Synthesis of immunodeterminant oligosaccharides of bacteria

theses of doctoral (PhD) dissertation

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1. Introduction

The interest in biologically-important oligosaccharides and glycoconjugates (e.g. glycolipids, glycoproteins) has increased in the last twenty years. Previously carbohydrates have been known only as structural and food storage materials and energy sources in the living organisms. Since then the important role that carbohydrates play in the biological processes was recognised. The glycoconjugates are involved in interactions of cells with other cells, bacteria, viruses, and toxins, and are of importance for cell-growth, cell differentiation, immunological response, tumor metastasis, inflammation, and bacterial viral infections.

Large amounts of oligosaccharides are needed for biochemical and pharmacological investigations. These experiments can lead to the development of new diagnostics and to vaccines and therapeutics for a number of diseases. This demand requires effective chemical synthesis of oligosaccharides with high yield and stereoselectivities.

Mycobacteria, in the diseases they cause, further remain a serious problem. The magnitude of worldwide incidence of tuberculosis (caused by *Mycobacterium tuberculosis*) and leprosy (*Mycobacterium leprae*) is enormous. But also the so called atypical *Mycobacteria* (for example members of the *Mycobacterium avium* serocomplex) are opportunistic pathogens and can cause serious infections. The synthesis of oligosaccharide haptens of the cell-surface antigens helps in diagnosis of *Mycobacterial* infection at early stage.

Shigella sonnei is a Gram-negative bacterium that can cause dysentery in humans, an acute inflammatory disease of the large intestine. Because all groups of *Shigella* acquired resistance to most available antibiotics, the effort to prevent shigellosis should go toward vaccine development. The O-SP is an essential virulence factor of *Shigellae* including *Shigella sonnei*. The O-SP of *Shigella sonnei* is a nonimmunogenic molecule that must be conjugated to an immunogenic protein carrier to induce antibodies.

2. Applied methods

The macro- and micro methods of the modern preparative organic chemistry were applied in the synthetic work.

The reactions were monitored by thin layer-chromatography, the purity of the substances and the ratios of the products were controlled by high pressure liquid-, and gas chromatography. The purification of the crude products and the separation of the isomers were carried out by crystallization or by column chromatography.

Elemental analysis, melting point- and optical rotation determination, one- and two dimensional ^{1}H and ^{13}C NMR spectroscopic methods and mass spectrometry were applied for the identification of the prepared compounds.

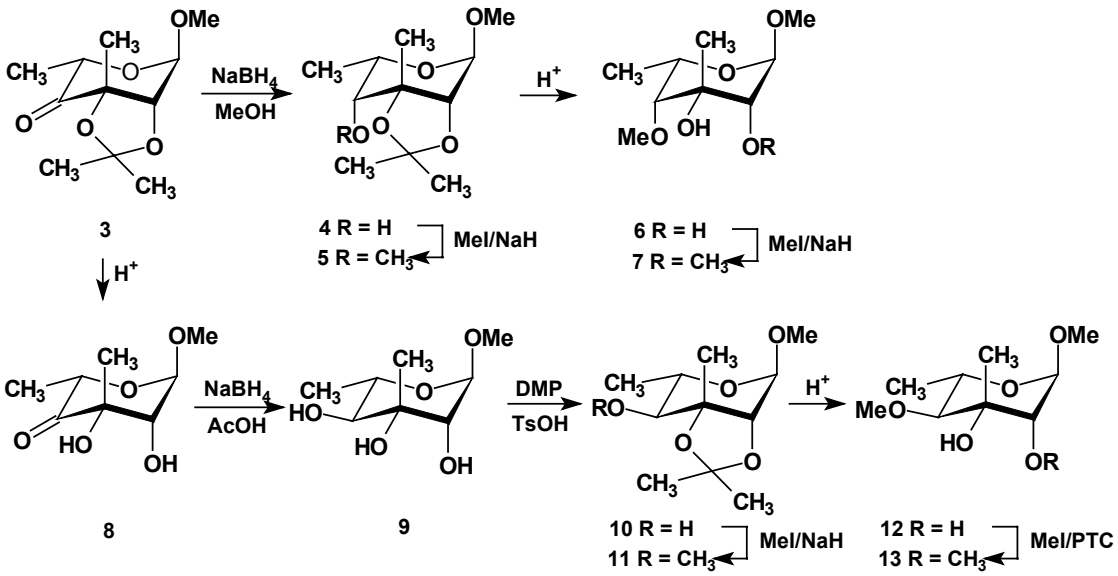
3. New scientific results of the dissertation

In the first part of the dissertation we present a chemical synthesis of the pentasaccharide hapten of the serovariant 19 of the *Mycobacterium avium* complex. The second part of the dissertation deals with the chemical synthesis of oligosaccharide fragments of the O-SP of the *Shigella sonnei*.

3.1. Synthesis of the pentasaccharide hapten of the serovariant 19 of the *Mycobacterium avium* complex

The pentasaccharide of the serovariant 19 contains a 3,4-di-*O*-methyl glucuronic acid and a 3-*C*-methyl-2,4-di-*O*-methyl branched sugar. While most of the structural features of the penultimate residue have been determined, ambiguity persists regarding the stereochemistry at C-4 of this unit.

Precursor for the synthesis of methyl 6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-α-L-talopyranoside (**7**) and methyl 3-*C*-methyl-2,4-di-*O*-methyl-α-L-rhamnopyranoside (**13**) was methyl 6-deoxy-3-*C*-methyl-2,3-*O*-isopropylidene-α-L-*lyxo*-hexopyrano-4-uloside (**3**) that was prepared with *C*-methylation (developed by Klemer) of methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-*lyxo*-hexopyranosid-4-ulose.

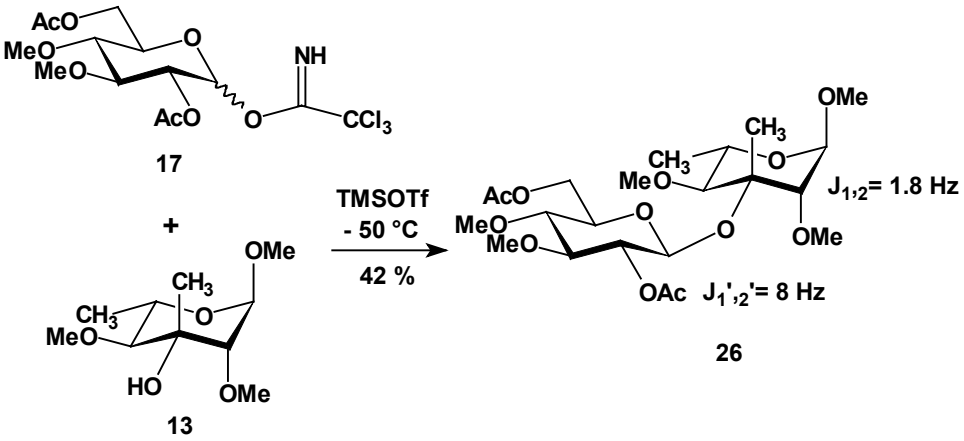


Reduction of compound **3** with $NaBH_4$ resulted in the 6-deoxy-*talo* isomer **4**. Methylation of compound **4** yielded **5** from which the isopropylidene group was removed

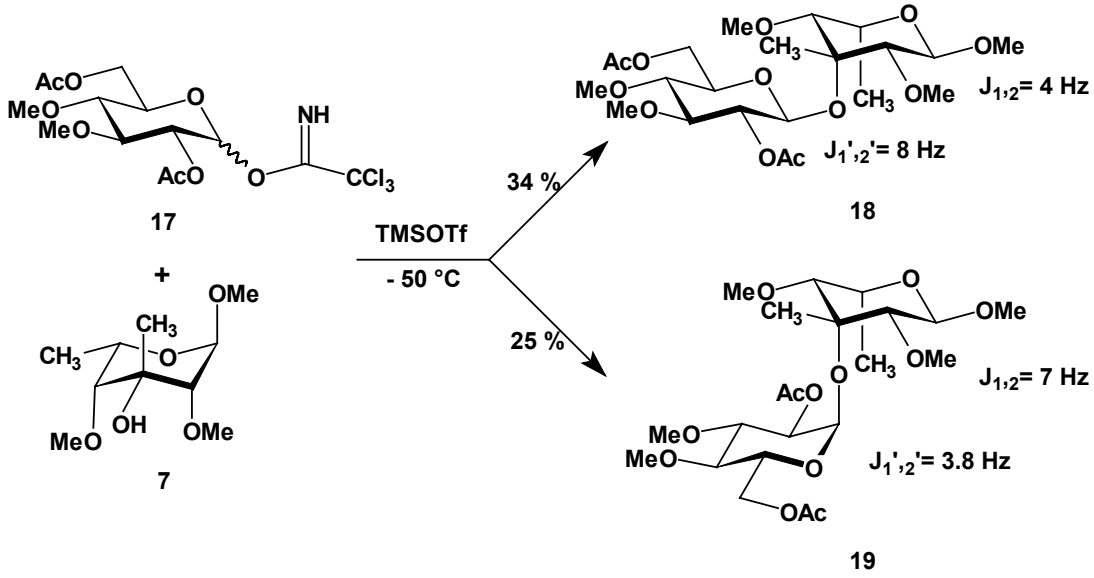
(→**6**). Methylation of the axial OH-2 of **6** gave **7**. To prepare the *rhamno* isomer (**9**) from compound **3**, its isopropylidene group was hydrolyzed, then the free ulose derivative (**8**) was treated with $NaBH_4$ in acetic acid. Isopropylidenation of **9** gave compound **10.** After methylation of **10** (→**11**) and acidic hydrolisis, the resulting diol **12** was selectively methylated at OH-2 under phase-transfer conditions to give **13**.

For the synthesis of terminal disaccharides glucose derivative (**17**) was used instead of the glucuronic acid derivative as a glycosyl donor. Glycosylation of acceptor **13** with trichloro-acetimidate donor **17** afforded exclusively the β-disaccharide **26**.

However, upon glycosylation of acceptor **7** with **17** two disaccharides have been



formed: one with β (**18**) and the second one with α interglycosidic linkage (**19**).



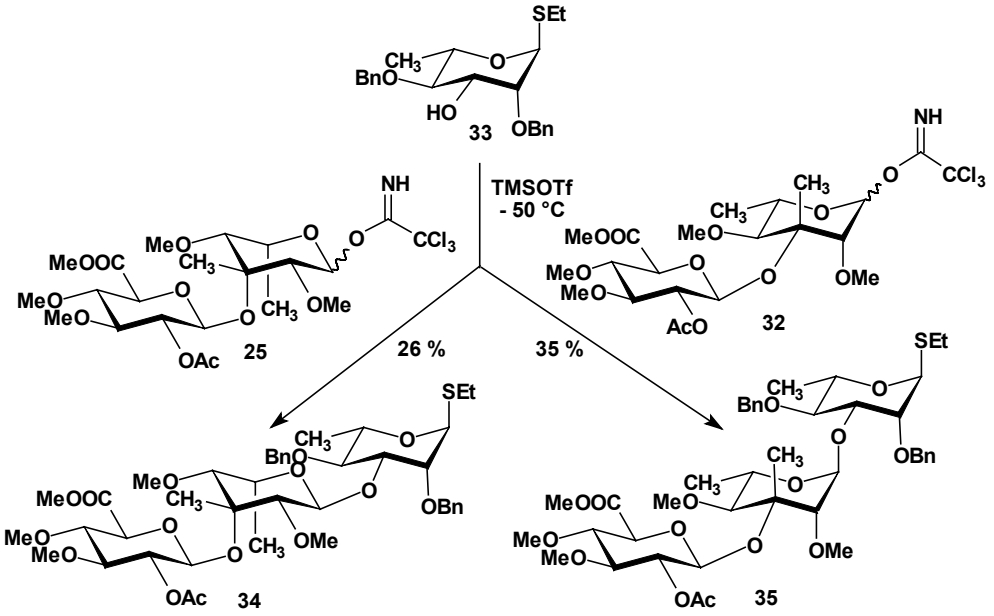
It was interesting that the conformation of the aglycone has been changed from $_4C^1$ (L) to $^4C_1$ (L) in the disaccharides (**18**, **19**).

For explanation of this phenomena we decided to synthesize each of the methyl ethers of the two sugars, and also to study and compare their conformational properties. All of the 6-

deoxy-3-*C*-methyl-α-L-mannopyranoside derivatives adopt the $^1C_4$ conformation: $J_{1,2} \leq 2$ Hz and $J_{4,5} \geq 9$ Hz. The situation in the case of the 6-deoxy-3-*C*-methyl-α-L-talopyranosides is completely different: for the fully substituted glycosides (**43**, **46**, **51**, **54** and **18**) the $^4C_1$ conformation is predominant, as proved by the coupling constants ($J_{1,2} \approx$ 5-6 Hz and $J_{4,5} \approx$ 4-4.5 Hz). However, it is to be noted that all of the mono- and disubstituted talopyranoside derivatives exist exclusively in the $_4C^1$ conformation.

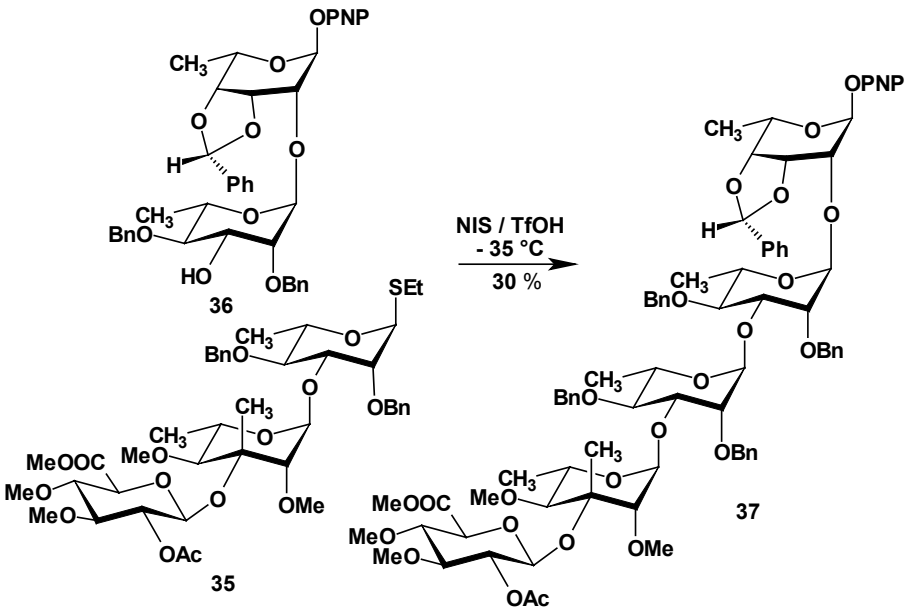
The fact that in the anomeric region of the ^{1}H NMR spectra of the tetraglycosyl alditol (isolated by the degradation of the antigene of the serovar 19) three ca. 1 Hz and one 7.75 Hz coupling constants could be determined, and that this last coupling constant (7.75 Hz) was assigned to the 3,4-di-*O*-methyl-β-D-glucuronic acid moiety proves that the building block next to the last one possesses L-*manno* (thus, not L-*talo*) configuration.

The NMR data of the trisaccharides (**34** and **35)** also supported this finding. First we converted two disaccharides to glycosyl donors (**25** and **32)** through deprotection, oxidation, acetolysis, deacetylation and trichloroacetimidate formation.

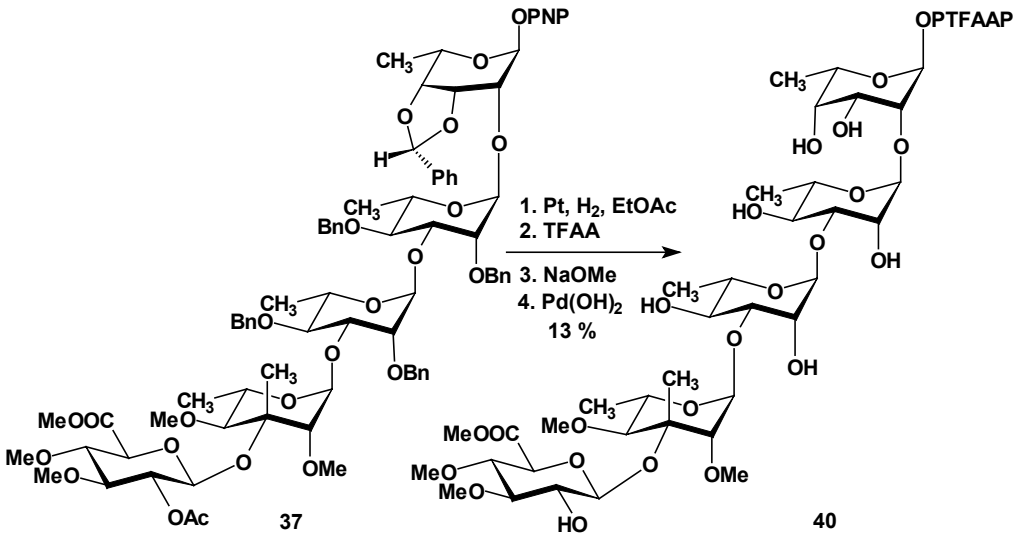


Glycosylation of ethyl 2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**33**) with trichloro-acetimidate donor **25** afforded trisaccharide **34**. Based on the ^{1}H spectra of the **34**, the 6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-L-talopyranose unit adopts the $^4C_1$ conformation: $J_{1',2'} = 3$ Hz. Therefore we used trisaccharide donor **35** for the synthesis of pentasaccharide. The trichloroacetimidate donor **32** and the acceptor **33** gave **35** in a yield of 35 %.

For the preparation of pentasaccharide we used the *p*-nitrophenyl 2,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-*endo*-3,4-*O*-benzylidene-6-deoxy-α-L-talopyranoside (**36)** as a glycosyl acceptor. Glycosylation of **36** with trisaccharide donor **35** gave the fully protected pentasaccharide **37** in a yield of 30 %.



The *p*-nitrophenyl aglycon of the pentasaccharide **37** was converted into *p*-trifluoro-acetamido phenyl by hydrogenation and subsequent treatment with trifluoroacetic anhydride. The removal of the acetyl group under Zemplén's condition we obtained 2-OH derivative.



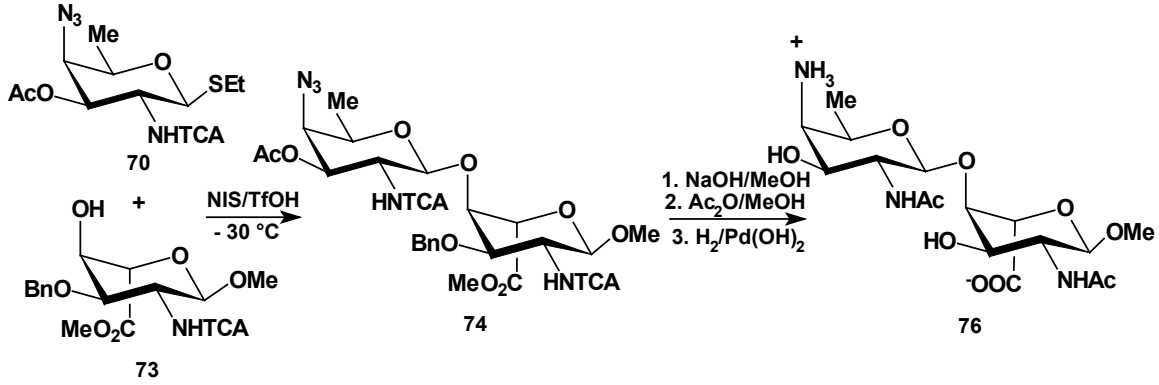
After the final hydrogenation we isolated the spacer-armed pentasaccharide **40**. The anomeric region of the ^{1}H NMR spectra of **40** was identical with that of the native hapten.

3.2. Synthesis of oligosaccharide fragments of the O-specific polisaccharide of *Shigella sonnei*

Kontrohr and Kene *et al.* established that the O-SP of *Shigella sonnei* is a linear heteropolysaccharide which is built up a disaccharide repeating unit consisting of rare monosaccharides, α (1→3)-linked 2-acetamido-2-deoxy-L-altruronic acid and β (1→4)-linked 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose.

The synthetic route started from ethyl 3-*O*-acetyl-4-azido-2-phthalimido-2, 4, 6-trideoxy-1-thio-β-D-galactopyranoside that was converted to the amine by treatment with ethylenediamine. Next a trichloroacetyl group was installed at the free amino group with trichloroacetyl chloride in the precence of triethylamine and after acetylation we obtained the glycosyl donor **70**.

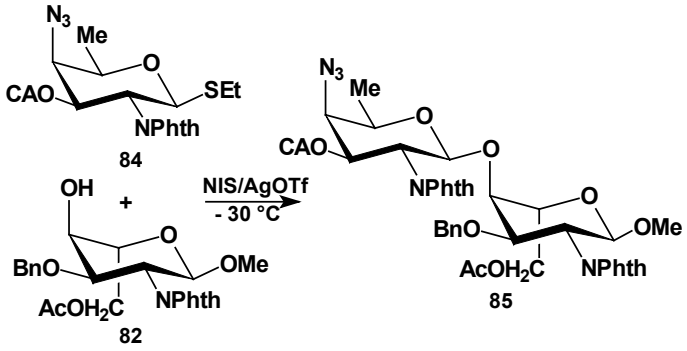
Then we reduced the azido group in methyl (methyl-2-azido-3-*O*-benzyl-2-deoxy-α-L-altropyranoside)-uronate to an amino group with H_2 over Pd/C to afford **72**. Subsequent treatment of **72** with trichloroacetyl chloride afforded **73**. Glycosylation of **73** with glycosyl donor **70** gave the fully protected disaccharide **74** in a yield of 85 %.



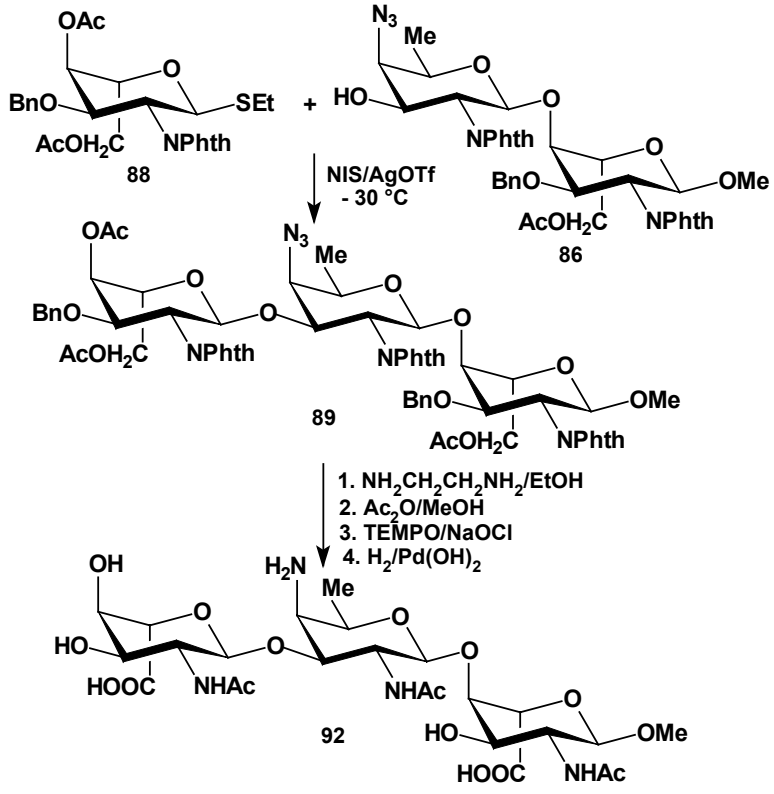
Treatment of **74** with NaOH in methanol removed the *N*-trichloroacetyl groups and afforded amine that was converted to the *N*-acetamido derivative **76** by acetylation. Hydrogenolytic cleavage of the benzyl groups that simultaneously reduced the azido group yielded the repeating disaccharide **76**.

Unfortunately, the removal of trichloroacetyl groups from higher-membered oligosaccharides was unsuccessful. Therefore we used *N*-phthaloyl group as *N*-protecting group for the synthesis of trisaccharide fragment of the O-SP of the *Shigella sonnei*. In this case the synthetic route started from methyl 2-azido-3-*O*-benzyl-2-deoxy-α-L-altropyranoside. In the first step we reduced this azido group to amino group with H_2 over Pd/C. The amine **80** was converted to phthalimido derivative by conventional procedures. Then regioselective acetylation of the diol gave the acceptor **82**. The chloroacetylation of the

ethyl 4-azido-2-phthalimido-2,4,6-trideoxy-1-thio-β-D-galactopyranoside afforded glycosyl donor **84**. Glycosylation of acceptor **82** with ethylthio glycoside **84** under NIS/AgOTf promotion gave the disaccharide **85** in an acceptable yield.



Then we converted disaccharide **85** to glycosyl acceptor through dechloroacetylation (→**86**). In the next step we transformed methyl-3-*O*-benzyl-2-deoxy-2-phthalimido-α-L-altropyranoside into ethylthio glycoside through acetolysis followed by Lewis-acid catalyzed thioglycoside formation (→**88**). Glycosylation of disaccharide acceptor **86** with glycosyl donor **88** afforded the trisaccharide **89**.



Phthalimido groups in compound **89** were converted to acetamido groups with ethylenediamine and subsequent *N*-acetylation. TEMPO oxidation of the triol **90** afforded the uronic acid derivative. Finally *O*-benzyl groups were removed by hydrogenolysis and

simultaneously the azido group was reduced to amino group and gave free trisaccharide (**92**) in a yield of 63 %.

4. Summary

We have developed a method for the stereoselective synthesis of methyl 6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-α-L-talopyranoside and methyl 3-*C*-methyl-2,4-di-*O*-methyl-α-L-rhamnopyranoside.

The prepared, spacer-armed, free pentasaccharide of *Mycobacterium avium* serovar 19 conjugated to immunogene protein can be applied to the serodiagnosis of *Mycobacterium* infections.

We have synthesized the repeating disaccharide unit and trisaccharide fragment of the O-specific polisaccharide of *Shigella sonnei*.

The antigenicity of the repeating disaccharide and those of the component monosaccharide was assayed by the passive hemolysis inhibition test. The results show that the disaccharide is a better inhibitor of the binding between the O-SP and a polyclonal antibody directed againts it than either one of its monosaccharide components of which the altruronic acid derivative is superior.

5. List of publications

5.1 Publications

1. K. Gyergyói, **A. Tóth**, I. Bajza, A. Lipták
   Unusual sugars of the GPL-type antigen of *Mycobacterium avium* serovar 19. Stereoselective synthesis of methyl 6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-α-L-mannopyranoside and its C-4 epimer
   Synlett., 127-128 (1998).

2. **A. Tóth**, A. Medgyes, I. Bajza, A. Lipták, Gy Batta, T. Kontrohr, K. Péterffy, V. Pozsgay
   Synthesis of the Repeating Unit of the O-specific Polysaccharide of *Shigella sonnei* and Quantitation of its Serologic Activity
   Bioorg. Med. Chem. Lett., **10,** 19-21 (2000).

3. Gy. Gyémánt, **A. Tóth**, I. Bajza, L. Kandra, A. Lipták
   Identification and structural analysis of synthetic oligosaccharides of *Shigella sonnei* using MALDI-TOF MS
   Carbohydr. Res. **334**, 315-322 (2001)

4. **A. Tóth**, J. Remenyik, I. Bajza, A. Lipták
   Synthesis of the methyl ethers of methyl 6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-α-L-talopyranoside and -α-L-mannopyranoside. Examination of the conformation and chromatographic properties of the compounds
   Arkivoc **V**, 28-45 (2003)

5.2. Lectures and posters

1. **Tóth A.**, Bajza I., Lipták A.
   A *Mycobacterium avium komplex* 19-es szerovariáns sejtfelszíni antigén diszacharid egységének előállítása
   XX. Kémiai Előadói Napok, Szeged, 1997. Október 13-15.

2. **A. Tóth**, K. Gyergyói, I. Bajza, A. Lipták
   Synthesis of the immunodeterminant pentasaccharide fragment of the cell surface GLP of *Mycobacterium avium serovar* 19
   2. German-East-European Carbohydrate Workshop, Güstrow/Rostock, March 24-28, 1999

3. **Tóth A.,** Bajza I., Pozsgay V. és Lipták A**.**
   A *Shigella sonnei* O-specifikus poliszacharidját felépítő oligoszacharid szekvenciák szintézise
   Vegyészkonferencia, Eger, 1999. Június 22-24.

4. **A. Tóth**, I. Bajza, V. Pozsgay, A. Lipták
Synthesis of di- and trisaccharide fragments of the O-specific polysaccharide of *Shigella sonnei*
10th European Carbohydrate Symposium, Galway, July 11-16, 1999

5. **A. Tóth**, I. Bajza, V. Pozsgay, A. Lipták
Synthesis of oligosaccharide fragments of the O-specific polysaccharide of *Shigella sonnei*
6th European Training Course on Carbohydrates, Debrecen, July 8-14, 2000

6. I. Bajza, **A. Tóth**, T. Kontrohr, V. Pozsgay, A. Lipták
Synthesis of higher-menbered oligosaccharide fragments of the LPS of *Shigella sonnei*
20th International Carbohydrate Symposium, Hamburg, August 27-September 1, 2000

7. **Tóth A. ,**Bajza I., Pozsgay V. és Lipták A**.**
A *Mycobacterium avium komplex* 19-es szerovariáns sejtfelszíni pentaszacharid haptén szintézise
Vegyészkonferencia, Hajdúszobszló, 2001. Június 22-24.

8. **A. Tóth**, I. Bajza, V. Pozsgay, A. Lipták
Synthesis of trisaccharide fragment of the O-specific polysaccharide of *Shigella sonnei*
Annual Meeting of the Committee of Carbohydrates of Chemistry of the Hungarian Academy of the Sciences, Mátrafüred, May 21-23, 2002

9. **A. Tóth**, I. Bajza, V. Pozsgay, A. Lipták
Synthesis of trisaccharide fragment of the O-specific polysaccharide of *Shigella sonnei*
Summer Course Glycosciences (7th European Training Course on Carbohydrates), Wageningen, June 23-27, 2002