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Asymmetric synthesis and σ receptor affinity of enantiomerically pure 1,4-disubstituted tetrahydro-1*H*-3-benzazepines

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ABSTRACT

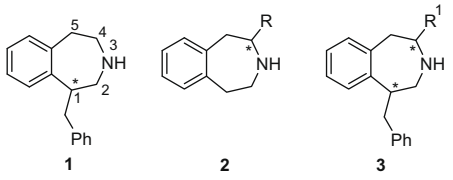
A very short (three steps) asymmetric synthesis of enantiomerically pure 1,4-disubstituted tetrahydro-1*H*-3-benzazepines **14** has been elaborated upon, starting from the *trans*- and *cis*-configured 11a-substituted 3-phenyl-2,3,11,11a-tetrahydro[1,3]oxazolo[2,3-*b*]-[3]-benzazepin-5(6*H*)-ones **6** and **7**. The stereoisomerically pure lactams **6** and **7** were benzylated to give 6-benzyl-substituted products **8** and **9**. NOE experiments showed a *trans*-configuration of the benzyl residue and the residue in the 11a-position indicated that the stereochemistry of the benzylation reaction was controlled by the stereocenter at the 11a-position. Reduction of the benzylated tricyclic benzolactams **8** and **9** with $AlCl_3/LiAlH_4$ (1/3) yielded the 1,3,4-trisubstituted 3-benzazepines **12** and **13**, which were formed stereoselectively with the retention of configuration. Finally, removal of the *N*-(2-hydroxy-1-phenylethyl) residue by hydrogenolytic cleavage resulted in the formation of enantiomerically pure 1,4-disubstituted 3-benzazepines **14**. The σ_1, σ_2, and NMDA receptor affinities of the enantiomerically pure 3-benzazepines **14** and *ent*-**14** were investigated in competitive receptor binding studies. The butyl derivative *ent*-**14c** showed a high affinity towards σ_1 and σ_2 receptors, with K_i-values of 26 nM and 41 nM, respectively.

1. Introduction

Over the past 30 years, tetrahydro-3-benzazepines, in particular 1-aryl-substituted derivatives, have been prepared and studied as dopamine receptor agonists and antagonists.[1–3] Several 3-benzazepines have been examined for pharmacological effects, which are not mediated by dopamine receptors[4–7] Furthermore, 3-benzazepines are active in animal models of various neurological disorders, for example, Parkinson's disease[8] and Alzheimer's disease.[9] Recently, we have published about the binding of racemic and enantiomerically pure 1-substituted and 2-substituted tetrahydro-3-benzazepines to σ-receptors and the PCP binding site of the NMDA receptor.[10–12]

Thirty years ago, σ receptors were first discovered and classified as an opioid receptor subtype.[13] But today σ receptors are well established as non-opioid, non-phencyclidine, and haloperidol-sensitive receptors with their own binding profile and characteristic distribution in the central nervous system (CNS), in the endocrine and immune systems as well as in some peripheral tissues, such as the kidney, liver, lung, and heart.[14,15]

The 1-benzyl-substituted 3-benzazepine **1** shows high stereoselective binding at the σ_1 receptor subtype[11] while the 2-substituted 3-benzazepines **2** also interact stereoselectively with the σ_1 receptor protein.[12] This prompted us to combine the two substitution patterns (1-Bn of **1** and 2-R of **2**) and to synthesize enantiomerically pure 1,4-disubstituted 3-benzazepines **3** in order to study their stereoselective σ_1 and σ_2 receptor interactions (Fig. 1).



1 2 3

Figure 1. Substituted tetrahydro-3-benzazepines.

Herein we report on the stereoselective synthesis of enantiomerically pure 1,4-disubstituted 3-benzazepines of type **3**, which were studied for their affinity to σ_1 and σ_2 receptors and the PCP binding site of the NMDA receptor. The key step of the synthesis is the stereoselective alkylation of tricyclic benzolactams *trans*-**6** and *cis*-**7**.[12]

2. Results and discussion

The tricyclic benzolactams *trans*-**6** and *cis*-**7** were prepared by condensation of keto acids **4a–d**[16] with (*R*)-phenylglycinol **5** in

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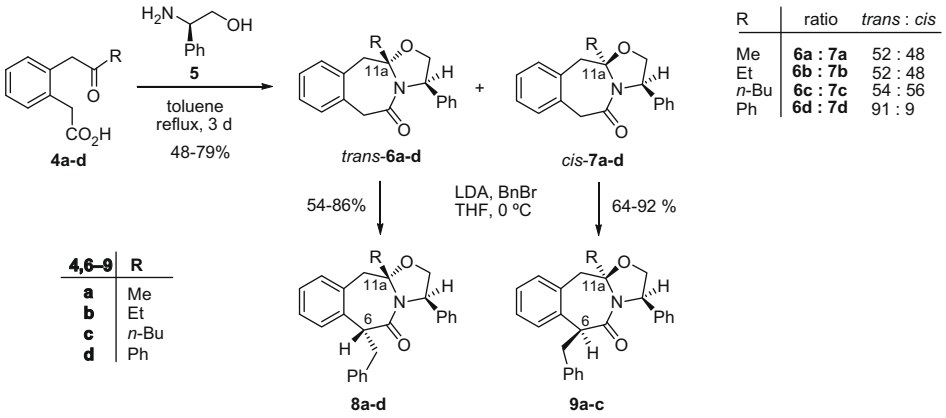


Scheme 1. Synthesis of tricyclic benzolactams and their benzylation.

refluxing toluene (Scheme 1).[12] The diastereomeric ratio of the methyl, ethyl, and *n*-butyl derivatives *trans*-**6a**–**c** to *cis*-**6a**–**c** was almost 50:50 whereas the phenyl derivative **4d** gave a ratio of *trans*-**6d** to *cis*-**7d** of 91:9. In the next step a substituent at the 6-position of the tricyclic ring system was introduced. The benzyl group was selected as a model for alkyl and arylalkyl substituents and the stereochemical outcome of benzylation was analyzed carefully.

For the benzylation of *trans*-**6a**–**d**, an enolate of the tricyclic benzolactam was generated by deprotonation with the strong base LDA at 0 °C. The enolate was reacted with benzyl bromide to afford the benzylated tricyclic benzolactams **8a**–**d**. The reaction took place with high diastereoselectivity and produced only one diastereomer. The benzylated tricyclic benzolactams **8a**–**d** were isolated in 54–97% yields.

The relative configuration of the newly formed stereogenic center at the 6-position was determined by NOE experiments. The NOE difference spectrum of compound **8a** shows an increase in the signal intensity at 4.14 ppm (6-H) after irradiation at 1.47 ppm (CH_3), indicating a *cis*-arrangement for these groups. In the control experiment with irradiation at 4.14 ppm (6-H) an increase in intensity of the signal at 1.47 ppm (CH_3) confirms the *cis*-arrangement of the two groups. Furthermore, irradiation at 3.20 ppm (CH_2Ph) did not result in an increase in the intensity of the signal at 1.47 ppm (CH_3), indicating *trans*-arrangement of the benzyl and the methyl groups in the molecule. The relative configuration at the 6-position of compound **8a** was shown unequivocally by the NOE experiment and hence the absolute configuration is (6*R*).

The configuration of the stereogenic center at the 6-position of compounds **8b** and **8c** was also proven by NOE experiments. In compounds **8b** and **8c**, the benzyl group is *trans*-oriented to the alkyl group at the 11a-position and the absolute configuration is (6*R*). The configuration of the phenyl derivative **8d** was deduced from the configuration of **8a**–**c**.

After deprotonation with LDA, the *cis*-configured tricyclic benzolactams *cis*-**7a**–**c** were benzylated with benzyl bromide in the same manner as *trans*-**6a**–**c**. Again, only one diastereomer **9a**–**c** was produced in 65–70% isolated yields (Scheme 1).

NOE experiments were performed in order to determine the configuration of the newly formed stereogenic center at the 6-position. The corresponding ^{1}H NMR spectra obtained from the NOE experiments of compound **9a** showed the *cis* arrangement of the methyl group at the stereocenter C-11a (1.63 ppm) and the proton at the stereocenter C-6 of the tricyclic benzolactams (4.07 ppm). Thus, the new benzyl group is *trans*-oriented relative to the methyl group at the 11a-position indicating a (6*S*)-configuration. Analogous NOE experiments performed with **9b** and **9c** also showed (6*S*)-configuration.

The stereochemistry observed during the benzylation of *trans*-**6a**–**d** and *cis*-**7a**–**c** can be explained by steric hindrance caused by the R group at the C-11a stereocenter. The enolate **10a**, which had been formed by the deprotonation of *trans*-**6a**, has a planar geometry at C-5 and C-6. Figure 2 clearly shows that the methyl group at the 11a-position is sterically shielding the *Si*-face of C-6 forcing the benzyl bromide to attack from the *Re*-face, which leads to the formation of the (6*R*)-configured benzylated product **8a**. This effect is supported by the C-3-phenyl ring above the ring plane, which also shields the *Si*-face at C-6.

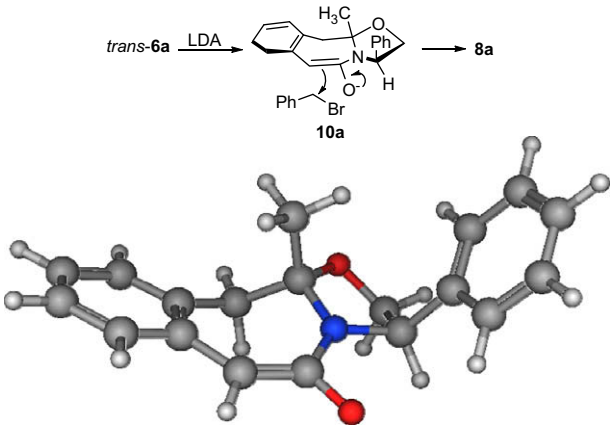


Figure 2. Energy minimized model of the enolate **10a** of *trans*-**8a**, calculated using the semiempirical program AM1 (using Molecular Operating Environment).

A similar model calculated for the enolate **11a** of *cis*-**7a** shows that the methyl group at the 11a-position shields the *Re*-face of C-6 and so benzyl bromide can only attack from the *Si*-face, opposite to the methyl group (Fig. 3). Obviously, the C-3-phenyl group above the ring plane has only little to no influence on the diastereoselectivity, since its distance to the reacting center at the 6-position is too far. The model shown in Figure 3 explains the observed high diastereoselectivity.

The benzylation of both types of tricyclic benzolactams *trans*-**6** and *cis*-**7** took place with high diastereoselectivity leading to high yields of **8** and **9**, respectively. The diastereomers formed in these reactions have a *trans*-arrangement of the C-11a-substituents and the new C-6-benzyl group. Thus, the C-11a stereocenter of the tricyclic benzolactams *trans*-**6** and *cis*-**7**, in particular the exocyclic

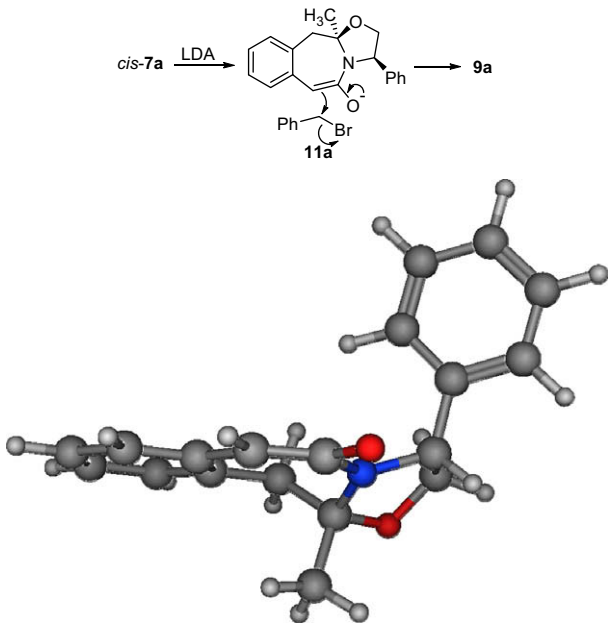
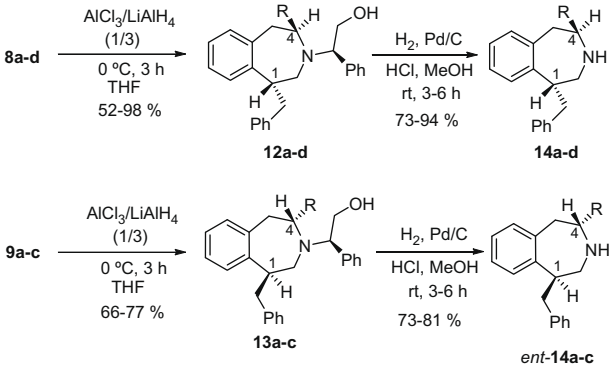


Figure 3. Energy minimized model of the enolate **11a** of *cis*-**7a**, calculated using the semiempirical program AM1 (using Molecular Operating Environment).

C-11a substituent, controlled the stereochemistry at the 6-position, whereas the original stereogenic center of phenylglycinol (C-3) was overruled by the new stereogenic center C-11a.

The oxazolidine moiety of the benzylated tricyclic benzolactams **8a–d** and **9a–c** was reductively opened with AlH_3,[11,12,17] which was generated in situ by mixing $AlCl_3$ and $LiAlH_4$ in a 1:3 ratio[18] and afforded trisubstituted 3-benzazepines **12a–d** and **13a–c** in 52–98% yields (Scheme 2).



Scheme 2. Synthesis of enantiomerically pure 1,4-disubstituted 3-benzazepines **14**.

It is assumed that the AlH_3 reduction of both diastereomers **8** and **9** took place with the retention of configuration at the original C-11a stereocenter.[19] In all the reactions, only a single diastereomer was detected and isolated, thus indicating a very high diastereoselectivity for the reduction step (Scheme 3).

In analogy to the mechanism described in the literature,[19] the following mechanism is proposed to explain the retention of configuration during the AlH_3 reduction. Coordination of the Lewis acid AlH_3 with the O-atom of the oxazolidine moiety weakens the adjacent C-O bond in **8**-AlH_3. Simultaneous delivery of the hydride from the same face as the departing oxygen forms the observed product **12** with the retention of configuration. The very high diastereoselectivity in the above reaction is supported by the benzyl group at the 6-position, which is *trans*-oriented to the R group at C-11a. The benzyl group at the benzazepine ring forces the R group to the opposite (*trans*) face in the final products and so favors the hydride delivery from the *Si*-face in 4-position. The precoordination of the reducing agent AlH_3 together with the benzyl moiety at the 6-position led to the formation of only one diastereomer **12** with the retention of configuration.

The same factors are responsible for the formation of the trisubstituted 3-benzazepines **13a–c** in stereochemically pure form. The configuration of the C-4 stereocenter was proved by an X-ray crystal structure analysis of the methyl derivative **13a**, which was crystallized as a HCl salt.[17] The absolute configuration of the C-1 and C-4 centers of chirality of the compounds **12a–d** and **13b–c** were assigned by analogy.

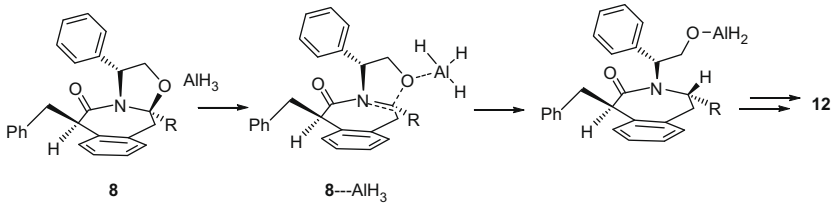
The last step for obtaining 1,4-disubstituted 3-benzazepines **14** was the removal of the (2-hydroxy-1-phenylethyl) residue. For the hydrogenolytic cleavage, compounds **12a–d** and **13a–c** were dissolved in MeOH. Pd/C and a small amount of HCl (1 M) and the mixtures were stirred under a H_2 atmosphere (1 bar) for 3–6 h. Flash chromatographic purification provided **14a–d** and *ent*-**14a–c** in 73–94% yields.

The condensation of **4d** with (*R*)-phenylglycinol gave only small amounts of the tricyclic benzolactam *cis*-**7d**.[12] In order to test all stereoisomers pharmacologically, the phenyl keto acid **4d** was reacted with (*S*)-phenylglycinol to obtain enantiomers *ent*-**6d** and *ent*-**7d**. The major diastereomer *ent*-**6d** was benzylated and reductively degraded to provide enantiomer *ent*-**14d**.

The ^1H NMR spectra and the specific rotations of the enantiomeric pairs **14a–d** and *ent*-**14a–d** match exactly with each other, and hence prove the enantiomeric relationship between **14** and *ent*-**14** (Table 1).

3. Receptor binding studies

The receptor affinities were investigated in competitive receptor binding studies. In the σ assays, the radioligands [^3H]-(+)-pentazocine (σ_1) and [^3H]-ditolylguanidine (σ_2) and membrane preparations from guinea pig brains (σ_1) and rat livers (σ_2) were used.[11,20] In addition to the σ receptor binding, the affinity toward the NMDA receptors was also investigated in this study, because some potent σ ligands also interact with the NMDA receptors and vice versa.[21,22] The affinity for the PCP binding site of the NMDA receptor was determined in competition experiments using the radioligand [^3H]-(+)-MK-801. Fresh pig brain cortex membrane preparations were employed as receptor material.[11]



Scheme 3. Mechanism of reduction with AlH_3.

Table 1
Yields and specific rotations of compounds **14a–d** and *ent*-**14a–d**

R	Compound	Yield (%)	Specific rotation	Compound	Yield (%)	Specific rotation
Me	**14a** (1*R*,4*R*)	94	+25.4	*ent*-**14a** (1*S*,4*S*)	73	−24.8
Et	**14b** (1*R*,4*R*)	76	+19.2	*ent*-**14b** (1*S*,4*S*)	81	−18.2
n-Bu	**14c** (1*R*,4*R*)	73	+21.0	*ent*-**14c** (1*S*,4*S*)	77	−23.5
Ph	**14d** (1*R*,4*S*)	85	+41.5	*ent*-**14d** (1*S*,4*R*)	47	−45.0

In the *ent*-series, the affinity toward the σ_1 receptor increases with increasing length of the C-4 residue R (*ent*-**14a** < *ent*-**14b** < *ent*-**14c**). However, the phenyl derivative *ent*-**14d** shows only a low σ_1 affinity. Increasing the chain length of the C-4 substituent increased not only the σ_1 affinity but also the eudismic ratio. Whereas the enantiomeric methyl compounds **14a** and *ent*-**14a** show almost the same σ_1 affinity (eudismic ratio = 1), the eudismic ratio of the butyl derivative **14c** is 11 with the (1*S*,4*S*)-configured enantiomer *ent*-**14c** being the eutomer with a K_i value of 26 nM.

The reason for the high enantioselective binding of *ent*-**14c** could be the involvement of both the benzyl and butyl substituents in the interaction with properly positioned hydrophobic regions of the σ_1 receptor protein. As a result, *ent*-**14c** with a long hydrophobic butyl chain interacts more strongly with the hydrophobic region of the σ_1 receptor than the corresponding methyl and ethyl derivatives *ent*-**14a** and *ent*-**14b** (Table 2).

Table 2
Affinity of enantiomerically pure 1,4-disubstituted 3-benzazepines **14** toward σ_1, σ_2, and NMDA receptors

Compound	K_i ± SEM (nM)			
	R	σ_1	σ_2	NMDA
14a	Me	1039	3078	1012
ent-**14a**		1058	1033	37%[a]
14b	Et	17%[a]	1028	10,000
ent-**14b**		208	205	5023
14c	*n*-Bu	289 ± 130	777 ± 335	30%[a]
ent-**14c**		26 ± 4.0	41 ± 2.45	0%[a]
14d	Ph	1053	2330	0%[a]
ent-**14d**		16%[a]	280 ± 171	46%[a]
(+)-Pentazocine	—	5.6 ± 2.2	—	—
Haloperidol	—	6.3 ± 1.6	78 ± 2.3	—
Ditolylguanidine	—	89.2 ± 29	57.5 ± 18	—
(*S*)-Ketamine	—	—	—	383 ± 41
(+)-MK-801	—	—	—	2.9 ± 0.6

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

The phenyl derivatives **14d** and *ent*-**14d** show only low σ_1 affinity, which might be due to the large lipophilic phenyl residue at the 4-position, which alters the molecular orientation in the receptor binding site.

For the σ_2 affinity, a similar trend was observed as for the σ_1 affinity: the σ_2 affinity increased with increasing C-4 side chain length from the methyl *ent*-**14a** over the ethyl *ent*-**14b** to the *n*-butyl derivative *ent*-**14c**. The butyl derivative *ent*-**14c** shows an eudismic ratio of 19 with *ent*-**14c** being the eutomer (K_i = 41 nM). Generally, in this series of compounds the (1*S*,4*S*)-configured derivatives have higher σ_2 affinity than their (1*R*,4*R*)-configured enantiomers. This observation is also true for the phenyl derivative *ent*-**14d**, although the stereodescriptors have been changed due to the CIP rules.

Surprisingly, the σ_1 and σ_2 receptor affinities of most of the 1,4-disubstituted 3-benzazepines **14** are quite similar indicating a poor differentiation between the two σ receptor subtypes. The low subtype selectivity is in clear contrast to the activity of other 3-benzazepines.[11,12]

The enantiopure 1,4-disubstituted 3-benzazepines **14** do not show remarkable affinity to the PCP binding site of the NMDA receptor. Even the K_i-value of 1012 nM of the most potent compound **14a** is too low to consider this compound as a potent antagonist of the NMDA receptor. Nevertheless, it should be mentioned that memantine, which is used for the treatment of severe Alzheimer's disease, is also a weak NMDA receptor antagonist binding with a similar affinity to the PCP binding site of the NMDA receptor.[23]

4. Conclusion

In conclusion, enantiomerically pure 1,4-disubstituted 3-benzazepines **14** were synthesized in three reaction steps starting from tricyclic benzolactams *trans*-**6** and *cis*-**7**. After deprotonation with LDA, mono benzylation at C-6 took place with high diastereoselectivity, which was controlled by the configuration of C-11a. In the formed products **8** and **9** the new benzyl moiety at C-6 is *trans*-oriented to the substituent R at the 11a-position. The butyl derivative *ent*-**14c** showed high affinity to σ_1 and σ_2 receptors, with K_i-values of 26 nM and 41 nM, respectively. Reduction of the chain length, replacement by a phenyl moiety, or changing of the configuration resulted in reduced σ_1 and σ_2 receptor affinities.

5. Experimental

5.1. General

Unless otherwise mentioned, THF was dried with sodium/benzophenone and was freshly distilled before use. Thin layer chromatography (tlc): Silica Gel 60 F254 plates (Merck). Flash chromatography (fc): Silica Gel 60, 40–64 μm (Merck); diameter of the column, eluent, fraction size, and R_f value are given in the parentheses. Melting point: Melting point apparatus SMP 3 (Stuart Scientific), uncorrected. MS: MAT GCQ (Thermo-Finnigan); EI = electron impact, ESI = electro spray ionization. HRMS: Micro-Tof (Bruker Daltronics, Bremen), Calibration with sodium formate clusters before measurement. IR: IR spectrophotometer 480Plus FT-ATR-IR (Jasco). ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz): Mercury plus 400 spectrometer (Varian); δ in parts per million related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution. HPLC method for determination of the product 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: 1.000 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: (A%): 0 min: 90%, 4 min: 90%, 29 min: 0%, 31 min: 0%, 31.5 min: 90%, 40 min: 90%.

5.2. General procedures

5.2.1. General procedure A for benzylation of tricyclic benzolactams

To a cooled solution (0 °C) of tricyclic benzolactam (1 equiv, 0.68 mmol) dissolved in THF (40 mL) under a N_2 atmosphere, LDA (2 M in THF, 1.2 equiv, 0.41 mL, 0.82 mmol) was added. After

1 h of stirring at 0 °C, benzyl bromide (80 μL, 1 equiv, 0.68 mmol) was added and the solution was stirred further for 2 h at 0 °C. Completion of the reaction was checked by tlc. Saturated NH_4Cl solution was added (10 mL) to destroy the excess of LDA and the mixture was extracted with diethyl ether (3 × 10 mL). The organic layer was further washed with NH_4Cl solution (10 mL) and then with water (10 mL). The aqueous layer was further extracted with diethyl ether (2 × 10 mL). The combined organic layer was dried (Na_2SO_4) and filtered, and the solvent was evaporated in vacuum to get the crude product. The product was purified by fc and further by recrystallization with CH_2Cl_2/*n*-hexane.

5.2.2. General procedure B for the reduction of tricyclic benzolactams using alane ($AlCl_3$/$LiAlH_4$)

At 0 °C, dry THF (8 mL) was added to anhydrous $AlCl_3$ (1.02 mmol, 1 equiv) under a N_2 atmosphere. The resulting clear colorless solution was stirred at 0 °C for 5 min. Then a solution of $LiAlH_4$ (1.0 M in THF, 3.05 mmol; 3 equiv) was added via syringe. The resulting clear, colorless solution was allowed to warm to rt and was stirred for 20 min to give a solution of alane (AlH_3). A solution of tricyclic benzolactam (1.02 mmol, 1 equiv) in dry THF (8 mL) was added to the stirred, cooled (0 °C) solution of alane in THF under a N_2 atmosphere. The resulting solution was stirred at 0 °C for 3 h and then warmed to rt over 30 min. The resulting clear solution was cooled to 0 °C before 1 M HCl (only few drops) was added carefully. The resulting slurry was diluted with water (10 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with 1 M NaOH and brine (15 mL). The combined organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuum to provide the crude product, which was further purified by fc.

5.2.3. General procedure C for the hydrogenolysis

A mixture of phenylethanol derivative and Pd/C (10% by wt) in methanol and 1 M HCl (1.5 mL) was stirred at rt under a H_2 atmosphere (balloon) for 4–6 h. The reaction mixture was filtered using a silica bed, the solvent was removed under reduced pressure to obtain a residue, which was dissolved in CH_2Cl_2 (10 mL) and washed with 1 M NaOH (3 × 4 mL), which was back extracted with CH_2Cl_2 (2×). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuum to provide an oily liquid which was purified by fc.

5.3. (3*R*,6*R*,11a*S*)-6-Benzyl-11a-methyl-3-phenyl-2,3,11,11a-tetrahydro[1,3]oxazolo [2,3-*b*]-[3]-benzazepin-5(6*H*)-one 8a

Following the general procedure A, *trans*-**6a** (200 mg, 0.68 mmol) was benzylated to afford 333.5 mg of crude product. The product was purified by fc (2 cm, EtOAc/cyclohexane 1/9, 15 mL, R_f = 0.35 (EtOAc/cyclohexane 3/7)) and was further purified by recrystallization (CH_2Cl_2/*n*-hexane). Colorless solid, mp 162–164 °C yield 194 mg (74%). $[\alpha]_{589}^{20} = -103.4$ (*c* 0.84, CH_2Cl_2). FT-IR (ATR, film): ν (cm^{-1}) = 3027 (w, arom C–H), 2928 (w, aliph C–H), 1652 (s, C=O). MS (EI): *m*/*z* (%) = 383 [M, 22], 340 [M−(CH_3, CO), 8], 292 [M−C_7H_7, 32], 120 [$PhCHCH_2O$, 67], 91 [C_7H_7, 39]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.46 (s, 3H, CH_3), 3.20 (dd, *J* = 13.7/5.8 Hz, 1H, CH_2Ph), 3.39 (2d, *J* = 15.3 Hz, 2H, 11-H), 3.75 (dd, *J* = 13.6/8.2 Hz, 1H, CH_2Ph), 3.75–3.79 (m, 1H, 2-H), 4.14 (dd, *J* = 8.2/5.8 Hz, 1H, 6-H), 4.33 (t, *J* = 8.8 Hz, 1H, 2-H), 5.20 (t, *J* = 8.4 Hz, 1H, 3-H), 6.99–7.02 (m, 2H, arom), 7.19–7.28 (m, 12 H, arom). ^{13}C NMR ($CDCl_3$): δ (ppm) = 26.7 (1C, CH_3), 34.9 (1C, CH_2Ph), 44.9 (1C, C-11), 50.5 (1C, C-6), 60.9 (1C, C-3), 69.0 (1C, C-2), 94.6 (1C, C-11a), 125.4, 126.0, 126.4, 127.2, 127.5, 128.1, 128.5, 128.7, 129.4, 130.4 (14C, Ph-*CH*), 133.9, 138.5, 140.3, 140.4 (4C, Ph-*C*), 168.5 (1C, *C*=*O*). HPLC: Purity 99.6%, t_R = 23.95 min.

5.4. (3*R*,6*R*,11a*S*)-6-Benzyl-11a-ethyl-3-phenyl-2,3,11,11a-tetrahydro[1,3]oxazolo [2,3-*b*]-[3]-benzazepin-5(6*H*)-one 8b

Following the general procedure A, *trans*-**6b** (292 mg, 0.95 mmol) was benzylated to provide 510.6 mg of crude product. The product was purified by fc (3 cm, EtOAc/cyclohexane 1.5/8.5, 25 mL, R_f = 0.53 (EtOAc/cyclohexane 4/6)). Colorless viscous liquid, yield 206 mg (54%). $[\alpha]_{589}^{20} = -134.3$ (*c* 1.07, CH_2Cl_2). FT-IR (ATR, film): ν (cm^{-1}) = 3027 (w, arom C–H), 2967 (w, aliph C–H), 1653 (s, C=O). MS (EI): *m*/*z* (%) = 397 [M, 14], 368 [M−CH_2CH_3, 32], 120 [$PhCHCH_2O$, 100], 91 [C_7H_7, 58]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.96 (t, *J* = 7.4 Hz, 3H, CH_3), 1.65 (dq, *J* = 14.6/7.4 Hz, 1H, CH_2CH_3), 1.81 (dq, *J* = 14.9/7.4 Hz, 1H, CH_2CH_3), 3.17 (dd, *J* = 13.6/5.1 Hz, 1H, CH_2Ph), 3.26 (d, *J* = 15.4 Hz, 1H, 11-H), 3.48 (d, *J* = 15.3 Hz, 1H, 11-H), 3.71 (t, *J* = 8.5 Hz, 1H, 2-H), 3.79 (dd, *J* = 13.6/8.9 Hz, 1H, CH_2Ph), 4.18 (dd, *J* = 8.9/5.2 Hz, 1H, 6-H), 4.31 (t, *J* = 8.8 Hz, 1H, 2-H), 5.19 (t, *J* = 8.5 Hz, 1H, 3-H), 6.96 (dd, *J* = 7.2/1.0 Hz, 2H, arom), 7.18–7.30 (m, 12H, arom). ^{13}C NMR ($CDCl_3$): δ (ppm) = 8.5 (1C, CH_2CH_3), 31.3 (1C, CH_2CH_3), 34.2 (1C, C-11), 41.0 (1C, CH_2Ph), 49.9 (1C, C-6), 60.7 (1C, C-2), 68.5 (1C, C-3), 97.1 (1C, C-11a), 125.3, 125.4, 126.4, 127.1, 127.5, 128.1, 128.5, 128.7, 129.5, 130.5 (14C, Ph-*CH*), 133.8, 138.7, 140.4, 140.6 (4C, Ph-*C*), 168.8 (1C, *C*=*O*). HPLC: Purity 97.8%, t_R = 23.47 min.

5.5. (3*R*,6*R*,11a*S*)-6-Benzyl-11a-butyl-3-phenyl-2,3,11,11a-tetrahydro[1,3]oxazolo [2,3-*b*]-[3]-benzazepin-5(6*H*)-one 8c

Following the general procedure A, *trans*-**6c** (157 mg, 0.46 mmol) was benzylated to afford 212 mg of crude product, which was purified by fc (2 cm, EtOAc/cyclohexane 1/9, 15 mL, R_f = 0.33 (EtOAc/cyclohexane 2/8)). Pale yellow viscous oil, yield 133 mg (67%). $[\alpha]_{289}^{20} = -99.6$ (*c* 0.66, CH_2Cl_2). FT-IR (ATR, film): ν (cm^{-1}) = 3062, 3024 (w, arom C–H), 2954, 2924 (w, aliph C–H), 1654 (s, C=O). MS: *m*/*z* = 425 [M, 5], 368 [M−C_4H_9, 100], 120 [$PhCHCH_2O$, 37], 91[C_7H_7, 21]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.80 (t, *J* = 7.1 Hz, 3H, CH_3), 1.08–1.50 (m, 4H, $CH_2CH_2CH_2CH_3$), 1.60 (m, 1H, $CH_2CH_2CH_2CH_3$), 1.74 (m, 1H, $CH_2CH_2CH_2CH_3$), 3.16 (dd, *J* = 13.5/4.9 Hz, 1H, CH_2Ph), 3.24 (d, *J* = 15.3 Hz, 1H, 11-H), 3.53 (d, *J* = 15.4 Hz, 1H, 11-H), 3.74 (t, *J* = 8.6 Hz, 1H, 2-H), 3.80 (dd, *J* = 13.5/9.1 Hz, 1H, CH_2Ph), 4.19 (dd, *J* = 9.0/4.9 Hz, 1H, 6-H), 4.29 (t, *J* = 8.8 Hz, 1H, 2-H), 5.19 (t, *J* = 8.5 Hz, 1H, 3-H), 6.95 (d, *J* = 6.9 Hz, 2H, arom), 7.20–7.29 (m, 12H, arom). ^{13}C NMR ($CDCl_3$): δ (ppm) = 14.2 (1C, CH_3), 22.9 (1C, CH_2CH_3), 26.6 (1C, $CH_2CH_2CH_3$), 34.1 (1C, $CH_2CH_2CH_2CH_3$), 38.2 (1C, C-11), 41.5 (1C, CH_2Ph), 49.9 (1C, C-6), 60.7 (1C, C-2), 68.4 (1C, C-3), 96.8 (1C, C-11a), 125.3, 126.4, 127.1, 127.4, 128.1, 128.5, 128.6, 129.5, 130.5 (14C, Ph-*CH*), 133.8, 138.7, 140.5, 140.6 (4C, Ph-*C*), 168.9 (1C, *C*=*O*). HPLC: purity 97.9%, t_R = 24.85 min.

5.6. (3*R*,6*R*,11a*R*)-6-Benzyl-3,11a-diphenyl-2,3,11,11a-tetrahydro[1,3]oxazolo [2,3-*b*]-[3]-benzazepin-5(6*H*)-one 8d

Following the general procedure A, *trans*-**6d** (500 mg, 1.46 mmol) was benzylated to afford 758 mg of crude product, which was purified by fc (3 cm, EtOAc/cyclohexane 1.5/8.5, 25 mL, R_f = 0.38 (EtOAc/cyclohexane 2/8)) and was further purified by recrystallization (CH_2Cl_2/*n*-hexane). Colorless solid, mp 131–133 °C yield 541.6 mg (86%). $[\alpha]_{589}^{20} = -42.3$ (*c* 0.82, CH_2Cl_2).). FT-IR (ATR, film): ν (cm^{-1}) = 3061, 3027 (w, arom C–H), 1656 (s, C=O). MS (EI): *m*/*z* (%) = 445 [M, 100], 221 [M-(C_7H_7, CH_2O, CH_2C(Ph)), 58], 105 [C_8H_9, 70], 91 [C_7H_7, 31]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.25 (dd, *J* = 13.6/5.7 Hz, 1H, CH_2Ph), 3.42 (d, *J* = 15.4 Hz, 1H, 11-H), 3.52–3.56 (m, 2H, 11-H/2-H), 3.84 (dd, *J* = 13.6/8.4 Hz, 1H, CH_2Ph), 4.24–4.26 (m, 1H, 2-H), 4.36 (dd, *J* = 8.3/5.6 Hz, 1H, 6-H), 5.11 (dd, *J* = 9.3/8.5 Hz, 1H, 3-H), 6.85–6.88 (m, 2H, arom), 7.09–7.34 (m, 17H, arom). ^{13}C NMR (CDCl3): δ (ppm) = 34.2 (1C,

C-6), 47.0 (1C, CH_2Ph), 50.5 (1C, C-11), 62.5 (1C, C-2), 69.0 (1C, C-3), 97.4 (1C, C-11a), 125.2, 125.5, 126.2, 127.0, 127.1, 127.2, 127.9, 128.0, 128.2, 128.6, 129.2, 130.1 (19C, Ph-CH), 133.4, 138.2, 138.7, 140.1, 143.1 (5C, Ph-*C*), 169.5 (1C, *C*=*O*). HPLC: purity 99.9%, t_R = 25.36 min.

5.7. (3*S*,6*S*,11a*S*)-6-Benzyl-3,11a-diphenyl-2,3,11,11a-tetrahydro[1,3]oxazolo[2,3-*b*]-[3]-benzazepin-5(6*H*)-one *ent*-8d

Following the general procedure A, *ent-trans*-**6d** (200 mg, 0.56 mmol) was benzylated to afford 310 mg of crude product, which was purified by fc (2 cm, EtOAc/cyclohexane 1.5/8.5, 15 mL, R_f = 0.40 (EtOAc/cyclohexane 2/8)) and further purified by recrystallization (CH_2Cl_2/*n*-hexane). Colorless solid, mp 131–133 °C, yield 233 mg (93%). $[\alpha]_{589}^{20} = +45.4$ (*c* 0.38, CH_2Cl_2). HPLC: purity 99.7%, t_R = 25.28 min.

5.8. (3*R*,6*S*,11a*R*)-6-Benzyl-11a-methyl-3-phenyl-2,3,11,11a-tetrahydro[1,3]oxazolo [2,3-*b*]-[3]-benzazepin-5(6*H*)-one (9a)

Following the general procedure A, *cis*-**7a** (127 mg, 0.43 mmol) was benzylated to afford 270 mg of crude product, which was purified by fc (2 cm, EtOAc/cyclohexane 1.5/8.5, 15 mL, R_f = 0.41 (EtOAc/cyclohexane 1.5/8.5)) and was further purified by recrystallization (CH_2Cl_2/*n*-hexane). Colorless solid, mp 207–209 °C yield 133.4 mg (80%). $[\alpha]_{589}^{20} = +40.3$ (*c* 1.24, CH_2Cl_2). FT-IR (ATR, film): ν (cm^{-1}) = 3028 (w, arom C–H), 2922 (w, aliph C–H), 1658 (carbonyl C=O). MS (EI): *m*/*z* (%) = 383 [M, 7], 368 [M−CH_3, 5], 120 [$PhCHCH_2O$, 100], 91 [C_7H_7, 54], 77 [C_6H_5, 17]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.63 (s, 3H, CH_3), 3.10 (dd, *J* = 14.0/5.1 Hz, 1H, CH_2Ph), 3.46 (d, *J* = 15.5 Hz, 1H, 11-H), 3.58 (d, *J* = 15.5 Hz, 1H, 11-H), 3.64 (dd, *J* = 14.0/8.9 Hz, 1H, CH_2Ph), 3.72 (dd, *J* = 9.2/1.8 Hz, 1H, 2-H), 4.07 (dd, *J* = 8.9/5.1 Hz, 1H, 6-H), 4.32 (dd, *J* = 9.2/7.5 Hz, 1H, 2-H), 4.89 (dd, *J* = 7.4/1.7 Hz, 1H, 3-H), 6.50 (dd, *J* = 8.1/1.0 Hz, 2H, arom), 6.94 (t, *J* = 7.6 Hz, 2H, arom), 7.01–7.06 (m, 1H, arom), 7.15–7.39 (m, 9H, arom). ^{13}C NMR ($CDCl_3$): δ (ppm) = 26.5 (1C, CH_3), 32.8 (1C, CH_2Ph), 45.5 (1C, C-11), 49.2 (1C, C-6), 60.2 (1C, C-3), 70.7 (1C, C-2), 94.3 (1C, C-11a), 125.4, 125.6, 126.3, 127.2, 127.3, 127.8 128.4, 128.6, 129.2, 130.4 (14C, Ph-*CH*), 135.1, 140.1, 140.5, 142.0 (4C, Ph-*C*), 168.2 (1C, *C*=*O*). HPLC: purity 99.9%, t_R = 23.32 min.

5.9. (3*R*,6*S*,11a*R*)-6-Benzyl-11a-ethyl-3-phenyl-2,3,11,11-tetrahydro[1,3]oxazolo[2,3-*b*]-[3]-benzazepin-5(6*H*)-one 9b

Following the general procedure A, *cis*-**7b** (285 mg, 0.93 mmol) was benzylated to afford 445 mg of crude product, which was purified by fc (3 cm, EtOAc/cyclohexane 1/9, 25 mL, R_f = 0.61 (EtOAc/cyclohexane 4/6)). Colorless viscous liquid, yield 237.6 mg (64%). $[\alpha]_{589}^{20} = +36.4$ (*c* 1.19, CH_2Cl_2). FT-IR (ATR, film): ν (cm^{-1}) = 3027 (w, arom C–H), 2922 (w, aliph C–H), 1654 (s, C=O). MS (EI): *m*/*z* (%) = 397 [M, 2], 368 [M-CH_2CH_3, 100], 120 [$PhCHCH_2O$, 25], 91 [C_7H_7, 14]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.07 (t, *J* = 7.4 Hz, 3H, CH_2CH_3), 1.79 (dq, *J* = 14.6/7.3 Hz, 1H, CH_2CH_3), 2.04 (dq, *J* = 14.8/7.5 Hz, 1H, CH_2CH_3), 3.08 (dd, *J* = 14.1/5.1 Hz, 1H, CH_2Ph), 3.42 (d, *J* = 15.4 Hz, 1H, 11-H), 3.48 (d, *J* = 15.4 Hz, 1H, 11-H), 3.63 (dd, *J* = 13.6/8.3 Hz, 1H, CH_2Ph), 3.66 (dd, *J* = 9.1/2.0 Hz, 1H, 2-H), 4.08 (dd, *J* = 8.8/5.2 Hz, 1H, 6-H), 4.24 (dd, *J* = 9.1/7.6 Hz, 1H, 2-H), 4.86 (dd, *J* = 7.6/1.9 Hz, 1H, 3-H), 6.47 (d, *J* = 8.4 Hz, 2H, arom), 6.93 (t, *J* = 7.6 Hz, 2H, arom), 7.04 (ddd, *J* = 7.2/4.0/1.1 Hz, 1H, arom), 7.14–7.39 (m, 9H, arom). ^{13}C NMR ($CDCl_3$): δ (ppm) = 8.2 (1C, CH_2CH_3), 31.7 (1C, CH_2CH_3), 32.8 (1C, C-11), 42.3 (1C, CH_2Ph), 49.1 (1C, C-6), 60.3 (1C, C-2), 70.5 (1C, C-3), 96.5 (1C, C-11a), 125.3, 125.4, 126.3, 127.1, 127.3, 127.7, 128.4, 128.6, 129.3, 130.6 (14C, Ph-*CH*), 135.0, 140.1, 140.5, 142.1 (4C, Ph-*C*), 168.3 (1C, *C*=*O*). HPLC: purity 99.8%, t_R = 23.03 min.

5.10. (3*R*,6*S*,11a*R*)-6-Benzyl-11a-butyl-3-phenyl-2,3,11,11a-tetrahydro[1,3]oxazolo [2,3-*b*]-[3]-benzazepin-5(6*H*)-one 9c

Following the general procedure A, *cis*-**7c** (100 mg, 0.30 mmol) was benzylated to afford 180 mg of crude product, which was purified by fc (2 cm, EtOAc/cyclohexane 1/9, 15 mL, R_f = 0.56 (EtOAc/cyclohexane 4/6)). Pale yellow viscous oil, yield 116 mg (91%). $[\alpha]_{289}^{20} = +25.5$ (*c* 0.59, CH_2Cl_2). FT-IR (ATR, film): ν (cm^{-1}) = 3027 (w, arom C–H), 2962, 2871 (w, aliph C–H), 1658 (s, C=O). MS (EI): *m*/*z* = 425 [M, 4], 368 [M−C_4H_9, 49], 120 [$PhCHCH_2O$, 37], 91[C_7H_7, 21]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.90 (t, *J* = 7.2 Hz, 3H, CH_3), 1.28–1.55 (m, 4H, $CH_2CH_2CH_3$), 1.77 (ddd, *J* = 14.0/11.5/4.5 Hz, 1H, $CH_2CH_2CH_2CH_3$), 1.96 (ddd, *J* = 14.1/11.6/4.8 Hz, 1H, $CH_2CH_2CH_2CH_3$), 3.07 (dd, *J* = 14.0/5.1 Hz, 1H, CH_2Ph), 3.46 (s, 2H, 11-H), 3.62 (dd, *J* = 12.8/7.6 Hz, 1H, 6-H), 3.65 (dd, *J* = 9.2/2.0 Hz, 1H, 2-H), 4.10 (dd, *J* = 8.9/5.1 Hz, 1H, CH_2Ph), 4.26 (dd, *J* = 9.1/7.6 Hz, 1H, 2-H), 4.85 (dd, *J* = 7.6/2.0 Hz, 1H, 3-H), 6.45 (d, *J* = 7.3 Hz, 2H, arom), 6.92 (t, *J* = 7.6 Hz, 2H, arom), 7.03 (m, 1H, arom), 7.14–7.38 (m, 9H, arom). ^{13}C NMR ($CDCl_3$): δ (ppm) = 14.2 (1C, CH_3), 23.0 (1C, CH_2CH_3), 26.0 (1C, $CH_2CH_2CH_3$), 32.8 (1C, CH_2Ph), 38.8 (1C, $CH_2CH_2CH_2CH_3$), 42.8 (1C, C-11), 49.2 (1C, 6-C), 60.4 (1C, C-2), 70.5 (1C, C-3), 96.3 (1C, C-11a), 125.3, 125.4, 126.3, 127.1, 127.3, 127.7, 128.4, 128.6, 129.3, 130.5 (14C, Ph-*CH*), 135.1, 140.1, 140.5, 142.1 (4C, Ph-C), 168.3 (1C, *C*=*O*). HPLC: purity 99.2%, t_R = 25.57 min.

5.11. (*R*)-2-[(1*R*,4*R*)-1-Benzyl-4-methyl-2,3,4,5-tetrahydro-*1H*-3-benzazepin-3-yl]-2-phenylethanol 12a

Following the general procedure B, **8a** (245 mg, 0.64 mmol, 1 equiv) was reduced to give 192 mg of the crude product, which was purified by fc (2 cm, EtOAc/cyclohexane 1/9, 15 mL, R_f = 0.67 (EtOAc/petroleum ether/NH_3 50/49.5/0.5)). Colorless viscous liquid, yield 125 mg (52%). $[\alpha]_{589}^{20} = -8.4$ (*c* 1.31, CH_2Cl_2). FT-IR (ATR, film): ν (cm^{-1}) = 3422 (w, OH), 3059, 3025 (w, arom C–H), 2928 (w, aliph C–H). MS (EI): *m*/*z* (%) = 372 [MH^+, 0.5], 340 [M−CH_2OH, 100], 91 [C_7H_7, 78]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.93 (s, broad, 3H, CH_3), 2.49–2.53 (m, 2H, 2-H/5-H), 2.69 (br s, 1H, OH), 2.93 (dd, *J* = 13.0/8.6 Hz, 1H, CH_2Ph), 3.04 (dd, *J* = 13.4/6.4 Hz, 1H, CH_2Ph), 3.15–3.27 (m, 3H, 2-H/4-H/5-H), 3.34 (dd, *J* = 14.5/7.4 Hz, 1H, 1-H), 3.57 (dd, *J* = 10.5/4.8 Hz, 1H, CH_2OH), 3.77 (t, *J* = 10.2 Hz, 1H, CH_2OH), 3.95 (dd, *J* = 10.1/4.8 Hz, 1H, N*CH*Ph), 6.97–7.15 (m, 8H, arom), 7.21–7.36 (m, 6H, arom). ^{13}C NMR ($CDCl_3$): δ (ppm) = 17.7 (1C, CH_3), 40.7 (1C, CH_2Ph), 41.9 (1C, C-1), 49.0 (1C, C-5), 49.5 (1C, C-4), 52.2 (1C, C-2), 61.1 (1C, CH_2OH), 64.6 (1C, N*CH*Ph), 126.2, 126.3, 126.8, 127.1, 128.5, 128.6, 129.4, 130.7 (14C, Ph-*CH*), 136.7, 138.1, 140.3, 142.1 (4C, Ph-*C*). HPLC: purity 99.9%, t_R = 20.85 min.

5.12. (*R*)-2-[(1*R*,4*R*)-1-Benzyl-4-ethyl-2,3,4,5-tetrahydro-*1H*-3-benzazepin-3-yl]-2-phenylethanol 12b

Following the general procedure B, **8b** (150 mg, 0.37 mmol, 1 equiv) was reduced to give 155 mg of the crude product, which was purified by fc (2 cm, EtOAc/cyclohexane 0.5/9.5, 15 mL, R_f = 0.45 (EtOAc/cyclohexane 2/8)). Colorless viscous oil, yield 86.5 mg (59.4%). $[\alpha]_{589}^{20} = -15.1$ (*c* 1.23, CH_2Cl_2). FT-IR (ATR, film): ν (cm^{-1}) = 3419 (w, O–H), 3059, 3025 (w, arom C–H), 2929, 2872 (w, aliph C–H). MS (EI): *m*/*z* (%) = 354 [M−CH_2OH, 100], 117 [PhCH=CHN, 8], 91 [C_7H_7, 19], 77 [C_6H_5, 40]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.80 (t, *J* = 7.3 Hz, 3H, CH_2CH_3), 1.11–1.18 (m, 2H, CH_2CH_3), 2.55–2.60 (m, br, 1H, 5-H), 2.70 (dd, *J* = 14.8/4.2 Hz, 1H, CH_2Ph), 2.88–2.97 (m, 2H, 5-H/2-H), 3.05–3.18 (m, 3H, CH_2Ph/2-H/4-H), 3.25–3.32 (m, 1H, 1-H), 3.57 (dd, *J* = 10.4/4.7 Hz, 1H, CH_2OH), 3.79 (t, *J* = 10.0 Hz, 1H, N*CH*Ph), 3.89 (dd, *J* = 9.6/4.7 Hz, 1H, CH_2OH), 6.99–7.14 (m, 8H, arom), 7.18–7.34 (m, 6H, arom). A signal for the OH proton could not be detected. ^{13}C NMR ($CDCl_3$): δ (ppm) = 11.1

(1C, CH_2CH_3), 22.4 (br s, 1C, CH_2CH_3), 37.7 (1C, C-5), 40.6 (1C, CH_2Ph), 48.8 (1C, C-1), 50.2 (1C, C-4), 58.1 (1C, C-2), 61.3 (1C, CH_2OH), 66.0 (1C, NCHPh), 126.3, 126.4, 126.7, 128.0, 128.5, 128.6, 129.4, 130.7 (14C, Ph-CH), 137.0, 138.5, 140.4, 142.1 (4C, Ph-C). HPLC: purity 98.2%, t_R = 21.55 min.

5.13. (*R*)-2-[(1*R*,4*R*)-1-Benzyl-4-butyl-2,3,4,5-tetrahydro-*1H*-3-benzazepin-3-yl]-2-phenylethanol 12c

Following the general procedure B, **8c** (290 mg, 0.68 mmol, 1 equiv) was reduced to give 255 mg of the crude product, which was purified by fc (2 cm, EtOAc/cyclohexane 1/9, 15 mL, R_f = 0.33 (EtOAc/cyclohexane 2/8)). Colorless viscous oil, yield 246.7 mg (87.5%). $[\alpha]_{589}^{20} = -39.6$ (*c* 0.89, CH_2Cl_2). FT-IR (ATR, film): *v* (cm^{-1}) = 3426 (w, O–H), 3060, 3024 (arom. C–H), 2927, 2857 (aliph C–H). MS (EI): *m*/*z* = 414 [MH, 2], 382 [M−CH_2OH, 24], 356 [M−C_4H_9, 5], 306 [M−CH(Ph)CH_2OH, 8], 117 [PhCH_2CHN, 32], 91 [C_7H_7, 100]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.79 (t, *J* = 7.0, 3H, CH_3), 1.08–1.33 (m, 6H, $CH_2CH_2CH_2CH_3$), 2.57 (t, *J* = 9.8 Hz, 1H, 2-H), 2.66 (dd, *J* = 14.8/4.3 Hz, 1H, 5-H), 2.91–2.95 (m, 2H, 4-H/CH_2Ph), 3.05 (dd, *J* = 13.4/6.4 Hz, 1H, 5-H), 3.12–3.17 (m, 2H, 2-H/CH_2Ph), 3.23–3.30 (m, 1H, 1-H), 3.56 (dd, *J* = 10.2/4.5 Hz, 1H, CH_2OH), 3.78 (t, *J* = 9.8 Hz, 1H, NCHPh), 3.87 (dd, *J* = 9.7/4.6 Hz, 1H, CH_2OH), 6.99–7.31 (m, 14H, arom). ^{13}C NMR ($CDCl_3$): δ (ppm) = 14.3 (1C, CH_3), 23.0 (1C, CH_2CH_3), 29.1 (1C, $CH_2CH_2CH_3$), 38.4 (1C, $CH_2CH_2CH_2CH_3$), 40.5 (1C, CH_2Ph), 48.9 (1C, C-1), 50.3 (1C, C-5), 56.2 (1C, C-2), 61.2 (2C, C-4/CH_2OH), 65.9 (1C, NCHPh), 126.2, 126.3, 126.7, 127.9, 128.4, 128.6, 129.3, 130.6 (14C, Ph-CH), 137.1, 138.4, 140.4, 142.0 (4C, Ph-C). HPLC: purity 96.4%, t_R = 23.26 min.

5.14. (*R*)-2-[(1*R*,4*S*)-1-Benzyl-4-phenyl-2,3,4,5-tetrahydro-*1H*-3-benzazepin-3-yl]-2-phenylethanol 12d

Following the general procedure B, **8d** (350 mg, 0.78 mmol, 1 equiv) was reduced to give 370 mg of the crude product, which was purified by fc (3 cm, EtOAc/petroleum ether 2/98, 15 mL, R_f = 0.36 (EtOAc/cyclohexane 2/8)). Colorless viscous liquid, yield 335 mg (98.3%). $[\alpha]_{589}^{20} = -93.0$ (*c* 2.00, CH_2Cl_2). FT-IR (ATR, film): *v* (cm^{-1}) = 3469 (w, O–H), 3059, 3024 (w, arom C–H), 2929 (w, aliph C–H). MS (ESI): *m*/*z* (%) = 434 [MH]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.38 (m, 1H, 2-H), 2.57 (d, *J* = 15.0 Hz, 1H, 5-H), 2.87 (dd, *J* = 13.3/8.8 Hz, 1H, CH_2Ph), 3.01 (dd, *J* = 13.1/5.5 Hz, 1H, CH_2Ph), 3.27–3.38 (m, 3H, 2 × 2-H/5-H), 3.55–3.63 (m, 1H, 1-H), 3.67–3.79 (m, 2H, NCHPh/CH_2OH), 3.89 (s, br, 1H, 4-H), 6.57 (d, *J* = 6.5 Hz, 1H, arom), 6.95–7.31 (m, 18H, arom). A signal for OH proton could not be detected. ^{13}C NMR ($CDCl_3$): δ (ppm) = 41.7 (1C, CH_2Ph), 48.2 (1C, C-5), 48.6 (1C, C-1), 61.0 (1C, C-2), 63.0 (2C, C-4/CH_2OH), 65.6 (1C, NCHPh), 126.0, 236.5, 127.0, 128.0, 128.3, 128.4, 128.5, 128.6, 129.1, 129.5 (19C, Ph-CH), 131.3, 135.9, 139.8, 142.1, 142.9 (5C, Ph-C). HPLC: purity 95.7%, t_R = 22.93 min.

5.15. (*S*)-2-[(1*S*,4*R*)-1-Benzyl-4-phenyl-2,3,4,5-tetrahydro-*1H*-3-benzazepin-3-yl]-2-phenylethanol *ent*-12d

Following the general procedure B, *ent*-**8d** (100 mg, 0.22 mmol, 1 equiv) was reduced to give 106 mg of the crude product, which was purified by fc (3 cm, EtOAc/petroleum ether 2/98, 15 mL, R_f = 0.36 (EtOAc/cyclohexane 2/8)). Colorless viscous oil, yield 96.6 mg (99.3%). $[\alpha]_{589}^{20} = +96.4$ (*c* 0.56, CH_2Cl_2). HPLC: purity 99.5%, t_R = 22.10 min.

5.16. (*R*)-2-[(1*S*,4*S*)-1-Benzyl-4-methyl-2,3,4,5-tetrahydro-*1H*-3-benzazepin-3-yl]-2-phenylethanol 13a

Following the general procedure B, **9a** (221.3 mg, 0.57 mmol, 1 equiv) was reduced to give 143 mg of the crude product, which was quite pure, a fc purification was not required. R_f = 0.67 (EtOAc/petroleum ether/NH_3 50/49.5/0.5). Colorless oil, 142.8 mg (66%). $[\alpha]_{589}^{20} = +77.03$ (*c* 0.36, CH_2Cl_2). FT-IR (ATR, film): *v* (cm^{-1}) = 3442 (w, OH), 3059, 3024 (w, arom C–H), 2960, 2928 (w, aliph C–H). MS (EI): *m*/*z* (%) = 340 [M−CH_2OH, 100], 91 [C_7H_7, 78]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.82 (s, br, 3H, CH_3), 2.74 (dd, *J* = 14.5/6.8 Hz, 2H, 5-H), 2.85–2.88 (m, 2H, 1-H/2-H), 3.03 (dd, *J* = 13.3/6.3 Hz, 1H, CH_2Ph), 3.20 (br s, 1H, 2-H), 3.34 (dd, *J* = 13.3/8.1 Hz, 1H, CH_2Ph), 3.47–3.54 (m, 2H, CH_2OH/4-H), 3.78–3.84 (m, 2H, CH_2OH/NCHPh), 7.10–7.34 (m, 14H, arom). A signal for the OH proton could not be detected. ^{13}C NMR ($CDCl_3$): δ (ppm) = 14.5 (1C, CH_3), 38.2 (1C, CH_2Ph), 43.9 (1C, C-5), 46.6 (2C, C-1/C-2), 56.4 (1C, C-4), 61.9 (1C, CH_2OH), 71.1 (1C, NCHPh), 126.1, 126.7, 127.9, 128.4, 128.6, 128.9, 129.1 (14C, Ph-CH), 138.6, 139.9, 141.0, 141.9 (4C, Ph-C). HPLC: purity 98.3%, t_R = 20.64 min.

5.17. (*R*)-2-[(1*S*,4*S*)-1-Benzyl-4-ethyl-2,3,4,5-tetrahydro-*1H*-3-benzazepin-3-yl]-2-phenylethanol 13b

Following the general procedure B, **9b** (190 mg, 0.48 mmol, 1 equiv) was reduced to give 202.5 mg of the crude product, which was purified by fc (2 cm, EtOAc/cyclohexane 0.5/9.5, 15 mL, R_f = 0.26 (EtOAc/cyclohexane 2/8)). Colorless viscous oil, yield 131 mg (71%). $[\alpha]_{589}^{20} = +13.3$ (*c* 0.57, CH_2Cl_2). FT-IR (ATR, film): *v* (cm^{-1}) = 3429 (w, O–H), 3059, 3024 (w, arom C–H), 2930 (w, aliph C–H). MS (EI): *m*/*z* (%) = 354 [M–CH_2OH, 100], 91 [C_7H_7, 26]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.74 (t, *J* = 7.4 Hz, 3H, CH_2CH_3), 1.14 (br s, 2H, CH_2CH_3), 2.61 (s, broad, 1H, 5-H), 2.72–2.76 (m, 2H, 2-H/5-H), 2.89 (dd, *J* = 13.4/6.4 Hz, 1H, CH_2Ph), 2.97 (s, br, 1H, 2-H), 3.07 (s, br, 1H, 1-H), 3.21 (m, 2H, CH_2Ph/4-H), 3.37 (s, br, 1H, CH_2OH), 3.62 (br s, 1H, CH_2OH), 3.72 (br s, 1H, NCHPh), 7.01–7.35 (m, 14 H, arom). A signal for OH proton could not be detected. ^{13}C NMR ($CDCl_3$): δ (ppm) = 11.2 (1C, CH_2CH_3), 29.9 (1C, CH_2CH_3), 38.1 (1C, C-1), 38.4 (1C, CH_2Ph), 40.4 (1C, C-5), 47.3 (1C, 4-C), 61.9 (1C, 2-C), 63.2 (1C, CH_2OH), 71.2 (1C, NCHPh), 126.1, 126.7, 127.8, 128.4, 128.6, 128.8, 129.0 (14C, Ph-CH), 140.6, 140.9, 142.0 (4C, Ph-C). HPLC: purity 97.8%, t_R = 20.49 min.

5.18. (*R*)-2-[(1*S*,4*S*)-1-Benzyl-4-butyl-2,3,4,5-tetrahydro-*1H*-3-benzazepin-3-yl]-2-phenylethanol 13c

Following the general procedure B, **9c** (65 mg, 0.15 mmol, 1 equiv) was reduced to give 48.5 mg of the crude product, which was purified by fc (1 cm, EtOAc/cyclohexane 0.5/9.5, 10 mL, R_f = 0.35 (EtOAc/cyclohexane 2/8)). Colorless viscous oil, yield 47.9 mg (77%). $[\alpha]_{589}^{20} = +30.0$ (*c* 0.52, CH_2Cl_2). FT-IR (ATR, film): *v* (cm^{-1}) = 3437 (b, alcoholic O–H), 3058, 3024 (w, arom C–H), 2953, 2923 (aliph. C–H). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.75 (t, *J* = 6.8 Hz, 3H, CH_3), 1.10–1.14 (m, 6H, $CH_2CH_2CH_2CH_3$), 2.54–2.64 (m, 2H, 5-H/CH_2Ph), 2.72–2.77 (m, 2H, 1-H/2-H), 2.89 (dd, *J* = 13.5/6.2 Hz, 1H, CH_2Ph), 2.93–2.98 (m, 1H, 5-H), 3.05–3.08 (m, br, 1H, 2-H), 3.19–3.24 (m, 1H, 4-H), 3.53 (dd, *J* = 10.9/5.5 Hz, 1H, CH_2OH), 3.61 (dd, *J* = 10.9/5.7 Hz, 1H, CH_2OH), 3.71 (t, *J* = 5.5 Hz, 1H, NCHPh), 6.92–7.21 (m, 14H, arom). A signal for the OH proton could not be detected. HRMS (ESI): $C_{29}H_{35}$NOH: Calcd 414.2791, found 414.2788. HPLC: purity 96.4%, t_R = 21.99 min.

5.19. (1*R*,4*R*)-1-Benzyl-4-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine 14a

Following the general procedure C, **12a** (105 mg, 0.28 mmol) on hydrogenolysis gave 79.9 mg of a crude yellow oil, which was purified by fc (1 cm, EtOAc/petroleum ether/NH_3 50/49.5/0.5, 10 mL, R_f = 0.16 (EtOAc/petroleum ether/NH_3 70/29.5/0.5)). Colorless oil, yield 66.7 mg (94%). $[\alpha]_{589}^{20} = +25.4$ (*c* 0.63, CH_2Cl_2). FT-IR (ATR,

film): ν (cm^{-1}) = 3365 (w, N–H), 3058, 3024 (w, arom C–H), 2957, 2922 (w, aliph C–H). MS (EI): m/z (%) = 252 [MH, 23], 222 [MH−CH_2OH, 21], 160 [M−C_7H_7, 100], 91 [C_7H_7, 27]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.13 (d, J = 5.7 Hz, 3H, *CH*$_3$), 2.61 (dd, J = 13.2/8.4 Hz, 1H, 2-H), 2.77 (s, br, 1H, *NH*), 2.84–2.95 (m, 4H, 4-H/5-H/*CH*$_2$Ph), 3.16 (dd, J = 13.3/2.4 Hz, 1H, *CH*$_2$Ph), 3.23 (dd, J = 13.7/5.4 Hz, 1H, 2-H), 3.30–3.34 (m, 1H, 1-H), 7.10–7.32 (m, 9H, arom). ^{13}C NMR ($CDCl_3$): δ (ppm) = 23.0 (br, 1C, *CH*$_3$), 38.4 (1C, *CH*$_2$Ph), 45.6 (1C, C-5), 47.4 (br, 1C, C-1), 50.4 (br s, 1C, C-2), 52.4 (1C, C-4), 126.2, 126.3, 126.6, 128.6, 129.4, 130.1 (9C, Ph-*CH*), 140.2, 140.7, 144.5 (3C, Ph-*C*). HRMS (ESI): $C_{18}H_{21}NH$: Calcd 252.1747, found 252.1741. HPLC: purity 99.5%, t_R = 17.23 min.

5.20. (1*S*,4*S*)-1-Benzyl-4-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (*ent*-14a)

Following the general procedure C, **13a** (139.5 mg, 0.37 mmol) on hydrogenolysis gave 94.4 mg of a crude product, which was purified by fc (1 cm, EtOAc/petroleum ether/NH_3 50/49.5/0.5, 10 mL, R_f = 0.16 (EtOAc/petroleum ether/NH_3 70/29.5/0.5)). Colorless oil, yield 69.1 mg (73%). $[\alpha]_{589}^{20} = -24.8$ (c 0.71, CH_2Cl_2). HRMS (ESI): $C_{18}H_{21}NH$: Calcd 252.1747, found 252.1753. HPLC: purity 98.0%, t_R = 17.23 min.

5.20.1. (1*R*,4*R*)-1-Benzyl-4-ethyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine 14b

Following the general procedure C, **12b** (65 mg, 0.16 mmol) on hydrogenolysis gave 52 mg of a crude product, which was purified by fc (1 cm, EtOAc/petroleum ether/NH_3 30/69.5/0.5, 10 mL, R_f = 0.19 (EtOAc/petroleum ether/NH_3 50/49.5/0.5)). Colorless oil, yield 34 mg (76%). $[\alpha]_{589}^{20} = +19.2$ (c 1.22, CH_2Cl_2). FT-IR (ATR, film): ν (cm^{-1}) = 3382 (w, N–H), 3058, 3020 (w, arom C–H), 2936 (w, aliph C–H). MS (EI): m/z (%) = 264 [M−H, 2], 250 [M−CH_3, 100], 91 [C_7H_7, 12]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.93 (t, J = 7.4 Hz, 3H, CH_2CH_3), 1.37–1.46 (m, 2H, *CH*$_2$CH$_3$), 1.91 (s, br, 1H, NH), 2.59 (dd, J = 13.3/8.1 Hz, 1H, *CH*$_2$Ph), 2.62–2.65 (m, 1H, 4-H), 2.83–2.97 (m, 2H, 2-H/5-H), 3.15 (dd, J = 13.3/2.8 Hz, 1H, 5-H), 3.20 (dd, J = 13.6/5.6 Hz, 1H, CH_2Ph), 3.20–3.24 (m, 1H, 2-H), 3.25–3.29 (m, 1H, 1-H), 7.09–7.22 (m, 7H, arom), 7.26–7.30 (m, 2H, arom). ^{13}C NMR ($CDCl_3$): δ (ppm) = 11.3 (1C, CH_2CH_3), 28.8 (1C, *CH*$_2$CH$_3$), 38.4 (1C, *CH*$_2$Ph), 43.3 (1C, C-5), 47.6 (1C, C-1), 49.8 (1C, C-2), 58.3 (1C, C-4), 126.2, 126.3, 126.5, 128.6, 129.4, 130.1 (9C, Ph-*CH*), 140.1, 140.8, 144.3 (3C, Ph-*C*). HRMS (ESI): $C_{19}H_{23}NH$: Calcd 266.1903, found 266.1914. HPLC: purity 98.5%, t_R = 17.73 min.

5.21. (1*S*,4*S*)-1-Benzyl-4-ethyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine *ent*-14b

Following the general procedure C, **13b** (75.5 mg, 0.19 mmol) on hydrogenolysis gave 62 mg of a crude product, which was purified by fc (1 cm, EtOAc/petroleum ether/NH_3 30/69.5/0.5, 10 mL, R_f = 0.19 (EtOAc/petroleum ether/NH_3 50/49.5/0.5)). Colorless oil, yield 42 mg (81%). $[\alpha]_{589}^{20} = -18.8$ (c 1.55, CH_2Cl_2). HRMS (ESI): $C_{19}H_{23}NH$: Calcd 266.1903, found 266.1908. HPLC: purity 99.0%, t_R = 17.73 min.

5.22. (1*R*,4*R*)-1-Benzyl-4-butyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine 14c

Following the general procedure C, **12c** (60 mg, 0.14 mmol) on hydrogenolysis gave 50 mg of a crude product, which was purified by fc (1 cm, EtOAc/petroleum ether/NH_3 15/84.5/0.5, 10 mL, R_f = 0.24 (EtOAc/petroleum ether/NH_3 30/69.5/0.5)). Colorless oil, yield 31 mg (73%). $[\alpha]_{289}^{20} = +21.0$ (c 0.87, CH_2Cl_2). FT-IR (ATR, film): ν (cm^{-1}) = 3058, 3023 (w, arom C–H), 2953, 2925 (w, aliph C–H), 1452 (m, C–N valence bond). MS: m/z = 293 [M, 11], 236 [M−C_4H_9, 100], 202 [M−C_7H_7, 62], 91 [C_7H_7, 49]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.89 (t, J = 6.8 Hz, 3H, CH_3), 1.26–1.41 (m, 6H, $CH_2CH_2CH_2CH_3$), 2.33 (s, br, 1H, NH), 2.60 (dd, J = 13.2/8.1 Hz, 1H, 2-H), 2.74 (s, br, 1H, 4-H), 2.85–2.97 (m, 3H, *CH*$_2$Ph,/5-H), 3.14–3.23 (m, 2H, *CH*$_2$Ph/2-H), 3.30 (s, br, 1H, 1-H), 7.09–7.30 (m, 9H, arom). ^{13}C NMR ($CDCl_3$): δ (ppm) = 14.3 (1C, *CH*$_3$), 22.9 (1C, *CH*$_2$CH$_3$), 28.8 (1C, *CH*$_2$CH$_2$CH$_3$), 35.6 (1C, *CH*$_2$CH$_2$CH$_2$CH$_3$), 38.4 (1C, *CH*$_2$Ph), 43.3 (1C, 5-C), 47.4 (1C, 1-C), 49.7 (1C, 2-C), 56.7 (1C, 4-C), 126.3, 126.4, 128.6, 129.4, 130.1 (9C, Ph-*CH*), 140.0, 140.7, 144.2 (3C, Ph-*C*). HRMS (ESI): $C_{21}H_{27}NH$: Calcd 294.2216, found 294.2228. HPLC: purity 99.9%, t_R = 20.35 min.

5.23. (1*S*,4*S*)-1-Benzyl-4-butyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine *ent*-14c

Following the general procedure C, **13c** (44.7 mg, 0.11 mmol) on hydrogenolysis gave 32.5 mg of a crude product, which was purified by fc (1 cm, EtOAc/petroleum ether/NH_3 15/84.5/0.5, 10 mL, R_f = 0.24 (EtOAc/petroleum ether/NH_3 30/69.5/0.5)). Colorless oil, yield 24.6 mg (77.6%). $[\alpha]_{289}^{20} = -23.5$ (c 0.84, CH_2Cl_2). HRMS (ESI): $C_{21}H_{27}NH$: Calcd 294.2216, found 294.2222. HPLC: purity 99.7%, t_R = 19.89 min.

5.24. (1*R*,4*S*)-1-Benzyl-4-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (14d)

Following the general procedure C, **12d** (39 mg, 0.089 mmol) on hydrogenolysis gave 32 mg of a crude product, which was purified by fc (1 cm, EtOAc/petroleum ether/NH_3 10/89.5/0.5, 10 mL, R_f = 0.26 (EtOAc/petroleum ether/NH_3 20/79.5/0.5)). Colorless oil, yield 24 mg (85%). $[\alpha]_{589}^{20} = +41.5$ (c 1.04, CH_2Cl_2). FT-IR (ATR, film): ν (cm^{-1}) = 3332 (w, NH), 3059, 3024 (w, arom C–H), 2965, 2923, 2852 (w, aliph C–H). MS (EI): m/z (%) = 313 [M, 7], 222 [M−C_7H_7], 118 [CH_2CH(Ph)N, 70], 91 [C_7H_7, 55]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.60 (dd, J = 12.7/8.7 Hz, 1H, 2-H), 2.80 (dd, J = 13.8/10.4 Hz, 1H, *CH*$_2$Ph), 2.90 (d, J = 14.1 Hz, 1H, 5-H), 3.15 (dd, J = 12.7/1.8 Hz, 1H, 2-H), 3.23 (dd, J = 13.9/5.2 Hz, 1H, *CH*$_2$Ph), 3.35 (dd, J = 14.2/9.4 Hz, 1H, 5-H), 3.42–3.46 (m, 1H, 1-H), 3.70 (d, J = 9.0 Hz, 1H, 4-H), 7.04 (t, J = 8.0 Hz, 1H, arom), 7.09 (d, J = 7.3 Hz, 1H, arom), 71.2–7.29 (m, 12H, arom). A signal for NH proton could not be detected. ^{13}C NMR ($CDCl_3$): δ (ppm) = 38.5 (1C, C-1), 45.9 (1C, C-5), 46.3 (1C, *CH*$_2$Ph), 52.0 (1C, C-2), 62.8 (1C, C-4), 125.6, 126.3, 126.4, 126.8, 127.4, 128.7, 129.5, 130.1 (14C, Ph-*CH*), 140.7, 144.9, 145.9 (4C, Ph-*C*). HRMS (ESI): $C_{23}H_{23}NH$: Calcd 314.1903, found 314.1906. HPLC: purity 98.8%, t_R = 20.58 min.

5.25. (1*S*,4*R*)-1-Benzyl-4-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine *ent*-14d

Following the general procedure C, *ent*-**12d** (66.4 mg, 0.15 mmol) on hydrogenolysis gave 32 mg of a crude product, which was purified by fc (1 cm, EtOAc/petroleum ether 10/90, 10 mL, R_f = 0.26 (EtOAc/petroleum ether/NH_3 20/79.5/0.5)). Colorless oil, yield 22.6 mg (47%). $[\alpha]_{589}^{20} = -45.0$ (c 0.82, CH_2Cl_2). HRMS (ESI): $C_{23}H_{23}NH$: Calcd 314.1903, found 314.1916. HPLC: purity 98.6%, t_R = 19.58 min.

6. Receptor binding studies

6.1. Materials and general procedures

The guinea pig brains and rat livers were commercially available (Harlan-Winkelmann, Borchen, Germany). The pig brains

were a donation of the local slaughterhouse (Coesfeld, Germany). Homogenizer: Elvehjem Potter (B. Braun Biotech International, Melsungen, Germany). Centrifuge: High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Fisher Scientific, Langenselbold, Germany). Filter: Printed Filtermat Types A and B (Perkin Elmer LAS, Rodgau-Jügesheim, Germany), presoaked in 0.5% aqueous polyethylenimine for 2 h at room temperature before use. The filtration was carried out with a MicroBeta FilterMate-96 Harvester (Perkin Elmer). The scintillation analysis was performed using Meltilex (Type A or B) solid scintillator (Perkin Elmer). The solid scintillator was melted on the filtermat at a temperature of 95 °C for 5 min. After solidifying of the scintillator at room temperature, the scintillation was measured using a MicroBeta Trilux scintillation analyzer (Perkin Elmer). The counting efficiency was 40%. All experiments were carried out in triplicates using standard 96-well multiplates (Diagonal, Muenster, Germany). The IC_{50}-values were determined in competition experiments with at least six concentrations of the test compounds and were calculated with the program GRAPHPAD PRISM® 3.0 (GRAPHPAD Software, San Diego, CA, USA) by non-linear regression analysis. The K_i-values were calculated according to the formula of Cheng and Prusoff.[24] The K_i-values are given as mean value ± SEM from three independent experiments.

6.2. Determination of the σ_1 receptor affinity[11,20]

6.2.1. Membrane preparation for the σ_1 assay

Five guinea pig brains were homogenized with the potter (500–800 rpm, 10 up-and-down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1200*g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 23,500*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*g* (20 min, 4 °C). This procedure was repeated twice. The final pellet was resuspended in 5–6 volumes of buffer, the protein concentration was determined according to the method of Bradford[25] using bovine serum albumin as standard, and subsequently the preparation was frozen (−80 °C) in 1.5 mL portions containing about 1.5 mg protein/mL.

6.2.2. Performance of the σ_1 assay

The test was performed with the radioligand [^{3}H]-(+)-pentazocine (32,2 Ci/mmol; Perkin Elmer LAS). The thawed membrane preparation (about 75 μg of the protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-pentazocine, and buffer (50 mM TRIS, pH 7.4) in a total volume of 200 μL for 150 min at 37 °C. The incubation was terminated by rapid filtration through the presoaked filtermats using a cell harvester. After washing each well five times with 300 μL of water, the filtermats were dried at 95 °C. Subsequently, the solid scintillator was placed on the filtermat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at rt. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The non-specific binding was determined with 10 μM unlabeled (+)-pentazocine. The K_d-value of (+)-pentazocine is 2.9 nM.[26]

6.3. Determination of the σ_2 receptor affinity[11,20]

6.3.1. Membrane preparation for the σ_2 assay

Two rat livers were cut into smaller 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 1200*g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 31,000*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*g* for 20 min at 4 °C. The final pellet was resuspended in 5–6 volumes of buffer, the protein concentration was determined according to the method of Bradford[25] using bovine serum albumin as standard, and subsequently the preparation was frozen (−80 °C) in 1.5 mL portions containing about 2 mg protein/mL.

6.3.2. Performance of the σ_2 assay

The test was performed with the radioligand [^{3}H]-ditolylguanidine (50 Ci/mmol; ARC, St. Louis, MO, USA). The thawed membrane preparation (about 100 μg of the protein) was incubated with various concentrations of test compounds, 3 nM [^{3}H]-ditolylguanidine, and buffer containing (+)-pentazocine (2 μM (+)-pentazocine in 50 mM TRIS, pH 8.0) in a total volume of 200 μL for 150 min at rt. The incubation was terminated by rapid filtration through the presoaked filtermats using a cell harvester. After washing each well five times with 300 μL of water, the filtermats were dried at 95 °C. Subsequently, the solid scintillator was placed on the filtermat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at room temperature. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The non-specific binding was determined with 10 μM unlabeled ditolylguanidine. The K_d-value of ditolylguanidine is 17.9 nM.[27]

6.4. Determination of the affinity to the phencyclidine binding site of the NMDA receptor

The preparation of the membranes and the assay were performed according to the literature.[11]

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