UNIVERSITY OF PARDUBICE
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Synthesis of Biological Active Nitrogen Derivatives of Three- to Five-Membered Carbocyclic Compounds

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References

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Abstract

This dissertation consists of a theoretical and experimental part. Introduction describes the preparation of biologically active or potentially biologically active substances using nitroaldol condensation and cyclopropanation reaction. The theoretical part also includes a chapter dealing with the inhibition of cholinesterases and the use of potential inhibitors as healing substances for the treatment of Alzheimer's disease. The experimental part focuses on the study of the asymmetric inter- and intramolecular Henry reaction, affording chiral β-nitroalcohols, which represent the intermediates for preparation of biologically active substances. Another part of dissertation targets on cyclopropanation reaction and its asymmetric version, for the preparation of a series of carbamates derived from the human drug Tranylcypromine.

Keywords

Henry reaction; β-nitroalcohol; imidazolidine-4-one derivatives; copper complexes; carbamates; cholinesterases inhibitors; cyclopropane derivatives; *in vitro* biological studies

Abstrakt

Tato disertační práce se skládá z teoretické a experimentální části. Úvod je věnován přípravě biologicky aktivních nebo potencionálně biologicky aktivních látek s využitím nitroaldolové kondenzace a cyklopropanační reakce. V teoretické části je také kapitola zabývající se inhibicemi cholinesteráz a uplatnění potenciálních inhibitorů jako léčivých substancí pro léčbu Alzheimerovy choroby. Experimentální část se zaměřuje na studium asymetrické inter- a intramolekulární Henryho reakce, pro přípravu biologicky aktivních β-nitroalkoholů. Další významnou kapitolou je studium cyklopropanační reakce a její asymetrické verze, pro přípravu série karbamátů odvozených od léčiva Tranylcyprominu.

Klíčová slova

Henryho reakce; β-nitroalkoholy; deriváty imidazolidin-4-onů; měďnaté komplexy, karbamáty; inhibitory cholinesteráz; deriváty cyklopropanu; *in vitro* biologické studie

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1 Introduction

At present, medicinal chemistry represents one of the most important area of chemical research. Particular attention is devoted to the preparation of new compounds, which possess some interesting biological activity. With regard to the fact, that almost all such compounds are chiral, stereoselective and stereocontrolled synthesis need to be utilized. This work is focused on chiral vicinal aminoalcohols, compounds bearing cyclopropane moiety and carbamate derivatives, because these classes of compounds belong among important building blocks for synthesis of many of human drugs, e.g.(*R*)-Salmeterol,[1] Tranylcypromine[2], Rivastigmine[3] (**Fig. 1**).

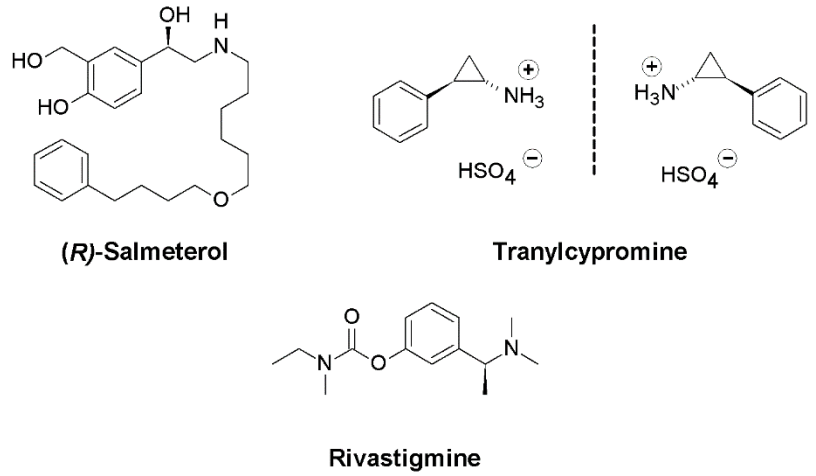


Fig. 1 The selected human drugs

2 Aim of thesis

This dissertation deals with the preparation of chiral β-nitroalcohols in non-racemic form as intermediates of biologically active vicinal aminoalcohols. Moreover, in further part, the dissertation deals with the preparation of cyclopropane derivatives and carbamates as potential inhibitors of cholinesterases.

The main aims of the thesis can be summarized in these points:

1) The preparation and characterization of new series of chiral ligands derived from 2-(pyridine-2-yl)imidazolidine-4-one derivatives and their copper(II) complexes.

2) The study of catalytic activity and enantioselectivity of copper(II) complexes of newly prepared 2-(pyridine-2-yl)imidazolidine-4-one derivatives in asymmetric Henry reaction of different aldehydes with nitromethane. The application of the most efficient catalyst for the preparation of a key intermediate in the synthesis of Salmeterol.

3) The study of intramolecular Henry reaction of 2-(2-nitroalkyl)benzaldehydes in terms of enantio- and diastereoselectivity. The reduction of obtained substituted 2-nitroindan-1-ols into corresponding 2-aminoindan-1-ols.

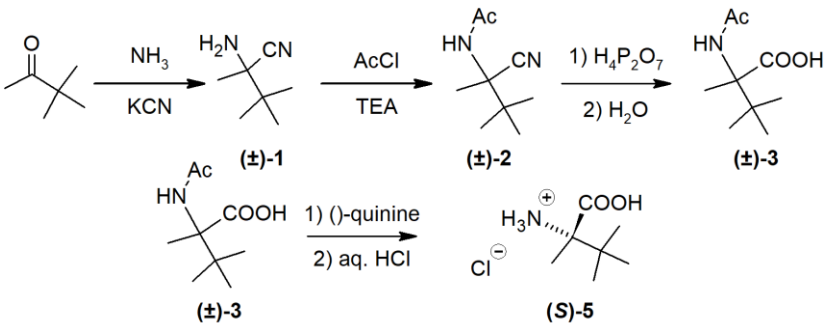
4) The preparation and characterization of a serie of carbamates derived from Tranylcypromine, both in racemic and non-racemic forms. The study of these carbamates as potential inhibitors of cholinesterases and determination of their cytotoxicity.

5) The preparation and characterization of an analogical serie of carbamates with modified structure, i.e. containing cyclobutane ring instead of cyclopropane ring, 4-halogenphenyl substitution instead of phenyl etc. The study of these carbamates as potential inhibitors of cholinesterases and determination of their cytotoxicity.

3 Results and discussion

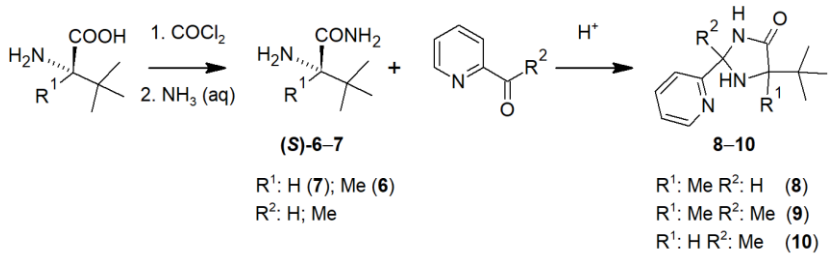
3.1 Asymmetric Henry reaction

The most successful catalysts suitable for asymmetric Henry reaction include the Cu(II) complexes of functionalized 2-(pyridine-2-yl)imidazolidine-4-ones.[4] It was found that their enantiocatalytic activity was distinctly affected by the substituents attached to stereogenic centers, among which the steric effect of the 5-alkyl substituent was important. Herein, the aim was the preparation and testing of the enantiocatalytic efficiency of a new serie of 2-(pyridine-2-yl)imidazolidine-4-ones containing a *tert*-butyl group at the 5-position of imidazolidine-4-one ring. The key intermediate for the synthesis of 2-(pyridine-2-yl)imidazolidine-4-one derivatives were the corresponding 2-aminoalkanamides, therefore, it was necessary to prepare the corresponding 2-amino-2,3,3-trimethylbutanamide[5] (α-methyl-*tert*-leucinamide) (**(*S*)-6**) and 2-amino-3,3-dimethylbutanamide (**(*S*)-7**) in enantiomerically pure form. While the starting L-*tert*-leucine was commercially available, (*S*)-*α*-methyl-*tert*-leucine **(*S*)-5** needs to be prepared by the synthetic procedure described in **Scheme 1** with >99% enantiomeric purity on a gram scale (**Scheme 1**).



Scheme 1 Synthesis of (*S*)-α-methyl-*tert*-leucine hydrochloride (**(*S*)-5**).

(*S*)-*α*-Methyl-*tert*-leucinamide (**(*S*)-6**) L-*tert*-leucinamide (**(*S*)-7**) were prepared by the treatment of aqueous ammonia solution on *N*-carboxyanhydrides (NCA) of the corresponding 2-amino acids (88–92%) (**Scheme 2**). Both NCA derivatives were easily accessible in virtually quantitative yields by the action of phosgene or triphosgene on the amino acid itself. The advantage of this method lay in the fact that the transformation into NCA not only activated the carboxylic group but also simultaneously protected the amino group. The subsequent reaction of NCA with nucleophiles proceeded very easily and regiospecifically on the carboxylic carbon atom of the amino acid moiety.[5]



Scheme 2 Synthesis of imidazolidine-4-one ligands **8–10**.

Imidazolidine-4-one derivatives **8–10** were prepared by condensation reaction of amides **6–7** with pyridine-2-carbaldehyde or 2-acetylpyridine (**Scheme 2**) using a modified procedure described previously.[4] The individual diastereomers were separated by column chromatography (**Fig. 2**). The relative configuration at the newly formed stereogenic center of ligands **8–10** was determined by means of ^{1}H NMR NOESY pulse sequence, and in the case of ligand **(2*R*,5*S*)-8**, it was also confirmed by X-ray diffraction. The catalytic activity of the *in situ* prepared Cu(II)-complexes of ligands **8–10** (**Fig. 2**) was studied in the asymmetric Henry reaction of four different aldehydes with nitromethane (**Tab. 1**). These aldehydes were chosen with respect to the possibility of comparison of catalytic parameters with those obtained with previously studied imidazolidine-4-one derivatives.[4]

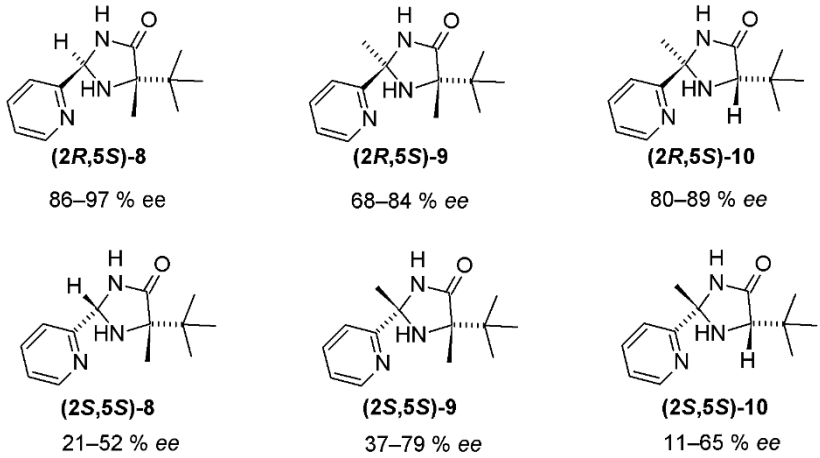
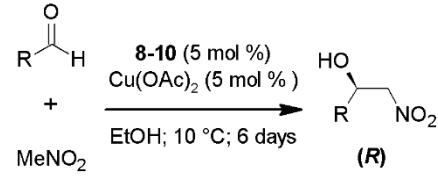


Fig. 2 Newly prepared imidazolidine-4-one ligands **8–10**

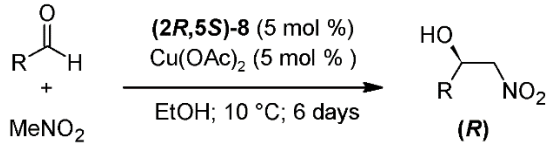
Table 1 Survey of experiments of asymmetric Henry reaction catalyzed by copper(II) complexes of ligands **8–10**



R	*t*Bu		4-$NO_2C_6H_4$		C_6H_5		2-$CH_3OC_6H_4$	
L	yield (%)	*ee* (%)	yield (%)	*ee* (%)	yield (%)	*ee* (%)	yield (%)	*ee* (%)
(2*R*,5*S*)-8	48	97	93	86	81	93	88	92
(2*S*,5*S*)-8	15	–51	31	–21	44	–45	42	–52
(2*R*,5*S*)-9	38	84	75	62	80	78	96	68
(2*S*,5*S*)-9	–	–	57	37	65	79	45	74
(2*R*,5*S*)-10	33	89	99	80	87	88	94	89
(2*S*,5*S*)-10	14	65	52	11	47	65	35	37

The experiments presented in **Table 1** showed that the most efficient ligand in serie **8–10** was derivative **(2*R*,5*S*)-8**. Therefore, its enantiocatalytic activity was further verified in Henry reaction of other aldehydes (aromatic or heteroaromatic and aliphatic) with nitromethane. The results obtained (**Table 2**) showed that the attained chemical yields correspond with the reactivity of aldehyde and enantioselectivity was in most cases higher than 90% *ee*, except for the two most reactive aldehydes (entry 1, 86% *ee* and entry 4, 88% *ee*).

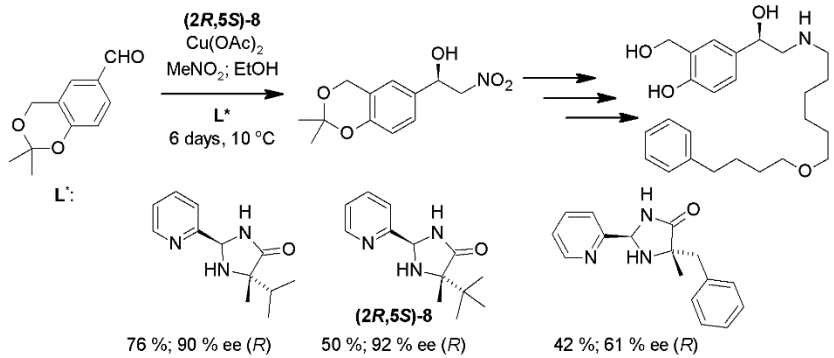
Table 2 Henry reaction of nitromethane with various aldehydes catalyzed by copper(II) complex of ligand **(2*R*,5*S*)-8**



Entry	Aldehyde	Yield (%)	*ee* (%)
1	4-CNC_6H_4	99	86
2	4-ClC_6H_4	90	91
3	4-PhC_6H_4	88	92
4	2-$NO_2C_6H_4$	96	88
5	Naphth-2-yl	87	90
6	Thien-2-yl	46	91
7	*n*-C_4H_9	67	90
8	$PhCH_2CH_2$	56	91
9	*c*-C_6H_{11}	65	92
10	PhCH=CH	43	90

Enantioselectivity of the Cu(II)-complex of the ligand **(2*R*,5*S*)-8** was compared with the previously prepared[4] analogous 5-benzyl and 5-isopropyl derivatives in the Henry reaction affording (*R*)-1-(2,2-

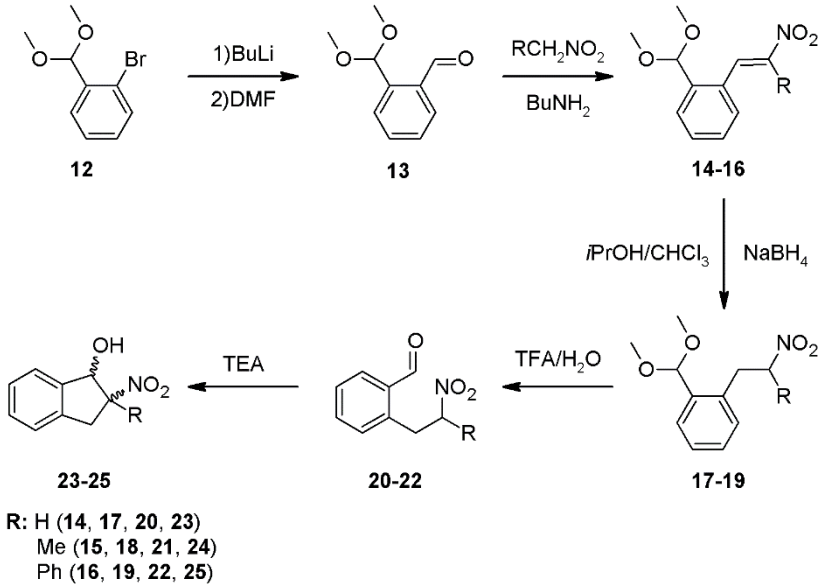
dimethyl-4*H*-benzo[*d*-1,3]dioxin-6-yl)-2-nitroethanol (**Scheme 3**). This compound represents the intermediate in the synthesis of (*R*)-Salmeterol, which is applied as a long-acting β2-adrenoreceptor agonist (sold under trade name Serevent®).[6] The highest enantiomeric purity was attained in case of 5-*tert*-butyl derivative **(2*R*,5*S*)-8** (92% *ee*), with a chemical yield of 50%. In comparison, the 5-isopropyl derivative gave only a slightly lower enantioselectivity (90% *ee*), but higher chemical yield (76%); in the case of 5-benzyl derivative, the enantioselectivity was unsatisfactory (61% *ee*).



Scheme 3 Enantioselectivity and catalytic activity of copper(II) complexes of imidazolidine-4-one derivatives with different alkyl groups at the 5-position in the preparation of the intermediate of the (*R*)-Salmeterol.

3.2 Intramolecular Henry reaction

Recently, several 2-substituted 2-nitroindan-1-ols *via* Henry reaction were prepared. (**Scheme 4**).[7,8] The synthetic procedure includes both, inter- and intramolecular version of Henry reaction.



Scheme 4 Synthesis of 2-substituted 2-nitroindan-1-ols **23–25**

At first, the reaction conditions of each steps in this reaction sequence for preparation of 2-nitroindan-1-ols **23–25** were optimized. Some reaction conditions were modified to achieve higher chemical yields. For example, the reaction time of intermolecular Henry reaction of **13** with the corresponding nitroalkane was prolonged from 5 to 24 h. Similarly, in the case of the reduction of alkenes **14–16**, optimized reaction conditions led to higher isolated yields (from 80% for **17**, 70% for **18** and 53% **19**) to over 90% for all synthesized alkanes. Deprotection of **17–19** was almost quantitative and intramolecular Henry reaction of **20–22** was performed using sub-stoichiometric amount of TEA in *i*-PrOH giving mixture of both diastereomers of **23–25** in 86–97% isolated yield. It was examined the verification of the relative stereochemistry using NOESY NMR experiment. Unfortunately, it was found that both diastereomers of **23–25** show NOE cross peak between hydrogen atoms on C1 and C2, CH_3, or *o*-Ph. Other possibility of determination of configuration of compounds **23–25** was using of X-ray

diffraction. Only in the case of **23** it was possible to isolate pure major diastereomer in crystalline form. The *trans*-configuration of major diastereomer of **23** was proved by this method (**Fig. 3**).

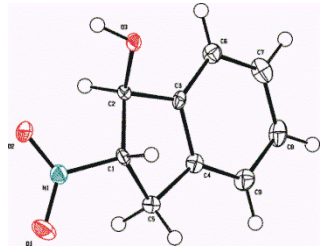
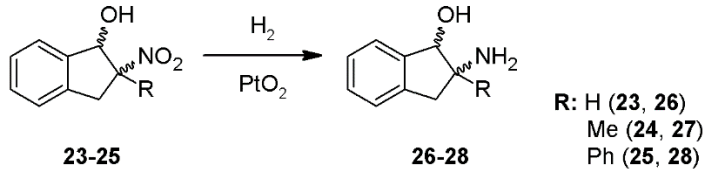


Fig. 3 ORTEP diagram of *trans*-2-nitroindan-1-ol (**23**)

Further, the asymmetric Henry reaction of 2-(2-nitroalkyl) benzaldehydes **20–22** catalyzed by selected enantioselective catalysts (e.g., Cu(II) acetate complex of ligand **(2*R*,5*S*)-8**, cinchonidine, β-cyclodextrine) was studied. Unfortunately, the corresponding 2-nitroindan-1-ols **23–25** were obtained in racemic form in all cases.

Next, the reduction of the compounds **23–25** (**Scheme 5**) was performed. The reduction was carried out in hydrogen atmosphere (1 bar) in the presence of Adams catalyst (PtO_2) in MeOH.[9] This mild conditions were used with regard to possible hydrogenolysis of the hydroxy group (benzyl type). However, under these conditions, only derivatives **26** and **28** were successfully prepared.



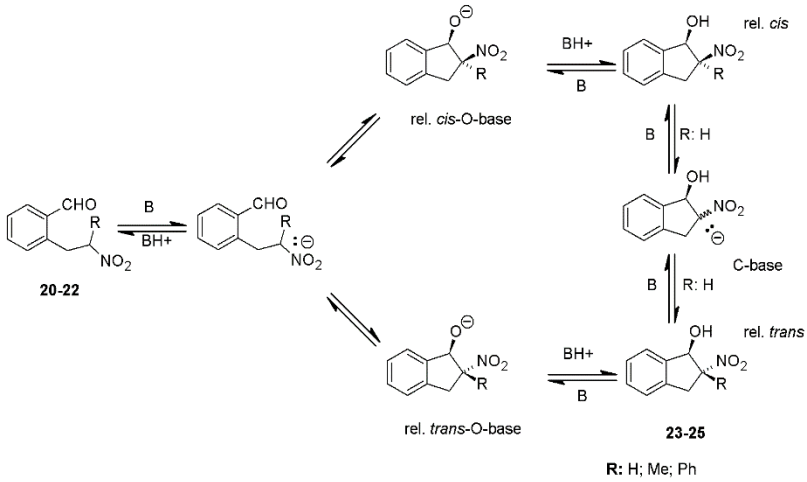
Scheme 5 Reduction of 2-substituted 2-nitroindan-1-ols **23–25**

Finally, the effect of used solvent and the base on reaction rate and diastereomeric ratio of the intramolecular Henry reaction of compounds **20–22** was studied. The kinetics measurements and determination of diastereomeric ratio were performed using ^{1}H NMR spectroscopy in non-aqueous solvents ($CDCl_3$, DMSO-d_6, benzene-d_6, acenitrile-d_3, CD_3OD) (0.5 mL) in the presence of base: TEA (3μL), pyridine (3μL) or *N*-methylmorpholine (3μL). Almost all measurements were catalyzed with TEA, only derivative **20** was studied in acetonitrile-d_3 with pyridine and *N*-methylmorpholine. Preferential formation of *trans*-**23-25** has been observed in all solvents with the highest ratio *trans*-/*cis*- in benzene-d_6 and CD_3OD (**Table 3**).

Table 3 Ratio of *cis*-/*trans*- isomers of **23–25** observed by TEA-catalyzed intramolecular Henry reaction of **20–22** in various deuterated solvents.

Solvent	**Ratio of *cis*-/*trans*-**		
	23	**24**	**25**
CD_3OD	20 : 80	24 : 76	36 : 64
$CDCl_3$	20 : 80	23 : 77	45 : 55
CD_3CN	26 : 74	31 : 69	43 : 57
DMSO-d_6	26 : 74	30 : 70	43 : 57
C_6D_6	17 : 83	19 : 81	45 : 55

Based on these kinetics measurements, the mechanism of intramolecular Henry reaction of **20–22** was proposed (**Scheme 6**).



Scheme 6 The proposed mechanism of intramolecular Henry reaction of compounds **20–22**

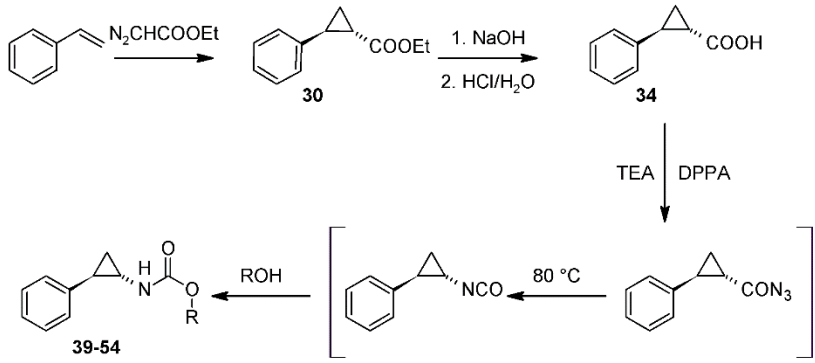
3.3 *N*-(2-Phenylcyclopropyl)carbamates 39–54 and 64–66

The further aim of this work was the preparation and characterization of a serie of *N*-(2-phenylcyclopropyl)carbamate derivatives **39–54** and **64–66**. These compounds, derived from well known (±)-*trans*-2-phenylcyclopropane-1-amine, used in human medicine as the MAO inhibitor,[10] contains two different pharmacophores – cyclopropane ring and carbamate functional group. The inhibitory activity of these compounds against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) respectively as well as their cytotoxicity were studied and evaluated (**Table 4.**).

With respect to the fact, that *N*-(2-phenylcyclopropyl)carbamate derivatives were chiral compounds, the most promising derivative **51**, possessing high inhibitory activity, was prepared in all of configuration forms (4 stereoisomers). The mutual comparison of inhibitory activity of all stereomers **51** enables the consideration of

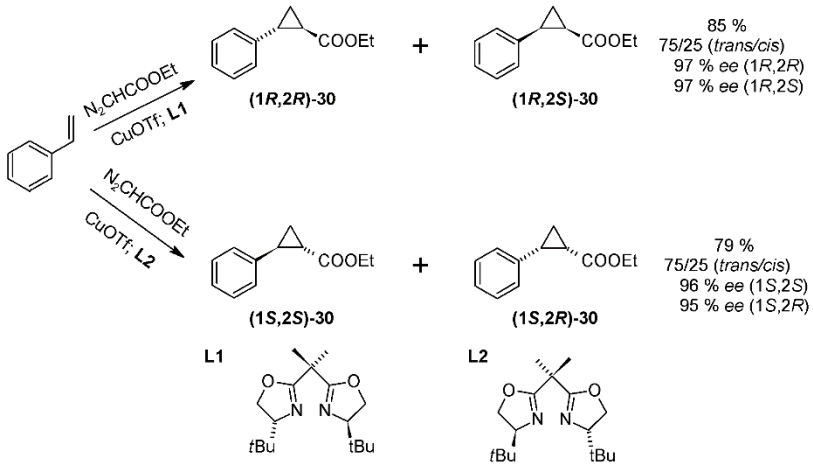
influence of absolute configuration at stereogenic centres of **51** on its resulting biological activity. From this point of view, only small differences of inhibitory activity of derivatives **51** were observed. The key intermediate for the synthesis of carbamate derivatives **39–54** was 2-phenylcyclopropanecarboxylic acid **34**. It was prepared by standard two-step synthesis. At first cyclopropane cycle was formed by action of ethyl diazoacetate onto styrene affording cyclopropanecarboxylate **30**. Subsequently, hydrolysis of ester group under basic condition was performed (**Scheme 7**). The carboxylic acid **34** was transformed to individual carbamates **39–54** by the action of diphenylphosphoryl azide (DPPA) in the presence of TEA and appropriate alcohol (**Scheme 7**).[11]

The optically pure forms of ester **30** were prepared according the protocol described by Evans[12] employing the enantioselective catalyst based on copper(I) complex of commercially available chiral bisoxazoline derivative (**Scheme 8**).



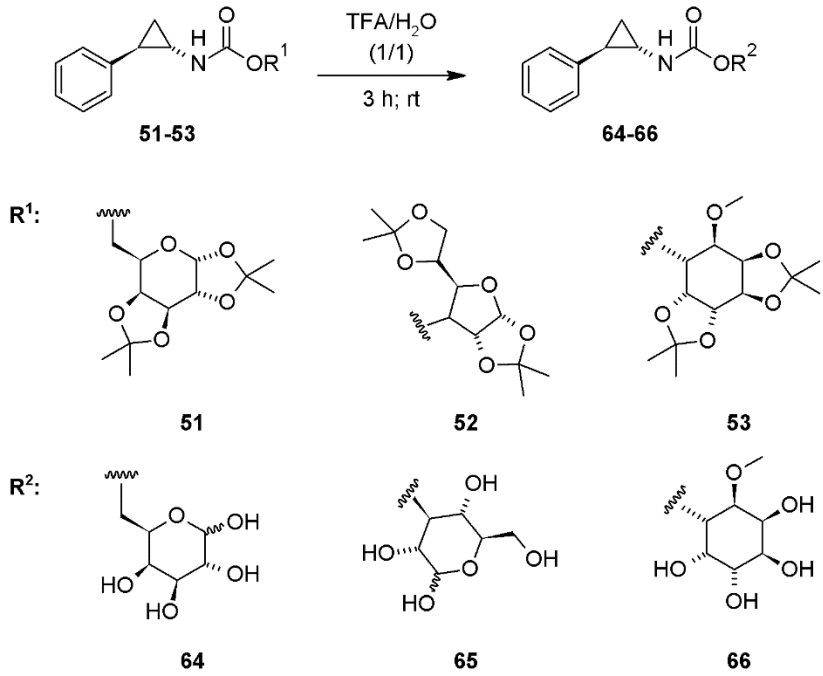
R: viz **Tab. 4**

Scheme 7 Three-step synthesis of carbamates **39–54**



Scheme 8 Enantioselective synthesis of ethyl ester **30**

The serie of prepared carbamates also included four members **51**–**54** derived from protected monosaccharides and their corresponding deprotected forms **64**–**66**. These carbamates were deprotected (deacetalisation of monosaccharide moiety) by the treatment of aqueous trifluoroacetic acid (TFA) (1/1) (**Scheme 9**).[13]



Scheme 9 Deprotection of *O*-glycosyl carbamate derivatives **51–53**; the survey of individual carbamates **64–66**

The ability of carbamates **39–54** and **64–66** to inhibit AChE from electric eel (*Electrophorus electricus*) and BChE from equine serum was determined *in vitro* using modified Ellman´s method.[14] Ellman's method[14] is widely used for measuring of cholinesterase activity and effectiveness of cholinesterase inhibitors and is usually expressed as IC_{50} value representing the concentration of an inhibitor, which is necessary for reduction of enzyme activity (or reaction rate) to 50 %. The obtained results are shown in **Table 4**.

Table 4 The survey of prepared carbamates **39–54** and **64–66** and their inhibitory activity against AChE and BChE and lipophilicity

Compound		IC_{50} (μM)		AChE/BChE selectivity	logP_{ow}
Code	R	AChE	BChE		
(±)-39	*i*Pr	72.70	33.64	2.16	4.61
(±)-40	Bu	66.32	17.40	3.81	5.17
(±)-41	*i*Bu	71.45	17.00	4.21	6.63
(±)-42	*t*Bu	62.87	21.06	2.99	2.90
(±)-43	*s*Bu	77.63	40.77	1.90	4.37
(±)-44	*c*Hex	62.78	29.05	2.16	4.87
45	(–)-menthyl	72.25	**9.05**	7.98	4.93
(±)-46	3-Ph$(CH_2)_3$–	60.51	27.15	2.22	5.18
(±)-47	2-Ph$(CH_2)_2$–	60.45	33.33	1.81	4.31
(±)-48	PhCH_2	54.93	33.29	1.65	3.57
(±)-49	4-BrPhCH_2	49.39	15.85	3.12	4.52
(±)-50	Geranyl	**36.07**	47.45	0.76	5.33
51	PG-galactos-6-yl	57.85	9.79	5.91	5.00
(1*S*,2*R*)-51	PG-galactos-6-yl	54.84	5.80	9.45	4.80
(1*R*,2*S*)-51	PG-galactos-6-yl	85.87	32.74	2.62	4.85
(1*S*,2*S*)-51	PG-galactos-6-yl	94.39	37.59	2.51	5.87
(1*R*,2*R*)-51	PG-galactos-6-yl	82.38	20.24	4.07	5.63
52	PG-glucos-3-yl	63.30	105.57	0.60	4.10
53	PG-pinitol-4-yl	83.41	109.13	0.76	4.84
54	PG-fructos-1-yl	**36.76**	120.61	0.30	3.50
64	Galactos-6-yl	89.63	–	–	1.78
65	Glucos-3-yl	75.59	152.46	0.50	1.70
66	Pinitol-4-yl	80.01	–	–	1.44
Rivastigmine[15]	–	56.10	38.42	1.46	–

All tested compounds could be separated in two groups: *Group 1* included compounds **39–50** (R = 12 alkyls) and *Group 2* included compounds **51–54** (R = 4 protected glycosyls) and **64–66** (R = 3 deprotected glycosyls). Based on obtained results, it is obvious, that all tested compounds in *Group 1* showed moderate inhibition of AChE and BChE. Generally, it is possible to conclude, that the compounds **39–49** showed higher selectivity to BChE, contrary to the compound **(±)-50**. The IC_{50} values for AChE inhibition were in range of 36.07–77.63 $\mu mol \cdot l^{-1}$, whereas the most potent inhibitor was the carbamate **(±)-50** (R = geranyl), while the least effective was the compound **(±)-43** (R = *s*Bu). The most effective BChE inhibitor was found the carbamate **45** (R = menthyl) with IC_{50} = 9.05 $\mu mol \cdot l^{-1}$.
All tested compounds in *Group 2* showed moderate inhibition of AChE (36.76–94.39 $\mu mol \cdot l^{-1}$) and BChE (9.79–152,46 $\mu mol \cdot l^{-1}$), with exception of compounds **64** and **66**, which exhibited low inhibitory activity against BChE. The compounds **51**, **(1*S*,2*R*)-51**, **(1*R*,2*S*)-51**, **(1*S*,2*S*)-51** and **(1*R*,2*R*)-51** showed higher selectivity to BChE. Interestingly, the **51** did not show significantly lower inhibitory effectiveness than the most active optically pure form **(1*S*,2*R*)-51**. The compounds **64–66** showed (if it is possible to determine) higher selectivity to AChE.

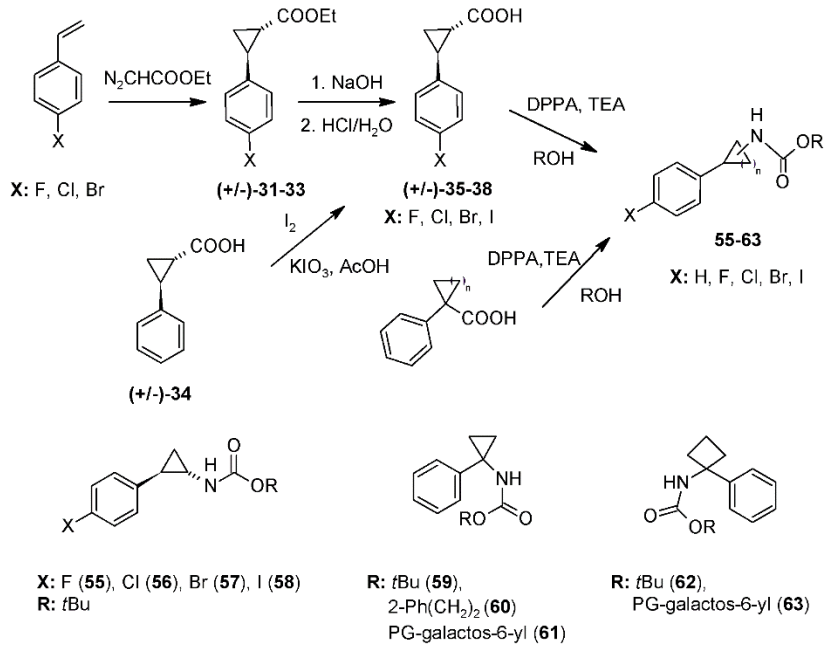
Generally, all tested compounds showed moderate inhibitory activity. The most effective inhibitor was found the derivative **45** (for BChE) resp. **(±)-50** (for AChE). However, evaluating cytotoxic activity of all derivatives, the derivative **(±)-50** was found as highly cytotoxic. From this point of view, the carbamate **54** can be considered as the most promising AChE inhibitor due to its potent inhibitory activity and concurrently low cytotoxicity against Jurkat cells.

3.4 Carbamates 55–63 and 70–72

From the above mentioned results of biological studies carried out on carbamates derived from racemic (±)-*trans*-2-phenylcyclopropane-1-amine **39–54** and **64–66**, it was found, that the inhibitory activity of the prepared derivatives against ChEs did not change dramatically with *O*-substitution. However, the cytotoxicity of the compounds **39–54** and **64–66** was significantly affected by this substitution. With

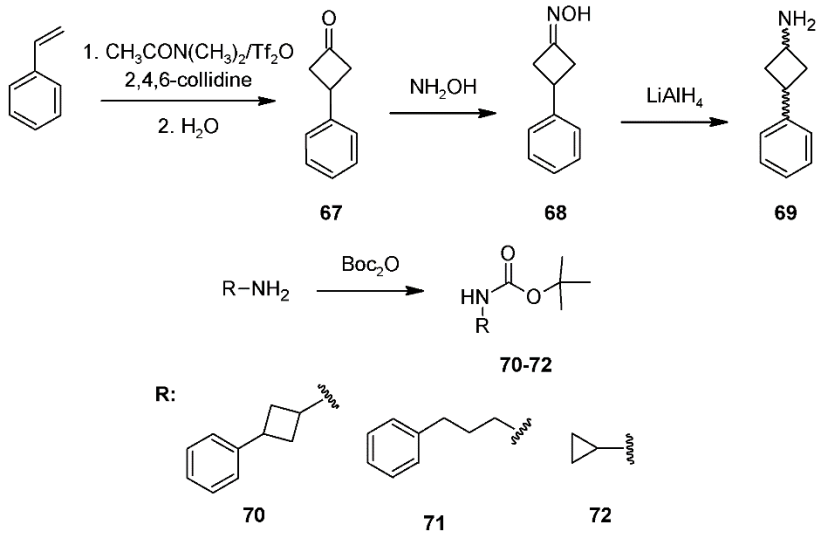
regard to these findings, this study was extended with new *N*-(2-phenylcyclopropyl)carbamates derived from some other alcohols. Moreover, structural modifications in *N*-(2-phenylcyclopropyl) moiety affording 1-phenyl isomers and non-substituted cyclopropyl derivative, extension of cyclopropane ring into cyclobutane derivatives and introduction of a halogen atom in the structure were performed to obtain another serie of carbamate derivatives **55–63** and **70–72**. Their inhibitory activity against cholinesterases as well as cytotoxicity was determined.

The survey of prepared carbamate derivatives **55–63** was summarized in **Scheme 10**. The compounds **55–63** were synthetized from individual carboxylic acid derivatives. While 1-phenyl cyclopropanecarboxylic acid and 1-phenylcyclobutanecarboxylic acid respectively were commercially available, 4-halogeno derivatives of 2-phenylcyclopropanecarboxylic acid **(±)-35–38** were synthetized according to previously described methods.[16] Hence, it was used standard two-step synthesis. At first, ethyl 2-phenylcyclopropanecarboxylates **31–33** as a mixture of *cis*/*trans* isomers in satisfactory yields (50–70 %) were formed by the action of ethyl diazoacetate onto corresponding styrene derivatives catalyzed by a copper(I) salt. The subsequent hydrolysis of esters **31–33** gave the required acids **(±)-35–37** in good yields (75–99 %). Differently, 4-iodo derivative **(±)-38** was prepared by direct iodination of *trans*-2-phenylcyclopropanecarboxylic acid (**(±)-30**) with moderate yield of 42 %.[17] Finally, the carboxylic acids **(±)-35–38** were transformed to carbamate derivatives **55–63** by treatment of diphenylphosphoryl azide in the presence of TEA and corresponding hydroxyl derivative in yields from 40 % to 86 % (**Scheme 10**).



Scheme 10 Syntheses of carbamates **55–63**.

The alternative synthetic strategy was used for preparation of the carbamates **70–72** containing *O-tert*-butyl group. In this case, the carbamate group was introduced by the action of di-*tert*-butyl dicarbonate onto primary amines (i.e., cyclopropylamine, 3-phenylpropane-1-amine and amine **69**[18]) (**Scheme 11**). The compound **69** was available by three-step synthesis, including at first 3-phenylcyclobutanone (**67**) formation (46% yield) via [2+2] cycloaddition of styrene with keteniminium salt.[19] Subsequently, the ketone **67** was transformed into corresponding oxime **68** with good yield of 85 % and finally, the reduction of oxime **68** by action of $LiAlH_4$ in THF afforded required amine **69** with moderate yield of 49 % as a mixture of *cis*/*trans* form.[20] Unfortunately, the separation of the individual diastereomers of amine **69** as well as its carbamate derivative **70** by means of column chromatography was unsuccessful.



Scheme 11 Syntheses of carbamates **70–72**.

The carbamates **55–63** and **70–72** were investigated for their inhibitory activity against AChE from electric eel and BChE from equine serum using modified Ellman's method. The results were compared with the inhibitory activity determined for the established cholinergic drug Rivastigmine. From the obtained results (**Table 5**), it was obvious, that all tested carbamates **55–63** and **70–72** showed a considerable inhibitory activity of both choline esterases.

Table 5 The inhibitory activity of prepared carbamates **55–63** and **70–72** against AChE and BChE and their lipophilicity

Compound	IC_{50} (μM)		AChE/BChE selectivity	logP_{ow}
Code	AChE	BChE		
(±)-55	44.67	96.13	0.46	3.02
(±)-56	78.63	168.58	0.47	4.27
(±)-57	50.43	215.39	0.23	3.91
(±)-58	73.17	89.44	0.82	3.96
59	59.49	53.33	1.12	3.37
60	84.93	179.50	0.47	4.20
61	54.89	**9.77**	**5.60**	5.48
62	75.45	45.66	1.65	3.59
63	45.00	47.38	0.95	3.38
70	**41.89**	30.65	1.36	3.25
71	53.18	97.20	0.55	3.16
72	**44.09**	106.64	0.41	2.07
Rivastigmine[15]	56.10	38.42	1.46	–

The IC_{50} values for AChE inhibition varied in the range of 41.89–84.93 μmol·l^{-1} and for BChE inhibition in the range of 9.8–215.4 μmol·l^{-1}. Markedly, the IC_{50} values for BChE are in a significantly broader range than for AChE. In comparison with the results of inhibitory activity of carbamate derivatives **39–54** and **64–66** tested in the previous study (Chap. 3.3; Table 4), it is obvious, that the introduction of halogen atom at position 4- of phenyl cycle did not change inhibitory activity against AChE (62.9 μmol·l^{-1} (non-substituted) vs. 44.7–78.6 μmol·l^{-1} for **(±)-55-58**) significantly, whereas inhibitory activity of **(±)-55–58** against BChE decreased (21.1 μmol·l^{-1} (non-substituted) vs. 89.4–215.4 μmol·l^{-1} for

(±)-55-58). All of *N*-1-phenylcyclopropyl derivatives **59–61**, *N*-1-phenylcyclobutyl derivatives **62–63** and *N*-3-phenylcyclobutyl derivative **70** exhibited favourable cytotoxicity, especially the carbamates derived from protected galactopyranose **61** and **63**. The structural modification of previously studied *O*-*tert*-butyl-*N*-(2-phenylcyclopropyl)carbamate (**(±)-42**) into the carbamates **71** (propyl group instead of cyclopropyl cycle) and **72** (without phenyl cycle) led to slight enhancement of inhibitory activities (increasing for AChE and decreasing for BChE respectively). Therefore, the derivative **72** can be considered as the most promising compound for further study due to its very low cytotoxicity and concurrently moderate inhibitory activity against cholinesterases.

4 Conclusion

The experimental part of this dissertation was focused on the preparation of potentially biologically active compounds. In this work, the serie of 5-*tert*-butyl-2-(pyridine-2-yl)imidazolidin-4-one ligands **8–10** was prepared and their copper(II) complexes were tested as enantioselective catalysts for asymmetric Henry reaction. The most successful catalyst for this reaction was evaluated the copper(II) complex of **(2*R*,5*S*)-8**. The enantioselectivity of this catalyst is comparable to the best enantioselective catalysts of Henry reaction, known at present. This catalyst was also used for the preparation of a key chiral intermediate of the synthesis of drug (*R*)-Salmeterol.

Further, the synthetic procedure for preparation of substituted 2-nitroindan-1-ols **23–25**, as intermediates for potentially biologically active 2-aminoindan-1-ols, was modified and corresponding amino derivatives **26** and **28** were newly prepared. The reaction rate and diastereoselectivity of the intramolecular Henry reaction of 2-(2-nitroalkyl)benzaldehydes **20–22** was studied using NMR spectroscopy. It was found out, that the reaction rate and diasteroselectivity of this reaction can be influenced by the choice of solvent and base.

In another part of this work, different series of carbamate derivatives were prepared and characterized. All of these carbamates were studied for their inhibitory activity against AChE from electric eel and BChE from equine serum. Furthermore, lipophilicity and cytotoxicity against Jurkat cell line of these carbamates was determined.

The first serie, including 17 new compounds **39–54** and **64–66**, was derived from *trans*-2-fenylcycloprop-1-ylamine (Tranylcypromine). The second serie of carbamates **55–63** and **70**–**72** arose from the structural modifications (i.e., substitution of phenyl group; modification/extension of the cyclopropane ring; etc.). It included 12 newly prepared and characterized compounds.

Generally, it is possible to conclude, that all tested compounds showed moderate inhibitory activity against cholinesterases. With regard to the results obtained by all of these biological and physicochemical studies (inhibitory activity, cytotoxicity and lipophilicity), the derivatives **45**, **54**, **61** and **72** were evaluated as the most promising compounds. These four substituted carbamates have the potential to be good candidates for utilization as AChE and BChE inhibitors and therefore, they should deserve further attention for their possible medicinal applications.

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6 List of Student's Published Works

- **Articles in impacted journals (associated with the thesis)**
  - K. Vorčáková, M. Majeková, E. Horáková, P. Drabina, M. Sedlák, Š. Štěpánková, *Bioorg. Chem.* **2018**, *78*, 280–289.
  - E. Horáková, P. Drabina, L. Brůčková, Š. Štěpánková, K. Vorčáková, M. Sedlák, *Montsh. Chem.* **2017**, *148*, 2143–2153.
  - E. Horáková, P. Drabina, B. Brož, Š. Štěpánková, K. Vorčáková, K. Královec, R. Havelek, M. Sedlák, *J. Enzym. Inhib. Med. Chem.* **2016**, *31*(sup3), 173–179.
  - P. Drabina, E. Horáková, Z. Růžičková, M. Sedlák, *Tetrahedron: Asymmetry* **2015**, *26*, 141–147.
  - E. Horáková, J. Valtr, K. Dostálová, P. Drabina, J. Váňa, A. Růžička, J. Hanusek, *the manuscript was sent to the Editorial office of the Journal ChemistrySelect* **2019**.

- **Articles in impacted journals (other)**
  - V. Macháček, E. Horáková P. Šimůnek J. Valtr, V. Bertolasi, B. Brož, *Monatsh. Chem.* **2016**, *147*, 1765–1777.

- **Presented lectures**
  - E. Horáková, P. Drabina, M. Sedlák: Příprava a studium (±)-*trans*-*N*-(2-fenylcyklopropyl)karbamátů jako potenciálních inhibitorů acetylcholinesterasy, XVI. Mezioborové setkání mladých biologů, biochemiků a chemiků, Milovy, 10.-13.5.2016.

- **Presented posters**
  - E. Horáková, P. Drabina, M. Sedlák: Henry reaction catalyzed by new series of 5-*tert*-butyl-2-(pyridine-2-yl)imidazolidine-4-one Cu-complexes, 19th European Symposium of Organic Chemistry (ESOC 2015), Lisabon, 12. – 16. 7. 2015.

- E. Horáková, P. Drabina, M. Sedlák: Synthesis and Biological Studies of (±)*Trans*-*N*-(2-Phenylcyclopropyl)Carbamates as Cholinesterase Inhibitors, 4th International Conference on Chemical Technology (ICCT), Mikulov, 25. – 27.4.2016.
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- V. Macháček, E. Horáková: Reactivity of 5-nitro-2,1-benzothiazole-3-diazonium hydrogensulfate towards OH and CH acids: structure, properties, tautomerism, 17th Blue Danube Symposium on Heterocyclic Chemistry (BDSHC17), Linz (A), 30.8. – 2.9.2017.
- E. Horáková, K. Vorčáková, P. Drabina, M. Sedlák: Synthesis and characterization of new *N*-phenylcarbamates as potential inhibitors of cholinesterases, 16th Belgian Organic Synthesis Symposium (BOSS/XVI), Brussels, 8. – 13.7.2018.