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Oxa-Pictet-Spengler reaction as key step in the synthesis of novel σ receptor ligands with 2-benzopyran structure

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Abstract

The Oxa-Pictet-Spengler reaction of methyl 3-hydroxy-4-phenylbutanoate (**8**) was explored to obtain novel σ receptor ligands. 1-Acyl protected piperidone ketals **10** and **11** reacted with phenylethanol **8** to yield spirocyclic compounds. Aliphatic aldehyde acetals **19** provided 1,3-disubstituted 2-benzopyrans **20** with high *cis*-diastereoselectivity. The intramolecular Oxa-Pictet-Spengler reaction of **24** led to the tricyclic compound **25**. The spirocyclic compounds **18** show high σ_1 affinity (K_i 20-26 nM) and σ_1/σ_2 selectivity (>9-fold), when a large substituent (*n*-octyl, benzyl, phenylpropyl) is attached to the piperidine N-atom. Opening of the piperidine ring to yield aminoethyl (**22**, **23**) or aminomethyl derivatives (**21**) resulted in reduced σ_1 affinity and σ_1/σ_2 selectivity.

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Key words: σ ligands; Oxa-Pictet-Spengler reaction; intramolecular Oxa-Pictet-Spengler reaction; 2-benzopyrans; spirocyclic piperidines; tricyclic systems; aryl-N-distance

1. Introduction

At first, the nature of the σ receptor was controversially discussed: originally it was regarded as opioid receptor subtype, then as binding site at the NMDA receptor and now it is accepted as unique receptor type without relationship to any other mammalian protein. There exist two subtypes termed σ_1 and σ_2 receptors, which differ in their molecular weight, tissue distribution and ligand binding profile.[1-5]

The σ_1 receptor is involved in various neurological disorders, including depression and anxiety, cognitive deficits, psychosis, pain and dependence (cocaine, ethanol).[6-8] The importance of σ_1 receptors is emphasized by the fact that several antipsychotics (e.g. haloperidol),[9] antidepressants (e.g. opipramol, fluvoxamine)[10] and anti-Alzheimer drugs (e.g. donepezil)[11] show in addition to their main activity interactions with σ_1 receptors. Additionally, σ_1 ligands are able to modulate the analgesic activity of opioid analgesics. The σ_1 agonist (+)-pentazocine antagonized the analgesic effects of morphine, whereas the σ_1 antagonist haloperidol reversed this effect and moreover led to potentiation of morphine analgesia.[12] The particular role of σ_1 receptors in the field of neuropathic pain was shown with σ_1 receptor knock-out mice.[13] The pyrazole derivative S1RA is currently under clinical investigation for the therapy of neuropathic pain.[14] A significantly increased level of σ_1 receptors was found in some human tumor cell lines including brain, breast, lung and prostate

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cancer cell lines. Since σ_1 receptor antagonists were shown to induce apoptosis in human tumor cell lines, selective targeting of σ_1 receptors represents a promising strategy for the development of a novel antitumor therapy.[15-18]

In addition to overexpression of σ_1 receptors, σ_2 receptors are also upregulated in various human tumor cell lines. It was shown that apoptotic processes could be induced by activation of σ_2 receptors highly expressed in rapidly proliferating tumor cells.[18-20]

During the past decades, substantial efforts have been spent on the generation of novel σ_1 and σ_2 receptor ligands. Based on pharmacophore models[21] and a 3D-homology model[22] of the σ_1 receptor, we have developed spirocyclic compounds showing high σ_1 receptor affinity. Both the benzofuran and benzopyran derivatives **1a** (K_i = 1.1 nM) and **1b** (K_i = 1.3. nM) display very high σ_1 receptor affinity and more than 1000-fold selectivity over the σ_2 subtype. (Figure 1) In the capsaicin assay, an animal model for neuropathic pain, the benzofuran **1a** sowed promising analgesic activity.[23-25] Starting with the high σ_1 affinity and selectivity of **1a** a series of homologous fluoroalkyl derivatives was designed as PET (positron emission tomography) tracers[26-31] resulting in the fluoroethyl derivative fluspidine (**2**). [^{18}F]-labeled (*S*)-configured fluspidine [^{18}F]-(*S*)-**2** is currently under clinical investigation as PET tracer for imaging of σ_1 receptors in the central nervous system.[28] Spirocyclic compounds with differently annulated thiophene rings (e.g. **3**) were prepared revealing high σ_1 affinity. Depending on the position of the phenyl moiety introduced at the very end of the synthesis, thiophenes **3** and regioisomers show reverse binding modes in the σ_1 receptor binding pocket.[32]

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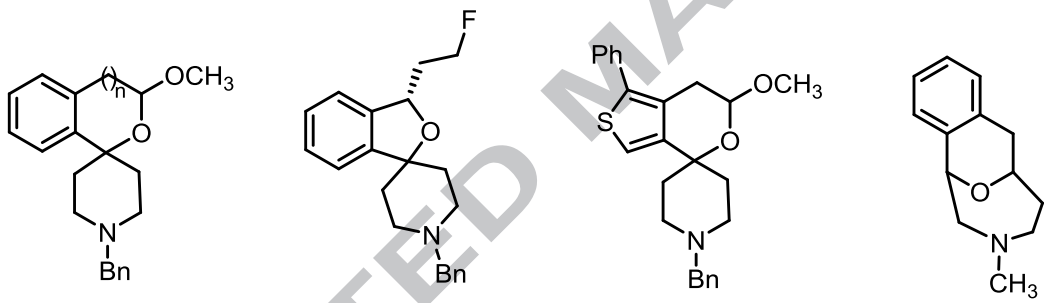
In the mouse acetic acid writhing assay the 1,6-epoxy-3-benzazonine **4** showed analgesic activity with an ED_{50} value of 20 mg /kg body weight.[33] (Figure 1) Both the spirocyclic σ_1 ligands **1b** and **3** and the epoxy-3-benzazonine **4** contain the benzopyran substructure. In both types of ligands the arylalkylamine pharmacophore is conformationally restricted: in **1**-**3** it is embedded in a spirocyclic system and in **4** in a tricyclic system. However, the distance between the benzene ring and the basic amino moiety is different in the spirocyclic compounds **1**-**3** compared to the tricyclic compound **4**.



1a: n = 0
1b: n = 1

(*S*)-**2**

3

4

Figure 1: Benzofuran and benzopyran based σ_1 ligands and analgesics with conformationally restricted arylalkylamine substructure.

The Oxa-Pictet-Spengler reaction is the condensation of a 2-arylethanol derivative with an aldehyde, ketone or derivatives thereof to produce 3,4-dihydro-2-1*H*-benzopyrans.[34-36] This oxygen variation of the Pictet-Spengler reaction is a versatile method to synthesize variously substituted 2-benzopyrans and larger fused ring systems. Moreover, intramolecular versions are known.[37] It is promoted by Broensted or Lewis acids, including $BF_3{\cdot}OEt_2$, TMSOTf, and $Bi(OTf)_3$,[38] and can be applied for the stereoselective synthesis of complex natural products.[39-41]

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In order to broaden the relationships between the structure of 1-aminoalkyl substituted 2-benzopyrans and their σ_1 affinity and selectivity over the σ_2 subtype, modifications of the side chain in 3-position and the aminoalkyl part of the compounds were envisaged. In order to get access to a side chain bearing an appropriate substituent, the Oxa-Pictet-Spengler reaction using 3-hydroxy-4-phenylbutanoate **8** as 2-phenylethanol component should be applied. Herein, the applicability of the β-hydroxyester **8** as arylethanol component in the inter- and intramolecular Oxa-Pictet-Spengler reaction should be investigated. Moreover, its reaction with various carbonyl compounds should lead to 2-benzopyrans with conformationally restricted or flexible phenylalkylamine substructures. The selection of the substituents at the 2-benzoparyn system was driven by the substitution pattern of known σ_1 lead compounds as shown in Figure 1.

2. Synthesis

The β-hydroxyester **8**[42,43] was the central building block of this project. It was prepared by $NaBH_4$ reduction of the β-ketoester **7**, which was obtained in a two-step, one-pot synthesis. At first, Meldrum's acid (**6**) was acylated with phenylacetyl chloride in the presence of pyridine. The product was then heated in methanol resulting in decomposition of the triacyl intermediate into an acylketene, which reacted with methanol to afford the β-ketoester **7**.[44] The β-hydroxyester **8** was obtained in 59 % yield over two steps from phenylacetyl chloride. (Scheme 1)

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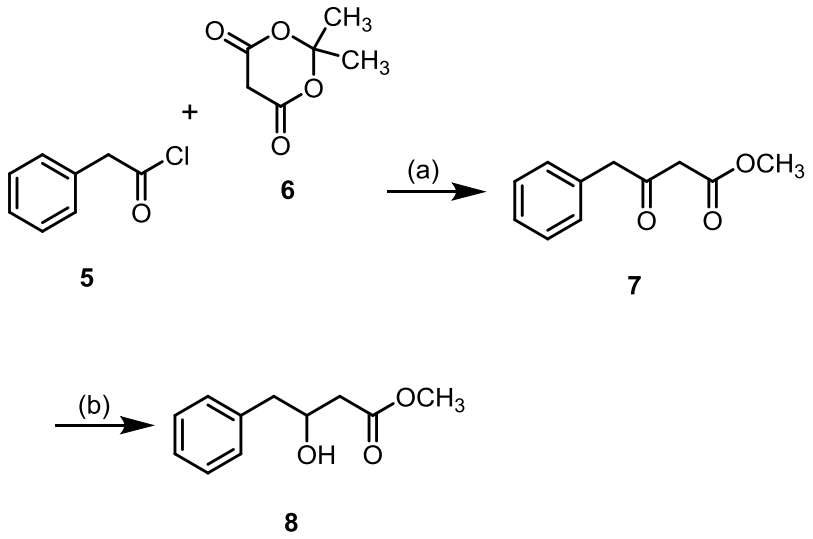

Scheme 1: Synthesis of β-hydroxyester **8**.
Reagents and reaction conditions: (a) 1. Pyridine, CH_2Cl_2, 2.5 h; 2. CH_3OH, reflux, 2 h, 72 %. (b) CH_3OH, $NaBH_4$, rt, 20 h, 82 %.

In order to synthesize spirocyclic benzopyran derivatives with a piperidine ring, 1-benzylpiperidin-4-one and its dimethyl ketal **9** were employed in the Oxa-Pictet-Spengler reaction with β-hydroxyester **8**. However, all attempts failed to give the corresponding spirocyclic benzopyrans. It was assumed that the basic piperidine ring reacted first with the added Broensted or Lewis acid, thus inhibiting the acid induced formation of a cation in 4-position of the piperidine ring.

Therefore, non-basic 1-acyl protected piperidin-4-ones were employed in the Oxa-Pictet-Spengler reaction. Since these piperidin-4-ones did not react with the β-hydroxyester **8**, the ketals **10** and **11** were prepared by ketalization of the corresponding ketones with methanol and trimethyl orthoformate. In the presence of $BF_3{\cdot}OEt_2$ the β-hydroxyester **8** reacted with the ketals **10** and **11** to afford the spirocyclic benzopyrans **12** and **13** in 86 % and 22 % yield, respectively.

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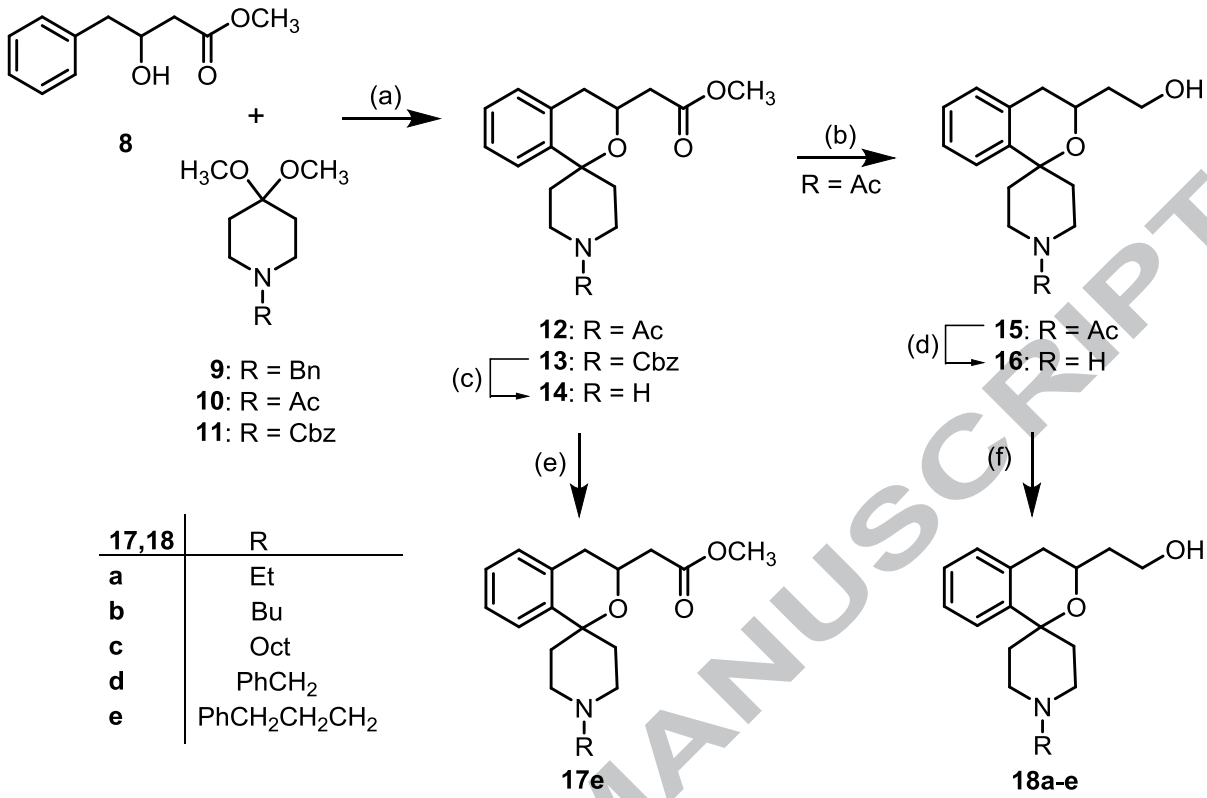

Scheme 2: Synthesis of 2-benzopyrans with spirocyclic connected piperidine ring. Reagents and reaction conditions: (a) $BF_3{\cdot}OEt_2$, toluene, rt, 6 h, 86 % (**12**), or 8 d, 22 % (**13**). (b) $LiBH_4$, THF, rt, 20 h, 76 %. (c) H_2 (balloon), Pd/C, CH_3OH, rt, 20 h, 89 %. (d) NaOH, reflux, 3 h, 53 %. (e) $BrCH_2CH_2CH_2Ph$, CH_3CN, K_2CO_3, reflux, 4 h, 69 %. (f) R-Br, CH_3CN, reflux, 4 h, 42-70 % (**18a**-**c**, **18e**); reflux, 20 h for Bn-Br, 17 % (**18d**). The compounds **8** and **12**-**18** were prepared as racemic mixtures.

The ester in the side chain of the N-acetyl derivative **12** was reduced with $LiBH_4$ to provide the primary alcohol **15** in 76 % yield. The Cbz- and Ac-protective groups of the ester **13** and the alcohol **15** were cleaved off hydrogenolytically and upon treatment with NaOH, respectively. The resulting secondary amines **14** and **16** were alkylated with various alkyl bromides in the presence of K_2CO_3 to obtain the N-substituted spirocyclic piperidine derivatives **17e** and **18a**-**e**. Since the Oxa-Pictet-Spengler reaction with the Cbz-protected ketal **11** provided spirocyclic 2-benzopyran **13** in only 22 % yield, only the phenylpropyl derivative **17e** with an ester side chin was prepared.

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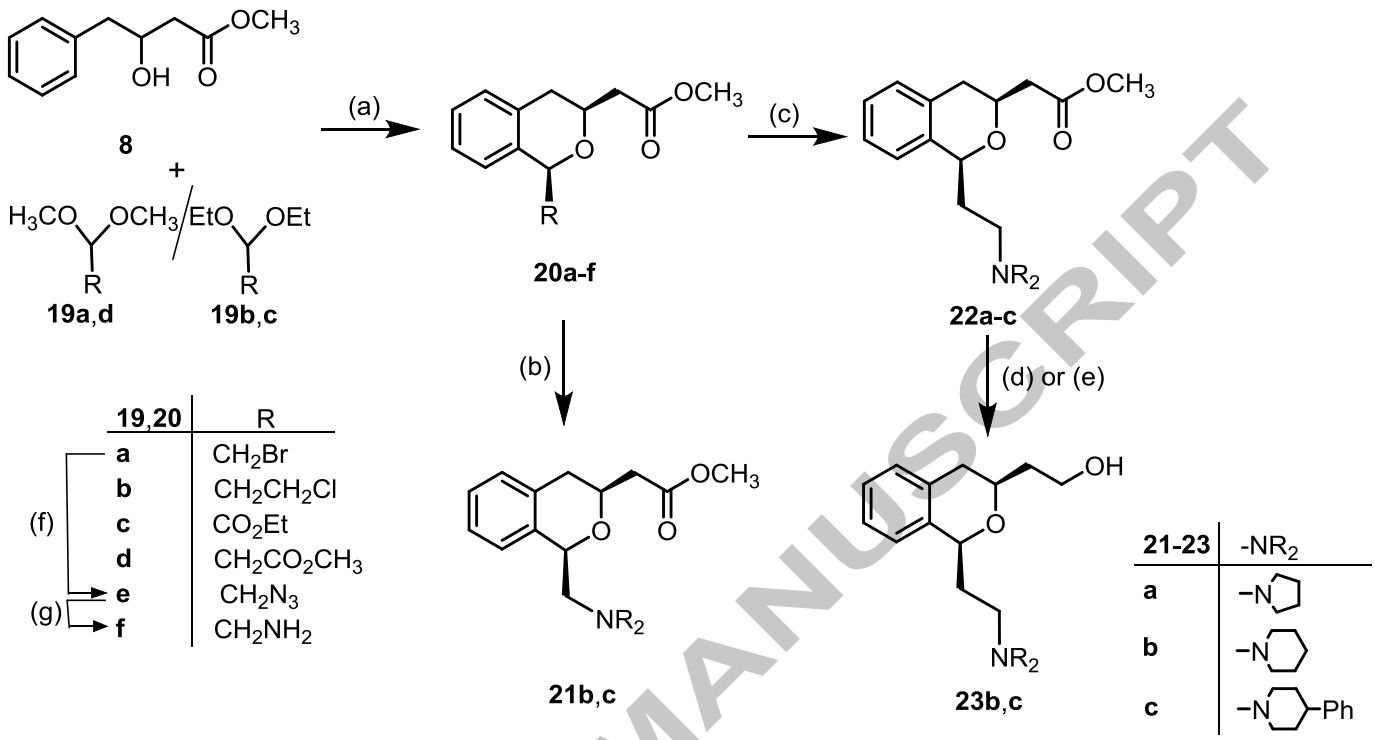

Scheme 3: Synthesis of 1-(aminoalkyl) substituted 2-benzopyrans.
Reagents and reaction conditions: (a) $BF_3{\cdot}OEt_2$, toluene, CH_2Cl_2, rt, 2-4 d, 84-99 % (**20a**,**c**,**d**), 32 % (**20b**). (b) **20a**, piperidine or 4-phenylpiperidine, Bu_4NI, K_2CO_3, CH_3CN, reflux, 20 h, 16 % (**21b**), 11 % (**21c**). (c) **20b**, pyrrolidine, piperidine, or 4-phenylpiperidine, Bu_4NI, K_2CO_3, CH_3CN, reflux, 20 h, 38-64 %. (d) $LiBH_4$, THF, rt, 20 h, 43 % (**23b**). (e) $LiAlH_4$, THF, 0 °C, 2 h, 66 % (**23c**). (f) NaN_3, DMF, 60-70 °C, 24 h, 28 %. (g) H_2, Pd/C, CH_3OH. Only one enantiomer of the racemic mixtures is shown, respectively.

The synthesis of analogs of **17** and **18** with linear substituents in 1-position of the 2-benzopyran ring was envisaged by reaction of β-hydroxyester **8** with aliphatic aldehyde acetals. In particular acetals with additional substituents in the side chain were considered, which should allow the introduction of basic amino moieties. For this purpose bromoacetaldehyde acetal **19a** and chloropropionaldehyde acetal **19b** were reacted with the β-hydroxyester **8** in the presence of $BF_3{\cdot}OEt_2$. The bromomethyl derivative **20a** was obtained in 86 % yield, whereas the chloroethyl

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derivative **20b** was isolated in only 32 % yield. The lower yield of the chloroethyl derivative **20b** might be explained by fast β-elimination. Although a second center of chirality in 1-position was established, only one diastereomer of the haloalkyl substituted 2-benzopyrans **20a** and **20b** was isolated after Oxa-Pictet-Spengler reaction. The relative configuration of the haloalkyl derivatives **20a** and **20b** was determined by nuclear Overhauser effect (NOE) experiments. For example for **20a**, irradiation with the resonance frequency of 3-H at 4.18 ppm resulted in an increased signal at 5.09 ppm (1-H) indicating the *cis*-orientation of these protons. Obviously, during the Oxa-Pictet-Spengler reaction thermodynamically more stable *cis*-configured diastereomers were formed with high diastereoselectivity.

Nucleophilic substitution of the bromomethyl and chloroethyl derivatives **20a** and **20b** with secondary amines (pyrrolidine, piperidine, 4-phenylpiperidine) led to the tertiary amines **21b**,**c** and **22a**-**c**. In order to increase the yields and the reaction rate, tetrabutylammonium iodide was added to the reaction mixture. The primary alcohols **23b** and **23c** were obtained by $LiBH_4$ and $LiAlH_4$ reduction of the esters **22b** and **22c**, respectively. The σ_1 and σ_2 affinities of the spirocyclic amines **17** and **18** as well as the tertiary amines **21**-**23** were tested in receptor binding studies.

The substituent in 1-position of the 2-benzopyran ring should be used to form tricyclic systems by connecting it with the ester group in 3-position of cis-configured 2-benzopyrans **20**. Subsequently, a basic functional group should be installed at or within the newly formed bridge. At first a Dieckmann condensation of diesters **20c** and **20d** was envisaged, which were prepared by Oxa-Pictet-Spengler reaction of β-hydroxyester **8** with acetals **19c** and **19d** and $BF_3{\cdot}OEt_2$. Only one diastereomer of **20c** and **20d** was found indicating high diastereoselectivity of the Oxa-Pictet-

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Spengler reaction. A positive NOE between the protons in 1-ppsition (5.23 ppm) and 3-position (4.14 ppm) confirmed the thermodynamically favored *cis*-configuration of **20d** and indirectly of **20c** as well.

Although the Dieckmann cyclization of diesters **20c** and **20d** was tried with different bases (NaH/CH_3OH, NaH/EtOH, $NaOCH_3/CH_3OH$, KO^{t}Bu, LHMDS; LDA) in different solvents under different reaction conditions, tricyclic systems could not be identified. Usually the starting diesters **20c** and **20d** were re-isolated, although *cis/trans*-isomerization with strong bases and transesterification with alcohols were observed.

According to the next idea, the bromomethyl derivative **20a** was transformed into the azidomethyl derivative **20e**. Reduction of the azide **20e** with H_2 and Pd/C led to the primary amine **20f**, which upon treatment with Lewis or Broensted acids did not react with the ester in 3-position to yield the lactam **25**.

Therefore, the strategy was changed into an intramolecular Oxa-Pictet-Spengler reaction. Aminolysis of the β-hydroxyester **8** with aminoacetaldehyde dimethyl acetal led to the amide **24** containing both the phenylethanol and acetal structural elements. The intramolecular Oxa-Pictet-Spengler reaction of **24** was catalyzed by $BF_3{\cdot}OEt_2$ affording the tricyclic lactam **25**. Several optimization experiments were performed, but the yield of the tricyclic lactam **25** did not exceed 10 %. The lactam **25** had been previously prepared by a Schmidt rearrangement of 1,4,5,6-tetrahydro-1,5-epoxybenzo[8]annulen-3(2*H*)-one. Whereas Schmidt rearrangement provided two regioisomers,[33] the intramolecular Oxa-Pictet-Spengler reaction yielded only one regioisomer, although in low yields. The transformation of the lactam **25** into the

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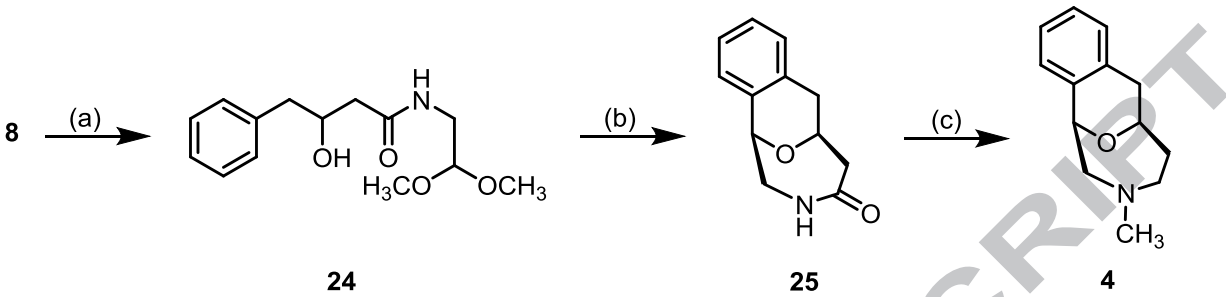
analgesically active amine **4** required $LiAlH_4$ reduction and methylation as reported in literature.[33]



24 **25** **4**

Scheme 4: Synthesis of tricyclic benzopyrans by intramolecular Oxy-Pictet-Spengler reaction.

Reagents and reaction conditions: (a) $H_2NCH_2CH(OCH_3)_2$, *p*TosOH, reflux, 69 %. (b) toluene, CH_2Cl_2, rt, 30 min, then addition of $BF_3{\cdot}OEt_2$ 40-50 °C, 2 d, 10 %. (c) two steps according to ref.[33]. The compounds **24**, **25** and **4** were prepared as racemic mixtures.

3. Receptor affinity

The σ_1 affinity of the 2-benzopyrans was tested in radioligand receptor binding studies with guinea pig brain membrane preparations and the radioligand [^{3}H]-(+)-pentazocine. In the σ_2 assay rat liver membrane preparations and [^{3}H]di-o-tolylguanidine were used. Since di-o-tolylguanidine also interacts with σ_1 receptors, an excess of (+)-pentazocine was added to mask the σ_1 receptors.[45-47] The affinity data of the tested 2-benzopyrans are summarized in Table 1.

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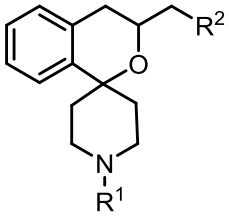
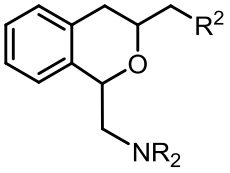
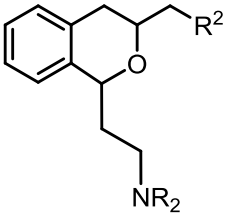
Table 1: Affinity of 2-benzopyrans towards σ_1 and σ_2 receptors.



17,18



21



22,23

compd.	R^1 or NR_2	R^2	K_i ± SEM (nM) (n = 3)[a]		σ_1/σ_2 selectivity
			σ_1	σ_2	
17e	$Ph(CH_2)_3$	CO_2CH_3	39 ± 5.0	610 ± 230	15
18a	Et	CH_2OH	0 %*	0 %*	--
18b	*n*-Bu	CH_2OH	60 ± 13	13 %*	--
18c	*n*-Oct	CH_2OH	26 ± 8	234 ± 28	9
18d	$PhCH_2$	CH_2OH	20 ± 6	0 %*	--
18e	$Ph(CH_2)_3$	CH_2OH	20 ± 2	617 ± 73	30
21b	Piperidin-1-yl	CO_2CH_3	510 ± 100	7 %*	--
21c	4-Ph-piperidin-1-yl	CO_2CH_3	673 ± 84	785 ± 65	1.2
22a	Pyrrolidin-1-yl	CO_2CH_3	>1000	0 %*	--
22b	Piperidin-1-yl	CO_2CH_3	519 ± 64	0 %*	--
22c	4-Ph-piperidin-1-yl	CO_2CH_3	38 ± 4.0	107 ± 50	2.8
23b	Piperidin-1-yl	CH_2OH	4 %*	14 %*	--
23c	4-Ph-piperidin-1-yl	CH_2OH	52 ± 19	20 ± 2.0	0.4
(+)-Pentazocine			2.2 ± 0.7	-	
Haloperidol			1.9 ± 0.2	78.1 ± 1.3	
Di-*o*-tolylguanidine			177 ± 4	20.2 ± 1.3	

[a] All K_i-values were recorded three times, the number of independent experiments is 3.

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* For low-affinity compounds only the inhibition of the radioligand binding (in %) at a test compound concentration of 1 µM is given.

The σ_1 affinity of the conformationally restricted spirocyclic alcohols **18** increased with increasing size of the N-substituent. The highest σ_1 affinity was found for the *n*-octyl (**18c**, K_i = 26 nM), benzyl (**18d**, K_i = 20 nM) and phenylpropyl derivative (**18e**, K_i = 20 nM). An ester moiety in the side chain in 3-position of the 2-benzopyran (**17e**) slightly reduced the σ_1 affinity compared to the primary alcohol **18e**. 2-Benzopyrans **22** and **23** with a flexible aminoethyl side chain in 1-position, but the same aryl-N-distance revealed slightly reduced σ_1 affinity compared to the more rigid spirocyclic compounds **18**. The K_i values of **22c** and **23c** with the large 4-phenylpiperidin-1-yl moiety are 38 nM and 52 nM, respectively. In this case ester and alcohol in the side chain led to almost the same σ_1 affinity. The smaller piperidin-1-yl moiety at the end of the 1-ethyl spacer (**22b**, **23b**) and shorter aminomethyl derivatives **21** resulted in more than 10-fold reduced σ_1 affinity.

The highest σ_2 affinity in the group of spirocyclic 2-benzopyrans was found for **18c** with the large *n*-octyl moiety at the N-atom (K_i = 234 nM). Nevertheless, **18c** showed still a 9-fold preference for the σ_1 over the σ_2 receptor. In the class of aminoalkyl substituted 2-benzopyrans only 4-phenylpiperidin-1-yl derivatives (**c**-series) revealed considerable σ_2 affinity. Whereas the aminomethyl derivative **21c** interacted equally with both σ receptor subtypes, the aminoethyl homolog **22c** showed a slight preference for the σ_1 receptor. However, the selectivity was reversed for the alcohol **23c** displaying a 2.5-fold preference for the σ_2 subtype.

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4. Conclusion

The Oxa-Pictet-Spengler reaction was used to synthesize a diverse set of benzopyrans with the aim to address σ receptors. Variations of the structure and length of the aminoalkyl substituent in 1-position, the N-substituent(s) and the group in 3-position of the 2-benzopyran ring were performed. It was found that spirocyclic compounds **18** show high σ_1 affinity (K_i = 20-26 nM) and σ_1/σ_2 selectivity (>9-fold), when large substituents are attached to the N-atom. A higher flexibility of the 1-substituent was realized with aminoethyl derivatives **22** and **23** resulting in slightly reduced σ_1 affinity. Shortening of the 2-benzopyran-N-distance to three bond lenghths as in **21** led to considerable reduction of σ_1 affinity. On the other hand more flexible side chains allowed better interactions with σ_2 receptors leading to reduced σ_1/σ_2 selectivity.

With respect to σ_1 affinity and σ_1/σ_2 selectivity the benzyl derivative **18d** ($K_i(\sigma_1)$ = 20 nM, ($K_i(\sigma_2)$ > 1 µM) represents one of the most promising σ_1 ligands of this series. Compared to related spirocyclic piperidines **1**-**3** its σ_1 affinity is 10-15-fold reduced. It is assumed that the reduced affinity is due to the 2-hydroxyethyl moiety in 3-position of the benzopyran system, but it remains to be elucidated, whether size and/or polarity of this moiety are responsible for this effect. However, due to the synthetic novel strategy of the Oxa-Pictet-Spengler reaction versatile substituents can be introduced in 3-position by starting with appropriate phenylethanol derivatives or modification of the existing acetate or ethanol side chain.

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5. Experimental

5.1. Chemistry, general methods

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. THF was dried with sodium/benzophenone and was freshly distilled before use. 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), ^{13}C NMR (100 MHz): Unity Mercury Plus 400 spectrometer (Varian); δ in ppm related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution. 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), 250-4 mm; flow rate: 1.00 mL/min; injection volume: 5.0 µL; detection at λ = 210 nm; solvents: A: water with 0.05% (v/v) CF_3CO_2H; B: CH_3CN with 0.05% (v/v) CF_3CO_2H: gradient elution: (A %): 0-4 min: 90 % , 4-29 min: gradient from 90 % to 0 %, 29-31 min: 0 %, 31-31.5 min: gradient from 0 % to 90 %, 31.5-40 min: 90 %.

5.2. Synthetic procedures

5.2.1. Methyl 3-oxo-4-phenylbutanoate (7)[44]

Under N_2 atmosphere, pyridine (4 g) was added over 15 min. at 0 °C to a solution of Meldrum's acid (**6**, 2.88 g, 20 mmol) in CH_2Cl_2 (10 mL). A solution of phenylacetyl chloride (**5**, 3.1 g, 20 mmol) in CH_2Cl_2 (8 mL) was added dropwise at 0 °C during 2 h. After stirring for 90 min at 0 °C and 60 min at rt, CH_2Cl_2 (5 mL) and 2 M HCl (14 mL) were added. The organic layer was separated and the aqueous layer was extracted

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with CH_2Cl_2 (3×10 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The obtained orange crystals were suspended in methanol (20 mL) and the mixture was heated to reflux for 3 h. Then the mixture was concentrated in vacuo and the residue was purified by fc (6 cm, cyclohexane : EtOAc = 9 : 1, 30 mL, R_f = 0.41). Colorless oil, yield 2.75 g (72 %). $C_{11}H_{12}O_3$ (192.2). MS (EI): m/z = 192 [M], 91 [$PhCH_2$]. IR**:** ν [cm^{-1}] = 2953 (C-H), 1745 (C=O, ester), 1715 (C=O, ketone), 729, 698 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 3.46 (s, 2H, $CH_2CO_2CH_3$), 3.71 (s, 3H, CO_2CH_3), 3.82 (s, 2H, PhCH_2), 7.18-7.38 (m, 5H, arom.).

5.2.2. Methyl (±)-3-hydroxy-4-phenylbutanoate (8)[42,43]

The β-ketoester **7** (2.75 g, 14.3 mmol) was dissolved in methanol (25 mL). $NaBH_4$ (543 mg, 14.3 mmol) was added under cooling and the mixture was stirred for 20 h at rt. Subsequently the solvent was removed in vacuo, the residue was dissolved in water (10 mL) and acidified with 2 M HCl. The aqueous layer was extracted with Et_2O (4×20 mL), the combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by fc (6 cm, cyclohexane : EtOAc = 4 :1, 30 mL, R_f = 0.29). Colorless oil, yield 2.29 g (82 %). $C_{11}H_{14}O_3$ (194.2). MS (EI): m/z = 194 [M], 91 [$PhCH_2$]. IR: ν [cm^{-1}] = 3457 (O-H), 2952 (C-H), 1730 (C=O), 699 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.46 (dd, J = 16.4/8.6 Hz, 1H, $CH_2CO_2CH_3$), 2.52 (dd, J = 16.4/3.7 Hz, 1H, $CH_2CO_2CH_3$), 2.77 (dd, J = 13.6/6.1 Hz, 1H, PhCH_2), 2.87 (dd, J = 13.6/7.1 Hz, 1H, PhCH_2), 3.69 (s, 3H, CO_2CH_3), 4.25 - 4.29 (m, 1H, *CH*OH), 7.20-7.33 (m, 5H, arom.). A signal for the OH proton is not seen.

5.2.3. 1-Acetylpiperidin-4-one dimethyl acetal (10)

A solution of 1-acetylpiperidin-4-one (2.82 g, 20 mmol), trimethyl orthoformate (10.6 g) and *p*-toluenesulfonic acid (190 mg, 1 mmol) in CH_3OH abs. (4 mL) was stirred at

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rt for 16 h. Then 10 % $NaHCO_3$ solution (40 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 25 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was used for the next reaction step without further purification. Colorless oil, yield 3.26 g (87 %). $C_9H_{17}NO_3$ (187.2). MS (EI): m/z = 187 [M], 144 [M-$COCH_3$]. IR: ν [cm^{-1}] = 2959 (C-H), 1640 (C=O), 1108, 1046 (C-O). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.44 – 1.52 (m, 4H, N(CH_2*CH_2*)$_2$), 2.20 (s, 3H, NCO*CH_3*), 3.49 (s, 6H, 2 x O*CH_3*), 3.74 – 3.90 (m, 4H, N(*CH_2*CH_2)$_2$).

5.2.4. Benzyl 4,4-dimethoxypiperidine-1-carboxylate (11)

A solution of benzyl 4-oxopiperidine-1-carboxylate (2.33 g, 8.9 mmol), trimethyl orthoformate (5.5 mL) and *p*-toluenesulfonic acid (95 mg, 0.5 mmol) in CH_3OH abs. (4 mL) was stirred at rt for 2 d. Then10 % $NaHCO_3$ solution (30 mL) was added and the mixture was extracted with CH_2Cl_2 (3×15 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was used without further purification. Yellow oil, yield 2.85 g (> 100 %, unpurified), R_f = 0.2 (cyclohexane : EtOAc = 4 : 1). $C_{15}H_{21}NO_4$ (279.3). MS (EI): m/z = 172 [M-$PhCH_2O$], 156 [M-Bn-OCH_3]. IR: ν [cm^{-1}] = 2960, 2830 (C-H), 1697 (C=O), 1230 (O-CH_3). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.68 - 1.76 (m, 4H, N(CH_2*CH_2*)$_2$), 3.18 (s, 6H, 2 x O*CH_3*), 3.50 (t, J = 5.7 Hz, 4H, N(*CH_2*CH_2)$_2$), 5.12 (s, 2H, O*CH_2*Ph), 7.28-7.33 (m, 5H, arom.).

5.2.5. Methyl (±)-2-(1'-acetyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)acetate (12)

Under N_2, a solution of dimethyl ketal **10** (1.83 g, 9.8 mmol in toluene (2 mL) was added to a solution of **8** (438 mg, 2.2 mmol) in CH_2Cl_2 (7 mL) and the mixture was stirred for 30 min at rt. Then, the solution was cooled to 0 °C, $BF_3{\cdot}Et_2O$ (2.6 mL, approx. 16 mmol) was added and the mixture was stirred for 6 d at rt. A 10 % solution

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of $NaHCO_3$ (20 mL) was added, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (4×16 mL). The combined organic layers were dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (4 cm, cyclohexane : EtOAc = 1 : 1, 20 mL, R_f = 0.18). Pale yellow oil, yield 602 mg (86 %). $C_{18}H_{23}NO_4$ (317.4). MS (EI): m/z = 317 [M], 274 [M-$COCH_3$]. IR: ν [cm^{-1}] = 2925 (C-H), 1736 (C=O ester), 1634 (C=O amide). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.64 – 1.75 (m, 2H, N(CH_2*CH_2*)$_2$), 1.99 (td, J = 13.2/4.9 Hz, 1H, N(CH_2*CH_2*)$_2$), 2.13 (s, 3H, NCO*CH_3*), 2.09 – 2.20 (m, 1H, N(CH_2*CH_2*)$_2$), 2.59 – 3.00 (m, 5H, *CH_2*CO_2CH_3 (2H), Ar*CH_2* (2H), N(*CH_2*CH_2)$_2$ (1H)), 3.40 – 3.56 (m, 1H, N(*CH_2*CH_2)$_2$), 3.59 – 3.68 (m, 1H, N(*CH_2*CH_2)$_2$), 3.73 (s, 3H, CO_2*CH_3*), 4.20 – 4.31 (m, 1H, ArCH_2*CH*), 4.50 – 4.58 (m, 1H, N(*CH_2*CH_2)$_2$), 7.03 – 7.09 (m, 2H, arom.), 7.14 – 7.21 (m, 2H, arom.).

5.2.6. Methyl (±)-2-(1'-benzyloxycarbonyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)acetate (13)

Under N_2, a solution of dimethyl ketal **11** (1.40 g, 5.0 mmol) in toluene (1 mL) was added to a solution of **8** (194 mg, 1.0 mmol) in CH_2Cl_2 (7 mL) and the mixture was stirred for 30 min at rt. The solution was cooled to 0 °C, $BF_3{\cdot}Et_2O$ (2.0 mL, ca. 12 mmol) was added and the mixture was stirred for 8 d at rt. A 10 % solution of $NaHCO_3$ (12 mL) was added, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (4×10 mL). The combined organic layers were dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (3 cm, cyclohexane : EtOAc = 4 : 1, 20 mL, R_f = 0.13). Colorless oil, yield 91.4 mg (22 %). $C_{24}H_{27}NO_7$ (409.5). MS (EI): m/z = 409 [M], 318 [M-$PhCH_2$], 274. [M-$PhCH_2CO_2$]. IR: ν [cm^{-1}] = 2925 (C-H), 1736 (C=O ester), 1694 (C=O carbamate), 1207, 1043 (C-O). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.64 - 1.78 (m, 2H N(CH_2*CH_2*)$_2$), 2.02 (td, J = 13.2/4.9 Hz, 1H, N(CH_2*CH_2*)$_2$), 2.09 (dd, J = 14.3/2.3 Hz, 1H, N(CH_2*CH_2*)$_2$), 2.61 (dd, J =

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15.3/4.4 Hz, 1H, $CH_2CO_2CH_3$), 2.69 (dd, J = 15.3/8.7 Hz, 1H, $CH_2CO_2CH_3$), 2.71 (dd, J = 15.8/3.3 Hz, 1H, ArCH_2CH), 2.78 (dd, J = 15.8/10.5 Hz, 1H, ArCH_2CH), 3.14 (td, J = 13.1/2.8 Hz, 1H, N($CH_2CH_2)_2$), 3.24 (td, J = 13.1/2.8 Hz, 1H, N($CH_2CH_2)_2$), 3.73 (s, 3H, CO_2CH_3), 4.04-4.15 (m, 2H, N($CH_2CH_2)_2$), 4.21 - 4.3 (m, 1H, ArCH_2CH), 5.17 (s, 2H, PhCH_2O), 7.04 - 7.09 (m, 2H, arom.), 7.14 - 7.22 (m, 2H, arom.), 7.29 - 7.41 (m, 5H, arom.).

5.2.7. Methyl (±)-2-(3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3yl)acetate (14)

13 (91.4 mg, 0.223 mmol) was dissolved in methanol (17 mL). Pd/C (8.7 mg) was added and the mixture was stirred under H_2 atmosphere for 20 h. The catalyst was removed by filtration and the solution was concentrated in vacuo. Colorless oil, yield 56.2 mg (89 %), R_f = 0.04 (cyclohexane : EtOAc = 4 : 1). $C_{16}H_{21}NO_3$ (275.35). MS (EI): m/z = 275 [M], 219 [M-$CHNHCH_2CH_2$]. IR: ν [cm^{-1}] = 2925 (C-H), 1735 (C=O), 1155, 1044 (C-O). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.68 (dd, J = 15.7/2.2 Hz, 1H, N($CH_2CH_2)_2$), 1.77 (td, J = 13.4/4.7 Hz, 1H, N($CH_2CH_2)_2$), 2.05 - 2.13 (m, 2H, N($CH_2CH_2)_2$), 2.4 (s, 1H, N*H*), 2.61 (dd, J = 15.1/4.5 Hz, 1H, $CH_2CO_2CH_3$), 2.65 - 2.74 (m, 2H, N($CH_2CH_2)_2$), 2.76 (dd, J = 15.7/10.4 Hz, 1H, $CH_2CO_2CH_3$), 2.88 - 3.04 (m, 3H, ArCH_2 (2H), N($CH_2CH_2)_2$ (1H)), 3.11 (td, J = 12.2/2.1 Hz, 1H, N($CH_2CH_2)_2$), 3.74 (s, 3H, CO_2CH_3), 4.21 - 4.28 (m, 1H, ArCH_2CH), 7.06 (d, J = 7.5 Hz, 1H, arom.), 7.11 - 7.23 (m, 3H, arom.).

5.2.8. (±)-2-(1'-Acetyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)ethanol (15)

Under N_2, **12** (602 mg, 1.9 mmol) was dissolved in THF (15 mL). The mixture was cooled to 0 °C and a solution of $LiBH_4$ (3.8 mL, 7.6 mmol, 2 M in THF) was added.

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The mixture was stirred at rt for 20 h, then is was concentrated in vacuo. The residue was dissolved in water (10 mL), 0.5 M HCl was added and the mixture was extracted with CH_2Cl_2 (4×12 mL). The combined organic layers were dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (2 cm, CH_2Cl_2 : methanol = 95 : 5, 10 mL, R_f = 0.24). Colorless oil, yield 415 mg (76 %). $C_{17}H_{23}NO_3$ (289.4). MS (EI): m/z = 289 [M], 246 [M-$COCH_3$]. IR: ν [cm^{-1}] = 3385 (O-H), 2923 (C-H), 1617 (C=O amide). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.63 – 2.03 (m, 6H, CH_2CH_2OH (2H), N($CH_2CH_2)_2$ (4H)), 2.08 (s, 3H, NCOCH_3), 2.60 (d, J = 15.9 Hz, 1H, ArCH_2), 2.77 (dd, J = 15.9/11.1 Hz, 1H, ArCH_2), 2.81 – 2.92 (m, 1H, N($CH_2CH_2)_2$), 3.37 – 3.49 (m, 1H, N($CH_2CH_2)_2$), 3.66 – 3.71 (m, 1H, N($CH_2CH_2)_2$), 3.79 – 3.91 (m, 2H, CH_2OH), 3.92 – 4.04 (m, 1H ArCH$_2$*CH*), 4.49 – 4.57 (m, 1H, N($CH_2CH_2)_2$), 6.97 – 7.06 (m, 2H, arom.), 7.07 – 7.15 (m, 2H, arom.). A signal for the OH proton is not seen in the spectrum.

5.2.9. (±)-2-(3,4-Dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)ethanol (16)

A solution of **15** (346 mg, 1.2 mmol) in 2 M NaOH (20 mL) was heated to reflux for 3 h. The mixture was cooled to rt and extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried (K_2CO_3) and concentrated in vacuo. The product was directly used without further purification. Colorless oil, yield 157.3 mg (53 %), R_f = 0.03 (CH_2Cl_2 : methanol = 95 : 5). $C_{15}H_{21}NO_2$ (247.3). MS (EI): m/z = 247 [M], 228 [M-H_3O^+], 199 [M-H_3O^+-$NHCH_2$], 184 [M-H_3O^+-$NH_2CH_2CH_2$]. IR: ν [cm^{-1}] = 3345 (O-H) (N-H), 2937 (C-H), 1488, 1422 (C-H), 1043 (C-O). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.62 – 2.11 (m, 6H, NH($CH_2CH_2)_2$ (4H), CH_2CH_2OH (2H)), 2.55 (dd, J = 15.8/2.6 Hz, 1H, PhCH_2), 2.75 (dd, J = 15.8/11.2 Hz, 1H, PhCH_2), 2.85 – 3.15 (m, 4H, NH($CH_2CH_2)_2$), 3.81 – 3.88 (m, 2H, CH_2CH_2OH), 3.92 – 4.01 (m, 1H, PhCH$_2$*CH*), 7.0 (d, J = 7.4 Hz, 1H, arom.) 7.05 – 7.17 (m, 3H, arom.). Signals for the OH and NH protons are not seen in the spectrum.

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5.2.10. Methyl (±)-2-[1'-(3-phenylpropyl)-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl]acetate (17e)

1-Bromo-3-phenylpropane (44.8 mg, 0.23 mmol) and K_2CO_3 (37 mg, 0.27 mmol) were added to a solution of **14** (50.8 mg, 0.18 mmol) in CH_3CN (8 mL). The mixture was heated to reflux for 4 h. The residue was filtered and concentrated in vacuo. The residue was purified by fc (1 cm, cyclohexane : EtOAc = 1 : 1 + 0.1 % NH_3, 5 mL, R_f = 0.36). Colorless oil, yield 49.2 mg (69 %). $C_{25}H_{31}NO_3$ (393.53). MS (EI): m/z = 393 [M], 288 [M-$PhCH_2CH_2$]. IR: ν [cm^{-1}] = 2944, 2812 (C-H), 1736 (C=O), 1155, 1046 (C-O). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.69 (dd, J = 13.5/2.6 Hz, 1H, N(CH_2*CH_2*)$_2$), 1.82 - 1.91 (m, 3H, N(CH_2*CH_2*)$_2$, (1H), *CH_2*CH_2Ph (2H)), 2.09 (dd, J = 14.3/2.5 Hz, 1H, N(CH_2*CH_2*)$_2$) 2.15-2.28 (m, 2H, N(CH_2*CH_2*)$_2$ (1H), N(CH_2CH_2*CH_2*Ph (1H)), 2.34 - 2.44 (m, 3H, N(*CH_2*CH_2)$_2$ (2H), NCH_2CH_2*CH_2*Ph (1H)), 2.59 (dd, J = 14.8/4.6 Hz, 1H, CH*CH_2*CO_2CH_3), 2.63 - 2.68 (m, 3H, CH*CH_2*CO_2CH_3 (1H), N*$CH_2CH_2CH_2$*Ph (2H)), 2.69 - 2.79 (m, 4H, Ar*CH_2*CH (2H), N(*CH_2CH_2*)$_2$ (2H)), 3.71 (s, 3H, CO_2*CH_3*), 4.20 – 4.25 (m, 1H, ArCH_2*CH*), 7.05 (d, J = 7.3 Hz, 1H, arom.), 7.11 – 7.23 (m, 6H, arom.), 7.25 - 7.30 (m, 2H, arom.). Purity determined by HPLC: 96 %.

5.2.11. (±)-2-(1'-Ethyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)ethanol (18a)

1-Bromoethane (44.6 mg, 0.40 mmol) and K_2CO_3 (69 mg, 0.5 mmol) were added to a solution of **16** (81.1 mg, 0.33 mmol) in CH_3CN (15 mL). The mixture was heated to reflux for 4 h, then it was filtered and concentrated in vacuo. The residue was purified by fc (1 cm, CH_2Cl_2 : methanol = 95 : 5 + 0.1 % NH_3, 5 mL, R_f = 0.19). Colorless oil, yield 43.5 mg, (48 %). $C_{17}H_{25}NO_2$ (275.4). MS (ESI): m/z = 276 [MH^+]. IR: ν [cm^{-1}] = 3395 (O-H), 2926 (C-H), 1061, 1045 (C-O). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.07 (t, J =

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7.2 Hz, 3H, CH_2CH_3), 1.68 (dd, J = 13.2/2.3 Hz, 1H, $N(CH_2CH_2)_2$), 1.79 – 1.93 (m, 3H, $N(CH_2CH_2)_2$ (1H), CH_2CH_2OH (2H)), 2.05 (dd, J = 14.4/2.7 Hz, 1H, $N(CH_2CH_2)_2$), 2.13 – 2.29 (m, 3H, $N(CH_2CH_2)_2$ (1H), $N(CH_2CH_2)_2$ (2H)), 2.43 (q, J = 7.2 Hz, 2H, NCH_2CH_3), 2.56 (dd, J = 15.9/2.6 Hz, 1H, $ArCH_2CH$), 2.73 – 2.84 (m, 3H, $N(CH_2CH_2)_2$ (2H), $ArCH_2CH$ (1H)), 3.84 (t, J = 5.5 Hz, 2H, CH_2CH_2OH), 3.92 – 3.99 (m, 1H, $ArCH_2CH$), 6.99 (d, J = 7.3 Hz, 1H, arom.) 7.05 – 7.16 (m, 3H, arom.). A signal for the OH proton is not seen in the spectrum. Purity determined by HPLC: 98 %.

5.2.12. (±)-2–(1'-Butyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)ethanol (18b)

1-Bromobutane (27.4 mg, 0.20 mmol) and K_2CO_3 (33 mg, 0.24 mmol) were added to a solution of **16** (39.5 mg, 0.16 mmol) in CH_3CN (10 mL). The mixture was heated to reflux for 4 h, then it was filtered and concentrated in vacuo. The residue was purified by fc (1 cm, cyclohexane : EtOAc = 1 : 1 + 0.1 % NH_3, 5 mL, R_f = 0.22). Colorless oil, yield 20.3 mg (42 %). $C_{19}H_{29}NO_2$ (303.5). MS (EI): m/z = 303 [M], 260 [M-$(CH_2)_2CH_3$] IR: ν [cm^{-1}] = 3392 (O-H), 2929, 2820 (C-H), 1098, 1044 (C-O). ^{1}H NMR ($CDCl_3$): δ [ppm] = 0.87 (t, J = 7.3 Hz, 3H, $(CH2)_3CH_3$), 1.27 (sext., J = 7.3 Hz, 2H $(CH_2)_2CH_2CH_3$), 1.42 – 1.50 (m, 2H, $CH_2CH_2CH_3$), 1.53 – 1.60 (s, 1H, OH), 1.67 (dd, J = 13.2/2.4 Hz, 1H, $N(CH_2CH_2)_2$), 1.80 – 1.93 (m, 3H, $N(CH_2CH_2)_2$ (1H), CH_2CH_2OH (2H)), 2.04 (dd, J = 14.4/2.6 Hz, 1H, $N(CH_2CH_2)_2$), 2.13 – 2.29 (m, 3H, $N(CH_2CH_2)_2$ (1H), $N(CH_2CH_2)_2$ (2H)), 2.31 – 2.36 (m, 2H, $NCH_2(CH_2)_2CH_3$), 2.56 (dd, J = 16.0/2.6 Hz, 1H, $ArCH_2CH$), 2.74 – 2.81 (m, 3H, $N(CH_2CH_2)_2$ (2H), $ArCH_2CH$ (1H)), 3.84 (t, J = 5.5 Hz, 2H, CH_2CH_2OH), 3.92 – 3.99 (m, 1H, $ArCH_2CH$), 6.99 (d, J = 7.4 Hz, 1H, arom.), 7.05 – 7.16 (m, 3H, arom.). Purity determined by HPLC: 97 %.

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5.2.13. (±)-2-(1'-Octyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)ethanol (18c)

1-Bromooctane (116 mg, 0.6 mmol) and K_2CO_3 (104 mg, 0.75 mmol) were added to a solution of **16** (122 mg, 0.49 mmol) in CH^3CN (15 mL). The mixture was heated to reflux for 4 h, then it was filtered and concentrated in vacuo. The residue was purified by fc (2.5 cm, cyclohexane : EtOAc = 1 : 1 + 0.1 % NH_3, 10 mL, R_f = 0.30). Colorless oil, yield 92.2 mg, (53 %). $C_{23}H_{37}NO_2$ (359.6). MS (EI): m/z = 359 [M], 260 [M-$(CH_2)_6CH_3$]. IR: ν [cm^{-1}] = 3214 (O-H), 2924, 2856 (C-H), 1098, 1043 (C-O). ^{1}H NMR ($CDCl_3$): δ [ppm] = 0.88 (t, J = 6.8 Hz, 3H, $(CH_2)_7$*CH_3*), 1.22 – 1.32 (m, 10H, *$(CH_2)_5$*CH_3), 1.48 – 1.55 (m, 2H, CH_2*CH_2*$(CH_2)_5CH_3$), 1.63 – 1.72 (s, 1H, OH) 1.73 (dd, J = 13.7/2.7, 1H, N(CH_2*CH_2*$)_2$), 1.83 – 2.0 (m, 3H, N(CH_2*CH_2*$)_2$ (1H), *CH_2*CH_2OH (2H)), 2.08 (dd,J = 14.4/2.6, 1H, N(CH_2*CH_2*$)_2$), 2.18 – 2.33 (m, 3H, N(CH_2*CH_2*$)_2$ (1H), N(*CH_2*$CH_2)_2$ (2H)), 2.36 – 2.41 (m, 2H, N*CH_2*$(CH_2)_6CH_3$), 2.62 (dd, 1H, J = 13.3/2.6, Ph*CH_2*CH), 2.78 – 2.87 (m, 3H, N(*CH_2*$CH_2)_2$ (2H), Ph*CH_2*CH (1H)), 3.90 (t, J = 5.5 Hz, 2H, CH_2*CH_2*OH), 3.98 – 4.05 (m, 1H, $PhCH_2$*CH*), 7.05 (d, J = 7.2, 1H, arom.), 7.12 – 7.22 (m, 3H, arom.). Purity determined by HPLC: 99 %.

5.2.14. (±)-2-(1'-Benzyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)ethanol (18d)

Benzyl bromide (68.4 mg, 0.40 mmol) and K_2CO_3 (62 mg, 0.45 mmol) were added to a solution of **16** (68.3 mg, 0.28 mmol) in CH_3CN (10 mL). The mixture was heated to reflux for 20 h, then it was filtered and the crude mixture was concentrated in vacuo. The residue was purified by fc (2.5 cm, cyclohexane : EtOAc = 1 : 1 + 0.1 % NH_3, 10 mL, R_f = 0.14). Colorless oil, yield 15.7 mg, (17 %). $C_{22}H_{27}NO_2$ (337.5). MS (EI): m/z = 337 [M], 246 [M – CH_2Ph], 91 [CH_2Ph]. IR**:** ν [cm^{-1}] = 3394 (O-H), 2939, 2821 (C-H), 1094, 1049 (C-O), 741, 700 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.71 (dd, J =

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13.4/2.7 Hz, 1H, N(CH_2CH_2)$_2$), 1.84 – 2.01 (m, 3H, N(CH_2CH_2)$_2$ (1H), CH_2CH_2OH (2H)), 2.08 (dd, J = 14.2/4.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.22 (td, J = 13.1/4.4 Hz, 1H, N(CH_2CH_2)$_2$), 2.30 – 2.44 (m, 2H, N(CH_2CH_2)$_2$), 2.61 (dd, J = 15.9/2.7 Hz, 1H, ArCH_2CH), 2.72 – 2.83 (m, 2H, N(CH_2CH_2)$_2$), 2.83 (dd, J = 15.9/11.3 Hz, 1H, ArCH_2CH), 3.57 (s, 2H, NCH_2Ph), 3.89 (t, J = 5.5 Hz, 2H, CH_2CH_2OH), 3.96 – 4.05 (m, 1H, ArCH_2CH), 7.04 (d, J = 7.0 Hz, 1H, arom.), 7.10-7.40 (m, 8H, arom.). A signal for the OH proton is not seen in the spectrum. Purity determined by HPLC: 98 %.

5.2.15. (±)-2-[1'-(3-Phenylpropyl)-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl]ethanol (18e)

1-Bromo-3-phenylpropane (156 mg, 0.78 mmol) and K_2CO_3 (133 mg, 0.96 mmol) were added to a solution of **16** (158 mg, 0.64 mmol) in CH_3CN (15 mL). The mixture was heated to reflux for 4 h, then it was filtered and concentrated in vacuo. 0.5 M HCl (10 mL) was added and the solution was washed with EtOAc (2×5 mL). The aqueous layer was alkalised, extracted with CH_2Cl_2 (3×10 mL) and the combined CH_2Cl_2 layers were concentrated in vacuo. The residue was purified by fc (2.5 cm, cyclohexane : EtOAc = 1 : 1 + 0.1 % NH_3, 10 mL, R_f = 0.25). Colorless oil, yield 164.8 mg (70 %). $C_{24}H_{31}NO_2$ (365.5). MS (EI): m/z = 365 [M], 260 [M – $(CH_2)_2C_6H_5$]. IR: ν [cm^{-1}] = 3024 (O-H), 2938, 2822 (C-H), 1062, 1045 (C-O), 753, 689 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.67 (dd, J = 13.3/2.6 Hz, 1H, N(CH_2CH_2)$_2$), 1.76 – 1.91 (m, 5H, N(CH_2CH_2)$_2$ (1H), CH_2CH_2OH (2H), CH_2CH_2Ph (2H)), 2.03 (dd, J = 14.3/2.6 Hz, 1H, N(CH_2CH_2)$_2$), 2.15 (td, J = 12.9/4.2 Hz, 1H, N(CH_2CH_2)$_2$), 2.20 – 2.29 (m, 2H, $NCH_2(CH_2)_2Ph$), 2.35 – 2.40 (m, 2H, N(CH_2CH_2)$_2$), 2.52 – 2.61 (m, 3H, ArCH_2CH (1H), N$(CH_2)_2CH_2$Ph (2H)), 2.63 (s, 1H, *OH*), 2.71 – 2.81 (m, 3H, N(CH_2CH_2)$_2$ (2H), ArCH_2CH (1H)), 3.79 – 3.88 (m, 2H, CH_2CH_2OH), 3.91 – 4.00 (m, 1H, ArCH_2CH),

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6.99 (d, J = 7.3 Hz, 1H, arom.), 7.04 – 7.15 (m, 5H, arom.), 7.18 – 7.38 (m, 3H, arom.). Purity determined by HPLC: 99 %.

5.2.16. Methyl *cis*-(±)-2-[1-(bromomethyl)-3,4-dihydro-1*H*-2-benzopyran-3-yl]acetate (20a)

Under N_2, **8** (194 mg, 1.0 mmol) was dissolved in CH_2Cl_2 abs. (14 mL). Bromoacetaldehyde dimethyl acetal (**19a**, 507 mg, 3.0 mmol, 50 % in toluene) was added and the mixture was stirred at rt for 30 min. Then solution was cooled to 0 °C, BF_3•Et_2O (1.3 mL, 8 mmol) was added and the mixture was stirred at rt for 2 d. 0.5 M HCl (15 mL) was added and the mixture was extracted with CH_2Cl_2 (3×15 mL). The combined organic layers were dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (4 cm, cyclohexane : EtOAc = 9 : 1, 20 mL, R_f = 0.44). Colorless oil, yield 258 mg (86 %). $C_{13}H_{15}BrO_3$ (299.2). MS (EI): m/z = 300 [^{81}Br-M],298 [^{79} Br-M]. IR: ν [cm^{-1}] = 2950 (C-H), 1734 (C=O), 1156, 1092 (C-O), 745 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.62 (dd, J = 15.5/5.6 Hz, 1H, *CH*$_2$CO$_2$CH$_3$), 2.72 – 2.88 (m, 3H, *CH*$_2$CO$_2$CH$_3$ (1H), Ar*CH*$_2$ (2H)), 3.64 (dd, J = 10.9/5.9 Hz, 1H, *CH*$_2$Br), 3.74 (s, 3H, CO$_2$*CH*$_3$), 3.87 (dd, J = 10.9/2.7 Hz, 1H, *CH*$_2$Br), 4.14 – 4.22 (m, 1H, ArCH$_2$*CH*), 5.09 (dd, J = 5.1/1.9 Hz, 1H, Ar*CH*O), 7.07 – 7.14 (m, 2H, arom.), 7.20 – 7.25 (m, 2H, arom.). The relative configuration was determined by NOE spectra.

5.2.17. Methyl *cis*-(±)-2-[1-(2-chloroethyl)-3,4-dihydro-1*H*-2-benzopyran-3-yl]acetate (20b)

Under N_2, **8** (388 mg, 2.0 mmol) was dissolved in CH_2Cl_2 abs. (14 mL). 3-Chloropropionaldehyde diethyl acetal (**19b,** 833 mg, 5.0 mmol in 1.5 mL toluene) was added and the mixture was stirred for 30 min at rt. The solution was cooled to 0 °C, BF_3•Et_2O (2.5 mL, ca. 16 mmol) was added and the mixture was stirred at rt for 4

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d. 0.5 M HCl (15 mL) was added and the mixture was extracted with CH_2Cl_2 (3×15 mL). The combined organic layers were dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (3 cm, cyclohexane : EtOAc 9 : 1, 20 mL, R_f = 0.33). Pale yellow oil, yield 1739 mg (32 %). $C_{14}H_{17}ClO_3$ (268.47). MS (EI): m/z = 205 [M-CH_2CH_2Cl], 129 [$C_{10}H_9$], 117 [C_9H_9]. IR: ν [cm^{-1}] = 2951 (C-H), 1738 (C=O), 1157, 1094 (C-O), 743 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.09-2.18 (m, 1H, *CH*$_2$CHCl), 2.40-2.44 (m, 1H, *CH*$_2$CH$_2$Cl), 2.59 (dd, J = 15.2/5.4 Hz, 1H, *CH*$_2$CO$_2$CH$_3$), 2.62-2.78 (m, 2H, Ph*CH*$_2$), 2.69 (dd, J = 15.2/7.8 Hz, 1H, *CH*$_2$CO$_2$CH$_3$), 3.57-3.64 (m, 1H, *CH*$_2$Cl), 3.73 (s, 3H, CO$_2$*CH*$_3$), 3.75-3.79 (m, 1H, *CH*$_2$Cl), 4.10-4.18 (m, 1H, PhCH$_2$*CH*), 4.95 (d, J = 7.9 Hz, 1H, Ph*CH*O) 7.07-7.22 (m, 4H, arom.). The relative configuration was determined by NOE spectra.

5.2.18. Ethyl *cis*-(±)-3-[1-(methoxycarbonyl)methyl]-3,4-dihydro-1*H*-2-benzopyran-1-carboxylate (20c)

Under N_2, **8** (486 mg, 2.5 mmol) was dissolved in CH_2Cl_2 (10 mL). Ethyl 2,2-diethoxyacetate (**19c**, 1.32 g, 7.5 mmol, 50 % in toluene) was added and the mixture was stirred for 30 min at rt. Then solution was cooled to 0 °C, BF_3•Et_2O (3.2 mL, 20 mmol) was added and the mixture was stirred at rt for 4 d. 0.5 M HCl (17.5 mL) was added and the mixture was extracted with CH_2Cl_2 (3×12.5 mL). The combined organic layers were dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (4 cm, petroleum ether : EtOAc = 4 :1, 20 mL, R_f = 0.48). Colorless oil, yield 618 mg (99 %). $C_{15}H_{18}O_5$ (278.3). MS (ESI): m/z = 278 [M]. IR: ν [cm^{-1}] = 2981, 2953 (C-H), 1734 (C=O), 1159, 1105 (C-O), 749 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.29 (t, J = 7.1 Hz, 3H, CO$_2$CH$_2$*CH*$_3$), 2.66 (dd, J = 15.9/5.7 Hz, 1H, *CH*$_2$CO$_2$CH$_3$), 2.77 (dd, J = 15.7/2.7 Hz, 1H, Ar*CH*$_2$), 2.88 (dd, J = 15.9/7.3 Hz, 1H, *CH*$_2$CO$_2$CH$_3$), 2.94 (dd, J = 15.7/11.2 Hz, 1H, Ar*CH*$_2$), 3.72 (s, 3H, CO$_2$*CH*$_3$), 4.18 – 4.29 (m, 3H,

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$CO_2CH_2CH_3$ (2H), $ArCH_2CH$ (1H)), 5.44 (s, 1H, ArCHO), 7.09 – 7.34 (m, 4H, arom.).

5.2.19. Dimethyl *cis*-(±)-3,4-dihydro-1*H*-2-benzopyran-1,3-diacetate (20d)

Under N_2, **8** (486 mg, 2.5 mmol) was dissolved in CH_2Cl_2 (10 mL). Methyl 3,3-dimethoxypropionat (**19d**, 1.11 g, 7.5 mmol, 50 % in toluene) was added and the mixture was stirred for 30 min at rt. The solution was cooled to 0 °C, BF_3•Et_2O (3.2 mL, 20 mmol) was added and the mixture was stirred at rt for 4 d. 0.5 M HCl (17.5 mL) was added and the mixture was extracted with CH_2Cl_2 (3×12.5 mL). The combined organic layers were dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (4 cm, petroleum ether : EtOAc = 4:1, 20 mL, R_f = 0.37). colorless solid, yield 582.8 mg (84 %), mp. 62.9 °C. $C_{15}H_{18}O_5$ (278.3). MS (EI): m/z = 278 [M], 205 [M-CH_2COOCH_3], 145 [M-(CH_2COOCH_3+$COOCH_3^+$)]. IR: ν [cm^{-1}] = 2947, 2879 (C-H), 1743, 1723 (C=O), 1159, 1103 (C-O), 750 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.57 (dd, J = 15.3/5.9 Hz, 1H, $ArCH_2CHCH_2CO_2CH_3$), 2.68 (dd, J = 15.1/9.2 Hz, 1H, $ArCH_2$), 2.70 (dd, J = 15.3/7.4 Hz, 1H, $ArCH_2CHCH_2CO_2CH_3$), 2.76 – 2.81 (m, 2H, $ArCHCH_2CO_2CH_3$), 2.94 (dd, J = 15.1/3.7 Hz, 1H, $ArCH_2$), 3.70 (s, 3H, CO_2CH_3), 3.71 (s, 3H, CO_2CH_3), 4.10 – 4.18 (m, 1H, $ArCH_2CH$), 5.23 (dd, J = 9.1/3.7 Hz, 1H, ArCHO), 7.01 – 7.21 (m, 4H, arom.). The relative configuration was determined by NOE spectra.

5.2.20. Methyl *cis*-(±)-2-[1-(azidomethyl)-3,4-dihydro-1*H*-2-benzopyran-3-yl]acetate (20e)

20a (330 mg, 1.1 mmol) and NaN_3 (286 mg, 4.4 mmol) were dissolved in DMF (20 mL). The mixture was heated to 60-70 °C for 24 h. After cooling to rt, water (180 mL) was added and the mixture was extracted with Et_2O (5 x 60 mL). The combined organic layers were extracted with water (3 x 150 mL), dried (Na_2SO_4) and

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concentrated in vacuo. The residue was purified by fc (4 cm, cyclohexane : EtOAc = 9 :1, 20 mL, R_f = 0.19). Colorless oil, yield 83.2 mg (29 %). $C_{13}H_{15}N_3O_3$ (261.3). MS (EI): m/z = 261 [M], 233 [M-N_2], 205 [M- CH_2N_3]. IR: ν [cm^{-1}] = 2951 (C-H); 2098 (-N_3), 1735 (C=O), 1157, 1099 (C-O), 746 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.62 (dd, J = 15.6/5.4 Hz, 1H, *CH*$_2$$CO_2CH_3$), 2.72 – 2.91 (m, 3H, *CH*$_2$$CO_2CH_3$ (1H), Ar*CH*$_2$ (2H)), 3.48 (dd, J = 13.1/ 6.4 Hz, 1H, *CH*$_2$$N_3$), 3.64 (dd, J = 13.1/2.7 Hz, 1H, *CH*$_2$$N_3$), 3.73 (s, 3H, CO_2*CH*$_3$), 4.15 – 4.23 (m, 1H, $ArCH_2$*CH*), 5.08 (d, J = 5.0 Hz, 1H, Ar*CH*O), 7.0 – 7.25 (m, 4H, arom.).

5.2.21. Methyl *cis*-(±)-2-{1-[(-piperidin-1-yl)methyl]-3,4-dihydro-1*H*-2-benzopyran-3-yl}acetate (21b)

20a (71.3 mg, 0.26 mmol), piperidine (0.03 mL, 0.3 mmol), K_2CO_3 (55 mg, 0.4 mmol) and a small amount of tetrabutylammonium iodide were dissolved in CH_3CN (10 mL). The mixture was heated to reflux for 20 h. The residue was filtered, concentrated in vacuo, the residue was dissolved in 0.5 M HCl (10 mL) and the mixture was extracted with EtOAc (2x5 mL). The aqueous layers were alkalized with 0.5 M NaOH and extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried (K_2CO_3) and concentrated in vacuo. The residue was purified by fc (1 cm, CH_2Cl_2 : CH_3OH = 98 : 2 + 0.1 % NH_3, 5 mL, R_f = 0.13 Pale yellow oil, yield 12.5 mg (16 %). $C_{18}H_{25}NO_3$ (303.4) MS (EI): m/z = 303 [M], 98 [$CH_2N(CH_2CH_2)_2CH_2^+$]. IR: ν [cm^{-1}] = 2931, 2852 (C-H), 1735 (C=O), 1154, 1089 (C-O), 743 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.40 – 1.51 (m, 2H, $N(CH_2CH_2)_2$*CH*$_2$), 1.59 – 1.71 (m, 4H, $N(CH_2$*CH*$_2)_2CH_2$), 2.50 – 2.87 (m, 9H, $N($*CH*$_2CH_2)_2CH_2$ (4H), *CH*$_2$$CO_2CH_3$ (2H), Ar*CH*$_2$ (2H), N*CH*$_2$ (1H)), 2.95 (d, J = 12.2 Hz, 1H, N*CH*$_2$), 3.71 (s, 3H, CO_2*CH*$_3$), 4.08 – 4.16 (m, 1H, $ArCH_2$*CH*), 5.02 (d, J = 5.5 Hz, 1H, Ar*CH*O), 7.02 – 7.28 (m, 4H, arom.). The relative configuration was determined by a NOESY spectrum. Purity determined by HPLC: 96 %.

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5.2.22. Methyl *cis*-(±)-2-{1-[(4-phenylpiperidin-1-yl)methyl]-3,4-dihydro-1*H*-2-benzopyran-3-yl}acetate (21c)

20a (274 mg, 1.02 mmol), 4-phenylpiperidine (242 mg, 1.5 mmol), K_2CO_3 (207 mg, 1.5 mmol) and a small amount of tetrabutylammonium iodide were dissolved in CH_3CN (20 mL). The mixture was heated to reflux for 20 h. Then it was filtered, concentrated in vacuo, the residue was dissolved in 0.5 M HCl (10 mL) and extracted with EtOAc (2x5 mL). The aqueous layers were alkalized with 0.5 M NaOH and extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (2.5 cm, cyclohexane : EtOAc = 1 : 1 + 0.1 % NH_3, 10 mL, R_f = 0.22). Pale yellow oil, yield 40.8 mg (11 %). $C_{24}H_{29}NO_3$ (379.5). MS (EI): m/z = 380 [M], 174 [$PhCH(CH_2CH_2)_2NCH_2$]. IR: ν [cm^{-1}] = 3025, 2929 (C-H), 1736 (C=O), 1155, 1092 (C-O), 744, 699 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.72 – 1.84 (m, 4H, $N(CH_2\mathit{CH_2})_2CHPh$), 2.15 – 2.29 (m, 2H, $N(\mathit{CH_2}CH_2)_2CHPh$), 2.39 – 2.49 (m, 1H, $N(CH_2CH_2)_2\mathit{CH}Ph$), 2.54 (dd, J = 15.2/5.3 Hz, 1H, $\mathit{CH_2}CO_2CH_3$), 2.64 – 2.81 (m, 4H, $\mathit{CH_2}CO_2CH_3$ (1H), Ar$\mathit{CH_2}$CH (2H), N$\mathit{CH_2}$ (1H)), 2.92 (dd, J = 13.4/3.5 Hz, 1H N$\mathit{CH_2}$), 3.06 (dd, J = 10.6/1.6 Hz, 1H, $N(\mathit{CH_2}CH_2)_2CHPh$), 3.20 (dd, J = 11.2/1.2 Hz, 1H, $N(\mathit{CH_2}CH_2)_2CHPh$), 3.64 (s, 3H, $CH_2CO_2CH_3$), 4.04 – 4.12 (m, 1H, $ArCH_2\mathit{CH}$), 4.96 (dd, J = 7.3/2.4 Hz, 1H, Ar*CH*O), 7.00 – 7.28 (m, 9H, arom.). The relative configuration was determined by a NOESY spectrum. Purity determined by HPLC: 98 %.

5.2.23. Methyl *cis*-(±)-2-{1-[2-(pyrrolidin-1-yl)ethyl]-3,4-dihydro-1*H*-2-benzopyran-3-yl}acetate (22a)

20b (102 mg, 0.38 mmol), pyrrolidine (0.05 mL, 0.43 mmol), K_2CO_3 (69 mg, 0.5 mmol) and a small amount of tetrabutylammonium iodide were dissolved in CH_3CN

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(10 mL). The mixture was heated to reflux for 20 h. It was filtered, concentrated in vacuo, the residue was dissolved in 0.5 M HCl (10 mL) and the mixture was extracted with EtOAc (2x5 mL). The aqueous layers were alkalized with 0.5 M NaOH and extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried (K_2CO_3) and concentrated in vacuo. The residue was purified by fc (1 cm, cyclohexane : EtOAc = 1 : 1 + 0.1% NH_3, 5 mL, R_f = 0.12). Pale yellow oil, yield 54.0 mg (47 %). $C_{18}H_{25}NO_3$ (303.4). MS (EI): m/z = 303 [M], 230 [M – $CH_2CO_2CH_3$], 84 [$CH_2N(CH_2CH_2)_2$]. IR: ν [cm^{-1}] = 2953 (C-H), 1738 (C=O), 1155, 1094 (C-O), 743 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.74 – 1.82 (m, 4H, $N(CH_2\mathit{CH_2})_2$), 1.91 – 2.01 (m, 1H, $NCH_2\mathit{CH_2}$), 2.20 – 2.30 (m, 1H, $NCH_2\mathit{CH_2}$), 2.46 – 2.62 (m, 6H, $N(\mathit{CH_2}CH_2)_2$ (4H), $N\mathit{CH_2}CH_2$ (2H)), 2.63 – 2.82 (m, 3H, $\mathit{CH_2}CO_2CH_3$ (1H), Ar$\mathit{CH_2}$ (2H)), 2.70 (dd, J = 15.7/7.6 Hz, 1H, $\mathit{CH_2}CO_2CH_3$), 3.72 (s, 3H, $CO_2\mathit{CH_3}$), 4.07 – 4.17 (m, 1H, $ArCH_2\mathit{CH}$), 4.86 (d, J = 7.9 Hz, 1H, Ar*CH*O), 7.04 – 7.20 (m, 4H, arom). Purity determined by HPLC: 98 %.

5.2.24. Methyl *cis*-(±)-2-{1-[2-(piperidin-1-yl)ethyl]-3,4-dihydro-1*H*-2-benzopyran-3-yl}acetate (22b)

20b (118 mg, 0.44 mmol), piperidine (0.05 mL, 0.5 mmol), K_2CO_3 (69 mg, 0.5 mmol) and a small amount of tetrabutylammonium iodide were dissolved in CH_3CN (15 mL). The mixture was heated to reflux for 20 h. It was filtered, concentrated in vacuo, the residue was dissolved in 0.5 M HCl (10 mL) and the mixture was extracted with EtOAc (2x5 mL). The aqueous layer was alkalized with 0.5 M NaOH and extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (2.5 cm, CH_2Cl_2 : CH_3OH = 98 : 2 + 0.1% NH_3, 10 mL, R_f = 0.20). Pale yellow oil, yield 53.1 mg (38 %). $C_{19}H_{27}NO_3$ (317.4). MS (EI): m/z = 317 [M], 244 [M – $CH_2CO_2CH_3$]. IR: ν [cm^{-1}] =

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2931, 2850 (C-H), 1738 (C=O), 1154, 1094 (C-O), 741 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.32 – 1.41 (m, 2H, N(CH_2CH_2)$_2$*CH_2*), 1.48 – 1.58 (m, 4H, N(CH_2*CH_2*)$_2$$CH_2$), 1.82 – 1.92 (m, 1H, N$CH_2$*$CH_2$*), 2.10 – 2.20 (m, 1H, NCH_2*CH_2*), 2.27 – 2.46 (m, 6H, N(*CH_2*CH_2)$_2$$CH_2$ (4H), N*CH_2*CH_2 (2H)), 2.50 (dd, J = 15.2/5.5 Hz, 1H, *CH_2*CO_2CH_3), 2.63 (dd, J = 15.2/7.7 Hz, 1H, *CH_2*CO_2CH_3), 2.64 – 2.75 (m, 2H, Ar*CH_2*), 3.65 (s, 3H, CO_2*CH_3*), 4.00 – 4.07 (m, 1H, ArCH_2*CH*), 4.77 (d, J = 6.8 Hz, 1H, Ar*CH*O), 6.98 – 7.14 (m, 4H, arom.). Purity determined by HPLC: 98 %.

5.2.25. Methyl *cis*-(±)-2-{1-[2-(4-phenylpiperidin-1-yl)ethyl]-3,4-dihydro-1*H*-2-benzopyran-3-yl}acetate (22c)

20b (59.5 mg, 0.22 mmol), 4-phenylpiperidine (64.5 mg, 0.4 mmol), K_2CO_3 (55 mg, 0.4 mmol) and a small amount of tetrabutylammonium iodide were dissolved in CH_3CN (8 mL). The mixture was heated to reflux for 20 h. It was filtered, concentrated in vacuo, the residue was dissolved in 0.5 M HCl (10 mL) and the mixture was extracted with EtOAc (2x5 mL). The aqueous layers were alkalized with 0.5 M NaOH and extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (1 cm, cyclohexane : EtOAc = 1 : 1 + 0.1 % NH_3, 5 mL, R_f = 0.25). Pale yellow oil, yield 54.7 mg, (64 %). $C_{25}H_{31}NO_3$ (393.5). MS (EI): m/z = 393 [M], 320 [M -$CH_2CO_2CH_3$], 174 [Ph-CH(CH_2CH_2)$_2$$NCH_2$]. IR: ν [cm^{-1}] = 2928 (C-H), 1738 (C=O), 1155, 1095 (C-O), 743, 699 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.65 - 1.80 (m, 4H, N(CH_2*CH_2*)$_2$), 1.85 – 1.97 (m, 2H, NCH_2*CH_2*) 2.0 – 2.08 (m, 1H, N(*CH_2*CH_2)$_2$), 2.14 – 2.24 (m, 1H, N(*CH_2*CH_2)$_2$), 2.38 – 2.44 (m, 2H, N(CH_2CH_2)$_2$*CH*Ph (1H), N*CH_2*CH_2 (1H)), 2.45 – 2.58 (m, 1H, N*CH_2*CH_2), 2.52 (dd, J = 15.2/5.5 Hz, 1H, *CH_2*CO_2CH_3), 2.64 (dd, J = 15.2/7.7 Hz, 1H, *CH_2*CO_2CH_3), 2.64 – 2.75 (m, 2H, Ph*CH_2*), 3.0 (d, J = 11.3Hz, 2H, N(*CH_2*CH_2)$_2$), 3.66 (s, 3H, CO_2*CH_3*), 4.0 – 4.09 (m, 1H, PhCH_2*CH*), 4.8 (d, J = 6.5

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Hz, 1H, Ph*CH*O), 7.01 (d, J = 8.4 Hz, 1H, arom.), 7.02 – 7.22 (m, 8H, arom.). Purity determined by HPLC: 96 %.

5.2.26. *cis*-(±)-2-{1-[2-(Piperidin-1-yl)ethyl]-3,4-dihydro-1*H*-2-benzopyran-3-yl}ethanol (23b)

Under N_2, **22b** (68.0 mg, 0.20 mmol) was dissolved in THF (8 mL). The mixture was cooled to 0 °C and a solution of $LiBH_4$ (0.4 mL, 0.8 mmol, 2 M in THF) was added. The mixture was stirred at rt for 20 h. It was concentrated in vacuo and the residue was dissolved in water (6 mL). The mixture was acidified with 0.5 M HCl and extracted with EtOAc (2x5 mL). The aqueous layers were alkalized with 0.5 M NaOH and extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (1 cm, cyclohexane : EtOAc = 1 : 1 + 0.1% NH_3, 5 mL, R_f = 0.20). Colorless oil, yield 25.1 mg (43 %). $C_{18}H_{27}NO_2$ (289.4). MS (EI): m/z = 289 [M], 98 [$CH_2N(CH_2CH_2)_2CH_2^+$]. IR: ν [cm^{-1}] = 3420 (O-H), 2935 (C-H), 1106, 1038 (C-O), 743 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.40 – 1.50 (m, 2H, $N(CH_2CH_2)_2CH_2$), 1.51 – 1.65 (m, 4H, $N(CH_2CH_2)_2CH_2$), 1.68 – 1.99 (m, 4H, CH_2CH_2OH (2H), NCH_2CH_2 (2H)), 2.13 – 2.24 (m, 2H, $N(CH_2CH_2)_2$), 2.48 – 2.67 (m, 2H, $N(CH_2CH_2)_2$), 2.67 – 2.96 (m, 4H, ArCH_2 (2H), NCH_2CH_2 (2H)), 3.81 (t, J = 5.6 Hz, 2H, CH_2OH), 3.82 – 3.89 (m, 1H, $ArCH_2CH$), 4.85 (d, J = 4.5 Hz, 1H, Ar*CH*), 6.99 – 7.18 (m, 4H, arom.). Purity determined by HPLC: 97 %.

5.2.27. *cis*-(±)-2-{1-[2-(4-Phenylpiperidin-1-yl)ethyl]-3,4-dihydro-1*H*-2-benzopyran-3-yl}ethanol (23c)

Under N_2, **22c** (50 mg, 0.12 mmol) was dissolved in THF (8 mL). The mixture was cooled to 0 °C and a solution of $LiAlH_4$ (0.12 mL, 0.12 mmol, 1 M in THF) was added.

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The mixture was stirred at 0 °C for 2 h. Then it was concentrated in vacuo and the residue was dissolved in water (10 mL). The mixture was extracted with CH_2Cl_2 (4×8 mL). The combined organic layers were dried (K_2CO_3) and concentrated in vacuo. The residue was purified by fc (1 cm, CH_2Cl_2 : CH_3OH = 95 : 5, 5 mL, R_f = 0.10). Pale yellow oil, yield 28.9 mg (66 %). $C_{24}H_{31}NO_2$ (365.5). MS (EI): m/z = 365 [M], 174 [$PhCH(CH_2CH_2)_2NCH_2$]. IR: ν [cm^{-1}] = 3430 (O-H), 2929 (C-H), 1099, 1062 (C-O), 741, 698 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.80 – 1.94 (m, 6H, $N(CH_2\mathit{CH_2})_2$ (4H), $NCH_2\mathit{CH_2}$ (2H)), 1.97 – 2.12 (m, 2H, $\mathit{CH_2}CH_2OH$), 2.12 – 2.22 (m, 1H, $N(\mathit{CH_2}CH_2)_2$), 2.27 – 2.36 (m, 1H, $N(\mathit{CH_2}CH_2)_2$), 2.46 – 2.60 (m, 2H, $N(CH_2CH_2)_2\mathit{CH}Ph$ (1H), $N\mathit{CH_2}CH_2$ (1H)), 2.65 (dd, J = 16.0/2.5 Hz, 1H, $Ar\mathit{CH_2}$), 2.67 – 2.75 (m, 1H, $N\mathit{CH_2}CH_2$), 2.86 (dd, J = 16.0/11.2 Hz, 1H, $Ar\mathit{CH_2}$), 3.08 – 3.22 (m, 2H, $N(\mathit{CH_2}CH_2)_2$), 3.89 (t, J = 5.6 Hz, 2H, $\mathit{CH_2}OH$), 3.91 – 3.97 (m, 1H, $ArCH_2\mathit{CH}$), 4.95 (d, J = 7.9 Hz, 1H, $Ar\mathit{CH}O$), 7.07 – 7.32 (m, 9H, arom.). Purity determined by HPLC: 98 %.

5.2.28. (±)-N-(2,2-Dimethoxyethyl)-3-hydroxy-4-phenylbutanamide (24)

A mixture of the β-hydroxyester **8** (214.7 mg, 1.11 mmol), *p*-toluenesulfonic acid (40 mg, 0.2 mmol) and aminoacetaldehyde dimethyl acetal (4.8 mL) was heated to reflux. Then CH_2Cl_2 (40 mL) was added and the mixture was extracted with 1 M HCl (2 x 20 mL) and saturated NaCl solution (2 x 20 mL). The organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (3 cm, cyclohexane : EtOAc = 1 : 1, 10 mL, R_f = 0.27). Colorless oil, yield 204.5 mg (69 %). $C_{14}H_{21}NO_4$ (267.3). MS (EI): m/z = 267 [M], 236 [M-OCH_3], 144 [$C_6H_9NO_3$]. IR: ν [cm^{-1}] = 3319 (O-H), 2935, 2834 (C-H), 1643 (C=O), 1056 (C-O), 699 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.28 (dd, J = 15.3/8.8 Hz, 1H, $\mathit{CH_2}CON$), 2.38 (dd, J = 15.3/2.8 Hz, 1H, $\mathit{CH_2}CON$), 2.75 (dd, J = 13.5/6.3 Hz, 1H, $Ph\mathit{CH_2}$), 2.88 (dd, J = 13.5/7.0 Hz, 1H, $Ph\mathit{CH_2}$), 3.380 (s, 3H, $O\mathit{CH_3}$), 3.382 (s, 3H, $O\mathit{CH_3}$), 3.34 – 3.46 (m, 2H, $CON\mathit{CH_2}$), 4.20 – 4.26 (m,

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1H, $PhCH_2CH$), 4.37 (t, J = 5.1 Hz, 1H, $CH(OCH_3)_2$), 5.9 (s, broad, 1H, *NH*), 7.19 – 7.33 (m, 5H, arom.). A signal for the OH proton is not seen in the spectrum.

5.2.29. (±)-1,6-Epoxy-1,2,3,5,6,7-hexahydro-3-benzazonin-4-one (25)

The amide **24** (121.5 mg, 0.45 mmol) was dissolved in a mixture of toluene and CH_2Cl_2 (1 : 1, 6 mL) and the solution was stirred at rt for 30 min. Then it was cooled to 0 °C, $BF_3{\cdot}Et_2O$ (0.72 mL, 4.5 mmol) was added and the mixture was stirred at rt for 1 h and at 40-50 °C for 2 d. Afterwards, 0.5 M HCl (10 mL) was added, the aqueous layer was separated and extracted with CH_2Cl_2 (3 × 10 mL) after addition of CH_3OH (10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by recrystallization with CH_3OH. Colorless crystals, yield 9.1 mg (10 %), mp. 228 °C (CH_3OH), R_f = 0.62 (petroleum ether : EtOAc : CH_3OH = 10 : 10 : 2). $C_{12}H_{13}NO_2$ (203.2). MS (EI): m/z = 203 [M], 91 [$PhCH_2$]. IR: ν [cm^{-1}] = 3302 (N-H), 2957, 2868 (C-H), 1659 (C=O), 1087 (C-O), 741 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.68 – 2.92 (m, 3H, CH_2CON (2H), ArCH_2(1H)), 2.97 (dd, J = 16.5/10.9 Hz, 1H, ArCH_2), 3.53 – 3.61 (m, 1H, NCH_2), 4.13 – 4.21 (m, 1H, $ArCH_2CH$), 4.32 – 4.39 (m, 1H, NCH_2), 4.94 (d, J = 7.5 Hz, 1H, Ar*CH*O), 7.18 – 7.36 (m, 5H, arom. (4H), *NH* (1H)). ^{13}C NMR ($CDCl_3$): δ [ppm] = 34.2 (1C, ArCH_2), 43.1 (1C, CH_2CON), 43.6 (1C, NCH_2), 71.0 (1C, $ArCH_2CH$), 77.2 (1C, Ar*C*HO), 124.3, 126.9, 127.7, 129.2, 133.1, 133.6 (6C, arom), 170.7 (*C*=O).

5.3. Receptor binding studies

5.3.1. Materials

The guinea pig brains and rat liver for the σ_1 and σ_2 receptor binding assays were commercially available (Harlan-Winkelmann, Borchen, Germany). Homogenizer: Elvehjem Potter (B. Braun Biotech International, Melsungen, Germany). Cooling

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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 workshop of the institute). 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).

5.3.2. 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.

5.3.3. 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

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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

5.3.4. Protein determination

The protein concentration was determined by the method of Bradford,[48] modified by Stoscheck.[49] 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 λ = 595 nm was measured with a platereader (Tecan Genios, Tecan, Crailsheim, Germany).

5.3.5. 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 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}

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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.[50] The K_i-values are given as mean value ± SEM from three independent experiments.

5.3.6. Determination of the σ_1 receptor affinity (guinea pig brain)

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.[51]

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5.3.7. Determination of the σ_2 receptor affinity (rat liver)

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.[52]

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