



Preparation of oligopeptides incorporating anomeric α -amino acids

Ph.D. Theses

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1. Introduction

The glycopeptides, which consist of a carbohydrate and a protein part, have various biological roles. In these glycoconjugates the carbohydrate part contributes to the determination of the biologically relevant conformation of the protein. In the natural derivatives the carbohydrate is attached by a hydrolytically less stable *O*- or *N*-glycosidic bond to the corresponding amino acid of the protein. Mimetics, which have analogous structure and/or effects with the glycoproteins were prepared for investigation and modification of their function in the living organism. In these mimetics the C-O and C-N connections were replaced by stable C-C bonds. The anomeric α -amino acids are special C-glycosides, in which the asymmetric centre of the amino acid is the anomeric carbon of the carbohydrate.

Two different methods are known for the preparation of anomeric α -amino acid containing oligopeptides. One of these is the acylation of the amino group in 2-amino-2-deoxy-ald-2-ulonic acid derivatives with various amino acids, which always leads to pairs of anomers. In the other procedure a ring opening of bicyclic compounds forms only one anomer of the expected peptides.

In the present thesis I report on the synthesis of anomeric α -amino acid containing oligopeptides. The aim of our work was to develop new selective synthetic routes for the incorporation of both epimers of an anomeric α -amino acid into peptides.

2. Applied methods

The macro-, semimicro- and micro methods of modern preparative organic chemistry were applied in the synthetic work. Reactions were monitored by thin layer chromatography, the isolation and purification of the crude products were carried out by crystallization or by column chromatography.

Elemental analyses, melting point, and optical rotation measurements, NMR spectroscopy, and mass spectrometry were applied for the identification and characterization of the compounds prepared.

3. Results

3.1. Preparation of 2,6-anhydro-aldonic acid esters, chlorides and *N*-(per-*O*-acyl-2,6-anhydro-aldonoyl)glycine methylesters

For protection and activation of the carboxyl group – considering the following peptide synthesis – esters and acid chlorides were synthesised by literature methods. *Tert*.-Butyl- **136**, **140** , 2,2,2-trichloroethyl- **138**, **142**, **146** , and active esters **137**, **141**, **145**, as well as acid chlorides **139**, **143** of *D-galacto* and *D-gluco* configuration were isolated by column chromatography in good yield. Acylated amino acid methylesters **147**, **147a**, **149**, **150** were obtained from active esters **137**, **141**, **145** by coupling with glycine and alanine methylesters (Table 1).

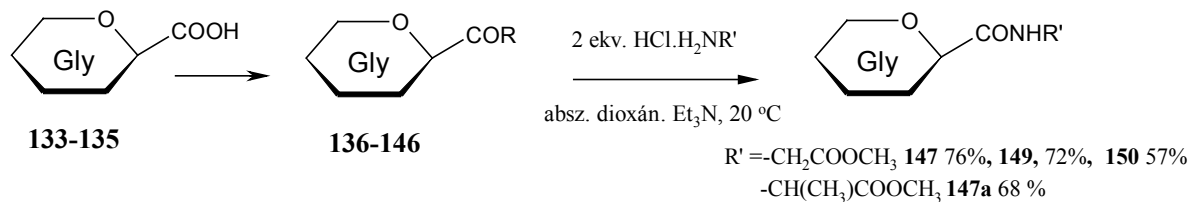
3.2. „Green” bromination conditions

For the bromination of 2,6-anhydro-aldonic acid derivatives CCl₄ was usually used as solvent (conditions **a**: Br₂, abs. CCl₄, h v, reflux and **b**: NBS, AIBN, abs. CCl₄, reflux). Since CCl₄ is carcinogen its use must be avoided. In those transformations, where CCl₄ was used it must be replaced by other solvents, which have similar properties and are less harmful to our health. Therefore, methods **d-f** (**d**: Na₂S₂O₄-KBrO₃, CH₂Cl₂-H₂O, 20 °C; **e**: Na₂S₂O₄-KBrO₃, CH₂Cl₂-H₂O, 20 °C; **f**: Br₂, PhCF₃, hv, K₂CO₃, reflux) were investigated for the bromination of known carbohydrate derivatives, and compared with literature observations made by methods **a** and **b**. From these results it was concluded that brominated products of carbohydrates with a capto-datively substituted carbon formed in excellent yield.

3.3. Preparation of 2-bromo-2-deoxy-ald-2-ulopyranosonic acid derivatives

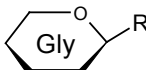
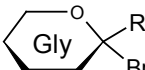
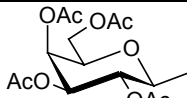
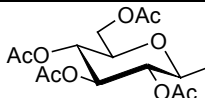
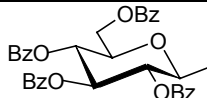
Bromination reactions of 2,6-anhydro-aldonic acid derivatives were investigated by methods **a**, **b** and **d** (Table 2). The bromination of acid **133** failed with these methods. Brominated active esters **153**, **173**, **174** were obtained in pure state in good yield. Brominated *tert*-butyl- **168**, **169** and trichloroethylesters **170**, **171**, **172** were not isolated in pure state, the crude products were immediately transformed into the corresponding azide. The bromination of acid chloride **139** was carried out under modified Hell-Volhard-Zelinsky conditions (**b**), and led to brominated acid chloride **176**, which wasn't isolated because of it's low stability.

Table 1: 2,6-anhydro-aldonic acid esters, acid chlorides and *N*-(per-*O*-acyl-2,6-anhydro-aldonoyl) amino acid methylesters



Gly	Reaction conditions	R	Yield %
 (133) (147, 147a)	t-BuOAc, cat. 60% HClO ₄ , 20 °C	-OtBu (136)	77
	3 eq. C ₆ Cl ₅ OH, 1 eq. DCC, abs. CH ₂ Cl ₂ , 20 °C	-OC ₆ Cl ₅ (137)	75
	3 eq. CCl ₃ CH ₂ OH, 1 eq. DCC, abs. CH ₂ Cl ₂ , 20 °C	-OCH ₂ CCl ₃ (138)	78
	1.1 eq. PCl ₅ , abs. Et ₂ O, reflux	-Cl (139)	75
 (134) (149)	t-BuOAc, cat. 60% HClO ₄ , 20 °C	-OtBu (140)	73
	3 eq. C ₆ Cl ₅ OH, 1 eq. DCC, abs. CH ₂ Cl ₂ , 20 °C	-OC ₆ Cl ₅ (141)	76
	3 eq. CCl ₃ CH ₂ OH, 1 eq. DCC, abs. CH ₂ Cl ₂ , 20 °C	-OCH ₂ CCl ₃ (142)	89
	1.1 eq. PCl ₅ , abs. Et ₂ O, reflux	-Cl (143)	75
 (135) (150)	t-BuOAc, cat. 60% HClO ₄ , 20 °C	-OtBu (144)	41
	3 eq. C ₆ Cl ₅ OH, 1 eq. DCC, abs. CH ₂ Cl ₂ , 20 °C	-OC ₆ Cl ₅ (145)	71
	3 eq. CCl ₃ CH ₂ OH, 1 eq. DCC, abs. CH ₂ Cl ₂ , 20 °C	-OCH ₂ CCl ₃ (146)	70

Table 2: Bromination of 2,6-anhydro-aldonic acid derivatives

 133-147	Applied methods a, b and d		 168-176
Gly (Method: Yield, [%])			
R			
-COOH (133)	(a,b,d : decomposition)	Not investigated	
-COOtBu (136,140)	(b :decomposition)	(b :decomposition)	
	168		
	(d :crude reaction mixture)		(b :83) ^b
	(d :85) ^b		169
	(d) ^c	(d) ^d	(d :72)
-COOCH ₂ CCl ₃ (138, 142, 146)	170	171	(d) ^d
-COOC ₆ Cl ₅ (137, 141, 145)	(a :99) 153	(a :99) 173	172
	(d :91)		(a :89) 174
-CONHCH ₂ CO ₂ CH ₃ (147)	(a,b :decomposition)		
	175		
-COCl (139, 143)	(b : not isolated) 176		
	Decomposition		
Applied methods:			
a: Br ₂ , hv, abs. CHCl ₃ , K ₂ CO ₃ , reflux			
b: NBS, AIBN, abs. CCl ₄ , reflux			
d: Na ₂ S ₂ O ₄ -KBrO ₃ (6-6 eq.), CH ₂ Cl ₂ -H ₂ O, 20 °C			

^a The minor hydroxy compound were separated by column chromatography.

^b Crude product.

^c Formed with an unidentified brominated compound, therefore it was not isolated in pure form.

^d Formed with the corresponding hydroxi derivative, which was isolated by column chromatography near the correspondig azide.

The bromination of glycine methylester **147** failed with the above procedures, because of it's two similarly active capto-dative centres (C-1, CH₂). Therefore, glycine and alanine amides **175**, **175a**, **177**, **178** were prepared from brominated active esters **153**, **173**, **174** by substitution with glycine and alanine methylesters (**Figure 1**). The bromine is in an axial position in these derivatives, which was proved by the values of the measured proton-carbon three bond heteronuclear couplings (³J_{H-2,COR} = ~1-2 Hz).

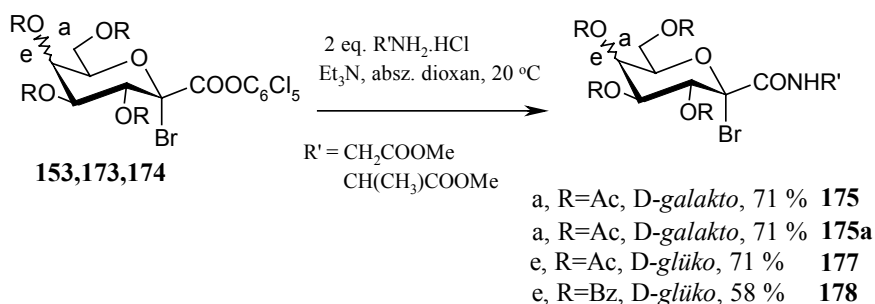
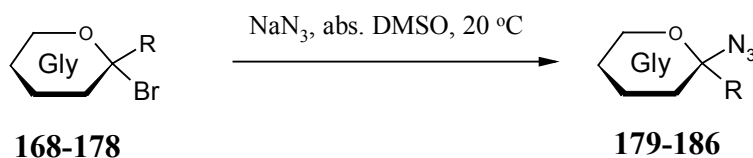


Figure 1

3.4. Preparation of 2-azido-2-deoxy-β-D-ald-2-ulosonic acid derivatives

The azide substitution of 2-bromo-2-deoxy-ald-2-ulopyranosyl acid derivatives was performed with NaN_3 in abs. DMSO or abs. DMF (**Table 3**). Except the active esters, which decomposed under these conditions we obtained the corresponding azides in good yield (**Table 3**). These derivatives contain equatorial azide according to the measured proton-carbon three bond heteronuclear couplings ($^3J_{\text{H-2,COR}}=4\text{-}6\text{ Hz}$).

Table 3: Preparation of 2-azido-2-deoxy-β-D-ald-2-ulosonic acid derivatives



Gly (Method: Yield, [%])			
R			
-COOtBu (168,169)	69(179)		56(180)
-COOCH ₂ CCl ₃ (170,171,172)	59 ^a (181)	60 ^a (182)	59 ^a (183)
-COOC ₆ Cl ₅ (153,173,174)	Decomposition	-	Decomposition
-CONHCH ₂ CO ₂ CH ₃ (175,177,178)	65(184)	69(185)	66(186)

^a The yield refer to for two steps: bromination and azide substitution.

3.5. Preparation of 2-azido-2-deoxy- α -D-ald-2-ulosonic acid derivatives

Brominated acid chloride **176** was transformed into azido ulosonic acid azide **187** under the azide substitution conditions. The axial position of the azide in this compound was proved by the proton-carbon three bond heteronuclear coupling ($^3J_{\text{H-2,CON}_3} = <1$ Hz) and the structure of the azido glycine derivative **199**, which was obtained from azide **187**. The hydrolysis of **187** in the presence of KOH led to azido ulosonic acid **198** (Figure 2).

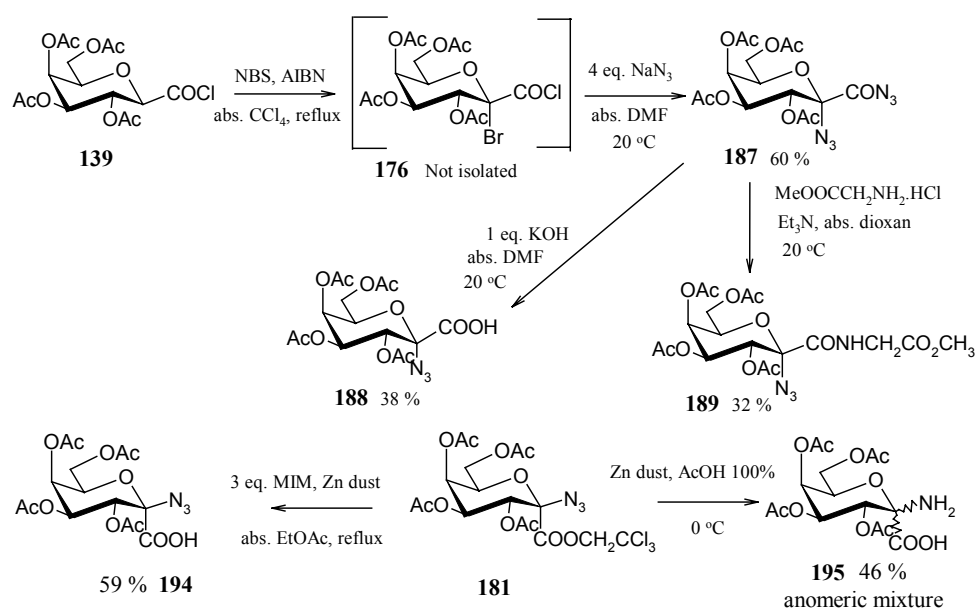


Figure 2

3.6. New method for the selective removal of 2,2,2-trichloroethyl-, and 2,2,2-trichloroethoxycarbonyl protective groups in the presence of acid sensitive and reducible groups

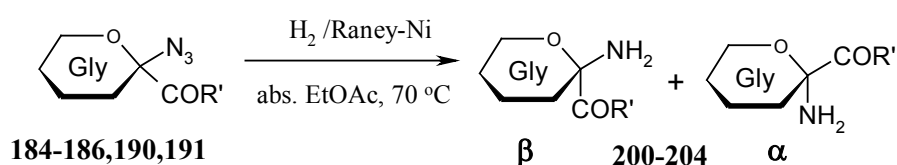
In order to get the anomer of azido ulosonic acid **198**, the cleavage reactions of trichloroethylester **181** were investigated. We obtained the azido ulosonic acid ester **192** from azide **181** by cleavage with Zn dust in abs. ethyl acetate in the presence of 3 eq. *N*-methylimidazole. When the cleavage was carried out with Zn dust in acetic acid the anomeric mixture of amino acid **191** was formed (Figure 2). We extended the new procedure to the cleavage of 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroethoxycarbonylamino- β -D-glucopyranosyl azide, and *N*-trichloroethoxycarbonyl-L-glycine *tert*-butylester. Cleavage of further substituted aromatic trichloroethylesters, –carbamates, and –carbonates was investigated by

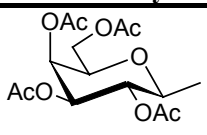
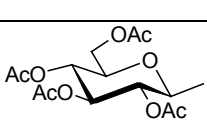
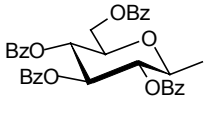
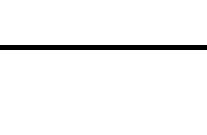
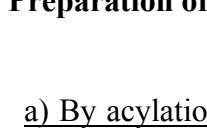
Edit Veres in her diploma thesis. These results demonstrate that 2,2,2-trichloroethyl based protecting groups can be selectively removed in the presence of azide, double bond, nitro-, phenacyl- and *tert.*-butyl groups.

3.7. Preparation of 2-amino-2-deoxy-ald-2-ulopyranosonic acid derivatives, and investigation of the equilibrium of the methyl esters

2-Azido-2-deoxy-ald-2-ulopyranosonic acid derivatives **184-186**, **190**, **191** were hydrogenated in the presence of Raney-Ni (**Table 4**) to give anomeric mixtures of the corresponding amines. The α -amin was the major component in the equilibrated mixtures of amines **200** (α/β 83 : 17) and **203** (α/β 91 : 9).

Table 4: Synthesis of 2-amino-2-deoxy-ald-2-ulopyranosonic acid derivatives



Gly	R'	Yield %
 (190)	-OCH ₃ (200)	84 % anomeric mixture $\alpha/\beta=12:88$ in 50 % the β -amine 200β crystallised from ethanol
 (184)	-NHCH ₂ COOCH ₃ (201)	79 % β -amin 201β
 (185)	-NHCH ₂ COOCH ₃ (202)	80 % anomeric mixture $\alpha/\beta=20:80$
 (191)	-OCH ₃ (203)	97 % anomeric mixture $\alpha/\beta=44:56$ in 89 % the α -amine 203α crystallised from ethanol
 (186)	-NHCH ₂ COOCH ₃ (204)	72 % anomeric mixture $\alpha/\beta=88:12$

3.8. Preparation of 2-acylamino-2-deoxy-ald-2-ulopyranosonic acid derivatives

a) By acylation of glycosylamines: during the acetylation of **200 β** and freshly prepared **203 α** amino methylesters with acetyl chloride in abs. pyridine (**Figure 3**) anomeric mixtures

(**205a β** and **206a β**) were formed in both reactions, which were separated by column chromatography.

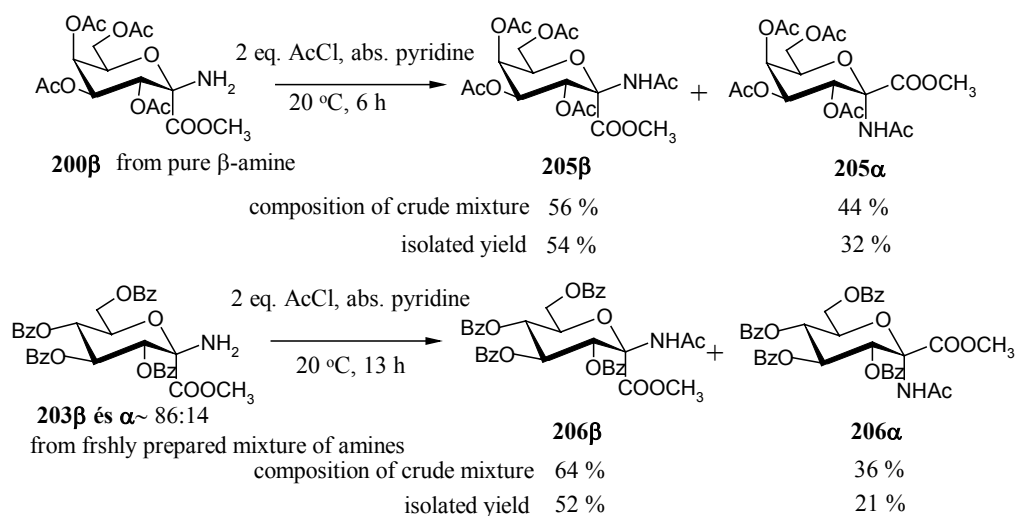


Figure 3

b) By Staudinger-reaction: Azide **191** was reacted with Me_3P , and then with acetyl chloride to give the triazene **207** together with amide anomers **206a α** and **206b β** in various ratios (**Figure 4**).

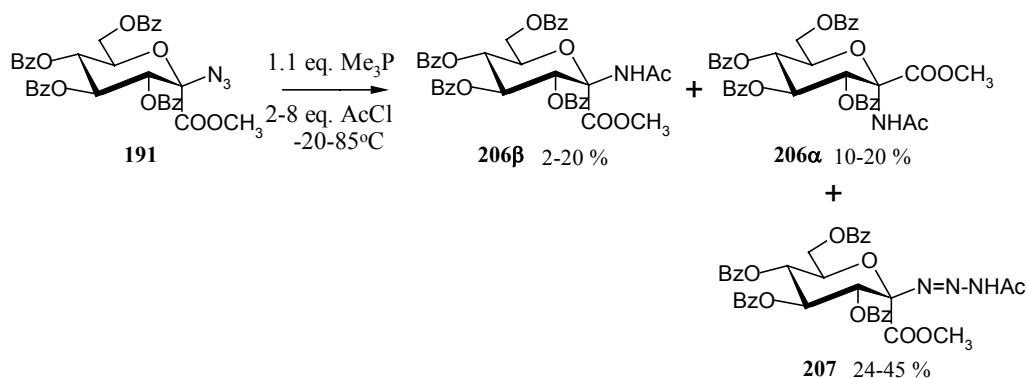
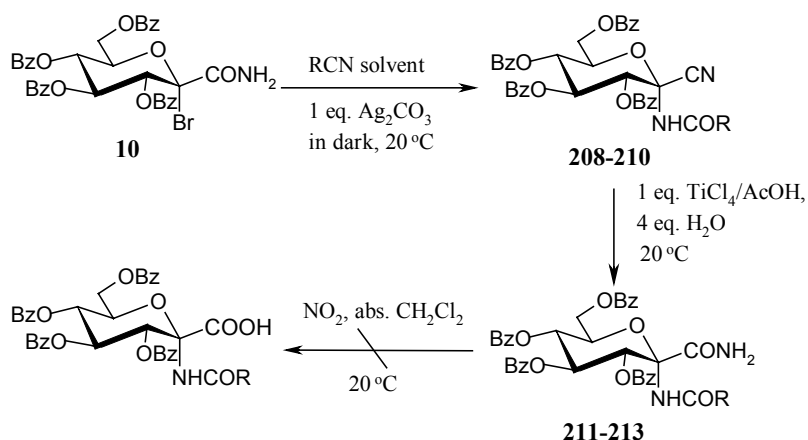


Figure 4

c) By Ritter-reaction: Starting from benzoylated bromide **10** we synthesised amides **208-210** in the presence of Ag_2CO_3 and different nitriles (**Table 5**). In every case only the axial anomer was formed.

Table 5: Preparation and transformation of per-*O*-benzoylated *N*-(1-cyano-D-glucopyranosyl)amides



R	Yield %	Yield %
-CH ₃	78 (208)	88 (211)
-CH ₂ CH ₃	57 (209)	75 (212)
-tBu	27 (210)	69 (213)

d) Investigation of the nitrile $\rightarrow$ carboxyl transformation: To get a free carboxyl group near the protected nitrogen function nitriles **208-210** were hydrolysed in the presence of TiCl₄ as the catalyst. The obtained carboxamides **211-213** decomposed under nitrosation with NO₂

Another possibility to obtain a carboxyl derivative is the Pinner reaction: **96** was transformed to **200a** by NaOMe catalysed addition of MeOH, followed by acid hydrolysis and acetylation. For the transformation of α -acylamino-nitriles to the corresponding acids was suggested the treatment with trifluoroacetic acid, which resulted in amide **215** in the case of **96**.

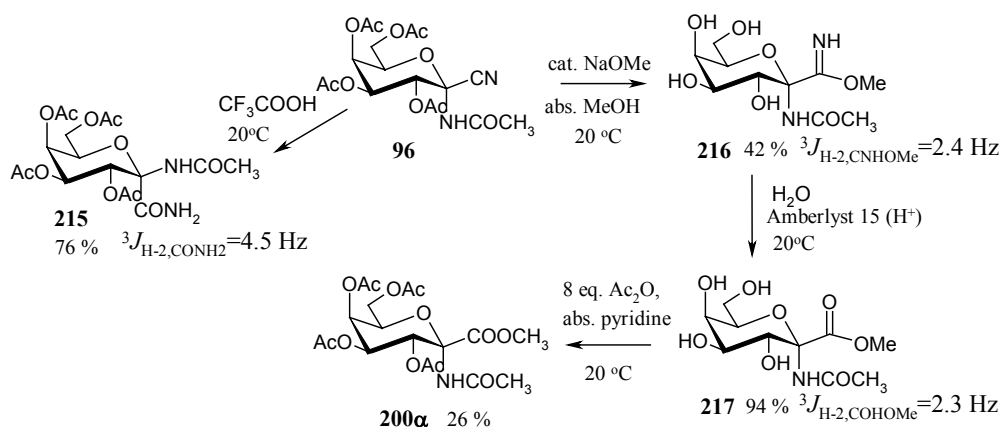


Figure 5

3.4. Di- and tripeptides

a) For the preparation of a tripeptide with β -anomeric configuration we reacted azido glycine methylester **184** with *tert.*-butoxycarbonyl-L-alanine (BOC-ala-OH) under Staudinger-conditions (**Figure 6**). With acetic anhydride dipeptide **219** was formed from azide **184**.

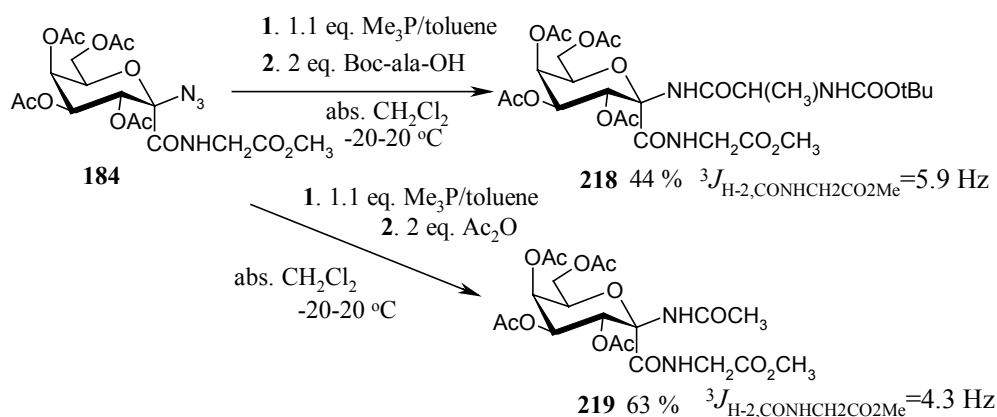


Figure 6

b) From glycine derivative **220** obtained from bromide **7** by a Ritter-reaction α -anomeric dipeptide **223** was isolated using the Pinner-reaction (**Figure 7**).

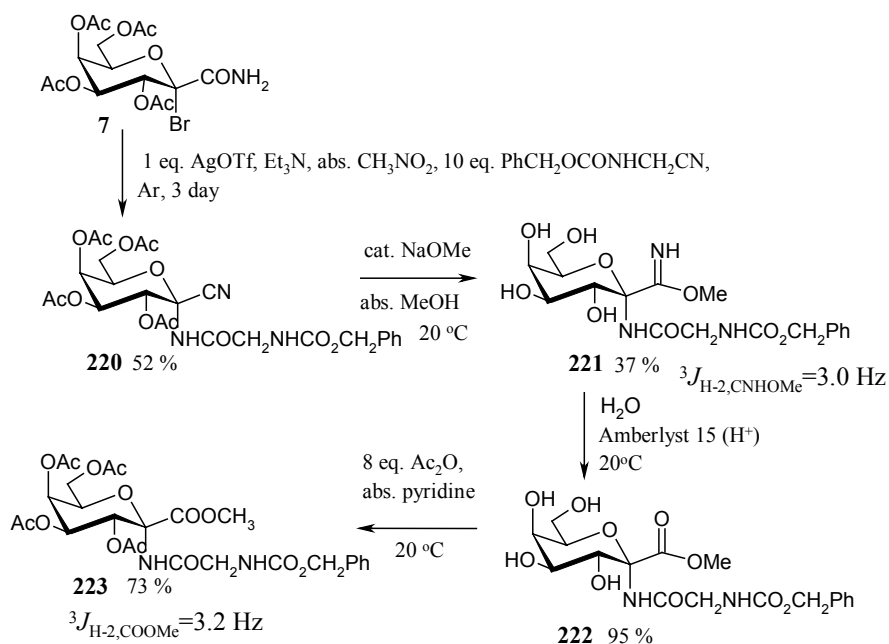


Figure 7

c) For the preparation of di- and tripeptides we investigated the extension of the Ritter-reaction for brominated glycine and alanine derivatives **175** and **175a**. In these reactions spiro-oxazolines **224-226** were formed (**Figure 8**). The ring opening of **224** and **226** in the presence of TFA resulted in tripeptide **228** and dipeptide **219**. To obtain **228** in a better yield the synthesis was carried out following route **B** without the isolation of **226**. The *N*-terminal part is in equatorial position in the both oligopeptides, in spite of the fact that oxazolines **224** and **226** show an opposite configuration. This can be explained with an anomerisation during the ring opening, which also happened in the ring opening of **225** with acetic acid to give dipeptide **227** in which the nitrogen substituent is again in an equatorial position.

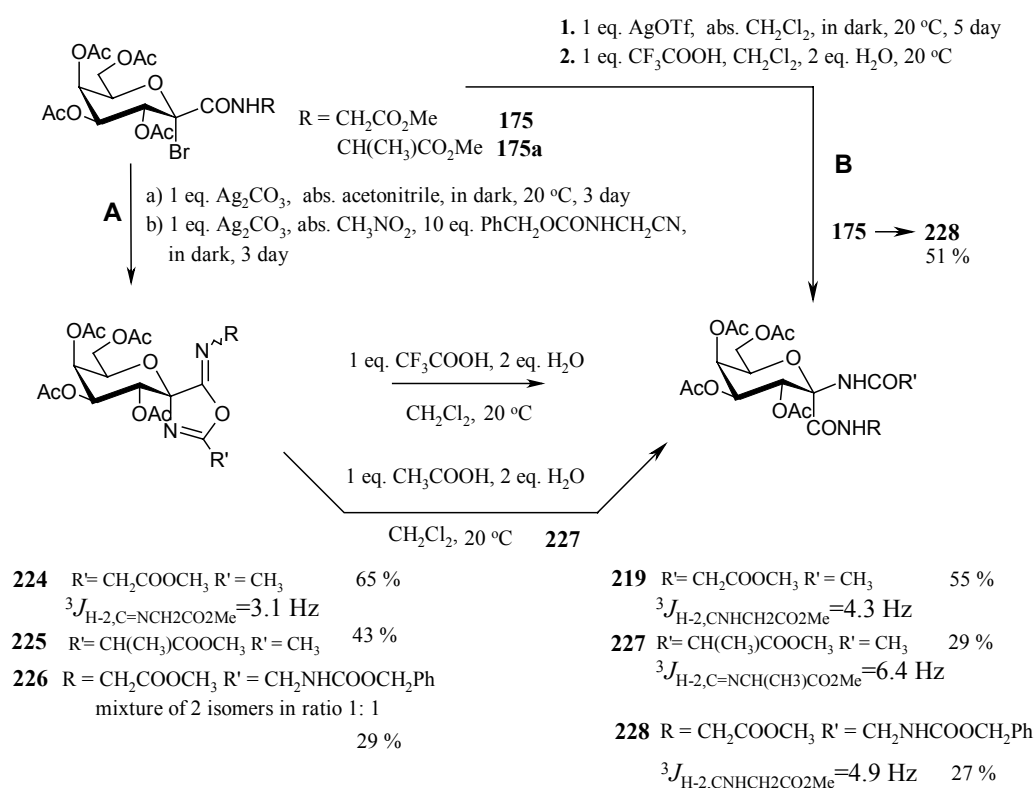


Figure 8

4. Possible application of the results

My work has been a basic research in order to synthesise potential glyco- and glycopeptidomimetics. Such derivatives can be used for biological investigations to clear the roles of carbohydrates and carbohydrate-peptide conjugates in living organisms.

Some derivatives of the prepared 2-*N*-substituted-2-deoxy- β -D-ald-2-ulopyranosonic acid were tested against glycogen phosphorylase enzymes, however, showed no significant inhibitory activity.

A possible application of the di- and tripeptides can be their incorporation into longer peptide chains in order to study their effect on the conformation of the peptide chain.

5. Publikációk/Publications

Az értekezés alapjául szolgáló közlemények/Papers underlying the Theses

1. Erzsébet Ősz, Katalin Czifrák, Tamás Deim, László Szilágyi, Attila Bényei, László Somsák

Preparation of 3,5-bis-(β -D-glycopyranosyl)-1,2,4-thiadiazoles from C-(β -D-glycopyranosyl)thioformamides
Tetrahedron, **2001**, 57, 5429-5434.

2. Katalin Czifrák, László Somsák

Radical-mediated bromination of carbohydrate derivatives: searching for alternative reaction conditions without carbon tetrachloride
Tetrahedron Lett., **2002**, 43, 8849-8852.

3. László Somsák, Katalin Czifrák, Edit Veres

Selective removal of 2,2,2-trichloroethyl- and 2,2,2-trichloroethoxycarbonyl protecting groups with Zn-N-methylimidazole in the presence of reducible and acid sensitive functionalities
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4. Katalin Czifrák, Péter Szilágyi, László Somsák

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Tetrahedron: Asymmetry, Közlésre beküldve.

Egyéb közlemények/Other papers

5. László Somsák, Katalin Czifrák, Tamás Deim, László Szilágyi, Attila Bényei

Studies into the preparation of 1-deoxy-1-thiocyanato-D-glycopyranosyl cyanides and the anomeric effect of the thiocyanate group
Tetrahedron: Asymmetry, **2001**, 12, 731-736.

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1. Erzsébet Ősz, Tamás Deim, Katalin Czifrák, László Szilágyi, László Somsák:

Unexpected formation of 3,5-bis-(β -D-glycopyranosyl)-1,2,4-thiadiazoles and 1-deoxy-1-thiocyanato-D-glycopyranosyl cyanides during studies into the preparation of glycopyranosylidene-spiro-dithiohydantoin

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2. Katalin Czifrák, László Somsák:

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MTA Kém. Tud. Oszt., Szénhidrátkémiai Munkabizottság előadóülése, Mátrafüred, 2001. május 15-16.

3. Czifrák Katalin

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Szeged, 2001. október 29-31.

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7. Czifrák Katalin, Somsák László

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8. Czifrák Katalin, Somsák László

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Hajdúszoboszló, 2003. június 26-28.

9. Katalin Czifrák, László Somsák:

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Eurocarb XI, July 6-11, 2003, Grenoble, France

10. Katalin Czifrák, László Somsák

Preparation and transformation sugar anomeric α -amino acid derivatives

1st Austrian-Hungarian Carbohydrate Conference, Sep 24-26, 2003, Burg Schlaining, Ausztria