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Synthesis and in vitro evaluation of new benzovesamicol analogues as potential imaging probes for the vesicular acetylcholine transporter

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Abstract—Our goal was to synthesize new stereospecific benzovesamicol analogues, which could potentially be used as SPECT or PET radioligands for the vesicular acetylcholine transporter (VAChT). This paper describes the chemical synthesis, resolution and determination of binding affinity for four enantiomeric pairs of derivatives. Their intrinsic affinities were determined by competition against binding of [^{3}H]vesamicol to human VAChT. Of the eight enantiomers, (*E*)-(*R*,*R*)-5-AOIBV [(*R*,*R*)-**3**], and (*R*,*R*)-5-FPOBV [(*R*,*R*)-**4**] displayed the highest binding affinities for VAChT (K_d = 0.45 and 0.77 nM, respectively), which indicated that an elongation of the chain from 5-idodo as in the case of 5-iodobenzovesamicol (5-IBVM), to a 5-(*E*)-3-iodoallyloxy or 5-fluoropropoxy substituent, as in 5-AOIBV and 5-FPOBV, respectively, was very well tolerated at the vesamicol binding site. The enantiomer (*R*,*R*)-4-MAIBV [(*R*,*R*)-**16**], which retains the basic structure of (−)-5-IBVM but possess an additional aminomethyl substituent in the 4-position of the piperidine ring, displayed lower binding affinity (K_d = 8.8 nM). Nevertheless, the result suggests that substitution at this position may be an interesting alternative to investigate for development of new benzovesamicol analogues. As expected, the corresponding (*S*,*S*) enantiomers displayed lower K_d values, they were approximately 10-fold lower in the case of (*S*,*S*)-5-FPOBV (K_d = 8.4 nM) and (*E*)-(*S*,*S*)-5-AOIBV (K_d = 4.3 nM). (*R*,*R*)-**3**, and (*R*,*R*)-**4** showed the same high affinity for VAChT as (−)-5-IBVM and may be suitable as imaging agents of cholinergic nerve terminals.

1. Introduction

Benzovesamicol (BVM) is an analogue of (−)-vesamicol (Fig. 1), a compound known to be a stereoselective inhibitor of acetylcholine uptake into pre-synaptic cholinergic vesicles. Vesamicol binds with high affinity to the vesicular acetylcholine transporter (VAChT) at an allosteric site.[1–4] Since (−)-vesamicol also binds to α-adrenoreceptors[5] and σ-receptors,[6] new efforts have been directed towards the development of more selective radiotracer molecules.[7–10] The interest in benzovesamicol derivatives as radioligands for Alzheimer's disease (AD) studies is based on the observation that as the disease progresses, the levels of VAChT change in parallel fashion and magnitude with other cholinergic marker proteins, in particular choline acetyltransferase (ChAT). Radiolabelled benzovesamicol analogues had already been used in the past as imaging probes in single photon emission computer tomography (SPECT) and positron emission tomography (PET) aimed for in vitro and in vivo studies of AD. Some of those radiotracers showed high selectivity and high binding affinity for the VAChT, but their pharmacokinetics and/or toxicity restricted their use as in vivo imaging agents.[10–15] Among those

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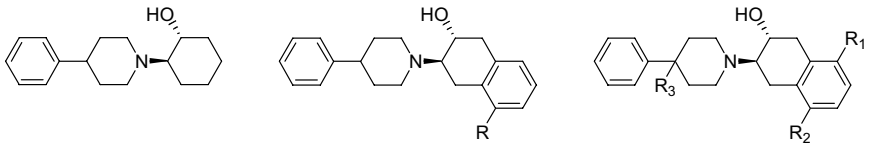


(–)-**vesamicol** R= I (–)-5-**IBVM** R= $O(CH_2)_2F$ **FEOBV** **New Analogues** : R_1= H, OCH_3 R_2= OCH_2CH=CHI, $O(CH_2)_3F$, or I R_3= H, CH_2NH_2

Figure 1. Chemical structure of (−)-vesamicol, (−)-5-iodobenzovesamicol, (−)-5-fluoroethoxybenzovesamicol and (*R*,*R*) new analogues.

radiotracers, (−)-(2*R*,3*R*)-2-hydroxy-3-(4-phenylpiperidino)-5-(2-[^{18}F]fluoroethoxy)tetralin [(−)-[^{18}F]-5-FEOBV][14] (Fig. 1) has proved to be an excellent imaging tool in rodent studies by PET,[16] but its use in human studies is missing so far. For SPECT studies, (−)-(2*R*,3*R*)-2-hydroxy-3-(4-phenylpiperidino)-5-[^{123}I]iodotetralin [(−)-[^{123}I]-5-IBVM] (Fig. 1) has been the more promising of the radioiodinated benzovesamicol analogues for the determination of VAChT density and atrophy in humans. Its gastrointestinal toxicity is not at issue due to the very small amount of cold compound to be injected, but its pharmacokinetics are far from ideal.[11–13]

For the development of new, more potent and selective benzovesamicol derivatives as radiotracers, two considerations are of uppermost importance: (a) in vivo studies in rats of the neuronal mapping potential of iodobenzovezamicol derivatives have revealed that considerable bulk tolerance exists at different structural positions of the benzovesamicol structure;[11,17] and (b) the binding to the VAChT is known to be highly enantioselective.

Of special consideration in the development for new VAChT probes is the method to be used in determination of their binding affinity. The previous method of choice utilized cholinergic synaptic vesicles purified from the marine ray *Torpedo*.[18] However, differences between elasmobranch and human VAChT affinities for analogues of vesamicol can be expected. Human VAChT has now been cloned and constitutes the preferred screen for development of clinically useful analogues.[19]

Our purpose was to search for new benzovesamicol derivatives with high affinity for the VAChT, lower toxicity and good pharmacokinetic characteristics. Our goal was to find analogues with potential use as radiolabelled probes for SPECT or PET with application in the detection of density changes at the pre-synaptic level, useful in early detection and follow-up of Alzheimer's disease in humans.

To that effect, we looked into how changes in lipophilicity and structural conformation could impact on radiotracer characteristics in comparison to (−)-5-IBVM. We chose to work with enantiomerically pure compounds and to look specifically at: (a) elongation of the substituent chain, (b) increase or decrease lipophilicity by placing additional substituents on the parent molecule, (c) the presence of an iodide versus fluoride atom in a substituent chain of similar length, (d) the effect of substitution in the 4-position of the piperidine ring; an alternative not previously examined in benzovesamicol series.

In this paper, we describe the chiral synthesis of four enantiomeric pairs of benzovesamicol derivatives (Fig. 1), and evaluation of in vitro binding to the VAChT radioligand binding experiments.

2. Results

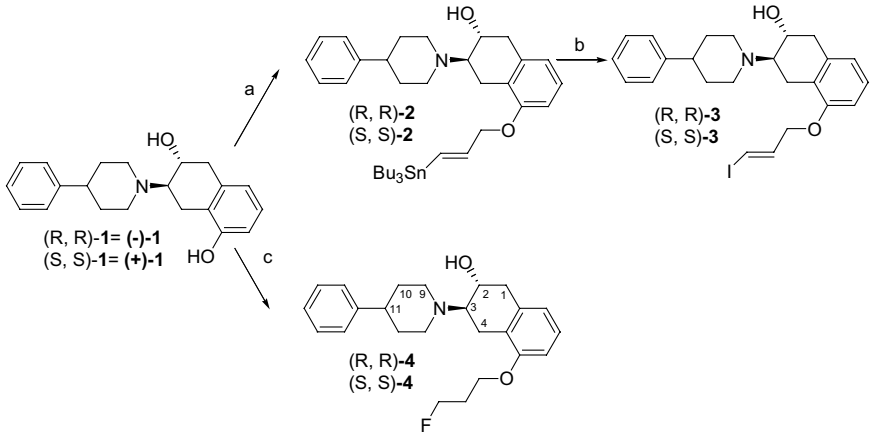
2.1. Chemistry

The syntheses of the four pairs of enantiomeric benzovesamicol derivatives, reported here, were accomplished via the routes illustrated in Schemes 1–3.

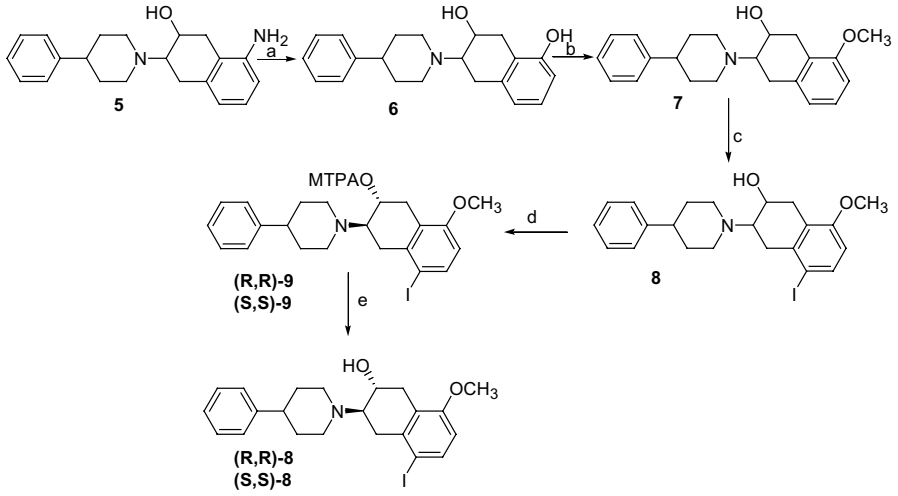
Synthesis and enantiomeric resolution of racemic 5-aminobenzovesamicol (5-ABV) was achieved as previously reported.[20] Sandmeyer reaction on each of the pure 5-ABV enantiomers, produced the corresponding 5-hydroxybenzovesamicol (5-HOBV; **1**) enantiomer[14] (Scheme 1). The *p*-toluenesulfonate ester of (*E*)-3-(tri-*n*-butylstannyl)prop-2-enol was synthesized as reported,[21] and later coupled with each enantiomer of the phenol **1**, leading to the allylstannylated precursor (5-Bu_3SnAOIBV; **2**) in 71–80% yield. Iododestannylation was accomplished under mild conditions with iodine or *N*-iodosuccinimide (NIS), with chemical yields of 67% and 77%, respectively, producing the iodoallyloxy derivative (5-AOIBV; **3**) (Scheme 1).

On the other hand, fluorination of propanediol ditosylate using tetra-*n*-butylammonium fluoride (TBAF) produced 3-fluoropropyltosylate [$F(CH_2)_3OTs$] in 35% yield. Reactions between this tosylate and the nucleophilic enantiomers of 5-HOBV, **1**, in acetonitrile led to authentic 5-fluoropropyloxybenzovesamicol (5-FPOBV; **4**) in 84–90% yield (Scheme 1).

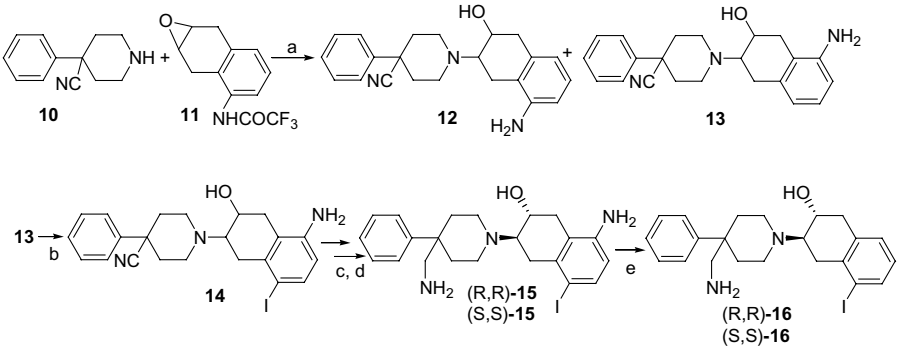
Scheme 2 describes the route followed for the synthesis of the enantiomeric derivatives 8-methoxy-5-iodobenzovesamicol (8-MOIBV; **8**). Through a Sandmeyer reaction, racemic 8-aminobenzovesamicol (8-ABV; **5**) was converted to racemic 8-hydroxybenzovesamicol (8-HOBV; **6**), which later was used to generate racemic 8-methoxybenzovesamicol (8-MOBV; **7**) in 67% yield.



Scheme 1. Synthesis of the enantiomeric pairs (*R*,*R*)/(*S*,*S*)-5-AOIBV, **3** and (*R*,*R*)/(*S*,*S*)-5-FPOBV, **4**. Reagents and conditions: (a) TBAOH, CH_3CN, (*E*)-$Bu_3SnCH{=}CH{-}CH_2OTs$, 80 °C; (b) from (*S*,*S*)-**2**: I_2, $CHCl_3$, rt, from (*R*,*R*)-**2**: NIS, THF, rt; (c) TBAOH, CH_3CN, $F(CH_2)_3OTs$, 80 °C.



Scheme 2. Synthesis of the enantiomeric pair (*R*,*R*)- and (*S*,*S*)-8-MOIBV, **8**. Reagents and conditions: (a) $NaNO_2$, H_2SO_4, THF, 0 °C then reflux; (b) TBAOH, CH_3CN, CH_3I, 80 °C; (c) ICl, CH_3CO_2H, rt; (d) (*R*)(−)MTPA-Cl, 4-DMAP, TEA, $CHCl_3$, rt; (e) 2 N NaOH, CH_3OH, THF, rt.



Scheme 3. Synthesis of the enantiomeric pair (*R*,*R*)- and (*S*,*S*)-MAIBV, **16**. Reagents and conditions: (a) EtOH, Et_3N, reflux, NaOH, rt; (b) ICl, CH_3CO_2H, rt; (c) (*R*)(−)MTPA-Cl, 4-DMAP, TEA, CH_3Cl, rt; (d) DIBAL-H, toluene, −78 °C, then rt; (e) H_3PO_2, HCl, $NaNO_2$, 0 °C, then rt.

Racemic 8-methoxyiodobenzovesamicol (8-MOIBV; **8**) was then obtained by iodination of **7** with iodine monochloride in glacial acetic acid, and the racemic mixture resolved through their MTPA esters, followed by 2 N NaOH hydrolysis, as reported for similar compounds.[14,17]

Scheme 3 details the synthetic path used in the preparation of enantiomeric 3-(4-methylamino-4-phenylpiperidino)-5-iodobenzovesamicol (MAIBV; **16**). Condensation in ethanol of 4-cyano-4-phenylpiperidine **10** with *N*-(trifluoroacetyl)-1-amino-5,8-dihydronaphthalene oxide, **11**, produced a mixture of racemic

regioisomers **12** and **13**. The regioisomers were separated via silica gel chromatography. Unfortunately, under standard conditions used for IBVM, the diazotization of the 5-amino-derivative **12** and reaction with potassium iodide did not lead to the desired iodo-derivative **16** (data not shown). So, an alternate approach was done: direct iodination of the regioisomer **13** with ICl in glacial acetic acid produced racemic **14**. During the chiral resolution, in the reductive cleavage step using DIBAL-H, as expected, the cyano group was reduced to aminomethyl (**15**). Subsequent deamination of the aryl amine with hypophosphorus acid (H_3PO_2) and sodium nitrite ($NaNO_2$) led to enantiomeric **16** (Scheme 3).

In each case, chiral resolution of racemic derivatives was achieved with (−)-(*R*)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (MTPA-Cl)[22] followed by deprotection with either 2 N NaOH or DIBAL-H (for the ester-amide obtained with **14**) as required. Reactions carried out with the (*R*,*R*)-enantiomers were repeated with the corresponding (*S*,*S*)-enantiomers.

2.2. Biological evaluation

Human VAChT stably expressed in the PC12 cell line,[23] which is a common model system for neurosecretory phenomena, was used to screen target compounds. Because the cell line expresses a relatively large amount of human VAChT (>2 pmol/mg postnuclear supernatant), displacement of bound [^{3}H]vesamicol by competing ligands is monophasic and simple. A low concentration of [^{3}H]vesamicol was used so that the IC_{50} value for displacement is essentially equal to the K_d value. Cells were grown in quantity, harvested and homogenized to prepare a low-speed, postnuclear supernatant rich in synaptic-like microvesicles containing human VAChT. Competition was allowed to proceed 24 h to ensure equilibration of low concentrations of target compounds. It could not be carried out at 37 °C due to the length of incubation. However, binding of vesamicol is not strongly temperature dependent.

3. Discussion

In the development of radiohalogenated benzovesamicol analogues as potential radiotracers for SPECT or PET, is necessary that the radiohalogen incorporated into the molecule does not alter the vesamicol-like inhibitory activity of the compound. Moreover, in cases where the binding to the receptor is enantioselective, enantiomeric purity of the tracer is a must. Here we report the chiral syntheses, and the in vitro determination of the binding affinity for the VAChT of enantiomeric compounds **3**, **4**, **8** and **16**. In fact, this route could also be used to generate pure enantiomeric precursors needed for radiolabelling: radioiodinated compounds could be obtained by iododestannylation of the corresponding tributyltin derivatives, which are usually prepared from the iodo precursors.

In each pair tested, the one with structure designation (*R*,*R*) consistently exhibit K_d value higher than the (*S*,*S*), in agreement with data previously reported[11,16,20] (Table 1).

Of the eight enantiomers synthesized, (*E*)-(*R*,*R*)-5-AOIBV [(*R*,*R*)-**3**] and (*R*,*R*)-5-FPOBV [(*R*,*R*)-**4**] displayed the highest binding affinities for VAChT (K_d = 0.45 and 0.77 nM, respectively), which suggest that elongation of the substituent chain from 5-idodo, as in the case of (−)-5-IBVM (K_d = 0.30 nM) to a five member 5-*O*-halogenoalkyl or alkenyl chain, had limited effect on affinity for VAChT. That bulkier ether substituents at this position does not impair the binding affinity to VAChT has been previously shown.[14,16] In this position, the electronegativity, and the halogen atom size does not influence the protein–ligand interaction. We chose a propyl chain to compare the activity with the fluoroethyloxy derivative (FEOBV).[14] The fluoropropyl may be more stable than the fluoromethyl or fluoroethyl compound in an in vitro metabolic and environment, and this type of radiotracer is hoped to be a better ligand in vivo. Moreover, we kept a consistent chain length between FEOBV and 5-AOIBV for comparison of their receptor–ligand interaction.

Substitution in the 4-position on the piperidine ring, which has not been extensively explored, with an aminomethyl group [(*R*,*R*)-**16**, K_d = 8.8 nM] slightly decreased the affinity compared to 5-IBVM, but this position might produce potent compounds having much more polarity.

On the other hand, (*R*,*R*)-**8**, which also maintains the basic structure of (−)-5-IBVM but with an additional methoxy group in the 8-position of the aromatic ring, showed very low activity for the target site (K_d around 100 nM); confirming the negative effect on receptor binding when substituents are present at this position as shown in studies by others.[17,20] An unsubstituted 8-position thus seems to be critical to the binding, specially since the lipophilicity (as inferred from their calculated log *P*, Table 1) appears to be of similar magnitude for both, 8-MOIBV (**8**) and 5-IBVM.

Table 1. Affinities (K_d ± SEM) of vesamicol, 5-IBVM, and selected analogues **3**, **4**, **8** and **16** for VAChT

	(*R*,*R*)-**3**	(*S*,*S*)-**3**	(*R*,*R*)-**4**	(*S*,*S*)-**4**	(*R*,*R*)-**8**	(*S*,*S*)-**8**	(*R*,*R*)-**16**	(*S*,*S*)-**16**	Vesamicol	5-IBVM
K_d (nM)[a]	0.45 ± 0.11	4.3 ± 1.4	0.77 ± 0.18	8.4 ± 1.9	102 ± 43	>100	8.8 ± 3.0	>100	20 ± 2.3	0.30 ± 0.11
Clog *P*[b]	5.5		4.4		5.2		3.8		3.5	5.2

[a] K_d values were determined by competition of derivatives against bound [^{3}H]vesamicol under equilibrium at 22 °C, unless stated; vesamicol and 5-IBVM were the pure (−)-enantiomer.

[b] Calculated log *P* (cLog*P*, ChemDraw Ultra 6.0.1, 2000).

4. Conclusion

We have synthesized eight new authentic optical isomer analogues of benzovesamicol. Of these, the iodinated derivative (*E*)-5-AOIBV [(*R*,*R*)-**3**] and the fluorinated derivative 5-FPOBV [(*R*,*R*)-**4**] displayed VAChT binding affinity of comparable magnitude to that of (−)-5-IBVM; suggesting its potential application, when radiohalogenated, as SPECT or PET imaging agents for studies involving cholinergic nerve terminals.

5. Experimental

5.1. Chemistry

NMR spectra were recorded on a Bruker DPX Avance 200 spectrometer (200 MHz for ^{1}H, 50.3 MHz for ^{13}C). $CDCl_3$ was used as solvent; chemical shifts are expressed in ppm relative to TMS as an internal standard. Melting points were determined in a Buchi B540 capillary melting point apparatus (Switzerland) and are uncorrected. Column chromatography was carried out using VWR silica gel (230–400 mesh), preparative TLC was carried out using 20 × 20 cm, glass backed silica gel F_{254} plates, 1 mm or higher thickness, from VWR. Thin-layer chromatography was performed using plastic backed sheets with silica gel F_{254} also from VWR. Chemicals and solvents ACS grade or higher, commercially available, were used without further purification. Elemental analyses of new compounds were within ±0.4% of theoretical values. Phenylpiperidine used in the synthesis of 5- and 8-aminobenzovesamicol (ABV),[20] was obtained by alkaline fusion from 4-cyano-4-phenylpiperidine,[24] (*E*)-3-(tri-*n*-butylstannyl)prop-2-en-1-ol *p*-toluene sulfonate was prepared from (*E*)-3-(tri-*n*-butylstannyl)-prop-2-en-1-ol and tosyl chloride in the presence of potassium trimethylsilanolate ($KOSiMe_3$) as described.[21]

(−)-5-IBVM was synthesized in the laboratory as previously described.[11,20]

5.1.1. (2*R*,3*R*)- and (2*S*,2*S*)-(*E*)-2-hydroxy-5-(tributylstannylallyloxy)-3-(4-phenylpiperidino)tetralin, (*E*)-5-Bu$_3$SnAOIBV, (*R*,*R*)- and (*S*,*S*)-2. A solution of (+)-5-HOBV;[14] (*S*,*S*)-**1**, (30.5 mg, 94 μmol) was dissolved in 3 mL of CH_2Cl_2 under inert atmosphere and 100 μL of 1 M tetrabutylammonium hydroxide (TBAOH) in methanol added; the solvent was removed by rotary evaporation at room temperature and to the residue 2 mL of dry CH_3CN added and the mixture rotoevaporated to dryness, to azeotropically remove traces of moisture. The process was repeated once more and then, under inert atmosphere, the mixture was resuspended in 3 mL of dry CH_3CN and (*E*)-3-(tri-*n*-butylstannyl)prop-2-en-1-ol, *p*-toluenesulfonate[21] (60 mg, 119 μmol) added. The mixture was heated, at 75 °C for 4 h, and then let slowly rise to room temperature overnight. After evaporation of the solvent, the crude product was partitioned between CH_2Cl_2 and water (20 and 10 mL, respectively) and the organic layer washed with 0.5 N NaOH, dried over anhydrous Na_2SO_4, concentrated and purified by preparative TLC on silica gel (30% EtOAc in hexane) to give 44 mg (71%) of the precursor (*S*,*S*)-**2** as a pale pink solid. The same procedure, starting with 37 mg (115 μmol) of the enantiomer (*R*,*R*)-**1**[14] produced 60 mg (80%) of the corresponding precursor (*R*,*R*)-**2** as a pale pink solid.

^{1}H NMR ($CDCl_3$): δ 0.95 (t, 9H, ^3J = 7.0 Hz, $3CH_3$), 1.25–1.68 [m, 18H, $(CH_3C_3H_6)_3Sn$], 1.72–2.06 (m, 4H, 4H-10), 2.44–3.19 (m, 9H, H-11, H-1, 2H-4, H-3, 4H-9), 3.30 (dd, 1H, ^3J = 5.6 Hz, ^3J = 16 Hz, H-1), 3. 88 (m, 1H, H-2), 4.60 (d, 2H, ^3J = 4.3 Hz, OCH_2), 6.13–6.44 (m, 2H, H-13, H-14), 6.66–6.76 (m, 2H, H-6, H-8), 7.09 (t, 1H, ^3J = 7.6 Hz, H-7), 7.19–7.38 (m, 5H, $5H_{Ar}$). ^{13}C NMR: δ 9.5 [$(CH_2)_3Sn$], 13.7 ($3CH_3$), 20.1 (C-4), 27.2 [$(CH_3CH_2C_2H_4)_3Sn$], 29.0 [$(CH_3CH_2$-$CH_2CH_2)_3Sn$], 33.9–34.4 (2C-10), 38.0 (C-1), 42.9 (C-11), 44.9, 53.6 (2C-9), 65.3 (C-2), 66.6 (C-3), 71.3 (OCH_2), 108.8 (CH_{Ar}), 121.3 (CH_{Ar}), 124.1 (CH_{Ar}), 126.2 (C_{Ar}), 126.6 (CH_{Ar}), 126.8 ($2CH_{Ar}$), 128.4 ($2CH_{Ar}$), 131.1 (SnCH=), 135.3 (C_{Ar}), 143.1 (CH=), 146.1 (C_{Ar}), 156.5 (C_{Ar}).

5.1.2. (2*S*,3*S*)-(*E*)-2-Hydroxy-5-(iodoallyloxy)-3-(4-phenylpiperidino)tetralin, (*E*)-5-AOIBV, (*S*,*S*)-(*E*)-3. Stannyl derivative (*S*,*S*)-**2** (34 mg, 52 μmol), was dissolved in $CHCl_3$ and cooled at −5 °C. A solution of iodine in CH_2Cl_2 (0.1 M, 532 μL) was then added in aliquots, under constant stirring, until a coloured solution resulted. The reaction mixture was washed with 12% $NaHSO_3$ (5 mL), the organic layer separated and the aqueous extracted with CH_2Cl_2 (3 × 10 mL); the combined organic layers were dried over anhydrous Na_2SO_4 and rotoevaporated off. The crude product was purified by preparative TLC on silica gel plates, with 30% EtOAc in hexane plus diethyl ether (7:3) to give 17 mg (67%) of (*S*,*S*)-**3**.

^{1}H NMR ($CDCl_3$): δ 1.74–1.96 (m, 4H, 4H-10), 2.48–2.75 (m, 3H, H-11, H-1, H-4), 2.78–3.15 (m, 6H, H-4, H-3, 4H-9), 3.33 (dd, 1H, ^3J = 5.6 Hz, ^3J = 16 Hz, H-1), 3.83–3.93 (m, 1H, H-2), 4.44 (s, OH), 4.50–4.53 (m, 2H, OCH_2), 6.52–6.687 (m, 2H, CH=, ICH=), 6.78–6.91 (m, 2H, H-6, H-8), 7.14 (t, 1H, J = 7.6 Hz, H-7), 7.25–7.41 (m, 5H, $5H_{Ar}$). ^{13}C NMR: δ 20.1 (C-4), 33.9–34.4 (2C-10), 37.9 (C-1), 42.8 (C-11), 44.8–53.5 (2C-9), 65.2 (C-2), 66.4 (C-3), 69.6 (OCH_2), 79.2 (ICH), 108.2 (CH_{Ar}), 121.9 (CH_{Ar}), 124.1 (C_{Ar}), 126.2 (CH_{Ar}), 126.7 ($2CH_{Ar}$), 126.8 ($2CH_{Ar}$), 128.4 (CH_{Ar}), 135.7 (C_{Ar}), 140.9 (CH=), 146.2 (C_{Ar}), 155.8 (C-5).

5.1.3. (2*R*,3*R*)-(*E*)-2-Hydroxy-5-(iodoallyloxy)-3-(4-phenylpiperidino)tetralin, (*E*)-5-AOIBV, (*R*,*R*)-(*E*)-3. The (*R*,*R*)-**2** precursor (38 mg, 58 μmol) was dissolved in degassed THF (5 mL) and stirred for 5 min under inert atmosphere. *N*-Iodosuccinimide (NIS, 13.8 mg, 61 μmol) dissolved in 0.5 mL degassed THF, was added and the mixture stirred for 20 min at room temperature. The solvent was rotoevaporated off, and the crude product purified by preparative TLC on silica gel, 30% EtOAc in hexane to give 22 mg (77%) of (*R*,*R*)-**3**.

5.1.4. Toluene-4-sulfonic acid-3-fluoropropyl ester: F(CH$_2$)$_3$OTs. Under inert atmosphere, propanediol ditosylate $TsO(CH_2)_3OTs$ (2.00 g, 5.20 mmol) was

dissolved in tetrabutylammonium fluoride 1 M solution in THF (TBAF) (13.4 mL). The mixture was heated, under inert atmosphere, at 80°C for 15 min. The solvent was removed by rotary evaporation. The crude product was portioned between Et_2O and 2 N Na_2CO_3 solution, the organic layer was washed with water, dried over Na_2SO_4, concentrated and purified by flash chromatography on silica gel with 50% CH_3Cl_3 in hexane to give 0.432 g (36%) of 3-(tosyloxy)-l-propyl fluoride [$F(CH_2)_3OTs$] as a colourless oil. NMR spectrum data matched the one previously reported for this compound.[25]

5.1.5. (2*R*,3*R*)-2-Hydroxy-3-(4-phenylpiperidino)-5-(3-fluoropropoxy)tetralin, 5-FPOBV, (*R*,*R*)-4. A solution of (−)-5-HOBV; (*R*,*R*)-**1** (30.5 mg, 94 μmol) was dissolved in 2 mL of CH_2Cl_2 under inert atmosphere and 100 μL of 1 M tetrabutylammonium hydroxide (TBAOH, 100 μmol) in methanol added; the solvent was removed by rotary evaporation at room temperature and the residue treated twice with 2 mL of dry CH_3CN followed each time by rotoevaporation to azeotropically remove traces of moisture. Under inert atmosphere, the phenoxide was resuspended in 4 mL of dry CH_3CN, and 3-fluoropropyltosylate in 2 mL dry CH_3CN was added; the reaction mixture was heated at 75 °C for 3 h. After rotoevaporation of the solvent, the crude product was partitioned between CH_2Cl_2 and water (20 and 10 mL, respectively) and the organic layer washed with 40 mL of 0.5 N NaOH, dried over anhydrous Na_2SO_4, concentrated to a yellow solid and purified by preparative TLC on silica gel plate and 50% EtOAc in hexane to give 30 mg (84%) of (*R*,*R*)-**4** as a white solid with mp 142–143 °C. The same procedure starting with 30 mg (93 μmol) of the (*S*,*S*)-**1** enantiomer produced 32 mg (90%) of (*S*,*S*)-**4** as a white solid.

^{1}H NMR ($CDCl_3$): δ 1.65–2.05 (m, 4H, 4H-10), 2.28 (qd, ^3J = 26.0, 5.9 Hz, CH_2), 2.42–3.14 (m, 9H, 4H-9, 2H-4, H-3, H-11, H-1), 3.33 (dd, 1H, ^3J = 16.0, 5.4 Hz, H-1), 3.82 (ddd, 1H, ^3J = 5.6, 10.2, 20.4 Hz, H-2), 4.15 (t, ^3J = 5.9 Hz, OCH_2), 4.73 (dt, ^3J = 47.0, 5.9 Hz, CH_2F), 6.70–6.81 (m, 2H, H-6, H-8), 7.16 (t, 1H, ^3J = 8.0 Hz, H-7), 7.25–7.41 (m, 5H, $5H_{Ar}$). ^{13}C NMR: δ 19.9 (C-4), 30.4 (CH_2, ^2J = 20 Hz), 33.9, 34.4 (2C-10), 38.0 (C-1), 42.1 (C-11), 44.9, 53.6 (2C-9), 63.4 (OCH_2, ^3J = 5.5 Hz), 65.3–66.5 (C-3, C-2), 80.9 (CH_2F, ^1J = 165 Hz), 108.0 (C-6), 121.5 (CH_{Ar}), 123.9 (C-4a), 126.2 (CH_{Ar}), 126.8 ($2CH_{Ar}$), 128.5 ($2CH_{Ar}$), 135.5 (C-8a), 146.2 (C_{Ar}), 156.4 (C-5).

5.1.6. (±)-*trans*-2,8-Dihydroxy-3-(4-phenylpiperidino)-tetralin, (±)-8-HOBV, (±)-6. A 1:2 dilution of concentrated sulfuric acid in water (6 mL) was added to a cooled solution of racemic 8-hydroxybenzovesamicol (8-ABV)[20] (185 mg, 574 μmol) in THF (5 mL), then a solution of sodium nitrite (47.5 mg, 690 μmol) in water (2 mL) was added dropwise while the temperature of the reaction mixture was maintained below 5 °C. Stirring was continued for 1 h. The resulting diazonium solution was then added carefully in small aliquots, to a second dilution of sulfuric acid (2 mL) in water (10 mL) at its boiling point. The mixture was boiled for 5 min after the addition was completed and then allowed to cool to room temperature. The solution was bought to pH = 9 with 5 N NaOH and extracted with ethyl acetate (3 × 50 mL). The combined extracts were dried over anhydrous $NaSO_4$ and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with 30% EtOAc in hexane to afford racemic 8-HOBV; (±)-**6** (181 mg, 97%) as a white solid (mp 206–207 °C).

^{1}H NMR ($CDCl_3$): δ 1.74–1.98 (m, 4H, 4H-10), 2.37–3.00 (m, 9H, 4H-9, 2H-4, H-3, H-11, H-1), 3.47 (dd, 1H, ^3J = 6.2 Hz, ^3J = 16.2 Hz, H-1), 3.92–3.98 (m, 1H, H-2), 6.63–6.67 (m, 2H, H-5, H-7), 7.07 (t, 1H, ^3J = 8 Hz, H-6), 7.22–7.41 (m, 5H, $5H_{Ar}$). ^{13}C NMR: δ 26.2 (C-4), 31.8 (C-1), 33.6–34.3 (2C-10), 42.9 (C-11), 45.0–53.6 (2C-9), 65.8 (C-3), 66.0 (C-2), 112.4 (C-7), 121.3 (C-5), 126.2 (C-8a), 126.8 (C-6), 126.9 ($2CH_{Ar}$), 128.3 ($2CH_{Ar}$), 137.0 (C-4a), 147.1 (C_{Ar}), 157.4 (C-8).

5.1.7. (±)-*trans*-2-Hydroxy-8-methoxy-3-(4-phenylpiperidino)tetralin, 8-MOBV, (±)-7. Racemic 8-hydroxybenzovesamicol, (±)-**6** (181 mg, 0.56 mmol) in 10 mL dry CH_3CN plus a few drops CH_2Cl_2, was rotoevaporated to dryness, 2 mL dry CH_3CN added and rotoevaporation repeated, to remove traces of moisture. To the residue, dissolved in 4 mL of dry CH_3CN, was added under inert atmosphere 70 μL of 1 M tetrabutylammonium hydroxide in methanol; the reaction mixture was stirred for 3–4 min followed by solvent rotoevaporation at room temperature. Traces of moisture were removed by azeotropically adding 2 mL of dry CH_3CN and rotoevaporation to dryness. Under inert atmosphere, the residue was resuspended in 6 mL of dry CH_3CN and CH_3I (160 mg, 1.12 mmol) added. The mixture was heated, at 85 °C for 1 h, and then let slowly rise to room temperature. After rotary evaporation of the solvent, the crude product was partitioned between CH_2Cl_2 and water (20 and 10 mL, respectively) and organic layer washed with 0.5 N NaOH (40 mL), dried over anhydrous Na_2SO_4, concentrated and purified by chromatography on silica gel (30% EtOAc in hexane) to give 127 mg (67%) of (±)-**7**, as a white solid (mp 202–203 °C).

^{1}H NMR ($CDCl_3$): δ 1.75–1.94 (m, 4H, 4H-10), 2.36–3.00 (m, 9H, 4H-9, 2H-4, H-3, H-11, H-1), 3.48 (dd, 1H, ^3J = 6 Hz, ^3J = 17 Hz, H-1), 3.78–3.82 (m, 4H, OCH_3, H-2), 6.70 (t, 2H, ^3J = 7 Hz, H-5, H-7), 7.12–7.41 (m, 6H, $5H_{Ar}$, H-6). ^{13}C NMR: δ 26.1 (C-4), 32.0 (C-1), 33.9–34.3 (2C-10), 42.9 (C-11), 45.0–53.6 (2C-9), 55.6 (OCH_3), 65.8 (C-3), 66.1 (C-2), 107.3 (C-7), 121.1 (C-5), 126.2 (C-8a), 126.6 (C-6), 126.8 ($2CH_{Ar}$), 128.4 ($2CH_{Ar}$), 136.3 (C-4a), 146.1 (C_{Ar}), 157.4 (C-8).

5.1.8. (±)-*trans*-2-Hydroxy-5-iodo-8-methoxy-3-(4-phenylpiperidino)tetralin, 8-MOIBV, (±)-8. To a solution of (±)-**7** (60 mg, 178 μmol) in glacial acetic acid (7 mL) was added dropwise a solution of iodine monochloride (65 mg, 400 μmol) in acetic acid (1 mL) and was heated at reflux overnight. The solvent was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (20 mL), and the solution was washed with saturated $NaHCO_3$ solution (20 mL) and the organic layer sepa-

rated. The aqueous layer was extracted with additional $CHCl_3$ (4×20 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude reaction mixture was purified by preparative TLC (silica gel and 30% ethyl acetate in petroleum ether) to afford 40 mg (47%) of (±)-**8** as a white solid.

^{1}H NMR ($CDCl_3$): δ: 1.78–1.94 (m, 4H, 4H-10), 2.42–3.00 (m, 9H, 4H-9, 2H-4, H-3, H-11, H-1), 3.33 (dd, 1H, ^3J = 16.9, 6 Hz, H-1), 3.78–3.87 (m, 4H, OCH_3, H-2), 6.48 (d, 1H, ^3J = 6.8 Hz, H-7), 7.23–7.38 (m, 5H, $5H_{Ar}$), 7.67 (d, 1H, ^3J = 6.8 Hz, H-6). ^{13}C NMR: δ 32.2, 33.0, 33.9, 34.3 (C-4, C-1, 2C-10), 42.9 (C-11), 45.0, 53.6 (2C-9), 55.6 (OCH_3), 65.4, 66.7 (C-3, C-2), 91.0 (C-5), 109.8 (C-7), 125.3 (CH_{Ar}), 125.2 (C-8a), 126.8 ($2CH_{Ar}$), 128.4 ($2CH_{Ar}$), 137.0 (C-6), 138.1 (C-4a), 146.1 (C_{Ar}), 157.1 (C-8).

5.1.9. Diastereomeric *O*-(*S*)-α-methoxy-α-trifluoromethylphenylacetyl derivatives of 8-MOIBV; (*R*,*R*)-9 and (*S*,*S*)-9. This procedure illustrates enantiomeric resolution in small scale, for molecules possessing only one reactive hydroxy group.

A mixture of racemic **8** (25 mg, 54 μmol), plus 4-(dimethylamino)pyridine, DMAP (18 μmol) and triethylamine (163 μmol) was prepared in dry $CHCl_3$ (2 mL). To the mixture was added in small portions and at room temperature, (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride; 18 mg (71 μmol). The reaction mixture was stirred for 18 h at room temperature, then poured into ethyl acetate (10 mL) and washed with saturated $NaHCO_3$ (15 mL). After separation of the organic layer, the aqueous layer was extracted with ethyl ether (3×20 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The two diastereomeric MTPA esters of **8** were separated by preparative TLC (silica gel; 5% EtOAc in petroleum ether, plate developed twice). After work up, 10.3 mg of the less polar (R_f = 0.25) diastereomer and 7 mg of the more polar (R_f = 0.17) were obtained. The less polar of the two MTPA esters, was presumed to be the (*S*,*S*)-**9** diastereomer and the more polar, the (*R*,*R*)-**9** diastereomer; by analogy with the TLC behaviour of the diastereomeric MPTA esters of 5-IBVM.[17]

5.1.10. Alkaline hydrolysis leading to (*R*,*R*)-8 and (*S*,*S*)-8. In separated reactions, each MTPA-ester (*S*,*S*)-**9** or (*R*,*R*)-**9**, (10 mg, 14.7 μmol and 7 mg, 10.3 μmol, respectively) was dissolved in THF (1 mL) and a solution of CH_3OH (0.5 mL) plus 2 N NaOH (2 mL) added. The reaction mixture was stirred at room temperature for 16 h. EtOAc (2.5 mL) was added, stirring for 10 min before transferring the mixture into a separatory funnel. The reaction vessel was washed with an additional 2.5 mL EtOAc and added to the same funnel. The organic layer was separated and the aqueous layer extracted with more EtOAc (2×5 mL) plus once with CH_2Cl_2 (5 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. After preparative TLC purification (30% EtOAc in petroleum ether), pure (*R*,*R*)-**8** was obtained in 71% yield and (*S*,*S*)-**8** in 97% yield.

5.1.11. (±)-*trans*-5-Amino-2-hydroxy-3-(4-cyano-4-phenylpiperidino)tetralin, 5-ACBV, (±)-12, (±)-*trans*-8-amino-2-hydroxy-3-(4-cyano-4-phenylpiperidino)tetralin, 8-ACBV, (±)-13. 4-Cyano-4-phenylpiperidine hydrochloride **10** (3.3 g, 14.5 mmol) was dissolved in ethanol (40 mL) containing triethyl amine (3.5 mL) and then added to *N*-(trifluoroacetyl)-1-amino-5,8-dihydronaphthalene oxide **11** (1.71 g, 6.66 mmol) dissolved in EtOH (20 mL). The mixture was refluxed for 20 h. The solvent was evaporated and the oil residue was dissolved in methanol (25 mL) and treated with 2 N NaOH (40 mL) and stirred at room temperature for 22 h. The reaction mixture was extracted with CH_2Cl_2 (2×50 mL). The organic layer was dried with anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel with CH_2Cl_2/MeOH/Et_3N: 195/5/1. (±)-**12** is the less polar, (R_f = 0.53, CH_2Cl_2/EtOH/TEA: 195/5/1) and is obtained in 36% yield. (±)-**13**, the more polar, (R_f = 0.29) is obtained in 28% yield.

(±)-**12**, ^{1}H NMR ($CDCl_3$): δ 2.12–2.53 (m, 4H, 4H-10), 2.85–3.35 (m, 9H, 2H-1, 2H-4, H-3, 4H-9), 3.66 (s, 2H, NH_2), 3.93–4.15 (m, 2H, H-2, OH), 6.20–6.24 (m, 2H, H-6, H-8), 7.04 (t, 1H, ^3J = 7.7 Hz, H-7), 7.39–7.58 (m, 5H, $5H_{Ar}$). ^{13}C NMR: δ 26.5 (C-4), 33.0 (C-1), 36.8, 37.1 (2C-10), 41.9, 50.1 (2C-9), 43.0 (C-11), 65.8, 65.9 (C-3, C-2), 112.6 (CH_{Ar}), 118.6 (CN), 119.3 (CH_{Ar}), 122.2 (C_{Ar}), 125.5 ($2CH_{Ar}$), 126.9 (CH_{Ar}), 128.2 (CH_{Ar}), 129.0 ($2CH_{Ar}$), 135.4 (C_{Ar}), 140.1 (C_{Ar}), 145.7 (C_{Ar}).

(±)-**13**, ^{1}H NMR ($CDCl_3$): δ 2.12–2.58 (m, 4H, 4H-10), 2.64–3.03 (m, 7H, 2H-4*, H-3, 4 H-9), 3.26 (m, 2H, 2H-1*), 3.71 (s, 2H, NH_2), 3.90–4.07 (m, 2H, H-2, OH), 6.59–6.65 (m, 2H, H-5, H-7), 7.04 (t, 1H, ^3J = 7.7 Hz, H-6), 7.39–7.59 (m, 5H, $5H_{Ar}$). ^{13}C NMR: δ 21.2 (C-4), 36.9, 37.3, 38.0 (2C-10, C-1), 43.0 (C-11), 41.9, 50.1 (2C-9), 65.3, 66.7 (C-3, C-2), 112.7 (CH_{Ar}), 119.1 (C_{Ar}), 119.5 (CH_{Ar}), 125.5 ($2CH_{Ar}$), 127.1 (CH_{Ar}), 128.3 (CH_{Ar}), 129.1 ($2CH_{Ar}$), 135.6 (C_{Ar}), 139.8 (C_{Ar}), 144.5 (C_{Ar}).

5.1.12. (±)-*trans*-8-Amino-2-hydroxy-5-iodo-3-(4-cyano-4-phenylpiperidino)tetralin, 8-ACIB, (±)-14. To a solution of (±)-8-aminocyanobenzovesamicol (**13**) (0.63 g, 1.82 mmol) in acetic acid (18 mL), at room temperature, was added dropwise a solution of iodine monochloride (0.3 g, 1.82 mmol) in acetic acid (2 mL). The reaction mixture was stirred overnight, and the solvent was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (20 mL), washed with saturated $NaHCO_3$ solution (15 mL) and extracted with $CHCl_3$ (3×30 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel with (35% ethyl acetate in petroleum ether) to afford 430 mg (50%) of (±)-**14** as a white solid (mp = 250–252 °C).

^{1}H NMR ($CDCl_3$): δ 2.16–2.26 (m, 4H, 4H-10), 2.54–3.00 (m, 7H, 2H-4*, H-3, H-1, 3H-9), 3.26 (m, 2H, H-1, H-9*), 3.71 (s, 2H, NH_2), 3.97–3.90 (m, 2H, H-2, OH), 6.36 (d, 1H, ^3J = 8.5 Hz, H-7), 7.37–7.57 (m, 6H,

5H_{Ar}, H-6). ^{13}C NMR: δ 21.6 (C-4), 36.8, 37.2 (2C-10), 41.9, 43.0 (C-1, C11), 44.4, 50.2 (2C-9), 65.9, 65.1 (C-3, C-2), 87.7 (C-5), 114.8 (CH_{Ar}), 121.3 (CN), 122.3 (C_{Ar}), 125.5 (2CH_{Ar}), 128.3 (CH_{Ar}), 129.1 (2CH_{Ar}), 137.1 (C_{Ar}), 137.1 (CH_{Ar}), 140.0 (C-4a), 144.8 (C_{Ar}).

5.1.13. Enantiomeric resolution of (±)-14, as a synthetic route to the enantiomeric pair 8-AMAIB: (*R*,*R*)- and (*S*,*S*)-15

5.1.13.1. Diastereomeric bis-*N*,*O*-(*S*)-α-methoxy-α-trifluoromethylphenylacetyl derivatives 14. Racemic **14** is an analogue possessing one hydroxy plus one amino reacting groups, its corresponding bis-*N*,*O*-(*S*)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) derivatives can be separated by chromatography by a similar method as that reported for 5-ABV.[14] Reductive cleavage of the protecting MTPA groups in each of the separated derivatives, using DIBAL-H, led to simultaneous reduction of the cyano into methylamino group.

A solution of (±)-**14** (0.41 mg, 0.87 mmol), plus 4-(dimethylamino)pyridine (63.4 mg, 0.52 mmol) and triethylamine (0.5 mL, 1.82 mmol) was prepared in dry $CHCl_3$ (15 mL). To the mixture was added in small portions and at room temperature, via syringe, (*R*)-(−)-MTPA chloride (503 mg, 2 mmol) in 2 mL dry $CHCl_3$. The reaction mixture was stirred for 19 h at ambient temperature, then poured into ethyl acetate (30 mL) and washed with saturated $NaHCO_3$ (30 mL) added carefully with constant stirring. After separation of the organic layer, the aqueous layer was extracted with ethyl acetate (2 × 40 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The two diastereomeric *N*,*O*-bis-MTPA isomers of racemic **14** were separated by chromatography (silica gel, CH_2Cl_2), to obtain 360 mg of the less polar compound (R_f = 0.53, EtOAc/CH_2Cl_2/petroleum ether: 1/2/7) and 260 mg of the more polar diastereomer (R_f = 0.36, EtOAc/CH_2Cl_2/petroleum ether: 1/2/7). By analogy with the TLC behaviour of the diastereomeric *N*,*O*-bis-MPTA derivatives of 5-ABV,[14] the less polar compound was presumed to be the (*S*,*S*)-*N*,*O*-bis-MTPA diastereomer of **14** (46% yield) and the more polar the (*R*,*R*)-MTPA diastereomer of **14** (33% yield).

5.1.14. Reductive cleavage to yield (*R*,*R*)- and (*S*,*S*)-15.

Procedure was similar to the one reported for 5-ABV,[14] except that the reaction vessel set up was flushed initially with nitrogen gas for 30 min. (*S*,*S*)-Diastereomer, (360 mg, 0.398 mmol) was dissolved in dry toluene (9 mL) and keep under inert atmosphere, stirring for 10 min and then cooled to −78 °C. Diisobutyl aluminium hydride (DIBAL-H, 2 mL of 1 M solution in cyclohexane; 2 mmol) was added dropwise via syringe. The resulting solution was stirred at −80 °C for 30 min and then 2 mL more of DIBAL-H were added to assure complete reduction of the cyano group. The reaction mixture was allowed to slowly warm to room temperature, quenched with 2.5 N HCl (10 mL) and left overnight at room temperature. The toluene layer was separated, the aqueous layer made alkaline (pH 10) with 6 N NaOH (about 8 mL) and extracted with CH_2Cl_2 (4 × 40 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (CH_2Cl_2/EtOAc/hexane/EtOH/Et_3N: 1.1/1/1/0.1/0.02). The larger fraction collected (about 110 mg) was re-purified using two preparative TLC 20 × 20 glass backed silica plates (about 55 mg in each) with EtOAc/hexane/EtOH/Et_3N (1/1/0.25/0.02) as mobile phase. The lowest band was scratched, extracted (MeOH), filtered (sintered funnel) and the solvent evaporated under reduced pressure. Recovered 80 mg of a white solid (mp = 230–231 °C). The enantiomer (*S*,*S*)-8-amino-2-hydroxy-5-iodo-3-(4-methylamino-4-phenylpiperidino)tetralin; (*S*,*S*)-8-AMAIB, (*S*,*S*)-**15** was obtained in 45% yield.

The (*R*,*R*)-**15** enantiomer was obtained, by a similar procedure, in 41% yield, starting with 260 mg (0.29 mmol) of the (*R*,*R*)-bis-MTPA diastereomer.

^{1}H NMR ($CDCl_3$): δ 1.38 (t, 2H, ^3J = 7.2 Hz, CH_2NH_2), 2.00–2.10 (m, 4H, 4H-10), 2.34–3.0 (m, 10H, H-1, H-3, 2H-4, 4H-9, CH_2NH_2), 3.35 (dd, 1H, ^3J = 16.7, 5.6 Hz, H-1), 3.58 (s, 2H, NH_2), 3.63 (s, 1H, OH), 3.75–3.88 (m, 1H, H-2), 6.32 (d, 1H, ^3J = 8.5 Hz, H-7), 7.29–7.53 (m, 7H, 5H_{Ar}, H-6, H-8). ^{13}C NMR: δ 32.9 (C-1), 33.8 (C-4), 34.8 (2C-10), 42.8 (C-11), 44.9 (2C-9), 50.9 (CH_2NH_2), 65.2, 67.2 (C-3, C-2), 102.9 (CI), 126.2 (CH_{Ar}), 126.8 (2CH_{Ar}), 127.8 (CH_{Ar}), 128.5 (2CH_{Ar}), 129.6 (CH_{Ar}), 135.8 (C_{Ar}), 137.1 (CH_{Ar}), 137.4 (C_{Ar}), 146.0 (C_{Ar}).

5.1.15. (*R*,*R*)- and (*S*,*S*)-2-Hydroxy-5-iodo-3-(4-aminomethyl-4-phenylpiperidino)tetralin, MAIBV, (*R*,*R*)- and (*S*,*S*)-16.

A solution of $NaNO_2$ (13 mg, 188 μmol) in H_2O (0.7 mL) was added dropwise to a cooled (5 °C) solution of (*S*,*S*)-**15** (82 mg, 172 μmol) in 50% hypophosphorous acid (4 mL) and concentrated HCl (1 mL) keeping the reaction temperature around 0 °C, stirring for 60 min and then allowed to warm to room temperature overnight, under continuous stirring. The reaction mixture was poured into saturated NaCl solution (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The aqueous layer was removed, carefully basified with $NaHCO_3$ (around 60 mL) and extracted with more CH_2Cl_2 (5 × 20 mL); the combined extracts were carefully washed with saturated $NaHCO_3$ (20 mL), and then with water (5 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was placed on two preparative TLC silica gel glass backed plates and run twice with EtOAc/hexane/EtOH/Et_3N (1.25/1.25/0.2/0.12). After work up, 73 mg (92%) of the enantiomer (*S*,*S*)-**16** was obtained as a solid (from MeOH). The same experimental conditions, starting with 56 mg (117 μmol) of (*R*,*R*)-**15** afforded 48 mg (88%) of the enantiomer (*R*,*R*)-**16**.

^{1}H NMR ($CDCl_3$): δ 1.31 (t, 2H, ^3J = 7.2 Hz, CH_2NH_2), 2.06–3.12 (m, 14H, 4H-10, 4H-9, 2H-4, H-3, H-1, CH_2NH_2), 3.38 (dd, 1H, ^3J = 6.0 Hz, ^3J = 16.8 Hz, H-1), 3.61 (s, 1H, OH), 3.73–3.96 (m, 1H, H-2), 6.81 (t, 1H, ^3J = 7.6 Hz, H-7), 7.02 (d, 1H, ^3J = 7.6 Hz, H-8), 7.28–7.48 (m, 5H, 5H_{Ar}), 7.71 (d, 1H, ^3J = 7.6 Hz, H-6). ^{13}C NMR: δ 27.2 (C-4), 31.5 (C-1), 42.5 (2C-10), 43.2 (C-11), 44.4 (2C-9), 47.6 (CH_2NH_2), 65.5, 66.6

(C-3, C-2), 102.1 (C-5), 126.6 (CH_{Ar}), 127.1 ($2CH_{Ar}$), 127.6 (CH_{Ar}), 128.9 ($2CH_{Ar}$), 129.2 (CH_{Ar}), 136.9 (CH_{Ar}), 136.2 (C_{Ar}), 137.2 (CH_{Ar}), 145.0 (C_{Ar}).

5.2. Biological evaluation: in vitro testing

Dissociation constants of novel compounds were determined by competition against the binding of 5 nM [^{3}H]vesamicol to postnuclear supernatant prepared from PC12 cells stably expressing human VAChT similarly as described.[26] Compounds were assayed in increments of 10-fold from 0.1 to 10,000 nM concentration. The surfaces of containers were precoated with Sigmacote. Samples containing 200 μg postnuclear supernatant in 200 μL were incubated at 22 °C for 24 h in 0.02% sodium azide. A volume of 90 μL was filtered in duplicate through GF/F glass fiber filters coated with polyethylenimine and washed. Filter-bound radioactivity was determined by liquid scintillation spectrometry. Averaged data were fit by regression with a rectangular hyperbola to estimate K_d. All compounds were independently assayed at least two times.

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