1. Introduction

Recognition of the essential roles of carbohydrates in various biological events has brought about an enormous development in synthetic carbohydrate chemistry. To get better insights into the action of carbohydrate derivatives (glycolipids and glycoproteins) in living organisms the molecules of natural origin as well as their counterparts with similar biological effects and/or chemical structure (the so-called mimetics) need to be prepared in large amounts by chemical synthesis.

Glucose has a central role in the energy supply of the body. Blood glucose levels are controlled by the continuous interaction of two metabolic pathways: the glucose and the glycogen metabolism. Insufficient operation of this complex system – regulated by enzymes and hormones – results in altered, usually chronically elevated blood sugar levels. This syndrome is *diabetes mellitus*, a serious disease becoming one of the main contributors to worldwide mortality through its long term complications. The end of the 20th century has seen a dramatic increase in the number of patients diagnosed with diabetes worldwide. Efficient causes and biological/biochemical backgrounds of diabetes formation are not known. Since all of its symptomes and complications originate from the altered, elevated blood glucose levels current treatments aim to maintain a constant, approximately normal blood glucose level.

The blood glucose levels may be adjustable by affecting of the enzymes of glycogen metabolism. One of the most important enzymes is glycogen phosphorylase which performes the degradation of glycogen. If the activity of these enzymes can be modified effectively with selective inhibitors a new possibility may open up in therapeutic treatment of *diabetes mellitus*.

The first goal of our work was to investigate the mechanism of reactions between *C*-(1-bromo-1-deoxy-D-glycopyranosyl)formamides and (thio)cyanate. Deprotected products of these reactions (e.g. glucopyranosylidene-spiro-(thio)hydantoins **1** and **2**) are among the most effective inhibitors of glycogen phosphorylases.

Another goal was to synthesize different glycomimetics (glycosyl amides, imides, carbodiimides and cyanamides) *via* transformation of glycosyl iminophosphoranes.

2. Methods

The macro- and micro methods of modern preparative organic chemistry were used in the synthetic work. Thin layer chromatography was applied to follow the reactions, and to control the purity of compounds. Column chromatography and crystallization were used for the purification of the products.

Melting points, elemental analyses, optical rotation measurements, mass-spectrometric and NMR spectroscopic methods were applied for structural elucidation, identification and characterization of the prepared substances.

Complete assignments of ^{1}H-, ^{13}C- and ^{15}N-spectra were achieved by the combined analysis of various 1D and 2D measurements such as ^{1}H-^{1}H COSY, TOCSY, ^{13}C-^{1}H HSQC, ^{13}C-^{1}H HMBC, ^{15}N-^{1}H HSQC and ^{15}N-^{1}H HMBC.

3. New scientific results

3.1. Mechanistic studies on the formation of glycopyranosylidene-spiro-(thio)hydantoins

We have investigated the ring closure reaction of *C*-(1-bromo-1-deoxy-D-glycosyl)formamides **35-38**[*] with (thio)cyanate. The main peculiarities of these reactions were as follows: *1)* The stereoselectivity of the ring closing step at the anomeric centre depended on the nucleophile: while with cyanate the highly selective formation of hydantoins **39-42** with retained "anomeric" configuration was observed, the ring closure with thiocyanate gave the inverted thiohydantoins **46-49** exclusively. *2)* The formation of *C*-(1-hydroxy-D-glycosyl)formamides **50-53** was unavoidable in each of these transformations.

The following experiments were accomplished in order to understand the mechanism of these reactions:

1. We first investigated the use of supported silver ions in the hope that back side attack with respect to the bromide and thereby inversion at the anomeric carbon can be forced in this way. Contrary to our expectations, in the reaction of 1-bromo-amide derivative **35** with potassium cyanate in the presence of supported silver ions the retained product **39**

[*] Numbering of compounds refers to that used in the dissertation.

remained the major product in the reaction mixtures and 1-hydroxy-amide derivative **50** was also observed.

2. Next, in order to push the reaction into an S_N2 fashion, we tried to enhance the nucleophilicity of cyanate by using the tetrabutylammonium salt in nitromethane, acetone, or acetonitrile, however also these reactions gave **39** and **50** in an unchanged ratio. No reaction took place with potassium cyanate in benzene in the presence of 18-crown-6.
3. Then reactions of **35** were performed under modified conditions (inert atmosphere, addition of radical- and radical anion traps) in order to test the possible involvement of radical intermediates.

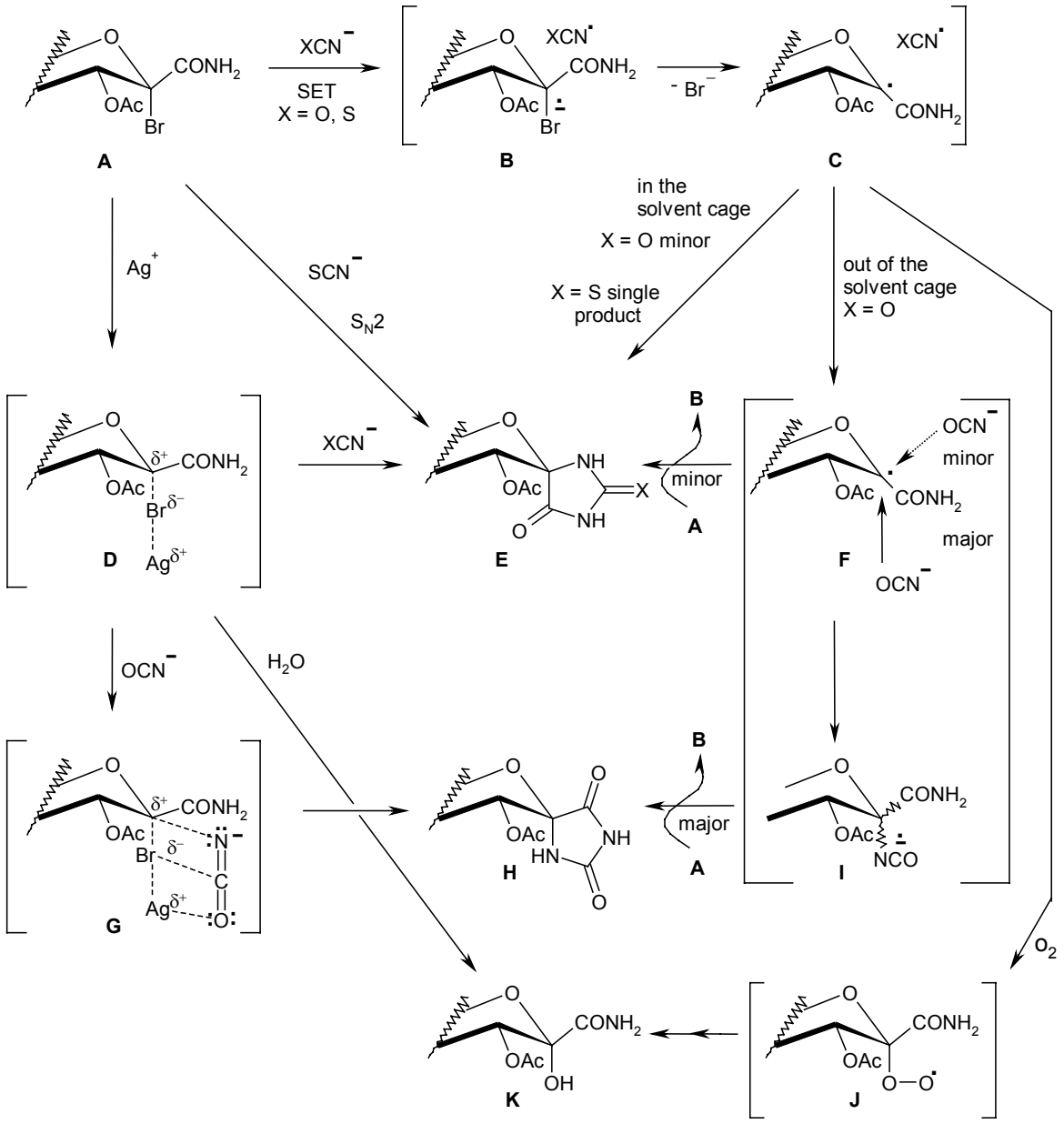
Our observations can be summarized as follows: *a,* the retained hydantoins **39-42** are formed mostly through radicals, whereas in the presence of silver ion a subordinate ionic pathway may exist *b,* the inverted thiohydantoins **46-49** are formed in a non-radical manner, however, the by-product arises *via* radicals even in these reactions; *c,* hydroxy-amide (**50-53**) formation involves radicals; *d,* the formation of hydroxy-amides in each reaction is mainly due to the presence of air oxygen, even in those cases where silver ion is present facilitating the reaction of 1-bromo-amides **35-38** with traces of water.

Based on our observations the following mechanism was proposed (Scheme 1):

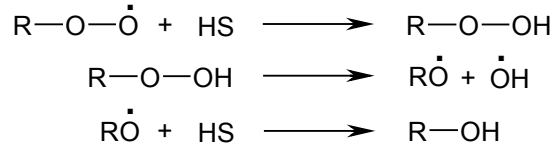
The most probable general reaction which may proceed independent of solvent and cation can be started by a single-electron-transfer (SET) from (thio)cyanate to bromo-amide **A** facilitated by the presence of the carboxamido group with low lying LUMO. The formed radical-anion **B** can split off a bromide to give the capto-dative radical **C**, which may combine with SCN radical in the solvent cage and the following ring closure gives inverted thiohydantoin **E** (X=S). This route is indistinguishable from an S_N2 substitution and **E** (X=S) may indeed be formed by the classical S_N2 route (**A**→**E**). If radical **C** escapes from the solvent cage it may react with cyanate ion to give radical anion **I**. Because of well known stereoelectronic reasons axial attack of OCN ion is favoured as shown in **F** (*major*) leading after ring closure to retained hydantoin **H** and inverted hydantoin **E** (X=O) can also be formed in these reactions as a minor product. Reduction of radical anion **I** may proceed by the starting material **A** thereby giving rise to a chain reaction. This is consistent with the finding that significantly lower than stoichiometric amounts of radical- or radical anion traps could completely inhibit the reaction.

Out of the solvent cage radical **C** may also be trapped by triplet oxygen to give the hydroperoxyl radical **J**. This can than abstract a hydrogen from the solvent and undergo known thermal decomposition to hydroxy-amide derivative **K** (Scheme 2).

A useful preparative tool arose from the mechanistic study to improve the yield (79% instead of 57%) of the biologically most efficient gluco-spirothiohydantoin **47** and the yield of side product **51** could be decreased from 19% to 4%.



Scheme 1



Scheme 2.

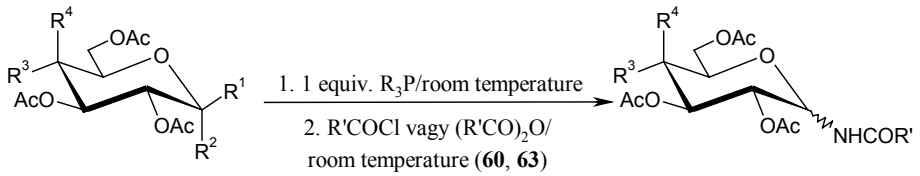
3.2. Synthesis of glycopyranosyl amides by a modified Staudinger reaction starting from glycopyranosyl azides

Starting from peracetylated glycopyranosyl azides (**60**, **63**, **74**, **97**) the expected phosphinimide derivatives were prepared with different trialkyl or triaryl phosphines (PR_3, R=Me, Et, Ph, nBu, NMe_2). Stability of these compounds was studied and it was established that the configuration of the anomeric carbon did not change during 1-2 days. After longer periods these compounds slowly transformed to anomeric mixtures.

The D-glycosyl phosphinimides were readily converted into 1-*N*-acyl-D-gluco- and galactopyranosyl amines (**57**, **81**, **89-96**, **98**, **100-101**) in high yields (50-90%) by using activated acid derivatives (acid chlorides or anhydrides). The reaction mixtures were evaporated to dryness and the final products were isolated by crystallization or column chromatography as white, crystalline substances (Table 1).

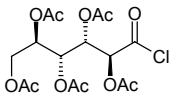
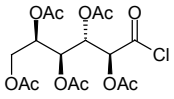
In most cases 1,2-*trans* glycopyranosyl amides were formed independently of the anomeric configuration of the starting materials. 1,2-*Cis* derivatives (**93**, **94**, **100**, **101**) could be synthesized only from trihalogenoacetic anhydrides.

Table 1. *Syntheses of 1-N-Acyl-(2,3,4,6-tetra-O-acetyl-D-glycopyranosyl)amines 1.*



60 $R^1 = N_3$, R^2 = H, R^3 = OAc, R^4 = H
63 $R_1 = N_3$, R^2 = H, R^3 = H, R^4 = OAc
74 R^1 = H, $R^2 = N_3$, R^3 = OAc, R^4 = H
97 R^1 = H, $R^2 = N_3$, R^3 = OAc, R^4 = H

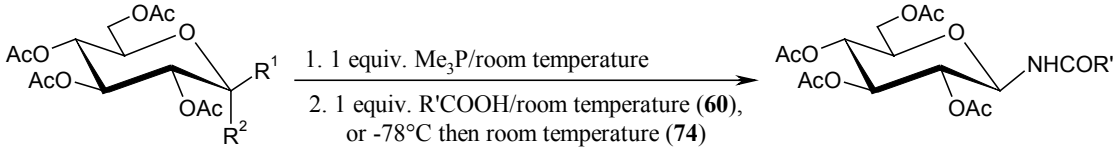
57, **81**, **89**-**96**, **98**, **100**-**101**

Entry	Start. azide	R	Solvent	Reagent (R'COCl or $(R'CO)_2O$)	Reaction time (min)	Product (isolated yield, %)	
						α-amide	β-amide
1.	**60**	Ph	CH_2Cl_2	PhCOCl	120	-	**81** (82)
2.	**74**	Me	CH_2Cl_2	$(PhCO)_2O$	180	-	**81** (74)
3.	**74**	Me	1,4-dioxane	$(PhCO)_2O$	600	-	**81** (87)
4.	**74**	Me_2N	CH_2Cl_2	$(PhCO)_2O$	20 hours	-	**81** (40)
5.	**60**	nBu	CH_2Cl_2	$(CH_3CO)_2O$	15	-	**89** (72)
6.	**74**	nBu	CH_2Cl_2	$(CH_3CO)_2O$	360	-	**89** (72)
7.	**60**	nBu	CH_2Cl_2	$(tBuCO)_2O$	7 days	-	**90** (15)
8.	**74**	nBu	CH_2Cl_2	$(tBuCO)_2O$	3 days	**91** (5)	**90** (21)
9.	**74**	Et	CH_2Cl_2	$(tBuCO)_2O$	3 days	-	**90** (15)
10.	**60**	nBu	CH_2Cl_2	$(CF_3CO)_2O$	15	-	**92** (76)
11.	**74**	nBu	CH_2Cl_2	$(CF_3CO)_2O$	30	**93** (52)	**92** (9)
12.	**74**	Ph	CH_2Cl_2	$(CF_3CO)_2O$	60	**93** (68)	**92** (19)
13.	**74**	Me	CH_2Cl_2	$(CCl_3CO)_2O$	10	**94** (69)	-
14.	**60**	Me	CH_2Cl_2		15	-	**95** (84)
15.	**60**	Me	CH_2Cl_2		15	-	**96** (55)
16.	**63**	Me	CH_2Cl_2	$(PhCO)_2O$	40	-	**97** (57)
17.	**97**	Me	CH_2Cl_2	$(PhCO)_2O$	40	-	**97** (57)
18.	**63**	Me	CH_2Cl_2	$(CH_3CO)_2O$	40	-	**57** (80)
19.	**97**	Me	CH_2Cl_2	$(CH_3CO)_2O$	60	-	**57** (59)
20.	**97**	Me	CH_2Cl_2	$(CF_3CO)_2O$	30	**100** (60)	-
21.	**97**	Me	CH_2Cl_2	$(CCl_3CO)_2O$	40	**101** (66)	-

Reactions of the strongly basic glycosyl trimethyl phosphinimides derived from azides and common aromatic acids or Boc-L-aspartic acid benzyl ester resulted in the corresponding 1,2-*trans* glycosyl amides in 52-85% yields under mild conditions (Table 2). The final products were isolated by crystallization.

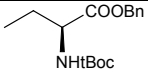
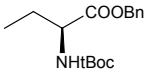
In these reactions the application of activated carboxylic acids, amino acids and complicated and expensive coupling agents is not necessary .

Table 2. *Syntheses of 1-N-Acyl-(2,3,4,6-tetra-O-acetyl-D-glycopyranosyl)amines 2.*



60 $R^1 = N_3$, $R^2 = H$
74 $R^1 = H$, $R^2 = N_3$

73, **81**, **89**, **103**-**106**

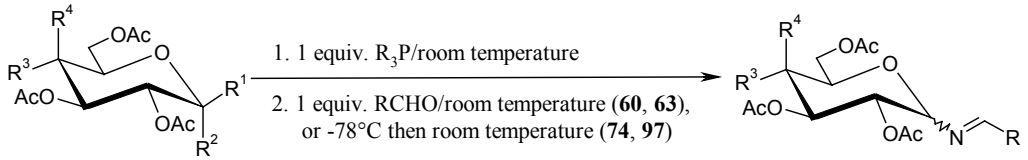
Entry	Start. azide	R'	Reaction time, hour	Product (Isolated yield, %)
1.	**60**		16	**73** (83)
2.	**74**		18	**73** (70)
3.	**60**	Ph	15	**81** (82)
4.	**74**	Ph	48	**81** (91)
5.	**60**	Me	3	**89** (58)
6.	**60**	pMe-C_6H_4	24	**103** (85)
7.	**74**	pMe-C_6H_4	18	**103** (41)
8.	**60**	pNO_2-C_6H_4	16	**104** (57)
9.	**74**	pNO_2-C_6H_4	48	**104** (23)
10.	**60**	pCl-C_6H_4	16	**105** (83)
11.	**74**	pCl-C_6H_4	19	**105** (47)
12.	**60**	Et	3	**106** (52)

3.3. Syntheses of *N*-glycopyranosyl imines (Schiff bases)

D-Glycopyranosyl trimethylphosphinimides prepared from peracetylated glycopyranosyl azides (**60**, **63**, **74**, **97**) and trimethylphosphine were *in situ* reacted with 1 equivalent of aliphatic and aromatic aldehydes to give 1-*N*-alkylidene and 1-*N*-arylidene derivatives, respectively (Table 3). The final products were isolated by crystallization or column chromatography.

In most cases the corresponding 1,2-*trans* Schiff-bases were formed (**109**, **124**, **125**, **127**-**131**) in good yields (70-95%). 1,2-*Cis* glycopyranosyl imines (**126**, **132**) could be synthesized only with aldehydes containing strong electron withdrawing groups (in 50-80% yields).

Table 3. *Syntheses of 1-N-arylidene(alkylidene)-[2,3,4,6-tetra-O-acetyl-D-gluco(galacto)-pyranosyl]imines*



60 $R^1 = N_3$, R^2 = H, R^3 = OAc, R^4 = H
63 $R_1 = N_3$, R^2 = H, R^3 = H, R^4 = OAc
74 R^1 = H, $R^2 = N_3$, R^3 = OAc, R^4 = H
97 R^1 = H, $R^2 = N_3$, R^3 = OAc, R^4 = H

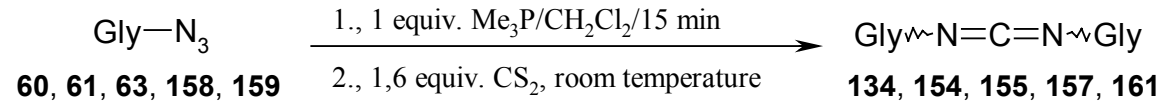
109, **124**-**132**

Entry	Starting material	R	Reaction time, min	Product (isolated yield, %)
1.	60	Ph	10	**109** (81), *trans*
2.	74	Ph	180	**109** (87), *trans*
3.	60	pCl-C_6H_4	10	**124** (73), *trans*
4.	74	pCl-C_6H_4	10	**124** (75), *trans*
5.	60	CBr_3	10	**125** (95), *trans*
6.	74	CBr_3	60	**126** (53), *cis*
7.	63	Ph	10	**127** (97), *trans*
8.	97	Ph	10	**127** (96), *trans*
9.	63	pCl-C_6H_4	10	**128** (90), *trans*
10.	63	1-$C_{10}H_7$	10	**129** (87), *trans*
11.	63	pCN-C_6H_4	5	**130** (90), *trans*
12.	63	CBr_3	5	**131** (69), *trans*
13.	97	CBr_3	60	**132** (80), *cis*

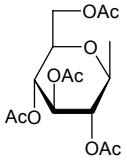
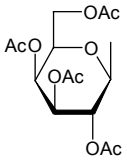
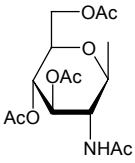
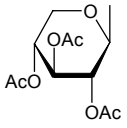
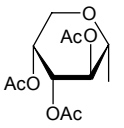
3.3. Synthesis of glycopyranosyl carbodiimides by a modified Staudinger reaction starting from glycopyranosyl azides

Symmetric glycopyranosyl carbodiimides (**134**, **154**, **155**, **157**, **161**) were obtained in reactions of 1,2-*trans* glycopyranosyl trimethyl phosphinimides with carbon disulfide under mild conditions (room temperature, short reaction time). The reaction mixtures were evaporated to dryness and the final products were isolated by crystallization in 65-95% yields (Table 4). Starting form 1,2-*cis* derivatives complex reaction mixtures were formed which could be purified neither by crystallization nor column chromatography.

Table 4. *Syntheses of 1,2 bis D-glycopyranosyl carbodiimides*



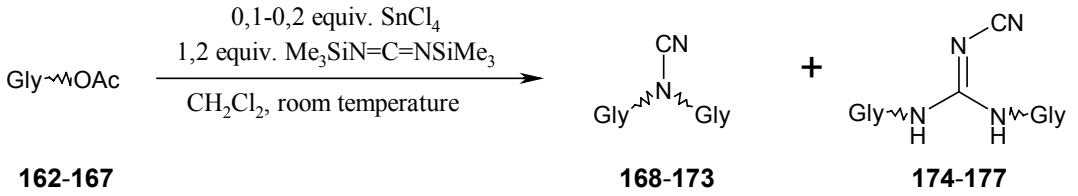
60, **61**, **63**, **158**, **159** → **134**, **154**, **155**, **157**, **161**

Entry	Starting azide		Reaction	Product
	No.	Gly	time	Isolated yield, (%)
1.	**60**		20 min	**134** (80)
2.	**63**		30 min	**154** (95)
3.	**61**		2 hours	**157** (71)
4.	**158**		12 hours	**155** (65)
5.	**159**		12 hours	**161** (76)

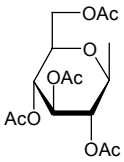
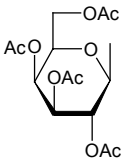
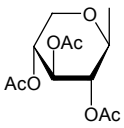
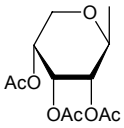
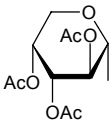
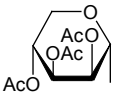
3.4. Syntheses of bis-glycosyl cyanamides and cyanoguanidines

Starting from peracetylated aldoses **162-167** and bis(trimethylsilyl)carbodiimide in the presence of $SnCl_4$ the corresponding *N,N*-bis(glycopyranosyl) cyanamides (**168-173**) and cyanoguanidines (**174-178**) were obtained instead of the expected symmetric carbodiimide derivatives (Table 5).

Table 5 *Syntheses of N,N-bis glycopyranosyl cyanamides and cyanoguanidines*



162-167 **168-173** **174-177**

Entry	Starting azide		Reaction	Products	
	No.	Gly	time	Isolated yield, (%)	
1.	**162**		48 hours	**168** (20)[a]	**174** (26)[a]
2.	**163**		48 hours	**169** (42)	**175** (20)
3.	**164**		12 hours	**170** (43)	**176** (29)
4.	**165**		12 hours	**171** (51)	**177** (11)
5.	**166**		12 hours	**172** (53)	**178** (18)[b]
6.	**167**		12 hours	**173** (58)	-

a: 90% conversion

b: *N*-(2,3,4-tri-O-acetyl-α-D-arabinopyranosyl) cyanamide was isolated as side product

Further experiments showed that the pure symmetrical glycosyl carbodiimides were transformed into the corresponding cyanamides under the influence of the Lewis acid. To the best of our knowledge, glycosyl cyanamides and cyanoguanidines are not known in the literature.

4. Possible applications of the results

This work is mainly a fundamental research in the field of the carbohydrate chemistry.

Investigation of the formation of glycopyranosylidene-spiro-(thio)hydantoins is important for the scaled-up syntheses of these compounds.

A novel method was elaborated for the preparation of glycosyl amides which can be applied successfully for the syntheses of various *N*-glycopeptides.

Sugar imines and carbodiimides, as chiral auxiliaries, are key intermediates in the syntheses of tetrahydropyridine, β-lactam and amino acid analogues and various glycoconjugates (trehazolin-type glycosidase inhibitors, glucocinnamoyl spermidine antibiotics).

5. List of publications

Papers related to the subject of the dissertation

1. László Somsák; **László Kovács**; Marietta Tóth; Erzsébet Ősz; László Szilágyi; Zoltán Györgydeák; Zoltán Dinya; Tibor Docsa; Béla Tóth; Pál Gergely:
   Synthesis of and a comparative study on the inhibition of muscle and liver glycogen phosphorylases by epimeric pairs of D-gluco- and D-xylopyranosylidene-spiro-(thio)hydantoins and N-(D-glucopyranosyl) amides.
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2. **László Kovács**; Erzsébet Ősz; Valéria Domokos; Wolfgang Holzer; Zoltán Györgydeák:
   An easy access to anomeric glycosyl amides and imines (Schiff bases) via transformation of glycopyranosyl trimethylphosphinimides.
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3. **László Kovács**; Erzsébet Ősz; Zoltán Györgydeák:
   Convenient syntheses of symmetrical and unsymmetrical glycosyl carbodiimides and N,N-bis(glycosyl)cyanamides.
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1. László Somsák; **László Kovács**; Viktor Gyóllai; Erzsébet Ősz:
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1. **László Kovács**, László Somsák:
   Mechanistic studies on the formation of glycosylidene-spiro-hidantoins
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   MKE, Vegyészkonferencia, Eger, 1999. június 22-24. (P)
5. **Kovács László**, Gyóllai Viktor, Somsák László:
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8. Tibor Docsa, Béla Tóth, Pál Gergely, Erzsébet Ősz, **László Kovács**, Marietta Tóth, László Somsák and László Szilágyi:
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11. **László Kovács**, Erzsébet Ősz, Valéria Domokos, Wolfgang Holzer, Zoltán Györgydeák:
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16. **László Kovács**, Katalin Czifrák, Marietta Tóth, László Somsák:
    Anomeric α-Amino Acid Derivatives as Glycogen Phosphorylase Inhibitors
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