solution (15 mL) was added and the aqueous layer was extracted with $CH_2Cl_2$ (5 x 10 mL). The combined organic layers were dried ($Na_2SO_4$), filtered and concentrated under reduced pressure. The residue was purified by fc (Ø 3 cm, h = 18 cm, v = 30 mL, cyclohexane:EtOAc = 2:1 → 1:1), $R_f$ = 0.27 (cyclohexane:EtOAc = 2:9)). Yellow-orange solid, mp 141-142 °C, yield 179 mg (43 %). $C_{25}H_{31}N_3O_4S$ (469.6). $[\alpha]_D^{20}$ = -39.9 (c = 0.21; $CH_3OH$). Purity by HPLC: 90.9 %, $t_R$ = 18.6 min. $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.14 (s, 9H, O=SC(C*H*$_3$)$_3$), 3.08 (dt, *J* = 16.5 / 5.6 Hz, 1H, CHC*H*$_2$CHN), 3.15 (dt, *J* = 16.4 / 3.6 Hz, 1H, CHC*H*$_2$CHN), 3.79 (s, 3H, OC*H*$_3$), 3.88 (d, *J* = 17.5 Hz, 1H, O=CC*H*$_2$N), 3.99 (d, *J* = 17.6 Hz, 1H, O=CC*H*$_2$N), 4.05 (d, *J* = 14.9 Hz, 1H, NC*H*$_2$Ar), 4.24 (dd, *J* = 5.7 / 4.1 Hz, 1H, C*H*CH$_2$CHN), 4.33 (d, *J* = 14.5 Hz, 1H, NC*H*$_2$Ph), 4.77 (d, *J* = 14.4 Hz, 1H, NC*H*$_2$Ph), 5.08 (d, *J* = 14.9 Hz, 1H, NC*H*$_2$Ar), 6.86 (d, *J* = 8.5 Hz, 2H, 3-*H*, 5-*H* $_{(p\text{-}methoxybenzyl)}$), 7.15 (d, *J* = 8.4 Hz, 2H, 2-*H*, 6-*H* $_{(p\text{-}methoxybenzyl)}$), 7.24 (d, *J* = 7.3 Hz, 2H, 2-*H*, 6-*H*$_{(benzyl)}$), 7.31 - 7.37 (m, 3H, 3-*H*, 4-*H*, 5-*H* $_{(benzyl)}$), 7.91 (t, *J* = 4.2 Hz, 1H, CHCH$_2$C*H*=N). $^{13}$C NMR

(101 MHz, $CDCl_3$): δ (ppm) = 22.5 (3C, O=SC($CH_3$)$_3$), 37.6 (1C, CH$CH_2$CHN), 46.8 (1C, N$CH_2$Ar), 49.3 (1C, O=C$CH_2$N), 49.8 (1C, N$CH_2$Ph), 55.4 (1C, O$CH_3$), 56.4 (1C, $CHCH_2CHN$), 57.2 (1C, S$C(CH_3)_3$), 114.6 (2C, *C*-3, *C*-5$_{(p\text{-methoxybenzyl})}$), 127.0 (1C, *C*-1$_{(p\text{-methoxybenzyl})}$), 128.4 (1C, *C*-4$_{(benzyl)}$), 128.6 (2C, *C*-2, *C*-6$_{(benzyl)}$), 129.1 (2C, *C*-3, *C*-5$_{(benzyl)}$), 129.7 (2C, *C*-2, *C*-6$_{(p\text{-methoxybenzyl})}$), 135.0 (1C, *C*-1$_{(benzyl)}$), 159.7 (1C, *C*-4$_{(p\text{-methoxybenzyl})}$), 163.7 (1C, *C*=O), 164.1 (1C, CH$CH_2$*C*H=N), 165.2 (1C, *C*H=O). Exact mass (APCI): m/z = 470.2123 (calcd. 470. 2108 for $C_{25}H_{32}N_3O_4S^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2928 (w, C-$H_{aliph.}$), 1647 (s, C=$O_{amide}$ / C=$N_{imine}$ ),1508 (m, C=$C_{aromat.}$), 1242 (s, C-$O_{ether}$), 1084 (s, C-$O_{ether}$), 845 (w, C-$H_{arom.\ disubst.}$), 733, 689 (m, C-$H_{arom.\ monosubst.}$).

**(*S*,*E*)-N-{2-[(*S*)-4-Benzyl-1-(4-methoxybenzyl)-3,6-dioxopiperazin-2-yl]ethylidene}-2-methylpropane-2-sulfinamide ((*S*,*S*)-9)**

(*S*)-2-Methylpropane-2-sulfinamide (116 mg, 0.96 mmol) and $Ti(OEt)_4$ (0.3 mL, 1.4 mmol) were added to a solution of (*S*)-**8** (232 mg, 0.63 mmol) in THF abs. (15 mL) and the reaction mixture was stirred for 3.5 h at room temperature. For work up, a 1:1 mixture of $CH_2Cl_2$ (15 mL) and saturated $NaHCO_3$-solution (15 mL) was added and the aqueous layer was extracted with $CH_2Cl_2$ (5 x 10 mL). The combined organic layers were dried ($Na_2SO_4$), filtered and concentrated under reduced pressure. The residue was purified by fc (Ø 2 cm, h = 20 cm, v = 10 mL, cyclohexane:EtOAc = 2:1 → 1:1, $R_f$ = 0.27 (cyclohexane:EtOAc = 2:9)). Yellow-orange solid, mp 116-117 °C, yield 190 mg (64 %). $C_{25}H_{31}N_3O_4S$ (469.6). $[\alpha]_D^{20}$ = +82.2 (c = 3.7 mg; $CH_2Cl_2$). Purity by HPLC: 82.3 %, $t_R$ = 19.7 min. $^1$H NMR (600 MHz, $CDCl_3$): δ (ppm) = 1.09 (s, 9H, O=SC(C$H_3$)$_3$), 3.05 (dt, *J* = 17.9 / 4.7 Hz, 1H, CHC$H_2$CHN), 3.24 (dt, *J* = 17.7 / 3.5 Hz, 1H, CHC$H_2$CHN ), 3.78 (s, 3H, OC$H_3$), 3.83 (d, *J* = 14.4 Hz, 1H, NC$H_2$Ar), 3.85 (d, *J* = 16.5 Hz, 1H, O=CC$H_2$N), 3.93 (d, *J* = 17.6 Hz, 1H, O=CC$H_2$N), 4.22 (d, *J* = 14.7 Hz, 1H, NC$H_2$Ph), 4.24 (t, *J* = 4.4 Hz, 1H, C*H*$CH_2$CHN), 4.89 (d, *J* = 14.5 Hz, 1H, NC$H_2$Ph), 5.30 (d, *J* =

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14.8 Hz, 1H, NC$H_2$Ar ), 6.85 (d, *J* = 8.5 Hz, 2H, 3-*H*, 5-*H*$_{(p\text{-methoxybenzyl})}$), 7.17 (d, *J* = 8.5 Hz, 2H, 2-*H*, 6-*H*$_{(p\text{-methoxybenzyl})}$), 7.20 (d, *J* = 7.7 Hz, 2H, 2-*H*, 6-*H*$_{(benzyl)}$), 7.28 – 7.35 (m, 3H, 3-*H*, 4-*H*, 5-*H*$_{(benzyl)}$), 8.02 (t, *J* = 3.5 Hz, 1H, CHCH$_2$C*H*=N). $^{13}$C NMR (151 MHz, $CDCl_3$): δ (ppm) = 22.4 (3C, O=SC(*C*$H_3$)$_3$), 36.9 (1C, CH*C*$H_2$CHN), 46.2 (1C, N*C*$H_2$Ar), 49.4 (1C, O=C*C*$H_2$N), 49.6 (1C, N*C*$H_2$Ph), 55.4 (2C, O*C*$H_3$, *C*HCH$_2$CHN), 57.0 (1C, *C*(CH$_3$)$_3$), 114.6 (2C, *C*-3, *C*-5$_{(p\text{-methoxybenzyl})}$), 126.9 (1C, *C*-1$_{(p\text{-methoxybenzyl})}$), 128.2, (1C, *C*-4$_{(benzyl)}$), 128.5 (2C, *C*-2, *C*-6$_{(benzyl)}$), 129.1 (2C, *C*-3, *C*-5$_{(benzyl)}$), 129.8 (2C, *C*-2, *C*-6$_{(p\text{-methoxybenzyl})}$), 134.9 (1C, *C*-1$_{(benzyl)}$), 159.7 (1C, *C*-4$_{(p\text{-methoxybenzyl})}$), 163.8 (1C, *C*=O), 164.3 (1C, CHCH$_2$*C*H=N), 164.9 (1C, *C*=O). Exact mass (ESI): m/z = 470.2107 (calcd. 470.2108 for $C_{25}H_{32}N_3O_4S^+$ [MH]$^+$). IR (neat): ũ [cm$^{-1}$] = 2963 (w, C-H$_{aliph.}$), 1655 (s, C=O$_{amide}$ / C=N$_{imine}$ ), 1512 (m, C=C$_{aromat.}$), 1246 (s, C-O$_{ether}$), 1076 (m, C-O$_{ether}$), 818 (w, C-H$_{arom.\ disubst.}$), 745, 702 (w, C-H$_{arom.\ monosubst.}$).

**(1*S*,4*S*,7*R*)-2,5-Dibenzyl-7-methoxy-7-(trimethylsiloxy)-2,5-diazabicyclo[2.2.2]octane-3,6-dione ((*S*,*S*,*R*)-11a)**

Under $N_2$, (*S*)-**6a** (1.6 g, 4.3 mmol) was dissolved in THF abs. (50 mL) and the solution was cooled to -78 °C. Then, sodium hexamethyldisilazane in THF (1.0 M, 12.9 mL, 12.9 mmol) was added. After 45 min, chlorotrimethylsilane (1.4 mL, 10.7 mmol) was added and the reaction mixture was stirred for 1 h at -78 °C and for 2 h at room temperature. For work-up, a saturated $NaHCO_3$ solution (50 mL) was added and the suspension was extracted with $CH_2Cl_2$ (4 x 25 mL). The combined organic layers were dried ($Na_2SO_4$), filtered and the solvent was removed in vacuo. The residue was adsorbed on silica gel and purified by fc (Ø 8 cm, h = 16 cm, v = 65 mL, cyclohexane:EtOAc = 4:1, $R_f$ = 0.49 (cyclohexane:EtOAc = 3:2)). Colorless solid, mp 131-132 °C, yield 733 mg (39 %). $C_{24}H_{30}N_2O_4Si$ (438.6). $[\alpha]_D^{20}$ = -3.0 (c = 0.17; $CH_2Cl_2$). Purity by HPLC: 83.6 %, $t_R$ = 22.9 min.

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**(1*R*,4*R*,7*S*)-2,5-Dibenzyl-7-methoxy-7-(trimethylsiloxy)-2,5-diazabicyclo[2.2.2]octane-3,6-dione ((*R*,*R*,*S*)-11a)**

Under $N_2$, (*R*)-**6a** (6.2 g, 16.9 mmol) was dissolved in THF abs. (220 mL) and cooled to -78 °C. Then, sodium hexamethyldisilazane in THF (1M, 50.7 mL, 50.7 mL) was added. After 30 min and 35 min, chlorotrimethylsilane (5.4 mL, 42.3 mmol) was added in two aliquots and the reaction mixture was stirred for 1 h at -78 °C and for 2 h at room temperature. For work-up, a saturated $NaHCO_3$ solution (60 mL) was added and the suspension was extracted with $CH_2Cl_2$ (4 x 25 mL). The combined organic layers were dried ($Na_2SO_4$), filtered and the solvent was removed in vacuo. The residue was adsorbed on silica gel and purified by fc (Ø 5.5 cm, h = 20 cm, v = 65 mL, cyclohexane:EtOAc = 4:1 → 1:1, $R_f$ = 0.49 (cyclohexane:EtOAc = 3:2)). Colorless solid, mp 128-129 °C, yield 1.2 g (16 %). $C_{24}H_{30}N_2O_4Si$ (438.6). $[\alpha]_D^{20}$ = +1.8 (c = 0.15; $CH_2Cl_2$). Purity by HPLC: 85.4 %, $t_R$ = 22.8 min.

Spectroscopic data of (*S*,*S*,*R*)-**11a** and (*R*,*R*,*S*)-**11a**

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.14 (s, 9H, OSi(C*H*$_3$)$_3$), 1.91 (dd, *J* = 13.6 / 3.8 Hz, 1H, 8-*H*), 2.12 (dd, *J* = 13.6 / 2.0 Hz, 1H, 8-*H*), 3.06 (s, 3H, OC*H*$_3$), 3.88 (dd, *J* = 3.9 / 2.0 Hz, 1H, 4-*H*), 3.97 (s, 1H, 1-*H*), 4.19 (d, *J* = 15.0 Hz, 1H, NC*H*$_2$Ph), 4.30 (d, *J* = 14.8 Hz, 1H, NC*H*$_2$Ph), 4.78 (d, *J* = 14.8 Hz, 1H, NC*H*$_2$Ph), 4.89 (d, *J* = 15.0 Hz, 1H, NC*H*$_2$Ph), 7.19 – 7.25 (m, 4H, Ar-*H*), 7.29 – 7.36 (m, 6H, Ar-*H*). $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) = 1.5 (3C, OSi(*C*H$_3$)$_3$), 39.8 (1C, *C*-8), 48.3 (1C, NC$H_2$Ar), 49.0 (1C, NC$H_2$Ar), 50.0 (1C, OC$H_3$), 58.6 (1C, *C*-4), 66.0 (1C, *C*-1), 102.6 (1C, *C*-7), 128.1 (1C, Ar-*C*), 128.2 (1C, Ar-*C*), 128.4 (2C, Ar-*C*), 128.5 (2C, Ar-*C*), 129.0 (4C, Ar-*C*), 136.0 (d, *J* = 6.6 Hz, Ar-*C*$_q$), 167.5 (1C, *C*=O), 168.9 (1C, *C*=O). Exact mass (APCI): m/z = 439.2041 (calcd. 439.2048 for $C_{24}H_{31}N_2O_4Si^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2924 (w, C-

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$H_{aliph.}$), 1674 (s, C=$O_{amide}$), 1254 (m, C-$O_{ether}$), 1099 (m, SiO), 837 (m, Si($CH_3$)$_3$) 733, 694 (s, C-$H_{arom.\ monosubst.}$).

**(1*S*,4*S*,7*R*)-5-Benzyl-7-methoxy-2-(4-methoxybenzyl)-7-(trimethylsilyloxy)-2,5-diazabicyclo[2.2.2]octane-3,6-dione ((*S*,*S*,*R*)-11b)**

Under $N_2$, (*S*)-**6b** (767 mg, 1.9 mmol) was dissolved in THF abs. (35 mL) and the solution was cooled to -78 °C. Then, sodium hexamethyldisilazane in THF (1 M, 5.8 mL, 5.8 mmol) was added. After 30 min and 35 min, chlorotrimethylsilane (0.62 mL, 4.8 mmol) was added in 2 aliquots and the reaction mixture was stirred for 1 h at -78 °C and for 2 h at room temperature. For work-up, a saturated $NaHCO_3$ solution (30 mL) was added and the suspension was extracted with $CH_2Cl_2$ (4 x 15 mL). The combined organic layers were dried ($Na_2SO_4$), filtered and the solvent was removed in vacuo. The residue was adsorbed on silica gel and purified by fc (Ø 4.5 cm, h = 18 cm, v = 65 mL, cyclohexane:EtOAc = 4:1, $R_f$ = 0.67 (cyclohexane:EtOAc = 1:1)). Colorless solid, mp 151-152 °C, yield 109 mg (12 %). $C_{25}H_{32}N_2O_5Si$ (468.6). $[\alpha]_D^{20}$ = -49.1 (c = 0.38; $CH_3OH$). Purity by HPLC: 82.3%, $t_R$ = 23.5 min. $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.14 (s, 9H, OSi(C*H*$_3$)$_3$), 1.89 (dd, *J* = 13.6 / 3.8 Hz, 1H, 8-*H*), 2.11 (dd, *J* = 13.6 / 1.7 Hz, 1H, 8-*H*), 3.07 (s, 3H, OC*H*$_3$), 3.80 (s, 3H, Ar-OC*H*$_3$), 3.84 – 3.88 (m, 1H, 4-*H*), 3.97 (s, 1H, 1-*H*), 4.11 (d, *J* = 14.8 Hz, 1H, NC*H*$_2$Ar), 4.28 (d, *J* = 14.8 Hz, 1H, NC*H*$_2$Ar), 4.77 (d, *J* = 14.8 Hz, 1H, NC*H*$_2$Ar ), 4.84 (d, *J* = 14.8 Hz, 1H, NC*H*$_2$Ar), 6.86 (d, *J* = 8.5 Hz, 2H, 3-*H*, 5-*H* $_{(p\text{-methoxybenzyl})}$), 7.13 (d, *J* = 8.4 Hz, 2H, 2-*H*, 6-*H* $_{(p\text{-methoxybenzyl})}$), 7.19 – 7.25 (m, 2H, Ar-*H*), 7.28 – 7.35 (m, 3H, Ar-*H*). $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) = 1.5 (3C, OSi(*C*$H_3$)$_3$), 39.9 (1C, *C*-8), 48.3 (1C, NC$H_2$Ar), 48.4 (1C, N*C*$H_2$Ar), 50.0 (1C, O*C*$H_3$), 55.4 (1C, Ar-O*C*$H_3$), 58.7 (1C, *C*-4), 65.6 (1C, *C*-1), 102.6 (1C, *C*-7), 114.4 (2C, *C*-3, *C*-5$_{(benzyl)}$), 127.9 (1C, Ar-*C*$_q$), 128.2 (1C, Ar-*C*), 128.5 (2C, Ar-*C*), 129.0 (2C, Ar-*C*), 129.8 (2C, *C*-2, *C*-6$_{(p\text{-methoxybenzyl})}$), 136.0 (1C, Ar-*C*$_q$), 159.5 (1C, *C*-4$_{(p\text{-methoxybenzyl})}$), 167.6

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(1C, *C*=O), 168.8 (1C, *C*=O). Exact mass (APCI): m/z = 469.2135 (calcd. 469.2153 for $C_{25}H_{33}N_2O_5Si^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2955 (w, C-$H_{aliph.}$), 1678 (s, C=$O_{amide}$), 1250 (s, C-$O_{ether}$), 1096, 1038 (m, SiO), 876 (m, C-$H_{arom.\ disubst.}$), 837 (m, $Si(CH_3)_3$), 745, 694 (s, C-$H_{arom.\ monosubst.}$).

**(1*S*,4*S*,7*R*)-5-Benzyl-2-(cyclohexylmethyl)-7-methoxy-7-(trimethylsilyloxy)-2,5-diazabicyclo[2.2.2]octane-3,6-dione ((*S*,*S*,*R*)-11c)**[19]

Under $N_2$, (*S*)-**6c** (2.8 g, 7.5 mmol) was dissolved in THF abs. (200 mL) and the solution was cooled to -78 °C. Then, sodium hexamethyldisilazane in THF (1 M, 22.5 mL, 22.5 mmol) was added. After 45 min, chlorotrimethylsilane (2.4 mL, 18.8 mmol) was added. The reaction mixture was stirred for 1 h at -78 °C and for 2 h at room temperature. For work-up, a saturated $NaHCO_3$ solution (40 mL) was added and the suspension was extracted with $CH_2Cl_2$ (4 x 25 mL). The combined organic layers were dried ($Na_2SO_4$), filtered and the solvent was removed in vacuo. The residue was adsorbed on silica gel and purified by fc (Ø 5.5 cm, h = 20 cm, v = 65 mL, cyclohexane:EtOAc = 1:1, $R_f$ = 0.64). Colorless solid, mp 129-130 °C, yield 797 mg (15 %). $C_{24}H_{36}N_2O_4Si$ (444.6). $[\alpha]_D^{20}$ = -13.9 (c = 0.40; $CH_3OH$). Purity by HPLC: 80.1 %, $t_R$ = 23.7 min. $^1H$ NMR (600 MHz, $CDCl_3$): δ (ppm) = 0.21 (s, 9H, $OSi(CH_3)_3$), 0.83 – 0.97 (m, 2H, $NCH_2C_6H_{11}$), 1.11 – 1.25 (m, 3H, $NCH_2C_6H_{11}$), 1.53 – 1.71 (m, 6H, $NCH_2C_6H_{11}$), 1.85 (dd, *J* = 13.5 / 3.8 Hz, 1H, 8-*H*), 2.07 (dd, *J* = 13.5 / 1.6 Hz, 1H, 8-*H*), 2.75 (dd, *J* = 13.8 / 6.6 Hz, 1H, $NCH_2C_6H_{11}$), 3.24 (s, 3H, $OCH_3$), 3.60 (dd, *J* = 13.8 / 7.7 Hz, 1H, $NCH_2C_6H_{11}$), 3.82 (dd, *J* = 3.7 / 1.8 Hz, 1H, 4-*H*), 3.96 (s, 1H, 1-*H*), 4.26 (d, *J* = 14.8 Hz, 1H, $NCH_2Ar$), 4.83 (d, *J* = 14.8 Hz, 1H, $NCH_2Ar$), 7.22 – 7.35 (m, 5H, Ar-*H*). $^{13}C$ NMR (151 MHz, $CDCl_3$): δ (ppm) = 1.62 (3C, $OSi(CH_3)_3$), 25.78 (1C, $NCH_2C_6H_{11}$), 25.87 (1C, $NCH_2C_6H_{11}$), 26.45 (1C, $NCH_2C_6H_{11}$), 30.49 (1C, $NCH_2C_6H_{11}$), 30.89 (1C, $NCH_2C_6H_{11}$), 37.13 (1C, $NCH_2C_6H_{11}$), 39.34 (1C, *C*-8), 48.29 (1C, $NCH_2Ar$), 50.40 (1C,

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OC$H_3$), 51.94 (1C, NC$H_2C_6H_{11}$), 58.84 (1C, *C*-4), 67.67 (1C, *C*-1), 102.76 (1C, C-7), 128.15 (1C, Ar-*C*), 128.53 (2C, Ar-*C*), 128.90 (2C, Ar-*C*), 136.02 (1C, Ar-$C_q$), 167.71 (1C, *C*=O), 168.92 (1C, *C*=O). IR (neat): ũ [$cm^{-1}$] = 2920 (w, C-$H_{aliph.}$), 1682 (s, C=$O_{amide}$), 1254 (m, C-$O_{ether}$), 1096 (m, SiO), 845 (m, Si($CH_3$)$_3$) 737, 698 (s, C-H $_{arom.\ monosubst.}$).

**(1*S*,4*S*,7*R*)-2-Benzyl-7-methoxy-5-(4-methoxybenzyl)-7-(trimethylsilyloxy)-2,5-diazabicyclo[2.2.2]octane-3,6-dione ((*S*,*S*,*R*)-11e)**

Under $N_2$, (*S*)-**6e** (244 mg, 0.62 mmol) was dissolved in THF abs. (50 mL) and the solution was cooled to -78 °C. Then, sodium hexamethyldisilazane in THF (2 M, 0.9 mL, 1.8 mmol) was added. After 45 min, chlorotrimethylsilane (0.2 mL, 1.5 mmol) was added and the reaction mixture was stirred for 1 h at -78 °C and for 2 h at room temperature. For work-up, a saturated $NaHCO_3$ solution (50 mL) was added and the suspension was extracted with $CH_2Cl_2$ (4 x 10 mL). The combined organic layers were dried ($Na_2SO_4$), filtered and the solvent was removed in vacuo. The residue was adsorbed on silica gel and purified by fc (Ø 2 cm, h = 12 cm, v = 10 mL, cyclohexane:EtOAc = 2:1 → 1:2, $R_f$ = 0.44 (cyclohexane:EtOAc = 2:3)). Colorless oil, yield 0.9 mg (0.3 %). $C_{25}H_{32}N_2O_5Si$ (468.6). $^1$H NMR (300 MHz, $CDCl_3$): δ (ppm) = 0.14 (s, 9H, OSi(C$H_3$)$_3$), 1.87 (dd, *J* = 13.6 / 3.8 Hz, 1H, 8-*H* ), 2.11 (dd, *J* = 13.6 / 1.7 Hz, 1H, 8-*H*), 3.05 (s, 3H, OC$H_3$), 3.80 (s, 3H, Ar-OC$H_3$), 3.86 – 3.90 (m, 1H, 4-*H*), 3.96 (s, 1H, 1-*H*), 4.19 (d, *J* = 15.0 Hz, 1H, NC$H_2$Ar), 4.22 (d, *J* = 14.6 Hz, 1H, NC$H_2$Ar), 4.73 (d, *J* = 14.6 Hz, 1H, NC$H_2$Ar), 4.88 (d, *J* = 15.0 Hz, 1H, NC$H_2$Ar), 6.85 (d, *J* = 8.5 Hz, 2H, 3-*H*, 5-*H* $_{(p\text{-methoxybenzyl})}$), 7.12 - 7.22 (m, 4H, Ar-*H*), 7.25 – 7.39 (m, 3H, Ar-*H*).

**(1*S*,4*S*)-2,5-Dibenzyl-2,5-diazabicyclo[2.2.2]octane-3,6,7-trione ((*S*,*S*)-12a)**

*p*-Toluenesulfonic acid monohydrate (140 mg, 0.7 mmol) was added to a solution of (*S*,*S*,*R*)-**11a** (358 mg, 0.8 mmol) in a THF / $H_2O$ mixture (45 / 5 mL). The reaction mixture

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was stirred for 16 h at room temperature. For work-up, the mixture was treated with saturated $NaHCO_3$ solution (30 mL) and the aqueous layer was extracted with $CH_2Cl_2$ (4 x 15 mL). The combined organic layers were dried ($Na_2SO_4$), filtered, the solvent was removed in vacuo and the residue was purified by fc (Ø 2 cm, h = 13 cm, v = 10 mL, cyclohexane:EtOAc = 3:2 →0:1 $R_f$ = 0.11(cyclohexane:EtOAc = 3:2)). Colorless solid, mp 196-197 °C, yield 193 mg (70 %). $C_{20}H_{18}N_2O_3$ (334.4). $[\alpha]_D^{20}$ = +2.1 (c = 0.09; $CH_3OH$). Purity by HPLC: 97.8 %, $t_R$ = 14.0 min.

**(1*R*,4*R*)-2,5-Dibenzyl-2,5-diazabicyclo[2.2.2]octane-3,6,7-trione ((*R*,*R*)-12a)**

*p*-Toluenesulfonic acid monohydrate (514 mg, 2.7 mmol) was added to a solution of (*R*,*R*,*S*)-**11a** (1.2 g, 2.7 mmol) in a THF:$H_2O$ mixture (45:5 mL). The reaction mixture was stirred for 16 h at room temperature. For work-up, the mixture was treated with saturated $NaHCO_3$ solution (30 mL) and the aqueous layer was extracted with $CH_2Cl_2$ (4 x 15 mL). The combined organic layers were dried ($Na_2SO_4$), filtered, the solvent was removed in vacuo and the residue was purified by fc (Ø 3.5 cm, h = 10 cm, v = 30 mL, cyclohexane:EtOAc = 1:1 →0:1, $R_f$ = 0.11 (cyclohexane:EtOAc = 3:2)). Colorless solid, mp 193-194 °C, yield 618 mg (69 %). $C_{20}H_{18}N_2O_3$ (334.4). $[\alpha]_D^{20}$ = -0.9 (c = 0.22; $CH_2Cl_2$). Purity by HPLC: 96.4 %, $t_R$ = 15.3 min.

Spectroscopic data of (*S*,*S*)-**12a** and (*R*,*R*)-**12a**

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 2.20 (dd, *J* = 18.5 / 3.2 Hz, 1H, 8-*H*), 2.50 (dd, *J* = 18.5 / 2.0 Hz, 1H, 8-*H*), 4.15 (dd, *J* = 3.3 / 2.1 Hz, 1H, 4-*H*), 4.21 (s, 1H, 1-*H*), 4.40 (d, *J* = 14.6 Hz, 1H, NC*H*$_2$Ph), 4.60 (s, 2H, NC*H*$_2$Ph), 4.83 (d, *J* = 14.6 Hz, 1H, NC*H*$_2$Ph), 7.15 – 7.17 (m, 2H, Ar-*H*), 7.20 – 7.22 (m, 2H, Ar-*H*), 7.31 – 7.39 (m, 6H, Ar-*H*). $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) = 36.8 (1C, *C*-8), 49.1 (1C, NC$H_2$Ph), 49.2 (1C, NC$H_2$Ph), 57.7 (1C, *C*-4), 71.1 (1C, *C*-1), 128.3 (2C, Ar-*C*), 128.5 (2C, Ar-*C*), 128.7 (1C,

*C*-4$_{(benzyl)}$), 128.8 (1C, *C*-4$_{(benzyl)}$), 129.3 (2C, Ar-*C*), 129.4 (2C, Ar-*C*), 134.8 (1C, *C*-1$_{(benzyl)}$), 134.9 (1C, *C*-1$_{(benzyl)}$), 164.3 (1C, *C*=O$_{amide}$), 166.9 (1C, *C*=O$_{amide}$), 196.5 (1C, *C*=O$_{ketone}$). Exact mass (APCI): m/z = 335.1383 (calcd. 335.1390 for $C_{20}H_{19}N_2O_3^+$ [MH]$^+$). IR (neat): ũ [cm$^{-1}$] = 3017 (w, C-H$_{arom.}$), 1748 (m, C=O$_{ketone}$), 1678 (s, C=O$_{amide}$), 729, 698 (m, C-H$_{arom.\ monosubst.}$).



**(1*S*,4*S*)-5-Benzyl-2-(4-methoxybenzyl)-2,5-diazabicyclo[2.2.2]octane-3,6,7-trione ((*S*,*S*)-12b)**

*p*-Toluenesulfonic acid monohydrate (49 mg, 0.30 mmol) was added to a solution of (*S*,*S*,*R*)-**11b** (133 mg, 0.3 mmol) in a THF / $H_2O$ mixture (30 mL, 9:1). The reaction mixture was stirred for 16 h at room temperature. For work-up, the mixture was treated with saturated $NaHCO_3$ solution (30 mL) and the aqueous layer was extracted with $CH_2Cl_2$ (4 x 15 mL). The combined organic layers were dried ($Na_2SO_4$), filtered, the solvent was removed in vacuo and the residue was purified by fc (Ø 2 cm, h = 10 cm, v = 10 mL, cyclohexane:EtOAc = 1:1 →0:1, $R_f$ = 0.16 (cyclohexane:EtOAc = 1:1)). Colorless solid, mp 135-136 °C, yield 97 mg (95 %). $C_{21}H_{20}N_2O_4$ (364.4). $[\alpha]_D^{20}$ = +5.0 (c = 0.31; $CH_2Cl_2$). Purity by HPLC: 95.0 %, $t_R$ = 16.0 min. $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 2.19 (dd, *J* = 18.4 / 3.4 Hz, 1H, 8-*H*), 2.48 (dd, *J* = 18.4 / 2.0 Hz, 1H, 8-*H*), 3.79 (s, 3H, OC*H*$_3$), 4.13 (dd, *J* = 3.3 / 2.1 Hz, 1H, 4-*H*), 4.21 (s, 1H, 1-*H*), 4.39 (d, *J* = 14.7 Hz, 1H, NC*H*$_2$Ph), 4.51 (d, *J* = 14.7 Hz, 1H, NC*H*$_2$Ar), 4.54 (d, *J* = 14.7 Hz, 1H, NC*H*$_2$Ar), 4.82 (d, *J* = 14.7 Hz, 1H, NC*H*$_2$Ph), 6.85 (d, *J* = 8.7 Hz, 2H, 3-*H*, 5-*H* $_{(p\text{-methoxybenzyl})}$), 7.09 (d, *J* = 8.7 Hz, 2H, 2-*H*, 6-*H* $_{(p\text{-methoxybenzyl})}$), 7.18 – 7.22 (m, 2H, 2-*H*, 6-*H* $_{(benzyl)}$), 7.34 (dd, *J* = 9.5 / 5.4 Hz, 3H, Ar-*H*). $^{13}$C NMR (151 MHz, $CDCl_3$): δ (ppm) = 36.8 (1C, *C*-8), 48.6 (1C, NC$H_2$Anisyl), 49.0 (1C, NC$H_2C_6H_5$), 55.4 (1C, OC$H_3$), 57.8 (1C, *C*-4), 70.9 (1C, *C*-1), 114.6 (2C, *C*-3, *C*-5$_{(p\text{-methoxybenzyl})}$), 126.9 (1C, *C*-1$_{(p\text{-methoxybenzyl})}$), 128.5 (2C, *C*-2, *C*-6$_{(benzyl)}$), 128.8 (1C, Ar-*C*-4$_{(benzyl)}$), 129.3 (2C, *C*-3, *C*-

$5_{(benzyl)}$), 129.7 (2C, *C*-2, *C*-$6_{(p\text{-}methoxybenzyl)}$), 134.8 (1C, *C*-$1_{(benzyl)}$), 159.9 (1C, *C*-$4_{p\text{-}methoxybenzyl)}$), 164.4 (1C, *C*=$O_{amide}$), 166.8 (1C, *C*=$O_{amide}$), 196.6 (1C, *C*=$O_{ketone}$). Exact mass (APCI): m/z = 365.1488 (calcd. 365.1496 for $C_{21}H_{21}N_2O_4^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2982 (w, C-$H_{arom.}$), 1748 (m, C=$O_{ketone}$), 1690, 1663 (m, C=$O_{amide}$), 737, 702 (m, C-$H_{arom.\ monosubst.}$).

**(1*S*,4*S*)-5-Benzyl-2-(cyclohexylmethyl)-2,5-diazabicyclo[2.2.2]octane-3,6,7-trione ((*S*,*S*)-12c)**[19]

(*S*,*S*,*R*)-**11c** (416 mg, 0.9 mmol) was dissolved in a 9:1 mixture (150 mL) of THF and an aqueous solution of 0.5 M HCl and the mixture was stirred for 16 h at room temperature. For work-up, the mixture was treated with water (15 mL) and the aqueous layer was extracted with $CH_2Cl_2$ (4 x 15 mL). The combined organic layers were dried ($Na_2SO_4$), filtered, the solvent was removed in vacuo and the residue was purified by fc (Ø 3 cm, h = 18 cm, v = 20 mL, cyclohexane:EtOAc = 3:2 → 0:1, $R_f$ = 0.2 (cyclohexane:EtOAc = 3:2)). Colorless solid, mp 143-144 °C, yield 234 mg (74 %). $C_{20}H_{24}N_2O_3$ (340.4). $[\alpha]_D^{20}$ = -20.8 (c = 0.35; $CH_3OH$). Purity by HPLC: 66.3 %, $t_R$ = 16.5 min. $^1H$ NMR (600 MHz, $CDCl_3$): δ (ppm) = 0.85 – 0.96 (m, 2H, $NCH_2C_6H_{11}$), 1.10 – 1.22 (m, 3H, $NCH_2C_6H_{11}$), 1.52 (s, 3H, $NCH_2C_6H_{11}$), 1.62 – 1.73 (m, 3H, $NCH_2C_6H_{11}$), 2.20 (dd, *J* = 18.5 / 3.3 Hz, 1H, 8-H), 2.52 (dd, *J* = 18.5 / 2.1 Hz, 1H, 8-*H*), 3.16 (dd, *J* = 13.9 / 6.9 Hz, 1H, $NCH_2C_6H_{11}$), 3.36 (dd, *J* = 13.9 / 6.8 Hz, 1H, $NCH_2C_6H_{11}$), 4.11 (dd, *J* = 5.0 / 1.9 Hz, 1H, 4-*H*), 4.20 (s, 1H, 1-*H*), 4.37 (d, *J* = 14.6 Hz, 1H, $NCH_2Ar$), 4.89 (d, *J* = 14.6 Hz, 1H, $NCH_2Ar$), 7.24 (d, *J* = 6.5 Hz, 2H, Ar-*H*), 7.27 – 7.39 (m, 3H, Ar-*H*). Exact mass (APCI): m/z = 341.1844 (calcd. 341.1860 $C_{20}H_{25}N_2O_3^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2920 (w, C-$H_{aliph.}$), 1744 (m, C=$O_{ketone}$), 1686, 1670 (s, C=$O_{amide}$), 737, 698 (m, C-$H_{arom.\ monosubst.}$).

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**(1*S*,4*S*,7*R*)- and (1*S*,4*S*,7*S*)-22,5-Dibenzyl-7-hydroxy-2,5-diazabicyclo[2.2.2]-octane-3,6-dione ((*S*,*S*,*R*)-13a and (*S*,*S*,*S*)-13a)**

Under ice cooling, $NaBH_4$ (11 mg, 0.3 mmol) was added to a solution of (*S*,*S*)-**12a** (63 mg, 0.2 mmol) in a THF : $CH_3OH$ mixture (50 mL, 8:2) and the reaction mixture was stirred for 30 min. For work-up, the solvent was removed in vacuo, the residue was dissolved in $H_2O$ (20 mL), and extracted with EtOAc (4 x 20 mL). The combined organic layers were dried ($Na_2SO_4$), filtered and the solvent was removed in vacuo. The residue was purified by fc (Ø 2 cm, h = 14 cm, v = 10 mL, EtOAc:cyclohexane = 3:2, $R_f$ = 0.22 (EtOAc:cyclohexane = 4:1)). Colorless solid, mp 197 – 198 °C, yield 53 mg (84 %). $C_{20}H_{20}N_2O_3$ (336.4). $[\alpha]_D^{20}$ = -35.4 (c = 0.71; $CH_3OH$). Purity by HPLC: 97.7 %, $t_R$ = 14.6 min. $^1$H NMR (400 MHz, $(CD_3)_2SO$): δ(ppm) = 1.46 (d, *J* = 13.9 Hz, 0.2H, 8-*H*$^{ND}$), 1.59 (d, *J* = 13.7 Hz, 0.8H, 8-*H*$^{HD}$), 2.20 (dd, *J* = 12.8 / 8.9 Hz, 0.8H, 8-*H*$^{HD}$), 2.30 – 2.39 (m, 0.2H, 8-*H*$^{ND}$), 3.92 (d, *J* = 3.8 Hz, 0.8H, 4-*H*$^{HD}$), 3.94 (d, *J* = 2.3 Hz, 0.2H, 4-*H*$^{ND}$), 3.96 (s, 0.2H, 1-*H*$^{ND}$), 3.99 (s, 0.8H, 1-*H*$^{HD}$), 4.02 – 4.05 (m, 0.2H, 7-*H*$^{ND}$), 4.06 – 4.14 (m, 0.8H, 7-*H*$^{HD}$), 4.23 (d, *J* = 14.8 Hz, 2 x 0.2H, 2 x NC*H*$_2$Ar$^{ND}$), 4.37 (d, *J* = 14.7 Hz, 2 x 0.8H, 2 x NC*H*$_2$Ar$^{HD}$), 4.54 (d, *J* = 14.9 Hz, 0.8H, NC*H*$_2$Ar$^{HD}$), 4.63 (d, *J* = 14.7 Hz, 0.2H, NC*H*$_2$Ar$^{ND}$), 4.66 (d, *J* = 15.1 Hz, 0.8H, NC*H*$_2$Ar$^{HD}$), 5.08 (d, *J* = 15.2 Hz, 0.2H, NC*H*$_2$Ar$^{ND}$), 5.45 (d, *J* = 3.8 Hz, 0.2H, O*H*$^{ND}$), 5.52 (d, *J* = 3.3 Hz, 0.8H, O*H*$^{HD}$), 7.11 - 7.42 (m, 10H, Ar-*H*$^{HD+ND}$). HD= major diastereomer, ND= minor diastereomer. The ratio of (*S*,*S*,*R*)-**13a** : (*S*,*S*,*S*)-**13a** is 80:20. $^{13}$C NMR (101 MHz, $(CD_3)_2SO$): δ(ppm) = 34.3 (1C, *C*-8$^{HD}$), 34.4 (1C, *C*-8$^{ND}$), 46.8 (1C, N*C*H$_2$Ar$^{ND}$), 46.9 (1C, N*C*H$_2$Ar$^{HD}$), 47.0 (1C, N*C*H$_2$Ar$^{HD}$), 48.9 (1C, N*C*H$_2$Ar$^{ND}$), 58.4 (1C, *C*-1$^{HD}$), 58.7 (1C, *C*-1$^{ND}$), 64.00 (1C, *C*-4$^{HD+ND}$), 66.3 (1C, *C*-7$^{ND}$), 66.6 (1C, *C*-7$^{HD}$), 127.3 (1C, Ar-*C*$_{para}$$^{ND}$), 127.36 (1C, Ar-*C*$_{para}$$^{HD}$), 127.41 (2C, Ar-*C*$^{ND}$), 127.5 (1C, Ar-*C*$_{para}$$^{ND}$), 127.6 (1C, Ar-*C*$_{para}$$^{HD}$), 127.65 (2C, Ar-*C*$^{ND}$), 127.67 (2C, Ar-*C*$^{HD}$), 127.8 (2C, Ar-*C*$^{HD}$), 128.4 (2C, Ar-*C*$^{HD}$), 128.47 (2C, Ar-*C*$^{ND}$), 128.51 (2C, Ar-*C*$^{ND}$), 128.6 (2C, Ar-*C*$^{HD}$), 136.9 (1C, Ar-*C*$_q$$^{ND}$), 137.0 (1C, Ar-*C*$_q$$^{HD}$),

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137.1 (1C, Ar-$C_q^{HD}$), 137.2 (1C, Ar-$C_q^{ND}$), 167.3 (1C, $C$=$O^{HD}$), 167.8 (1C, $C$=$O^{ND}$), 168.3 (1C, $C$=$O^{ND}$), 168.7 (1C, $C$=$O^{HD}$). HD= major diastereomer, ND= minor diastereomer. Exact mass (APCI): m/z = 337.1562 (calcd. 337.1547 for $C_{20}H_{21}N_2O_3^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 3256 (w, O-H), 2924 (w, C-$H_{aliph.}$), 1663 (m, C=$O_{amide}$), 733, 694 (m, C-$H_{arom.\ monosubst.}$).

**(1*S*,4*S*,7*R*)-2,5-Dibenzyl-3,6-dioxo-2,5-diazabicyclo[2.2.2]octane-7-yl methanesulfonate ((*S*,*S*,*R*)-14a)**

A mixture of (*S*,*S*,*R*)-**13a** and (*S*,*S*,*S*)-**13a** (ratio 80:20, 13 mg, 0.04 mmol) was dissolved in $CH_2Cl_2$ (5 mL) and the solution was cooled to 0 °C. Methanesulfonyl chloride (6 µL, 0.08 mmol), 4-(dimethylamino)pyridine (5.0 mg, 0.04 mmol) and triethylamine (12 µL, 0.09 mmol) were added subsequently and the reaction mixture was stirred for 30 min at 0 °C and for 16 h at room temperature. For work up, the mixture was poured into cold water and the aqueous layer was extracted with $CH_2Cl_2$ (4 x 10 mL). The combined organic layers were dried ($Na_2SO_4$), filtered and the solvent was removed in vacuo. The residue was purified by fc (Ø 2 cm, h = 10 cm, v = 10 mL, EtOAc:cyclohexane = 1:1 →4:1, $R_f$ = 0.56 (EtOAc:cyclohexane = 4:1)) Colorless solid, mp 152-153 °C, yield 13 mg (78 %). $C_{21}H_{22}N_2O_5S$ (414.5). $[\alpha]_D^{20}$ = -15.6 (c = 0.14; $CH_3OH$). Purity by HPLC: 99.5 %, $t_R$ = 19.0 min. $^1$H NMR (600 MHz, $CDCl_3$): δ(ppm) = 1.83 (dt, *J* = 14.7 / 2.9 Hz, 1H, 8-*H*), 2.27 - 2.37 (m, 1H, 8-*H*), 2.99 (s, 3H, $SO_3CH_3$), 3.94 – 4.01 (m, 1H, 4-*H*), 4.31 (d, *J* = 4.2 Hz, 1H, 1-*H*), 4.35 (t, *J* = 14.5 Hz, 2H, $NCH_2Ar$), 4.70 – 4.77 (m, 2H, $NCH_2Ar$, 7-*H*), 4.86 (d, *J* = 14.7 Hz, 1H, $NCH_2Ar$), 7.24 (d, *J* = 7.2 Hz, 4H, Ar-*H*), 7.30 – 7.40 (m, 6H, Ar-*H*). $^{13}$C NMR (151 MHz, $CDCl_3$): δ(ppm) = 33.6 (1C, *C*-8), 39.2 (1C, $SO_3CH_3$), 48.7 (2C, $NCH_2Ar$), 58.3 (1C, *C*-4), 61.8 (1C, *C*-1), 73.1 (1C, *C*-7), 128.4 (2C, Ar-*C*), 128.5 (2C, Ar-*C*), 128.6 (1C, *C*-4$_{(benzyl)}$), 128.8 (1C, *C*-4$_{(benzyl)}$), 129.2 (2C, Ar-*C*), 129.4 (2C, Ar-*C*), 135.40 (1C, *C*-1$_{(benzyl)}$), 135.42 (1C, *C*-1$_{(benzyl)}$), 165.7 (1C, *C*=O), 168.2 (1C,

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*C*=O). Exact mass (APCI): m/z = 415.1333 (calcd. 415.1322 for $C_{21}H_{23}N_2O_5S^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2940 (w, C-$H_{aliph.}$), 1682 (m, C=$O_{amide}$), 1354, 1173 (m, S=O), 729, 698 (m, C-$H_{arom.\ monosubst.}$).

**(1*S*,4*S*,7*R*)-2,5-Dibenzyl-7-(pyrrolidin-1-yl)-2,5-diazabicyclo[2.2.2]octane-3,6-dione ((*S*,*S*,*R*)-15a) and (1*S*,4*S*,7*S*)-2,5-Dibenzyl-7-(pyrrolidin-1-yl)-2,5-diazabicyclo[2.2.2]octane-3,6-dione ((*S*,*S*,*S*)-15a)**

Method A: Under $N_2$, (*S*,*S*)-**12a** (393 mg, 1.2 mmol) was dissolved in THF (15 mL). $NaBH(OAc)_3$ (397 mg, 1.9 mmol), freshly distilled pyrrolidine (0.1 mL, 1.2 mmol) and AcOH (67 µL, 1.2 mmol) were added subsequently and the reaction mixture was stirred at room temperature for 3 d. For work-up, the mixture was treated with 0.5 M NaOH (25 mL) and the aqueous layer was extracted with $CH_2Cl_2$ (4 x 20 mL). The combined organic layers were dried ($Na_2SO_4$), filtered and the solvent was removed in vacuo. The residue was adsorbed on silica gel and purified by fc (Ø 3 cm, h = 20 cm, v = 30 mL, cyclohexane:EtOAc = 2:1 → 1:1 → 1:2 → 0:1). At first (*S*,*S*,*R*)-**15a**, then (*S*,*S*,*S*)-**15a** was eluted.

(*S*,*S*,*R*)-**15a** ($R_f$ = 0.18, cyclohexane : EtOAc = 1 : 4): Pale yellow solid, mp 118-119 °C, yield 257 mg (56 %). $C_{24}H_{27}N_3O_2$ (389.5). $[\alpha]_D^{20}$ = +6.7 (c = 0.61; $CH_2Cl_2$). Purity by HPLC: 90.5 %, $t_R$ = 15.5 min.

(S,S,S)-**15a** ($R_f$ = 0.04, cyclohexane : EtOAc = 1 : 4): Pale grey solid, mp 140-141 °C, yield 26 mg (6 %). $C_{24}H_{27}N_3O_2$ (389.5). $[\alpha]_D^{20}$ = -5.5 (c = 0.19; $CH_2Cl_2$). Purity by HPLC: 89.6 %, $t_R$ = 15.3 min.

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Method B: Under $N_2$, freshly distilled pyrrolidine (62 µL, 0.8 mmol) was dissolved in $CH_2Cl_2$ (5 mL). Then, $Ti(OiPr)_4$ (0.4 mL, 1.3 mmol) was added and the reaction mixture was cooled to 0 °C. At this temperature, a solution of (*S*,*S*)-**12a** (212 mg, 0.6 mmol) in $CH_2Cl_2$ (10 mL) was added, followed by the addition of $NaBH_3CN$ (119 mg, 1.9 mmol). The reaction mixture was then stirred at room temperature for 3 d. For work-up, the mixture was treated with 0.5 M NaOH (25 mL) and the aqueous layer was extracted with $CH_2Cl_2$ (4 x 20 mL). The combined organic layers were dried ($Na_2SO_4$), filtered and the solvent was removed in vacuo. The residue was adsorbed on silica gel and purified by fc (Ø 3.5 cm, h = 22 cm, v = 30 mL, cyclohexane:EtOAc = 2:1 → 1:1 → 1:2 → 0:1). At first (*S*,*S*,*R*)-**15a**, then (*S*,*S*,*S*)-**15a** was eluted.

(*S*,*S*,*R*)-**15a** ($R_f$ = 0.18, cyclohexane : EtOAc = 1 : 4): Pale yellow solid, yield 135 mg (55 %).

(S,S,S)-**15a** ($R_f$ = 0.04, cyclohexane : EtOAc = 1 : 4): Pale grey solid, yield 21 mg (9 %).

**(1*R*,4*R*,7*S*)-2,5-Dibenzyl-7-(pyrrolidin-1-yl)-2,5-diazabicyclo[2.2.2]octane-3,6,-dione ((*R*,*R*,*S*)-15a) and**

**(1*R*,4*R*,7*R*)-2,5-Dibenzyl-7-(pyrrolidin-1-yl)-2,5-diazabicyclo[2.2.2]octane-3,6,-dione ((*R*,*R*,*R*)-15a)**

Under $N_2$, (*R*,*R*)-**12a** (236 mg, 0.7 mmol) was dissolved in THF (50 mL). $NaBH(OAc)_3$ (237 mg, 1.1 mmol), freshly distilled pyrrolidine (60 µL, 0.7 mmol) and AcOH (60 µL, 1.1 mmol) were added subsequently and the reaction mixture was stirred at room temperature for 3 d. For work-up, the mixture was treated with 0.5 M NaOH (15 mL) and the aqueous layer was extracted with $CH_2Cl_2$ (4 x 20 mL). The combined organic layers were dried ($Na_2SO_4$), filtered and the solvent was removed in vacuo. The residue was adsorbed on silica gel and purified by fc (Ø 2.5 cm, h = 21 cm, v = 20 mL,

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EtOAc:cyclohexane:triethylamine = 8:2:0.1). At first (*R*,*R*,*S*)-**15a**, then (*R*,*R*,*R*)-**15a** was eluted.

(*R*,*R*,*S*)-**15a** ($R_f$ = 0.47, EtOAc : $CH_3OH$ = 9 : 1): Colorless solid, mp 113-114 °C, yield 218 mg (80 %). $C_{24}H_{27}N_3O_2$ (389.5). $[\alpha]_D^{20}$ = -7.6 (c = 0.35; $CH_2Cl_2$). Purity by HPLC: 99.8 %, $t_R$ = 15.8 min.

(*R*,*R*,*R*)-**15a** ($R_f$ = 0.22, EtOAc : $CH_3OH$ = 9 : 1): Pale yellow solid, mp 141-142 °C, yield 32 mg (12 %). $C_{24}H_{27}N_3O_2$ (389.5). $[\alpha]_D^{20}$ = +3.5 (c = 0.27; $CH_2Cl_2$). Purity by HPLC: 91.6 %, $t_R$ = 15.8 min.

Spectroscopic data of (*S*,*S*,*R*)-**15a** and (*R*,*R*,*S*)-**15a**

$^1$H NMR (600 MHz, $CDCl_3$): δ (ppm) = 1.64 – 1.77 (m, 5H, 3-C*H*$_{2\,pyr}$, 4-C*H*$_{2\,pyr}$, 8-*H*), 1.81 – 1.94 (m, 1H, 8-*H*), 2.20 – 2.33 (m, 2H, 2-C*H*$_{pyr}$, 5-C*H*$_{pyr}$), 2.40 (s, 1H, 7-*H*), 2.44 – 2.54 (m, 2H, 2-C*H*$_{pyr}$, 5-C*H*$_{pyr}$), 3.90 (dd, *J* = 3.4 / 1.9 Hz, 1H, 4-*H*), 4.06 (d, *J* = 3.5 Hz, 1H, 1-*H*), 4.27 (d, *J* = 14.6 Hz, 1H, N$^2$C*H*$_2$Ph), 4.31 (d, *J* = 14.7 Hz, 1H, N$^5$C*H*$_2$Ph), 4.79 (d, *J* = 14.6 Hz, 1H, N$^2$C*H*$_2$Ph), 4.87 (d, *J* = 14.9 Hz, 1H, N$^5$C*H*$_2$Ph), 7.22 – 7.25 (m, 2H, Ar-*H*), 7.26 – 7.37 (m, 8H, Ar-*H*). $^{13}$C NMR (151 MHz, $CDCl_3$): δ (ppm) = 23.5 (2C, *C*-3$_{pyr}$, *C*-4$_{pyr}$), 31.6 (1C, *C*-8), 48.3 (1C, N$^5$*C*H$_2$Ph), 48.5 (1C, N$^2$*C*H$_2$Ph), 52.2 (2C, *C*-2$_{pyr}$, *C*-5$_{pyr}$), 59.4 (1C, *C*-4), 63.1 (1C, *C*-1), 63.3 (1C, *C*-7), 128.0 (1C, Ar-*C*$_{para}$), 128.3 (1C, Ar-*C*$_{para}$), 128.52 (2C, Ar-*C*), 128.54 (2C, Ar-*C*), 128.7 (2C, Ar-*C*), 129.1 (2C, Ar-*C*), 136.1 (1C, Ar-*C*$_q$), 136.2 (1C, Ar-*C*$_q$), 168.0 (1C, *C*=O), 169.5 (1C, *C*=O). Exact mass (APCI): m/z = 390.2179 (calcd. 390.2176 for $C_{24}H_{28}N_3O_2^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2959, 2928 (w, C-H$_{aliph.}$), 1682 (s, C=O$_{amide}$), 729, 698 (m, C-H$_{arom.\ monosubst.}$).

Spectroscopic data of (*S*,*S*,*S*)-**15a** and (*R*,*R*,*R*)-**15a**

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$^1$H NMR (600 MHz, $CDCl_3$): δ (ppm) = 1.63 – 1.75 (m, 5H, 3-C$H_{2\ pyr}$, 4-C$H_{2\ pyr}$, 8-*H*), 1.91 – 1.98 (m, 1H, 8-*H*), 2.30 – 2.37 (m, 2H, 2-C$H_{2\ pyr}$, 5-C$H_{2\ pyr}$), 2.40 – 2.49 (m, 2H, 2-C$H_{2\ pyr}$, 5-C$H_{2\ pyr}$), 2.66 (ddd, *J* = 8.8 / 4.2 / 1.7 Hz, 1H, 7-*H*), 3.92 (dd, *J* = 4.0 / 1.7 Hz, 1H, 4-*H*), 4.09 (d, *J* = 1.7 Hz, 1H, 1-*H*), 4.14 (d, *J* = 15.2 Hz, 1H, N$^2$C$H_2$Ph), 4.35 (d, *J* = 14.7 Hz, 1H, N$^5$C$H_2$Ph), 4.69 (d, *J* = 14.7 Hz, 1H, N$^5$C$H_2$Ph), 5.01 (d, *J* = 15.2 Hz, 1H, N$^2$C$H_2$Ph), 7.21 (d, *J* = 6.9 Hz, 4H, Ar-*H*), 7.27 – 7.36 (m, 6H, Ar-*H*). $^{13}$C NMR (151 MHz, $CDCl_3$): δ (ppm) = 23.5 (2C, *C*-3$_{pyr}$, *C*-4$_{pyr}$), 31.9 (1C, *C*-8), 48.3 (1C, N$^5$*C*H$_2$Ph), 49.2 (1C, N$^2$*C*H$_2$Ph), 52.7 (2C, *C*-2$_{pyr}$, *C*-5$_{pyr}$), 59.2 (1C, *C*-4), 62.6 (1C, *C*-7), 63.2 (1C, *C*-1), 128.0 (1C, Ar-*C*$_{para}$), 128.2 (2C, Ar-*C*), 128.3 (1C, Ar-*C*$_{para}$), 128.3 (2C, Ar-*C*), 128.9 (2C, Ar-*C*), 129.1 (2C, Ar-*C*), 136.1 (1C, Ar-*C*$_q$), 136.1 (1C, Ar-*C*$_q$), 169.0 (1C, *C*=O), 169.4 (1C, *C*=O). Exact mass (APCI): m/z = 390.2197 (calcd. 390.2176 for $C_{24}H_{28}N_3O_2^+$ [MH]$^+$). IR (neat): ũ [cm$^{-1}$] = 2928, 2778 (w, C-H$_{aliph.}$), 1674 (s, C=O$_{amide}$), 729, 694 (m, C-H$_{arom.\ monosubst.}$).

**X-ray crystal structure analysis of (*R*,*R*,*S*)-15a**

A colorless prism-like specimen of $C_{24}H_{27}N_3O_2$, approximate dimensions 0.142 mm x 0.266 mm x 0.288 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured. A total of 1182 frames were collected. The total exposure time was 11.91 h. The frames were integrated with the Bruker SAINT software package using a wide-frame algorithm. The integration of the data using a tetragonal unit cell yielded a total of 25621 reflections to a maximum θ angle of 68.39° (0.83 Å resolution), of which 3636 were independent (average redundancy 7.046, completeness = 99.4 %, $R_{int}$ = 2.59%, $R_{sig}$ = 1.75 %) and 3579 (98.43 %) were greater than 2σ($F^2$). The final cell constants of a = 13.7270(3) Å, b = 13.7270(3) Å, c = 10.9453(3) Å, volume = 2062.43(11) Å$^3$, are based upon the refinement of the XYZ-centroids of 9895 reflections above 20 σ(I) with 10.33° < 2θ < 136.5°. Data were corrected for absorption effects using the multi-

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scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.913. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.8370 and 0.9150. The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group $P4_3$, with Z = 4 for the formula unit, $C_{24}H_{27}N_3O_2$. The final anisotropic full-matrix least-squares refinement on $F^2$ with 262 variables converged at R1 = 2.60%, for the observed data and wR2 = 6.43% for all data. The goodness-of-fit was 1.103. The largest peak in the final difference electron density synthesis was 0.107 $e^-/Å^3$ and the largest hole was -0.156 $e^-/Å^3$ with an RMS deviation of 0.034 $e^-/Å^3$. On the basis of the final model, the calculated density was 1.254 $g/cm^3$ and F(000), 832 $e^-$. Flack parameter was refined to 0.01(5). The data were deposited under CCDC 1546596.

**(1*S*,4*S*,7*R*)-5-Benzyl-2-(4-methoxybenzyl)-7-(pyrrolidin-1-yl)-2,5-diazabicyclo[2.2.2]octane-3,6-dione ((*S*,*S*,*R*)-15b) and (1*S*,4*S*,7*S*)-5-Benzyl-2-(4-methoxybenzyl)-7-(pyrrolidin-1-yl)-2,5-diazabicyclo[2.2.2]octane-3,6-dione ((*S*,*S*,*S*)-15b)**

Under $N_2$, (*S*,*S*)-**12b** (125 mg, 0.3 mmol) was dissolved in THF (20 mL). $NaBH(OAc)_3$ (117 mg, 0.6 mmol), freshly distilled pyrrolidine (28 µL, 0.3 mmol) and AcOH (19 µL, 0.3 mmol) were added subsequently and the reaction mixture was stirred at room temperature for 18 h. For work-up, the mixture was treated with 0.5 M NaOH (15 mL) and the aqueous layer was extracted with $CH_2Cl_2$ (5 x 10 mL). The combined organic layers were dried ($Na_2SO_4$), filtered and the solvent was removed in vacuo. The residue was adsorbed on silica gel and purified by fc (Ø 3 cm, h = 23 cm, v = 20 mL, EtOAc:cyclohexane = 6:4 → 1:1 → 1:0). At first (*S*,*S*,*R*)-**15b**, then (*S*,*S*,*S*)-**15b** was eluted.

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(*S*,*S*,*R*)-**15b** ($R_f$ = 0.49, EtOAc : $CH_3OH$ = 9 : 1): Pale yellow oil, yield 113 mg (79 %). $C_{25}H_{29}N_3O_3$ (419.5). $[\alpha]_D^{20}$ = +9.3 (c = 0.34; $CH_2Cl_2$). Purity by HPLC: 90.4 %, $t_R$ = 16.0 min. $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.64 – 1.79 (m, 5H, 3-C*H*$_{2\ pyr}$, 4-C*H*$_{2\ pyr}$, 8-*H*), 1.83 – 1.93 (m, 1H, 8-*H*), 2.24 – 2.37 (m, 2H, 2-C*H*$_{pyr}$, 5-C*H*$_{pyr}$), 2.38 – 2.46 (m, 1H, 7-*H*), 2.46 – 2.57 (m, 2H, 2-C*H*$_{pyr}$, 5-C*H*$_{pyr}$), 3.80 (s, 3H, OC*H*$_3$), 3.86 – 3.92 (m, 1H, 4-*H*), 4.07 (d, *J* = 2.8 Hz, 1H, 1-*H*), 4.20 (d, *J* = 14.5 Hz, 1H, NC*H*$_2$Ar), 4.33 (d, *J* = 14.5 Hz, 1H, NC*H*$_2$Ar), 4.73 (d, *J* = 14.5 Hz, 1H, NC*H*$_2$Ar), 4.85 (d, *J* = 14.9 Hz, 1H, NC*H*$_2$Ar), 6.86 (d, *J* = 8.5 Hz, 2H, 3-*H*, 5-*H* $_{(p\text{-methoxybenzyl})}$), 7.17 (d, *J* = 8.5 Hz, 2H, 2-*H*, 6-*H* $_{(p\text{-methoxybenzyl})}$), 7.27 – 7.31 (m, 5H, Ar-*H*). $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) = 23.5 (2C, *C*-3$_{pyr}$, *C*-4$_{pyr}$), 31.4 (1C, *C*-8), 47.9 (1C, NC$H_2$Ar), 48.3 (1C, NC$H_2$Ar), 52.2 (2C, *C*-2$_{pyr}$, *C*-5$_{pyr}$), 55.4 (1C, OC$H_3$), 59.4 (1C, *C*-4), 62.7 (1C, *C*-1), 63.2 (1C, *C*-7), 114.4 (2C, *C*-3, *C*-5$_{(p\text{-methoxybenzyl})}$), 128.0 (1C, *C*-4$_{(benzyl)}$), 128.1 (1C, *C*-1$_{(p\text{-methoxybenzyl})}$), 128.5 (2C, *C*-2, *C*-6$_{(benzyl)}$), 128.7 (2C, *C*-3, *C*-5$_{(benzyl)}$), 129.9 (2C, *C*-2, *C*-6$_{(p\text{-methoxybenzyl})}$), 136.2 (1C, *C*-1$_{(benzyl)}$), 159.6 (1C, *C*-4$_{(p\text{-methoxybenzyl})}$), 169.3 (1C, *C*=O), 171.3 (1C, *C*=O). Exact mass (APCI): m/z = 420.2281 (calcd. 420.2282 for $C_{25}H_{30}N_3O_3^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2963, 2793 (w, C-$H_{aliph.}$), 1682 (s, C=$O_{amide}$), 737, 698 (m, C-$H_{arom.\ monosubst.}$).

(*S*,*S*,*S*)-**15b** (($R_f$ = 0.22, EtOAc : $CH_3OH$ = 9 : 1): Pale yellow oil, yield 12 mg (8 %). $C_{25}H_{29}N_3O_3$ (419.5). $[\alpha]_D^{20}$ = -26.5 (c = 4.5 mg; $CH_2Cl_2$). Purity by HPLC: 97.9 %, $t_R$ = 15.9 min. $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.63 – 1.84 (s, 5H, 3-C*H*$_{2\ pyr}$, 4-C*H*$_{2\ pyr}$, 8-*H*), 1.90 – 2.02 (m, 1H, 8-*H*), 2.36 – 2.61 (m, 4H, 2-C*H*$_{2\ pyr}$, 5-C*H*$_{2\ pyr}$), 2.66 – 2.83 (s, 1H, 7-*H*), 3.79 (s, 3H, OC*H*$_3$), 3.89 – 3.95 (m, 1H, 4-*H*), 4.09 (d, *J* = 15.5 Hz, 1H, NC*H*$_2$Ar), 4.13 (s, 1H, 1-*H*), 4.34 (d, *J* = 14.7 Hz, 1H, NC*H*$_2$Ar), 4.67 (d, *J* = 14.7 Hz, 1H, NC*H*$_2$Ar), 4.97 (d, *J* = 14.9 Hz, 1H, NC*H*$_2$Ar), 6.86 (d, *J* = 8.5 Hz, 2H, 3-*H*, 5-*H* $_{(p\text{-methoxybenzyl})}$), 7.15 (d, *J* = 8.2 Hz, 2H, 2-*H*, 6-*H* $_{(p\text{-methoxybenzyl})}$), 7.16 – 7.24 (m, 2H, Ar-*H*), 7.28 – 7.39 (m, 3H, Ar-*H*). $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) = 23.5 (2C, *C*-

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$3_{pyr}$, *C*-$4_{pyr}$), 31.4 (1C, *C*-8), 48.3 (1C, N*C*$H_2$Ar), 48.6 (1C, N*C*$H_2$Ar), 52.7 (2C, *C*-$2_{pyr}$, *C*-$5_{pyr}$), 55.4 (1C, O*C*$H_3$), 59.2 (1C, *C*-4), 62.4 (1C, *C*-7), 62.6 (1C, *C*-1), 114.3 (2C, *C*-3, *C*-$5_{(p\text{-methoxybenzyl})}$), 128.0 (1C, *C*-$1_{(p\text{-methoxybenzyl})}$), 128.3 (3C, Ar-*C*), 129.1 (2C, Ar-*C*), 129.7 (2C, *C*-2, *C*-$6_{(p\text{-methoxybenzyl})}$), 136.0 (1C, *C*-$1_{(benzyl)}$), 159.5 (1C, *C*-$4_{(p\text{-methoxybenzyl})}$), 168.7 (1C, *C*=O), 169.1 (1C, *C*=O). Exact mass (APCI): m/z = 420.2260 (calcd. 420.2282 for $C_{25}H_{30}N_3O_3^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2963, 2793 (w, C-$H_{aliph.}$), 1682 (s, C=$O_{amide}$), 1242 (m, C-N), 737, 698 (m, C-$H_{arom.\ monosubst.}$).

**(1*R*,4*S*,7*R*)-2,5-Dibenzyl-7-(pyrrolidin-1-yl)-2,5-diazabicyclo[2.2.2]octane ((*R*,*S*,*R*)-16a)**

Under $N_2$, (*S*,*S*,*R*)-**15a** (323 mg, 0.8 mmol) was dissolved in THF (10 mL) and cooled to 0 °C. $LiAlH_4$ (157 mg, 4.1 mmol) was added in portions and the mixture was stirred for 10 min at 0 °C and for 16 h under reflux. After cooling to 0 °C, $H_2O$ was added slowly as long as formation of $H_2$ could be observed. Afterwards, the mixture was heated to reflux for 30 min. After cooling to room temperature, the suspension was filtered and the solvent was removed in vacuo. The residue was adsorbed on silica gel and purified by fc (Ø 3 cm, h = 18 cm, v = 20 mL, EtOAc:triethylamine = 10: 0.1, $R_f$ = 0.67 (EtOAc:triethylamine = 9:1)). Yellow oil, yield 202 mg (67 %). $C_{24}H_{31}N_3$ (361.5). $[\alpha]_D^{20}$ = -25.3 (c = 0.22; $CH_2Cl_2$). Purity by HPLC: 98.6 %, $t_R$ = 14.5 min.

**(1*S*,4*R*,7*S*)-2,5-Dibenzyl-7-(pyrrolidin-1-yl)-2,5-diazabicyclo[2.2.2]octane ((*S*,*R*,*S*)-16a)**

As described for the synthesis of (*R*,*S*,*R*)-**16a**, (*R*,*R*,*S*)-**15a** (513 mg, 1.3 mmol) was reduced with $LiAlH_4$ (250 mg, 6.6 mmol) in THF (25 mL). Yellow oil, yield 332 mg (71

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%). $C_{24}H_{31}N_3$ (361.5). $[\alpha]_D^{20}$ = +9.4 (c = 0.21; $CH_2Cl_2$). Purity by HPLC: 93 %, $t_R$ = 14.2 min.

Spectroscopic data of (*R*,*S*,*R*)-**16a** and (*S*,*R*,*S*)-**16a**

$^1$H NMR (600 MHz, $CDCl_3$): δ (ppm) = 1.73 – 1.80 (m, 4H, 3-C*H*$_{2\ pyr}$, 4-C*H*$_{2\ pyr}$), 1.80 - 1.85 (m, 1H, 8-*H* ), 1.92– 1.99 (m, 1H, 8-*H*), 2.35 – 2.45 (m, 2H, 2-C*H*$_{2\ pyr}$, 5-C*H*$_{2\ pyr}$), 2.51 – 2.59 (m, 3H, 2-C*H*$_{2\ pyr}$, 5-C*H*$_{2\ pyr}$, 7-*H*), 2.61 – 2.67 (m, 2H, 4-*H*, 6-*H*), 2.67 – 2.72 (m, 1H, 1-*H*), 2.97 – 3.07 (m, 2H, 3-$H_{ax}$, 3-$H_{eq}$), 3.08– 3.13 (d, *J* = 9.4 Hz, 1H, 6-*H*), 3.80 (s, 2H, N$^5$C*H*$_2$Ph), 3.86 (s, 2H, N$^2$C*H*$_2$Ph), 7.21 – 7.27 (m, 2H, Ar-*H*), 7.28 – 7.37 (m, 4H, Ar-*H*), 7.40 (d, *J* = 6.7 Hz, 4H, Ar-*H*). $^{13}$C NMR (151 MHz, $CDCl_3$): δ (ppm) = 23.4 (2C, *C*-3$_{pyr}$, *C*-4$_{pyr}$), 31.3 (1C, *C*-8), 49.3 (2C, *C*-3, *C*-4), 52.7 (2C, *C*-2$_{pyr}$, *C*-5$_{pyr}$), 53.0 (1C, *C*-1), 53.6 (1C, *C*-6), 59.3 (1C, N$^5$*C*H$_2$Ph), 59.5 (1C, N$^2$*C*H$_2$Ph), 61.4 (1C, *C*-7), 126.9 (1C, Ar-*C*$_{para}$), 127.0 (1C, Ar-*C*$_{para}$), 128.28 (2C, Ar-*C*), 128.33 (2C, Ar-*C*), 128.7 (2C, Ar-*C*), 128.9 (2C, Ar-*C*), 139.8 (2C, Ar-$C_q$). Exact mass (APCI): m/z = 362.2593 (calcd. 362.2591 for $C_{24}H_{32}N_3^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2940, 2789 (w, C-H$_{aliph.}$), 729, 698 (m, C-H$_{arom.\ monosubst.}$).

**(1*R*,4*S*,7*S*)-2,5-Dibenzyl-7-(pyrrolidin-1-yl)-2,5-diazabicyclo[2.2.2]octane ((*R*,*S*,*S*)-16a)**

As described for the synthesis of (*R*,*S*,*R*)-**16a**, (*S,S,S*)-**15a** (44 mg, 0.1 mmol) was reduced with $LiAlH_4$ (21 mg, 0.6 mmol) in THF (10 mL). The residue was adsorbed on silica gel and purified by fc (Ø 2 cm, h = 15 cm, v = 10 mL, EtOAc:cyclohexane: triethylamine = 7:3:0.1 $R_f$ = 0.50 (EtOAc:triethylamine = 9:1)). Yellow oil, yield 13 mg (31 %). $C_{24}H_{31}N_3$ (361.5). $[\alpha]_D^{20}$ = +6.8 (c = 0.12; $CH_2Cl_2$).Purity by HPLC: 95.3 %, $t_R$ = 13.5 min.

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**(1*S*,4*R*,7*R*)-2,5-Dibenzyl-7-(pyrrolidin-1-yl)-2,5-diazabicyclo[2.2.2]octane ((*S*,*R*,*R*)-16a)**

As described for the synthesis of (*R*,*S*,*R*)-**16a**, (*R*,*R*,*R*)-**15a** (35 mg, 0.1 mmol) was reduced with $LiAlH_4$ (17 mg, 0.4 mmol) in THF (10 mL). The residue was adsorbed on silica gel and purified by fc (Ø 2 cm, h = 13 cm, v = 10 mL, EtOAc:cyclohexane:triethylamine = 8:2:0.1 $R_f$ = 0.50 (EtOAc:triethylamine = 9:1)). Yellow oil, yield 19 mg (57 %). $C_{24}H_{31}N_3$ (361.5). $[\alpha]_D^{20}$ = -0.7 (c = 0.1; $CH_2Cl_2$). Purity by HPLC: 94.5 %, $t_R$ = 13.3 min.

Spectroscopic data of (*R*,*S*,*S*)-**16a** and (*S*,*R*,*R*)-**16a**

$^1$H NMR (600 MHz, $CDCl_3$): δ (ppm) = 1.65 – 1.75 (m, 5H, 3-C*H*$_{2pyr}$, 4-C*H*$_{2\ pyr}$, 8-*H*), 2.10 – 2.25 (m, 3H, 8-*H*, 2-C*H*$_{2(pyr)}$, 5-C*H*$_{2(pyr)}$), 2.30 – 2.47 (m, 3H, 7-*H*, 2-C*H*$_{2(pyr)}$, 5-C*H*$_{2(pyr)}$), 2.62 – 2.64 (m, 1H, 4-*H*), 2.65 – 2.68 (m, 2H, 6-*H*, 1-*H*), 2.90 (dt, *J* = 10.3 / 2.0 Hz, 1H, 3-*H*), 3.00 (dd, *J* = 10.6 / 2.0 Hz, 1H, 6-*H*), 3.14 (dd, *J* = 10.2 / 2.8 Hz, 1H, 3-*H*), 3.67 (d, *J* = 12.9 Hz, 1H, N$^5$C*H*$_2$Ph), 3.72 (s, 2H, N$^2$C*H*$_2$Ph), 3.85 (d, *J* = 12.9 Hz, 1H, N$^5$C*H*$_2$Ph), 7.20 – 7.41 (m, 10H, Ar-*H*). $^{13}$C NMR (151 MHz, $CDCl_3$): δ (ppm) = 23.2 (2C, *C*-3$_{pyr}$, *C*-4$_{pyr}$), 33.3 (1C, *C*-8), 50.2 (1C, *C*-6), 50.8 (1C, *C*-1), 51.6 (1C, *C*-4), 52.2 (2C, *C*-2$_{pyr}$, *C*-5$_{pyr}$), 53.1 (1C, *C*-3), 58.9 (1C, N$^2$*C*H$_2$Ph), 59.45 (1C, N$^5$CH$_2$Ph), 64.5 (1C, *C*-7), 127.0 (1C, *C*-4$_{(benzyl)}$), 127.1 (1C, *C*-4$_{(benzyl)}$), 128.2 (2C, Ar-*C*), 128.4 (2C, Ar-*C*), 128.7 (2C, Ar-*C*), 129.5 (2C, Ar-*C*), 139.2 (1C, *C*-1$_{(benzyl)}$), 139.7 (1C, *C*-1$_{(benzyl)}$). Exact mass (APCI): m/z = 362.2596 (calcd. 362.2591 for $C_{24}H_{32}N_3^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2951, 2789 (w, C-H$_{aliph.}$), 729, 698 (m, C-H$_{arom.\ monosubst.}$).

**(1*R*,4*S*,7*R*)-5-Benzyl-2-(4-methoxybenzyl)-7-(pyrrolidin-1-yl)-2,5-diazabicyclo[2.2.2]octane ((*R*,*S*,*R*)-16b)**

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As described for the synthesis of (*R*,*S*,*R*)-**16a**, (*S*,*S*,*R*)-**15b** (64 mg, 0.2 mmol) was reduced with $LiAlH_4$ (29 mg, 0.8 mmol) in THF (10 mL). The residue was adsorbed on silica gel and purified by fc (Ø 2 cm, h = 20 cm, v = 10 mL, EtOAc:cyclohexane:triethylamine = 8:2:0.1, $R_f$ = 0.29 (EtOAc:$CH_3OH$:triethylamine = 9:1:0.1)). Yellow oil, yield 18 mg (30 %). $C_{25}H_{33}N_3O$ (391.5). $[\alpha]_D^{20}$ = +11.0 (c = 0.18; $CH_2Cl_2$). Purity by HPLC: 91.2 %, $t_R$ = 14.8 min. $^1$H NMR (600 MHz, $CDCl_3$): δ (ppm) = 1.71 – 1.82 (m, 5H, 3-C$H_{2\ pyr}$, 4-C$H_{2\ pyr}$, 8-*H*), 1.89 – 1.97 (m, 1H, 8-*H*), 2.33 – 2.42 (s, 2H, 2-C$H_{2\ pyr}$, 5-C$H_{2\ pyr}$), 2.48 – 2.56 (m, 3H, 7-*H*, 2-C$H_{2\ pyr}$, 5-C$H_{2\ pyr}$), 2.58 – 2.64 (m, 2H, 3-*H,* 4-*H*), 2.65 – 2.70 (m, 1H, 1-*H*), 2.94 – 3.04 (m, 2H, 6-$H_{ax}$, 6-$H_{eq}$), 3.04 – 3.10 (m, 1H, 3-*H*), 3.74 – 3.79 (m, 4H, NC$H_2$Ar), 3.80 (s, 3H, OC$H_3$), 6.85 (d, *J* = 8.5 Hz, 2H, 3-*H*, 5-*H* $_{(p\text{-methoxybenzyl})}$), 7.19 - 7.25 (m, *J* = 7.8 / 7.3 Hz, 1H, Ar-*H*), 7.26 – 7.35 (m, 4H, 3-*H*$_{(benzyl)}$, 5-*H* $_{(benzyl)}$, 2-*H*$_{(p\text{-methoxybenzyl})}$, 6-*H* $_{(p\text{-methoxybenzyl})}$), 7.39 (d, *J* = 7.3 Hz, 2H, 2-*H*, 6-*H* $_{(benzyl)}$). $^{13}$C NMR (151 MHz, $CDCl_3$): δ(ppm) = 23.4 (2C, *C*-$3_{pyr}$, *C*-$4_{pyr}$), 31.3 (1C, *C*-8), 49.3 (1C, *C*-4), 49.4 (1C, *C*-6), 52.7 (1C, *C*-1), 52.8 (2C, *C*-$2_{pyr}$, *C*-$5_{pyr}$), 53.6 (1C, *C*-3), 55.4 (1C, O*C*$H_3$), 58.8 (1C, N*C*$H_2$Ar), 59.3 (1C, N*C*$H_2$Ar), 61.3 (1C, *C*-7), 113.7 (2C, *C*-3, *C*-$5_{(p\text{-methoxybenzyl})}$), 126.9 (1C, *C*-$4_{(benzyl)}$), 128.3 (2C, *C*-3, *C*-$5_{(benzyl)}$), 128.9 (2C, *C*-2, *C*-$6_{(benzyl)}$), 129.9 (2C, *C*-2, *C*-$6_{(p\text{-methoxybenzyl})}$), 131.8 (1C, *C*-$1_{(p\text{-methoxybenzyl})}$), 139.9 (1C, *C*-$1_{(benzyl)}$), 158.7 (1C, *C*-$4_{(p\text{-methoxybenzyl})}$). Exact mass (APCI): m/z = 392.2708 (calcd. 392.2696 for $C_{25}H_{34}N_3O^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2932, 2789 (w, C-$H_{aliph.}$), 1508 (m, C=$C_{arom.}$), 1242 (s, C-N), 733, 698 (m, C-$H_{arom.\ monosubst.}$).

**(1*R*,4*S*,7*R*)-7-(Pyrrolidin-1-yl)-2,5-diazabicyclo[2.2.2]octane ((*R*,*S*,*R*)-17)**

(*R*,*S*,*R*)-**16a** (115 mg, 0.3 mmol) was dissolved in a 1:1 mixture of THF and $H_2O$ (25 mL). HCl conc. (0.5 mL) and Pd/C (10 %, 56.2 mg) were added and the reaction mixture was stirred for 16 h under $H_2$ (1 atm, balloon) at room temperature. For work-up, it was filtered through Celite® and THF was removed in vacuo. The pH value of the aqueous

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solution was set to pH 13-14 by the addition of NaOH platelets and the mixture was extracted with $CH_2Cl_2$ (5 x 10 mL). The aqueous layer was diluted with brine and extracted with a solution of $CH_2Cl_2$ and $CH_3OH$ (5 x 15 mL, 2:1). The combined organic layers were dried ($K_2CO_3$), filtered and the solvent was removed in vacuo. Pale yellow oil, yield 56 mg (99.9 %). $C_{10}H_{19}N_3$ (181.3). $[\alpha]_D^{20}$ = -15.6 (c = 0.14; $CH_3OH$). Exact mass (APCI): m/z = 182.1649 (calcd. 182.1652 for $C_{10}H_{20}N_3^+$ $[MH]^+$).

**(1*S*,4*R*,7*S*)-7-(Pyrrolidin-1-yl)-2,5-diazabicyclo[2.2.2]octane ((*S*,*R*,*S*)-17)**

As described for the synthesis of (*R*,*S*,*R*)-**17**, a mixture of (*S*,*R*,*S*)-**16a** (241 mg, 0.7 mmol), THF/$H_2O$ (29 mL, 1:1), HCl conc. (1.0 mL) and Pd/C (10 %, 118 mg) was stirred under $H_2$ (1 atm, balloon). Pale yellow oil, yield 90.5 mg (74 %). $C_{10}H_{19}N_3$ (181.3). $[\alpha]_D^{20}$ = +5.6 (c = 0.12; $CH_3OH$). Exact mass (APCI): m/z = 182.1664 (calcd. 182.1652 for $C_{10}H_{20}N_3^+$ $[MH]^+$).

Spectroscopic data of (*R*,*S*,*R*)-**17** and (*S*,*R*,*S*)-**17**

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.67– 1.84 (m, 5H, 3-C*H*$_{2\ pyr}$, 4-C*H*$_{2\ pyr}$, 8-*H*), 1.84 – 1.94 (m, 1H, 8-*H*), 2.48 – 2.26 (m, 3H, 2-C*H*$_{2\ pyr}$, 5-C*H*$_{2\ pyr}$, 7-*H*), 2.50 – 2.60 (m, 2H, 2-C*H*$_{2\ pyr}$, 5-C*H*$_{2\ pyr}$), 2.81– 2.86 (m, 1H, 4-*H*), 2.88 – 2.97 (m, 2H, 1-*H*, 6-*H*), 3.04 – 3.14 (m, 1H, 3-*H*), 3.20 – 3.30 (m, 1H, 6-*H*), 3.32 – 3.44 (m, 1H, 3-*H*). Signals for the N*H*-protons are not seen. $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) = 23.3 (2C, *C*-3$_{pyr}$, *C*-4$_{pyr}$), 35.0 (1C, *C*-8), 43.8 (1C, *C*-3), 44.2 (1C, *C*-1), 46.9 (1C, *C*-4), 47.6 (1C, *C*-6), 52.6 (2C, *C*-2$_{pyr}$, *C*-5$_{pyr}$), 64.0 (1C, *C*-7). IR (neat): ũ [$cm^{-1}$] = 3291 (w, N-H), 2959 (w, C-H $_{aliph.}$).

**1,1'-((1*R*,4*S*,7*R*)-7-(Pyrrolidin-1-yl)-2,5-diazabicyclo[2.2.2]octane-2,5-diyl)bis(2-(3,4-dichlorophenyl)ethanone) ((*R*,*S*,*R*)-18)**

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Under $N_2$, (*R*,*S*,*R*)-**17** (32 mg, 0.2 mmol) was dissolved in $CH_2Cl_2$ (5 mL). (3,4-Dichlorophenyl)acetyl chloride (97 mg, 0.4 mmol) was added and the reaction mixture was stirred for 16 h at room temperature. For work-up, 2 M NaOH (10 mL), was added and the mixture was stirred for 1 h. The aqueous layer was extracted with $CH_2Cl_2$ (4 x 10 mL). The combined organic layers were dried ($K_2CO_3$), filtered and the solvent was removed in vacuo. The residue was purified by fc (Ø 2 cm, h = 20 cm, v = 10 mL, EtOAc:$CH_3OH$:triethylamine = 9.5: 0.45: 0.05, $R_f$ = 0.36)). Colorless solid, mp 165-166 °C, yield 42 mg (45 %). $C_{26}H_{27}Cl_4N_3O_2$ (555.3). $[\alpha]_D^{20}$ = -22.4 (c = 0.16; $CH_2Cl_2$). Purity by HPLC: 93.0 %, $t_R$ = 20.2 min.

**1,1'-((1*S*,4*R*,7*S*)-7-(Pyrrolidin-1-yl)-2,5-diazabicyclo[2.2.2]octane-2,5-diyl)bis(2-(3,4-dichlorophenyl)ethanone) ((*S*,*R*,*S*)-18)**

A described for the synthesis of (*R*,*S*,*R*)-**18** (32 mg, 0.2 mmol), enantiomer (*S*,*R*,*S*)-**17** (18 mg, 0.1 mmol) dissolved in $CH_2Cl_2$ (5 mL) was acylated with (3,4-dichlorophenyl)acetyl chloride (90 mg, 0.4 mmol). Colorless solid, mp 160-161 °C, yield 37 mg (66 %). $C_{26}H_{27}Cl_4N_3O_2$ (555.3). $[\alpha]_D^{20}$ = +9.2 (c = 0.17; $CH_2Cl_2$). Purity by HPLC: 91.8 %, $t_R$ = 20.4 min.

Spectroscopic data of (*R*,*S*,*R*)-**18** and (*S*,*R*,*S*)-**18**

$^1$H NMR (400 MHz, $(CD_3)_2SO$): δ(ppm) = 1.59 – 1.71 (m, 5H, 3-C*H*$_{2\ pyr}$, 4-C*H*$_{2\ pyr}$, 8-*H*), 1.90 – 2.04 (m, 1H, 8-*H*), 2.19 – 2.26 (m, 1H, 7-*H*$^{R1}$), 2.27 – 2.49 (m, 5H, 2-C*H*$_{2\ pyr}$, 5-C*H*$_{2\ pyr}$, 7-*H*$^{R2}$), 3.37 – 3.89 (m, 8H, 3-*H*$_{eq}$, 3-*H*$_{ax}$, 6-*H*$_{eq}$, 6-*H*$_{ax}$, O=CC*H*$_2$Ar, O=CC*H*$_2$Ar), 4.17 – 4.24 (m, 0.5H, 4-*H*$^{HR}$), 4.26 – 4.31 (m, 0.25H, 4-*H*$^{NR1}$), 4.32 – 4.37 (m, 0.25H, 4-*H*$^{NR2}$), 4.57 – 4.61 (m, 0.25H, 1-*H*$^{NR1}$), 4.62 – 4.65 (m, 0.25H, 1-*H*$^{NR2}$), 4.66 – 4.73 (m, 0.5H, 1-*H*$^{HR}$), 7.17 – 7.28 (m, 2H, 6-*H*$_{Ar1+2}$), 7.46 – 7.62 (m, 4H, 2-*H*$_{Ar1+2}$, 5-*H*$_{Ar1+2}$). HR = major rotamer, NR = minor rotamer. $^{13}$C NMR (101 MHz, $(CD_3)_2SO$): δ(ppm) = 22.78,

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22.81 (2 x 1C, *C*-$3_{pyr}$, *C*-$4_{pyr}$), 32.2, 32.3, 32.9, 33.0 (2C, *C*-8), 37.71, 37.75, 37.78, 37.85, 38.16, 38.21, 38.6, 38.7 (4C, O=C*C*$H_2$Ar, O=C*C*$H_2$Ar), 42.0, 42.4 (2 x 0.3C, *C*-$1^{R1+2}$), 43.5, 44.0, 44.5, 44.7, 44.8 (5 x 0.4C, *C*-3), 45.0 (0.4C, *C*-$1^{HR}$), 46.3, 46.8 (0.7C, *C*-4), 47.9, 48.4, 48.9, 49.25, 49.31 (2C, *C*-6), 49.5 (0.3C, *C*-4), 51.58, 51.64 (2C, *C*-$2_{pyr}$, *C*-$5_{pyr}$), 60.4, 61.0 (2 x 0.5C, *C*-$7^{HR+NR}$), 128.9 – 129.0 (2C, *C*-$Cl^{HR+NR}$), 129.9, 130.0, 130.06, 130.08 (2C, *C*-$6_{Ar}^{HR+NR}$), 130.1, 130.17, 130.18, 130.3 (2C, Ar-$C^{HR+NR}$), 130.46, 130.48, 130.5, 130.54, 130.57, 130.73, 130.74 (2C, *C*-$Cl^{HR+NR}$), 131.38, 131.4, 131.49, 131.51, 131.7 (2C, Ar-$C^{HR+NR}$), 136.8, 136.83, 136.9, 137.09, 137.13 (*C*-$1_{Ar}^{HR+NR}$), 167.69, 167.73, 167.95, 168.08, 168.42, 168.44, 168.57, 168.71 (2C, *C*=$O^{HR+NR}$). HR = major rotamer, NR = minor rotamer. Exact mass (APCI): m/z = 554.0930 (calcd. 554.0949 for $C_{26}H_{28}Cl_4N_3O_2^+$ $[MH]^+$). IR (neat): ũ [$cm^{-1}$] = 2967, 2793 (w, C-H $_{aliph.}$), 1636 (s, C=$O_{amide}$), 1408 (s, C=$C_{arom}$), 883, 783 (w, C-H $_{arom.\ disubst.}$).

**X-ray crystal structure analysis of 18**

A colorless needle-like specimen of $C_{26}H_{27}Cl_4N_3O_2$, approximate dimensions 0.015 mm x 0.051 mm x 0.228 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured. A total of 4178 frames were collected. The total exposure time was 29.64 hours. The frames were integrated with the Bruker SAINT software package using a narrow-frame algorithm. The integration of the data using a monoclinic unit cell yielded a total of 31737 reflections to a maximum θ angle of 68.36° (0.83 Å resolution), of which 4563 were independent (average redundancy 6.955, completeness = 99.8 %, $R_{int}$ = 8.60%, $R_{sig}$ = 4.68 %) and 3427 (75.10 %) were greater than $2\sigma(F^2)$. The final cell constants of a = 24.6806(6) Å, b = 6.2455(2) Å, c = 16.1174(4) Å, β = 93.5410(10)°, volume = 2479.64(12) $Å^3$, are based upon the refinement of the XYZ-centroids of 9893 reflections above 20 σ(I) with 7.176° < 2θ < 136.5°. Data were corrected for absorption effects using the multi-scan method (SADABS). The ratio of

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minimum to maximum apparent transmission was 0.730. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.4210 and 0.9340. The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group *P*2/*c*, with Z = 4 for the formula unit, $C_{26}H_{27}Cl_4N_3O_2$. The final anisotropic full-matrix least-squares refinement on $F^2$ with 316 variables converged at R1 = 5.51 %, for the observed data and wR2 = 14.25 % for all data. The goodness-of-fit was 1.055. The largest peak in the final difference electron density synthesis was 0.618 $e^-/Å^3$ and the largest hole was -0.443 $e^-/Å^3$ with an RMS deviation of 0.068 $e^-/Å^3$. On the basis of the final model, the calculated density was 1.487 $g/cm^3$ and F(000), 1152 $e^-$). The data were deposited under CCDC 1546597.

## 6. Molecular modelling

The conformational analysis was performed with force field MMFF94x of the Molecular Modelling Program MOE (molecular operating environment), version 2013.08 (Chemical Computing Group AG).

## 7. Experimental, Pharmacological

### 7.1. Materials

The guinea pig brains for KOR, MOR, and $\sigma_1$ receptor binding assays, rat brains for DOR binding assay and rat liver for $\sigma_2$ binding assay were commercially available (Harlan-Winkelmann, Borchen, Germany). Homogenizer: Elvehjem Potter (B. Braun Biotech International, Melsungen, Germany) and Soniprep 150, MSE, London, UK). Centrifuges: Cooling centrifuge model Rotina 35R (Hettich, Tuttlingen, Germany) and High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Fisher Scientific, Langenselbold, Germany). Multiplates: standard 96-well multiplates (Diagonal, Muenster, Germany). Shaker: self-made device with adjustable temperature and tumbling speed (scientific

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workshop of the institute). Vortexer: Vortex Genie 2 (Thermo Fisher Scientific, Langenselbold, Germany). Harvester: MicroBeta FilterMate-96 Harvester. Filter: Printed Filtermat Typ A and B. Scintillator: Meltilex (Typ A or B) solid state scintillator. Scintillation analyzer: MicroBeta Trilux (all Perkin Elmer LAS, Rodgau-Jügesheim, Germany). Chemicals and reagents were purchased from different commercial sources and of analytical grade.



**7.2. Preparation of membrane homogenates from guinea pig brain**

Five guinea pig brains were homogenized with the potter (500-800 rpm, 10 up-and-down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1200 x *g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 23500 x *g* for 20 min at 4 °C. The pellet was resuspended in 5-6 volumes of buffer (50 mM TRIS, pH 7.4) and centrifuged again at 23500 x g (20 min, 4 °C). This procedure was repeated twice. The final pellet was resuspended in 5-6 volumes of buffer and frozen (−80 °C) in 1.5 mL portions containing about 1.5 mg protein/mL.

**7.3. Preparation of membrane homogenates from rat brain**

5 rat brains (Sprague Dawley rats) were homogenized with the potter (500-800 rpm, 10 up-and-down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1,200 x *g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 23,500 x *g* for 20 min at 4 °C. The pellet was resuspended in 5-6 volumes of buffer (50 mM TRIS, pH 7.4) and centrifuged again at 23,500 x *g* (20 min, 4 °C). This procedure was repeated twice. The final pellet was resuspended in 5-6 volumes of buffer and stored at -80,°C in 1.5 mL portions containing about 1.5 mg protein/mL.

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### 7.4. Preparation of membrane homogenates from rat liver

Two rat livers were cut into small pieces and homogenized with the potter (500-800 rpm, 10 up-and-down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1,200 x *g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 31,000 x *g* for 20 min at 4 °C. The pellet was resuspended in 5-6 volumes of buffer (50 mM TRIS, pH 8.0) and incubated at room temperature for 30 min. After the incubation, the suspension was centrifuged again at 31,000 x *g* for 20 min at 4 °C. The final pellet was resuspended in 5-6 volumes of buffer and stored at -80,°C in 1.5 mL portions containing about 2 mg protein/mL.

### 7.5. Protein determination

The protein concentration was determined by the method of Bradford,[36] modified by Stoscheck.[37] The Bradford solution was prepared by dissolving 5 mg of Coomassie Brilliant Blue G 250 in 2.5 mL of EtOH (95%, v/v). 10 mL deionized $H_2O$ and 5 mL phosphoric acid (85%, m/v) were added to this solution, the mixture was stirred and filled to a total volume of 50.0 mL with deionized $H_2O$. The calibration was carried out using bovine serum albumin as a standard in 9 concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0 and 4.0 mg /mL). In a 96-well standard multiplate, 10 µL of the calibration solution or 10 µL of the membrane receptor preparation were mixed with 190 µL of the Bradford solution, respectively. After 5 min, the UV absorption of the protein-dye complex at $\lambda$ = 595 nm was measured with a platereader (Tecan Genios, Tecan, Crailsheim, Germany).

### 7.6. General procedures for the binding assays

The test compound solutions were prepared by dissolving approximately 10 µmol (usually 2-4 mg) of test compound in DMSO so that a 10 mM stock solution was obtained. To obtain the required test solutions for the assay, the DMSO stock solution

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was diluted with the respective assay buffer. The filtermats were presoaked in 0.5 % aqueous polyethylenimine solution for 2 h at room temperature before use. All binding experiments were carried out in duplicates in the 96-well multiplates. The concentrations given are the final concentration in the assay. Generally, the assays were performed by addition of 50 µL of the respective assay buffer, 50 µL of test compound solution in various concentrations ($10^{-5}$, $10^{-6}$, $10^{-7}$, $10^{-8}$, $10^{-9}$ and $10^{-10}$ mol/L), 50 µL of corresponding radioligand solution and 50 µL of the respective receptor preparation into each well of the multiplate (total volume 200 µL). The receptor preparation was always added last. During the incubation, the multiplates were shaken at a speed of 500-600 rpm at the specified temperature. Unless otherwise noted, the assays were terminated after 120 min by rapid filtration using the harvester. During the filtration each well was washed five times with 300 µL of water. Subsequently, the filtermats were dried at 95 °C. The solid scintillator was melted on the dried filtermats at a temperature of 95 °C for 5 minutes. After solidifying of the scintillator at room temperature, the trapped radioactivity in the filtermats was measured with the scintillation analyzer. Each position on the filtermat corresponding to one well of the multiplate was measured for 5 min with the [$^3$H]-counting protocol. The overall counting efficiency was 20 %. The $IC_{50}$-values were calculated with the program GraphPad Prism® 3.0 (GraphPad Software, San Diego, CA, USA) by non-linear regression analysis. Subsequently, the $IC_{50}$ values were transformed into $K_i$-values using the equation of Cheng and Prusoff.[38] The $K_i$-values are given as mean value ± SEM from three independent experiments.

### 7.7. Determination of KOR affinity (guinea pig brain)[16,17]

The assay was performed with the radioligand [$^3$H]-U-69593 (55 Ci/mmol, Amersham, Little Chalfont, UK). The thawed guinea pig brain membrane preparation (about 100 µg of the protein) was incubated with various concentrations of test compounds, 1 nM [$^3$H]-

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U-69593, and TRIS-$MgCl_2$-Puffer (50 mM, 8 mM $MgCl_2$, pH 7.4) at 37 °C. The non-specific binding was determined with 10 µM unlabeled U-69593. The $K_d$-value of U-69593 is 0.69 nM.

### 7.8. Determination of MOR affinity (guinea pig brain)[23]

The assay was performed with the radioligand [$^3$H]-DAMGO (51 Ci/mmol, Perkin Elmer LAS). The thawed guinea pig brain membrane preparation (about 100 µg of the protein) was incubated with various concentrations of test compounds, 3 nM [$^3$H]-DAMGO, and TRIS-$MgCl_2$-Puffer (50 mM, 8 mM $MgCl_2$, pH 7.4) at 37 °C. The non-specific binding was determined with 10 µM unlabeled Naloxon. The $K_d$-value of DAMGO is 0.57 nM.

### 7.9. Determination of DOR affinity (rat brain)[23]

The assay was performed with the radioligand [$^3$H]-DPDPE (69 Ci/mmol, Amersham). The thawed rat membrane preparation (about 75 µg of the protein) was incubated with various concentrations of test compounds, 3 nM [$^3$H]-DPDPE, and TRIS-$MgCl_2$-PMSF-buffer (50 mM, 8 mM $MgCl_2$, 400 µM PMSF, pH 7.4) at 37 °C. The non-specific binding was determined with 10 µM unlabeled Morphine. The $K_d$-value of DPDPE is 0.65 nM.

### 7.10. Determination of the $\sigma_1$ receptor affinity (guinea pig brain)[28-30]

The assay was performed with the radioligand [$^3$H]-(+)-pentazocine (22.0 Ci/mmol; Perkin Elmer). The thawed membrane preparation of guinea pig brain cortex (about 100 µg of the protein) was incubated with various concentrations of test compounds, 2 nM [$^3$H]-(+)-Pentazocine, and TRIS buffer (50 mM, pH 7.4) at 37 °C. The non-specific binding was determined with 10 µM unlabeled (+)-pentazocine. The $K_d$-value of (+)-pentazocine is 2.9 nM.[39]

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### 7.11. Determination of the $\sigma_2$ receptor affinity (rat liver)[28-30]

The assays were performed with the radioligand [$^3$H]-di-*o*-tolylguanidine ([$^3$H]DTG, specific activity 50 Ci/mmol; ARC, St. Louis, MO, USA). The thawed membrane preparation of rat liver containing 100 µg protein was incubated with various concentrations of the test compound, 3 nM [$^3$H]DTG and buffer containing (+)-pentazocine (500 nM (+)-pentazocine in 50 mM TRIS, pH 8.0) at room temperature. The non-specific binding was determined with 10 µM non-labeled DTG. The $K_d$ values is 17.9 nM.[40]

### 7.12. [$^{35}$S]GTPγS binding assay, agonistic activity at KOR[31,32]

The [$^{35}$S]-guanosine-5´-3-O-(thio)triphosphate (GTPγS) assay was carried out as described in ref.[41,42]. The receptor material was obtained from human HEK 293 (human embryonic kidney) cells. Vehicle 1.00 % DMSO. Incubation time 30 min. Incubation temperature 30 °C. Incubation buffer 20 mM HEPES pH 7.4, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, 1 mM EDTA. Quantification of bound [$^{35}$S]GTPγS. Significance criteria for an agonists: >50 % increase of bound [$^{35}$S]GTPγS relative to U-69,593 response. The $EC_{50}$ values were determined by a non-linear, least squares regression analysis using MathIQTM (ID Business Solutions Ltd., UK). Reference standards were run as an integral part of each assay to ensure the validity of the results obtained.

### Supporting Information Available

Supporting Information includes data for the X-ray crystal structure analysis and $^1$H and $^{13}$C NMR spectra.

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There is no conflict of interest.

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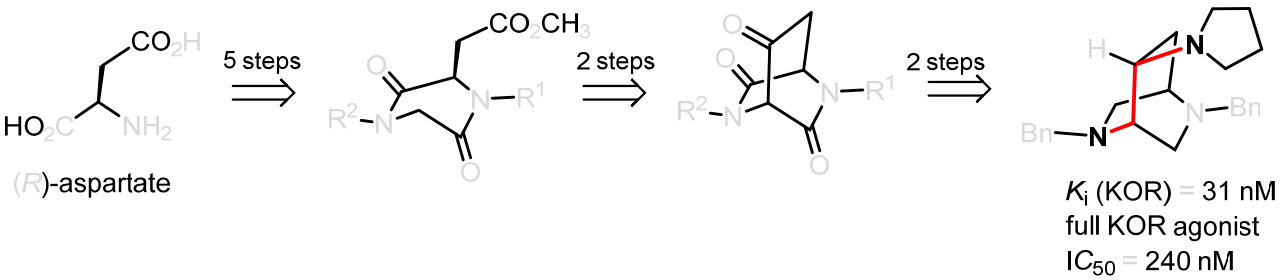


(*R*)-aspartate

$K_i$ (KOR) = 31 nM
full KOR agonist
$IC_{50}$ = 240 nM

### Contents entry text

A Dieckmann analogous cyclization represents the key step in the synthesis of bicyclic KOR agonists with high KOR affinity and agonistic activity.