New propranolol analogues: binding and chiral discrimination by cellobiohydrolase Cel7A

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Novel propranolol analogues have been designed and synthesised and their enantioselective binding to the cellulose degrading enzyme, Cel7A, has been evaluated. Affinity and enantioselectivity have been determined by capillary electrophoresis experiments. Ligands with significantly improved affinity and selectivity have been obtained and an analysis of the results has led to insights concerning the relation between the changes in ligand structure and selectivity as well as affinity to the protein.

Introduction

Propranolol (**1**) is a chiral, adrenergic β-blocker that binds enantioselectively to the active site of cellobiohydrolase Cel7A[1] produced by the cellulose degrading fungus *Trichoderma reesei.* The enzyme Cel7A hydrolyses the β-1,4-glycosidic linkage of cellulose from the reducing end.[2] Several three-dimensional crystallographic structure determinations are available for Cel7A, including mutants without catalytic capacity.[1,3–5] The enzyme is characterized by the presence of a 50 Å long tunnel, which binds 7 glucosyl units before the catalytically active site and an exit opening for its hydrolytic product cellobiose (Fig. 1).[1] The active site has been localized through structural studies of complexes with stable ligands and the importance and function of the catalytic amino acid residues have been determined through point mutations at Glu212, Asp214 and Glu217.[6]

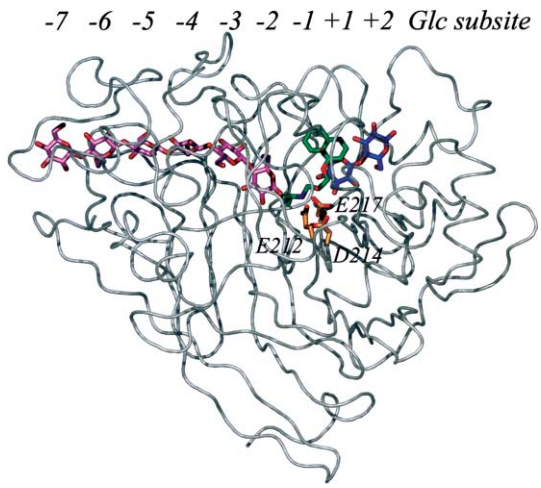

Fig. 1 Cel7A with cellulose (pink) placed in the entrance tunnel, propranolol (**1**) (green) at the active site, catalytic amino acids (orange) and cellobiose (blue). Reprinted from ref. 1 with permission from Elsevier.

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The use of Cel7A as a chiral stationary phase for enantiomer separation of various chiral drugs is well established.[7,8] The (*S*)-enantiomer of propranolol, the active β-adrenoceptor blocking enantiomer,[9] binds more strongly to the active site in Cel7A than the (*R*)-enantiomer.[10] The side chain of propranolol carrying the stereogenic atom binds to the catalytic amino acids. This interaction has been shown to be responsible for the chiral discrimination.[11,12] The X-ray crystallographic study of Cel7A with bound (*S*)-propranolol opens for rational design of analogues with higher binding abilities and stereoselectivities. These compounds will be used to provide a better understanding of the chiral recognition mechanism of Cel7A, which is the objective of this study.

Design of analogues

The binding site of (*S*)-propranolol (**1**) was explored by preliminary molecular mechanics computations using the AMBER* force field in Macromodel version 6.5. The analysis reveals several possible molecular modifications to affect the binding as shown in Fig. 2: The isopropyl group resides in a comparatively hydrophilic but constrained region built up by the polar or ionized residues Asp173 and Tyr145 as well as the catalytic amino acid residues Glu212, Asp214 and Glu217. The introduction of small polar groups on the ligand isopropyl side chain may give further electrostatic interactions, including hydrogen bonding to these amino acid residues (1). Thus, an analogue substituted at the methyl groups by hydroxyl groups was subjected to a conformational search using the Monte Carlo multiple minimum (MCMM) method. The protein was stripped of water molecules and free movement with respect to all degrees of freedom was allowed for the ligand. A shell of protein residues at a distance of 6 Å from the ligand was subjected to restricted flexibility using the default force constant (100 kJ $Å^{-1}$), whereas the rest of the protein was kept fixed. In the resulting low energy conformation both methyl bound hydroxyl groups were hydrogen bonded to Asp173 and Asp214, respectively (Fig. 3). The hydroxyl group calculated to be hydrogen bonded to Asp214 is also close to Glu217 (O–O distance being 2.71 and 2.83 Å, respectively). The position of the remaining part of the molecule was only slightly modified. In the crystal structure the naphthalene ring is positioned in the tunnel and stacks with the indole ring of Trp376. Additional

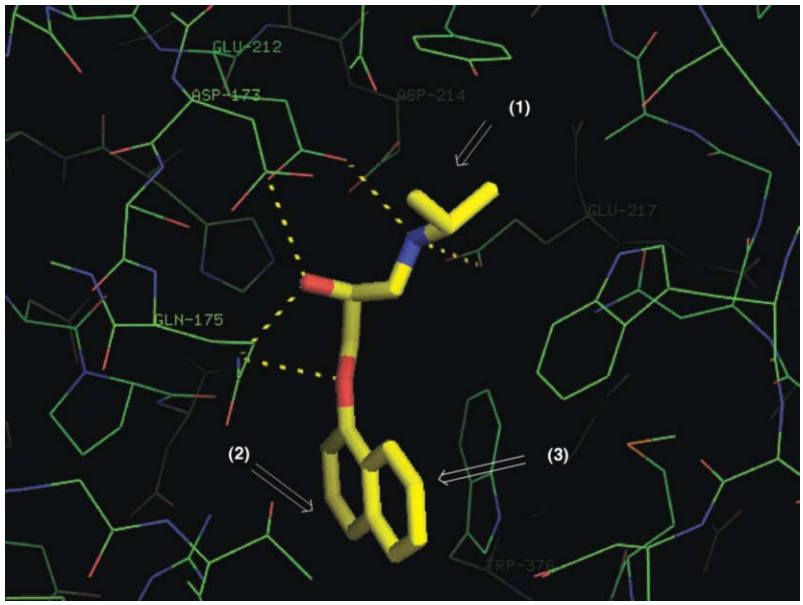


Fig. 2 The binding site of propranolol in Cel7A from the X-ray diffraction structure. Hydrogen bonding to the oxygen atoms and nitrogen atom of propranolol are indicated. The three positions on propranolol identified for structural modifications are indicated by (1–3).

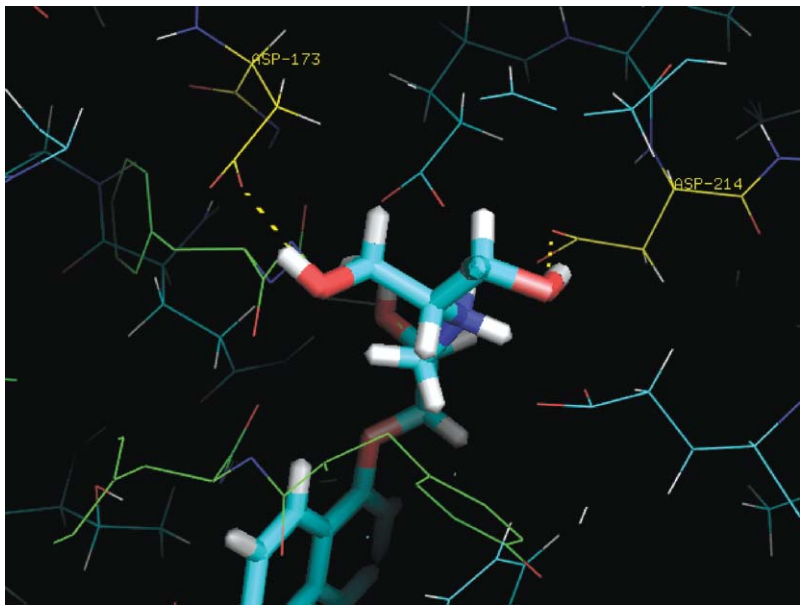


Fig. 3 Minimized structure of the dihydroxyl analogue **2** showing hydrogen bonds to the amino acid residues Asp173 and Asp214. For computational details see the text.

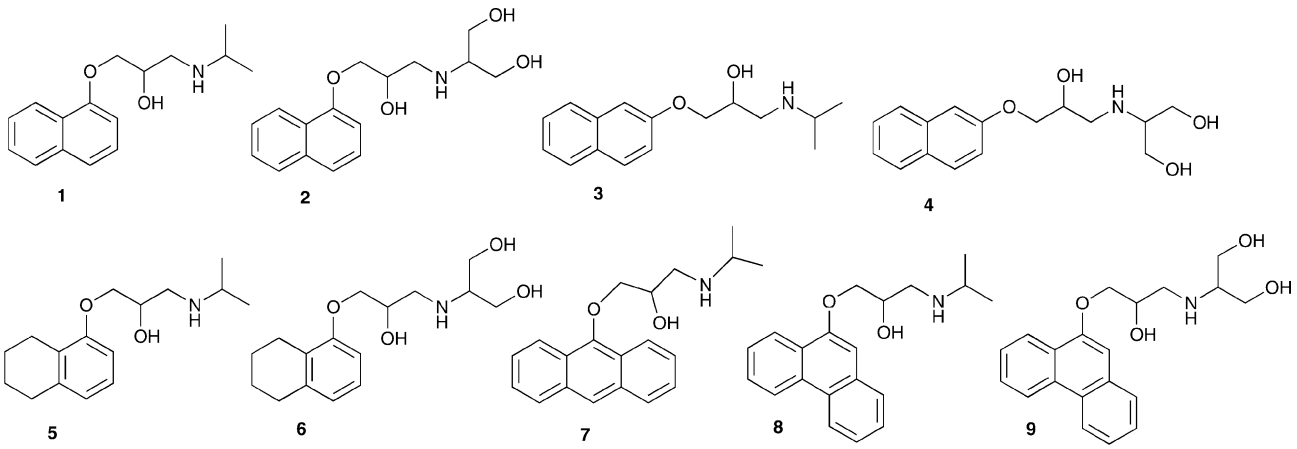
binding to other amino acid residues in the tunnel could be envisioned from modifications of the aromatic ring system (2) or by adding suitable substituents (3). Preliminary modelling studies suggest that anthracene or phenanthrene ring systems could be accommodated in this position and enhance stacking and hydrophobic effects. Exploring further effect of substitution in region (3) will be described in later work.

Fig. 4 shows a superposition of propranolol and its phenanthrene analogue minimized as described above in the protein complex. The results of this computational modelling directed us to synthesise a series of novel propranolol analogues (Scheme 1).

Results

Synthesis

The general synthetic routes used in the preparation of the new ligands are shown in Scheme 2. The glycidyl ethers have previously been synthesised in several different ways. However, the two main approaches use either CsF or NaH as base. It has been reported that CsF gives better yields and selectivities,[13,14] since it activates the epoxide as well as functioning as a base.[15] In this study we chose to try both bases for all compounds (Table 1). The use of CsF gave equivalent or higher yields for all compounds except for **13**. This



Scheme 1 Ligands synthesised.

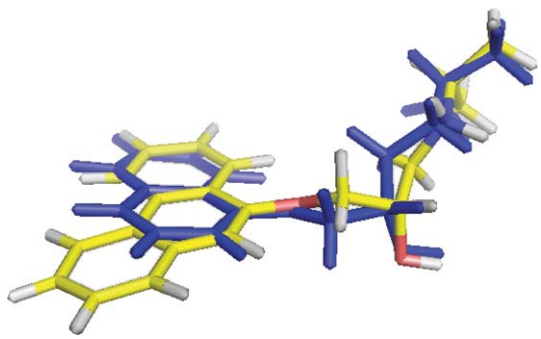
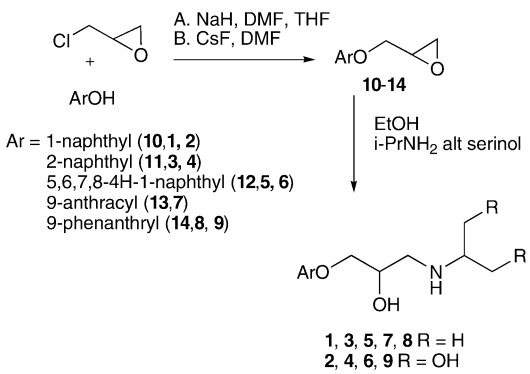


Fig. 4 Superimposition of propranolol **1** and its phenanthrene analogue **8** minimized in the binding site of Cel7A. For computational details see the text.



Scheme 2 General synthesis of amino alcohols.

could be explained by the activation of the aryl alcohol by CsF.[16] Initially, this increases the proton-accepting capabilities of the base as well as providing a counter ion to the resulting aryloxide, facilitating the desired reaction. This activation cannot be as easily achieved in the case of **13**, due to the increased distance between the carbonyl oxygen, and the acidic proton to be abstracted in anthrone.

Further, purified glycidyl ether as well as crude product have been used in the following amination step, as reported in Table 2, and no major differences in overall yields were noted for most compounds.

Table 1 Yields of glycidyl ethers, using two different bases

	Ar–OH	NaH (%)[a]	CsF (%)[b]
	10	50	72
	11	49	82
	12	71	87
	13	46	34
	14	78	82

[a] Method A. [b] Method B.

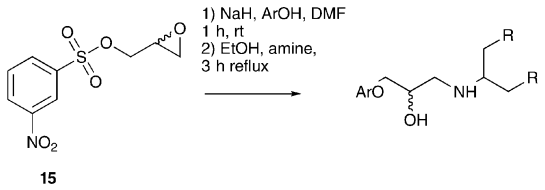
Table 2 Amination of glycidyl ethers with two different procedures

Ligand	Yield (%)[a]	Yield A (%)[b]	Yield B (%)[c]
1	82	64	65
2	39	17	14
3	97	68	63
4	51	31	39
5	74	38	52
6	18	19	14
7	95	47	64
8	64	65	49
9	67	22	16

[a] Yield from purified glycidyl ether. [b] Overall yield, using the direct route from glycidyl ether Method A, NaH as base. [c] Overall yield, using the direct route from glycidyl ether Method B, CsF as base.

To be able to determine the elution order of the enantiomers, highly enriched enantiomers of the compounds had to be produced. In the first step of the racemic compounds, epichlorohydrine was used. According to previous investigations of the mechanism, the nucleophilic attack occurs in both the 1- and 3-position and is rather slow with long reaction times, since both epoxide and chloride are relatively poor leaving groups.[17] To circumvent these problems when synthesising pure enantiomers, nosylate **15** was chosen as the reagent and NaH as the base (Scheme 3), which afforded a greater preference for reaction at the 1-position.[18]

A combination of DMF and THF was used as solvent when NaH was used as base in the reaction with epichlorohydrin to obtain better yields. THF increases solubility of starting materials and may also have a solvent effect.[17] However, all reactions with



Scheme 3 Synthesis of enantiomers, using nosylate (**15**) as reagent.

the more reactive nosylate **15**, worked well with only DMF as a solvent.

The direct route described in the Experimental section, has a simple and straightforward workup procedure. A silica filtration was included only to remove remaining DMF in order to simplify purification of the final product. All pure enantiomers were synthesised using the direct route, since the purity of the glycidyl ethers seemed to be of less importance in the subsequent amination step.

Purification of all compounds with the additional dihydroxyl groups was difficult and a lot of product was probably lost in the extraction prior to the recrystallisation. However, the extraction was necessary to achieve a solid product of sufficient purity for recrystallisation.

To verify the stereochemistry of the enantiomers, propranolol was used as a reference compound to confirm selectivity of the reaction with nosylate as the reagent. The ee, and therefore the reaction selectivity, as well as the retention order of enantiomers, was determined by HPLC, using the chiral stationary phase OD–H silica. The (*R*)-enantiomer elutes first in all cases and the ee was >99%.

Binding studies

Methods based on capillary electrophoresis, CE, were utilised to determine affinity constants as well as enantioselectivity. An important reason for choice of method, is the possibility to study the protein ligand interaction in free solution, *i.e.*, without previous manipulation of the protein structure, such as its immobilisation on a support particle. This ensures a protein with a natural fold and without other possible structural disturbances, maintaining full dynamics in solution. The partial-filling technique (Fig. 5) was applied as previously described,[19] as it demands very small amounts of protein and facilitates UV-detection without the interference of protein. **1**–**6**, **8**, **9** had a chemical purity of >97% as determined by NMR and **7** had a chemical purity of 91% as determined by HPLC.

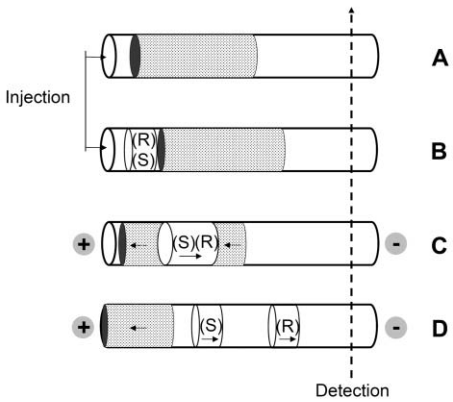


Fig. 5 A description of the partial-filling technique using CE. (A) Injection of selector, Cel7A. (B) Injection of racemic compound. (C) Applying an electrical current will drive the oppositely charged species in different directions and the analyte, compound **1**–**9**, interacts with the selector, Cel7A. (D) The separated enantiomers are detected and migration times determined.

Affinity

To obtain K_d-values for the binding of ligands to Cel7A, migration times of the racemic ligands, through a series of different plug lengths of Cel7A, were measured. As the binding strength of a ligand increased, the concentration of protein was decreased, keeping plug lengths constant.[20] The differences in migration times compared to when no protein was present, were plotted *versus* the amount of Cel7A. The amount of protein was calculated from the absorbance and therefore K_d-values are only related to total amount of protein, not the activity of the enzyme. The K_d-values of the (*R*)- and (*S*)-enantiomers of all compounds were calculated according to eqn (1)[21] and are given in Table 3.

$$\frac{1}{K_d} = \frac{\pi \times r^2 \times l_{det}}{t_{0,det}} \times \frac{d\Delta t}{dn} \quad (1)$$

Where r is the radius and l_{det} is the length of capillary to detection window, $t_{0,det}$ is the migration time of the analyte when no protein is present and $d\Delta t/dn$ is the slope of the linear correlation between the increase in migration time, Δt (s), and the total amount of protein n (mol Cel7A).

Two structural modifications of propranolol (**1**) proposed by the design, turned out to lead to significant increase in affinity.

Table 3 K_d-values and binding relative to **1** at pH 5.0 (25 °C) as well as calculated intrinsic selectivity, α^i

Solute	$K_d(R)$/μM[a]	Relative affinity[c]	$K_d(S)$/μM[a]	Relative affinity[c]	α^i [b]
1	620	1.00	245	1.00	2.53
2	134	4.63	41	5.98	3.27
3	720	0.86	330	0.74	2.18
4	338	1.83	68	3.60	4.97
5	887	0.70	367	0.67	2.42
6	410	1.51	72	3.40	5.69
7	316	1.96	170	1.44	1.86
8	133	4.66	12.7	19.3	10.47
9	30	20.7	1.8	136	16.67

[a] Calculated according to eqn (1). [b] Calculated according to eqn (2). [c] In comparison to **1**.

First, an increase in the size of the aromatic system increases binding. However, this enhancement is much more pronounced with phenanthrene (**8**) than with anthracene (**7**). Looking at the known structure of the protein with propranolol in the active site, an aromatic stacking is indicated between the naphthyl ring and the indole of Trp376. The difference in binding of anthracene and phenanthrene probably arises from the positioning of the ring systems, where the phenanthrene ring system has a different directional positioning. This may facilitate interactions with a second tyrosine (Trp367) as well as increasing the interaction with Trp376, hence the difference in K_d-values. On the other hand, when the aromatic system was reduced (**5**) or position of the side chain changed (**3**) in comparison to the naphthyl in **1**, a decrease in binding was observed.

Secondly, the introduction of two hydroxyls at the isopropyl (**2**, **4**, **6** and **9**) increased the affinity. This is probably due to further electrostatic interactions with increased number of amino acid residues. Generally, the dihydroxy function increased binding of the (*S*)-enantiomer in all cases. This indicates an additional interaction which is independent of the changes in the aromatic system. However, this increase in affinity is somewhat different for the (*R*)-enantiomers, which show a variation through the series. A combination of the two structural modifications leading to enhanced affinity results in **9**. This compound has the strongest affinity.

Selectivity

It has previously been shown that both enantiomers of propranolol bind to the active site (no other strong selective bindings have been accounted for). The chiral selectivity is maintained throughout the series of compounds, confirming a stereospecific interaction between the active site and the amino alcohol moiety, which is common for all ligands studied. Also, through correlating the synthesis, the HPLC analysis and CE measurements, we can determine that it is the (*S*)-enantiomer which is most strongly retained in all cases, as is the usual case for all investigated β-blockers.

To compare enantiomeric selectivities, the intrinsic selectivity, α^i, was calculated [eqn (2)]. The results are given in Table 3.

$$\alpha^i = \frac{K_d(R)}{K_d(S)} \quad (2)$$

There is a general trend that the intrinsic selectivity increases with affinity, with the only exception of compound **7**. Dihydroxy substitution in the two methyl groups leads to an increase of both affinity and selectivity and could be compared throughout the series. Interestingly, this effect seems to be of greater importance when the hydrophobic interactions are weakened. When **4** and **6** are compared to their analogues, **3** and **5**, respectively, a major increase in selectivity is revealed when related to the increase in selectivity in the case of **2** compared to **1**. However, the change in affinity due to the addition of the two hydroxyl groups is not as favourable in the cases of **4** and **6** as for **2**. Also, it could be noted that the increased selectivity mainly is related to the reduced increase of the affinity of the (*R*)-enantiomer.

A striking decrease in selectivity was noticed in **7**, even if the affinity is increased compared to **1**, **3** and **5**. On the other hand, both **8** and **9** show a major increase in selectivity along with an increase in binding, even if the selectivity contribution from the dihydroxy function is less pronounced in this case. These findings indicate an interesting combinatorial effect on the selectivity, caused by the dihydroxy functionality and the derivatisation of the ring system.

With respect to the changes in affinity of the (*S*)-enantiomers, it is implied that the two different interactions are without any obvious conformational restraints on each other, neither in the ligand nor in the protein. Hence, enough flexibility is present in this complex to allow for some movement in the bound ligand.

On the other hand, the affinity of the (*R*)-enantiomers are affected in a way which indicates an interconnected dependency of the two interaction points. This may be due to some kind of locking of conformation in the enzyme or in the ligand.

These results also shows that the intrinsic selectivity is not directly connected to the general increase in binding strength.

Conclusion

We have designed and synthesised new ligands, which bind to the active site of Cel7A, based on the previous knowledge of the enzyme structure, using computational modelling as an important tool for the design. Through careful selection of compounds, the importance of the hydrophobic effects and stacking interactions between ligand and Trp376 have been shown, as well as the possibility to displace water in the protein structure to achieve further electrostatic interactions with the amino acid residues Asp173, Glu212, Glu217 and Asp214. Interestingly, both of these interactions have an effect on the enantiomeric selectivity. These interactions also seem to be interconnected. However, the increase in affinity and the increase in selectivity are not strictly correlated.

The two main features giving the strongest increase in binding were combined in **9**, which has an affinity strong enough to be expected to cause inhibition of enzyme activity. However, further investigation of the binding of ligands could provide more information about the effects causing the changes in selectivity. Such studies, as well as enzyme inhibition experiments, are in progress.

Experimental

Purchased chemicals were used as received and solvents for dry conditions were kept over activated 4 Å molecular sieves. Flash column chromatography was carried out using Matrex (0.063–0.200 mm) under pressure. Analytical thin layer chromatography (TLC) was performed on glass plates pre-coated with Merck Kieselgel 60 F_{254} and visualised by ultra-violet irradiation (254 nm). All compounds were dried under vacuum until all solvent was removed before analyses. Melting points were performed on a Electrothermal IA9100 Digital Melting Point Instrument, and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 LC polarimeter using a sodium lamp (589 nm) as the light source. Infra-red spectra were obtained on a Nicolet Impact 410 spectrometer, using KBr plates or paraffin. High resolution mass spectra were obtained on a Jeol JMS SX-102, by FAB+. HPLC analyses were performed on a Varian ProStar apparatus, Daicel OD–H column 4.6 × 250 mm, with 80 : 20 hexane–*i*PrOH as eluent, at a flow of 0.5 mL min^{-1}, with a Varian Prostar PDA detector at 254 nm. ^1H NMR spectra were recorded at ambient

temperature on a Bruker DRX400 NMR spectrometer at 400 MHz with residual protic solvents $CHCl_3$ ($\delta_H = 7.28$ ppm) or d_5-pyridine ($\delta_H = 8.74$ ppm) as the internal reference; chemical shifts (δ) are given in parts per million (ppm) and coupling constants (J) are given in Hertz (Hz). The proton spectra are reported as follows δ/ppm (number of protons, multiplicity, coupling constants J/Hz, assignment). For concentration dependent compounds, ^{1}H NMR spectra were taken at a concentration of 1 mg/0.6 mL for **1**, **5**, **8** 3 mg/0.6 mL for **2**, **4**, **6**, **9** and 5 mg/0.6 mL for **3**. ^{13}C NMR spectra were recorded at ambient temperature on the same spectrometer at 100 MHz, with the central peak of $CHCl_3$ ($\delta_C = 77.23$ ppm) or the central peak of d_5-pyridine ($\delta_C = 150.4$ ppm) as the internal reference. HMQC NMR spectroscopy were used where appropriate, to aid in the assignment of signals in the ^{1}H and ^{13}C NMR spectra. Where coincident coupling constants have been observed in the ^{1}H NMR spectrum, the apparent multiplicity of the proton resonance concerned has been reported. Elemental analyses were performed by A. Kolbe, Mikroanalytisches Laboratorium, Germany. Evaporation refers to the removal of solvent under reduced pressure.

Glycidyl ethers, method A[22]

Aryl alcohol was added to NaH (1.1–1.3 eq.) dissolved in dry DMF (1.5 mL/mmol ArOH) and dry THF (0.3 mL/mmol ArOH) under inert conditions and heated at 50 °C for 10–30 min (colour change). Epichlorohydrine (1.2 eq.) was added and the mixture was left stirring at 50 °C, followed by TLC (EtOAc–heptane 30 : 70) until no more reaction (11–22 h, colour change and sometimes precipitate formed). The mixture was allowed to reach rt and H_2O (10–25 mL/mmol) was added, which gave a strong precipitation, and stirred for 20 min at rt. The mixture was extracted with EtOAc and the organic layers were dried (Na_2SO_4), filtered and evaporated. Short filtration through silica (EtOAc–heptane 50 : 50) gave a crude product which could be used directly. Flash column chromatography afforded pure product.

2-(Naphthalen-1-yloxymethyl)oxirane (10). From 1-naphthol (5.77 g, 40.04 mmol), stirred at 50 °C for 16 h, workup gave a brown oil, which was purified by flash column chromatography (EtOAc–heptane 10 : 90) and afforded **10** as a clear oil (4.01 g, 50%). ν_{max}(paraffin)/cm^{-1} 3062, 2720, 1582, 1506, 1401, 1273, 1245, 1102, 1069, 1021, 917, 865, 793, 770, 727. δ_H(400 MHz; $CDCl_3$) 2.88 (1H, dd, J 2.6 and 4.9, *trans*-CHCH_2O), 3.00 (1H, app t, J 4.5, *cis*-CHCH_2O), 3.51–3.55 (1H, m, CH_2CH(O)CH_2), 4.18 (1H, dd, J 5.5 and 11.0, CHCH_2OAr), 4.43 (1H, dd, J 3.1 and 11.0, CHCH_2OAr), 6.84 (1H, d, J 7.5, 2-H), 7.38 (1H, app t, J 7.9, 3-H), 7.46–7.53 (3H, m, 4-H, 6-H, 7-H), 7.81–7.83 (1H, m, 5-H), 8.31–8.33 (1H, m, 8-H). δ_C(100 MHz; $CDCl_3$) 44.7, 50.2, 68.9, 104.9, 120.8, 122.0, 125.3, 125.5, 125.7, 126.5, 127.4, 134.5, 154.2.

2-(Naphthalen-2-yloxymethyl)oxirane (11). From 2-naphthol (2.88 g, 20.0 mmol), stirred at 50 °C for 22 h, workup gave a brown mixture, which was purified by flash column chromatography (EtOAc–heptane 5 : 95) and afforded **11** as white crystals (1.94 g, 49%). mp = 63.5–64.4 °C. (Found: C, 77.9; H, 6.0. $C_{13}H_{12}O_2$ requires C, 78.0; H, 6.0%.) ν_{max}(KBr)/cm^{-1} 3062, 2929, 1630, 1592, 1511, 1255, 1226, 1927, 913, 842, 737. δ_H(400 MHz; $CDCl_3$) 2.84 (1H, dd, J 2.6 and 4.9, *trans*-CHCH_2O), 2.97 (1H, app t, J 4.5, *cis*-CHCH_2O), 3.43–3.47 (1H, m, CH_2CH(O)CH_2), 4.11 (1H, dd, J 5.7 and 11.0, CHCH_2OAr), 4.37 (1H, dd, J 3.2 and 11.0, CHCH_2OAr), 7.16 (1H, d, J 2.5, 1-H), 7.21 (1H, dd, J 2.5 and 8.9, 3-H), 7.35–7.39 (1H, m, 6-H), 7.44–7.48 (1H, m, 7-H), 7.74–7.80 (1H, m, 4-H, 5-H, 8-H). δ_C(100 MHz; $CDCl_3$) 45.0, 50.3, 69.0, 107.1, 119.0, 124.1, 126.7, 127.0, 127.9, 129.4, 129.8, 134.6, 156.7. HRMS (FAB+) found 200.0833 ([M]$^+$ $C_{16}H_{21}NO_2$ requires 200.0837).

2-(5,6,7,8-Tetrahydro-naphthalen-1-yloxymethyl)oxirane (12)[23]. From 5,6,7,8-tetrahydro-1-naphthol (148 mg, 1.00 mmol), stirred at 50 °C for 19 h, workup gave a light yellow oil, which was purified by flash column chromatography (EtOAc–heptane 5 : 95) and afforded **12** as a clear oil (1.45 g, 71%). ν_{max}(paraffin)/cm^{-1} 2720, 1587, 1340, 1259, 1102, 926, 865, 855, 765, 727. δ_H (400 MHz; $CDCl_3$) 1.75–1.83 (4H, m, 6-H, 7-H), 2.71 (2H, t, J 6.0, 8-H), 2.77 (2H, t, J 5.8, 5-H), 2.81 (1H, dd, J 2.7 and 5.0, *trans*-CHCH_2O), 2.92 (1H, dd, J 4.2 and 5.0, *cis*-CHCH_2O), 3.36–3.40 (1H, m, CH_2CH(O)CH_2), 4.00 (1H, dd, J 5.3 and 11.1, CHCH_2OAr), 4.23 (1H, dd, J 3.1 and 11.1, CHCH_2OAr), 6.64 (1H, d, J 8.1, 2-H), 6.74 (1H, d, J 7.6, 4-H), 7.06 (1H, app t, J 7.9, 3-H). δ_C(100 MHz; $CDCl_3$) 23.0, 23.0, 23.3, 29.8, 44.9, 50.6, 68.7, 108.2, 122.2, 125.8, 126.6, 139.0, 156.4.

2-(Anthracene-9-yloxymethyl)oxirane (13)[24]. From anthrone (966 mg, 4.97 mmol), stirred at 50 °C for 18 h, workup gave a dark brown oil which was purified by flash column chromatography (EtOAc–heptane 10 : 90, twice) and afforded **13** as a light yellow crystals (576 mg, 46%). mp = 97.7–98.8 °C. ν_{max}(KBr)/cm^{-1} 3053, 2920, 1361, 1327, 1285, 1091, 915, 859, 740. δ_H(400 MHz; $CDCl_3$) 2.85 (1H, dd, J 2.7 and 4.9, *trans*-CHCH_2O), 2.99 (1H, dd, J 4.2 and 4.9, *cis*-CHCH_2O), 3.59–3.63 (1H, m, CH_2CH(O)CH_2), 4.19 (1H, dd, J 6.2 and 11.3, CHCH_2OAr), 4.51 (1H, dd, J 2.8 and 11.3, CHCH_2OAr), 7.47–7.54 (4H, m, 3-H, 9-H, 4-H, 8-H), 8.01–8.03 (2H, m, 5-H, 7-H), 8.27 (1H, s, 6-H), 8.33–8.38 (2H, m, 2-H, 10-H). δ_C(100 MHz; $CDCl_3$) 45.0, 51.1, 76.6, 122.6, 123.1, 125.0, 125.9, 126.0, 127.7, 128.9, 132.8, 134.6, 150.8. HRMS (FAB+) found 250.0992 ([M]$^+$ $C_{17}H_{14}O_2$ requires 250.0994).

2-(Phenanthren-9-yloxymethyl)oxirane (14). From phenanthrol (194 mg, 1.00 mmol), stirred at 11.5 h, workup gave a brown liquid which was purified by flash column chromatography (EtOAc–heptane 5 : 95) and afforded **14** as light yellow crystals (194 mg, 78%). mp = 81.8–84.2 °C. (Found: C, 81.7; H, 5.6. $C_{17}H_{14}NO_2$ requires C, 81.6; H, 5.6%.) ν_{max}(KBr)/cm^{-1} 3052, 2919, 1625, 1596, 1449, 1307, 1221, 1126, 1098, 865, 832, 770, 728. δ_H(400 MHz; $CDCl_3$) 2.93 (1H, dd, J 2.7 and 4.9, *trans*-CHCH_2O), 3.03 (1H, dd, J 4.2 and 4.8, *cis*-CHCH_2O), 3.56–3.60 (1H, m), 4.27 (1H, dd, J 5.6 and 10.9, CHCH_2OAr), 4.53 (1H, dd, J 3.1 and 10.9), 7.00 (1H, s, 10-H), 7.49–7.59 (2H, m, 2-H, 7-H), 7.62–7.74 (2H, m, 3-H, 6-H), 7.77–7.79 (1H, m, 1-H), 8.42–8.44 (1H, dd, J 1.2 and 8.1, 8-H), 8.60–8.69 (2H, m, 4-H, 5-H). δ_C(100 MHz; $CDCl_3$) 45.0, 50.4, 69.0, 103.3, 122.7, 122.7 122.8, 124.7, 126.6, 126.7, 126.9, 127.2, 127.5, 127.6, 131.5, 132.9, 152.6. HRMS (FAB+) found 250.0993 ([M]$^+$ $C_{17}H_{14}O_2$ requires 250.0994).

Glycidyl ethers, method B[14]

Epichlorohydrine (1.2 eq.) to a mixture of aryl alcohol and CsF (3 eq.) in dry DMF (1.5 mL/mmol) while stirring at rt under inert conditions. Heated to 50 °C and left stirring, followed by

TLC (EtOAc–heptane 30 : 70) until no more reaction (13–20 h). The mixture was allowed to reach rt, H_2O (10–25 mL/mmol) was added, which gave a strong precipitation, and left stirring at rt until the solution was clear over the precipitation. The mixture was extracted with EtOAc and the combined organic layers were dried (Na_2SO_4), filtered and evaporated. Short filtration through silica (EtOAc–heptane 50 : 50) gave a crude product which could be directly continued with. Flash column chromatography afforded pure product.

2-(Naphthalen-1-yloxymethyl)oxirane (10). From 1-naphthol (0.153 mg, 1.06 mmol), stirred at 50 °C for 19.5 h, workup gave a brown liquid, which was purified by flash column chomatography (EtOAc–heptane 10 : 90), affording **10** as a clear oil (764 mg, 72%).

2-(Naphthalen-2-yloxymethyl)oxirane (11). From 2-naphthol (290 mg, 2.01 mmol), stirred at 50 °C for 17.5 h, workup gave a yellow solid, which was purified by flash column chromatography (EtOAc–heptane 10 : 90), affording **11** as white crystals (331 mg, 82%).

2-(5,6,7,8-Tetrahydro-naphthalen-1-yloxymethyl)oxirane (12). From 5,6,7,8-tetrahydro-1-naphthol (303 mg, 2.04 mmol), stirred at 50 °C for 16 h, workup gave a light yellow oil, which was purified by flash column chromatography (EtOAc–heptane 10 : 90), affording a clear oil (363 mg, 87%).

2-(Anthracen-9-yloxymethyl)oxirane 13. From anthrone (390 mg, 2.01 mmol), stirred at 50 °C for 13 h, workup gave an orange solid, which was purified by flash column chromatography (EtOAc–heptane 10 : 90), affording a light yellow solid (178 mg, 34%).

2-(Phenanthren-9-yloxymethyl)oxirane 14. From phenanthrol (196 mg, 1.01 mmol), stirred at 50 °C for 13 h, workup gave a brown liquid, which was purified by flash column chromatography (EtOAc–heptane 10 : 90), affording white crystals (208 mg, 82%).

Amination, method A[25]

Glycidyl ether was mixed with isopropylamine (2.5 eq.) in EtOH (2 mL/mmol), refluxed for 3 h and left stirring at rt over night. The solvent was evaporated and the product purified through flash column chromatography (EtOAc–MeOH–14%NH_3(aq) 98 : 1 : 1).

Amination, method B

Glycidyl ether was mixed with serinol (2.5 eq.) in EtOH (2 mL/mmol) and refluxed for 3 h and left stirring at rt overnight forming a precipitate. The solvent was evaporated and the product was dissolved in equivalent amounts of EtOAc and 1 M aqueous HCl and the organic layer was extracted with a second portion of 1 M aqueous HCl. The combined aqueous layers were carefully made basic, using 2 M NaOH, until precipitation formed (pH *ca.* 9) and extracted with EtOAc, until the organic layer was colourless. The combined organic layers were dried (Na_2SO_4), filtered and evaporated to give a solid which was recrystallised from a mixture of 2-propanol and heptane (50 : 50). The product could also be purified through flash column chromatography (EtOAc–MeOH–14%NH_3(aq) 98 : 1 : 1) with some difficulty.

Propranolol 1. Method A from **10** (153 mg, 0.76 mmol) gave **1** as white crystals (162 mg, 82%). mp = 90.8–92.5 °C. ν_{max}(KBr)/cm^{-1} 3280, 2967, 2938, 1591, 1506, 1463, 1401, 1283, 1245, 1112, 789, 765. δ_H(400 MHz $CDCl_3$) 1.31 (3H, d, *J* 3.3, CHCH_3), 1.33 (3H, d, *J* 3.3, CHCH_3), 3.06 (1H, dd, *J* 8.7 and 12.3, CH(OH)CH_2NH), 3.13–3.20 (1H, m, NHCH(CH_3)$_2$), 3.23 (1H, dd, *J* 3.3 and 12.3, CH_2NHCH), 4.16 (1H, dd, *J* 5.6 and 9.6, CHCH_2OAr), 4.23 (1H, dd, *J* 5.0 and 9.6, CHCH_2OAr), 4.47–4.50 (1H, m, CH_2CH(OH)CH_2), 6.81 (1H, d, *J* 7.1, 2-H), 7.35–7.56 (4H, m, 3-H, 4-H, 6-H, 7-H), 7.80–7.84 (1H, m, 5-H), 8.26–8.27 (1H, m, 8-H). δ_C(100 MHz; $CDCl_3$) 23.1, 23.3, 49.3, 49.7, 68.7, 70.9, 105.1, 120.9, 122.0, 125.5, 125.8, 126.0, 126.7, 127.8, 134.7, 154.6. HRMS (FAB+) found 260.1648 ([M + H]$^+$ $C_{16}H_{21}NO_2$ requires 260.1651). HPLC: Daicel OD-H. Hexane–*i*PrOH, 80 : 20, 0.5 mL min^{-1}, 254 nm: t_r (*R*) = 16.12 min, t_r (*S*) = 24.40 min.

2-[2-Hydroxy-3-(naphthalen-1-yloxy)-propylamino]propane-1,3-diol (2). Method B from **10** (456 mg, 2.28 mmol) gave **2** as a light white crystals (260 mg, 39%). mp = 105.6–106.3 °C. (Found: C, 66.0; H, 7.3; N, 4.8. $C_{16}H_{21}NO_4$ requires C, 66.0; H, 7.3; N, 4.8%.) ν_{max}(KBr)/cm^{-1} 3422, 3289, 2929, 2872, 1587, 1458, 1401, 1268, 1245, 1107, 1069, 1031, 789, 765. δ_H(400 MHz; d_5-pyridine) 2.81 (1H, br s, CH_2NHCH), 3.27–3.36 (2H, m, NHCH(CH_2OH)$_2$, CH(OH)CH_2NH), 3.42 (1H, dd, *J* 4.3 and 11.8, CH(OH)CH_2NH), 4.11–4.19 (4H, m, 2 × CHCH_2OH), 4.47 (2H, dd, *J* 2.9 and 5.3, CH(OH)CH_2OAr), 4.66 (1H, app br s, CH_2CH(OH)CH_2), 6.07 (1H, br s, CH_2OH), 6.13 (1H, br s, CH_2OH), 6.92 (1H, app br d, *J* 3.4, CH_2CH(OH)CH_2), 6.98 (1H, d, *J* 7.6, 2-H), 7.38–7.54 (4H, m, 3-H, 4-H, 6-H, 7-H), 7.88 (1H, d, *J* 8.1, 5-H), 8.49 (1H, d, *J* 8.4, 8-H). δ_C(100 MHz; d_5-pyridine) 52.2, 63.2, 63.2, 63.4, 70.5, 72.8, 106.1, 121.0, 123,2, 125.9, 126.8, 127.1, 127.3, 128.4, 135.6, 155.9. HRMS (FAB+) found 292.1528 ([M + H]$^+$ $C_{16}H_{21}NO_4$ requires 292.1549). HPLC: Daicel OD–H. Hexane–*i*PrOH, 80 : 20, 0.5 mL min^{-1}, 254 nm: t_r (*R*) = 22.76 min, t_r (*S*) = 57.42 min.

1-Isopropylamino-3-(naphthalen-2-yloxy)propan-2-ol (3). Method A from **11** (1.47 g, 7.36 mmol) gave **3** as white crystals (1.84 g, 97%). mp = 132.8–135.7 °C. (Found: C, 74.0; H, 8.3; N, 5.3. $C_{16}H_{21}NO_2$ requires C, 74.1; H, 8.2; N, 5.4%.) ν_{max}(KBr)/cm^{-1} 3052, 2976, 1629, 1601, 1473, 1264, 1221, 1116, 1026, 836, 741. δ_H(400 MHz; $CDCl_3$) 1.15 (6H, d, *J* 6.3, 2 × CHCH_3), 2.52 (2H, br s, CH_2CH(OH)CH_2, CH_2NHCH), 2.83 (1H, dd, *J* 7.2 and 12.3, CH(OH)CH_2NH), 2.88–2.94 (1H, m, NHCH(CH_3)$_2$), 2.99 (1H, dd, *J* 3.3 and 12.1, CH(OH)CH_2NH), 4.11–4.17 (3H, m, CH_2CH(OH)CH_2, CH(OH)CH_2OAr), 7.18–7.21 (2H, m, 1-H, 3-H), 7.36–7.38 (1H, m, 6-H), 7.46–7.47 (1H, m, 7-H), 7.73–7.79 (3H, m, 4-H, 5-H, 8-H). δ_C(100 MHz; $CDCl_3$) 23.0, 23.1, 49.4, 49.4, 68.5, 70.7. 107.1, 119.0, 124.0, 126.6, 127.0, 127.9, 129.3, 129.7, 134.7, 156.8. HRMS (FAB+) found 260.1655 ([M + H]$^+$ $C_{16}H_{21}NO_2$ requires 260.1651). HPLC: Daicel OD-H. Hexane–*i*PrOH, 80 : 20, 0.5 mL min^{-1}, 254 nm: t_r (*R*) = 12.58 min, t_r (*S*) = 17.83 min.

2-[2-Hydroxy-3-(naphthalen-2-yloxy)-propylamino]-propane-1,3-diol (4). Method B from **11** (331 mg, 1.65 mmol) gave **4** as white crystals (244 mg, 51%). mp = 138.6–139.2 °C. (Found: C, 66.1; H, 7.3; N, 4.8. $C_{16}H_{21}NO_4$ requires C, 66.0; H, 7.3; N, 4.8%.) ν_{max}(KBr)/cm^{-1} 3299, 2948, 2910, 1629, 1596, 1520, 1473, 1397, 1354, 1268, 1221, 1188, 1102, 1031, 836, 746. δ_H(400 MHz; d_5-pyridine) 2.78 (1H, br s, CH_2NHCH), 3.23–3.30 (2H, m,

NHC*H*(CH_2OH)$_2$, CH(OH)C*H*$_2$NH), 3.36 (1H, dd, *J* 4.3 and 11.8, CH(OH)C*H*$_2$NH), 4.09–4.23 (4H, m, 2 × CHC*H*$_2$OH), 4.43 (2H, d, *J* 5.4, CH(OH)C*H*$_2$OAr), 4.57 (1H, app br s, CH_2C*H*(OH)CH_2), 6.07 (1H, app t, *J* 4.9, CH_2O*H*), 6.13 (1H, app t, *J* 4.8, CH_2O*H*), 6.92 (1H, br d, *J* 4.5, CH_2CH(O*H*)CH_2), 7.32 (1H, dd, *J* 2.5 and 8.9, 3-H), 7.38 (1H, t, *J* 7.6, 6-H), 7.44 (1H, d, *J* 2.3, 1-H), 7.49 (1H, t, *J* 7.6, 7-H), 7.81 (1H, d, *J* 9.0, 4-H), 7.85 (2H, d, *J* 8.5, 5-H, 8-H). δ_C(100 MHz; $CDCl_3$) 52.0, 63.2, 63.2, 63.3, 70.4, 72.6, 107.9, 120.0, 124.5, 127.3, 127.7, 128.6, 130.0, 130.2, 135.7, 158.2. HRMS (FAB+) found 292.1547 ([M + H]$^+$ $C_{16}H_{21}NO_4$ requires 292.1549). HPLC: Daicel OD-H. Hexane–*i*PrOH, 80 : 20, 0.5 mL min^{-1}, 254 nm: t_r (*R*) = 24.17 min, t_r (*S*) = 42.69 min.

1-Isopropylamino-3-(5,6,7,8-tetrahydro-naphthalen-1-yloxy)-propan-2-ol (5)[23]. Method A from **12** (90 mg, 0.44 mmol) gave **5** as white crystals (86 mg, 74%). mp = 82.9–84.5 °C. ν_{max}(KBr)/cm^{-1} 3280, 2976, 2919, 2853, 1582, 1463, 1335, 1259, 1102, 1002, 912, 888, 765. δ_H(400 MHz; $CDCl_3$) 1.29 (6H, dd, *J* 2.6 and 6.4, 2 × CHC*H*$_3$), 1.73–1.80 (4H, m, 6-H, 7-H), 2.67 (2H, t, *J* 5.9, 8-H), 2.76 (2H, t, *J* 5.8, 5-H), 2.95 (1H, dd, *J* 8.5 and 12.3, CH(OH)C*H*$_2$NH), 3.09–3.15 (2H, m, NHC*H*(CH_3)$_2$, CH(OH)C*H*$_2$NH), 3.97 (1H, dd, *J* 5.6 and 9.6, CH(OH)C*H*$_2$OAr), 4.05 (1H, dd, *J* 5.1 and 9.6, CH(OH)C*H*$_2$OAr), 4.30–4.33 (1H, m, CH_2C*H*(OH)CH_2), 6.64 (1H, d, *J* 8.0, 2-H), 6.73 (1H, d, *J* 7.5, 4-H), 7.02–7.54 (1H, m, 3-H). δ_C(100 MHz; $CDCl_3$) 23.0, 23.0, 23.0, 23.1, 23.4, 29.8, 49.3, 49.6, 68.6, 70.6, 108.0, 122.0, 126.0, 126.2, 138.9, 156.5. HRMS (FAB+) found 264.2982 ([M + H]$^+$ $C_{16}H_{25}NO_2$ requires 264.1964). HPLC: Daicel OD–H. Hexane–*i*PrOH, 80 : 20, 0.5 mL min^{-1}, 254 nm: t_r (*R*) = 8.63 min, t_r (*S*) = 21.25 min.

2-[2-Hydroxy-3-(5,6,7,8-tetrahydro-naphthalen-1-yloxy)propylamino]propane-1,3-diol (6). Method B from **12** (363 mg, 1.78 mmol) gave **6** as white crystals (92 mg, 18%). mp = 106.1–106.8 °C. (Found: C, 64.9; H, 8.5; N, 4.8. $C_{16}H_{25}NO_4$ requires C, 65.1; H, 8.5; N, 4.7%.) ν_{max}(KBr)/cm^{-1} 3422, 3280, 2929, 2872, 1582, 1458, 1335, 1249, 1097, 1059, 1031, 898, 855, 760. δ_H(400 MHz; d_5-pyridine) 1.32 (4H, t, *J* 3.2, 5-H, 8-H), 2.65–2.75 (4H, m, 6-H, 7-H), 3.24–3.31 (2H, m, CH(OH)C*H*$_2$NH, NHC*H*(CH_2OH)$_2$), 3.37 (1H, dd, *J* 4.1 and 11.8, CH(OH)C*H*$_2$NH), 4.11 (2H, dd, *J* 5.6 and 10.8, CHC*H*$_2$OH), 4.16–4.22 (2H, m, CHC*H*$_2$OH), 4.26–4.34 (2H, m, CH(OH)C*H*$_2$OAr), 4.51–4.55 (1H, m, CH_2C*H*(OH)CH_2), 6.09 (2H, br s, 2 × CH_2O*H*), 6.74 (1H, d, *J* 7.6, 2-H), 6.83 (1H, d, *J* 8.1, 4-H), 7.12 (1H, app t, *J* 7.8, 3-H). δ_C(100 MHz; d_5-pyridine) 23.5, 23.6, 23.9, 30.3 52.1, 63.1, 63.3, 70.4, 72.2, 109.0, 122.2, 126.6, 126.7, 138.9, 157.8. HRMS (FAB+) found 296.1850 ([M + H]$^+$ $C_{16}H_{25}NO_4$ requires 296.1862). HPLC: Daicel OD-H. Hexane–*i*PrOH, 80 : 20, 0.5 mL min^{-1}, 254 nm: t_r (*R*) = 12.21 min, t_r (*S*) = 51.38 min.

1-(Anthracen-9-yloxy)-3-isopropylaminopropan-2-ol (7). Method A from **13** (47 mg, 0.19 mmol) gave **7** as light orange semi solid (55 mg, 95%). ν_{max}(KBr)/cm^{-1} 3431, 1672, 1592, 1335, 1307, 1283, 1179, 936, 808, 694. δ_H(400 MHz; $CDCl_3$) 0.97 (6H, d, *J* 6.3, 2 × CHC*H*$_3$), 2.71–2.76 (2H, m, CH(OH)C*H*$_2$NH, NHC*H*(CH_3)$_2$), 2.85 (1H, dd, *J* 3.8 and 11.9, CH(OH)C*H*$_2$NH), 4.03 (2H, d, *J* 5.0, CH(OH)C*H*$_2$OAr), 4.16–4.19 (1H, m, CH_2C*H*(OH)CH_2), 7.25–7.31 (4H, m, 2-H, 3-H, 6-H, 7-H), 7.78–7.81 (2H, m, 4-H, 5-H), 8.03 (1H, s, 10-H), 8.16–8.18 (2H, m, 1-H, 8-H). δ_C(100 MHz; $CDCl_3$) 22.7, 22.8, 49.3, 49.6, 69.3, 78.1, 122.4, 122.7, 124.7, 125.6, 125.7, 127.5, 128.7, 132.6, 134.3, 150.7. HRMS (FAB+) found 310.1821 ([M + H]$^+$ $C_{20}H_{23}NO_2$ requires 310.1807). HPLC: Daicel OD-H. Hexane–*i*PrOH, 80 : 20, 0.5 mL min^{-1}, 254 nm: t_r (*R*) = 13.41 min, t_r (*S*) = 16.69 min.

1-Isopropylamino-3-(phenanthren-9-yloxy)propan-2-ol (8). Method A from **14** (93 mg, 0.37 mmol) gave **8** as light yellow crystals (73 mg, 64%). mp = 125.7–127.1 °C. (Found: C, 77.6; H, 7.4; N, 4.5. $C_{20}H_{23}NO_2$ requires C, 77.6; H, 7.5; N, 4.5%.) ν_{max}(KBr)/cm^{-1} 3280, 3081, 2976, 2929, 1629, 1596, 1454, 1311, 1240, 1150, 1126, 1097, 831, 765, 746, 727. δ_H(400 MHz; $CDCl_3$) 1.39 (6H, dd, *J* 4.8 and 6.5, 2 × CHC*H*$_3$), 3.14 (1H, dd, *J* 9.1 and 12.3, CH(OH)C*H*$_2$NH), 3.25–3.30 (1H, m, NHC*H*(CH_3)$_2$), 3.32 (1H, dd, *J* 3.0 and 12.3, CH(OH)C*H*$_2$NH), 4.24 (1H, dd, *J* 5.7 and 9.7, CH(OH)C*H*$_2$OAr), 4.33 (1H, dd, *J* 4.9 and 9.6, CH(OH)C*H*$_2$OAr), 4.62–4.64 (1H, m, CH_2C*H*(OH)CH_2), 6.94 (1H, s, 10-H), 7.50–7.67 (5H, m, 1-H, 2-H, 3-H, 6-H, 7-H), 8.34–8.37 (1H, m, 8-H), 8.55–8.62 (2H, m, 4-H, 5-H). δ_C(100 MHz; $CDCl_3$) 23.0, 23.2, 49.7, 49.8, 68.6, 70.9, 103.5, 122.8, 122.9, 123.0, 124.9, 126.9, 127.0, 127.4, 127.5, 127.6, 127.8, 131.7, 133.2, 152.8. HRMS (FAB+) found 310.1821 ([M + H]$^+$ $C_{20}H_{23}NO_2$ requires 310.1807). HPLC: Daicel OD–H. Hexane–*i*PrOH, 80 : 20, 0.5 mL min^{-1}, 254 nm: t_r (*R*) = 37.09 min, t_r (*S*) = 59.78 min.

2-[2-Hydroxy-3-(phenanthren-9-yloxy)propylamino]propane-1,3-diol (9). Method B from **14** (194 mg, 0.78 mmol) gave **9** as orange crystals (178 mg, 67%). mp = 132.8–135.1 °C. (Found: C, 70.5; H, 6.8; N, 4.0. $C_{20}H_{23}NO_4$ requires C, 70.4; H, 6.8; N, 4.1%.) ν_{max}(KBr)/cm^{-1} 3318, 3062, 2938, 2872, 1625, 1596, 1449, 1312, 1236, 1122, 1098, 1065, 1036, 827, 766, 728. δ_H(400 MHz; d_5-pyridine) 2.89 (1H, br s, CH_2N*H*CH), 3.30–3.40 (2H, m, CH(OH)C*H*$_2$NH, NHC*H*(CH_2OH)$_2$), 3.46 (1H, dd, *J* 4.3 and 11.8, CH(OH)C*H*$_2$NH), 4.12–4.25 (4H, m, 2 × CHC*H*$_2$OH), 4.56–4.61 (2H, m, CH(OH)C*H*$_2$OAr), 4.71–4.72 (1H, m, CH_2C*H*(OH)CH_2), 6.10 (1H, br s, CH_2O*H*), 6.15 (1H, br s, CH_2O*H*), 6.98 (1H, br s, CH_2CH(O*H*)CH_2), 7.31 (H, s, 10-H), 7.55–7.62 (4H, m, 2-H, 3-H, 6-H, 7-H), 7.68–7.72 (1H, m, 1-H), 8.59–8.61 (1H, m, 8-H), 8.76–8.82 (2H, m, 4-H, 5-H). δ_C(100 MHz; d_5-pyridine) 52.2, 63.2, 63.2, 63.4, 70.4, 72.6, 104.0, 123.4, 123.6, 123.6, 125.2, 127.2, 127.4, 127.6, 127.9, 128.1, 128.3, 132.3, 134.1, 153.8. HRMS (FAB+) found 342.1702 ([M + H]$^+$ $C_{20}H_{23}NO_4$ requires 342.1705). HPLC: Daicel OD-H. Hexane–*i*PrOH, 80 : 20, 0.5 mL min^{-1}, 254 nm: t_r (*R*) = 44.28 min, t_r (*S*) = 54.78 min.

(*S*)-Glycidyl-3-nitrobenzenesulfonate ((*S*)-15)[18]. NEt_3 (3.1 mL, 22.2 mmol) and (*R*)-glycidol (1.33 mL, 20.0 mmol) was added to toluene (60 mL) at −10 °C. 3-Nitrobenzenesulfonylchloride (4.48 g, 20.2 mmol) was added and the reaction mixture was left stirring for 12 h. The mixture was allowed to reach rt, washed with 5% H_2SO_4 (2 × 10 mL), sat $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4) and evaporation gave white crystals. Recrystallisation, once from Et_2O–heptane and once from EtOH, afforded (*S*)-**15** as white crystals (1.62 g, 31%). mp = 61.1–63.6 °C. $[\alpha]_D^{22}$ +24.1 (*c* 2.06 in $CHCl_3$). δ_H(400 MHz; $CDCl_3$) 2.65 (1H, dd, *J* 2.6 and 4.6, *trans*-CHC*H*$_2$O), 2.87 (1H, app t, *J* 4.4, *cis*-CHC*H*$_2$O), 3.23–3.26 (1H, m, CH_2C*H*(O)CH_2), 4.07 (1H, dd, *J* 6.4 and 11.6, CHC*H*$_2$OAr), 4.51 (1H, dd, *J* 3.2 and 11.6), 7.83

(1H, t, *J* 8.1, 5-H), 8.27–8.30 (1H, m, 6-H), 8.54–8.56 (1H, m, 4-H), 8.80 (1H, s, 2-H).

(*R*)-Glycidyl-3-nitrobenzenesulfonate ((*R*)-15)[18]. Using same procedure as for (*S*)-**15**, (*S*)-glycidol (0.33 mL, 4.97 mmol) gave (*R*)-**15** as white crystals (159 mg, 12%). mp = 61.1–63.6 °C. $[\alpha]_D^{22}$ −23.6 (*c* 2.12 in $CHCl_3$).

General procedure for (*R*)- and (*S*)-amino alcohols[18]

NaH (1.2 eq.) and ArOH were mixed in dry DMF (10 mL/mmol) and left stirring at rt for 30 min. (*R*)-alt (*S*)-**15** (0.95 eq.) in DMF (5 mL/mmol) was added and the reaction mixture was stirred for 1–1.5 h, followed by TLC (EtOAc–heptane 30 : 70). Sat NH_4Cl (3 mL/mmol) and water (50 mL/mmol) was added and the solution was extracted with EtOAc and the combined organic layers were dried ($MgSO_4$), evaporated and filtered through silica (EtOAc–heptane 50 : 50). The crude product was dissolved in EtOH (2 mL/mmol) and amine (2.5 eq.) was added. The reaction mixture was refluxed at 80 °C for 3 h and left stirring at rt over night. Workup in accordance to corresponding racemic compound.

(*R*)-Propranolol 1. From 1-naphthol (145 mg, 1.01 mmol), purification through flash column chromatography (EtOAc–MeOH–14%NH_3(aq) 98 : 1 : 1), (*R*)-**1** was obtained as white crystals (192 mg, 75%). mp = 69.4–70.9 °C.

2-[(2*S*)-Hydroxy-3-(naphthalen-1-yloxy)propylamino]propane-1,3-diol ((*S*)-2). From 1-naphthol (306 mg, 2.12 mmol), purified through recrystalisation from a mixture of 2-propanol and heptane (50 : 50), (*S*)-**2** was obtained as white crystals (126 mg, 22%). mp = 91.4–92.9 °C. $[\alpha]_D^{20}$ −9.0° (*c* 1.0 in EtOH).

(2*S*)-1-Isopropylamino-3-(phenanthren-9-yloxy)propane-2-ol ((*S*)-8). From phenanthrol (355 mg, 1.83 mmol), purification through flash column chromatography (EtOAc–MeOH–14%NH_3(aq) 98 : 1 : 1), (*S*)-**8** was obtained as light yellow crystals (200 mg, 36%). mp = 98.8–103.5 °C. $[\alpha]_D^{20}$ −6.4° (*c* 1.0 in EtOH).

2-[(2*S*)-Hydroxy-3-(phenanthren-9-yloxy)propylamino]propane-1,3-diol (*S*)-9. From phenanthrol (352 mg, 1.81 mmol), purified through recrystalisation from a mixture of 2-propanol and heptane (50 : 50), (*S*)-**9** was obtained as light orange crystals (103 mg, 17%). mp = 110.5–114.6 °C. $[\alpha]_D^{20}$ −5.9° (*c* 0.99 in EtOH).

Binding studies

Bis-Tris and *rac*-Propranolol were purchased from Sigma-Aldrich. The water used was of Millipore quality. All other chemicals were of analytical grade.

Apparatus

All CE experiments were performed on a Hewlett Packard[3D] Capillary Electrophoresis system (Agilent Technologies, Waldbronn, Germany) using ChemStation Version A.06.01 for system control, data collection and data analysis. Separation was performed in polyvinyl alcohol (PVA)-coated capillaries, made essentially in accordance with previously described methods,[26] of 33 cm (effective length 24.5 cm) and 50 μm ID. UV detection was carried out at 214 nm for **1**–**4**, 204 nm for **5**–**6** and 254 nm for **7**–**9**. 10 mM Bis-Tris–AcOH, pH 5.0, was used as background electrolyte (BGE). The sample solutions, protein and BGE were hydrodynamically injected at the anode at a pressure of 34.5 mbar (instrumental setting). A constant temperature of 25 °C and a constant current of 8 μA was used.

Samples were dissolved in BGE at a concentration of 15 μM, degassed and filtered through 0.45 μm syringe filters prior to analysis. Protein was dissolved in BGE and concentration calculated from UV, using ε_{280} = 78 800. Protein concentrations used were 176 μM for **5**, 83 μM for **1**, **3**, **7**, 37 μM for **4**, **6**, 18 μM for **2**, **8** and 2 μM for **9**.

All runs were made in triplicate and the mean value of each response calculated.

Method

The capillaries were rinsed with BGE for 5 min before each run. Migration times for each analyte were measured at 5 different plug lengths of protein, *i.e.* 0, 21, 42, 64 and 85% of the effective length of the capillary (equal to a pressure of 34.5 mbar for 0, 50, 100, 150 and 200 s), keeping the protein concentration constant for each analyte. A plug of protein was first injected, followed by analyte (5 s) and finally a short plug of BGE (5 s) to prevent diffusion back to the anode.

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