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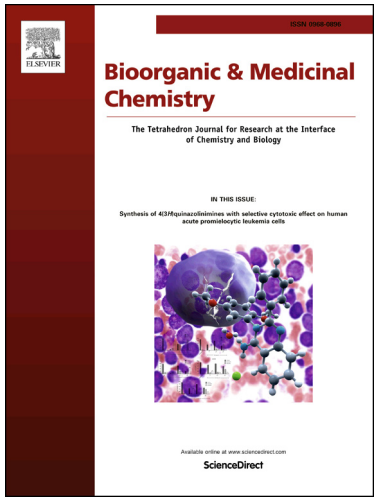

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Lipase-catalyzed kinetic resolution as key step in the synthesis of enantiomerically pure σ ligands with 2-benzopyran structure

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Dedicated to Prof. Dr. Dr. h.c. Gottfried Blaschke on the occasion of his 80th birthday

Abstract

In order to obtain enantiomerically pure σ_1 receptor ligands with a 2-benzopyran scaffold an Oxa-Pictet-Spengler reaction with the enantiomerically pure 2-phenylethanol derivatives (*R*)-**4** and (*S*)-**4** was envisaged. The kinetic resolution of racemic alcohol (±)-**4** using Amano Lipase PS-C II and isopropenyl acetate in *tert*-butyl methyl ether led to the (*R*)-configured alcohol (*R*)-**4** in 42 % yield with an enantiomeric excess of 99.6 %. The (*S*)-configured alcohol (*S*)-**4** was obtained by Amano Lipase PS-C II catalyzed hydrolysis of enantiomerically enriched acetate (*S*)-

5 (76.9 % *ee*) and provided (*S*)-**4** in 26 % yield and 99.7 % *ee*. The absolute configuration of alcohol (*R*)-**4** was determined by exciton coupled CD spectroscopy of the bis(bromobenzoate) (*R*)-**7**. The next important step for the synthesis of 2-benzopyrans **2** and **3** was the Oxa-Pictet-Spengler reaction of the enantiomerically pure alcohols (*R*)-**4** and (*S*)-**4** with piperidone ketal **8** and chloropropionaldehyde acetal **12**. The conformationally restricted spirocyclic 2-benzopyrans **2** revealed higher σ_1 affinity than the more flexible aminoethyl derivatives **3**. The (*R*)- and (*R*,*R*)-configured enantiomers (*R*)-**2** and (*R*,*R*)-**3** represent the eutomers of this class of compounds with eudismic ratios of 4.8 (**2b**) and 4.5 (**2c**). High σ_1/σ_2 selectivity (>49) was found for the most potent σ_1 ligands (*R*)-**2b**, (*R*)-**2c**, (*R*)-**2d**, and (*S*)-**2d** ($K_i(\sigma_1)$ 9-15 nM).

Key words: σ ligands; lipases; kinetic resolution; Oxa-Pictet-Spengler reaction; simulation; 2-benzopyrans; spirocyclic compounds; exciton coupled CD spectroscopy; sterol Δ^8/Δ^7-isomerase; late stage diversification.

1. Introduction

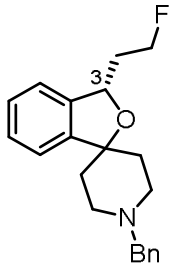
The class of σ receptors consists of two subtypes, which are termed σ_1 and σ_2 receptor.[1-4] Cloning of the σ_1 receptor provided a gene encoding for a 223 amino acid protein with a molecular weight of 25.3 kDa. It is a unique protein, which does not show any similarity to other mammalian proteins, but shares 30 % similarity with the yeast enzyme sterol Δ^8/Δ^7-isomerase. However, the σ_1 receptor does not have any sterol isomerase activity.[5-7]

The σ_1 receptor is associated with several neuropsychiatric disorders including depression, psychosis, neuropathic pain, cocaine and alcohol dependence as well as memory and cognition deficits. Moreover, it plays a role in neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and amyotrophic lateralsclerosis.[8-10] Therefore the modulation of the σ_1 receptor activity is of major interest for the treatment of these diseases. Moreover, several clinically used drugs such as haloperidol[11] (antipsychotic), fluvoxamine,[12] sertraline[12] (antidepressants), opipramol[13] (anxiolytic), pentazocine[14-16] (analgesic) and donepezil[17] (anti-Alzheimer drug) show high affinity towards the σ_1 receptor in addition to their main activity.

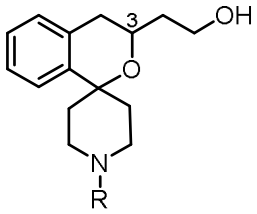
Very recently we have reported on the development of the fluorinated σ_1 receptor ligand fluspidine **1** (Figure 1).[18,19] The 18-F-labeled (*S*)-configured fluspidine enantiomer is currently investigated as positron emission tomography (PET) tracer for non-invasive imaging of σ_1 receptors in the central nervous system of humans (clinical study). The (*R*)-configured enantiomer (*R*)-**1** (K_i = 0.57 nM) has higher σ_1 affinity than the (*S*)-enantiomer (*S*)-**1** (K_i = 2.3 nM). However, in animal studies (*R*)-**1** showed an unexpected quasi-irreversible binding to σ_1 receptors in the brain without washout over the time. In contrast, the less affine (*S*)-enantiomer (*S*)-**1** displayed a normal uptake into the central nervous system and a normal washout over the time.[18-23]

Obviously, the enantiomers of σ_1 receptor ligands of type **1**, which were obtained by resolution of the racemic mixture, show different pharmacodynamic and pharmacokinetic behavior in animal experiments.[20-22] In addition to the 2-benzofuran fluspidine (**1**) the spirocyclic 2-benzopyrans **2** and the corresponding derivatives **3**

with a flexible 2-aminoethyl side chain in 1-postion represent promising σ_1 receptor ligands as well.[24] A common feature of the lead compounds **1**-**3** is the side chain with two C-atoms in 3-position adjacent to the ether O-atom of the ring system. 2-Benzopyrans **2** and **3** were prepared by an Oxa-Pictet-Spengler reaction of a 2-phenylethanol derivative with piperidone ketals or haloalkanal acetals.



(*S*)-**1**



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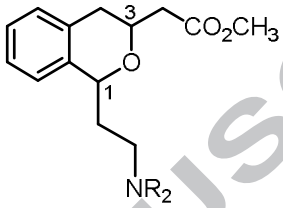

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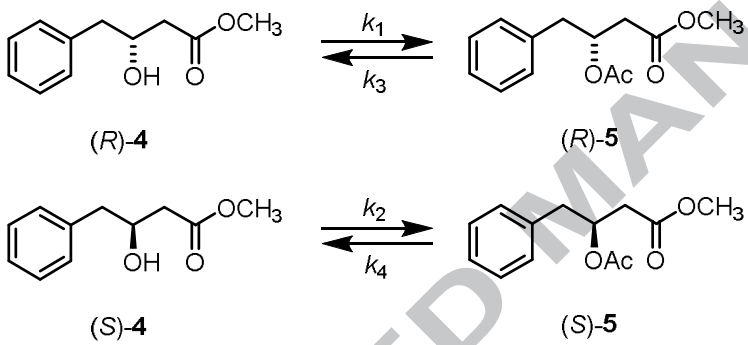
Figure 1: Potent σ_1 receptor ligands with 2-benzofuran and 2-benzpyran substructure.

The synthesis of tetrahydroisoquinolines by condensation of 2-arylethylamines with aldehydes or ketones is termed Pictet-Spengler reaction. In the Oxa-Pictet-Spengler reaction a 2-arylethanol derivative is reacted with an aldehyde, ketone or derivatives thereof to afford 3,4-dihydro-1*H*-2-benzopyrans.[25-27] This transformation represents a versatile method for the synthesis of substituted 2-benzopyrans and even larger fused ring systems. Moreover, intramolecular versions of the Oxa-Pictet-Spengler reaction have been described,[24,28] and it has been used for the stereoselective synthesis of complex natural products.[29-31] Usually it is promoted by Brønsted or Lewis acids, e.g. $BF_3{\cdot}OEt_2$, TMSOTf, and $Bi(OTf)_3$.[32]

Herein, we report the synthesis of enantiomerically pure spirocyclic and aminoethyl substituted 2-benzopyrans **2** and **3** by an Oxa-Pictet-Spengler reaction of enantiomerically pure 2-phenylethanol building blocks (*R*)-**4** and (*S*)-**4** as key step.

Synthesis of the enantiomeric alcohols (*R*)-**4** and (*S*)-**4** has already been reported by enantiodifferentiating reduction of *β*-ketoesters using H_2/tartaric acid modified Raney nickel[33] or a recombinant *S. coelicolor* A 3(2) strain.[34] Very recently, the asymmetric hydrogenation of *β*-ketoesters using chiral Ir-[35] and Ru-catalyst[36] was reported. In order obtain both enantiomers of the secondary alcohol **4** with high enantiomeric excess (>98 % *ee*) with easily accessible reagents, but avoiding metal reagents, a lipase-catalyzed kinetic resolution of racemic secondary alcohol (±)-**4** was envisaged.

2. Synthesis



Scheme 1: Acetylation of secondary alcohols **4** and hydrolysis of acetates **5** including the definition of the rate constants k_1-k_4 of the involved reactions.

The starting point of this study was racemic methyl 3-hydroxy-4-phenylbutanoate (±)-**4**,[24,37,38] which was obtained by acylation of Meldrum's acid with phenylacetyl chloride, heating of the product in methanol[39] and subsequent $NaBH_4$ reduction.[40,41] The racemic ester (±)-**5** was prepared by acetylation of (±)-**4** with Ac_2O (Scheme 1).

2.1. Chiral HPLC

In order to analyze the results obtained by lipase-catalyzed reactions a chiral HPLC method had to be developed for the simultaneous quantitative determination of (*R*)-**4**, (*S*)-**4**, (*R*)-**5**, and (*S*)-**5**.

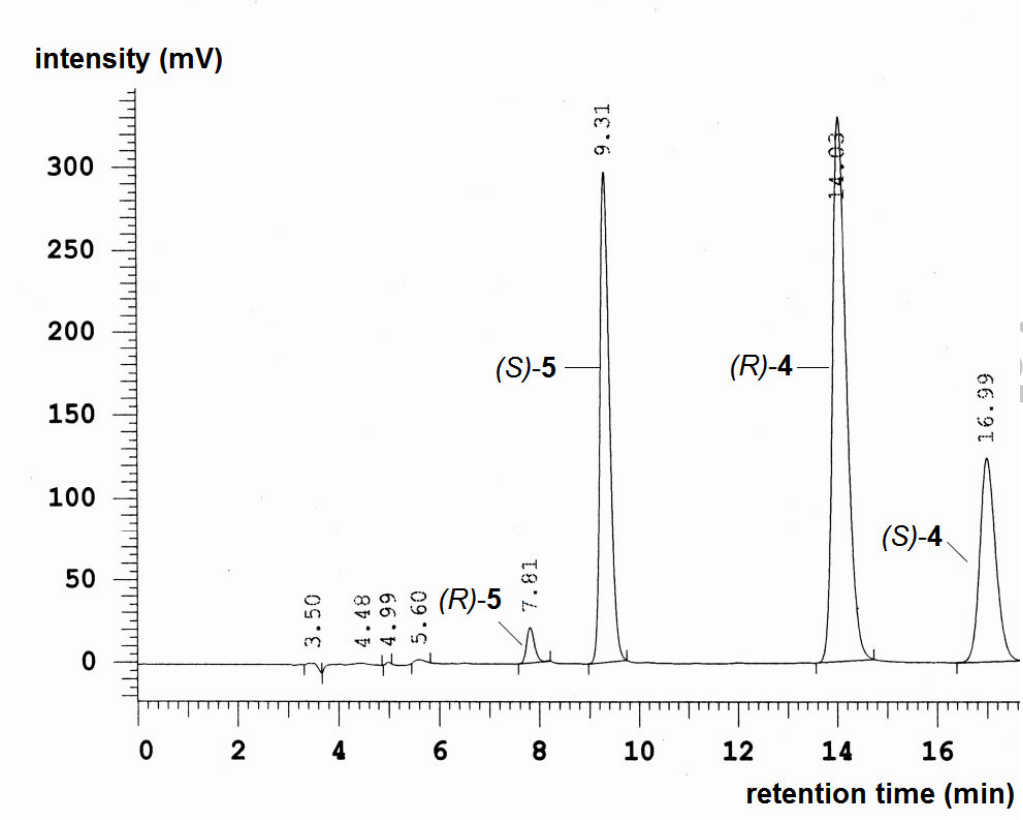


Figure 2: Chromatogram of a mixture of (*R*)-**4**, (*S*)-**4**, (*R*)-**5**, and (*S*)-**5**. Chiral HPLC: Chiralpak® IB, *n*-hexane : isopropanol = 98 : 2, flow rate 1.0 mL/min, UV detection λ = 210 nm, resolution: R_s ((*R*)-**5**:(*S*)-**5**) = 4.8; R_s ((*S*)-**5**:(*R*)-**4**) = 10.4; R_s ((*R*)-**4**:(*S*)-**4**) = 5.1.

In Figure 2 the chromatogram obtained from a mixture containing enantiomeric alcohols (*R*)-**4** and (*S*)-**4** and enantiomeric acetates (*R*)-**5**, and (*S*)-**5** is shown. With the Chiralpak® IB column the separation of the four compounds was performed within a very short analysis time of 18 min and excellent resolution of the four components. This method allowed the simultaneous analysis of the ratio of enantiomers (*R*)-**4** : (*S*)-**4** and (*R*)-**5** : (*S*)-**5** and moreover, the progress of the transformation during acetylation of alcohol (±)-**4** and hydrolysis of acetate (±)-**5**.

2.2. Lipase-catalyzed acetylation of alcohol 4

In a first screening nine lipases[41] were tested in the solvent *tert*-butyl methyl ether (TBME) at room temperature to catalyze the acetylation of racemic alcohol (±)-**4**. The definition of the lipases used in this study is given in Table 4 in the experimental section. Due to the higher activity of immobilized lipases (Chirazyme L-5, Lipozyme, Amano Lipase PS-C II) only 200 mg were used instead of 400 mg of native lipases. Isopropenyl acetate was employed as irreversible acetylating agent, as less side reactions were expected compared to vinyl acetate. In order to avoid mechanical damage of lipases a KPG stirrer was used instead of a magnetic bar.

Table 1:

Lipase-catalyzed enantioselective acetylation of racemic secondary alcohol (±)-**4**.

Entry	Lipase	Solvent	amount of lipase [mg]	time [h]	*ee* ((*R*)-**4**) [%]	*ee* ((*S*)-**5**) [%]	conv.[a]. [%]	*E*[b]
1	Chirazyme L-2	TBME	400	44	12.2	64.6	16	5.2
2	Chirazyme L-3	TBME	400	48	34.0	68.0	33	7.3
3	Chirazyme L-5[c]	TBME	200	4.2	16.2	20.8	44	1.8
4	Chirazyme L-7	TBME	400	95	--	--	--	--
5	Candida rugose	TBME	400	24	3.8	44.2	7.9	2.7
6	Lipozyme[d]	TBME	200	192	23.4	94.6	20	42
7	Amano Lipase PS	TBME	400	95	24.4	96.0	20	62
8	Amano Lipase PS	*i*Pr_2O	200	192	28.0	97.1	22	89
9	Amano Lipase PS	toluene (+H_2O)[g]	200	144	2.4	96.0	2.4	50
10	Amano Lipase PS	Acetone	200	671	6.6	87.2	7.0	16
11	Amano Lipase AK	TBME	400	443	13.5	92.8	13	31
12	Amano Lipase AK	*i*Pr_2O	200	97	6.0	97.4	5.8	81

13	Amano Lipase AK	toluene (+H_2O)[g)]	200	121	6.8	94.0	6.7	35
14	Amano Lipase AK	Acetone	200	407	4.0	92.0	4.2	25
15	Amano Lipase PS-C II[e)]	TBME	200	17	92.4	93.6	50	101
16	Amano Lipase PS-C II[e)]	*i*Pr_2O	100	18	54.0	94.4	36	60
17	Amano Lipase PS-C II[e)]	Acetone	100	480	20.2	98.1	17	127
18	Amano Lipase PS-C II[e)f)]	TBME	100	257	41.6	98.7	30	230

Standard reaction conditions; (±)-**4** (192 mg), isopropenyl acetate (0.5 mL, 4.5 equiv.), native lipase (400 mg) or immobilized lipase (200 mg), solvent (10 mL), rt, KPG stirrer.

[a)] conv. [%] = conversion [%] = { *ee* ((*R*)-**4**) / [*ee* ((*R*)-**4**) + *ee* ((*S*)-**5**)]} x 100 %.[41-43]

[b)] *E* = enantioselectivity = ln [1 – conv · (1 + *ee* ((*S*)-**5**))] / ln [1 – conv · (1 - *ee* ((*S*)-**5**))].[41-43]

[c)] immobilized on plastic particles.

[d)] immobilized on anion exchange resin.

[e)] immobilized on ceramic particles.

[f)] reaction was performed at -15 °C.

[g)] the amount of water was approx. 3-5 %.

The results in Table 1 clearly indicate that the lipases Chirazyme L-2, L-3 and, L-5 as well as *Candida rugosa* lipase did not provide high enantioselectivity (E-values < 8), the lipase Chirazyme L-7 did not lead to a conversion of racemic alcohol (±)-**4** (entries 1-5).

For optimization of the conversion and enantioselectivity Amano Lipase PS, Amano Lipase AK and Amano Lipase PS-C II were investigated. At first the conversion and E-values in different solvents were analyzed. In dry toluene a conversion could not be observed. Although addition of one drop of water resulted in clean acetylation of (*S*)-

4, the reaction times in the solvents toluene (+ H_2O) and acetone were very long (entries 9, 10, 13, 14, 17). Therefore, these solvents were not considered any longer even though the enantioselectivity was partly higher than in TBME. The conversion rates in *i*Pr_2O and TBME were very similar. Since for the most promising Amano Lipase PS-C II the enantioselectivity in TBME was higher than in *i*Pr_2O (entries 15, 16), TBME was selected as preferred solvent for the acetylation of (±)-**4**. All lipases led to a preferred acetylation of (*S*)-**4**.

The highest enantioselectivity (*E* = 101) and conversion (conv. = 50 %) were achieved with the Amano Lipase PS-C II in TBME at rt (entry 15). In order to further improve the *E*-value the temperature was reduced to -15 °C (entry 18). Although an excellent enantioselectivity was achieved at -15 °C (*E* = 230) the very long reaction time (257 h) and the low conversion (conv. = 30 %) were not appropriate to produce enantiomerically pure alcohol (*R*)-**4** or acetate (*S*)-**5**. Nevertheless, it is remarkable that the immobilized Amano Lipase PS-C II is still active at such a low temperature. In literature only few examples are reported, where lipases were used at such low temperature.[44-46]

In conclusion, under optimized reaction conditions (Amano Lipase PS-C II, isopropenyl acetate, TBME, 20 °C) (*R*)-configured alcohol (*R*)-**4** and (*S)*-configured acetate (*S*)-**5** were formed in >90 % *ee* at a conversion of 50 % (entry 15). Therefore, upon non-enzymatic hydrolysis of (*S*)-**5** into (*S*)-**4** it should be possible to obtain both alcohols (*R*)-**4** as well as (*S*)-**4** with >90 % *ee* and yields of almost 50 % via chiral resolution of (±)-**4**. However, it was our aim to produce both alcohols in >99.5 % *ee*.

A simulation[47] of the reaction course indicates that the *ee* value of alcohol (*R*)-**4**

should increase significantly during progress of the reaction, whereas the yield decreases only to a very low extent (Figure 3, see also Figure SI1 in Supporting Information). Therefore, the preparative scale conversion of (±)-**4** was monitored by HPLC and continued until (*R*)-**4** reached >99.5 % *ee*. The reaction was carried out for 145 h and after workup (*R*)-**4** was isolated with a yield of 42 % and 99.6 % *ee*. Additionally, (*S*)-**5** was obtained with 52 % yield and 76.9 % *ee*, which should be a valuable starting material for the production of (*S*)-**4**. Hydrolysis of the enantiomerically enriched acetate (*S*)-**5** should result in a higher *ee* of (*S*)-**4** than hydrolysis of racemic (±)-**5** assuming that the investigated lipases keep their enantiopreference in aqueous media.

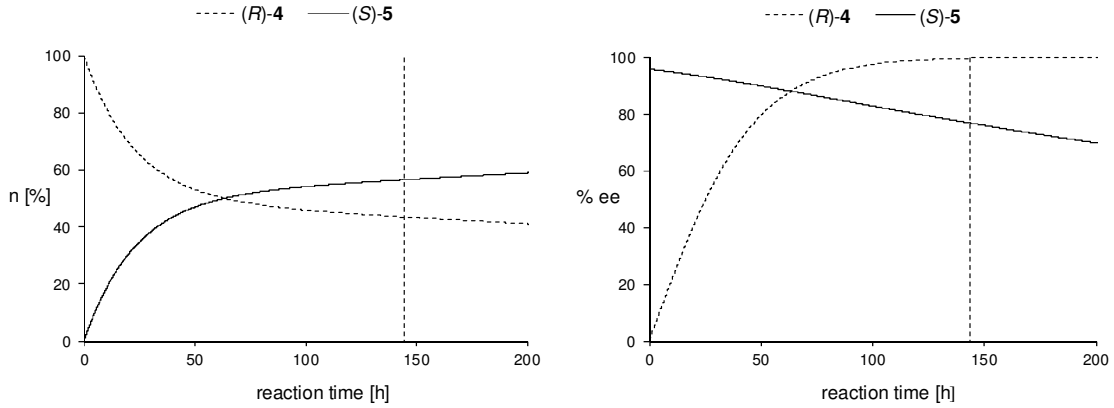


Figure 3: Simulation of the lipase-catalyzed acetylation of racemic alcohol (±)-**4** and comparison with experimental results. Left: amount of compounds (*R*)-**4** and (*S*)-**5**; right: *ee* of (*R*)-**4** and (*S*)-**5**. The reaction was carried out using (±)-**4** (1.70 g), Amano lipase PS-C II (1.70 g) and isopropenyl acetate (8.5 mL) in TBME (120 mL) and was stopped after 145 h leading to (*R*)-**4** in 42 % yield with 99.6 % *ee* (dotted line). Rate constants used for the simulation: k_1 = 45.5, k_2 = 1, k_3 = 0.00455, k_4 = 0.0001 (definition of k_1-k_4 see Scheme 1).

2.3. Lipase-catalyzed hydrolysis of acetate 5

The enantioselective hydrolysis of the racemic acetate (±)-**5** in phosphate buffer pH 7.0 with methanol as cosolvent was investigated using the five most promising

lipases from the acetylation reaction. Table 2 displays clearly that Amano lipase AK and Amano Lipase PS-C II converted (±)-**5** with high enantioselectivity (E = 108 and E = 263). However, the reaction rate of both transformations was rather low (entries 4 and 5). After 123 h, Amano Lipase PS-C II provided (*R*)-configured acetate (*R*)-**5** and (*S)*-configured alcohol (*S*)-**4** in 83.8 % *ee* and 98.0 % *ee*, respectively (entry 5).

Table 2

Lipase-catalyzed enantioselective hydrolysis of racemic acetate (±)-**5**.

Entry	Lipase	amount of lipase [mg]	Time [h]	*ee* ((*R*)-**5**) [%]	*ee* ((*S*)-**4**) [%]	conv.[a)] [%]	*E*[b)]
1	Chirazyme L-5[c)]	100	3	1.6	6.6	20	1.2
2	Lipozyme[d)]	100	48	2.8	31.0	8.3	2.0
3	Amano Lipase PS	200	74	40.7	95.2	30	61
4	Amano Lipase AK	200	95	23.6	97.7	20	108
5	Amano Lipase PS-C II[e)]	100	123	83.8	98.0	46	263

Standard reaction conditions; (±)-**5** (118 mg), phosphate buffer pH 7.0 (5 mL), lipase (200 mg) or immobilized lipase (100 mg), cosolvent methanol (2 mL), rt, magnetic bar for native lipases (entries 3 and 4), KPG stirrer for immobilized lipases (entries 1, 2, and 5).

[a)] conv. [%] = conversion [%] = { *ee* ((*R*)-**5**) / [*ee* ((*R*)-**5**) + *ee* ((*S*)-**4**)]} x 100 %.[41-43]

[b)] *E* = enantioselectivity = ln [1 – conv · (1 + *ee* ((*S*)-**4**))] / ln [1 – conv · (1 - *ee* ((*S*)-**4**))].[41-43]

[c)] immobilized on plastic particles.

[d)] immobilized on anion exchange resin.

[e)] immobilized on ceramic particles.

For the preparative scale production of (*S*)-**4** the enantiomerically enriched acetate (*S*)-**5** (76.9 % *ee*) obtained from the Amano Lipase PS-C II catalyzed acetylation (see above) was hydrolyzed in phosphate buffer pH 7.4 using the same enzyme. Again, it

was an important feature to find the optimal end point to stop the transformation. A comparison of the screening reactions shows that the enantioselectivity *E* of the Amano Lipase PS-C II for the hydrolysis in phosphate buffer is even higher than for the acetylation in TBME (Table1 entry 15, Table 2 entry 5). Simulation[47] of the reaction course demonstrates that hydrolysis of enantiomerically enriched acetate (*S*)-**5** should result in a high *ee* of alcohol (*S*)-**4**, which slightly decreases over the time, whereas the yield increases during the progress of the reaction (Figure 4, see also Figure SI2 in Supporting Information). However, the activity of the lipase was very low leading to very long reaction times. The hydrolysis was stopped after 311 h leading to (*S*)-**4** in 26 % yield upon workup, but with an excellent enantiopurity of 99.7 % *ee*.

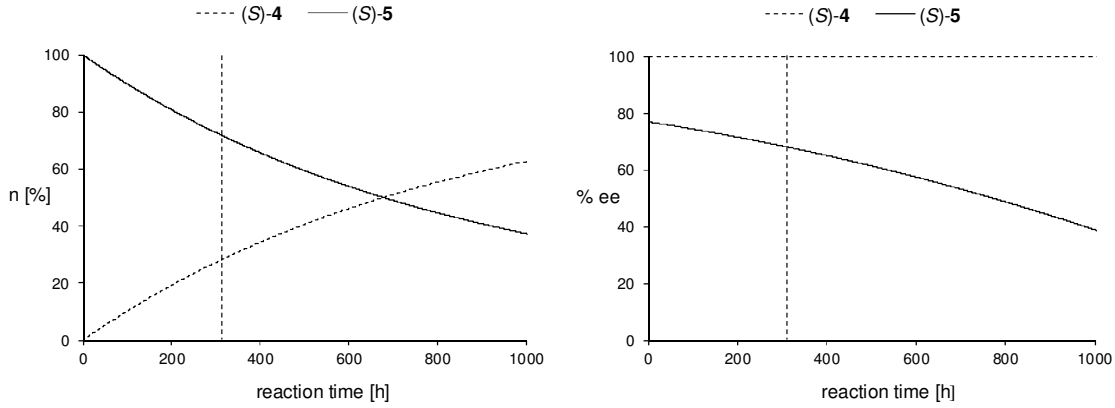


Figure 4: Simulation of the lipase-catalyzed hydrolysis of enantiomerically enriched acetate (*S*)-**5** and comparison with experimental results. left: amount of compounds (*S*)-**4** and (*S*)-**5**; right: *ee* of (*S*)-**4** and (*S*)-**5**. The reaction was carried out using (*S*)-**5** (1.06 g, 76.9 % *ee*), Amano lipase PS-C II (1.0 g) and methanol (22 mL) in phosphate buffer pH 7.4 (55 mL) and was stopped after 311 h leading to (*S*)-**4** in 26 % yield with 99.7 % *ee* (dotted line). rate constants used for the simulation: k_1 = 0.012, k_2 = 0.0001, k_3 = 120, k_4 = 1 (definition of k_1-k_4 see Scheme 1).

As an alternative strategy for the production of alcohol (*S*)-**4**, enantiomerically enriched acetate (*S*)-**5** could be hydrolyzed non-enzymatically to afford

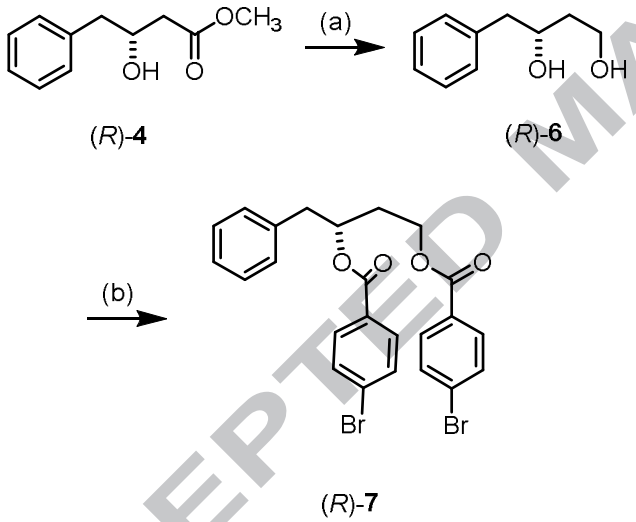
enantiomerically enriched alcohol (*S*)-**4** followed by a second lipase-catalyzed acetylation and a second non-enzymatic hydrolysis. This kind of kinetic resolution, however, comprises two additional reaction steps, i.e. non-enzymatic hydrolysis using H_2SO_4 in methanol and provided (*S*)-**4** in an overall yield of only 7 % with 99.2 % *ee*. These results demonstrate that the lipase-catalyzed hydrolysis of enantiomerically enriched acetate (*S*)-**5** is the most advantageous strategy.

2.4. Determination of the absolute configuration

According to the rule of Kazlauskas[42,48] the size of the two additional substituents at the secondary alcohol define the selectivity of the lipase during acetylation and hydrolysis. On condition the larger substituent has higher priority according to the CIP rules than the smaller substituent the (*R*)-enantiomer is transformed preferentially. It is assumed that the benzyl moiety of alcohol **4** and acetate **5** is larger than the methoxycarbonylmethyl moiety. Thus, the (*S*)-configured enantiomers (*S*)-**4** and (*S*)-**5** should be acetylated and hydrolyzed preferentially, since the larger benzyl moiety has a lower CIP priority than the smaller methoxycarbonylmethyl group. The results of our experiments correlate nicely with this rule.

The synthesis of the enantiomeric alcohols (*R*)-**4** and (*S*)-**4** has been reported (see Introduction).[33-36] In the first paper a specific optical rotation $[\alpha]_D^{20}$ = -8.6 (c = 1.2, $CHCl_3$, optical yield 88 %). was given for (*R*)-configured alcohol (*R*)-**4**.[33] The second paper reports $[\alpha]_D^{20}$ = +1.2 (c = 0.9, CH_2Cl_2) for (*S*)-**4**.[34] An enantiomeric excess of 85 % was given for this sample.[34] In the very recent publication,[35] the (*R*)-configured β-hydroxyester (*R*)-**4** was obtained by asymmetric hydrogenation of the corresponding β-ketoester. The enantiomeric excess of (*R*)-**4** was determined by chiral HPLC (98 % *ee*, $[\alpha]_D^{25}$ = -10.0 (c = 1.0, $CHCl_3$). The enantiomeric alcohols (*R*)-**4** and (*S*)-**4**

prepared by lipase-catalyzed kinetic resolution in this work possess the following specific rotations: (*R*)-**4**: $[\alpha]_D^{20}$ = +9.1 (c = 1.0, CH_3OH, 99.6 % *ee*); (*S*)-**4**: $[\alpha]_D^{20}$ = -8.8 (c = 1.0, CH_3OH, 99.7 % *ee*). The sign of the optical rotation is opposite to the reported one. Since the specific optical rotation depends considerably on the solvent, the measurement of the optical rotation of the (*S*)-configured alcohol (*S*)-**4** was repeated in the solvent CH_2Cl_2, which resulted in $[\alpha]_D^{20}$ = +10.3 (c = 1.63, CH_2Cl_2, 99.7 % *ee*). The plus-sign of prepared (*S*)-**4** correlates with the plus-sign reported for (*S*)-**4** (CH_2Cl_2)[34] and the minus-sign determined for (*R*)-**4** (CH_2Cl_2).[35] However, a proof of the absolute configuration of the prepared alcohols is missing in the reports.



Scheme 2: Synthesis of bis(4-bromobenzoate) (*R*)-**7**.
Reagents and reaction conditions: (a) $LiBH_4$, THF, rt, 16 h, 87 %. (b) 4-bromobenzoyl chloride, pyridine, CH_2Cl_2, rt, 16 h, 76 %.

In order to prove the absolute configuration of the enantiomeric alcohols (*R*)-**4** and (*S*)-**4** unequivocally, exciton-coupled CD spectroscopy of a bis(4-bromobenzoate) should be used. For this purpose (*R*)-**4** was reduced with $LiBH_4$ to afford the diol (*R*)-**6**, which was acylated with 4-bromobenzoyl chloride to provide bis(4-bromobenzoate)

(*R*)-**7** (Scheme 2). The UV- and CD-spectra of the bis(4-bromobenzoate) (*R*)-**7** were recorded in *n*-hexane (Figure 5). The exciton-coupled CD spectrum of (*R*)-**7** displays a negative Cotton effect at longer wave length (λ = 252 nm) and a positive Cotton effect at shorter wave length (λ = 235 nm). The position of the positive and negative Cotton effects in this exciton coupled CD spectrum proves unequivocally (*R*)-configuration of the center of chirality of (*R*)-**7** and thus (*R*)-configuration of the starting alcohol (*R*)-**4** as well.[49]

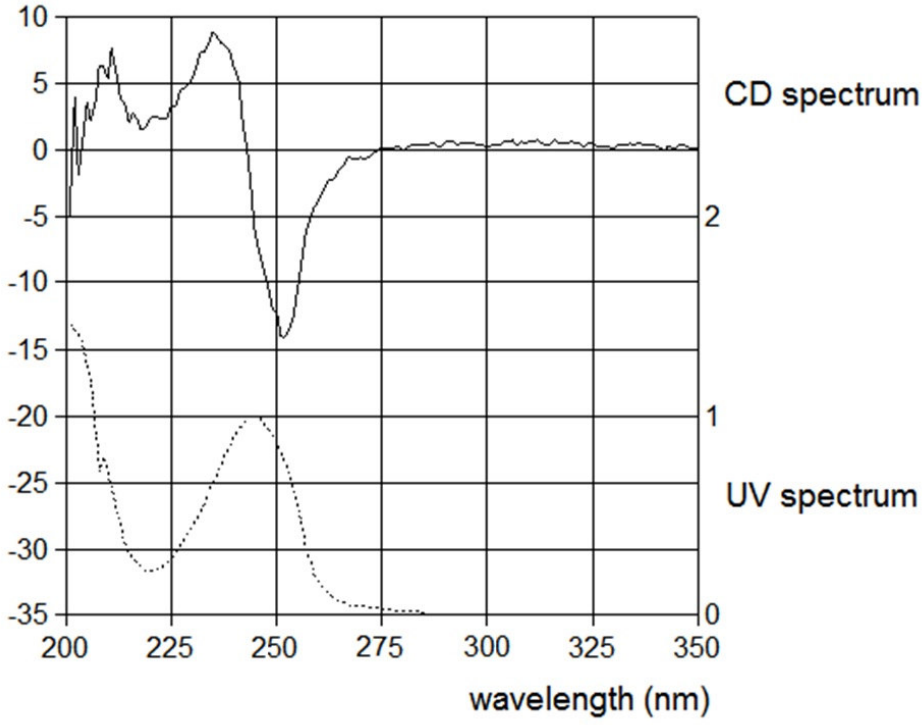
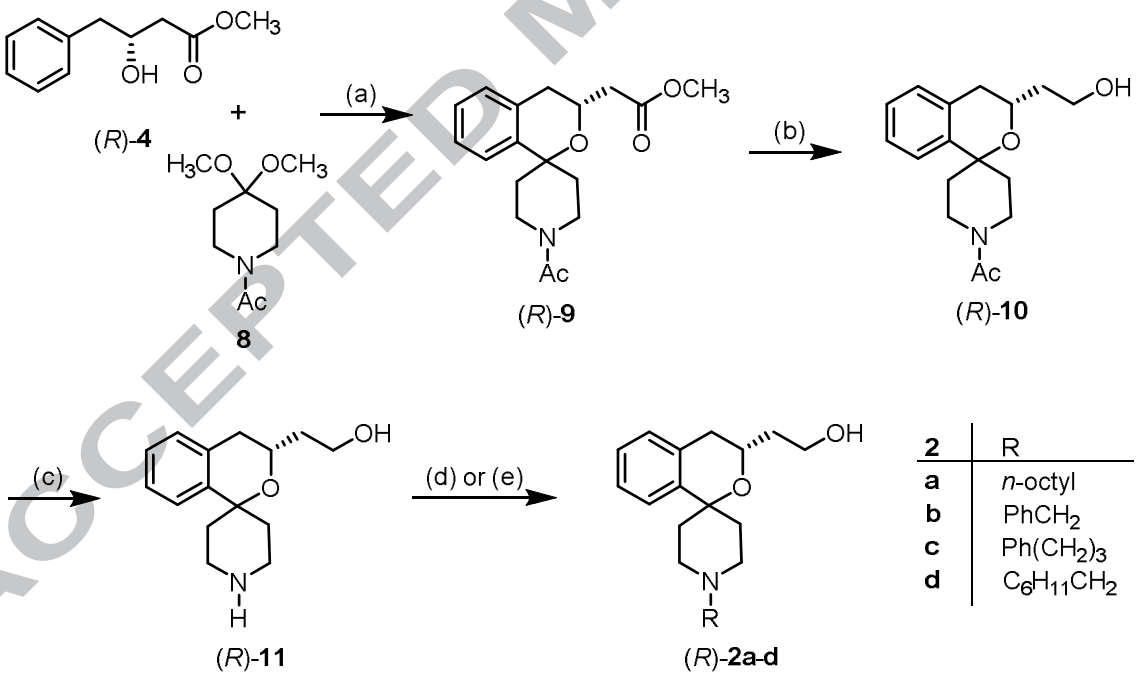


Figure 5: CD and UV spectra (*n*-hexane) of the bis(4-bromobenzoate) (*R*)-**7**.

2.5. Synthesis of enantiomerically pure σ ligands by Oxa-Pictet-Spengler reaction

The Oxa-Pictet-Spengler reaction of racemic alcohol (±)-**4** with piperidone ketal **8** and the subsequent transformation of (±)-**9** into racemic spirocyclic σ ligands (±)-**2** have

already been reported.[24] For the synthesis of the enantiomerically pure spirocyclic compounds (*R*)-**2** and (*S*)-**2** the same reaction conditions were applied (Scheme 3). The $BF_3{\cdot}OEt_2$ catalyzed Oxa-Pictet-Spengler reaction of enantiomerically pure alcohols (*R*)-**4** and (*S*)-**4** with piperidone dimethyl ketal **8** led to the spirocyclic piperidines (*R*)-**9** and (*S*)-**9**. The ester moiety was reduced with $LiBH_4$ and the *N*-acetyl protecting group of the resulting alcohols (*R*)-**10** and (*S*)-**10** was cleaved off by NaOH. The piperidine derivatives (*R*)-**11** and (*S*)-**11** represent valuable building blocks, which allow the introduction of diverse substituents at the very end of the synthesis.[50,51] Herein the secondary amines (*R*)-**11** and (*S*)-**11** were alkylated with bromoalkanes or reductively alkylated with aldehydes and $NaBH(OAc)_3$ to yield the enantiomerically pure spirocyclic ligands (*R*)-**2a-d** and (*S*)-**2a-d**.



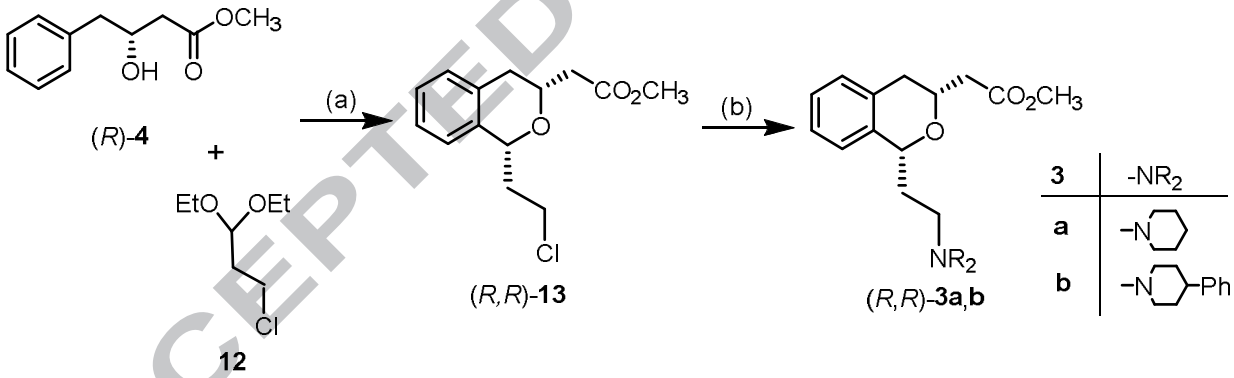
Scheme 3: Synthesis of enantiomerically pure spirocyclic σ ligands.

Reagents and reaction conditions: (a) $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt, 7 d, 78 % ((*R*)-**9**), 91 % ((*S*)-**9**). (b) $LiBH_4$, THF, rt, 16 h, 47 % ((*R*)-**10**), 37 % ((*S*)-**10**). (c) NaOH, 100 °C, 3 h, 53 % ((*R*)-**11**), 78 % ((*S*)-**11**). (d) $CH_3(CH_2)_7$-Br or $Ph(CH_2)_3$-Br or $C_6H_{11}CH_2$-Br,

CH_3CN, K_2CO_3, reflux. (e) PhCH=O, $NaBH(OAc)_3$), CH_2Cl_2, rt. In the Scheme the transformations are shown for (*R*)-configured compounds, the corresponding (*S*)-configured enantiomers were prepared in the same manner.

The Oxa-Pictet-Spengler reaction of (*R*)-**4** with chloropropionaldehyde acetal **12** and $BF_3{\cdot}OEt_2$ provided the 1-(chloroethyl) substituted 2-benzopyran (*R*,*R*)-**13** with high diastereoselectivity,[24] only the thermodynamically more stable *cis*-configured diastereomer (*R*,*R*)-**13** could be isolated (Scheme 4). The last step of the synthesis comprises nucleophilic substitution of (*R*,*R*)-**13** with various amines leading to diverse 1-(aminoethyl) substituted 2-benzopyrans (*R*,*R*)-**3a** and (*R*,*R*)-**3b**. The enantiomeric 2-benzopyrans (*S*,*S*)-**3a** and (*S*,*S*)-**3b** were prepared in the same manner starting with (*S*)-configured hydroxyester (*S*)-**4**.

Scheme 4



Scheme 4: Synthesis of enantiomerically pure aminoethyl substituted 2-benzopyrans. Reagents and reaction conditions: (a) $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt, 5 d, 32 % ((*R*,*R*)-**13**), 27 % ((*S*,*S*)-**13**). (b) piperidine or 4-phenylppiperidine, Bu_4NI (cat.), K_2CO_3, CH_3CN, reflux, 20 h.

The enantiomeric purity of the final products was analyzed exemplarily for the benzyl substituted spirocyclic piperidine enantiomers (*R*)-**2b** and (*S*)-**2b**. Among the different

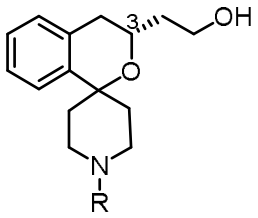
chiral stationary phases, only the column Chiralcel OJ-H allowed the separation of the enantiomers. The chromatograms of both enantiomers did not show significant signals for the other enantiomer ((ee > 98 %, see Supporting Information) indicating negligible racemization during the synthesis. Comparable enantiopurity results for the test compounds **2a**-**d** and **3a**-**b**, since they were prepared according to the same method. The opposite values for the specific rotation of enantiomeric pairs (see Supporting Information) confirm the high enantiomeric purity of the test compounds.

3. Biological activity

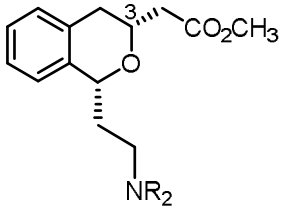
The σ receptor affinity of the enantiomerically pure spirocyclic 2-benzopyrans (*R*)-**2a-d** and (*S*)-**2a-d** as well as the aminoethyl substituted benzopyrans (*R*,*R*)-**3a**,**b** and (*S*,*S*)-**3a**,**b** was investigated with radioligand receptor binding studies. In the σ_1 assay guinea pig brain homogenates were used as receptor material and [^{3}H]-(+)-pentazocine as σ_1 selective radioligand. Rat liver homogenates served as receptor source in the σ_2 assay. Since a σ_2 selective radioligand for labeling of σ_2 receptors is not commercially available, [^{3}H]-di-o-tolylguanidine was used as radioligand. In order to achieve σ_2 selectivity an excess of (+)-pentazocine was added to mask σ_1 receptors.[52-54]

Table 3

σ_1 and σ_2 receptor affinity of enantiomerically pure 2-benzopyrans.



(*R*)-**2**



(*R,R*)-**3**

compd.	R	K_i ± SEM (nM)[a)]		σ_1/σ_2 selectivity	eudismic ratio (σ_1 affinity)	eudismic ratio (σ_2 affinity)
		σ_1	σ_2			
(*R*)-**2a**	*n*-octyl	24 ± 4	68 ± 29	2.9	2.3	3.8
(*S*)-**2a**	*n*-octyl	56 ± 6	260 ± 50	4.6		
(*R*)-**2b**	$PhCH_2$	10 ± 4	21 % *	>100	4.8	-
(*S*)-**2b**	$PhCH_2$	48 ± 15	9 % *	>20		
(*R*)-**2c**	$Ph(CH_2)_3$	15 ± 4	1220 ± 445	79	4.5	2.4
(*S*)-**2c**	$Ph(CH_2)_3$	68 ± 14	519 ± 122	7.6		
(*R*)-**2d**	$C_6H_{11}CH_2$	10 ± 1	510 ± 224	49	0.9	1.2
(*S*)-**2d**	$C_6H_{11}CH_2$	9.0 ± 2	614 ± 241	69		
(*R,R*)-**3a**	piperidin-1-yl	556 ± 184	13 % *	>1.7	1.5	-
(*S,S*)-**3a**	piperidin-1-yl	860 ± 188	39 %*	>1.6		
(*R,R*)-**3b**	4-phenyl-piperidin-1-yl	33 ± 9	525 ± 130	16	1.3	0.06
(*S,S*)-**3b**	4-phenyl-piperidin-1-yl	43 ± 17	32 ± 19	0.7		
(+)-pentazocine		4.2 ± 1.1	-			
Haloperidol		3.9 ± 1.5	78 ± 2.0			
di-*o*-tolylguanidine		61 ± 18	42 ± 8.1			

[a)] The reported K_i-values are mean values of three independent experiments (n = 3). For low affinity compounds, values in % (marked with an asterisk*) are given, indicating the inhibition of specific radioligand binding at a test compound concentration of 1 µM.

Table 3 displays clearly that (*R*)-configured spirocyclic 2-benzopyrans (*R*)-**2** and (*R*,*R*)-configured aminoethyl substituted 2-benzopyrans (*R*,*R*)-**3** show higher σ_1 affinity than their enantiomers (*S*)-**2** and (*S*,*S*)-**3,** although the low eudismic ratios of **2d**, **3a** and **3b** are not significant. The highest eudismic ratios were found for the most potent benzyl and phenylpropyl substituted spirocyclic 2-benzopyrans **2b** (4.8) and **2c** (4.5). They result from the high σ_1 affinity of (*R*)-**2b** (K_i = 10 nM) and (*R*)-**2c** (K_i = 15 nM). The cyclohexylmethyl substituted spirocyclic compound (*R*)-**2d** has high σ_1 affinity (K_i = 10 nM) as well, but due to the increased σ_1 affinity of the (*S*)-configured enantiomer (*S*)-**3d** *(*K_i = 9 nM) the eudismic ratio is reduced.

In general, the more rigid 2-benzopyrans **2** show higher σ_1 affinity than the 2-benzopyrans **3** with a flexible 2-aminoethyl side chain. In particular the spirocyclic compound (*R*)-**2c** with a phenylpropyl substituent (K_i = 15 nM) and the 2-aminoethyl derivative with a 4-phenylpiperidine moiety (*R*,*R*)-**3b** (K_i = 33 nM) represent constitutional isomers considering the amino moiety bearing substituents at 1-position of the 2-benzopyran ring, but differ considerably in their σ_1 affinity.

With exception of (*S*,*S*)-**3b** ($K_i(\sigma_2)$ = 32 nM) all ligands show high selectivity (e.g. (*R*)-**2b**, (*R*)-**2c**, (*R*)-**2d**, (*S*)-**2d**) or at least a preference (e.g. (*R*)-**2a**, (*S*)-2a, (*R*,*R*)-**3a**) for the σ_1 receptor over the σ_2 subtype. The highest σ_1/σ_2 selectivity was found for the very potent σ_1 ligand (*R*)-**2b**: ($K_i(\sigma_1)$ = 10 nM, σ_1/σ_2 = 100.

Since some ligands for σ_1 receptors and the phencyclidine (PCP) binding site within the channel pore of the N-methyl-*D*-aspartate (NMDA) receptor are often closely related,[55-57] the PCP affinity of the 2-benzopyrans **2** and **3** was also investigated in

this study. However, at a concentration of 10 µM the compounds did not compete considerably with the radioligand [^{3}H]-(+)-MK-801[55,58] indicating IC_{50} values higher than 10 µM. Obviously the 2-benzopyrans **2** and **3** with a basic substituent are highly selective for the σ_1 receptor over the PCP binding site of the NMDA receptor.

Due to the structural similarity of the σ_1 receptor with the yeast enzyme sterol-Δ^8/Δ^7-isomerase,[5-7] the inhibition of this enzyme by racemic 2-benzopyrans (±)-**2a**, (±)-**2c** and (±)-**3b** was investigated exemplarily in a cellular assay.[59] Even at a concentration of 4 µg/mL these compounds did not at all inhibit the growth or change the sterol pattern of the model yeast *Yarrowia lipolytica*. This finding indicates the high selectivity for the σ_1 receptor over the fungal sterol-Δ^8/Δ^7-isomerase. Further, the compounds were subjected to a cellular assay for inhibition of post-squalene enzymes in cholesterol biosynthesis.[60] In HL-60 cells no changes in sterol pattern were detected at a test concentration of 1 µM. At an extremely high concentration of 50 µM, a slight accumulation of cholesta-8,14-dienol was observed (see Supporting Information, Figure SI3), indicating a very weak inhibition of human sterol-Δ^8/Δ^7-isomerase.

4. Conclusion

The Oxa-Pictet-Spengler reaction of enantiomerically pure alcohols (*R*)-**4** and (*S*)-**4** was employed for the synthesis of enantiomerically pure spirocyclic and flexible aminoethyl substituted σ_1 ligands (*R*)-**2a-d**, (*S*)-**2a-d**, (*R*,*R*)-**3a**,**b**, and (*S*,*S*)-**3a**,**b** based on a 2-benzopyran scaffold. The synthesis of the enantiomerically pure alcohols (*R*)-**4** and (*S*)-**4** was performed by lipase-catalyzed kinetic resolution. The absolute configuration of prepared (*R*)-**4** was assigned by exciton-coupled CD-spectroscopy of its bis(4-bromobenzoate) derivative (*R*)-**7**. In general (*R*)-configured

spirocyclic compounds (*R*)-**2** and (*R*,*R*)-configured aminoethyl substituted derivatives (*R*,*R*)-**3** reveal higher σ_1 affinity than their enantiomers. The very potent σ_1 ligand (*R*)-**2c** (K_i = 15 nM) is 4.5-fold more affine than its enantiomer (*S*)-**2c** and shows high selectivity over the σ_2 subtype, the PCP binding site of the NMDA receptor and the sterol-Δ^8/Δ^7-isomerases in both yeast and human cells.

5. Experimental

5.1. Chemistry, General Methods

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

5.2. Instruments

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

5.3. HPLC method for the determination of the purity

Merck Hitachi Equipment; UV detector: L-7400; autosampler:L-7200; pump: L-7100; degasser: L-7614; column: LiChrospher® 60 RP-select B (5 µm); LiCroCART® 250-4 mm cartridge; flow rate: 1.00 mL/min; injection volume: 5.0 µL; detection at λ = 210 nm; solvents: A: water with 0.05% (v/v) trifluoroacetic acid; B: acetonitrile with 0.05% (v/v) trifluoroacetic acid: gradient elution: 0.0 min: 90.0 % of A, 10.0 % of B; 4.0 min: 90.0 % of A, 10.0 % of B; 29.0 min: 0.0 % of A, 100.0 % of B; 31.0 min: 0.0 % of A, 100.0 % of B; 31.5 min: 90.0 % of A, 10.0 % of B; 40.0 min: 90.0 % of A, 10.0 % of B.

5.4. HPLC method for the determination of enantiomeric excess

Merck Hitachi Equipment; pump: L-6200A; UV detector, autosampler, degasser: as described in Part 5.3; column: Chiralpak® IB (5 µm); temperature: 20 °C; flow rate: 1.000 mL/min; injection volume: 20.0 µL; detection at λ = 210 nm; isocratic elution, eluent: *n*-hexane : isopropanol 98 : 2.

5.5. Lipases used in this project

Table 4: Characterization of lipases used in this project

Name	Supplier	origin	preparation
Chirazyme L-2	Roche	*Candida antarctica* fraction B (fungus)	lyophilized
Chirazyme L-3, pur.	Roche	*Candida rugosa* (fungus)	lyophilized
Chirazyme L-5	Roche	*Candida antarctica* fraction A (fungus)	immobilized on plastic particles
Chirazyme L-7	Roche	Pig pancreas (mammal)	solid
Candida rugose Lipase	Fluka	*Candida rugosa* (fungus)	solid
Lipozyme	Fluka	*Mucor miehei* ((thermophilic fungus)	immobilized on anion exchange resin
Amano Lipase PS	Amano Lipases Inc.	*Burkholderia cepacia* (bacterium)	solid

Amano Lipase AK	Amano Lipases Inc.	*Pseudomonas fluorescens* (bacterium)	solid
Amano Lipase PS- C II	Sigma-Aldrich	*Burkholderia cepacia* (bacterium)	immobilized on ceramic particles

5.6. Synthetic procedures

5.6.1. (±)-Methyl 3-acetoxy-4-phenylbutanoate ((±)-5)

A solution of racemic alcohol (±)-**4** (50 mg, 0.26 mmol) NEt_3 (0.125 mL, 0.9 mmol), Ac_2O (0.055 mL, 0.56 mmol) and 4-(dimethylamino)pyridine (5.1 mg, 0.042 mmol) in $CH_2Cl_{2\ abs}$ (2 mL) was stirred at rt for 3 h. Et_2O (2 mL) was added and the mixture was washed with 0.5 M HCl (2 mL) and H_2O (2 mL). The aqueous layer was extracted with Et_2O (2 x 2 mL) and the combined organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (2 cm, cyclohexane : ethyl acetate = 9 : 1, 20 mL, R_f = 0.16). Colorless oil, yield 33 mg (54 %). $C_{13}H_{16}O_4$ (236.3). MS (ESI): m/z = 236 [M]. IR: ν [cm^{-1}] = 2952 (C-H), 1735 (C=O), 1027 (C-O), 700 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.00 (s, 3H, CO*CH*$_3$), 2.53 (dd, J = 15.7/5.7 Hz, 1H, *CH*$_2$CO$_2$CH$_3$), 2.57 (dd, J = 15.7/7.2 Hz, 1H, *CH*$_2$CO$_2$CH$_3$), 2.87 (dd, J = 13.7/6.8 Hz, 1H, Ph*CH*$_2$), 2.98 (dd, J = 13.7/6.4 Hz, 1H, Ph*CH*$_2$) , 3.65 (s, 3H, CO$_2$*CH*$_3$), 5.37 – 5.45 (m, 1H, *CH*OAc), 7.18 – 7.32 (m, 5H, arom.).

5.6.2. Methyl (*R*)-3-hydroxy-4-phenylbutanoate ((*R*)-4) and Methyl (*S*)-3-acetoxy-4-phenylbutanoate ((*S*)-5)

A mixture of racemic alcohol (±)-**4** (1.70 g, 8.75 mmol), isopropenyl acetate (8.5 mL) and Amano Lipase PS-C II (1.70 g) in *tert*-butyl methyl ether (120 mL) was shaken at rt for 145 h. The immobilized enzyme was filtered off and the solvent was removed in vacuo. Alcohol (*R*)-**4** and acetate (*S*)-**5** were separated by fc (3 cm, at first cyclohexane : ethyl acetate = 90 : 10, 20 mL, R_f = 0.16 (*S*)-**5**, then cyclohexane : ethyl acetate = 80 : 20, 20 mL, R_f = 0.29 (*R*)-**4**).

(*S*)-**5**: Colorless oil, yield 1.065 g, (52 %); *ee* = 76.9 %.

(*R*)-**4**: Colorless oil, yield 719.6 mg, (42 %); $[\alpha]_D^{20}$ = +9.13 (c = 1.00, CH_3OH); *ee* = 99.6 %.

5.6.3. Methyl (*S*)-3-hydroxy-4-phenylbutanoate ((*S*)-4)

Enantiomerically enriched acetate (*S*)-**5** (76.9 % *ee*, 1.06 g, 4.5 mmol) was dissolved in MeOH (22 mL) and phosphate buffer pH 7.4 (55 mL). Amano Lipase PS-C II (1.0 g) was added and the mixture was shaken at rt for 13 d. The immobilized enzyme was filtered off and the filtrate was extracted with Et_2O (5 x 25 mL). The combined organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (3 cm, at first: cyclohexane : ethyl acetate = 90 : 10, then cyclohexane : ethyl acetate = 80 : 20, 20 mL, R_f = 0.29). Colorless oil, yield 224 mg, (26 %); $[\alpha]_D^{20}$ = -8.79 (c = 1.00, MeOH); $[\alpha]_D^{20}$ = +10.3 (c = 1.63, CH_2Cl_2); *ee* = 99.7 %.

5.6.4. (*R*)-4-Phenylbutane-1,3-diol ((*R*)-6)

Under N_2 and cooling with ice, 2 M $LiBH_4$ (1 mL in THF, 2 mmol) was added to a solution of (*R*)-**4** (97.1 mg, 0.5 mmol) in THF_{abs} (8 mL). The mixture was stirred at rt for 16 h. Then it was concentrated in vacuo and the residue was dissolved in H_2O (8 mL) and 0.5 M HCl (2 mL). The solution was extracted with CH_2Cl_2 (3 x 8 mL). The combined organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (2.5 cm, cyclohexane : ethyl acetate = 50 : 50, 10 mL, R_f = 0.29). Colorless oil, yield 72.0 mg (87 %). $C_{10}H_{14}O_2$ (166.2). MS (ESI): m/z = 166 [M]. IR: ν [cm^{-1}] = 3366 (O-H); 2917 (C-H); 1330, 1280 (O-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.72 – 1.80 (m, 2H, *CH*$_2$$CH_2OH$), 2.38 (s, broad, 2H, *OH*), 2.76 (dd, J = 13.3/7.7 Hz, 1H, Ph*CH*$_2$), 2.82 (dd, J = 13.3/5.2 Hz, 1H, Ph*CH*$_2$), 3.78 – 3.92 (m, 2H, CH_2*CH*$_2$OH), 4.05 – 4.13 (m, 1H, $PhCH_2$*CH*OH), 7.20 – 7.35 (m, 5H, arom.). $[\alpha]_D^{20}$ = -15.3 (c =

1.10, MeOH).

5.6.5. (*R*)-(4-Phenylbutane-1,3-diyl) bis-(4-bromobenzoate) ((*R*)-7)

A solution of diol (*R*)-**6** (63.8 mg, 0.38 mmol), 4-bromobenzoyl chloride (301 mg, 1.2 mmol) and pyridine (0.01 mL, 0.1 mmol) in $CH_2Cl_{2\ abs}$ (8 mL) was stirred at rt for 16 h. 0.5 M HCl was added (pH 2), the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (3 cm, cyclohexane : ethyl acetate = 90 : 10, 10 mL, R_f = 0.35). Colorless oil, yield 153 mg (76 %). $C_{24}H_{20}Br_2O_4$ (532.2). MS (EI): m/z = 532 [M], 185/183 COC_6H_4Br. IR: ν [cm^{-1}] = 3027, 2922 (C-H), 1714 (CO). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.11 - 2.18 (m, 2H, CH_2Ph), 2.97 - 3.13 (m, 2H, CHCH_2CH_2), 4.32 - 4.47 (m, 2H, CH_2CH_2O), 5.48 - 5.55 (m, 1H, CH_2CHCH_2), 7.19 - 7.31 (m, 5H, Ph), 7.49 - 7.55 (m, 4H, arom.), 7.77 - 7-83 (m, 4H, arom.). $[\alpha]_D^{20}$ = -17.7 (c = 1.32, CH_2Cl_2).

5.6.6. Methyl (*R*)-2-(1'-acetyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)-acetate ((*R*)-9)

Under N_2, a solution of **8** (1.87 g, 10 mmol) in $toluene_{abs}$ (2 mL) was added to a solution of (*R*)-**4** (388 mg, 2.0 mmol) in $CH_2Cl_{2\ abs}$ (15 mL) and the mixture was stirred at rt for 30 min. The solution was cooled to 0 °C, BF_3•Et_2O (2.6 mL, approx. 16 mmol) was added and the mixture was stirred at rt for 7 d. 10 % $NaHCO_3$ solution (15 mL) was added, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (3 cm, cyclohexane : ethyl acetate = 1 : 1, 20 mL, R_f = 0.18). Pale yellow oil, yield 491.1 mg (78 %). $[\alpha]_D^{20}$ = +28.6 (c = 0.96, MeOH).

Methyl (*S*)-2-(1'-acetyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)-acetate ((*S*)-9)

As described for the synthesis of (*R*)-**9**, (*S*)-**4** (220 mg, 1.13 mmol) was reacted with **8** (1.07 g, 5.7 mmol and BF_3•Et_2O (1.5 mL, approx. 9 mmol) in $CH_2Cl_{2\ abs}$ (1 mL). Pale yellow oil, yield 327.8 mg (91 %). $[\alpha]_D^{20}$ = -30.7 (c = 1.60 , MeOH).

Spectroscopic data of (*R*)-**9** and (*S*)-**9** are identical with the data for (±)-**9**.[24] $C_{18}H_{23}NO_4$ (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.).

5.6.7. (*R*)-2-(1'-Acetyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)ethanol ((*R*)-10)

Under N_2 at 0 °C, $LiBH_4$ solution (2 M in THF, 3 mL 6 mmol) was added to a solution of (*R*)-**9** (468.5 mg, 1.5 mmol) in THF_{abs} (25 mL). The mixture was stirred at rt for 20 h. Then it was concentrated in vacuo, the residue was diluted with H_2O (10 mL), 0.5 M HCl was added (pH 2) and the solution was extracted with CH_2Cl_2 (4 x 10 mL). The combined organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (2 cm, CH_2Cl_2 : MeOH = 95 : 5, 10 mL, R_f = 0.24). Colorless oil, yield 204.4 mg (47 %). $[\alpha]_D^{20}$ = +46.5 (c = 0.90, MeOH).

(*S*)-2-(1'-Acetyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)ethanol ((*S*)-10)

As described for the synthesis of (*R*)-**10**, (*S*)-**9** (327 mg, 1.04 mmol) was reduced with $LiBH_4$ (2 M in THF, 2.1 mL, 4.2 mmol) in THF_{abs}. Colorless oil, yield 111.3 mg (37 %). $[\alpha]_D^{20}$ = -46.0 (c = 0.71, MeOH).

Spectroscopic data of (*R*)-**10** and (*S*)-**10** are identical with the data for (±)-**10**.[24] $C_{17}H_{23}NO_3$ (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_2*CH_2OH (2H), N(CH_2*CH_2*)$_2$ (4H)), 2.08 (s, 3H, NCO*CH_3*), 2.60 (d, J = 15.9 Hz, 1H, Ar*CH_2*), 2.77 (dd, J = 15.9/11.1 Hz, 1H, Ar*CH_2*), 2.81 – 2.92 (m, 1H, N(*CH_2*CH_2)$_2$), 3.37 – 3.49 (m, 1H, N(*CH_2*CH_2)$_2$), 3.66 – 3.71 (m, 1H, N(*CH_2*CH_2)$_2$), 3.79 – 3.91 (m, 2H, *CH_2*OH), 3.92 – 4.04 (m, 1H ArCH$_2$*CH*), 4.49 – 4.57 (m, 1H, N(*CH_2*CH_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.

5.6.8. (*R*)-2-(3,4-Dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)ethanol ((*R*)-11)

A solution of (*R*)-**10** (190.2 mg, 0.66 mmol) in 2 M NaOH (20 mL) was heated under reflux for 3 h. The mixture was cooled to rt and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layer was dried (K_2CO_3), concentrated in vacuo and the residue was used directly without further purification. Colorless oil, yield 131.1 mg, (53 %), R_f = 0.03 (CH_2Cl_2 : MeOH = 95 : 5). $[\alpha]_D^{20}$ = +45.5 (c = 0.74, MeOH).

(*S*)-2-(3,4-Dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)ethanol ((*S*)-11)

As described for the synthesis of (*R*)-**11**, (*S*)-**10** (111 mg, 0.38 mmol) was hydrolyzed with 2 M NaOH (20 mL). Colorless oil, yield 73.3 mg (78 %). $[\alpha]_D^{20}$ = -47.1 (c = 0.72,

MeOH).

Spectroscopic data of (*R*)-**11** and (*S*)-**11** are identical with the data for (±)-**11**.[24] $C_{15}H_{21}NO_2$ (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_2*CH_2*)$_2$ (4H), *CH_2*CH_2OH (2H)), 2.55 (dd, J = 15.8/2.6 Hz, 1H, Ph*CH_2*), 2.75 (dd, J = 15.8/11.2 Hz, 1H, Ph*CH_2*), 2.85 – 3.15 (m, 4H, NH(*CH_2*CH_2)$_2$), 3.81 – 3.88 (m, 2H, CH_2*CH_2*OH), 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.

5.6.9. (*R*)-2–(1'-Octyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)ethanol ((*R*)-2a)

1-Bromooctane (34.7 mg, 0.18 mmol) and K_2CO_3 (30.3 mg, 0.22 mmol) were added to a solution of (*R*)-**11** (36.0 mg, 0.15 mmol) in acetonitrile$_{abs}$ (8 mL). The mixture was heated under reflux for 4 h. Then it was filtered and the solution was concentrated in vacuo. The residue was purified by fc (1 cm, cyclohexane : ethyl acetate = 1 : 1 + 0.1 % NH_3, 5 mL, R_f = 0.30). Colorless oil, yield 44.9 mg, (83 %). $[\alpha]_D^{20}$ = +36.2 (c = 1.76, MeOH). Purity determined by HPLC: 98.7 %.

(*S*)-2–(1'-Octyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)ethanol ((*S*)-2a)

As described for the synthesis of (*R*)-**2a**, (*S*)-**11** (21.7 mg, 0.09 mmol) was alkylated with 1-bromooctane (23.2 mg, 0.12 mmol) in the presence of K_2CO_3 (20.7 mg, 0.15 mmol). Colorless oil, yield 22.9 mg (71 %). $[\alpha]_D^{20}$ = -36.1 (c = 0.88, MeOH). Purity determined by HPLC: 99.4 %.

Spectroscopic data of (*R*)-**2a** and (*S*)-**2a** are identical with the data for (±)-**2a**.[24] $C_{23}H_{37}NO_2$ (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, $(CH_2)_7CH_3$), 1.22 – 1.32 (m, 10H, $(CH_2)_5CH_3$), 1.48 – 1.55 (m, 2H, $CH_2CH_2(CH_2)_5CH_3$), 1.63 – 1.72 (s, 1H, OH) 1.73 (dd, J = 13.7/2.7, 1H, $N(CH_2CH_2)_2$), 1.83 – 2.0 (m, 3H, $N(CH_2CH_2)_2$ (1H), CH_2CH_2OH (2H)), 2.08 (dd, J = 14.4/2.6, 1H, $N(CH_2CH_2)_2$), 2.18 – 2.33 (m, 3H, $N(CH_2CH_2)_2$ (1H), $N(CH_2CH_2)_2$ (2H)), 2.36 – 2.41 (m, 2H, $NCH_2(CH_2)_6CH_3$), 2.62 (dd, 1H, J = 13.3/2.6, $PhCH_2CH$), 2.78 – 2.87 (m, 3H, $N(CH_2CH_2)_2$ (2H), $PhCH_2CH$ (1H)), 3.90 (t, J = 5.5 Hz, 2H, CH_2CH_2OH), 3.98 – 4.05 (m, 1H, $PhCH_2CH$), 7.05 (d, J = 7.2, 1H, arom.), 7.12 – 7.22 (m, 3H, arom.). ^{13}C NMR ($CDCl_3$): δ [ppm] = 14.2 ($(CH_2)_5CH_3$), 22.8 ($(CH_2)_5CH_3$), 27.2 ($(CH_2)_5CH_3$), 27.9 ($(CH_2)_5CH_3$), 29.4 ($(CH_2)_5CH_3$), 29.7 ($(CH_2)_5CH_3$), 31.9 (CH_2CH_2OH), 35.1 ($N(CH_2CH_2)_2$), 35.5 ($N(CH_2CH_2)_2$), 38.1 (NCH_2CH_2), 39.2 ($ArCH_2$), 49.6 ($N(CH_2CH_2)_2$), 49.7 ($N(CH_2CH_2)_2$), 59.3 (NCH_2), 60.9 (CH_2CH_2OH), 68.0 ($OCHR_2$), 74.5 ($ArCR_2O$), 125.4 (C-Ar), 126.4 (C-Ar), 126.4 (C-Ar), 128.7 (C-Ar), 133.4 (C_q-Ar), 141.5 (C_q-Ar).

5.6.10. (*R*)-2-(1'-Benzyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)ethanol ((*R*)-2b)

Under N_2, a solution of (R)-**11** (18.4 mg, 0.07 mmol), benzaldehyde (20 mg, 0.18 mmol) and $NaBH(OAc)_3$ (36.0 mg, 0.17 mmol) in $CH_2Cl_{2\ abs}$ (8 mL) was stirred at rt for 3 h. Then, 0.5 M HCl (8 mL) was added and the organic layer was separated. The aqueous layers were alkalized with 0.5 M NaOH (12 mL) 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 : ethyl acetate = 1 : 1 + 0.1 %

NH_3, 5 mL, R_f = 0.14). Colorless oil, yield 20.4 mg, (87 %). $[\alpha]_D^{20}$ = +33.7 (c = 0.88, MeOH), 98.68 % ee. Purity determined by HPLC: 97.3 %.

(*S*)-2-(1'-Benzyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl)ethanol ((*S*)-2b)

As described for the synthesis of (*R*)-**2b**, (*S*)-**11** (21.1 mg, 0.08 mmol) was reacted with benzaldehyde (20 mg, 0.18 mmol) and $NaBH(OAc)_3$ (36.2 mg, 0.17 mmol) in $CH_2Cl_{2\ abs}$ (8 mL). Colorless oil, yield 23.3 mg (69 %). $[\alpha]_D^{20}$ = -33.0 (c = 0.46, MeOH), 100 % ee. Purity determined by HPLC: 95.7 %.

Spectroscopic data of (*R*)-**2b** and (*S*)-**2b** are identical with the data for (±)-**2b**.[24]

$C_{22}H_{27}NO_2$ (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). ^1H NMR ($CDCl_3$): δ [ppm] = 1.71 (dd, J = 13.4/2.7 Hz, 1H, $N(CH_2\mathit{CH_2})_2$), 1.84 – 2.01 (m, 3H, $N(CH_2\mathit{CH_2})_2$ (1H), $\mathit{CH_2}CH_2OH$ (2H)), 2.08 (dd, J = 14.2/4.5 Hz, 1H, $N(CH_2\mathit{CH_2})_2$), 2.22 (td, J = 13.1/4.4 Hz, 1H, $N(CH_2\mathit{CH_2})_2$), 2.30 – 2.44 (m, 2H, $N(\mathit{CH_2}CH_2)_2$), 2.61 (dd, J = 15.9/2.7 Hz, 1H, $Ar\mathit{CH_2}CH$), 2.72 – 2.83 (m, 2H, $N(\mathit{CH_2}CH_2)_2$), 2.83 (dd, J = 15.9/11.3 Hz, 1H, $Ar\mathit{CH_2}CH$), 3.57 (s, 2H, $N\mathit{CH_2}Ph$), 3.89 (t, J = 5.5 Hz, 2H, $CH_2\mathit{CH_2}OH$), 3.96 – 4.05 (m, 1H, $ArCH_2\mathit{CH}$), 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.

5.6.11. (*R*)-2-[1'-(3-Phenylpropyl)-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl]ethanol ((*R*)-2c)

1-Bromo-3-phenylpropane (73.7 mg, 0.37 mmol) and K_2CO_3 (62.2 mg, 0.45 mmol) were added to a solution of (*R*)-**11** (73.5 mg, 0.3 mmol) in $acetonitrile_{abs}$ (8 mL) and

the mixture was heated under reflux for 4 h. The mixture was filtered and the filtrate was concentrated in vacuo. 0.5 M HCl (10 mL) was added to the residue and the solution was washed with ethyl acetate (2 × 5 mL). The aqueous layer was alkalized with 0.5 M NaOOH (15 mL), 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 (1 cm, cyclohexane : ethyl acetate = 1 : 1 + 0.1 % NH_3, 5 mL, R_f = 0.25). Colorless oil, yield 47.4 mg (43 %). $[\alpha]_D^{20}$ = +37.7 (c = 1.93, MeOH). Purity determined by HPLC: 99.5 %.

(*S*)-2-[1'-(3-Phenylpropyl)-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl]ethanol ((*S*)-2c)

As described for the synthesis of (*R*)-**2c**, (*S*)-**11** (73.5 mg, 0.30 mmol) was reacted with 1-bromo-3-phenylpropane (73.7 mg, 0.37 mmol) and K_2CO_3 (62.2 mg, 0.45 mmol) in acetonitrile$_{abs}$ (8 mL). Colorless oil, yield 49.1 mg (45 %). $[\alpha]_D^{20}$ = -38.0 (c = 1.70, MeOH). Purity determined by HPLC: 99.3 %.

Spectroscopic data of (*R*)-**2c** and (*S*)-**2c** are identical with the data for (±)-**2c**.[24]

$C_{24}H_{31}NO_2$ (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_2*CH_2*)$_2$), 1.76 – 1.91 (m, 5H, N(CH_2*CH_2*)$_2$ (1H), *CH_2*CH_2OH (2H), *CH_2*CH_2Ph (2H)), 2.03 (dd, J = 14.3/2.6 Hz, 1H, N(CH_2*CH_2*)$_2$), 2.15 (td, J = 12.9/4.2 Hz, 1H, N(CH_2*CH_2*)$_2$), 2.20 – 2.29 (m, 2H, N$CH_2(CH_2)_2$Ph), 2.35 – 2.40 (m, 2H, N(*CH_2*CH_2)$_2$), 2.52 – 2.61 (m, 3H, Ar*CH_2*CH (1H), N$(CH_2)_2$*CH_2*Ph (2H)), 2.63 (s, 1H, *OH*), 2.71 – 2.81 (m, 3H, N(*CH_2*CH_2)$_2$ (2H), Ar*CH_2*CH (1H)), 3.79 – 3.88 (m, 2H, CH_2*CH_2*OH), 3.91 – 4.00 (m, 1H, ArCH_2*CH*), 6.99 (d, J = 7.3 Hz, 1H, arom.), 7.04 – 7.15 (m, 5H, arom.), 7.18 – 7.38 (m, 3H, arom.). ^{13}C NMR ($CDCl_3$): δ

(ppm) = 28.9 (CH_2CH_2OH), 33.9 ($NCH_2CH_2CH_2Ph$), 35.0 ($N(CH_2CH_2)_2$), 35.4 ($N(CH_2CH_2)_2$), 38.1 ($ArCH_2$), 39.1 ($ArCH_2$), 49.5 ($N(CH_2CH_2)_2$), 49.5 ($N(CH_2CH_2)_2$), 58.5 (NCH_2), 60.6 (CH_2CH_2OH), 67.7 ($OCHR_2$), 74.4 ($ArCR_2O$), 125.3 (C-Ar), 125.8 (C-Ar), 126.3 (C-Ar), 126.4 (C-Ar), 128.3 (C-Ar), 128.4 (2C, C-Ar), 128.7 (2C, C-Ar), 133.4 (C_q-Ar), 141.4 (C_q-Ar), 142.1 (C_q-Ar).

5.6.12. (*R*)-2-[1'-(Cyclohexylmethyl)-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl]ethanol ((*R*)-2d)

(Bromomethyl)cyclohexane (34 mg, 0.19 mmol) and K_2CO_3 (33.2 mg, 0.24 mmol) were added to a solution of (*R*)-**11** (33.1 mg, 0.16 mmol) in acetonitrile$_{abs}$ (8 mL) and the mixture was heated under reflux for 4 h. It was filtered, concentrated in vacuo and the residue was purified by fc (1 cm, cyclohexane : ethyl acetate = 1 : 1 + 0.1 % NH_3, 5 mL, R_f = 0.15). Colorless oil, yield 23.9 mg, (44 %). $[\alpha]_D^{20}$ = +39.0 (c = 0.73, MeOH). Purity determined by HPLC: 98.5 %.

(*S*)-2-[1'-(Cyclohexylmethyl)-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-3-yl]ethanol ((*S*)-2d)

As described for the synthesis of (*R*)-**2d**, (*S*)-**11** (32 mg, 0.16 mmol), was reacted with (bromomethyl)cyclohexane (28.3 mg, 0.16 mmol) and K_2CO_3 (27 mg, 0.2 mmol) in acetonitrile$_{abs}$ (8 mL). Colorless oil, yield 34.9 mg, (64 %). $[\alpha]_D^{20}$ = -38.1 (c = 1.18, MeOH). Purity determined by HPLC: 95.0 %.

Spectroscopic data of (*R*)-**2d** and (*S*)-**2d**

$C_{22}H_{33}NO_2$ (343.5). MS (EI): m/z = 343 [M], 260 [M-C_6H_{11}]. IR: ν [cm^{-1}] = 3357 (O-H); 2920, 2848 (C-H); 1062, 1045 (C-O); 754, 731 (C-H). ^{1}H NMR ($CDCl_3$): δ [ppm] = 0.84 – 0.95 (m, 2H, $NCH_2CH(CH_2CH_2)_2CH_2$), 1.14 – 1.29 (m, 4H,

$NCH_2CH(CH_2\mathit{CH_2})_2CH_2$), 1.46 – 1.54 (m, 1H, $NCH_2\mathit{CH}(CH_2CH_2)_2CH_2$), 1.64 – 1.74 (m, 4H, $NCH_2CH(\mathit{CH_2}CH_2)_2CH_2$), 1.80 (d, J = 13.8, 2H, $N(CH_2\mathit{CH_2})_2$), 1.85 – 1.96 (m, 2H, $\mathit{CH_2}CH_2OH$), 2.07 (dd, J = 14.3/2.5 Hz, 1H $N(CH_2\mathit{CH_2})_2$), 2.18 (d, J = 7.05 Hz, 2H, $N\mathit{CH_2}C_6H_{11}$), 2.2 – 2.28 (m, 3H, $N(CH_2\mathit{CH_2})_2$ (1H), $N(\mathit{CH_2}CH_2)_2$ (2H)), 2.62 (dd, J = 15.9/2.6 Hz, 1H, $Ph\mathit{CH_2}$), 2.70 – 2.79 (m, 2H, $N(\mathit{CH_2}CH_2)_2$), 2.83 (dd, J = 15.9/11.1 Hz, $Ph\mathit{CH_2}$), 3.9 (t, J = 5.3 Hz, 2H $CH_2\mathit{CH_2}OH$), 3.98 – 4.05 (m, 1H, $PhCH_2\mathit{CH}$), 7.05 (d, J = 7.3 Hz, 1H, Aromat), 7.12 – 7.23 (m, 3H, Aromat).

5.6.13. Methyl 2-[(1*R*,3*R*)-1-(2-chloroethyl)-3,4-dihydro-1*H*-2-benzopyran-3-yl]acetate ((*R*,*R*)-13)

Under N_2, a solution of chloropropionaldehyde acetal **12** (747 mg, 4.5 mmol) in $toluene_{abs}$ (1.5 mL) was added to a solution of (*R*)-**4** (291.4 mg, 1.5 mmol) in $CH_2Cl_{2\ abs}$ (14 mL) and the solution was stirred at rt for 30 min. The solution was cooled to 0 °C, $BF_3{\bullet}Et_2O$ (2.25 mL, 14 mmol) was added and the mixture was stirred at rt for 5 d. After addition of 0.5 M HCl (15 mL), 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 : ethyl acetate 9 : 1, 20 mL, R_f = 0.33). Pale yellow oil, yield 128.4 mg (32 %). $[\alpha]_D^{20}$ = +80.7 (c = 2.46, MeOH).

Methyl 2-[(1*S*,3*S*)-1-(2-chloroethyl)-3,4-dihydro-1*H*-2-benzopyran-3-yl]acetate ((*S*,*S*)-13)

As described for the synthesis of (*R*,*R*)-**13**, (*S*)-**4** (194.2 mg, 1.0 mmol) in $CH_2Cl_{2\ abs}$ (14 mL) was reacted with chloropropionaldehyde acetal **12** (498 mg, 3.0 mmol) in $toluene_{abs}$ (1.5 mL) and $BF_3{\bullet}Et_2O$ (1.5 mL, 9 mmol). Pale yellow oil, yield 71.6 mg (27 %). $[\alpha]_D^{20}$ = -80.2 (c = 0.675, MeOH).

Spectroscopic data of (*R*,*R*)-**13** and (*S*,*S*)-**13** are identical with the data for (±)-**13**.[24] $C_{14}H_{17}ClO_3$ (268.5). 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.).

5.6.14. Methyl 2-{(1*R*,3*R*)-1-[2-(piperidin-1-yl)ethyl]-3,4-dihydro-1*H*-2-benzopyran-3-yl}acetate ((*R,R*)-3a)

A mixture of (*R*,*R*)-**13** (58.5 mg, 0.20 mmol), piperidine (0.025 mL, 0.25 mmol), K_2CO_3 (34.5 mg, 0.25 mmol) and Bu_4NI (catalytic amount) in acetonitrile$_{abs}$ (8 mL) was heated under reflux for 20 h. The mixture was filtered, concentrated in vacuo and the residue was dissolved in 0.5 M HCl (10 mL). After extraction with ethyl acetate (2 x 5 mL), the aqueous layer was alkalized with 0.5 M NaOH (15 mL) 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 : ethyl acetate = 1 : 1 + 0.1 % NH_3, 5 mL, R_f = 0.25). Pale yellow oil, yield 23.9 mg (38 %). $[\alpha]_D^{20}$ = +66.4 (c = 0.59, CH_2Cl_2). Purity determined by HPLC: 96.1 %.

Methyl 2-{(1*S*,3*S*)-1-[2-(piperidin-1-yl)ethyl]-3,4-dihydro-1*H*-2-benzopyran-3-yl}acetate ((*S,S*)-3a)

As described for the synthesis of (*R*,*R*)-**3a**, a mixture of (*S*,*S*)-**13** (66.0 mg, 0.24 mmol), piperidine (0.03 mL, 0.3 mmol), K_2CO_3 (41.5 mg, 0.30 mmol) and Bu_4NI (catalytic amount) in acetonitrile$_{abs}$ (8 mL) was heated under reflux and worked-up.

Pale yellow oil, yield 37.6 mg (49 %). $[\alpha]_D^{20}$ = -65.2 (c = 2.10, CH_2Cl_2). Purity determined by HPLC: 98.5 %.

Spectroscopic data of (*R*,*R*)-**3a** and (*S*,*S*)-**3a** are identical with the data for (±)-**3a**.[24] $C_{19}H_{27}NO_3$ (317.4). MS (EI): m/z = 317 [M], 244 [M – $CH_2CO_2CH_3$]. IR: ν [cm^{-1}] = 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*)$_2CH_2$), 1.82 – 1.92 (m, 1H, NCH_2*CH_2*), 2.10 – 2.20 (m, 1H, NCH_2*CH_2*), 2.27 – 2.46 (m, 6H, N(*CH_2*CH_2)$_2CH_2$ (4H), NCH_2*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.). ^{13}C NMR ($CDCl_3$): δ (ppm) = 24.4 (C-4_{pip}), 25.9 (2C, C-3_{pip}, C-5_{pip}). 33.0 (OCH*C*H_2), 34.6 (Ar-CH_2), 41.2 (2C, C-2_{pip}, C-6_{pip}), 51.8 (CO_2*C*H_3), 54.7 (*C*H_2CO_2R), 55.3 (NCH_2), 71.0 (O*C*HR_2), 76.0 (Ar*C*R_2O), 124.4 (C-Ar), 126.5 (2C, C-Ar), 128.9 (C-Ar), 133.6 (C_q-Ar), 137.7 (C_q-Ar), 171.7 (C=O).

5.6.15. Methyl 2-{(1*R*,3*R*)-1-[2-(4-phenylpiperidin-1-yl)ethyl]-3,4-dihydro-1*H*-2-benzopyran-3-yl}acetate ((*R,R*)-3b)

A mixture of (*R*,*R*)-**13** (128.4 mg, 0.47 mmol), 4-phenylpiperidine (88.7 mg, 0.55 mmol), K_2CO_3 (76.0 mg, 0.55 mmol) and Bu_4NI (catalytic amount) in $acetonitrile_{abs}$ (12 mL) was heated under reflux for 20 h. The mixture was filtered, the filtrate was concentrated in vacuo and the residue was purified by fc (2 cm, cyclohexane : ethyl acetate = 1 : 1 + 0.1 % NH_3, 10 mL, R_f = 0.25). Pale yellow oil, yield 56.3 mg (30 %). $[\alpha]_D^{20}$ = +61.3 (c = 1.04, CH_2Cl_2). Purity determined by HPLC: 97.1 %.

Methyl 2-{(1*S*,3*S*)-1-[2-(4-phenylpiperidin-1-yl)ethyl]-3,4-dihydro-1*H*-2-benzopyran-3-yl}acetate ((*S,S*)-3b)

As described for the synthesis of (*R*,*R*)-**3b**, a mixture of (*S*,*S*)-**13** (38.0 mg, 0.14 mmol), 4-phenylpiperidine (40.3 mg, 0.25 mmol), K_2CO_3 (34.5 mg, 0.25 mmol) and Bu_4NI (catalytic amount) in acetonitrile$_{abs}$ (8 mL) was heated under reflux and worked-up. Pale yellow oil, yield 28.6 mg, (52 %). $[\alpha]_D^{20}$ = -61.3 (c = 1.28, CH_2Cl_2). Purity determined by HPLC: 96.7 %.

Spectroscopic data of (*R*,*R*)-**3b** and (*S*,*S*)-**3b** are identical with the data for (±)-**3b**.[24] $C_{25}H_{31}NO_3$ (393.5). MS (EI): m/z = 393 [M], 320 [M -$CH_2CO_2CH_3$], 174 [Ph-$CH(CH_2CH_2)_2NCH_2$]. 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, $N(CH_2\mathit{CH_2})_2$), 1.85 – 1.97 (m, 2H, $NCH_2\mathit{CH_2}$) 2.0 – 2.08 (m, 1H, $N(\mathit{CH_2}CH_2)_2$), 2.14 – 2.24 (m, 1H, $N(\mathit{CH_2}CH_2)_2$), 2.38 – 2.44 (m, 2H, $N(CH_2CH_2)_2\mathit{CH}$Ph (1H), $N\mathit{CH_2}CH_2$ (1H)), 2.45 – 2.58 (m, 1H, $N\mathit{CH_2}CH_2$), 2.52 (dd, J = 15.2/5.5 Hz, 1H, $\mathit{CH_2}CO_2CH_3$), 2.64 (dd, J = 15.2/7.7 Hz, 1H, $\mathit{CH_2}CO_2CH_3$), 2.64 – 2.75 (m, 2H, Ph$\mathit{CH_2}$), 3.0 (d, J = 11.3Hz, 2H, $N(\mathit{CH_2}CH_2)_2$), 3.66 (s, 3H, $CO_2\mathit{CH_3}$), 4.0 – 4.09 (m, 1H, $PhCH_2\mathit{CH}$), 4.8 (d, J = 6.5 Hz, 1H, PhCHO), 7.01 (d, J = 8.4 Hz, 1H, arom.), 7.02 – 7.22 (m, 8H, arom.). ^{13}C NMR ($CDCl_3$): δ (ppm) = 32.9 (OCH$\mathit{C}H_2$), 33.0 (2C, C-3_{pip}, C-5_{pip}), 35.1 (Ar-CH_2), 37.9 (C-4_{pip}), 42.5 (2C, C-2_{pip}, C-6_{pip}), 54.0 ($CO_2\mathit{C}H_3$), 55.0 ($\mathit{C}H_2CO_2R$), 61.2 (NCH_2), 74.7 (O$\mathit{C}HR_2$), 75.7 (Ar$\mathit{C}R_2O$), 124.3 (C-Ar), 126.3(C-Ar), 126.5 (C-Ar), 126.6 (C-Ar), 127.0 (2C, C-Ar), 128.5 (2C, C-Ar), 129.0 (C-Ar), 133.9 (C_q-Ar), 137.4 (C_q-Ar), 146.0 (C_q-Ar), 176.3 (very small, C=O).

5.7. Receptor binding studies

The affinity of the 2-benzopyrans **2** and **3** towards σ_1, and σ_2 receptors was recorded as described in lit.[52-54]. The affinity towards the PCP binding site of the NMDA receptor was recorded as reported in lit.[55,58,] Details of the assay are given in the Supporting Information.

Supporting Information available

Supporting Information is available free of charge via the Internet. It includes Figures showing the experiments analyzing the development of *ee* -values during lipase-catalyzed reactions, experimental details of the receptor binding studies, and results of the cholesterol biosynthesis assay.

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Graphical Abstract