A citoplazmában előforduló glikoprotein pentaszacharid szénhidrátrészének szintézise

doktori (PhD) értekezés tézisei

Szabó Zoltán

(Témavezető: Dr. Lipták András)

Synthesis of the pentasaccharide-segment of a glycoprotein found in the cytoplasma

theses of doctoral (PhD) dissertation

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Debreceni Egyetem
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1. Introduction and objectives

Carbohydrates in living organisms are not only known as sources of energy or skeletal materials, but also known as carriers of information of biological importance (glycoconjugates). This finding was facilitated by the enormous advance of techniques and instruments, that are used for the purification and the elucidation (HPLC, MS, GC-MS, NMR) of natural and synthetic substances.

The oligoglycosyl moiety, found in glycoconjugates, can fulfill many roles, namely it protects a protein against the attack of proteases, it fixes the protein in a definite conformation with secondary interactions, and enables a cytoplasmic or nuclear protein to play its biological role. The oligosaccharide sequences found on the surface of the plasma membrane function as receptors. The investigation of glycoconjugates helped the researchers to understand the function of the sugar moiety, the interactions between cells and the cell and the matrix.

The recognition of the biological roles of carbohydrates means a novel challenge for chemists. The need for the preparation of higher-membered, and branched oligosaccharides demanded the elaboration of newer block syntheses, protecting group strategies and new stereospecific glycosylation methods. The latter brought about the development of techniques used for elucidation.

Long-standing research on the synthesis of sugars found in natural glycoconjugates is carried out in the Research Group for Carbohydrates of The Hungarian Academy of Sciences. The preparation of oligosaccharides with various linkages was significantly promoted by Lipták and co-workers who elaborated a new, acetal/ether protecting group strategy. It was recognised, that benzylidene acetals of carbohydrates, depending on their location on the sugar moiety, can be opened regio-, or stereoselectively in a reductive manner to give rise to the formation of benzylated sugar derivatives with a free OH group. This method enabled the researchers to prepare oligosaccharide building blocks, having a hydroxyl function at the desired position.

In year 2000 I joined the Research Group as an undergraduate student, and my task was to investigate the (2-naphthyl)methylene acetal and (2-naphthyl)methyl ether protective groups. This groups can be used for the selective protection of carbohydrates analogously to the benzylidene acetal / benzyl ether strategy, but can be manipulated under more mild conditions.

The use of (2-naphthyl)methylene acetal and (2-naphthyl)methyl(NAP) ether for the synthesis of monosaccharide building blocks and for the synthesis of isomeric oligosaccharides was targeted. The attained results are discussed in this dissertation.

2. Applied methods

The macro-, semi-micro-, and micro-methods of the modern preparative organic chemistry were used in the synthetic work. The purity of the substances, the ratio of products were controlled and the reactions were monitored by thin-layer chromatography. Purification of the crude products and separation of the isomers were carried out either by crystallization, or by column chromatography. The characterisation and the elucidation of the compounds were carried out by elemental analysis, melting point- and optical rotation determination, and by one and two-dimensional (^{1}H-^{1}H-COSY, TOCSY, ^{13}C-^{1}H-HSQC) NMR spectroscopy and MALDI/ESI-TOF mass spectrometric methods, respectively.

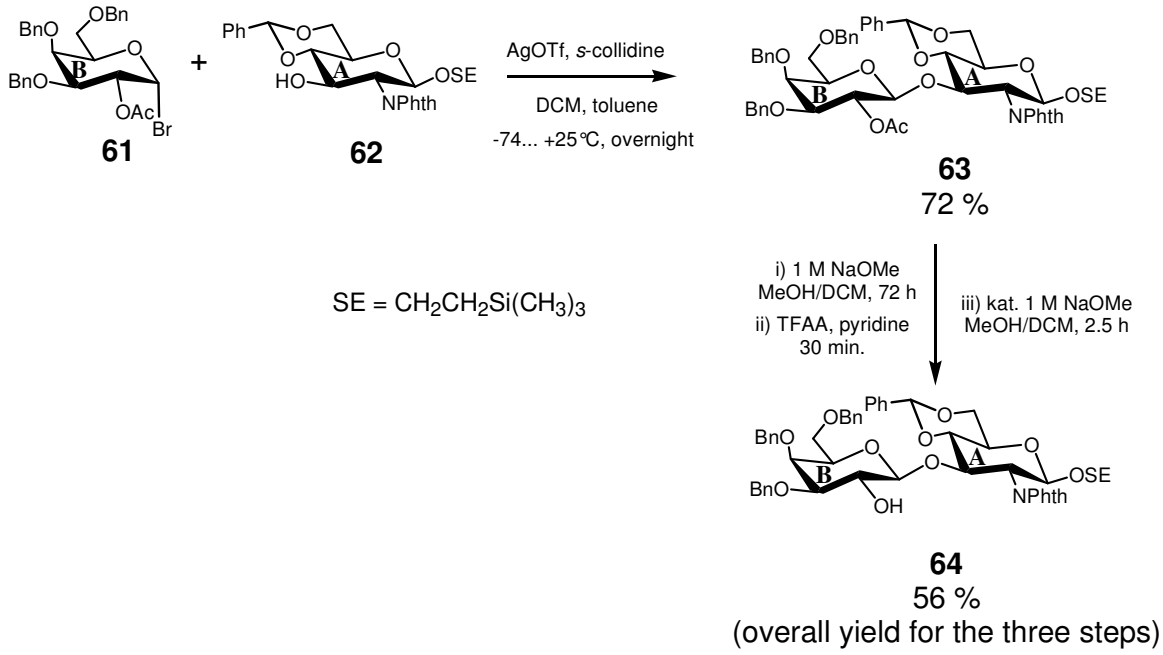
3. The new scientific results of the dissertation

3.1 The preparation of the pentasaccharide building blocks

The 143th amino acid of the Skp1 protein found in *Dictyostelium discoideum* is glycosylated with a pentasaccharide. In the course of the investigation of the pentasaccharide chain West and co-workers could not unambiguously determine the exact linkage position of the Galpα(1→6)Galpα(1→?) (**E+D**) cap disaccharide on the fucose moiety of the [Fucα(1→2)Galpβ(1→3)GlcNAc(1→)HyPro; **C+B+A**] reducing-end trisaccharide. As a solution to this problem the synthesis of three regioisomeric pentasaccharides, having different linkages on the fucose unit, was planned.

The **B+A** and **E+D** blocks can be built up of the appropriate monosaccharide units. The preparation of the **C** units demanded a more watchful design. Such derivatives, that can be transformed into suitable acceptors, following the fucosylation step, are necessary. The solution of this problem was planned by the aid of the (2-naphthyl)methyl protecting group, since it can be selectively removed in the presence of benzyl ethers.

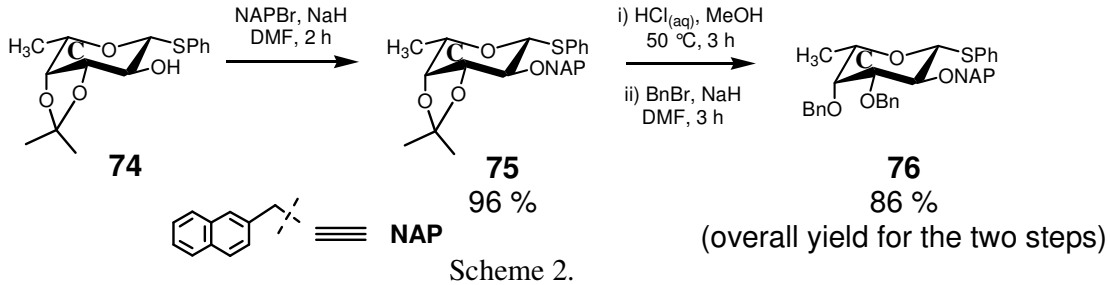
The B+A dissaccharide fragment was prepared by linking the known **61** and **62** compounds. The 2'-OAc group of the formed **63** was cleaved according to Zemplén-method to give acceptor **64** (Scheme 1.). In the course of the deacetylation step the reaction of the phthalimido-group was also observed, and its reclosure made the preparation of **64** difficult.



Scheme 1.

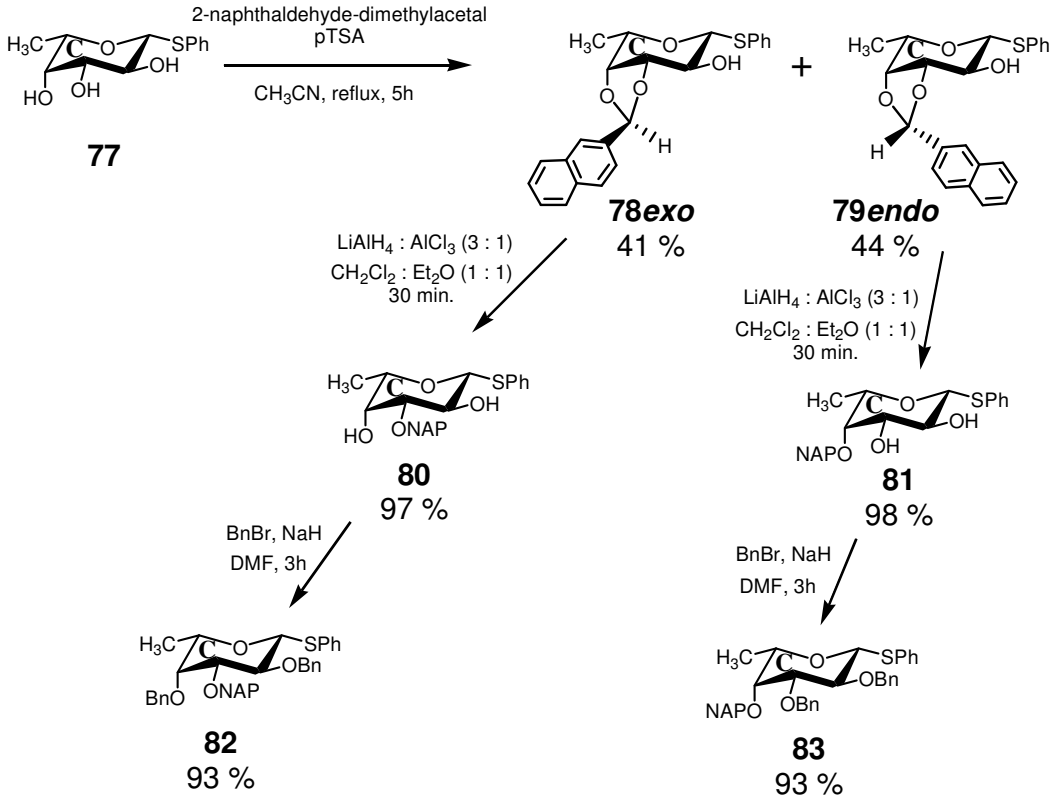
In order to avoid the extra synthetic steps in the preparation of **64** the synthesis of the disacharide was carried out with **62** acceptor and such donors, that bear a 2-*O*-chloroacetyl group. Unfortunately, in the latter experiments the α-glycoside was always the main product.

The C2, C3 and C4 fucoside compounds, that have a (2-naphthyl)methyl protective group at different positions were prepared from **74**, or from **77** known compounds. The introduction of the NAP-group was carried out in three ways: by direct alkylation, the reductive ring-opening of diastereomeric (2-naphthyl)methylene acetals, and by alkylation via stannylene acetal.



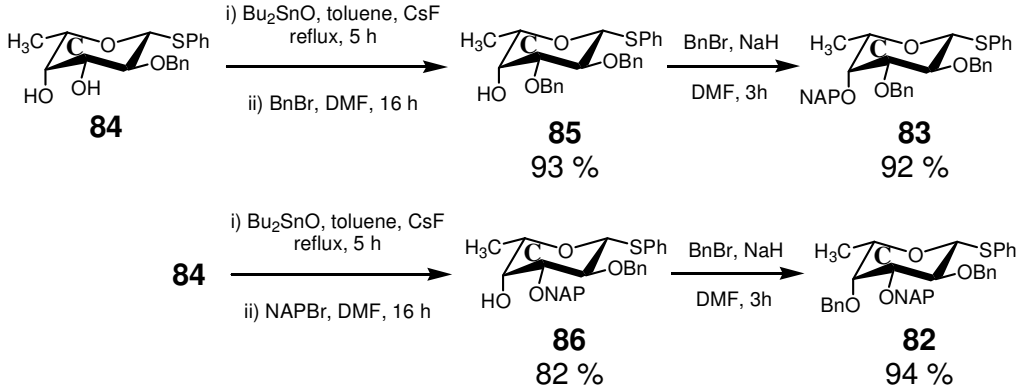
Scheme 2.

The 2-ONAP moiety of **76** was formed by direct alkylation (Scheme 2.). The preparation of the 3-ONAP and 4-ONAP compounds was accomplished by the ring-opening of the (2-naphthyl)methylene acetal having the appropriate stereochemistry (Scheme 3.)



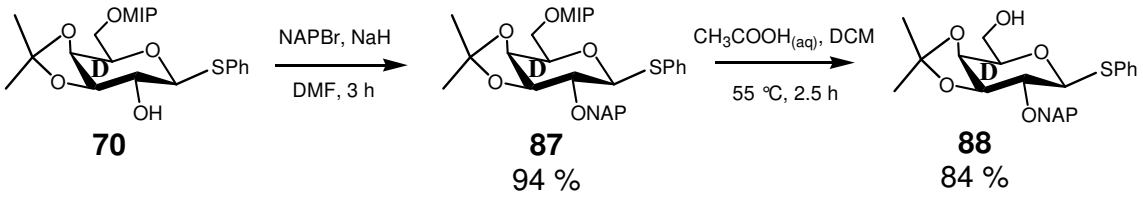
Scheme 3.

The synthesis of **82** and **83** was also carried out by alkylation via stannylene acetal. These reactions resulted in better yields, even with more synthetic steps, than the acetal-ring opening method (Scheme 4.).



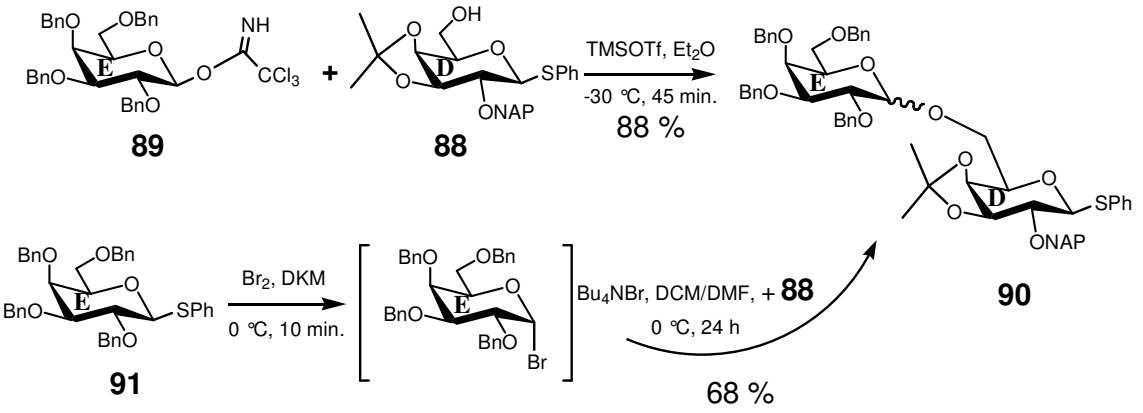
Scheme 4.

The preparation of the E+D disaccharide donor was made by linking **88**, a thioglycoside acceptor having a free 6-OH (Scheme 5.), and a tetra-*O*-benzyl-galactopyranosyl donor. The glycosylation was investigated with two known donor compounds (**89** and **91**), but neither reactions gave the desired product in an anomeric pure form (**90**, Scheme 6.).



70 **87** 94 % **88** 84 %

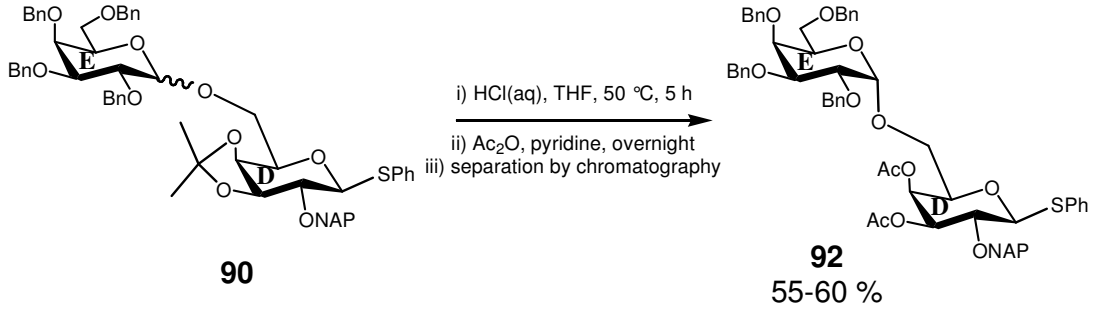
Scheme 5.



89 **88** **90** **91**

Scheme 6.

Compound **90** could be purified after a protecting group exchange to give the necessary α-glycoside (Scheme 7.).



90 **92** 55-60 %

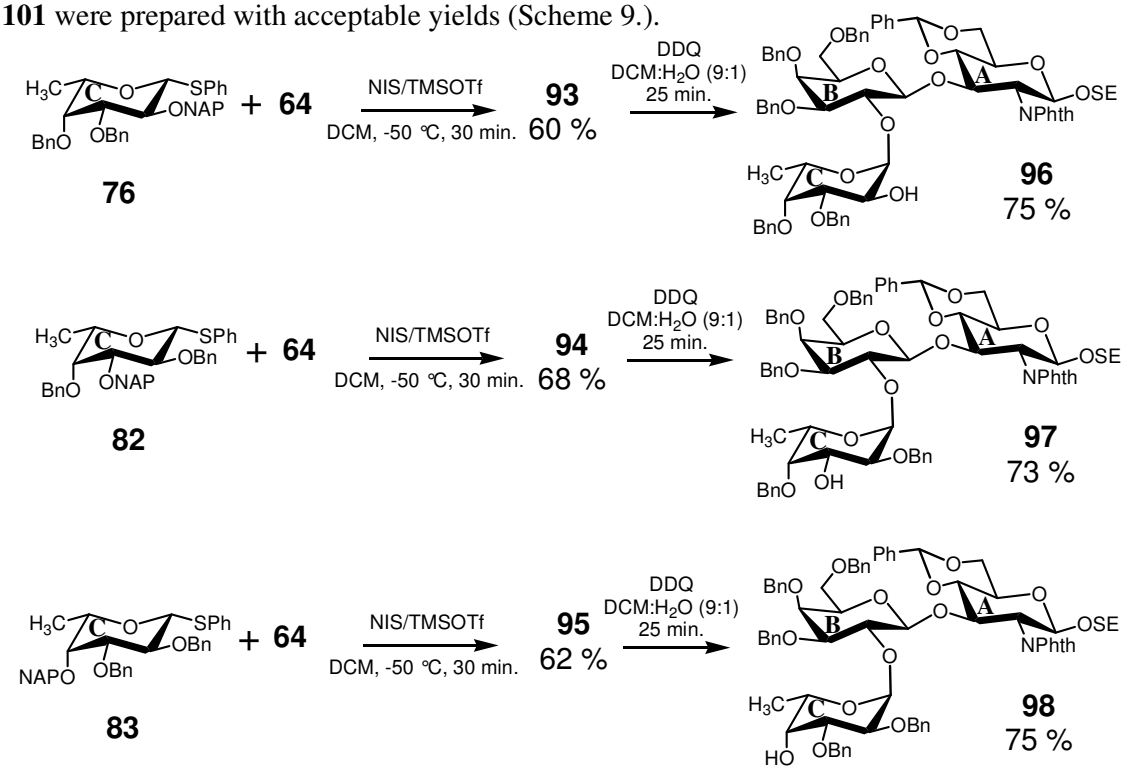
Scheme 7.

3.2 Glycosylation reactions

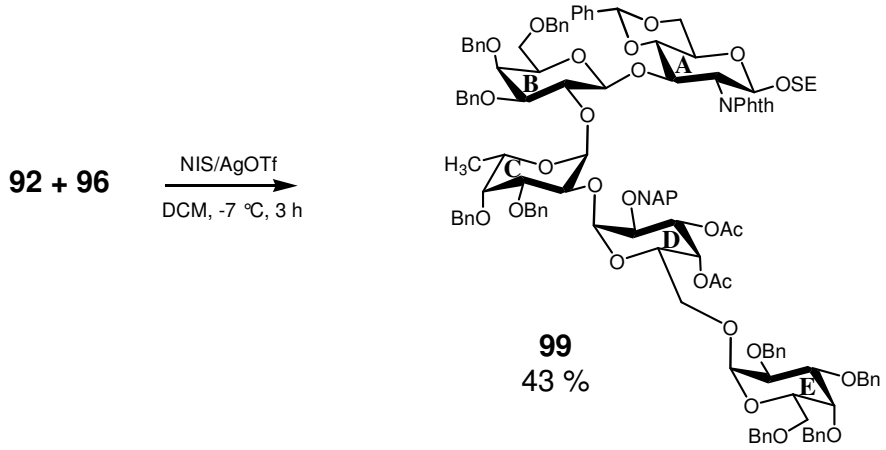
The preparation of the reducing-end trisaccharides were carried out by attaching the appropriate fucoside donor (**76**, **82**, **83**) to acceptor **64**. The synthesis and protecting group transformation of the three isomeric compounds were run under the same reaction conditions, since no significant difference in their taking-place was observed. Following the fucosylation step and the selective cleavage of the (2-naphthyl)methyl-group, **96**, **97,** and **98** trisaccharide acceptors were isolated (Scheme 8.).

The fully protected pentasaccharides were prepared by the glycosylation of the isomeric trisaccharide acceptors [**96** (2''-OH), **97** (3''-OH), **98** (4''-OH)] with **92**

disaccharide donor. The reactions were promoted by NIS/AgOTf, and compounds **99**, **100** and **101** were prepared with acceptable yields (Scheme 9.).



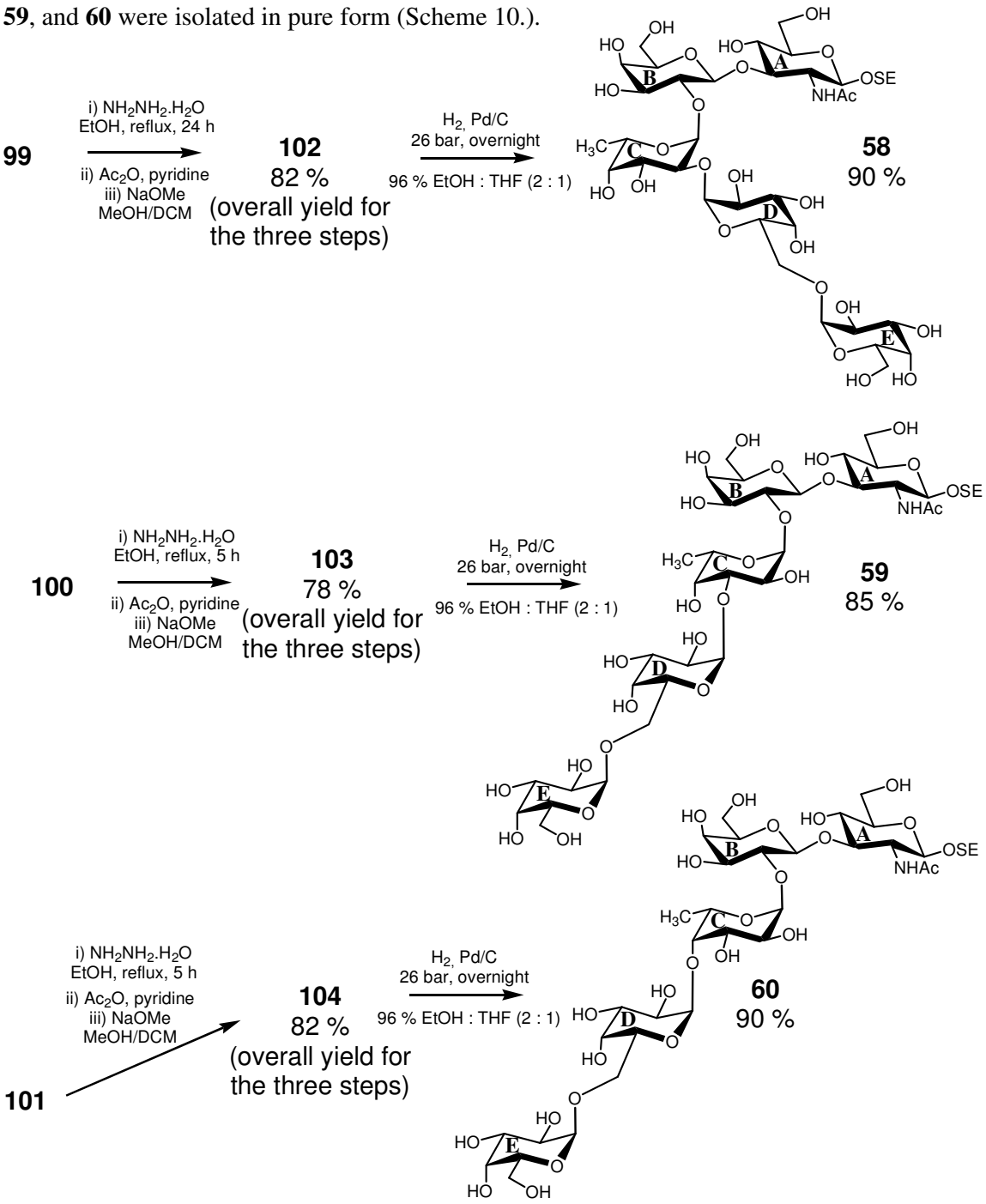
Scheme 8.



Scheme 9.

3.3 Removal of the protecting groups from the pentasaccharides

The transformation of the phthaloyl-group into an *N*-acetyl on compounds **99**, **100,** and **101** was carried out by treating them with hydrazine hydrate, and acetic anhydride, respectively. The obtained substances were then deacetylated with the Zemplén method to give **102**, **103**, and **104** compounds, respectively. The reducable protecting groups were removed by catalytic hydrogenation by the use of pressure and the target pentasaccharides **58**, **59**, and **60** were isolated in pure form (Scheme 10.).



Scheme 10.

4. Summary

The targeted, free, regioisomeric pentasaccharides were successfully prepared in an amount of, ~60-100 mgs. The compounds synthesized are not known in the literature. To perform our aim known glycosylation methods and the (2-naphthyl)methylene acetal / (2-naphthyl)methyl ether protecting group strategy, developed in our group, was used. The (2-naphthyl)methyl group could be introduced into any position of the fucose monosaccharide unit, and also could be cleaved in the presence of benzyl ethers. The NAP protective group was present in the C-2 position in case of two donors and functioned as a non-participating group, as it was expected.

The compounds synthesized might be able to support or to disprove the structure of the pentasaccharide of natural origin.

5. List of Publications

5.1 Publications

1.) A. Borbás, **Z. B. Szabó**, L. Szilágyi, A. Bényei, A. Lipták: **Dioxane-type (2-naphthyl)-methylene acetals of glycosides and their hydrogenolytic transformation into 6-O- and 4-O-(2-naphthyl)methyl (NAP) ethers**, *Tetrahedron* **2002**, 58, 5723-5732.

2.) A. Borbás, **Z. B. Szabó**, L. Szilágyi, A. Bényei, A. Lipták: **Stereoselective (2-naphthyl)-methylation of sugars by hydrogenolysis of diastereomeric dioxolane-type (2-naphthyl)methylene acetals**, *Carbohydr. Res.*, **2002**, 337, 1941-1951.

3.) T. Kurtán, A. Borbás, **Z. B. Szabó**, A. Lipták, A. Bényei, S. Antus: **Circular Dichroism of 1,3-Dioxane-Type (2'-Naphthyl)Methylene Acetals of Glycosides**, *Chirality* **2004**, 16, 244-250.

4.) Magdolna Csávás, **Zoltán B. Szabó**, Anikó Borbás, András Lipták; *2-Naphthylmethyl bromide*: *Electronic Encyclopaedia of Reagents for Organic Synthesis*, (2004).

5.) Anikó Borbás, Magdolna Csávás, **Zoltán B. Szabó**, András Lipták; *2-(Dimethoxymethyl)naphthalene*: *Electronic Encyclopaedia of Reagents for Organic Synthesis*, (2004).

6.) **Zoltán B. Szabó**, Anikó Borbás, István Bajza, András Lipták: **Synthesis of fully protected α-L-fucopyranosyl-(1→2)-β-D-galactopyranosides with a single free hydroxy group at position 2', 3' or 4' using O-(2-naphthyl)methyl (NAP) ether as a temporary protecting group**, *Tetrahedron: Asymmetry* **2005**, 16, 83-95.

5.2 Lectures (L) and posters (P)

1.) A. Lipták, A. Borbás, **Z. B. Szabó**, L. Jánossy, L. Szilágyi: Preparation of (2-naphthyl)methylene acetals of glycosides and their hydrogenolytic transformation into 2-naphthylmethyl(NAP) ethers, *20th International Carbohydrate Symposium, Hamburg, Germany, 27 August – 01 September, 2000*. B-224 (**P**) (p. 170, Abstract book)

2.) **Szabó Zoltán**: Glikozidok (2-naftil)metilén acetáljainak szintézise és hidrogenolitikus átalakításuk (2-naftil)metil éterekké, *XXV. OTDK Kémiai és Vegyipari Szekció, Szerves Kémia "A" tagozat, Gödöllő, 2001. április 10-12.* (**L**)

3.) **Szabó B. Z.**, Lipták A., Borbás A., Szilágyi L., Bényei A.: Glikozidok (2-naftil)metilén-acetáljainak szintézise és hidrogenolitikus átalakításuk (2-naftil)metil(NAP) éterekké, *MKE Vegyészkonferencia, Hajdúszoboszló, 2001. június 27-29.* P-99 (**P**) (abstract book p. 133.)

4.) A. Lipták, A. Borbás, **Z. B. Szabó**, L. Szilágyi: Preparation of (2-naphthyl)methylene acetals of glycosides and their selective hydrogenolysis into (2-naphthyl)methyl ethers, *11th European Carbohydrate Symposium, Lisboa, Portugal, 2-7 September, 2001*. PA035 (**P**) (abstract book, p. 163)

5.) **Z. B. Szabó**, A. Lipták, L. Jánossy: L-Fucoside building blocks suitable for oligosaccharide synthesis, *12th European Carbohydrate Symposium, Grenoble, France, 6-11 July, 2003*. PB-025 (**P**)

6.) **Z. B. Szabó**, A. Lipták, L. Jánossy: L-Fucoside building blocks suitable for oligosaccharide synthesis, *First Austrian-Hungarian Carbohydrate Conference, Burg-Schlaining, Austria, 24-27 September 2003*. (**L**)

7.) **Szabó B. Zoltán**, Lipták András, Jánossy Lóránt: α-L-Fukopiranozil kötések kialakítására alkalmas védett tioglikozidok szintézise, *XXVI. Kémiai Előadói Napok, Szeged, 2003. október 27-29*. (**L**) (abstract book p. 23.)

8.) **Zoltán B. Szabó**, Anikó Borbás, András Lipták: L-Fucose Building Blocks Suitable For Oligosaccharide Synthesis, *First German-Hungarian Workshop, Hannover, Germany, 5-6 July 2004*. (**L**)

9.) **Zoltán B. Szabó**, Mihály Herczeg, Magda Csávás, Anikó Borbás, Gyula Batta, András Lipták: Synthetic Studies on the Pentasaccharide Side-Chain of the Skp1 Glycoprotein Found in *Dictyostelium discoideum*, *Second German-Hungarian Workshop, Debrecen,Hungary, 4-9 April 2006*. (**L**)