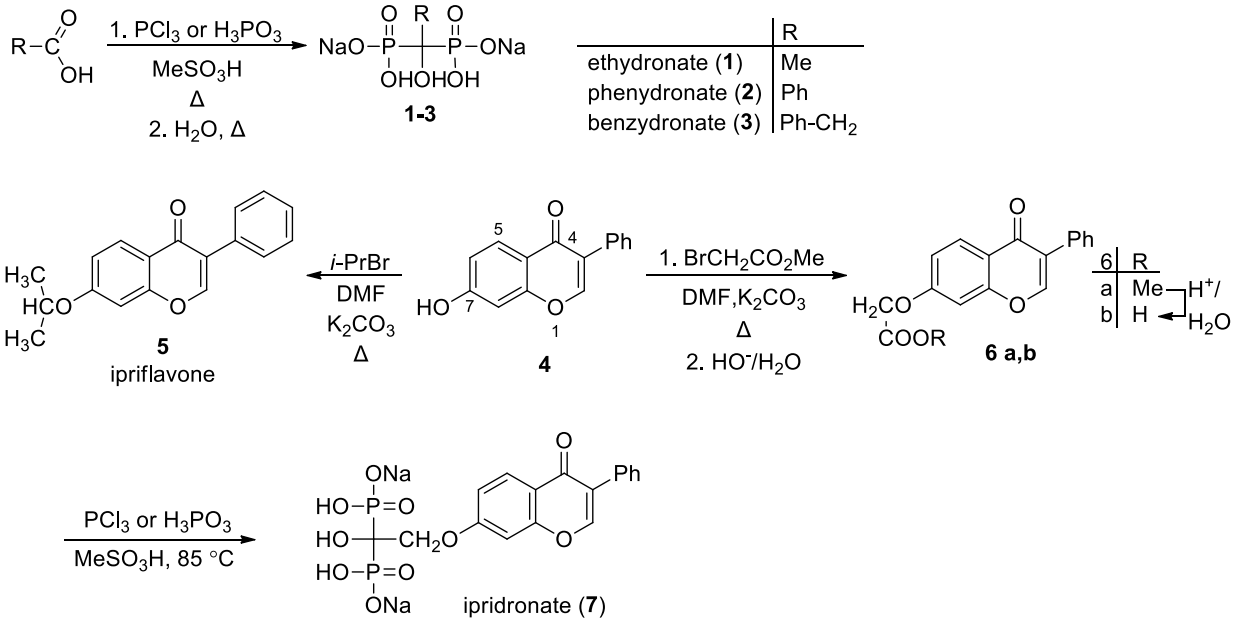
Final report on the research of the OTKA project K-112951 (01.09.2014 - 31.12.2018)

As a continuation of our research activities of the OTKA project K-81701, the achievements of the present research project were the following:

1) ***Synthesis of isoflavonoides with potential anti-osteoporotic activity***

In the past two decades, the α-hydroxy-methylene-bisphosphonate derivatives [ethydronate (**1**), phenydronate (**2**) and benzydronate (**3**)] were proved to be the most efficient oral anti-osteoporotic agents. By surveying the literature for the synthesis of these compounds, we supposed that their structural unit can be readily attached to the ipriflavone moiety [7-isopropyloxy-isoflavone (**5**)] by a simple synthesis. 7-Isopropyloxy-isoflavone [ipriflavone (**5**)] has been prepared from inexpensive starting materials, *i.e.* resorcinol and phenylacetyl chloride in three steps. The last step of this synthesis is the alkylation of 7-hydroxy-isoflavone (**4**) with isopropylbromide, which resulted in **5** as shown in Scheme 1. Similarly, **4** was alkylated with methyl α-bromoacetate affording **6a** whose hydrolysis gave **6b**, which could be transformed into ipridronate (**7**) in 65% yield.



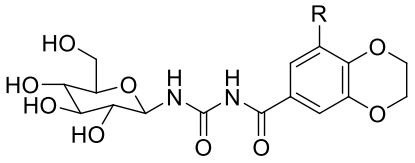
Scheme 1. Synthesis of ipriflavone (**4**) and iprodronate (**7**)

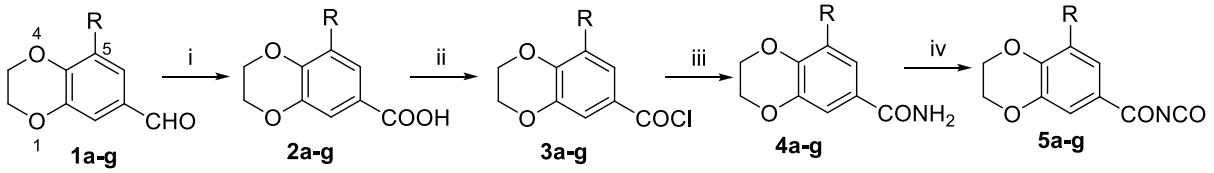
Ipriflavone (**5)** is the active constituent of Osteochin® introduced to the market as an anti-osteoporotic drug by Chinoin. In the course of the privatization of the Chinoin factory, this drug had been withdrawn by Sanofi from the market. It was hoped that the anti-osteoporotic effects of the two different active ingredients with different mode of actions mutually reinforce each other favourably in the ipridonate conjugate (**7**). In this case, the ipridronate (**7**) may be considered a new oral anti-osteoporotic agent, which should be patented first and then its synthesis can be published. The simple synthesis of ipridronate (**7**) has been successfully achieved by introduction of the ethydronate moiety on the C-7 position of the 7-hydroxyflavone. Further improvement of the last step of the synthesis (**6b→7)** and determination of anti-osteoporotic activity of **7** are in progress.

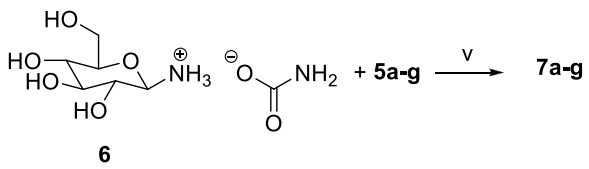
2) ***Synthesis of glucopyranosyl-1,4-benzodioxane-type glycogen phosphorylase inhibitors for the treatment of types II diabetes mellitus***.

It is well-known that the glycogen phosphorylase enzyme catalysing the metabolism of glycogen has a crucial role in the treatment of type II diabetes. As a continuation of our SAR studies in this field, the preparation of N-(β-D-glucopyranosyl)urea derivatives (**7a-g**) possessing a C-5 substituted 1,4-benzodioxane moiety was carried out as shown in Scheme 2.

7	R	K_i (μM)
a	H	0.71
b	OMe	0.98
c	Me	0.50
d	F	?
e	Cl	?
f	Br	0.37
g	I	0.98







Scheme 2. The synthesis of **7a-g**. Reactions and conditions: (i) $KMNO_4$, acetone-H_2O Δ, (ii) SO_2Cl_2, kat. DMF, rt. , (iii) 25% NH_4OH, rt., (iv) $(COCl)_2$ DKE, rt., (v) dry pyridine, rt.

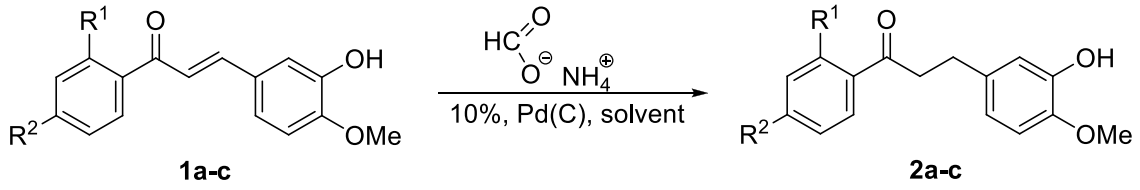
According to the literature, the C-5 substituted 1,4-benzodioxan-7-carbaldehydes (**1a-g**) were oxidized with $KMnO_4$ and the corresponding carboxylic acid derivatives (**2a-g**) could be obtained in good yield (75-80%). Subsequently, they were transformed into the corresponding acyl isocyanates (**5a-g**) in 3 steps (**2a-g→3a-g→4a-g→5a-g**). In the last step of the synthesis, the β-D-glucopyranosyl ammonium-carbamate (**6**), obtained from D-glucose and ammonium carbamate at 37 ^{0}C, was treated with isocyanates (**5a-g**) to afford N-(5-substituted-1,4-benzodioxane-7-carbonyl-N'-(β-D–glucopyranosyl)-carbamides (**7a-g**). Determination of K_i (μM) of **7d** and **7e** is in progress and the publication in Bioorg. Med. Chem. is in preparation.

3) ***Synthesis of calorie-free artificial sweeteners***

As a continuation our research started in the previous project on the novel synthesis and SAR study of the CH-401 dihydrochalcone derivative (DHC, **2c**) with 1000-fold sweeter taste than sucrose, we have the following goals:

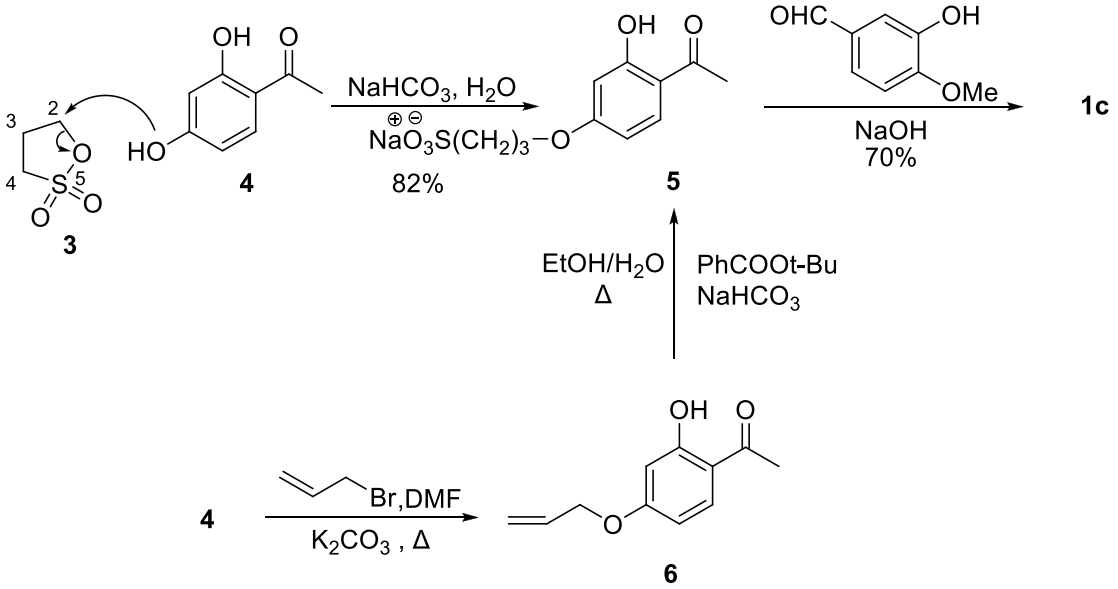
a) The third step of the synthesis of CH-401(**2c**) is the reduction of the corresponding 2'-hydroxychalcone derivative (**1c**) with atmospheric hydrogen in the presence of 10% Pd(C) catalyst in aqueous solution at room temperature. The risk of an accident during the use of hydrogen gas has to be eliminated by the application of alternative reducing agent, which can be used in aqueous solution. The catalytic transfer hydrogenation (CTH) is an important method for the reduction of α,β-unsaturated ketones and esters It can generally performed with $HCONH_2$ as a hydrogen source and 10% Pd(C) in various organic solvents (MeOH, THF,1,4-dioxane) but in some cases water can be used as solvent as well. We observed that the α,β-double bond of the **1a** chalcone derivative and its 2',4'-dihydroxy derivative (**1b**) could be selectively reduced in the presence of ammomium formate as a hydrogen donor and 10% Pd(C)/methanol at 65 ^{o}C and the corresponding α,β-dihydro-chalcone derivatives (**2a,b)** obtained in good yield (80-85%), respectively. On the basis of these results, the analogue reduction of the water soluble chalcone derivative **1c** was also performed. The transformation took place without difficulties and it resulted in CH-401 (**2c**) in 65 % yield (Scheme 3).

b) In the patented transformation of the CH401 (**2c**), the (3-sulpho-propyloxy-) sodium salt side-chain of the rezacetophenone (**4**) has been introduced by propane-sulton (**3**) produced by the Shell company. The **3** is a strong electrophile, and therefore it possesses teratogenic properties, which made difficult to handle it as a reagent.



1a-c **2a-c**

1,2	R^1	R^2	solvent	t °C	yield%
a	H	H	MeOH	rt.	85
b	OH	OH	MeOH	rt.	80
c	OH	-O-$(CH_2)_3$-$SO_3^{\ominus}Na^{\oplus}$	H_2O	rt.	65



Scheme 3. Synthesis of CH-401 (**2c**).

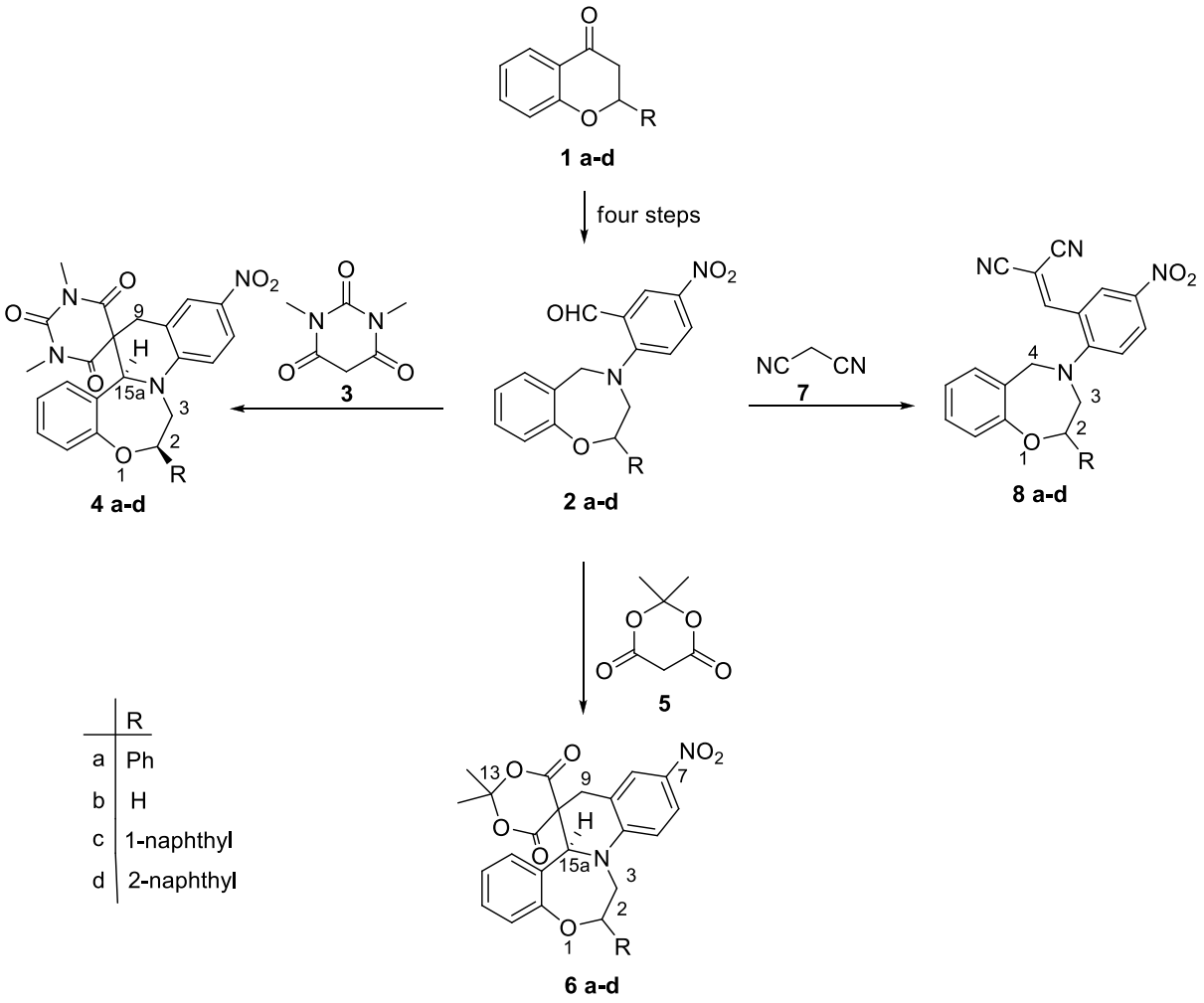
Due to safety reasons, a new route for the synthesis of **5** has been elaborated by us starting from resacetophenone (**4**). It was first alkylated regioselectively with allyl-bromide in DMF in the presence of K_2CO_3 and 2-hydroxy-4-allyloxy acetophenone (**6**) was obtained, whose treatment with *t*-butyl-benzoyl-peroxide in the presence of $NaHSO_3$ in ethanol at reflux resulted in **5** in 58% yield, which has been found identical in every respect with that obtained from the reaction of **4** with **3.**

After improving the yield for the last step (**6→5**) of the new synthesis for the CH-401 (**2c**), the results can be patented and published**.**

4) ***Synthesis, chiroptical study and neuropotective activity of 1,4- and 1,5-benzoxazepine derivatives***

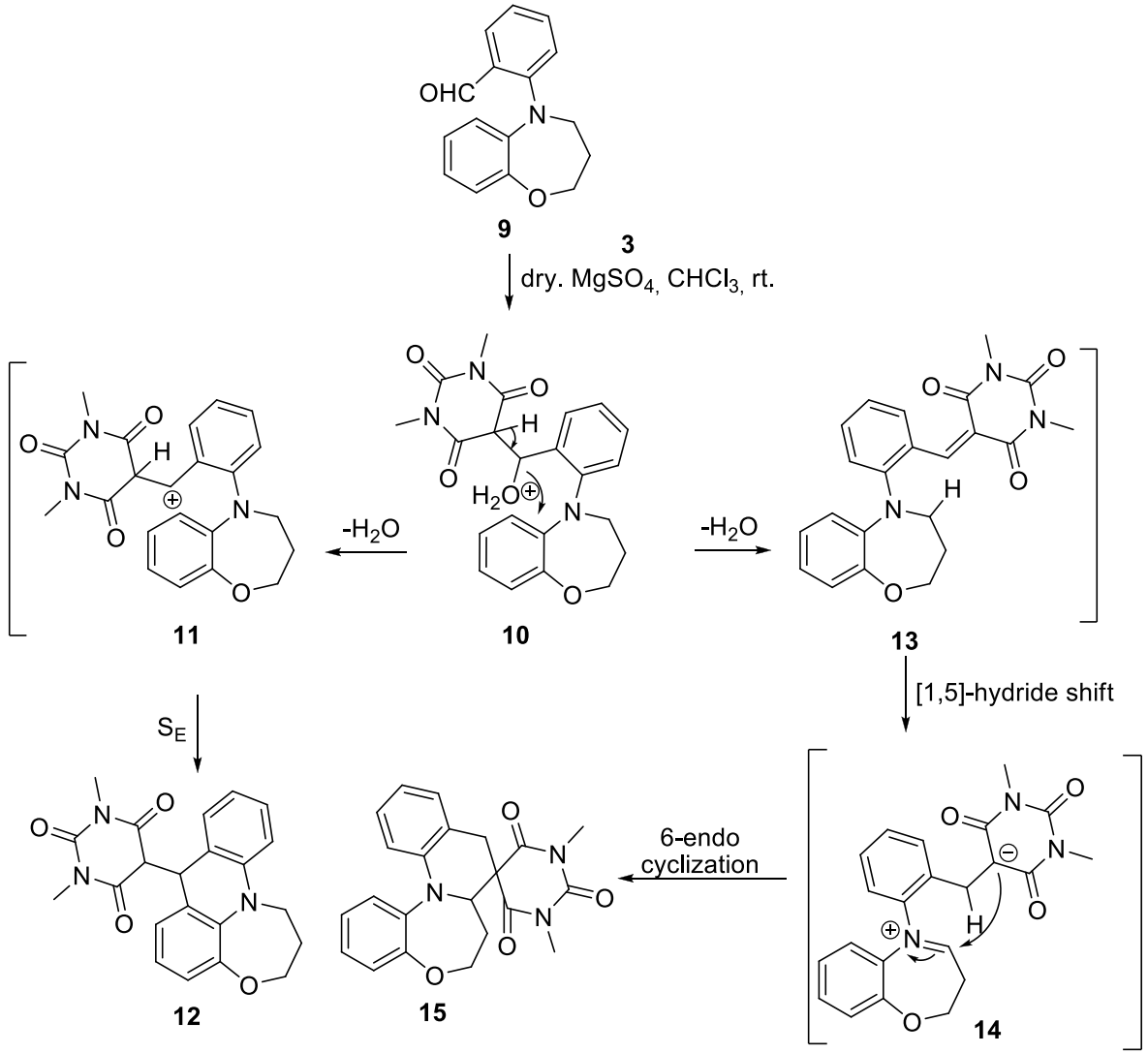
As a continuation of our research in the field of the synthesis of 1,4-and 1,5-benzoxazepine derivatives of potential neuroprotective activity, we have found similar manner as observed

by us earlier (*rac*-**2a**→*rac*-**4a** and *rac*-**2a**→ r*ac*-**6a)** that *rac*-*trans*-**4b-d** and **-6b-d** condensed *O,N*-heterocycles possessing 1,2,8,9-tetrahydro-7bH-quinolino[1,2-d][1,4]benzoxazepine skeleton, could be also prepared by the regioselective domino Knoevenagel-[1,5]-hydride shift-cyclization of the readily available 2-phenyl-4-(2'-formyl-4'-nitro)phenyl-2,3,4,5-tetrahydo-1,4-benzoxazepine (*rac*-**2b-d**) in high (80-96%) (Scheme 4.).



Scheme 4.

It is noteworthy that this type of domino reaction of *rac*-**2c-d** using malononitrile (**7**) as active methylene source stopped after the formation of the Knoevenagel product (**8c-d**) derivative as shown in Scheme 4.



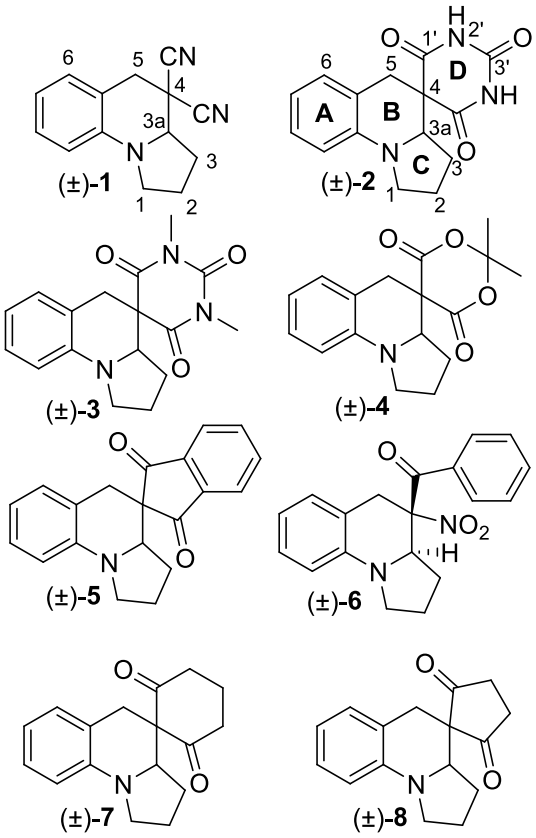
Scheme 5. Extension of the cyclization to *N*-(2-formyl)-phenyl-2,3,4,5-tetrahydro-1,5-benzoxazepine (**9**).

The enantiomers of *rac*-**4a-d** and **-6a-d** were separated and characterized by HPLC-ECD data, which allowed their configurational assignment on the basis of TDDFT-ECD calculation of the solution conformers. The extension of this cyclization to *N*-(2-formyl)-phenyl-2,3,4,5-tetrahydro-1,5-benzoxazepine (**9**) resulted unexpectedly in the **12** 2,3,4,5-terahydro-1,5-benzoxazepine instead of **15**.

The neuroprotective activities *rac*-**4a** and *rac*-**6a** were tested. These compounds showed neoroprotective activities against hydrogen peroxide or β-amiolides$_{25\text{-}35}$ (A$\beta_{25\text{-}35}$)-induced cellular injuries in human neuroblastoma SH-SY5Y cells with 16.4% and 22.8% increase in cell viability at 10μM concentrations, respectively. The determination of neuropoective activity of *rac*-**4c-d,** r*ac*-**6c-d** and *rac*-**12** is in progress.

5) HPLC-ECD and TDDFT-ECD study of hexahydropyrrolo [1,2-a]quinolone derivatives

Synthesis of *rac*-hexahydropyrrolo[1,2-a]quino-[1,5]quinoline derivatives (**1-8**) was achieved by utilizing the Knoevenagel-[1,5]-hydride shift-cyclization domino reaction.

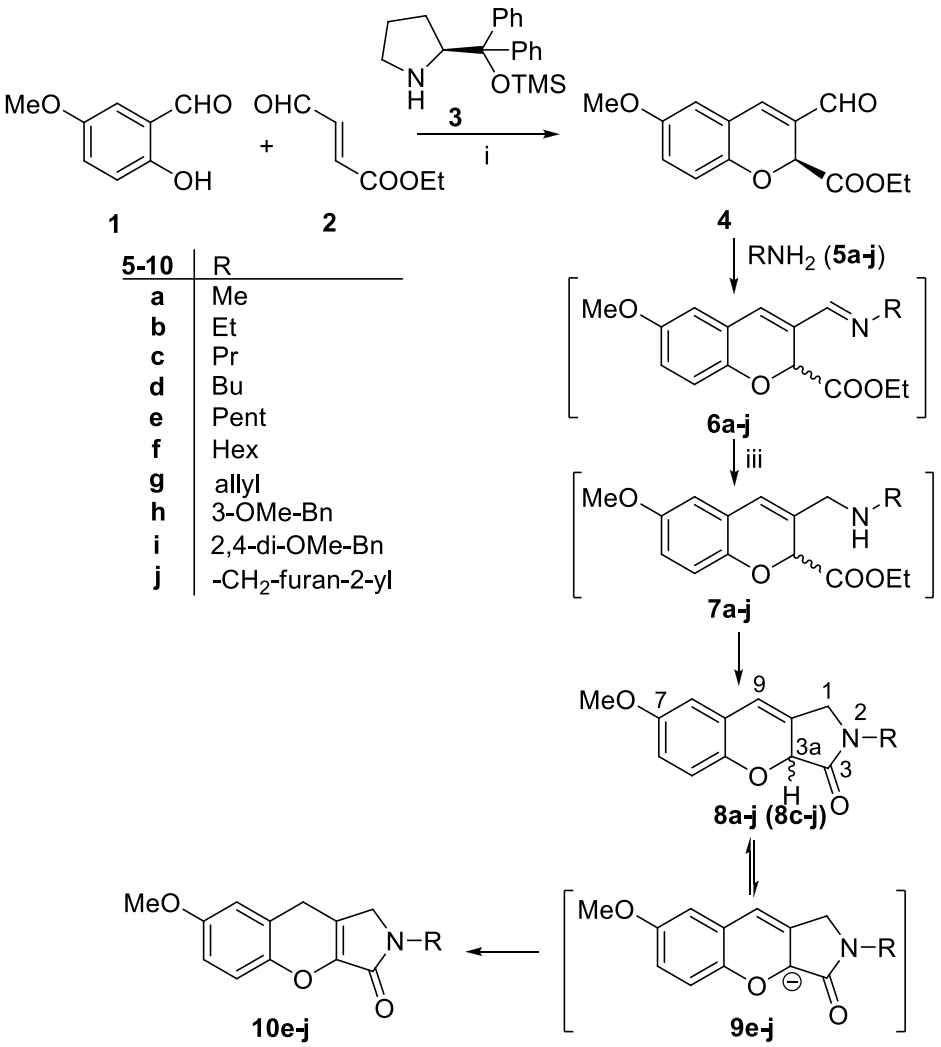


Scheme 6. Structures of studied hexahydropyrrolo[1,2-a]quinolone derivatives (*rac*-**1-8**).

Separation of the enantiomers of the chiral products (**1-8**) was carried out by chiral high-performance liquid chromatography and electronic circular dichroism (ECD) spectra were recorded to elucidate the absolute configuration by comparing the experimental and time-dependent density theory-ECD spectra obtained at various theoretical levels. For compound **6** having two newly formed chirality centers, the time-dependent density functional theory-ECD calculation allowed determining both the relative and the absolute configuration by distinguishing the four stereoisomers. One of compounds with *spiro* - 1,3-cyclohexaedione moiety (**7**) possessed moderate acetylcholinesterase inhibitory activity, while **3** showed neuroprotective *activity* in oxygen-glucose deprivation-induced neurotoxicity in human SH-SY5Y cells. The results are published in the journal Chirality.

6) An efficient synthesis of 1,2-dihydrochromeno[2,3-c]pyrrol-3-one derivatives of potential pharmacological activity and HPLC-ECD analysis.

Starting from commercially available 2-hydroxy-5-methoxybenzaldehyde (**1**) and (*E*)-4-oxo-but-2-enoic acid ethyl ester (**2**) optically active 3-formyl-6-methoxy-(2*H*)chromene-2-caboxylic acid ethyl ester (*S*-**4**) using (*S*)-α,α-diphenyl-2-pyrrolidinemethanol trimetylsilyl ether (**3**) as organocatalyst has been synthesized which could be transformed by **5a-j** primary amines under the condition of reductive amination into *N*-substituted 7-methoxy-1,2-dihydrochromeno[2,3-c]pyrrol-3(3a*H*)-ones (**8a-j**) in tree consecutive steps of a one pot reaction.



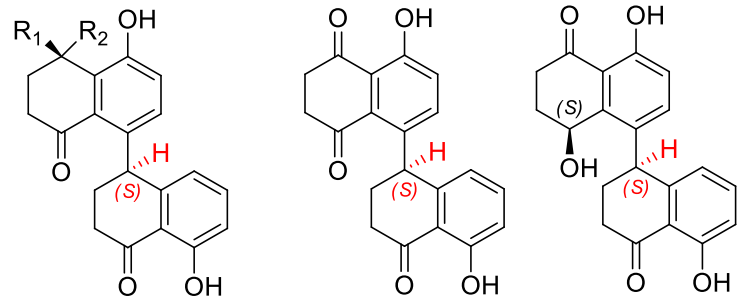
Scheme 7. Synthesis of 1,2-dihydrochromeno[2,3-c]pyrrol-3-one derivatives

Among them, the *N*-substituted 7-methoxy-1,2-dihydrochromeno[2,3-c]pyrrol–3(9*H*)-ones (**8e-j**) spontaneously isomerized to *rac*-**10e-j** as shown in Scheme 7. Isomerization and the inherently labile chirality center were studied and HPLC-ECD analysis of a chiral 1,2-

dihydrochromeno[2,3-c]pyrol-3(*3aH*)-one derivative aided by TDDFT-ECD calculation allowed configurational assignment of separated enantiomers. The antiproliferative activity of chiral *rac*-**8a-d** and **10e-g** was tested on CaCo-2 human epithelial colorectal adenocarcinoma cell line and (*2H*)-chromene derivative **10e** and **10f** showed modest antiprolifarative activity with 17 μM IC_{50} values. The results are published in the journal Synlett.

7) Characterization of cladosporols from the marine algal-derived endphylic fungus Cladosporiumcladosporiodes EN_399 and configurational revision of the previously reported cladosporol.

In collaboration with the Laboratory of Marine Biology and Biotechnology, National Laboratory for Marine Science and Technology, Key Laboratory of Experimental Marine Biology and Institute of Oceanology and University of Chinese Academy of Sciences (People's Republic of China), four new cladosporol derivatives, cladosporols F-I (**1-4**), the known cladosporol C (**5**), and its new epimercladosporol J (**6**) were isolated and identified from the marine algal-derived endophytic fungus *Cladosporium cladosporiodes* EN-399.



1 R_1 = H, R_2 = OMe
2 R_1 = OMe, R_2 = H
5 R_1 = H, R_2 = OH (revised)
6 R_1 = OH, R_2 = H

Scheme 8. The structures of cladasporonols **(1-6)**

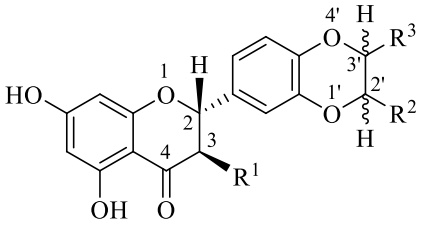
Their structures were determined by detailed interpretation of NMR and MS data and the absolute configuration were established on the basis of TDDFT-ECD and OR calculation. The configurational assignment of cladosporols-F (**1**) and -G(**2**) showed that the previously reported absolute configuration of cladosporol A all the reported cladosporols need to be revised from (4'*R*) to (4'*S*).

Compounds **1-6** showed antibacterial activity against *Esherichiacoli*, *Mirococcusluteus* and *Vibrio harveyi* with MIC values ranging from 4 to 128 μg /mL. Compound **3** showed significant cytotoxicity against A549, Huh7, and LM3 cell lines with IC_{50} values of 5.0, 1.0,

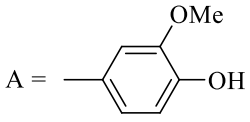
and 4.1 μ M, respectively, and compound **5** showed activity against H446 cell line with IC_{50} value of 4.0 μ M. The results are published in J. Org. Chem.

8) Evaluation of synthetic routes to (2R,3R)-3-hydroxymethyl-2-(4-hydroxy-3-methoxyphenyl)-1,4-benzodioxane-6-carbaldehyde.

Flavanolignans possessing a 1,4-benzodioxane subunit represent a small class of natural products with hepatoprotective activity. The first members of this family, (+)-silybin A (**1a**) and (–)-silybin B (**1b**)**,** were isolated from the violet-flowered *Silybum marianum* L in 1968 as a 1:1 mixture of **1a** and **1b**. They are the most active constituents of *Legalon*® (Madaus AG. Köln) used in the therapy of liver diseases (Scheme 9).



1a,b-4a,b

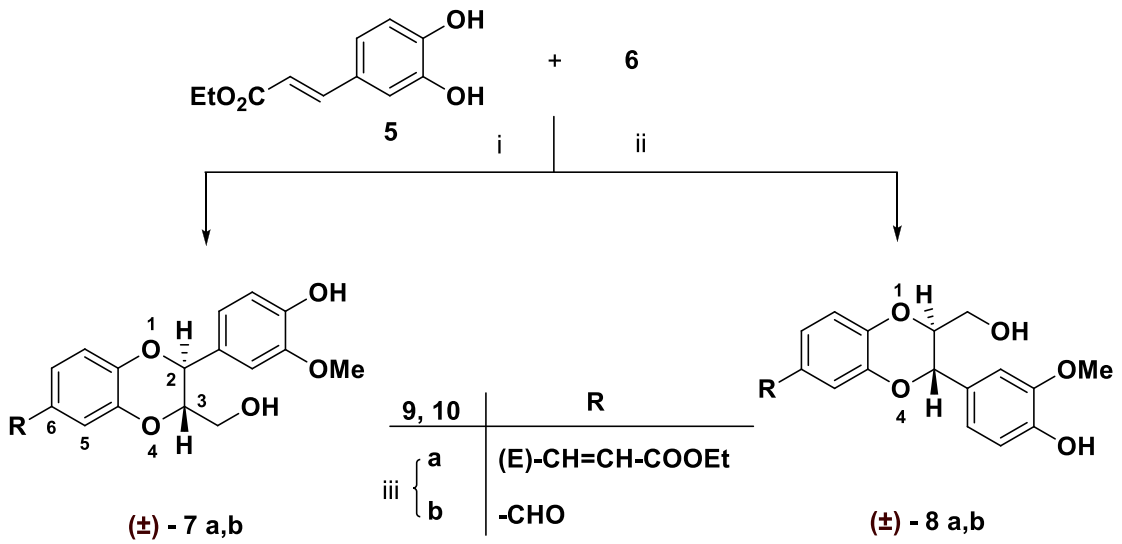


B = -CH_2OH

	R^1	R^2	R^3	configuration
(+)-silybin A (**1a**)	OH	A	B	(2*R*,3*R*,2'*R*,3'*R*)
(–)-silybin B (**1b**)	OH	A	B	(2*R*,3*R*,2'*S*,3'*S*)
(+)-isosilybin A (**2a**)	OH	B	A	(2*R*,3*R*,2'*R*,3'*R*)
(–)-isosilybin B (**2b**)	OH	B	A	(2*R*,3*R*,2'*S*,3'*S*)
(+)-silandrin A (**3a**)	H	B	A	(2*S*,2'*R*,3'*R*)
(–)-silandrin B (**3b**)	H	B	A	(2*S*,2'*S*,3'*S*)
(+)-isosilandrin A (**4a**)	H	A	B	(2*S*,2'*R*,3'*R*)
(–)-isosilandrin B (**4b**)	H	A	B	(2*S*,2'*R*,3'*S*)

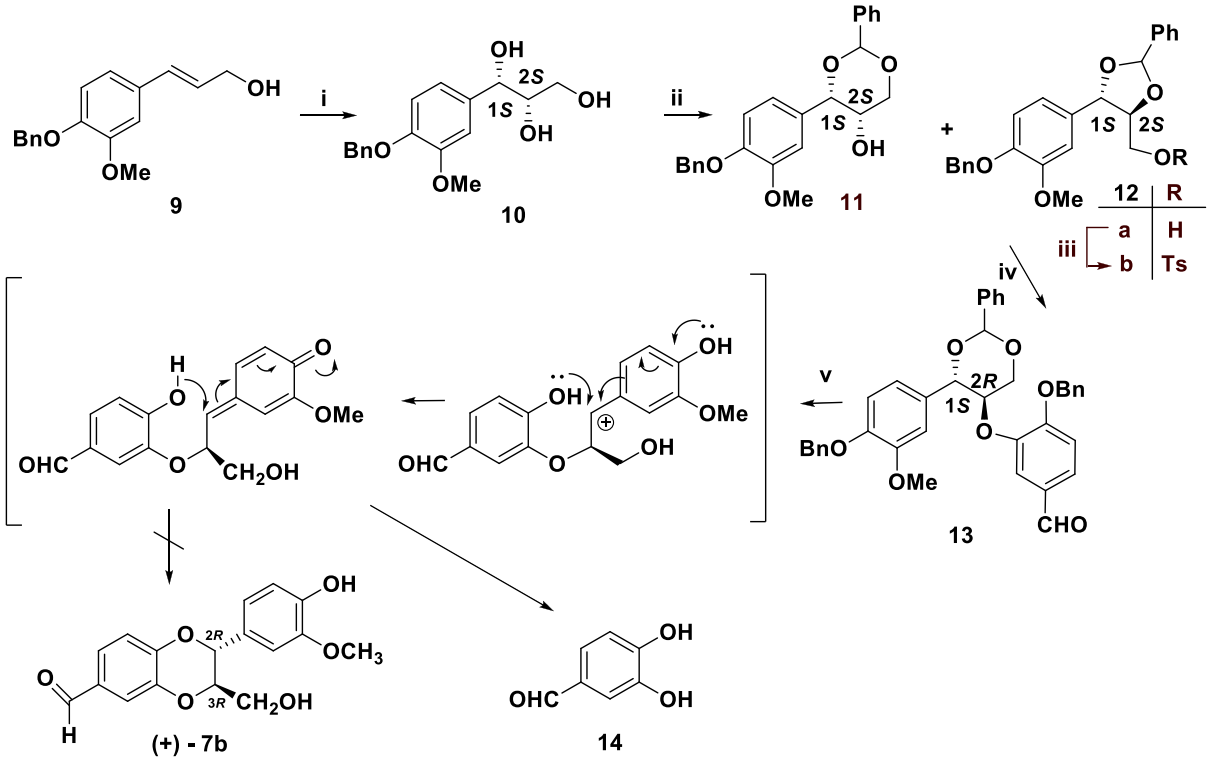
Scheme 9.

Recently, it was demonstrated that these flavanolignane stereoisomers showed fundamentally different activity against human prostate carcinoma *in vitro* and skin cancer *in vivo.* In order to study the pharmacological properties of flavanolignanes in detail, it was essential to evaluate the different possibilities for their large scale preparation. The synthesis of *rac*-**7b** and *rac*-**8b** was achieved by one of us in a regioselective oxidative coupling of caffeic acid ethyl ester (**5**) and coniferyl alcohol (**6**) as shown in Scheme 10.



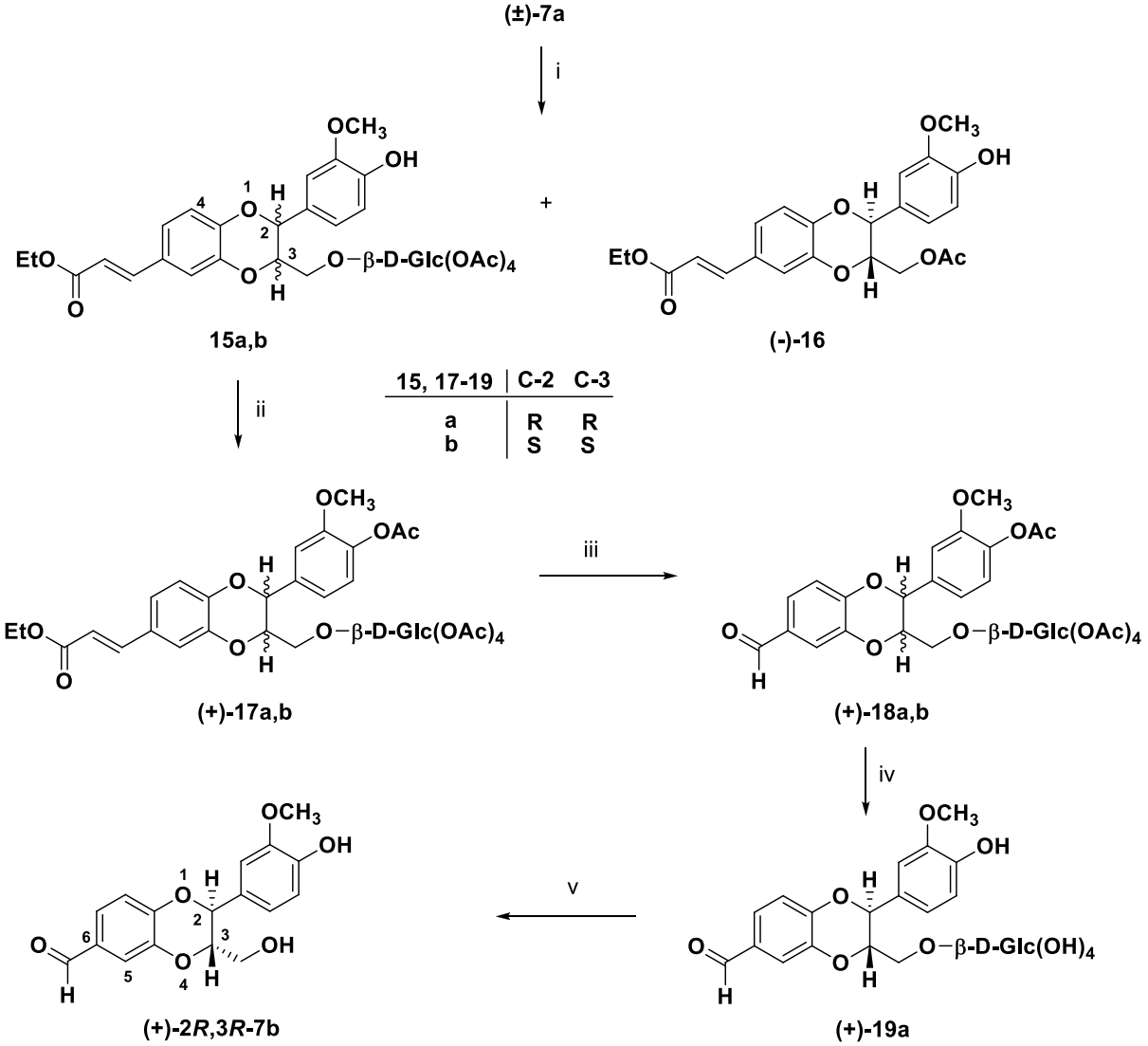
Scheme 10. Reactions and conditions: (i) NaOAc, $K_4Fe(CN)_6$, acetone/water 1:1, rt, 5h. (72%), (ii) Ag_2O or $AgCO_3$, benzene/acetone 1:1, rt, 24 h

Recently, Pan *et.al*. published the enantioselective synthesis of (+)-(2*R*,3*R*)-**7b** in five steps starting from 4-*O*-benzylconiferyl alcohol (**9**) with 13 % overall yield (Scheme 11).



Scheme 11. Reactions and conditions : (i) AD-mix-α, $MeSO_2NH_2$, *t*-BuOH, H_2O, 20 h,(94%) (ii) $PhCH(OMe)_2$, *p*-TsOH, CH_2Cl_2, rt, 2.5 h.(64%) (iii) TsCl, Et_3N, CH_2Cl_2, 25 ^oC, 8h, (iv) 4-benzyloxy-3-hydroxybenzaldehyde, Ph_3P, DEAD, PhH, Ar, 70 ^oC, 24 h (70%) (v) 36% HCl, AcOH, 65^o C (53%).

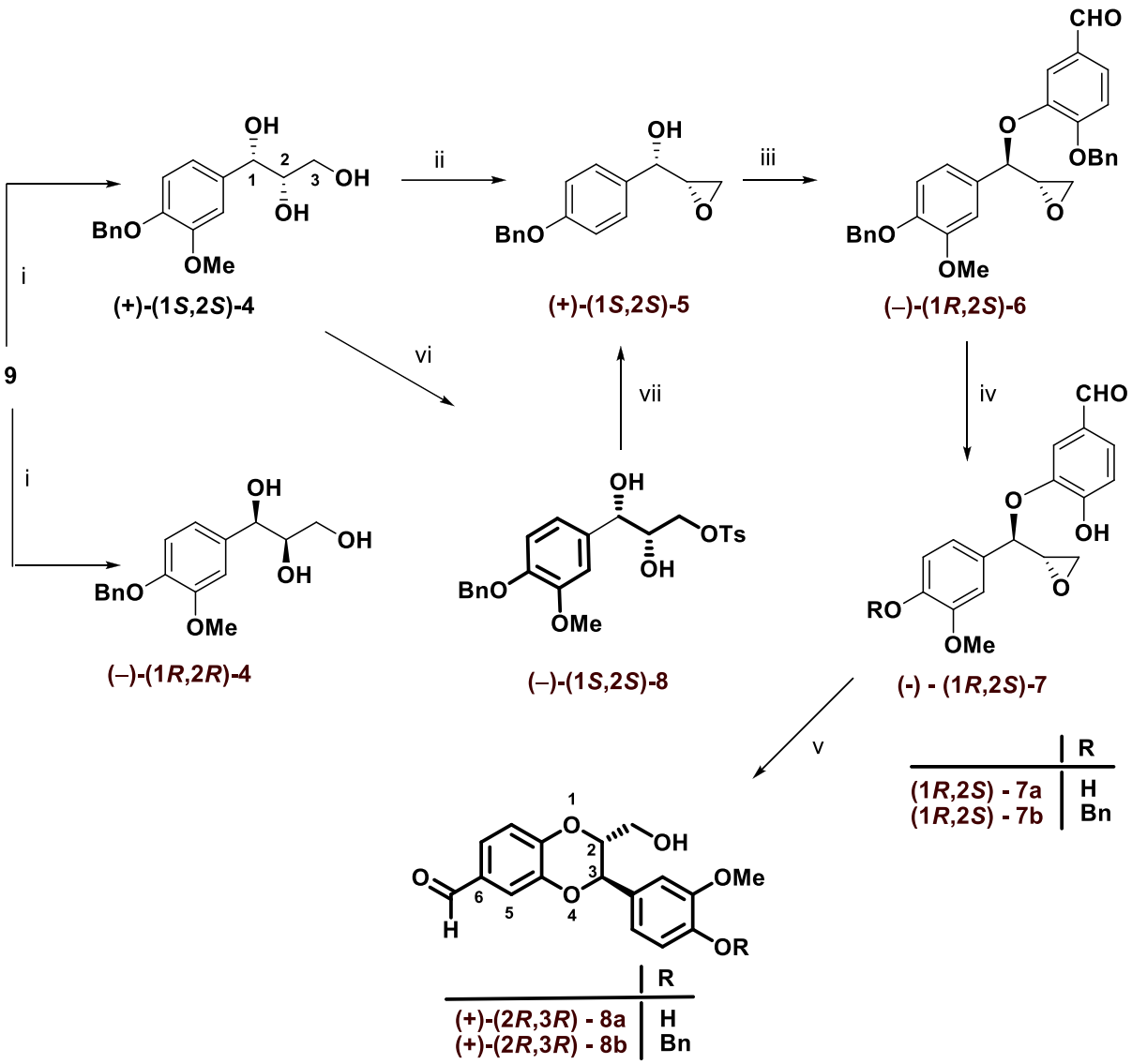
Since the last step of the synthesis of (+)-(2*R*,3*R*)-**7b** could not be reproduced by us, we performed the resolution of *rac*-**7a** as shown in Scheme 12 and the absolute configuration was determined by ECD and TDDFT-ECD calculation.



Scheme 12. Reactions and conditions: (i) 2,3,4,6-tetra-O-acetyl-a-D-glucopyranosyl trichloroacetimidate, TMSOTf, dry CH_2Cl_2, -25 ^oC, N_2,(20%) (ii) Ac_2O, dry pyridine, r.t., 12 h,(61%) (iii) $NaIO_4$-OsO_4, 1,4-dioxane, H_2O, r.t., 24h, (92%) (iv) NaOMe, MeOH, r.t.(71%), (v) MeOH, 10% HCl, reflux, 20 h (74%).

9) Re-examination of the enantioselctive synthesis of (2R,3R)-2-hydroxymethyl-3-(4-hydroxy -3-methoxyphenyl)-1,4-benzodioxane-6-carbaldehyde

Previously we reported that Pan's synthesis of (+)-(2*R*,3*R*)-**7b** could not be reproduced by us, therefore it seemed obvious to re-examine the synthesis of (*R*,3*R*)-**8b** which has the same strategy (Scheme 13) as used before and shown in Scheme 11.



Scheme 13. Reaction and conditions. (i) AD-mix-α, t-BuOH, H_2O, 0°C,20 h. (74%) [94% rep. Pan et.al], (iii) *N*-tosylimidazole , NaH, THF, rt., 2h. (13%), [71%] (iv) PPh_3,THF, DEAD, rt., 4-benzyloxy-3-hydroxybenzaldehyde, N_2, 12h., (53%), [83%] (v) 5% Pd-C, EtOAc, rt.(23% for **7b**) [90% for **7a**], (vi) K_2CO_3, MeOH, rt., 3h.(18% for **8b**) [93% for **8a**].

The sequence **9** → **4** → **5** → **6** →**7a** did not result in **7a** in our hand, because instead of **7a** a complex mixture was obtained. Its purification by preparative TLC afforded a colourless oil in 23% yield,whose structure could be identified as (1*R*,2*S*)-**7b** by its H^1-NMR spectrum. This fact could be also confirmed by its treatment with potassium carbonate in methanol at room temperature giving the 2-hydroxymethyl-3-(4-benzyloxy-3-methoxyphenyl)-1,4-bezodioxane-6-carbaldehyde (**8b**) as colourless oil with 18% yield, whose (2*R*,3*R*) absolute configuration was also proved by comparison of the experimental ECD spectrum with that of TDDFT-ECD calculation.

Summary

The patentable synthesis of the ipridronate conjugate, a potential new oral anti-osteoporotic agent, possessing ethydronate and ipriflavone moieties has been elaborated.

The preparation of new N-(β-D-glucopyranosyl)urea derivatives possessing a C-substituted 1,4-benzodioxane moiety as glycogen phosphorylase inhibitors were carried out.

A new patentable route for the preparation of a calorie-free artificial sweetener (CH-401) has been elaborated.

1,4- and 1,5-benzoxazepine derivatives possessing neuroprotective activity were prepared by regioselective domino Knoevenagel-[1,5]-hidride shift-cyclisation sequence.

Synthesis of *rac*-hexahydropyrrolo[1,2-a]quinoline derivatives with moderate acetylcholinesterase inhibitory activity was achieved and HPCL-ECD, TDFDFT-ECD analysis were performed.

An efficient synthesis of 1,2-dihydrochromeno[2,3-c]pyrrol-3-one derivatives with antiproliferative activity has been elaborated starting from 2-hydroxy-5-methoxybenzaldehyde in a one pot reaction.

Four new cladosporol derivatives have been isolated from the marine-derived fungus and their structures were determined by spectroscopic methods, on the basis of which the absolute configuration of the cladosporol family has been revised.

The preparation of 2*R*,3*R*-3-hydroxymethyl-1,4-benzodioxane-6-carbaldehyde derivative was achieved by the separation of its β-D-glucosyl derivatives.

An efficient rout to the 2*R*,3*R*-2-hydroxymethyl-1,4-benzodioxane-6-carbaldehyde derivative was achieved.

Összefoglalás

Az etidronát és ipriflavon egységeket tartalmazó, potenciálisan orális csontritkulásgátló hatású ipridonát konjugátum szabadalmazható szintézisét dolgoztam ki.

A glikogén-foszforiláz gátló hatású, C-szubsztituált 1,4-benzodioxán egységet tartalmazó új N-(β-D-glükopiranozil)karbamid származékok szintézisét valósítottam meg.

A kalóriamentes mesterséges édesítőszer (CH-401) előállítására egy új szabadalmaztatható szintézist dolgoztam ki.

Idegsejtvédő hatású 1,4- és 1,5-benzoxazepin származékokat állítottam elő regioszelektív domino Knoevenagel-[1,5]-hidrid vándorlás- gyűrűzárás szekvenciával.

Acetilkolinészteráz-gátló hatású *rac*-hexahidropirrolo[1,2-a]kinolin származékokat állítottam elő és HPCL-ECD és TDFDFT-ECD vizsgálatukat végeztem el.

Sejtosztódásgátló hatású 1,2-dihidrokroméno[2,3-c]pirrol-3-on származékok hatékony szintézisét dolgoztam ki egy lombikos reakcióban 2-hidroxi-5-metoxibenzaldehidből

Négy új kladosporol származékot izoláltam tengeri eredetű gombából, melyek szerkezetét spektroszkópiai módszerekkel határoztam meg, melynek alapján a kladosporol család abszolút konfigurációját felülvisgáltam.

A 2*R*,3*R*-3-hidoximetil-1,4-benzodioxán-6-karbaldehid származék szintézisét a β-D-glükozil-3-hidroximetil-származékok előállítását követő elválasztásával dolgoztam ki.

A 2*R*,3*R*-2-hidoximetil-1,4-benzodioxán-6-karbaldehid származék enantioszelektiv szintésének módosításával e vegyület hatékonyabb előallítását oldottam meg.