Short thesis for the degree of doctor of philosophy (PhD)

Stereoselective synthesis and structural analysis of *bis*-isochroman derivatives

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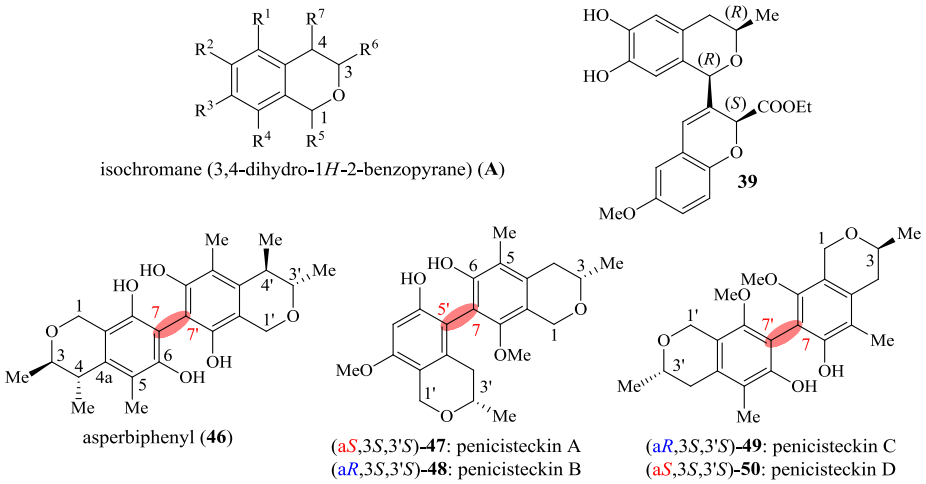
1. Introduction and objectives

Chiral substituted isochroman (3,4-dihydro-1*H*-2-benzopyran) derivatives (**A**) belong to a group of naturally occurring secondary metabolites. These *O*-heterocyclic compounds majorly contain hydroxy or alkoxy functional groups on the condensed aromatic ring or at the C-1, C-3, C-4 positions. Some of their natural and synthetic derivatives exhibit significant pharmacological activity (e.g. antibacterial, antioxidant, anti-inflammatory, neuroprotective, cytotoxic, antiproliferative activity). Our research group is experienced in the synthesis of isochroman derivatives. The optically active synthetic isochroman-2*H*-chromene hybrid molecule (**39**) with neuroprotective activity and its possible isomers were synthetized and their structures were investigated by ECD and VCD spectroscopy. Despite the fact that the benzene ring of naturally occurring isochroman compounds often contains activating substituents (hydroxyl and/or alkoxy groups), a few examples of biaryl-type *bis*-isochroman derivatives formed by oxidative coupling are known in the literature. Asperbiphenyl (**46**) was the first axially chiral *bis*-isochroman which was isolated from a marine fungus. The structures of several biologically active, axially chiral *bis*-isochromans (**47**-**50**) were determined by our research group in collaboration (Scheme 1). Considering the remarkable pharmacological activity of this type of compounds and the challenge of determining their structures, our aim was to synthetize optically active, axially chiral *bis*-isochromans and to carry out their complete analytical, respectively pharmacological investigation.



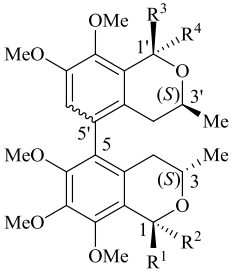
Scheme 1. Isochroman derivatives (**A**, **39**) and *bis*-isochromans with axial chirality (**46**-**50**).

2. Applied methodes

Thin-layer chromatography (TLC) was used to monitor the reactions and check the purity of the materials. Separation and purification of the compounds were usually performed by column chromatography (conventional/flash) and/or preparative HPLC. The physical constants (melting point, retention factor) of the synthesized new compounds were characterized. The structures of the compounds were determined by 1D and 2D NMR measurements, infrared spectroscopy, mass spectrometry, single crystal X-ray diffraction and chiroptical measurements (OR, ECD, VCD). The relative and absolute configurations of the compounds were determined by 2D ^{1}H-^{1}H NOESY and ROESY NMR, ECD, VCD measurements and single crystal X-ray crystallography. *In vitro* pharmacological studies of the prepared target compounds **Y** were performed in cooperation with the Institute of Medical Microbiology of the Semmelweis University in Budapest using four Gram-positive *(B. subtilis*, MSSA, MRSA and *E. faecalis*) and one Gram-negative (*A. baumannii*) bacterial strains.

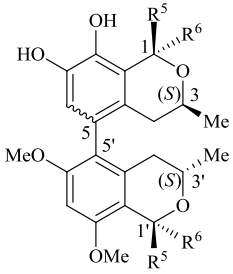
3. New scientific results of the dissertation

Bis-isochroman derivatives with 6,7,8,7',8'-pentamethoxy (**X**) and 7,8-dihydroxy-6',8'-dimethoxy (**Y**) substitution patterns were prepared by using two different synthetic strategies (Scheme 2.).



X

R^1, R^2: H, 4-F-C_6H_4
R^3, R^4: H, COOEt, CH_2COOEt



Y

R^5, R^6: H, 4-F-C_6H_4; 4-Br-C_6H_4; 3,4,5-$(OMe)_3$-C_6H_2; 3,4-(OCH_2O)-C_6H_3

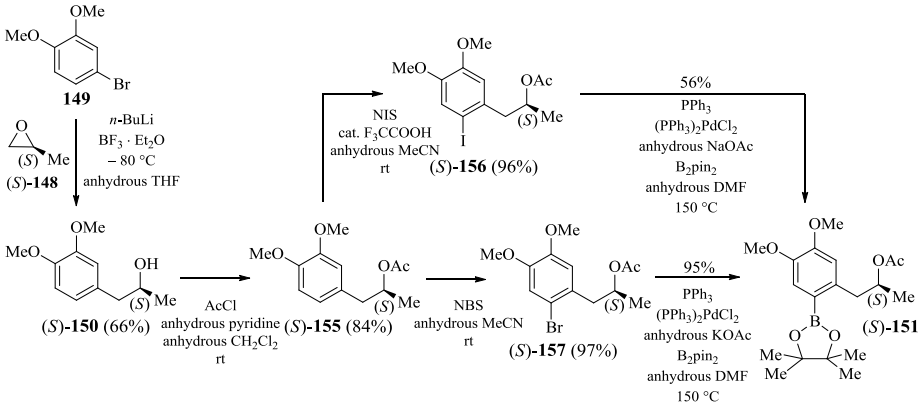
Schme 2. Prepared *bis*-isochroman target compounds (**X**, **Y**).

The chiral isochroman moiety was first evolved during the synthesis of the target compounds **X**, which was followed by the formation of the biaryl axis. In the synthesis of target compounds **Y**, the formation of the biaryl axis preceded the formation of the isochroman subunits.

3.1 Preparation of the target compounds X

3.1.1 Optically active 1-arylpropan-2-ol boronate ester and 1-aryl-5-iodoisochroman coupling partners for the Suzuki cross-coupling reaction were prepared in several steps.

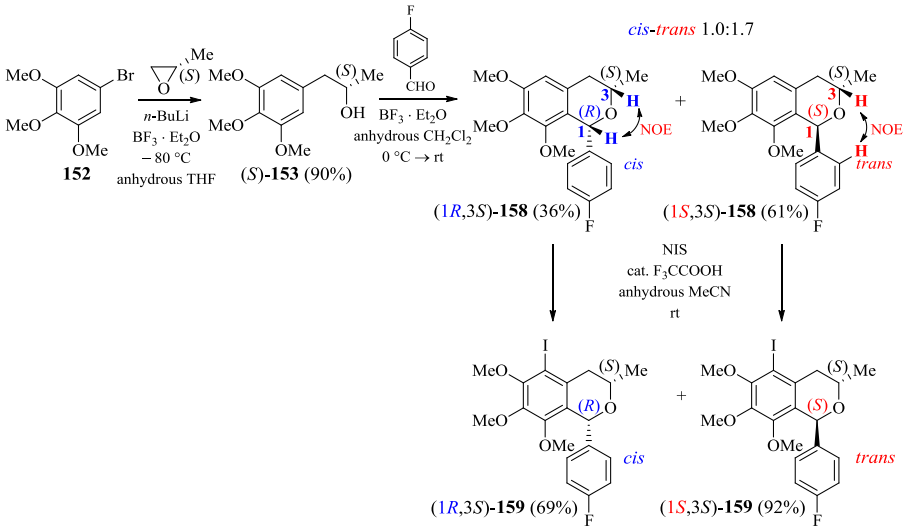
First, the corresponding (*S*)-1-arylpropan-2-ol derivative [(*S*)-**150**] was synthetized by regioselective ring-opening reaction of (*S*)-propylene oxide with aryl lithium reagent obtained from 1-bromo-3,4-dimethoxybenzene (4-bromeveratrol, **149**) *in situ*. Then, the secondary hydroxyl group was protected by acetyl group [(*S*)-**150** → (*S*)-**155**)]. The chirality centre was not affected by the epoxide ring-opening reaction, therefore it was appeared with unchanged (*S*) absolute configuration in the product. A regioselective iodination was performed on the protected derivative with *N*-iodosuccinimide (NIS) under acid catalysis [(*S*)-**155** → (*S*)-**156**]. The boronate ester coupling partner was obtained by Miyaura borylation with *bis*(pinacolato)diboron [(*S*)-**156** → (*S*)-**151**] with 56% yield. The reason of the low yield was the side reaction of the iodo compound (*S*)-**156** under the conditions of the borylation reaction in which iodine-hydrogen exchange was taken place. To increase the yield of the reaction (*S*)-**157** bromo compound obtained by regioselective bromination [(*S*)-**155** → (*S*)-**157**] of the acetyl protected derivative [(*S*)-**155**] with *N*-bromosuccinimide (NBS) was used to synthetize boronate ester derivative. Miyaura borylation of the brominated derivative resulted in the desired coupling partner [(*S*)-**157** → (*S*)-**151**] with excellent yield (95%) (Scheme 3).



Scheme 3. Preparation of the optically active 1-arylpropan-2-ol boronate ester [(*S*)-**151**] coupling partner from bromine and iodo derivatives.

To prepare the 1-aryl-5-iodoisochroman diastereomeric coupling partners [(1*R*/*S*,3*S*)-**159**], first the alcohol derivative (*S*)-**153** was prepared by the ring-opening reaction of 1-bromo-3,4,5-

trimethoxybenzene (**152**) and (*S*)-propylene oxide. Oxa-Pictet-Spengler cyclization of the (*S*)-1-arylpropan-2-ol derivative [(*S*)-**153**] was carried out with 4-fluorobenzaldehyde catalyzed by Lewis acid to result in *cis*/*trans* diastereomers [(*S*)-**153** → *cis*-(1*R*,3*S*)-**158** and *trans*-(1*S*,3*S*)-**158**] in the ratio of 1:1.7. The *cis*-(1*R*,3*S*)-**158** and *trans*-(1*S*,3*S*)-**158** diastereomers were separated by column chromatography and their relative and absolute configurations were determined by 2D ^{1}H-^{1}H NOESY NMR measurements based on NOESY correlation of *cis pseudoaxial* H-1 and H-3 protons. The 5-iodoisochroman coupling partners [(1*R*,3*S*)-**159**, (1*S*,3*S*)-**159**] were obtained by regioselective iodination with NIS reagent [(1*R*,3*S*)-**158** → (1*R*,3*S*)-**159**, and (1*S*,3*S*)-**158** → (1*S*,3*S*)-**159**] with good/medium and excellent yields (Scheme 4).



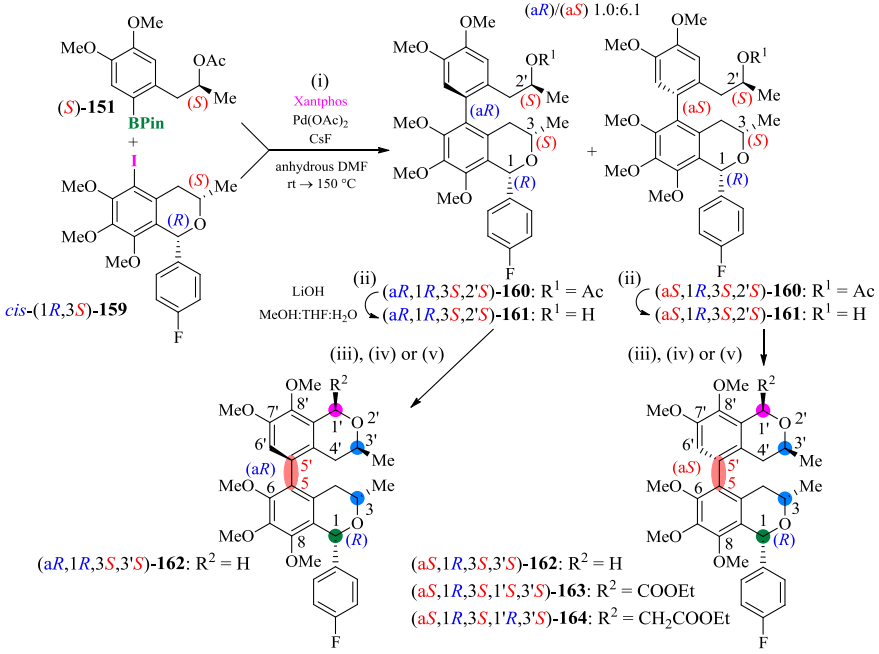
Scheme 4. Preparation of stereopure 1-(4-fluorophenyl)-5-iodoisochroman diastereomeric coupling partners [(1*R*,3*S*)-**159**, (1*S*,3*S*)-**159**] for Suzuki reaction.

3.1.2 The Suzuki cross-coupling reaction was performed by using the coupling partners with known absolute configuration. After removing the protecting groups, the second oxa-Pictet-Spengler cyclization reaction was carried out.

Suzuki cross-coupling reactions of the 5-iodoisochroman diastereoisomers [(1*R*,3*S*)-**159**, (1*S*,3*S*)-**159**] and the boronate ester [(*S*)-**151**] were performed separately (Scheme 5-6).

When the cross-coupling reaction was carried out with *cis*-(1*R*,3*S*)-**159** 5-iodoisochroman, chiral induction from the central to the axial chirality element was observed. The formation of the atropisomer (a*S*,1*R*,3*S*,2'*S*)-**160** was proved to be preferred with the ratio of 1.0:6.1 (a*R*/a*S*).

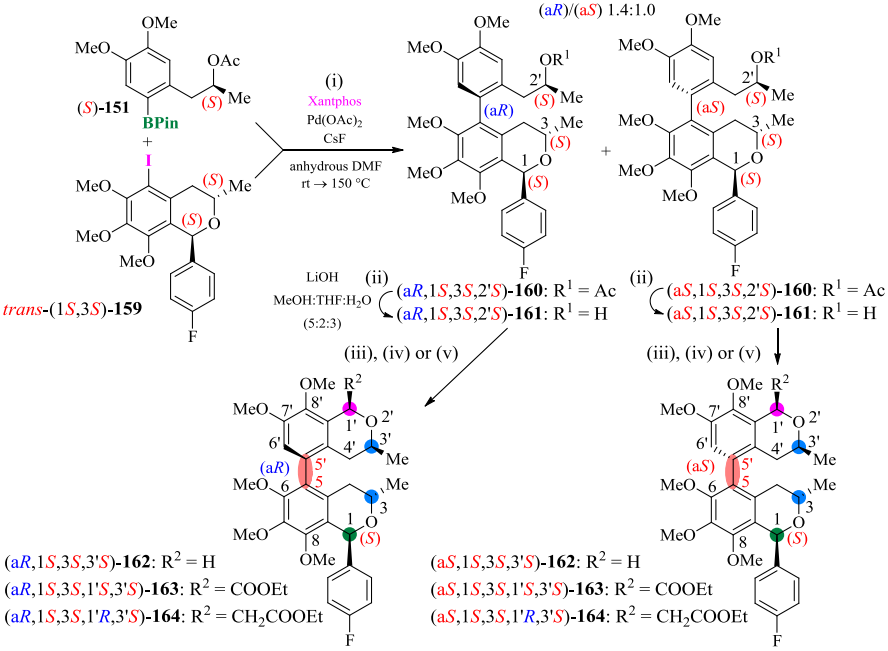
The (a*S*,1*R*,3*S*,2'*S*)-**160** and (a*R*,1*R*,3*S*,2'*S*)-**160** atropodiastereomers formed in the coupling reaction were separated by column chromatography. After removing the acetyl protecting groups, stereopure compounds were used in the second ring closure reactions which resulted in (a*S*,1*R*,3*S*,1'*S*,3'*S*)-**163** and (a*S*,1*R*,3*S*,1'*R*,3'*S*)-**164** compounds diastereoselectively with *cis*-1',3' configurations (Scheme 5).



Scheme 5. Suzuki cross-coupling reaction of the *cis*-(1*R*,3*S*)-**159** and the boronate ester [(***S***)-151]. After removing the protecting groups, the second oxa-Pictet-Spengler cyclization reaction was carried out. Reagents and conditions: (i) (a) *cis*-(1*R*,3*S*)-**159**, Xantphos, $Pd(OAc)_2$, Ar/N_2, DMF, rt, 1 hour; (b) (*S*)-**151**, CsF, Ar/N_2, DMF, rt 30 minutes; (c) a + b, Ar/N_2, 150 °C, 1.5 hours, (a*R*,1*R*,3*S*,2'*S*)-**160** (11%), (a*S*,1*R*,3*S*,2'*S*)-**160** (68%) (*dr* 1.0:6.1). (ii) LiOH, MeOH:THF:H_2O (1.0:0.8:0.5), rt, 4 hours, (a*R*,1*R*,3*S*,2'*S*)-**161** (94%), MeOH:THF:H_2O (5:2:3), rt, 4 hours (a*S*,1*R*,3*S*,2'*S*)-**161** (96%). (iii) MOMCl, $ZnCl_2$, Et_2O, 0 °C, 3 hours, (a*R*,1*R*,3*S*,3'*S*)-**162** (89%), 4 hours, (a*S*,1*R*,3*S*,3'*S*)-**162** (97%). (iv) $(Et_2O)_2CHCOOEt$, $BF_3{\cdot}Et_2O$, toluene, (SI) (a*S*,1*R*,3*S*,1'*S*,3'*S*)-**163** (23%). (v) $(Et_2O)_2CHCH_2COOEt$, $BF_3{\cdot}Et_2O$, toluene, 0 °C → rt, 16 hours, (a*S*,1*R*,3*S*,1'*R*,3'*S*)-**164** (91%).

In case of the Suzuki cross-coupling reaction of *trans*-(1*S*,3*S*)-**159** iodoisochroman and (*S*)-**151** boronate ester, (a*R*,1*S*,3*S*,2'*S*)-**160** atropisomer was observed with weak diastereoselectivity [(a*R*/a*S*) 1.4:1.0] in the absence of chiral induction. After removing the acetyl protecting groups, (a*R*,1*S*,3*S*,2'*S*)-**161** and (a*S*,1*S*,3*S*,2'*S*)-**161** atropodiastereomers could be separated with column chromatography. Thus, the formation of the second isochroman unit could be performed with

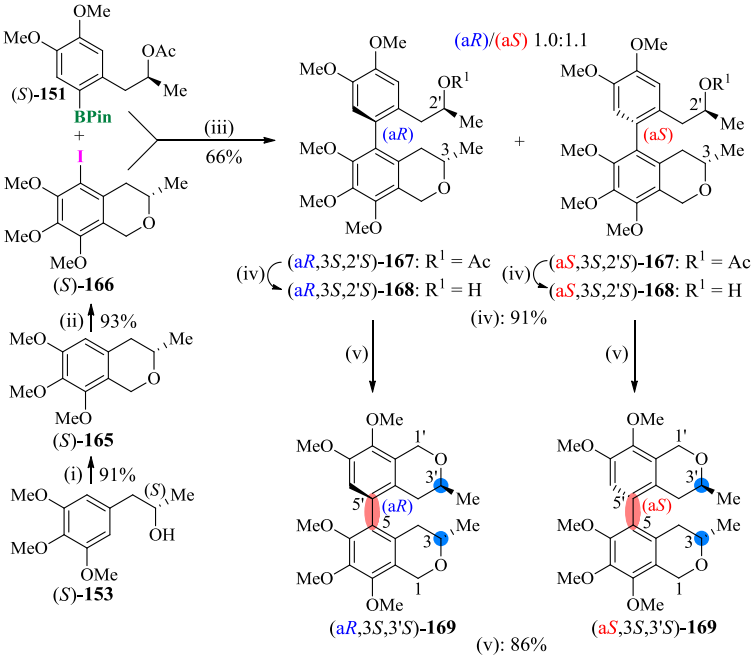
stereopure compounds to result in (a*S*/a*R*,1*S*,3*S*,1'*S*,3'*S*)-**163** and (a*S*/a*R*,1*S*,3*S*,1'*R*,3'*S*)-**164** diastereoselectively also with *cis*-configurations (Scheme 6).



Scheme 6. Suzuki cross-coupling reaction of the *trans*-(1*S*,3*S*)-**159** and the boronate ester [(***S***)-151]. After removing the protecting groups, the second oxa-Pictet-Spengler cyclization reaction was carried out. Reagents and conditions: (i) (a) *trans*-(1*S*,3*S*)-**159**, Xantphos, $Pd(OAc)_2$, Ar/N_2, DMF, rt, 1 hour; (b) (*S*)-151, CsF, Ar/N_2, DMF, rt 30 minutes; (c) a + b, Ar/N_2, 150 ◦C, 1.5 hours, atropodiastereomeric mixture of (a*R*,1*S*,3*S*,2'*S*)-**160** and (a*S*,1*S*,3*S*,2'*S*)-**160** (79%) (*dr* 1.4:1.0). (ii) LiOH, MeOH:THF:H_2O (5:2:3), rt, 4 hours, (a*S*,1*S*,3*S*,2'*S*)-**161** (40%), (a*R*,1*S*,3*S*,2'*S*)-**161** (53%). (iii) MOMCl, $ZnCl_2$, Et_2O, 0 °C, 4 hours, (a*R*,1*S*,3*S*,3'*S*)-**162** (99%), 6 hours, (a*S*,1*S*,3*S*,3'*S*)-**162** (96%). (iv) $(Et_2O)_2CHCOOEt$, $BF_3{\cdot}Et_2O$, toluene, (SI) (a*R*,1*S*,3*S*,1'*S*,3'*S*)-**163** (71%), (a*S*,1*S*,3*S*,1'*S*,3'*S*)-**163** (38%). (v) $(Et_2O)_2CHCH_2COOEt$, $BF_3{\cdot}Et_2O$, toluene, 0 °C → rt, 3 hours, (a*R*,1*S*,3*S*,1'*R*,3'*S*)-**164** (94%), 4 hours, (a*S*,1*S*,3*S*,1'*R*,3'*S*)-**164** (90%).

The Suzuki cross-coupling reaction was also performed with C-1 unsubstituted 5-iodoisochroman derivative [(*S*)-**166**]. Following the oxa-Pictet-Spengler cyclization reaction [(*S*)-**153** → (*S*)-**165**] of the corresponding 1-arylpropan-2-ol derivative [(*S*)-**153**] with chloromethyl methyl ether, S_EAr iodination reaction was carried out to result in (*S*)-**166**. The Suzuki biaryl coupling reaction of the C-1 unsubstituted iodo compound [(*S*)-**166**] and protected boronate ester derivative [(*S*)-**151**] gave an inseparable mixture of atropisomers [(a*S*/a*R*,3*S*,2'*S*)-**167**] with the ratio of nearly 1:1. After the deacetylation reaction of the atropodiastereomeric mixture [(a*S*/a*R*,3*S*,2'*S*)-**167** → (a*S*/a*R*,3*S*,2'*S*)-**168**)], the formation of the second isochroman unit was performed by MOMCl to afford 5,5'-linked *bis*-isochromans [(a*S*/a*R*,3*S*,3'*S*)-**169**]

containing methylene groups at the C-1 and C-1' positions (Scheme 7). After chiral preparative HPLC separation using Chiralpak IC column, stereopure (a*S*) and (a*R*) atropisomers were used for chiroptical analysis.



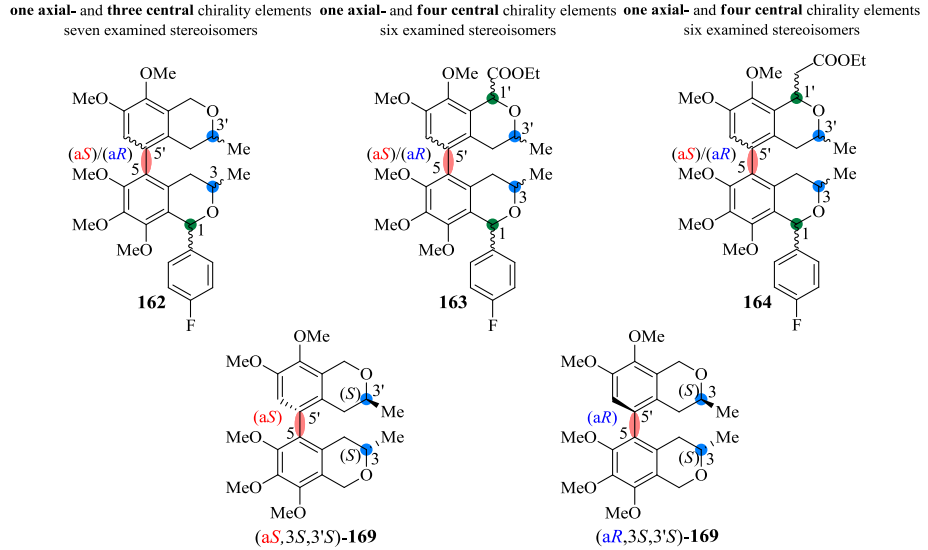
Scheme 7. Synthesis of 5,5'-linked *bis*-isochromans (**169**) containing methylene groups at the C-1 and C-1' positions. Reagents and conditions: (i) MOMCl, $ZnCl_2$, Et_2O, 0 °C, 4 hours, (*S*)-**165** (91%). (ii) NIS, TFA, MeCN, rt, 1.5 hours, (*S*)-**166** (93%). (iii) (a) (*S*)-**166**, SPhos, $Pd(OAc)_2$, Ar, DMF, rt, 1 hour; (b) (*S*)-**151**, CsF, Ar, DMF, rt, 30 minutes; (c) a + b, Ar, 150 ◦C, 1.5 hours, atropodiastereomeric mixture of (a*R*,3*S*,2'*S*)-**167** and (a*S*,3*S*,2'*S*)-**167** (66%) (*dr* 1.0:1.1). (iv) LiOH, MeOH:THF:H_2O (7:2:2), rt, 3 hours, (a*R*,3*S*,2'*S*)-**168** and (a*S*,3*S*,2'*S*)-**168** (91%). (v) MOMCl, $ZnCl_2$, Et_2O, 0 °C, 4 hours, atropodiastereomeric mixture of (a*R*/a*S*,3*S*,2'*S*)-**169** (86%), separated by HPLC using Chiralpak IC column.

3.1.3 Based on the previously described sequence, enantiomers of (162, 163, 164) were also prepared from (*R*)-propylene oxide, whose ECD and VCD spectra were used to determine the central and axial chirality elements side by side. VCD measurements of the enantiomer pairs allowed the validation of low-intensity VCD transitions and the identification of measurement artefacts.

3.1.4 We performed chiroptic analysis of the target compounds X in order to draw conclusions regarding to the determination of axial and central chirality side by side.

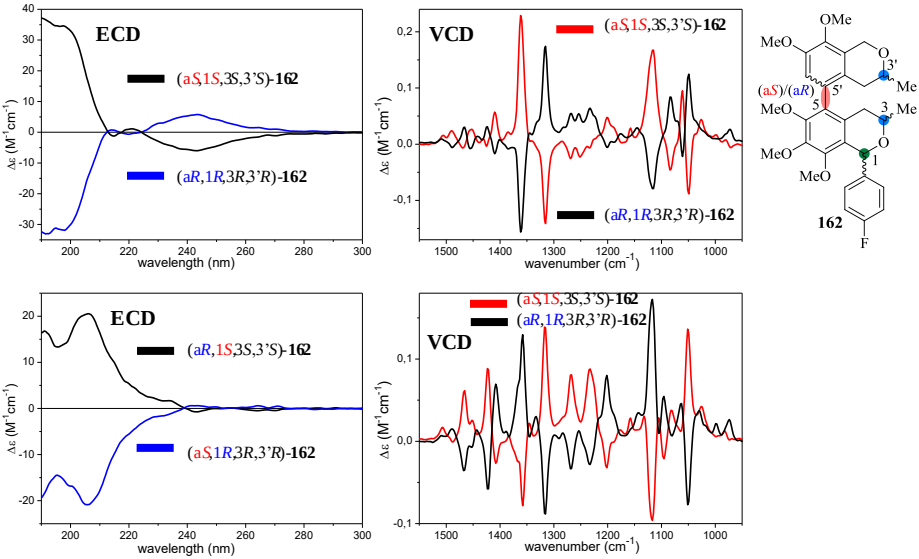
Seven stereoisomers of **162** were used as model compounds for ECD, VCD, OR measurements and calculations to determine the axial and central chirality elements side by

side. All six stereoisomers of **163** and **164** containing five known stereogenic centres were also compared with each other from the aspect of chiroptical spectroscopy. (a*S*/a*R*)-**169** atropisomers with (3*S*,3'S) configuration and without stereogenic centres at the C-1 and C-1' positions were also investigated, which differ only in axial chirality (Scheme 8).



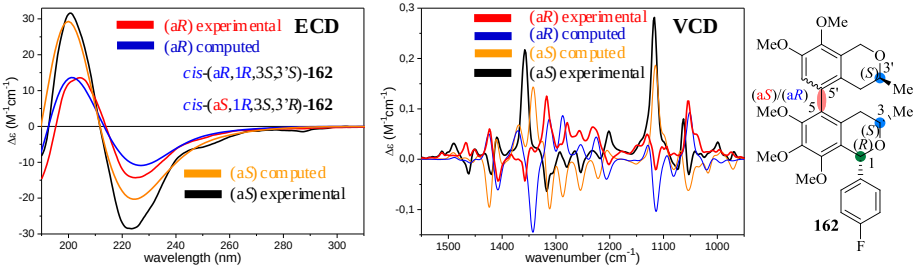
Scheme 8. Demonstration of target compounds **X** investigated by chiroptical spectroscopy**.**

The enantiomers of **162** and **164** showed near mirror image ECD and VCD spectra, which allowed the validation of the weak Cotton effects (CEs), artefacts and VCD measurement method. Measured ECD and VCD spectra of two pairs of enantiomers of **162** [(a*S*,1*S*,3*S*,3'*S*)-**162** and (a*R*,1*R*,3*R*,3'*R*)-**162**, respectively (a*R*,1*S*,3*S*,3'*S*)-**162** and (a*S*,1*R*,3*R*,3'*R*)-**162**] are presented to show the practically mirror image correlation (Scheme 9).



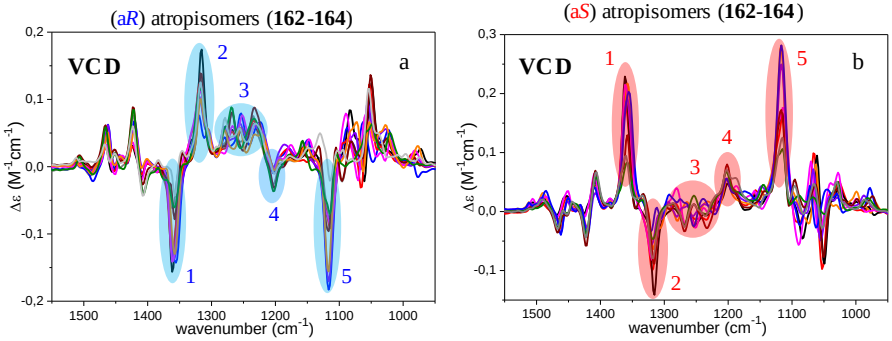
Scheme 9. Pairs of enantiomers [(a*S*,1*S*,3*S*,3'*S*)-**162**–(a*R*,1*R*,3*R*,3'*R*)-**162**] and [(a*R*,1*S*,3*S*,3'*S*)-**162**–(a*S*,1*R*,3*R*,3'*R*)-**162**] showed practically mirror image ECD and VCD spectra.

The measured and computed ECD, VCD spectra of *cis*-(a*R*,1*R*,3*S*,3'*S*)-**162** and *cis*-(a*S*,1*R*,3*S*,3'*S*)-**162** atropodiastereomers are shown in Scheme 10 as further example. The (a*R*) and (a*S*) atropisomers exhibited nearly identical ECD spectra, which is confirmed by the good agreement with the B3LYP/TZVP PCM/MeCN calculations. It can be concluded that the assignment of axial chirality is not feasible with ECD measurements. Measured and computed VCD spectra of the atropisomers of (a*R*/a*S*,1*R*,3*S*,3'*S*)-**162** gave a near mirror image agreement in the wavenumber range 1100-1450 cm^{-1}, which derives from the different axial chirality of the atropodiastereomers. The axial chirality can be clearly assigned from the VCD spectra due to the good agreement between the experimental and computed VCD spectra.



Scheme 10. Nearly identical measured and computed ECD, VCD spectra of *cis*-(a*R*,1*R*,3*S*,3'*S*)-**162** and *cis*-(a*S*,1*R*,3*S*,3'*S*)-**162** atropodiastereomers.

The measured VCD spectra of the atropisomers of **162-164** (a*R*) and (a*S*) with characteristic VCD transitions typical of the axial chirality were compared with each other (Scheme 11). Based on our experience, regardless of the substitution at position C-1' (H, COOEt or CH_2COOEt) and the absolute configuration of the central chirality elements (C-1, C-1', C-3, C-3'), the axial chirality of the stereogenic biaryl axis can be assigned from the measured VCD spectra. Five main characteristic wavenumber ranges can be identified, but additional characteristic bands can also be observed above 1400 cm^{-1} and below 1100 cm^{-1}.



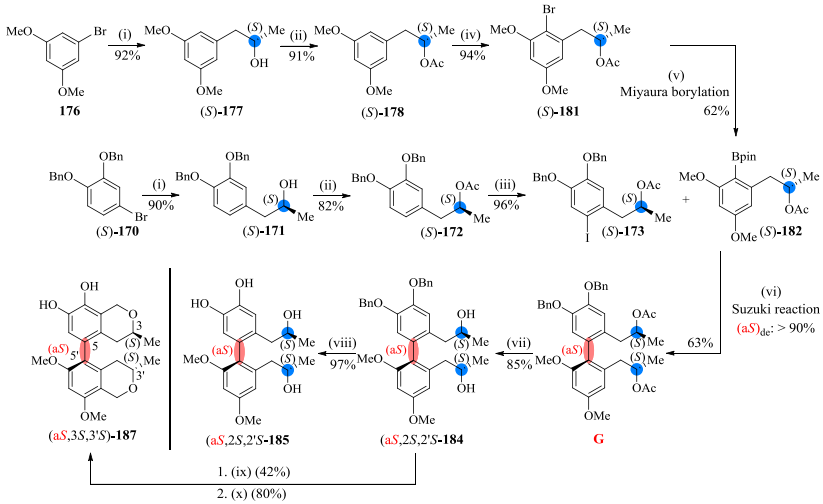
Scheme 11. Comparison of VCD spectra of (a*R*, a) és (a*S*, b) atropisomers (**162-164**) with identical axial chirality.

In general, it can be stated that ECD spectra of the target compound **X** provide information about the central chirality, while VCD measurements provide information about the axial chirality.

4.1 Preparation of the target compounds Y

4.1.1 The iodoisochroman and boronate ester derivatives with the corresponding substitution pattern were prepared similarly according to the reaction scheme described above. The Suzuki cross-coupling reaction was carried out, then removing the protecting groups led to an activated biaryl alcohol derivative which was used for the oxa-Pictet-Spengler cyclization reaction to obtain the isochroman subunit. The preparation of C-1 and C-1' unsubstituted *bis*-isochroman was also performed.

Suzuki coupling of (*S*)-**173** iodoisochroman and (*S*)-**182** boronate ester compounds was carried out at 150 °C with high diastereoselectivity by chiral induction (Scheme 12).

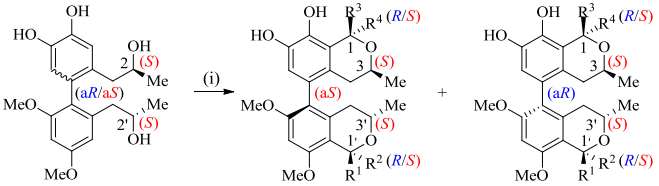


Scheme 12. Synthesis of coupling partners and preparation for the oxa-Pictet-Spengler cyclization reaction. Reagents and conditions: (i) *n*-BuLi, (*S*)-propylene oxide, $BF_3{\cdot}Et_2O$, anhydrous THF, − 78 °C, (*S*)-**177** (92%), (*S*)-**171** (90%). (ii) anhydrous pyridine, AcCl, anhydrous CH_2Cl_2, rt, (*S*)-**178** (91%), (*S*)-**172** (82%). (iii) NIS, TFA, anhydrous MeCN, rt, (*S*)-**173** (96%). (iv) NBS, anhydrous MeCN, rt, (*S*)-**181** (94%). (v) PPh_3, $(PPh_3)_2PdCl_2$, anhydrous KOAc, B_2pin_2, anhydrous DMF, 150 °C, (*S*)-**182** (62%). (vi) (a) (*S*)-**173**, Xantphos, $Pd(OAc)_2$, anhydrous DMF, rt; (b) (*S*)-**182**, CsF, anhydrous DMF, rt; a + b, 150 °C, **G** (63%). (vii) LiOH, MeOH, rt, (a*S*,2*S*,2'*S*)-**184** (85%). (viii) H_2/Pd-C, THF, rt, (a*S*,2*S*,2'*S*)-**185** (97%). (ix): MOMCl, $ZnCl_2$, anhydrous THF, rt, (42%). (x): H_2/Pd-C, THF, rt, (a*S*,3*S*,3'*S*)-**187** (80%).

When forming the stereogenic biaryl axis, the known (*S*) absolute configuration coupling partners resulted in compound **G** with high atropodiastereomer excess [$(aS)_{de}$ > 90%]. The (a*S*) axial chirality was confirmed by X-ray diffraction and VCD measurements on the some target compounds. The acetyl and benzyl protecting groups of the **G** biaryl compound were cleaved by basic hydrolysis, respectively catalytic hydrogenation. In consequence, the Brønsted acid catalysed oxa-Pictet-Spengler cyclization reaction was promoted by the obtained electron donating phenolic hydroxyl groups of **185** diol derivative. The cyclization reaction was also carried out with the benzyl-protected **184** derivative using chloromethyl methyl ether, and removing the benzyl protecting groups resulted in C-1, C-1' unsubstituted *bis*-isochroman (**187**).

4.1.2 The oxa-Pictet-Spengler cyclization reaction was performed with various aromatic aldehydes to prepare *bis*-isochroman derivatives with 7,8-dihydroxy-6',8'-dimethoxy substitution pattern.

Four different aromatic aldehydes (4-fluorobenzaldehyde; 4-bromobenzaldehyde; 3,4,5-trimethoxybenzaldehyde and piperonal) were used to form heterodimeric *bis*-isochroman derivatives by oxa-Pictet-Spengler reaction (Scheme 13).



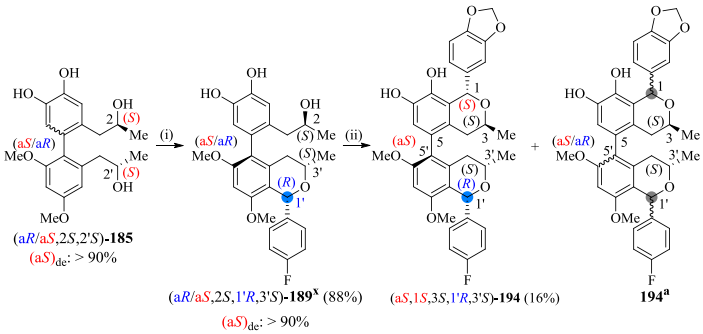
Compound	R^1	R^2	R^3	R^4	Yield
(aS,1R,3S,1'R,3'S)-**190**[x,y]	H	4-F-C_6H_4	4-F-C_6H_4	H	**59%**
(aS,1R,3S,1'R,3'S)-**191**[x]	H	4-Br-C_6H_4	4-Br-C_6H_4	H	**66%**
(aS,1R,3S,1'S,3'S)-**191**	H	4-Br-C_6H_4	H	4-Br-C_6H_4	**8%**
(aS,1S,3S,1'R,3'S)-**191**	4-Br-C_6H_4	H	4-Br-C_6H_4	H	**6%**
(aR,1R,3S,1'R,3'S)-**191**	H	4-Br-C_6H_4	4-Br-C_6H_4	H	**2%**
(aS,1R,3S,1'R,3'S)-**192**	H	3,4,5-$(MeO)_3$-C_6H_2	3,4,5-$(MeO)_3$-C_6H_2	H	**5%**
(aS,1R,3S,1'S,3'S)-**192**[x,y]	H	3,4,5-$(MeO)_3$-C_6H_2	H	3,4,5-$(MeO)_3$-C_6H_2	**12%**
(aS,1S,3S,1'R,3'S)-**192**	H	3,4,5-$(MeO)_3$-C_6H_2	3,4,5-$(MeO)_3$-C_6H_2	H	**5%**
(aS,1S,3S,1'S,3'S)-**192**	3,4,5-$(MeO)_3$-C_6H_2	H	H	3,4,5-$(MeO)_3$-C_6H_2	**6%**
(aS,1S,3S,1'R,3'S)-**194**	3,4-(OCH_2O)-C_6H_3	H	3,4-(OCH_2O)-C_6H_3	H	**10%**
(aS,1S,3S,1'S,3'S)-**194**	3,4-(OCH_2O)-C_6H_3	H	H	3,4-(OCH_2O)-C_6H_3	**36%**
(aR,1S,3S,1'S,3'S)-**194**	3,4-(OCH_2O)-C_6H_3	H	H	3,4-(OCH_2O)-C_6H_3	**3%**

Scheme 13. Target compounds **Y** were prepared by optimized oxa-Pictet-Spengler reaction. Reagents and conditions: (i) ArCHO, (1*S*)-(+)-10-camphorsulfonic acid, toluene:MeOH (4:1), 80 °C. [x]: structure was confirmed by X-ray crystallography. [y]: fluorescent compound.

Stereopure major products of 4-fluorophenyl [(aS,1*R*,3S,1'*R*,3'*S*)-**190** (59%)], 4-bromophenyl [(aS,1*R*,3S,1'*R*,3'*S*)-**190** (66%)] and 3,4-methylenedioxyphenyl [(aS,1*S*,3S,1'*S*,3'*S*)-**194** (36%)] derivatives could be isolated by column chromatography, while other stereoisomers required to use preparative HPLC for purification. The Brønsted acid-catalysed oxa-Pictet-Spengler cyclizations mainly depended on the structure and stereochemistry of the substrates. Under the certain conditions, the formation of stereoisomers and their ratios were determined jointly by steric and electronic effects (*I, M*).

4.1.3 The greater reactivity of the 3,5-dimethoxyaryl subunit of compound 185 made feasible preparation of heterodimeric *bis*-isochroman derivatives with versatile substitution patterns.

Based on our experience, the 3,5-dimethoxy subunit of molecule **185** was selectively reacted with one equivalent 4-fluorobenzaldehyde in the cyclization reaction, which allowed to prepare *bis*-isochroman derivatives with versatile substitution patterns. The mono-cyclization reaction was carried out under optimized conditions which afforded selectively the expected *cis*-**189** with a high yield (88%). The second isochroman moiety was formed with piperonal to obtain mixed heterodimeric *bis*-isochroman (a*S*,1*S*,3*S*,1'*R*,3'*S*)-**194** (16%) which was successfully separated by column chromatography (Scheme 14). Diastereomers of **194** with different configurations were observed larger than expected in the cyclization, which can be explained by the epimerization/isomerization during the reaction.



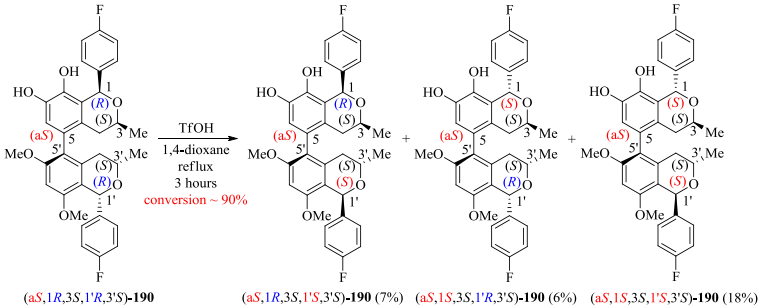
Scheme 14. Oxa-Pictet-Spengler cyclization reaction with *cis*-**189** to prepare *bis*-isochromans with versatile substitution patterns. **194**[a]: possible stereoisomers by isomerization. Reagents and conditions: (i) 1.2 eq. 4-fluorobenzaldehyde; 0.5 eq. (+)-camphorsulfonic acid; toluene:MeOH (4:1); 80 °C. (ii) 2 eq. piperonal; 1 eq. (+)-camphorsulfonic acid; toluene:MeOH (4:1); 80 °C. [x]: structure was confirmed by X-ray crystallography.

4.1.4 By developing epimerization/isomerization reactions, the investigation of possible stereoisomers which were produced in limited amounts during the cyclizations were achieved. Complete analytical and pharmacological studies of stereoisomers became possible that could not have been performed under the given ring-closure reaction.

Stereopure target compounds (a*S*,1*R*,3*S*,1'*R*,3'*S*)-**190** and (a*S*,1*R*,3*S*,1'*R*,3'*S*)-**191** with *cis*-*cis* configuration were used to examine the epimerization/isomerization processes during oxa-Pictet-Spengler cyclization reactions. The effects of temperature, the quality and the amount of Brønsted acid, respectively reaction time were investigated. Based on our experimental results, the isomerization reaction of (a*S*,1*R*,3*S*,1'*R*,3'*S*)-**190** was successfully produced the other three

diastereomers with 90% of conversion. However, a high amount of decomposition was also observed, which was responsible for the lower yields (Scheme 15).

Parameters examined	Effects
Temperature	The isomerization process was greatly enhanced by using the corresponding solvent, substrate and reaching the critical temperature in the presence of Brønsted acid.
Quality of Brønsted acid	Trifluoromethanesulfonic acid proved to be the most effective one using the corresponding solvent and temperature. TfOH > HCl > (1*S*)-(+)-10-camphorsulfonic acid.
Quantity of Brønsted acid	Higher amount of Brønsted acid accelerated the process of isomerization.
Reaction time	Using the corresponding Brønsted acid and temperature longer reaction time was required for the entire process.



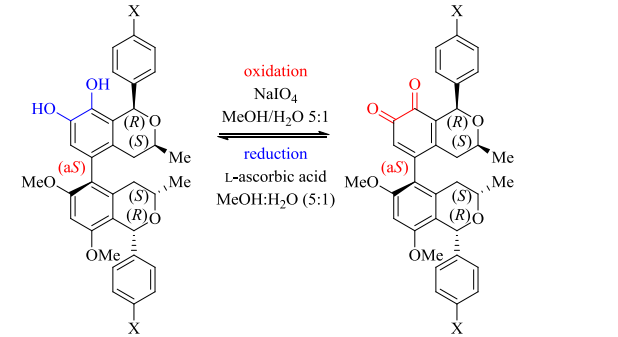
(aS,1*R*,3*S*,1'*R*,3'*S*)-**190** (aS,1*R*,3*S*,1'*S*,3'*S*)-**190** (7%) (aS,1*S*,3*S*,1'*R*,3'*S*)-**190** (6%) (aS,1*S*,3*S*,1'*S*,3'*S*)-**190** (18%)

Scheme 15. Epimerization/isomerization reaction wascarried out with stereopure (aS,1*R*,3*S*,1'*R*,3'*S*)-**190** 4-fluorophenyl *bis*-isochroman derivative.

Further studies revealed that decomposition can be decreased by using water/acetic acid 9:1 as a solvent with TfOH at 100 °C. Decomposition was not detected at around 80% conversion and the reaction was monitored by thin layer chromatography.

4.1.5 *Ortho*-quinone derivatives were prepared reversibly by redox chemical reactions.

The oxidation of (aS,1*R*,3*S*,1'*R*,3'*S*)-**190** and (aS,1*R*,3*S*,1'*R*,3'*S*)-**191** with $NaIO_4$ was performed to produce biaryl derivatives containing a reactive *ortho*-quinone moiety for further pharmacological studies. The obtained *o*-quinone derivatives can be reduced by L-ascorbic acid back to the original pyrocatechol compunds. The optimized oxidation and reduction processes took place as instantaneous reactions and did not cause changes in other structural units of the molecule. The use of L-ascorbic acid may also provide an opportunity to avoid oxidation processes because oxidation occurs spontaneously to a certain extent in solution. *O*-quinone derivatives are not only valuable compounds in pharmacological studies but also in organic chemical transformations (Scheme 16).

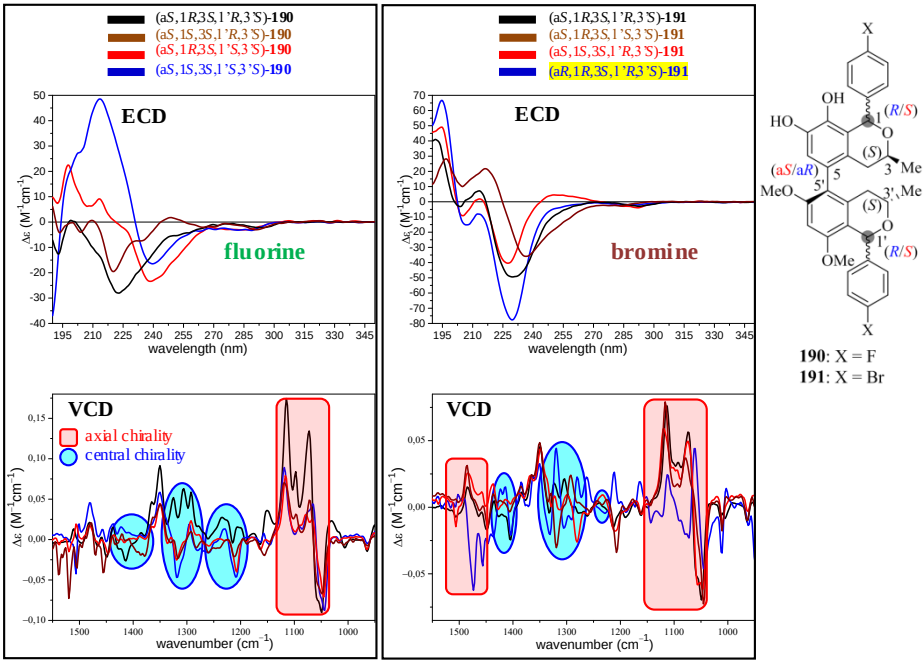


(a*S*,1*R*,3*S*,1'*R*,3'*S*)-**190**^{x,y}: X = F
(a*S*,1*R*,3*S*,1'*R*,3'*S*)-**191**^{x}: X = Br
(a*S*,1*R*,3*S*,1'*R*,3'*S*)-**195**^{x} (96%): X = F
(a*S*,1*R*,3*S*,1'*R*,3'*S*)-**196**^{x} (98%): X = Br

Scheme 16. Preparation and reduction of *ortho*-quinone derivatives. ^{x}: structure was confirmed by X-ray crystallography. ^{y}: fluorescent compound.

4.1.6 Similar conclusions can be drawn for the determination of central and axial chirality of the Y target compounds based on the results of the cyroptical analysis of the X compounds. ECD measurements mainly provide information on central chirality, while VCD measurements provide information on axial chirality.

Measured ECD and VCD spectra of the synthesized 4-fluorophenyl (a*S*,1*R*/*S*,3*S*,1'*R*/*S*,3'*S*)-**190** and 4-bromophenyl (a*S*/a*R*,1*R*/*S*,3*S*,1'*R*/*S*,3'*S*)-**191** *bis*-isochroman derivatives are shown in Scheme 17. Surprisingly, the ECD spectra of 4-fluorophenyl **190** compounds with different halogen atoms (F, Br) showed a significant difference. This can be explained by the size difference of the size and electronegativity of the F and Br atoms, which can generate different electron transitions in the aromatic chromophore system at position C-1 and C-1' (4-fluorophenyl or 4-bromophenyl units). In case of the VCD spectra, in addition to the previously identified characteristic wavenumber range of 1150-1025 cm^{-1} determining axial chirality, an additional region related to axial chirality was also identified. The axial chirality of four-four stereoisomers of (a*S*,1*R*/*S*,3*S*,1'*R*/*S*,3'*S*)-**190** and (a*S*/a*R*,1*R*/*S*,3*S*,1'*R*/*S*,3'*S*)-**191** could be clearly determined based on VCD spectra. We were able to draw conclusions about the central chirality (absolute configuration of C-1 and C-1') of the 4-fluorophenyl (a*S*,1*R*/*S*,3*S*,1'*R*/*S*,3'*S*)-**190** stereoisomers, while the 4-bromophenyl (a*S*/a*R*,1*R*/*S*,3*S*,1'*R*/*S*,3'*S*)-**191** derivatives could not be reliably distinguished by ECD measurements.



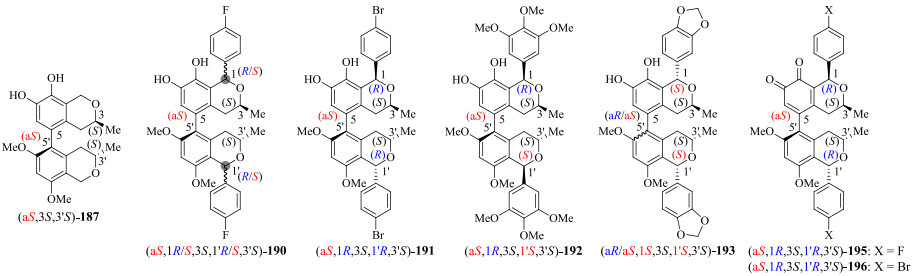
Scheme 17. Measured ECD and VCD spectra of 4-fluorophenyl (aS,1*R*/*S*,3*S*,1'*R*/*S*,3'*S*)-**190** and 4-bromophenyl (aS/aR,1*R*/*S*,3*S*,1'*R*/*S*,3'*S*)-**191** *bis*-isochroman derivatives.

4.1.7 *In vitro* antibacterial activity tests on the produced target compounds Y were performed in cooperation. Ten target compounds proved to have remarkable antibacterial activity against the *E. faecalis* ATCC 51299 strain.

In general, some activities were observed in case of two Gram-positive bacterial strains (*B. subtilis* ATCC 6633 and *E. faecalis* ATCC 51299). Three of the compounds containing 4-fluorophenyl moieties [(aS,1*R*/*S*,3*S*,1'*R*/*S*,3'*S*)-**190**] showed significant activities (MIC = 4, 1 and 0.5 μg/ml) selectively on *E. faecalis* ATCC 51299 strain, but (aS,1*R*,3*S*,1'*R*,3'*S*)-**190** was found to be inactive (MIC > 64 μg/ml). It is noteworthy that only (aS,1*R*,3*S*,1'*R*,3'*S*)-**191** from the 4-bromophenyl **191** derivatives had potent activity (MIC = 1 μg/ml) also selectively on *E. faecalis* ATCC51299. In case of compounds **190** and **191**, the difference in halogen atoms (F, Br) has a major impact not only on the analytical but also on the pharmacological results. The (aS,1S,3S,1'S,3'S)-**193** and (aR,1S,3S,1'S,3'S)-**193** atropisomers which differ only in axial chirality were found to be active. Moreover, (aR,1S,3S,1'S,3'S)-**193** was more potent on *E. faecalis* ATCC 51299 strain with MIC value of 1 μg/ml than the (aS) atropodiastereomer (MIC = 4 μg/ml). Only (aS,1*R*,3*S*,1'S,3'S)-**192** of the 3,4,5-trimethoxyphenyl **192** derivatives

exhibited moderate antibacterial activity. Weaker antibacterial activity was also observed in case of the C-1/C-1' unsubstituted (aS,3S,3'S)-**187** derivative (Scheme 18).

Compound Y	MIC [μg/mL]				
	B. subtilis ATCC 6633	MSSA ATCC 29213	MRSA ATCC 33591	*E. faecalis* ATCC 51299	*A. baumannii* ATCC BAA1605
(aS,3S,3'S)-**187**	8	> 64	> 64	4	> 64
(aS,1S,3S,1'*R*,3'S)-**190**	16	> 64	> 64	0.5	> 64
(aS,1*R*,3S,1'S,3'S)-**190**	4	> 64	> 64	4	> 64
(aS,1S,3S,1'S,3'S)-**190**	4	> 64	> 64	0.5	> 64
(aS,1*R*,3S,1'*R*,3'S)-**191**	4	> 64	> 64	1	> 64
(aS,1*R*,3S,1'S,3'S)-**192**	4	> 64	> 64	4	> 64
(aS,1S,3S,1'S,3'S)-**193**	4	> 64	> 64	4	> 64
(a*R*,1S,3S,1'S,3'S)-**193**	16	> 64	> 64	1	> 64
(aS,1*R*,3S,1'*R*,3'S)-**195**	8	> 64	> 64	8	> 64
(aS,1*R*,3S,1'*R*,3'S)-**196**	8	> 64	> 64	8	> 64
teicoplanin *	0.64	0.32	0.32	4	> 64
vancomycin *	0.64	0.64	0.64	128	> 64
ciprofloxacin *	0.05	0.25	2	2	> 64
colistin (polymyxin E) *	> 64	> 64	> 64	> 64	1



Scheme 18. Target compounds **Y** with remarkable pharmacological activity. Teicoplanin, vancomycin: glycopeptid; ciprofloxacin: fluoroquinolon, colistin (polymyxin E): cyclolipopeptid antibiotics. *: positive control.

5. Possible applications of the results

40 *bis*-isochroman derivatives with 6,7,8,7',8'-pentamethoxy (21 target compounds **X**) and 7,8-dihydroxy-6',8'-dimethoxy (19 target compounds **Y**) substitution pattern were prepared using two different synthetic strategies. The effect of the existing central/axial chirality elements to the newly formed axial/central chirality elements were also investigated. Preparations of heterodimeric *bis*-isochroman derivatives with versatile substitution patterns were carried out. The number of synthesized stereoiomers was increased by developing epimerization/isomerization reactions. *Ortho*-quinone derivatives were prepared reversibly by redox chemical reaction. The structure and stereochemistry of the products were determined by

1D and 2D NMR (COSY, HSQC, HMBC, ROESY, NOESY) and extensive spectroscopic methods such as ECD, VCD, OR supported by single crystal X-ray diffraction analysis and DFT calculations. *In vitro* antibacterial activity tests of the produced target compounds **Y** were performed in cooperation with the Institute of Medical Microbiology of the Semmelweis University of Budapest. Ten target compounds proved to have remarkable antibacterial activity against the *E. faecalis* ATCC 51299 strain.

6. List of publications and lectures

Publications related to the subject of the thesis:

1. Z. Czenke, A. Mándi, S. B. Király, A. Kiss-Szikszai, A. Kónya-Ábrahám, A. Kurucz-Szabados, K. Cserepes, A. Bényei, C. Zhang, M. Kicsák, T. Kurtán: VCD Analysis of Axial Chirality in Synthetic Stereoisomeric Biaryl-Type *bis*-Isochroman Heterodimers with Isolated Blocks of Central and Axial Chirality; *Int. J. Mol. Sci.*; **2024**, *25*, 9657. doi: 10.3390/ijms25179657.

2. Z. Czenke, A. Mándi, G. M. Fedics, R. A. Barta, A. Kiss-Szikszai, A. Kurucz-Szabados, I. Timári, A. Bényei, S. B. Király, E. Ostorházi, C. Zhang, M. Kicsák, T. Kurtán: Stereoselective Synthesis of Axially Chiral 5,5'-Linked *bis*-1-Arylisochromans with Antibacterial Activity; *Int. J. Mol. Sci.*; **2025**, *26*, 7777. doi: 10.3390/ijms26167777.

Patent:

1. T. Kurtán, M. Kicsák, Z. Czenke, E. Ostorházi, S. B. Király, L. Magyar, **Biaryl-type heterocyclic compounds and anti-bacterial uses thereof**, PCT/HU2024/050130.

Lectures:

1. Czenke Zoltán, Kurtán Tibor: *Bisz*-izokromán származékok sztereoszelektív szintézise és szerkezetvizsgálata. Zechmeister László előadói díj (Budapest, 2022.11.18.).

2. Czenke Zoltán, Kicsák Máté, Mándi Attila, Timári István, Kovács Maximilián, Kurtán Tibor. *Bisz*-izokromán származékok sztereoszelektív szintézise és szerkezetvizsgálata. Alkaloid- és Flavonoidkémiai Munkabizottság ülése (Mátrafüred, 2022.10.06-07.).

3. Czenke Zoltán, Kicsák Máté, Mándi Attila, Timári István, Varga Flóra Judit, Kurtán Tibor: *Bisz*-izokromán származékok sztereoszelektív szintézise és szerkezetvizsgálata. MKE Vegyészkonferencia (Eger, 2022.06.15-17.).

4. Czenke Zoltán, Kicsák Máté, Mándi Attila, Kurtán Tibor: *Bisz*-izokromán származékok sztereoszelektív szintézise és szerkezetvizsgálata. Alkaloid- és Flavonoidkémiai Munkabizottság ülése (Mátrafüred, 2021.10.07-08.).

5. Czenke Zoltán, Kicsák Máté, Mándi Attila, Kurtán Tibor. *Bisz*-izokromán származékok sztereoszelektív szintézise és szerkezetvizsgálata. Gyógyszerkémiai és Gyógyszertechnológiai Szimpózium (Herceghalom, 2021.09.20-21.).

6. Zoltán Czenke, Máté Kicsák, Attila Mándi, Tibor Kurtán: Preparation of biaryl isochroman derivatives and structural analysis. Young Researchers' International Conference on Chemistry and Chemical Engineering, YRICCCE III (Online konferencia, 2021.06.04-05.).

7. Czenke Zoltán, Kurtán Tibor: Axiális és centrális kiralitáselemet tartalmazó biaril származékok előállítása és szerkezetvizsgálata. Alkaloid- és Flavonoidkémiai Munkabizottság ülése (Mátrafüred, 2020.10.01-02.).

8. Czenke Zoltán, Antus Sándor, Kurtán Tibor: Naftil származékok átalakítása biaril- és kalkon analógokká. Alkaloid- és Flavonoidkémiai Munkabizottság ülése (Mátrafüred, 2019.04.11-12.).

9. Czenke Zoltán, Kurtán Tibor, Szappanos Ádám: Morfolin- és tiazol gyűrűvel kondenzált 2-arilkromán származékok előállítása. Alkaloid- és Flavonoidkémiai Munkabizottság ülése (Mátrafüred, 2018.04.12-13.).

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Subject: PhD Publication List

Candidate: Zoltán Czenke
Doctoral School: Doctoral School of Chemistry
MTMT ID: 10101361

List of publications related to the dissertation

Foreign language scientific articles in international journals (2)

1. **Czenke, Z.**, Mándi, A., Fedics, G. M., Barta, R. A., Kiss-Szikszai, A., Szabados, A., Timári, I., Bényei, A., Király, S. B., Ostorházi, E., Zhang, C., Kicsák, M., Kurtán, T.: Stereoselective Synthesis of Axially Chiral 5,5'-Linked *bis*-1-Arylisochromans with Antibacterial Activity. *Int. J. Mol. Sci. 26* (16), 1-34, 2025. ISSN: 1661-6596.
   DOI: http://dx.doi.org/10.3390/ijms26167777
   IF: 4.9 (2024)
2. **Czenke, Z.**, Mándi, A., Király, S. B., Kiss-Szikszai, A., Ábrahám, A., Szabados, A., Cserepes, K., Bényei, A., Zhang, C., Kicsák, M., Kurtán, T.: VCD Analysis of Axial Chirality in Synthetic Stereoisomeric Biaryl-Type *bis*-Isochroman Heterodimers with Isolated Blocks of Central and Axial Chirality.
   Int. J. Mol. Sci. 25 (17), 1-22, 2024. ISSN: 1661-6596.
   DOI: http://dx.doi.org/10.3390/ijms25179657
   IF: 4.9

Total IF of journals (all publications): 9,8
Total IF of journals (publications related to the dissertation): 9,8

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