A glikozidos-kötés kiterjesztése: diszulfid- és -szulfénamid típusú glikozidok szintézise és szerkezetvizsgálata

Doktori (Ph.D.) értekezés tézisei

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Témavezető: Dr. Szilágyi László

Extension of the glycosidic bond: syntheses and structural studies of diglycosyl disulfides and glycosyl sulfenamides

Ph.D. Theses

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Debreceni Egyetem, Természettudományi Kar

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1. Introduction

Carbohydrates occur in Nature in great abundance and in astonishing diversity. Although *monosaccharides* are involved in various biological processes and playing significant roles, most of the important physiological functions are associated with *glycosides* and *oligo*- or *polysaccharides*. These compounds are built of subunits and their building blocks are usually linked together through an *oxigen* atom via *two-bond* (sigma) linkages.

Analogues of oligosaccharides in which an N, S, Se or C-atom replaces the glycosidic O-atom are well known and have been investigated in detail. On the other hand, few structures featuring a *three-bond* interglycosidic linkage (3BIGL, C-X-Y-C, with X, Y = O, N, C), in place of the natural two-bond coupling between monosaccharide units, are known. The most important examples of natural three-bond interglycosidic linkage are the 1→6 bonds in oligo-and polysaccharides like in amylopectin. Some representatives of three-bond interglycosidic linkage with X=N, Y=O also occur in Nature as components of important antitumor antibiotics such as the *calicheamicin-esperamicin* family.

In the present thesis I will report on the syntheses of novel carbohydrate derivatives wherein the subunits are typically interconnected by three-bond interglycosidic linkage containing *two hetroatoms* different from oxygen.

Disulfide linkage (X=Y=S) play an essential role in stabilising tertiary structures of proteins, in the formation of cyclopeptides and in many biologically relevant systems. This structural motif is, however, virtually nonexistent within carbohydrates of either synthetic or natural origin.

We have therefore reasoned that the design a novel type of oligosaccharide scaffold wherein a disufide bridge replaces the interglycosidic oxygen would be of interest to affect properties that are involved in biological interactions of carbohydrates.

Another three-bond glycosidic connecting motif we have explored is the *sulfenamide* bond (X=S, Y=N). Although sulfenamides are synthetically useful and structurally interesting compounds with a number of practical applications, scant references can only be found in the literature among carbohydrate derivatives.

As a first step towards introducing the sulfenamide bond as an interglycosidic linkage the syntheses of some N-substituted S-glycosyl sulfenamides will be reported here using novel approaches.

The structures and conformational features of the new derivatives were investigated by NMR, X-ray and CD techniques in solution and in solid state as well.

2. Applied methods

The macro-, semimicro- and micro methods of modern preprative 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, mass spectrometry and X-ray crystallography were applied for the identification and characterization of the compounds prepared.

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.

The chiroptical properties of disulfide bond (S-S-) were studied by CD technique.

3. Results

The syntheses and structural studies of unsymmetrical *diglycosyl-disulfides* and *glucosyl-sulfenamides* are summerized below.

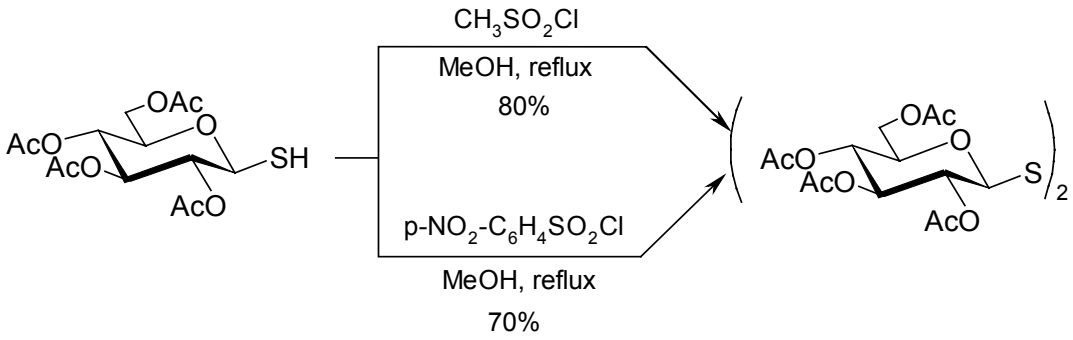
3.1. Syntheses of mixed disulfide disaccharides

S-S-Linked disaccharide model compounds were readily synthesised by adapting a general procedure to prepare unsymmetrical disulfides using alkylthiolsulfonate esters for transferring RS-groups to thiols:

$$R\text{-}SSO_2CH_3 + R'\text{-}SH \rightarrow R\text{-}S\text{-}S\text{-}R' + CH_3SO_2H$$

3.1.1. From 2,3,4,6-tetra-*O*-acetyl-β–D–glucopyranosyl methanetiolsulfonate (7)

For the synthesis of **7**[1] we reacted 2,3,4,6-tetra-*O*-acetyl-1-tio-β-D-glucopyranose **(20)** with methanesulfonyl chloride (A: CH_3SO_2Cl) but the reaction mixture contained the symmetrical diglucosyl-disulfide **19** as a major component. Similar results were obtained under various reaction conditions (see Table 1). Experiments were also conducted using p-NO_2-phenylsulfonyl chloride in reactions with **20**, but the expected p-NO_2-phenyl analogue (B: p-NO_2-$C_6H_4SO_2Cl$) of **7** could not be isolated either (Table 1).



20 **19**

Table 1

Reactions of thiol **20** with alkyl- and aryl- sulfonyl chlorides

Thiol	Sulfonyl-chloride	R. conditions	Thiol: Sulfonylchloride	R. mixture
20	A	MeOH, 4ºC, Et_3N	1 : 1	Complex
	A	MeOH, 4ºC, Et_3N	1 : 3	Complex
	A	MeOH, rt.	1 : 1	**19** (major prod.)
	A	MeOH, rt., Et_3N	1 : 1	**19** (major prod.)
	A	MeOH, rt., Et_3N	1 : 5	Complex
	A	DMF, 4ºC, Et_3N	1 : 3	**19** (major prod.)
	B	MeOH, rt., Et_3N	1 : 1	**19** (major prod.)
	B	MeOH, 4ºC, Et_3N	1 : 3	Complex

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

7 was therefore prepared from acetobromoglucose by reaction with $NaSSO_2CH_3$, as described in the literature, and reacted with 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-aldopyranoses (**23**: *Man*, **30**: *Gal*, **31**: *Gal(1→4)Glc*, **32**:*GlcNAc*) to furnish the protected β,β-(1,1')-dithia-disaccharides **(38-41)** in fair (78-80%) yields. These products could be smoothly deacetylated under Zemplén's conditions and unprotected S-S-disaccharides **(42-45)** were obtained in near quantitative yields (see Table 2).

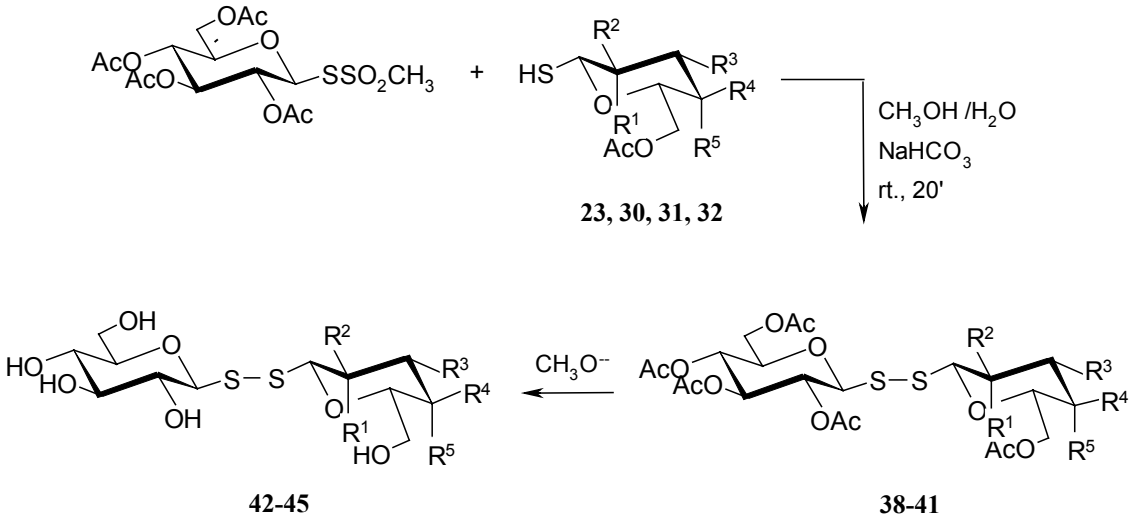


Table 2

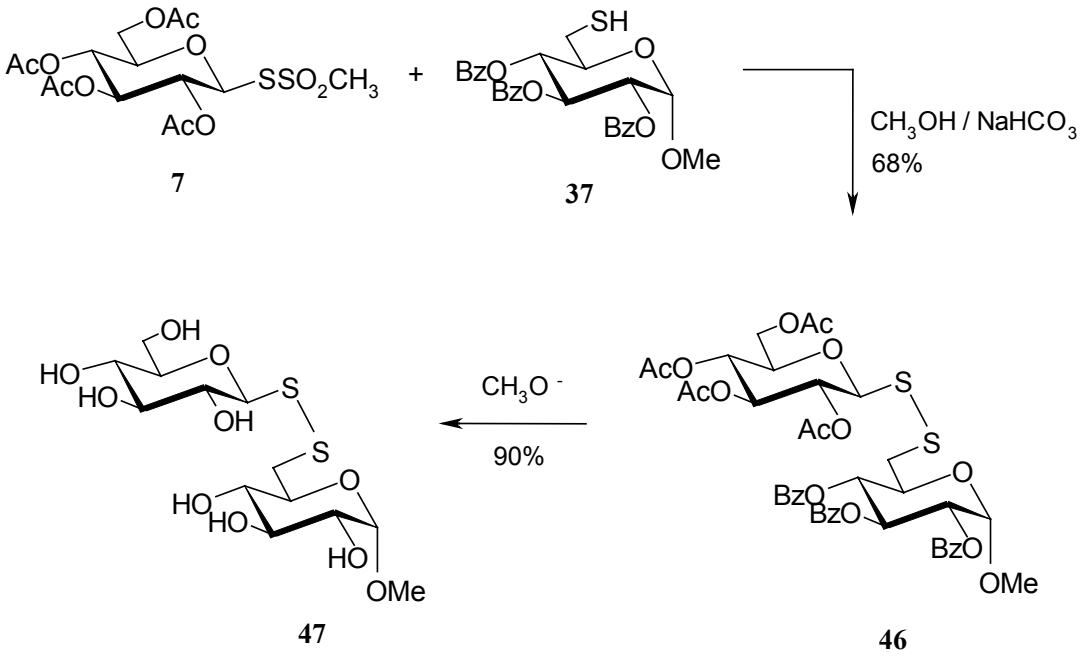
Disulfide-disaccharides

Comp.	R^1	R^2	R^3	R^4	R^5	R^6	Yield (%)
38	H	NHAc	OAc	OAc	H	Ac	78
42	H	NHAc	OH	OH	H	H	98
39	OAc	H	OAc	OAc	H	Ac	73
43	OH	H	OH	OH	H	H	98
40	H	OAc	OAc	H	OAc	Ac	80
44	H	OH	OH	H	OH	H	92
41	H	OAc	OAc	[a]	H	Ac	80
45	H	OH	OH	[b]	H	H	90

[a] R^4=2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl

[b] R^4= β-D-galactopyranosyl

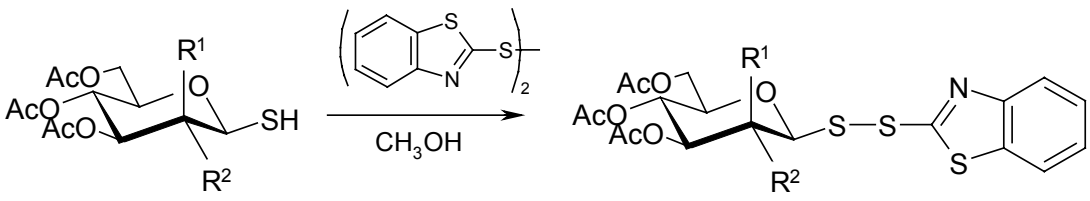
Analogous reaction with methyl 6-thio-2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside **(37)** as the thiol component furnished an 1→6-SS-linked disaccharide **(46)**, featuring a *four-bond* interglycosidic linkage (4BIGL).



Exploratory experiments were conducted to synthesise novel derivatives that are either more conveniently accessible than, and/or possessing glycosylthio-transfer properties superior to **7.** These derivatives containing an electrophilic divalent sulfur attached to the anomeric carbon are either glycosyl-aryl disulfides (Gly-SS-Ar) or S-glycosyl-N-acyl sulfenamides. The electrophilic character of the sulfur mentioned is due to the electron withdrawing effect of the substituents attached to it.

3.1.2. 2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl- aryl disulfides

Reactions of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose **(20)** and 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-mannopyranose **(23)** with 2,2'-dithiobis-(benzothiazol) **(21)** furnished the glycosyl-aryl disulfides **22** and **24**.



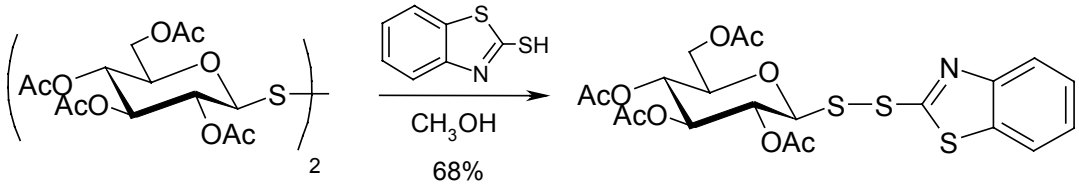
20 R^1= H, R^2= OAc

23 R^1= OAc, R^2= H

22 R^1= H, R^2= OAc (65%)

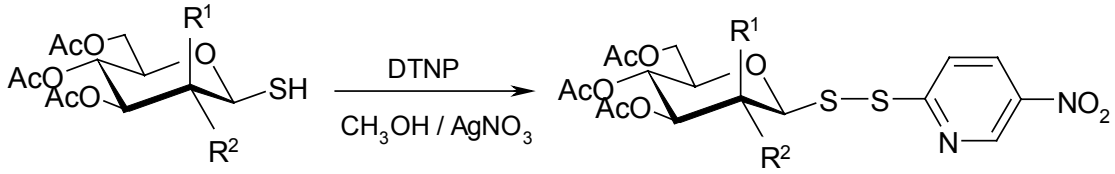
24 R^1= OAc, R^2= H (74%)

The synthesis of **22** was also carried out from bis-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-disulfide **(19)** with 2-thio-benzothiazol, under similar reaction conditions.



19 **22**

Experiments were conducted to synthesise new glycosyl-aryl mixed disulfides in the reactions of glycosyl thiols with 2, 2'-ditiobis-(5-nitropyridin) (DTNP) (see Table 3) as well.



20 R^1= H, R^2= OAc

23 R^1= OAc, R^2= H

25 R^1= H, R^2= OAc

26 R^1= OAc, R^2= H

Table 3

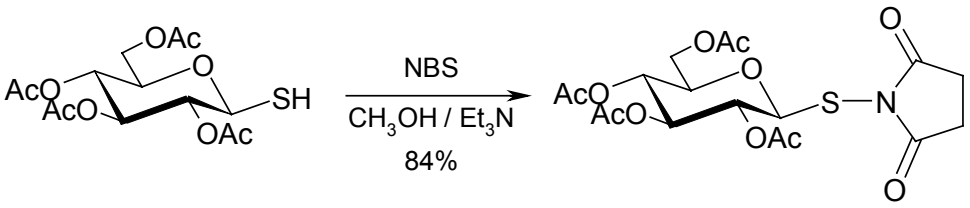
Reactions of 2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl tiols with DTNP[a]

Thiol	Product	Yield (%)	
20	**25**	58[b]	35[c]
23	**26**	53[b]	30[c]

[a] Thiol: DTNP ratio 1:1.1, [b] in methanol, in the presence of 1 eq. $AgNO_3$, [c] in methanol, in the absence of $AgNO_3$

2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-succinimidoyl sulfide (27)

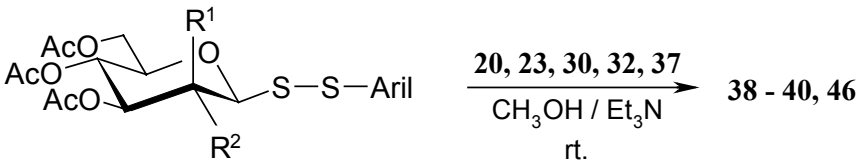
The sulfenamide type sulfenyl-transfer reagent tetra-*O*-acetyl-β-D-glucopyranosyl-succinimidoyl sulfide **(27)** was easily prepared from tetra-*O*-acetyl-1-thio-β-D-glucopyranose and N-Br-succinimide:



20 **27**

3.1.3. Synthses of disulfide-disaccharides from glycosyl- aryl disulfides

The efficiency of the new glycosylsulfenyl-transfer reagents, with respect to glycosyl thiols was systematically investigated (see Table 4).



Aryl: 2-benzthiazolyl **22** R^1= H, R^2= OAc

24 R^1= OAc, R^2= H

Aryl: 5-nitro-2-pyridyl **25** R^1= H, R^2= OAc

26 R^1= OAc, R^2= H

It was established that the yields of the target (1,1')-dithia-disaccharides, using reagents of the mixed disulfide type Gly-SS-Ar, are comparable to those obtained with **7** under similar reaction conditions. The mixed disulfides Gly-SS-Ar are, however, more convenient to synthesise than **7** by taking advantage of disulfide – thiol exchange reactions between symmetrical aryl disulfides (Ar-SS-Ar) and glycosyl thiols (Gly-SH).

Table 4

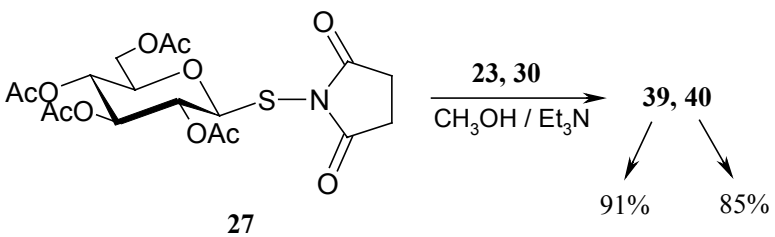
Synthses of disulfide-disaccharides from glycosyl- aryl disulfides [a, b]

Thiol donor	Thiol	Product	Yield (%)
24	**20**	**39**	41
22	**23**	**39**	45
22	**30**	**40**	43
22	**32**	**38**	40
22	**37**	**46**	40
26	**20**	**39**	50
25	**23**	**39**	53
25	**30**	**40**	48
25	**37**	**46**	45

[a] Thiol: disulfide ratio1:1

[b] Reaction. time 20'

On the other hand, the sulfenamide type sulfenyl-transfer reagent tetra-*O*-acetyl-β-D-glucopyranosyl-succinimidoyl sulfide **(27)** gave rise to the formation of unsymmetrical diglycosyl disulfides **(39, 40),** in yields significantly higher than with **7,** in reactions with glycosyl thiols under mild conditions.



27

3.1.4. Disulfide – thiol exchange with diglucosyl disulfide 19

$$\underset{A}{(GlcS)_2} + \underset{B}{Glc'\text{-}SH} \rightleftharpoons \underset{C}{Glc\text{-}S\text{-}S\text{-}Glc'} + \underset{D}{Glc\text{-}SH}$$

19 was reacetd with various glycosyl thiols (**23**: *Man*, **30**: *Gal*, **31**: *Gal(1→4)Glc*, **32**:*GlcNAc*) in methanol; the pH of the reaction mixture being controlled by addition of aqueous buffers. The ratios of the symmetrical / unsymmetrical diglycosyl disulfies (A/C) in the equilibrium mixture were determined from ^{1}H NMR spectra (see Table 5).

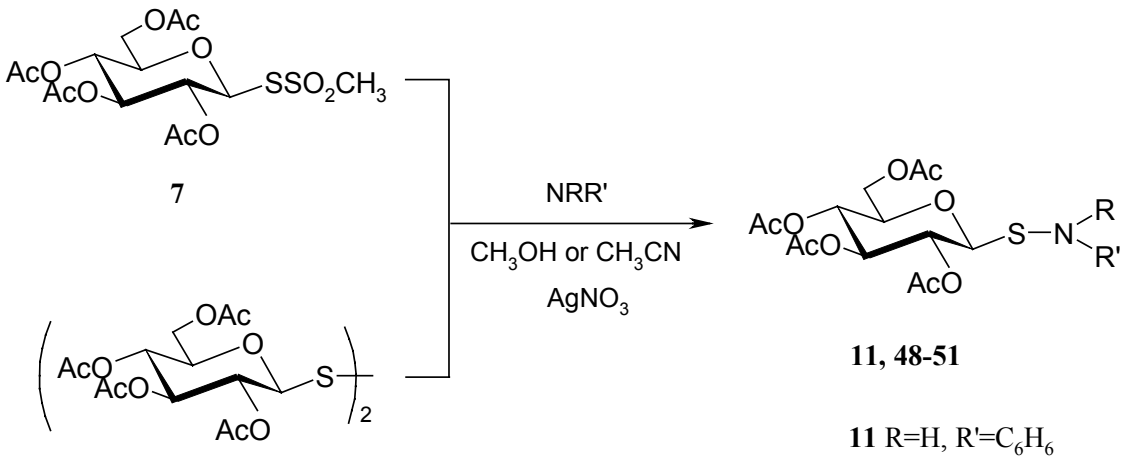
Table 5

Disulfide - thiol exchange with disulfide **19**

Thiol	Thiol: disulfide ratio	pH	A/C ratio
30	1 : 1	8. 5	45: 55
23	1 : 1	8. 5	45: 55
31	1 : 1	8. 5	45: 55
32	1 : 1	8. 5	45: 55
30	1.5 : 1	10. 0	40: 60
23	2 : 1	10. 0	25: 75

3.2. Syntheses of *N*-substituted *S*-glucosyl-sulfenamides

We have started to synthesise N-substituted S-glucosyl sulfenamides from **7** through nucleophilic displacement reactions with simple aliphatic and aromatic amines. Benzylamine reacts readily with **7** under mild conditions furnishing S-(tetra-*O*-acetyl-β-D-glucopyranosyl)-N-benzyl sulfenamide **(48)** in good yield. The reactions were more sluggish with secondary amines or with the aromatic aniline, whereas no reaction at all occurred with the sterically hindered 1-adamantylamine (Ad-NH_2) (see Table 6).



11 R=H, R'=C_6H_6

48 R=H, R'=C_6H_6CH

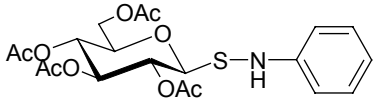
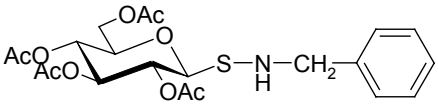
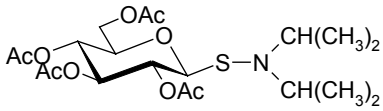
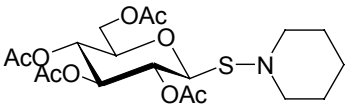
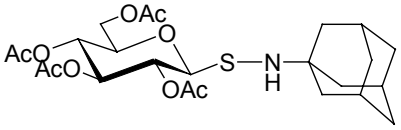
49 R=R'=$CH(CH_3)_2$

50 R=-$CH_2(CH_2)_3$-CH_2-

51 R=H, R'=1-adamantyl

Table 6

Glucosyl sulfenamides and reaction conditions

Comp	Sulfenamides	Route	Reagents[a]	Solvent	Yield (%)
11		6	1 : 2	CH_3OH	32
		7	1 : 8	CH_3CN	51
48		6	1 : 4	CH_3OH	45[b]
		6	1 : 8	CH_3CN	57[b]
		7	1 : 4	CH_3OH	60
		7	1 : 8	CH_3CN	87
49		6	1 : 4	CH_3OH	41
		7	1 : 4	CH_3CN	43
50		6	1 : 4	CH_3OH vagy CH_3CN	51
		7	1 : 4	CH_3OH	53
51		6	1 : 4	CH_3OH	15

[a] Thiol donor: amine ratio; 1 eq. $AgNO_3$

[b] In absence of $AgNO_3$ we achieved comparable yields

In another approach, the cleavage of the S-S-bond in symmetrical disulfides with amines, under silver ion activation, was studied (see Table 6). Bis-(tetra-*O*-acetyl-β-D-glucopyranosyl) disulfide **(19)** underwent smooth reactions with various amines and, those which showed low reactivity towards **7** proved to be much more reactive with **19** under these conditions. It was furthermore found that silver ion activation significantly enhanced the reactivity of **7** so as to enable formation of the sterically highly hindered sulfenamide with Ad-NH_2, albeit in low yield (see Table 6).

3.3. Structure analysis of the new coumpounds .

The anomeric configurations were determined from the $^{3}J_{H1\text{-}H2}$ couplings except in the case of mannose derivatives where the $^{2}J_{H1\text{-}C2}$ and $^{3}J_{H1\text{-}C3}$ values provided unequivocal confirmation for the same. The primary structures of the *S*-glycosyl-*N*-substituted sulfenamides were proved by ^{15}N-HMBC measurements; the connectivity across the SS-bond in the asymmetric disulfide-disaccharides was ascertained by NOE and X-ray measurements in addition to standard analytical data.

Conformational features of these derivatives were probed by NMR, X-ray and CD techniques. In solution, spatial proximity of the glycopyranosyl rings, rather than extended conformations, were revealed by interannular NOE contacts. In solid phase X-ray data showed the C1-S-S-C1' torsional angle to be close to the value (~ 80^{o}) found in unconstrained disulfides and prevailing of the exo-anomeric effect for both rings.

CD measurements have established the predominance of *M* chirality for the S-S bond both in solution and in solid phase.

4. Summary

We have elaborated new procedures to synthesise unsymmetrical disulfide-di-(and tri-) saccharides. The methanetiolsulfonate reagent **7** furnished the desired products in reactions with 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-aldopyranoses in good yields. Experiments were conducted to synthesise novel glycosyl-aryl disulfides derivatives and sulfenamide type sulfenyl-transfer reagents and the efficiency of the new glycosylsulfenyl-transfer reagents was investigated with respect to glycosyl thiols. The disulfide – thiol exchange with **19** was also studied in the synthesis of disulfide-disaccharides.

New methods were elaborated for the syntheses of N-substituted S-glucosyl sulfenamides from **7.** The cleavage of the S-S-bond in the diglucosyl disulfide **19** with amines, under silver ion activation, was studied.

The structures and conformational features of the new derivatives were investigated by NMR, X-ray and CD techniques in solution and in solid phase.

4. List of publications

Papers related to the subject of the dissertation

1. László Szilágyi, **Tünde-Zita Illyés**, Pál Herczegh
   Elaboration of a novel type of interglycosidic linkage: syntheses of disulfide disaccharides,
   Tetrahedron Lett. **2001**, *42*, 3901-3903

2. **Tünde-Zita Illyés**, Dóra Molnár-Gábor, László Szilágyi
   Novel approaches to the syntheses of *N*-substituted *S*-glycosyl-sulfenamides,
   Carbohydr. Res. **2004**, accepted for publication

Other paper

3. László Szilágyi, **Tünde-Zita Illyés,** Zoltán Györgydeák, György Szabó, András Karácsony
   Syntheses of partially hydrogenated [1, 2, 4]-triazolo-[4, 5-a]-pyrimidin-4-ones through cyclization of 2-arylidenhydrazino-6-methyl-4-pyrimidones,
   ARKIVOC, **2004**, accepted for publication

Lectures (L) and posters (P) related to the subject of the dissertation

1. László Szilágyi, Pál Herczegh, Tünde-Zita Illyés
   Elaboration of a novel type of interglycosidic linkage: syntheses of disulfide oligosaccharides: 8th Belgian Organic Syntheses Symposium, Ghent, July10-14, 2000, Belgium (P)

2. Illyés Tünde-Zita, Herczegh Pál, Bényei Attila, Szilágyi László
   Diszulfid diszacharidok szintézise és szerkezetvizsgálata: MTA Kém. Tud. Oszt., Szénhidrátkémiai Munkabizottság Előadóülése, Mátrafüred, 2001. május 14-16. (L)

3. Illyés Tünde-Zita, Herczegh Pál, Bényei Attila, Szilágyi László
   Diszulfid diszacharidok szintézise és szerkezetvizsgálata: Vegyészkonferencia, Hajdúszoboszló, 2001. június 27-29. (P)

4. Tünde-Zita Illyés, László Szilágyi, Pál Herczegh
   Syntheses of disulfide-linked disaccharide mimics: 11th European Carbohydrate Symposium, Lisbon, September 2-7, 2001, Portugal (P)

5. László Szilágyi, Krisztina Fehér, Attila Bényei, Tünde-Zita Illyés
   Conformational preferences in diglycosyl disulfides: 11th European Carbohydrate Symposium, Lisbon, September 2-7, 2001, Portugal (P)

6. Illyés Tünde-Zita, Molnár-Gábor Dóra, Szilágyi László
   A glikozid kötés kiterjesztése: glikozil-diszulfidok és-szulfénamidok: VIII. Nemzetközi Vegyészkonferencia, Kolozsvár, 2002. november 15-17. (L)

7. Forgó Péter, Fehér Krisztina, Illyés Tünde-Zita, Szilágyi László
   Diszulfidok-diszacharidok konformációjának vizsgálata MNR módszerekkel: Vegyészkonferencia, Hajdúszoboszló, 2003. június 26-28. (P)

8. Kurtán Tibor, Illyés Tünde-Zita, Bényei Attila, Antus Sándor, Szilágyi László
   Dikalkogenid-diszacharidok térszerkezetének vizsgálata oldatban és szilárd Fázisban: Vegyészkonferencia, Hajdúszoboszló, 2003. június 26-28. (P)

9. Tünde-Zita Illyés, Dóra Molnár-Gábor, László Szilágyi
   Extension of the glycosidic bond: glycosyl disulfides and sulfenamides: 13th European Symposium on Organic Chemistry, Cavtat-Dubrovnik, September 10- 15, 2003, Croatia (P)

Other lectures and posters

10. Hadady Zsuzsa, Illyés Tünde-Zita, Szilágyi László, Somsák László, Docsa Tibor, Tóth Béla, Gergely Pál
    Hetreocyclic derivatives of D-glucose as potential inhibitors of glycogen phosphorylase enzymes: Ann. Meeting Carbohydr. Discussion Group, H.A.S. Mátrafüred, 2002. may 21-23 (L)

11. Katalin E. Kövér, Krisztina Fehér, Tünde-Zita Illyés, László Szilágyi, Gyula Batta, Stefan Berger
    Accurate determination of small one-bond heteronuclear residual dipolar couplings bymodified heteronuclear correlation experiments: 44th ENC Conference, Savannah, GA, USA, March 30 – April 4, 2003 (P)