Theses of doctoral (Ph.D.) dissertation

SYNTHESIS OF A NATURALLY OCCURING AND ANALOGUE OF GLYCOGEN PHOSPHORYLASE INHIBITORS

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1. Introduction

The number of patients suffering from diabetes mellitus (DM) is dramatically increasing. Nowadays more than 360 million people suffer from Diabetes mellitus worldwide, and more than 90% of the diagnosed cases belong to the type 2 or non-insulin dependent diabetes mellitus. Among several investigational fields to diminish hepatic glucose output in T2DM, glycogen phosphorylase (GP) as a main regulatory enzyme of glycogen metabolism has become a validated target. Several molecules of high structural diversity were reported to bind to the allosteric site of GP, such as derivatives of acyl urea, dihydropyridine dicarboxylic acid, pentanedioic acid, phthalic acid, *N, N*'-diaryl-urea, and pentacyclic triterpenoids.

FR258900, is bis-O-(*p*-coumaroylated) 2,3-dihydroxypentanedioic acid derivative (**49**), was isolated from the fermentation broth of fungi No. 138354. The compound was shown to inhibit glycogen phosphorylases and to bind to the allosteric site of GP. The aim of my PhD thesis was the synthesis of new analogues of **49** and study the structure-activity relationships of these new compounds. The synthesis of 2,3-dihydroxypentanedioic acid is unknown in the literature, the core unit was planned to be replaced by easily available L-, D- and *meso*-tartaric acids (**50-52**), L-malic acid (**53**), L-glutamic acid (**54**) and 3-hydroxypentanedioic acid (**55**). Furthermore, the substitution pattern of the aromatic rings was also modified with cinnamic acid (**56**), *p*-coumaric acid (**57**), ferulic acid (**58**) (Figure 1.).

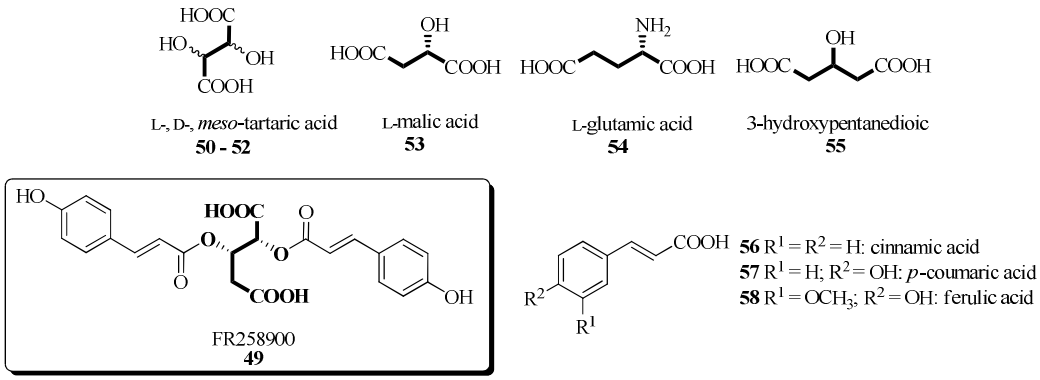


Figure 1. Planned modification of FR258900

The second aim of my PhD research was the preparation of new glucose analogue inhibitors of GP by the bioisosteric replacements of the "third" NHCO moieties of *N*-acyl-*N*'-β-D-glucopyranosyl urea type GP inhibitors with 1,3,4-oxadiazole and 1,2,4-triazole heterocycles and the study of their effects on inhibitor activity (Figure 2. **26c**, **26d**).

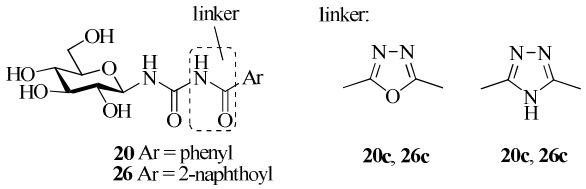


Figure 2. Planned modifications of *N*-acyl-*N'*-(β-D-glucopyranosyl)-urea

There were two pathways for the synthesis of *N*-(β-D-glucopyranosyl)-heteroaryl-carboxamides derivatives (Figure 3. „A", „B" pathways).

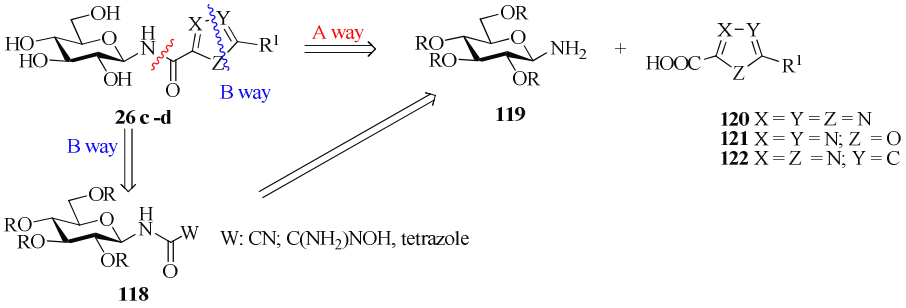


Figure 3. Retrosynthetic analysis of *N*-(β-D-glucopyranosyl)-heteroaryl-carboxamides derivatives

2. Methods

In our work macro, semimicro and micro methods of modern preparative organic chemistry were applied. Reactions were monitored by thin-layer chromatography. Products of the reactions were purified by coloumn chromatography and/or crystallization.

New compounds were characterized by their physical properties (melting point, optical rotation) and their structures were elucidated by ^{1}H NMR and ^{13}C NMR methods as well as mass spectrometry.

3. New scientific results

3.1. Synthesis of FR258900 analogues

Since the synthesis of 2,3-dihydroxy-pentanedioic acid (**49**) is unknown in the literature, our synthesis was based on the synthesis of chicoric acid (**65**).

Chicoric acid (**65**) is a biscoumaroylated tartaric acid derivative with several known synthetic procedures. Synthesis of **65** was replayed from **59** by using the benzylic protection of both functionalities (**79**), but in contrast with Lamidey's procedure, the removal of benzylic protective groups with equimolar amount of $Pd(OAc)_2$ was unsuccessful (Figure 4.).

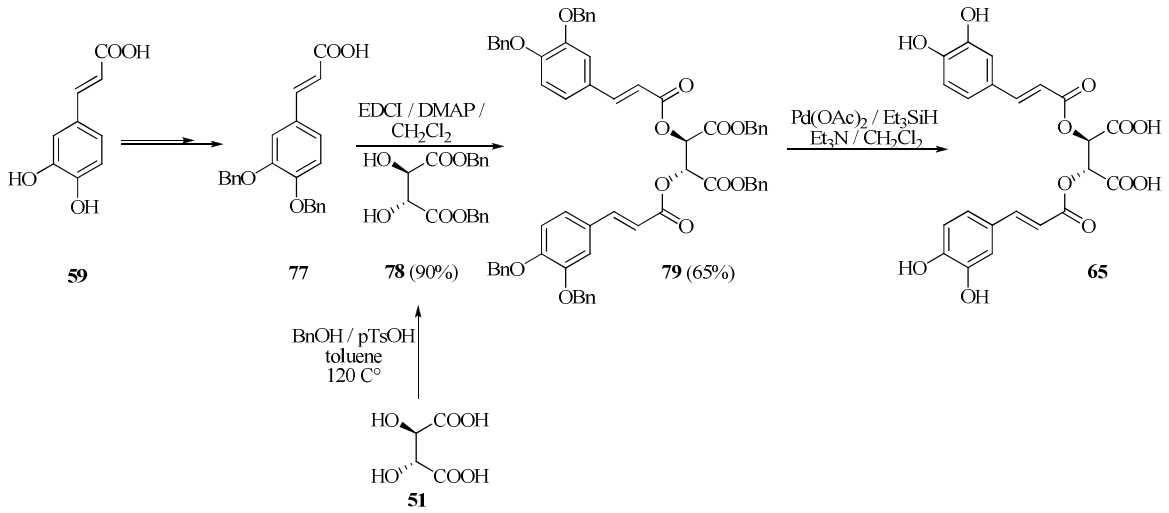
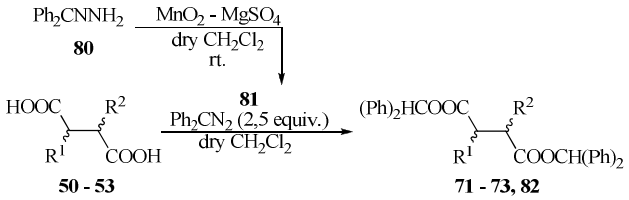


Figure 4. Synthesis of chichoric acid using benzylic protection of both functionalities

In continuation of our project we used the King's method, where COOH groups of L-, D- and *meso*-tartaric acids (**50 - 52**) and L-malic (**53**) acid were protected as diphenylmethyl- (DPM) and the phenolic OH group as methoxycarbonyl esters.

3.1.2. Synthesis of L-, D- and *meso*-tartaric acids and L-malic acid analogues

L-, D-, *meso*-tartaric acids (**50-52**) and L-malic (**53**) acid were reacted with diphenyldiazomethane (**81**) (generated *in situ* from benzophenonehydrazone (**80**) by oxidation with activated MnO_2 in CH_2Cl_2) and the desired DPM esters **71 – 73**, **82** were isolated in excellent yields (82 - 86 %) as white crystals and used further without any purification (Figure 5.).

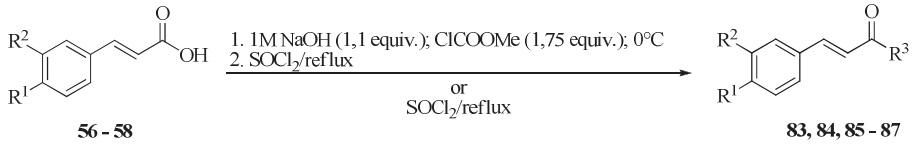


Starting compound	Product	Configuration	R^1	R^2	Yield (%)
50	**71**	(2*S*,3*S*); D	OH	OH	85
51	**72**	(2*R*,3*R*); L	OH	OH	83
52	**73**	(2*R*,3*S*); *meso*	OH	OH	82
53	**82**	(2*R*); L	OH	-	86

Figure 5. Preparation of DPM esters **71 - 73**, **82**

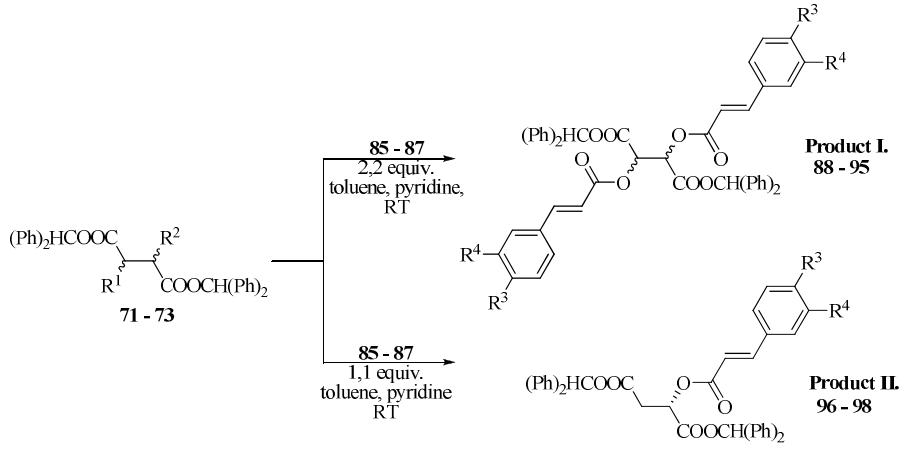
Next, acid-chlorides **85 - 87** were prepared from commercially available cinnamic (**56**), *p*-coumaric (**57**), and ferulic acids (**58**), respectively (Figure 6.), whereby phenolic OH groups of **57** and **58** were protected as methyl-carbonates **83** and **84**, respectively. Thionyl-chloride treatment of carboxylic acids **56**, **83**, **84** gave acid-chlorides **85 - 87** which were used for acylations without further purification.

Acylations of **71 -73**, **82** were carried out in dry toluene using 2.2 equiv. (1.1 equiv./OH) of acid-chlorides **85 - 87** and 2.2 equiv. (1.1 equiv. /OH) dry pyridine as base. The fully protected **88 - 98** derivatives were isolated by column chromatography in acceptable yields (Figure 7.).



	R^1	R^2	R^3	Yield
56	H	H		
57	H	OH		
58	OCH_3	OH		
83	H	$OCOOCH_3$	OH	84
84	OCH_3	$OCOOCH_3$	OH	88
85	H	H	Cl	quantitative
86	H	$OCOOCH_3$	Cl	quantitative
87	OCH_3	$OCOOCH_3$	Cl	quantitative

Figure 6. Preparation of acid chlorides **85 - 87**.



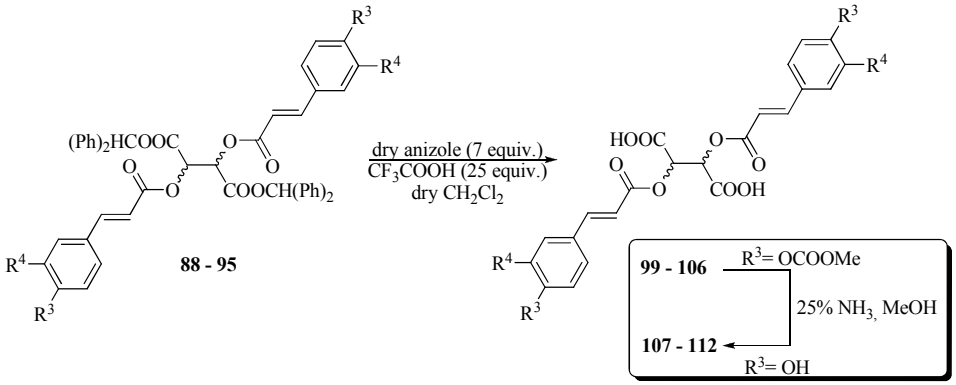
R^3	R^4	Starting compound R^1=R^2=OH		Product I.	Yield (%)	Starting compound R^1=OH;R^2=H	Product II.	Yield (%)
H	H	**71**	D	**88**	63	**82**	**96**	79
H	H	**72**	L	**89**	52			
OCOOMe	H	**71**	D	**90**	54	**82**	**97**	66
OCOOMe	H	**72**	L	**91**	48			
OCOOMe	H	**73**	*mezo*	**92**	83			
OCOOMe	OMe	**71**	D	**93**	48	**82**	**98**	66
OCOOMe	OMe	**72**	L	**94**	50			
OCOOMe	OMe	**73**	*mezo*	**95**	43			

Figure 7. Acylations of D-, L-, *meso* tartaric acids (**71 - 73**) and L-malic acid (**82**) with acid chlorides **85 - 87**.

Subsequent deprotections following the suggested protocol (removal of methoxycarbonyl groups with Na_2CO_3/aq. THF and cleavage of DPM esters with 70% aq. AcOH) caused in our hands total decomposition of the molecules, irrespective of the order of the deprotection steps.

Therefore a new protocol for the cleavage of protecting groups in **88 - 98** was developed. The DPM esters could be cleaved by using dry anisole–TFA reagent in dry CH_2Cl_2 at room temperature.

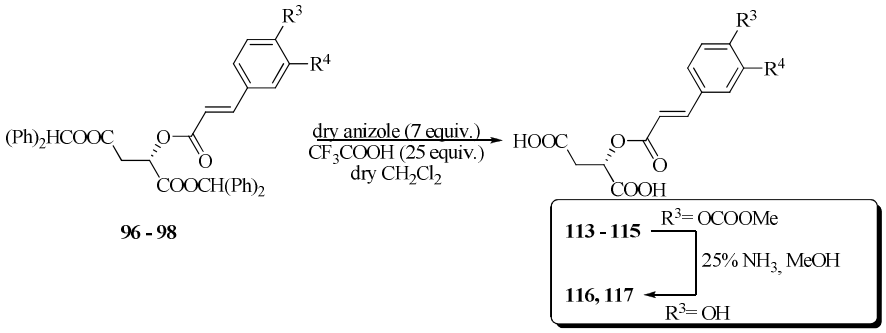
Purification of the crude products by column chromatography gave **99 - 106**, **113 - 115** in good to excellent yields (85–92 %) (Figure 8. and 9.).



Starting compound	Product	R^3	R^4	Yield (%)	Product	R^3	R^4	Yield (%)
88	**99**	H	H	86				
89	**100**	H	H	83				
90	**101**	OCO_2Me	H	82	**107**	OH	H	89
91	**102**	OCO_2Me	H	88	**108**	OH	H	85
92	**103**	OCO_2Me	H	82	**109**	OH	H	30
93	**104**	OCO_2Me	OMe	79	**110**	OH	OMe	81
94	**105**	OCO_2Me	OMe	85	**111**	OH	OMe	69
95	**106**	OCO_2Me	OMe	92	**112**	OH	OMe	88

Figure 8. Cleavage of the protective groups from tartartic acids derivatives

Hydrolysis of the methoxycarbonyl esters was achieved by using an aq. solution of NH_3 in MeOH and the products **107 - 112, 116, 117** were isolated in good to excellent yields (Figure 8. and 9.) Reversing of the sequence resulted in decomposition of the molecules in the first step.



Starting compound	Product	R^3	R^4	Yield (%)	Product	R^3	R^4	Yield (%)
96	**113**	H	H	85				
97	**114**	OCO_2Me	H	90	**116**	OH	H	85
98	**115**	OCO_2Me	OMe	87	**117**	OH	OMe	88

Figure 9. Cleavage of the protective groups from malic acid derivatives

3.2.1. Synthesis of *N*-(β-D-glucopyranosyl)-heteroarene-carboxamides by the formation of amides

Starting from commercially available carboxylic acid (**166**, **167**) and *N*-(β-D-glucopyranosyl)-azide (**165**) the necessary **163** amide was prepared under the Staudinger conditions ($PMe_3/CH_2Cl_2/RCOOCl$)(Figure 10. III., IV.) in moderate yield. In the case of benzimidazol-2-carboxylic acid (**164**) using same conditions resulted in a complex, unseparable reaction mixture, and the acylation of **162** glycopyranosyl-amine was unsuccessful (Figure 10. I., II.).

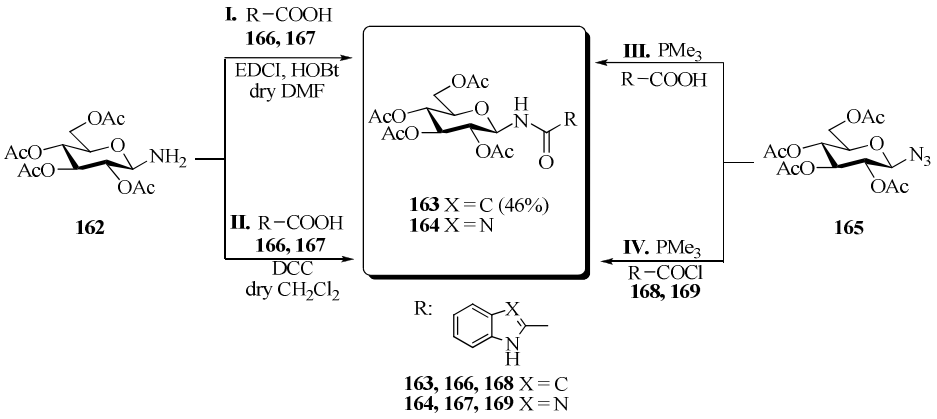


Figure 10. Synthesis of *N*-(β-D-glucopyranosyl)-amides

O-Deacetylation of **163** was performed by the Zemplén protocol to give the unprotected indole derivative (**170**) in good yields (Figure 11.).

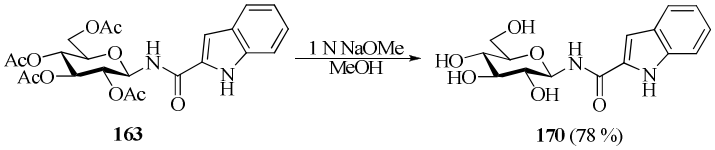


Figure 11. *O*-Deacetylation reation by Zemplén method

3.2.2. Synthesis of *N*-(β-D-glucopyranosyl)-heteroarene-carboxamides by the formation of the heterocycle

The *N*-cyanocarbonyl (**171**) derivative was converted to tetrazole (**172**) with Me_3SiN_3–Bu_2SnO in good yield (88%), which was used as the starting material for the preparation of *N*-(β-D-glucopyranosyl)-5-aryl-1,3,4-oxadiazole-2-carboxamides and *N*-(β-D-glucopyranosyl)-5-aryl-1,2,4-triazole-3-carboxamides (Figure 12.).

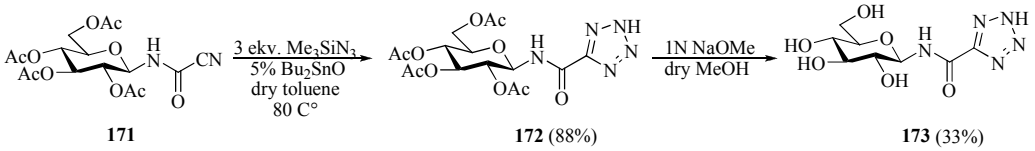


Figure 12. Synthesis of tetrazole (**172**)

The tetrazole **172** was reacted with *N*-benzyl-carboximidoyl chlorides (**179**, **180**), obtained from the corresponding *N*-benzyl-carboxamides **177**, **178** by $SOCl_2$, to provide *N*-(β-D-glucopyranosyl)-5-aryl-1,2,4-triazole-3-carboxamide (**181**, **182**) (Figure 13.).

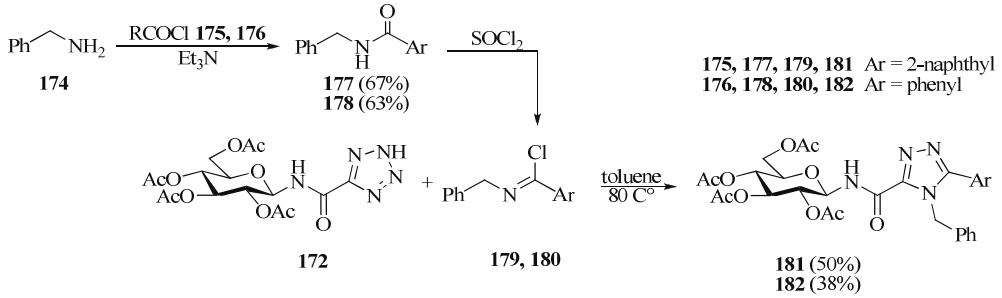


Figure 13. Synthesis of *N*-β-D-glucopyranosyl-5-aryl-1,2,4-triazole-3-carboxamides

Deprotection of these derivatives was readily carried out by the well known methods. Benzyl groups were removed by catalytic hydrogenation (**183, 184**), followed by deacetylation under Zemplén conditions to give the target 1,2,4-triazoles (**186**, **187**) in moderate yields (Figure 14.).

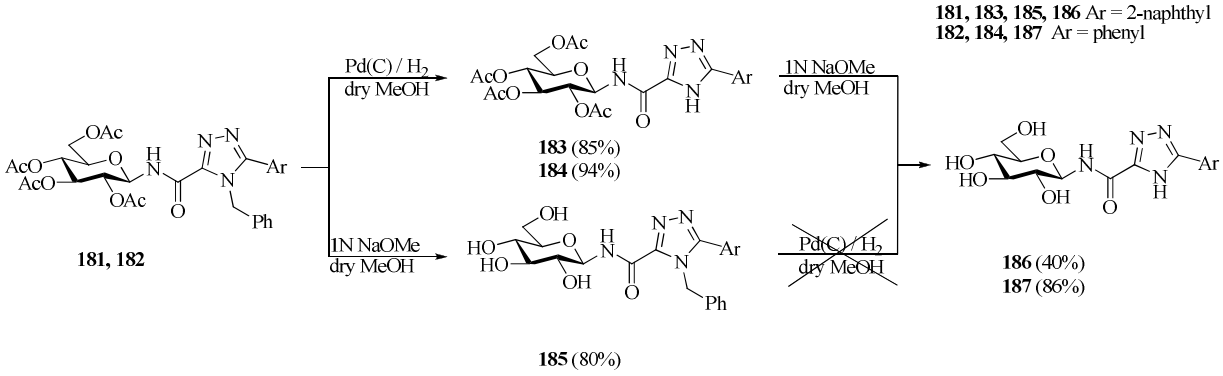


Figure 14. Cleavage of *O* and *N* protecting groups

The desired 5-aryl-1,3,4-oxadiazole-2-carboxamides (**188**, **189**) were obtained from the reaction of tetrazole **172** with the corresponding aroyl-chlorides in dry toluene at elevated temperature in moderate to good yields (**188:** 74%; **189:** 80%). *O*-Deacetylations were performed by the Zemplén protocol to give the unprotected 1,3,4-oxadiazole derivatives **190**, **191** in good yields (Figure 15.).

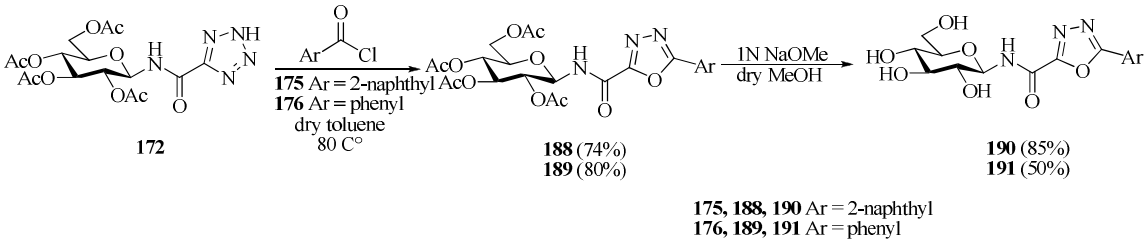


Figure 15. Synthesis of 1,3,4-oxadiazoles

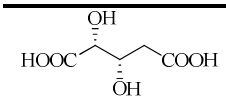
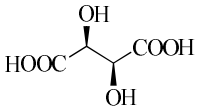
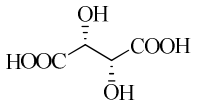
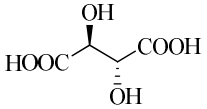
3.3 Enzyme kinetic studies

The synthesized derivatives were evaluated as inhibitors of rabbit muscle glycogen phosphorylase b (RMGPb) at the Department of Medical Chemistry of University of Debrecen.

3.3.1. GP inhibitor activity of FR258900 analogues

The synthesized derivatives were evaluated as inhibitors of rabbit muscle glycogen phosphorylase b (RMGPb), and the results are summarized in Table 1. For comparison FR258900 (**49**) was also tested under our conditions. Compound **49** proved a competitive inhibitor against AMP and the obtained K_i of 0.2 μM showed a good agreement with the literature value. When tested against G1P, **49** appeared as a non-competitive inhibitor with a K_i of 5.47 μM. Similar conclusions could be drawn from the kinetic studies of compounds **99, 100, 107 - 112**, as well, thereby indicating that in general the tartaric acid derivatives bound to the same site as FR258900 (**49**) (Table 1.).

Table 1. GP inhibitor activity of FR258900 analogues

Dikarbonsav	Conf.	$R^1 = R^2 = H$			R^1 = OH, R^2 = H			R^1 = OH, R^2 = OMe		
			G1P	AMP		G1P	AMP		G1P	AMP
	2R,3S (L)[a]	-	-	-	**49**	5.47	0.2 0.46	-	-	-
	2S,3S	**99**	800[b]	-	**107**	109	26.4	**110**	29.1	19.0
	2R,3R	**100**	NI[c]	-	**108**	300[b]	-	**111**	28.5	2.68
	2S,3R	-	-	-	**109**	71.4	5.68	**112**	3.36	2.0

[a]FR258900 configuration. [b]$K_i = IC_{50}/(1 + [S]/Km)$ calculated from IC_{50} values by the Cheng-Prusoff equation. [c] no inhibition

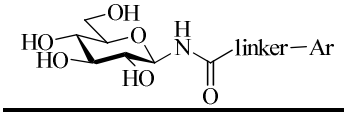
As non-competitive inhibitors against G1P, the cinnamoyl derivatives **99, 100**, lacking the 4-OH substituents characteristic of the natural product, proved practically inefficient. In the *p*-coumaroyl (**107 - 109**) and feruloyl (**110 - 112**) series the *meso*-configured compounds **109** and **112** proved most efficient. The latter demonstrated that introduction of an additional substituent in the aromatic rings (3-CH_3O) was very advantageous and **112** proved equipotent with **49**. As competitive inhibitors against AMP, beside the *meso*-configured **109** and **112**, the L-configured **110** proved most efficient (Table 1.).

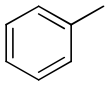
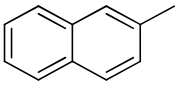
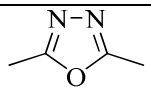
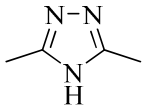
3.3.2. GP inhibitor activity of *N*-(β-D-glucopyranosyl)-heteroarene-carboxamides

The new glucose analogue inhibitors of GP were synthesized by the bioisosteric replacements of the "third" NHCO moieties of *N*-acyl-*N*'-(β-D-glucopyranosyl)-urea type GP inhibitors with 1,3,4-oxadiazole and 1,2,4-triazole heterocycles to study their effects on the inhibitor activity. The 1,3,4-oxadiazolecarboxamide and 1,3,4-triazolecarboxamide derivatives were less active than the "lead" compounds. While in the series of 1,3,4-oxadiazoles the 2-naphtyl derivative (**190**) (K_i = 30 μM), in the case of 1,2,4-triazole compounds the phenyl derivative (**187**) were the best (K_i = 1.0 μM) (Table 2.).

N-(β-D-glucopyranosyl)-indole-2-carboxamide inhibitor activity K_i =5.76 μM is one of the best in the series of N-(β-D-glucopyranosyl)-amide type GP inhibitors.

Table 2. GP inhibitor activity of *N*-(β-D-glucopyranosyl)-heteroarene-carboxamide derivatives



Linker				
NHCO	**20**	4.6	**26**	0.35
	**191**	545	**190**	30
	**187**	1	**186**	9.26

4. Scientific publications

List and data of scientific articles

1. **G. Varga**, T. Docsa, P. Gergely, L. Juhász, L. Somsák: Synthesis of tartaric acid analogues of FR258900 and their evaluation as glycogen phosphorylase inhibitors. *Bioorganic and Medicinal Chemistry Letters*, **23**(6), 1789 – 1792 (2013); IF: 2,554

2. M. Polyák, **G. Varga**, B. Szilágyi, L. Juhász, T. Docsa, P. Gergely, J. Begum, J. M. Hayes, L. Somsák: Synthesis, enzyme kinetics and computational evaluation of N-(β-D-glucopyranosyl) oxadiazolecarboxamides as glycogen phosphorylase inhibitors; *Bioorganic and Medicinal Chemistry*, **28**(18), 5738-5747 (2013); IF: 2,921

3. L. Juhász, **G. Varga,** A. Sztankovics, F. Béke, T. Docsa, P. Gergely, J. Kóňa, I. Tvaroška, L. Somsák: Structure-activity relationships of glycogen phosphorylase inhibitor FR258900 and its analogues: a combined synthetic, enzyme kinetic and computational study (*Bioorganic and Medicinal Chemistry*, beküldve)

Other publications

1. L. Somsák, É.,Bokor, M. Tóth, L. Juhász, K. Czifrák, B. Kónya, S. Kun, A. Páhi, B. Szőcs, **G. Varga**, L. Kóder, K. Nagy, P. Gergely, T. Docsa : Glikogén foszforiláz inhibitorok. P1100602/P1200475 Hungarian patent application. 2011.PCT/HU2012/000116 International patent application

List of conference presentations

Oral presentations

1. Juhász L., **Varga G.** Czakó Z.; Synthesis and study of biologically active O-heterocyclic compounds; *3rd. German – Hungarian Workshop* – Paderborn, (2008)
2. **Varga G.,** Juhász L.; Benzodioxán vázas troglitazon analógok szintézise; *MTA Heterociklusos Kémiai Munkabizottság Előadó Ülése*; Balatonszemes (2008)
3. **Varga G.,** Juhász L., Somsák L.; Borkősav származékok, mint potenciális glikogén foszforiláz inhibitorok előállítása; *MTA Heterociklusos Kémiai Munkabizottság Előadó Ülése*; Balatonszemes (2010)
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