ACCESS TO *C*-GLYCOSYL-IMINE TYPE COMPOUNDS AND *C*-GLYCOSYL-METHYLENE CARBENES AND INVESTIGATION OF THEIR PROPERTIES

Ph.D. theses

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1. Introduction and aim of the dissertation

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 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.

The formation and cleavage of the glycosidic bonds are essential in the construction and degradation of biologically important carbohydrate derivatives. These processes are catalysed by glycosyl-transferases and glycoside-hydrolases (glycosidases). Inhibitors of these enzymes are widely used in the study of the molecular mechanism of the enzyme function and for mapping the active site. Inhibitor compounds are potent drug candidates in a variety of diseases.

It is known from the literature that azasugars with different ring sizes, among them seven-membered ones are good glycosidase inhibitors. Expectedly seven-membered ring sugars may also exert similar effects.

Based on this background we envisaged to investigate the generation and ensuing reactions of glycosyl-methylene carbenes offering a possibility for a new synthetic route toward seven-membered ring sugars.

Another goal of our work was to synthesize carbohydrate derivatives with a C=N bond on the exocyclic carbon next to the anomeric center. Although there are lots of possibilities for the transformation of these derivatives – nucleophilic additions to the C=N bond, inter- and intramolecular addition of radicals to the C=N bond, cycloaddition reactions, Mannich-type reactions, Bamford-Stevens reaction of tosylhydrazones etc. – this type of molecules is little known in the carbohydrate field.

2. Applied methods

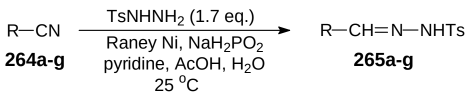
In the course of our work we have applied macro-, semimicro- and micro methods of modern preparative organic chemistry. Reactions were monitored by thin-layer chromatography. The isolation and purification of the products were carried out by crystallization or by column chromatography. Products were identified by classical (elemental analysis, melting point and optical rotation measurement) and modern analitical methodes (IR, ^{1}H-,^{13}C-NMR, X-ray crystallography).

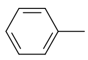
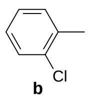
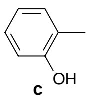
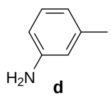
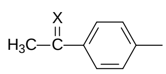
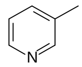
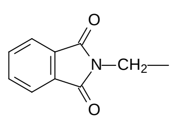
3. New results of the dissertation

3.1. Conversion of nitriles to aldehyde-tosylhydrazones

To achieve our goals first we worked out a general method for the synthesis of aldehyde-tosylhydrazones from nitriles. In the new *one-pot* reaction nitriles were reduced by Raney-Ni and sodium-hypophosphite in water-acetic-acid-pyridine in the presence of tosylhydrazine, as trapping agent at room temperature or at 40° C. The method was applied for an aliphatic (**264g**[1]), some aromatic (**264a-f**) (table 1), and several anhydro-aldononitriles (**210a-g**) (table 2). The desired products (**265a-e,g** and **266a-g**), with the exception of the **265f** pyridine derivative (20%), were isolated in good yields (55-100%).

table 1 Conversion of aliphatic and aromatic nitriles to aldehyde-tosylhydrazones

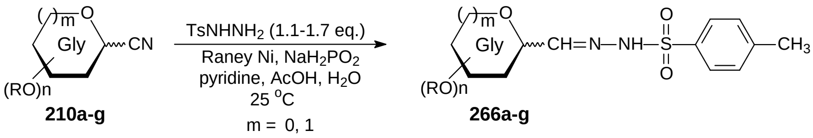


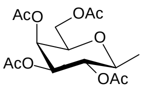
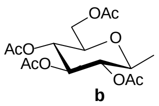
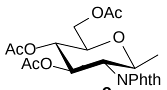
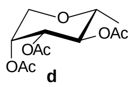
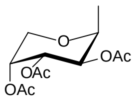
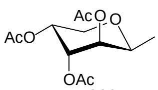
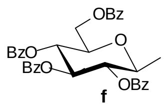
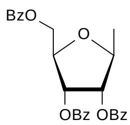
Entry	R	Isolated yield (%)
1	 **a**	96
2	 **b**	85
3	 **c**	88
4	 **d**	83
5	 **e** X = O in the starting material X = NNHTs in the product	80[a]
6	 **f**	20[b]
7	 **g**	75

[a] 3 equivalents. of $TsNHNH_2$ were used.
[b] The reaction was performed in acetic acid-water mixture.

[1] Numbering of compounds refers to one in the dissertation.

table 2 Conversion of anhydro-aldononitriles to anhydro-aldose-tosylhydrazones



Entry	Gly	$TsNHNH_2$ (eq.)	Isolated yield (%)
1	 **a**	1.7	55
2		1.1	90[a]
3	 **b**	1.7	60[b]
4	 **c**	1.2	58
5	 **d**	1.2	73
6	 **210e**  **266e**	1.2	69
7	 **f**	1.7	complex reaction mixture
8		1.7	64[c]
9	 **g**	1.7	100[a]

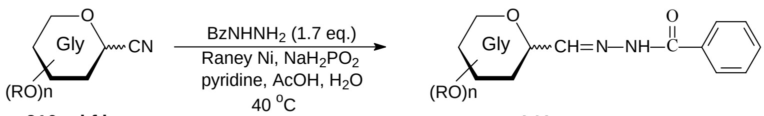
[a] Crude product.
[b] Conversion: 94%.
[c] Reaction temperature: 40 °C.

The method could not be applied for the transformation of the 1-substituted glycosyl-cyanides (1-substituents: **267**, NHAc; **268**, OAc; **105**, N_3; **269**, F) and the **270** 1-cyano-galactal to the corresponding tosylhydrazones. Reactions of **105** and **268-270** resulted in complex reaction mixtures, from which tosylhydrazones could not be isolated. The **267** cyano-acetamide could not be transformed even at higher temperature (60 °C).

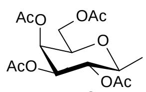
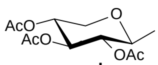
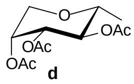
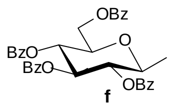
3.2. Conversion of anhydro-aldononitriles to anhydro-aldose-benzoylhydrazones and -semicarbazones

Using benzoylhydrazine as trapping agent anhydro-aldononitriles (**210a,d,f,h**) were transformed to the corresponding benzoylhydrazone derivatives (**271a,d,f,h**) in 58-90% yields (table 3), while with semicarbazide the **272a,d,f,h** anhydro-aldose-semicarbazones were prepared in 54-89% yields (table 4).

table 3 Conversion of anhydro-aldononitriles to anhydro-aldose-benzoylhydrazones

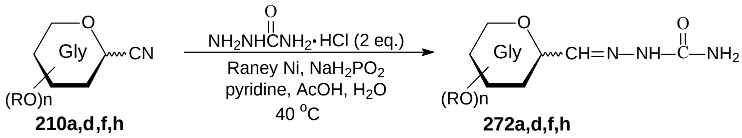


210a,d,f,h **271a,d,f,h**

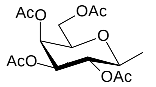
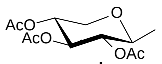
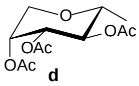
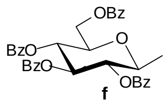
Entry	Gly	Isolated yield (%)
1	 **a**	86
2	 **h**	58
3	 **d**	84[a]
4	 **f**	90[a]

[a] Crude product.

table 4 Conversion of anhydro-aldononitriles to anhydro-aldose-semicarbazones



210a,d,f,h **272a,d,f,h**

Entry	Gly	Isolated yield (%)
1	 **a**	54
2	 **h**	89[a]
3	 **d**	65[a]
4	 **f**	63

[a] Crude product.

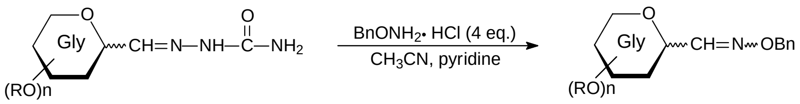
The method proved not suitable for the synthesis of oximes and Schiff-bases. The reaction of 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-D-glycero-L-manno-heptononitrile (**210a**) with hydroxylamine-hydrochloride, *O*-benzyl-hydroxylamine-hydrochloride and benzylamine provided the **263** 1-formyl-galactal (15-69%) and in some cases the starting material (**210a**) (5-38%). Transformations with aniline and phenylhydrazine-hydrochloride gave complex reaction mixtures unseperable by column chromatography.

3.3. Synthesis of *O*-benzyl-(anhydro-aldose)-oximes, anhydro-aldose-oximes and -thiosemicarbazones

The synthesis of *O*-benzyl-(anhydro-aldose)-oximes and anhydro-aldose-oximes was achieved by transimination reactions of anhydro-aldose-semicarbazones. In these syntheses the anhydro-aldose-semicarbazones (**272a,d,f**) were reacted with *O*-benzyl-hydroxylamine-hydrochloride and hydroxylamine-hydrochloride in acetonitrile-pyridine at room temperature. The corresponding *O*-benzyl-(anhydro-aldose)-oximes (**273a,d,f**) (table 5) and anhydro-aldose-oximes (**186a,g,f**) (table 7) could be isolated in good yields (65-96%) as mixtures of diastereomers. Separation of the diastereomers could not be carried out by column chromatography.

To make the reaction more simple **272a** and **272d** were reacted with *O*-benzyl-hydoxylamine-hydrochloride in glacial acetic-acid, the corresponding diastereomeric *O*-benzyl-oximes were isolated in 90-79% yields (table 6).

table 5 Conversion of anhydro-aldose-semicarbazones to *O*-benzyl-(anhydro-aldose)-oximes I.



272a,d,f **273a,d,f**

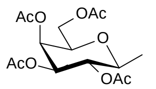
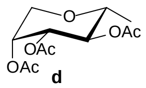
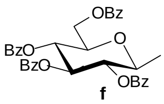
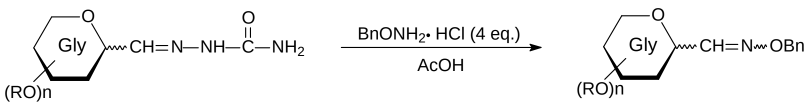
Entry	Gly	Reaction time (h)	Diastereomeric ratio (*E*/*Z*)	Isolated yield (%)
1	 **a**	27	13:1	95
2	 **d**	19	5:1	79
3	 **f**	19	7:1	84

table 6 Conversion of anhydro-aldose-semicarbazones to *O*-benzyl-(anhydro-aldose)-oximes II.



272a,d **273a,d**

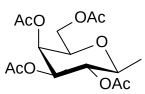
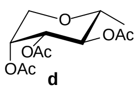
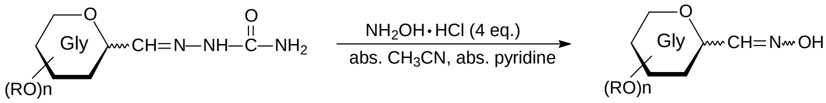
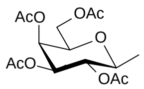
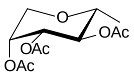
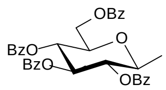
Entry	Gly	Reaction time (h)	Diastereomeric ratio (*E*/*Z*)	Isolated yield (%)
1	 **a**	24	11:1	90
2	 **d**	24	10:1	79

table 7 Conversion of anhydro-aldose-semicarbazones to anhydro-aldose-oximes



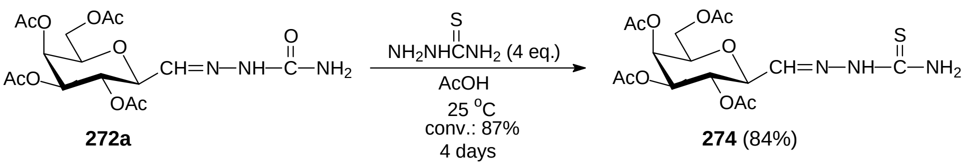
272a,d,f **186a,g,f**

Entry	Gly	Reaction time (h)	Diastereomeric ratio (*E/Z*)	Isolated yield (%)
1	 **a**	24	7:1	65
2	 **272d, 186g**	24	5:1	96[a]
3	 **f**	24	20:1	91

[a] Crude product.

The synthesis of Schiff-bases could not be achieved by the transimination reaction of anhydro-aldose-semicarbazones. Reaction of the **272a** semicarbazone with aniline- and benzylamine-hydrochloride could be performed neither in acetonitrile-pyridine mixture, nor in glacial acetic-acid even using 10 equivalents of the amines.

Thiosemicarbazone (**274**) was synthesized by the transimination reaction of 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-D-glycero-L-manno-heptose-semicarbazone (**272a**). Good yield (84%, conv.: 87%) was achieved only when glacial acetic-acid was used as solvent instead of the acetonitrile-pyridine mixture (scheme 1).



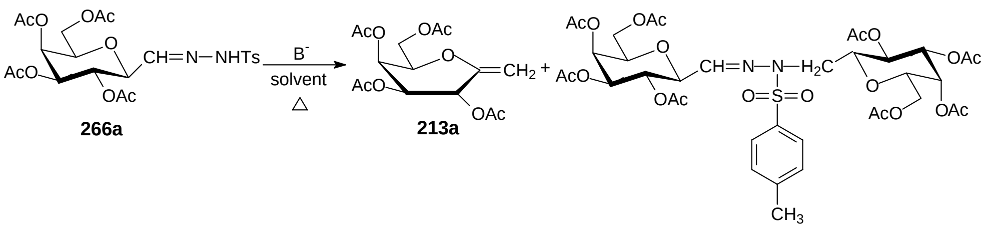
scheme 1

3.4. Synthesis of *exo*-glycals (2,5- or 2,6-anhydro-1-deoxy-hex- or -hept-1-enoses)

Study of the thermolytic version of the Bamford-Stevens reaction with **266a** using different bases (1.2-5 eq. Bu_4NOH, NaH, NaOMe, KO*t*-Bu) and different solvents (nitrobenzene, *t*-BuOH, 1,4-dioxane, diglyme) at different reaction temperatures showed the formation of two products, the **213a** *exo*-glycal (7-59%) and the **275a** tosylhydrazone derivative (14-31%) (table 8).

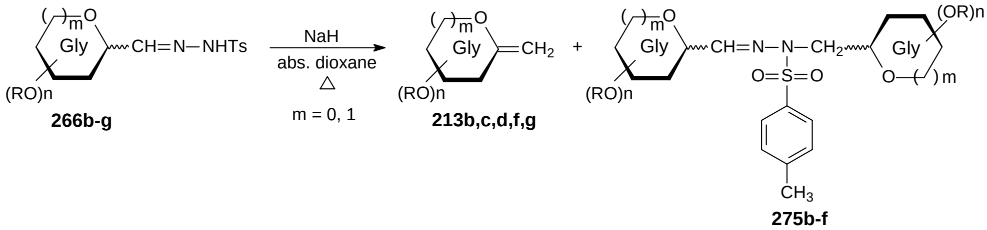
On optimization of the reaction conditions (10 eq. NaH, 1,4-dioxane, reflux temperature) *exo*-glycals (**213a,c,d,f**) (72-86%) were isolated as sole products from the **266a,c,d,e,f** anhydro-aldose-tosylhydrazones. There were only two exceptions: in the case of 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-D-glycero-D-gulo-heptose-tozylhydrazone (**266b**) beside the **213b** *exo*-glycal (39%) the **275b** tosylhydrazone derivative (22%) was also isolated even when 10 equivalents of sodium-hydride were used, otherwise in the case of 3,4,6-tri-*O*-benzoyl-2,5-anhydro-D-allo-hexose-tozylhydrazone (**266g**) the **213g** *exo*-glycal was isolated as the sole porduct in 50% yield when only 2 equivalents of sodium-hydride were applied
(table 9).

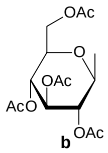
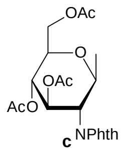
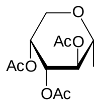
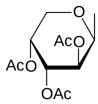
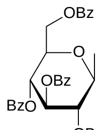
table 8 Thermolysis of the salts of the **266a** anhydro-aldose-tosylhydrazone



Entry	Base (eq.)	Solvent	T (°C)	Isolated yield (%)	
				213a	**275a**
1	Bu_4NOH (1.5)	nitrobenzene	210	not isolated	
2	KO*t*-Bu (2)	*t*-BuOH	80	7	not isolated
3	NaOMe (2)	1,4-dioxane	100	26	19
4	NaH (1.2)	1,4-dioxane	100	52	17
5	NaH (2.4)	diglyme	110	8	18
6	NaOMe (3)	diglyme	110	15	14
7	NaH (1.2)	1,4-dioxane	40	no transformation	
8	NaH (1.2)	1,4-dioxane	60	no transformation	
9	NaH (1.2)	1,4-dioxane	80	10	31
10	NaH (5)	1,4-dioxane	100	59	not isolated
11	NaH (10)	1,4-dioxane	100	77	not formed

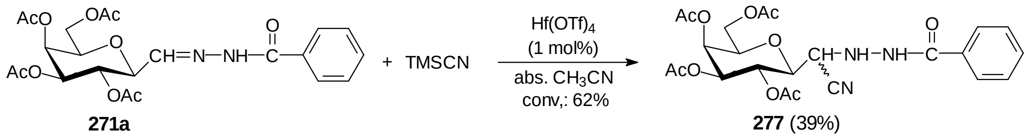
table 9 Thermolysis of the salts of anhydro-aldose-tosylhydrazones



Entry	Gly	NaH (eq.)	Isolated yield (%)	
			213	**275**
1		1.7	11	59
2	**b**	10	39	22
3	 **c**	10	74	not formed
4		1.2	25	30
5	**d**	10	86	not formed
6		2	18	not isolated
7	**266e, 213d**	10	7	not formed
8		2.2	16	45
9	**f**	10	72	not formed
10		2	50	not formed

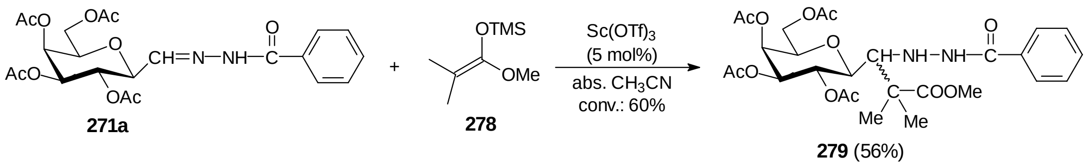
3.5. Study of nucleophilic addition reactions of *C*-nucleophiles to the C=N bond of 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-D-glycero-L-manno-heptose-benzoylhydrazone

Preliminary experiments were carried out in the field of nucleophilic additions to the C=N bond of anhydro-aldose-benzoylhydrazones. The reaction of 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-D-glycero-L-manno-benzoylhydrazone (**271a**) with trimethyl-silyl-cyanide in the presence of $Hf(OTf)_4 \cdot H_2O$ provided an α-amino-α-(β-D-galactopyranosyl)-acetic-acid derivative (**277**) in 39% yield (conv.: 62%) (scheme 2).



scheme 2

The Mannich-type reaction of the **271a** anhydro-aldose-benzoylhydrazone with the **278** ketene-silyl-acetal in the presence of $Sc(OTf)_3$ resulted in the formation of an α-(β-D-galactopyranosyl)-β-amino carboxylicacid derivative (**279**) in 56% yield (conv.: 60%) (scheme 3).



scheme 3

4. Possible utilization of the results

The work presented is a basic research in the field of carbohydrate chemistry resulting in methodological developments. The new synthetic methods for *C*-glycosyl-imine derivatives represent new alternative procedures which facilitate to avoid the use of strongly toxic tin compounds.

Addition reactions to the C=N double bond can provide new routes to the synthesis of many glycobiologically important derivatives, such as *C*-glycosyl-amino-acids, *C*-glycosides, *C*-disaccharides and the potential glycosyl-transferase inhibitor *C*-glycosyl-methyl-phosphonates as well. Cycloaddition reactions to the C=N bond can also be important in the synthesis of sugar-β-lactam derivatives.

The new route leading to *exo*-glycals makes especially the acylated derivatives easily available.

5. Publications

1. **Marietta Tóth,** László Somsák
   One-pot transformation of nitriles into aldehyde tosylhydrazones
   Tetrahedron Letters **2001**, *42*, 2723-2725

2. **Marietta Tóth**, László Somsák
   exo-Glycals from glycosyl cyanides. First generation of *C*-glycosylmethylene carbenes from 2,5- and 2,6-anhydroaldose tosylhydrazones
   J. Chem. Soc., Perkin Trans. 1 **2001**, 942-943

3. 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
   J. Med. Chem. **2001**, *44*, 2843-2848

4. Tünde Kiss, Andrea Székely, **Marietta Tóth**, László Somsák, László Kiss
   Inhibition of β-D-xylosidase (EC 3.2.1.37) from *Aspergillus carbonarius* by β-D-xylopyranose and D-xylal derivatives
   manuscript in preparation

5. **Marietta Tóth**, Katalin E. Kövér, Attila Bényei, László Somsák
   C-Glycosylmethylene Carbenes: Synthesis of Anhydro-aldose-tosylhydrazones as Precursors, Generation, and a New Route to *exo*-Glycals
   manuscript in preparation

6. **Marietta Tóth**, László Somsák
   Synthesis of *C*-glycosylimine derivatives
   manuscript in preparation

6. Lectures, posters

1. **Marietta Tóth**, László Somsák
   Studies on the generation and reactivity of glycosyl-methylene carbenes
   MTA Szénhidrátkémiai Munkabizottság előadóülése, Mátrafüred, 1999. máj. 26-27., lecture

2. **Marietta Tóth**, Veronika Nagy, László Somsák
   Synthesis of D-gluco- and D-xylopyranosylidene-spiro(thio)hydantoins and their effect on muscle and liver glycogen phosphorylases
   MTA Szénhidrátkémiai Munkabizottság előadóülése, Mátrafüred, 1999. máj. 26-27., lecture

3. Somsák László, Ősz Erzsébet, Kovács László, Gyóllai Viktor, **Tóth Marietta**, Szilágyi László
Glikozilidén-spiro-heterociklusok: a glikomimetikumok újabb képviselői
MTA Heterociklusos Kémiai Munkabizottság előadóülése, Balatonszemes, 1999. máj. 27-28., lecture

4. Kovács László, **Tóth Marietta**, Ősz Erzsébet, Szilágyi László, Docsa Tibor, Tóth Béla, Gergely Pál
Glikopiranozilidén-spiro-(tio)hidantoinok szintézise és glikogén-foszforiláz inhibíciós hatásuk vizsgálata
MKE, Vegyészkonferencia, Eger, 1999. jún. 22-24., 58. o., poster

5. Tibor Docsa, Béla Tóth, Pál Gergely, Erzsébet Ősz, László Kovács, **Marietta Tóth**, László Somsák and László Szilágyi
Inhibition of muscle and liver glycogen phosphorylases by glycopyranosylidene-spiro-(thio)hydantoins – *in vitro and in vivo* studies
Eurocarb 10, Galway, Ireland, July 11-16 1999, PD013, p 385., poster

6. **Tóth Marietta**
Glikozil-metilén-karbének generálása és reakcióik vizsgálata
MKE, XXII. Kémiai Előadói Napok, Szeged, 1999. nov. 1-3., lecture

7. **Marietta Tóth**, László Somsák
Synthesis and transformation of *C*-glycosyl-aldehyde-hydrazones
MTA Szénhidrátkémiai Munkabizottság előadóülése, Mátrafüred, 2000. máj. 31-jún. 1., lecture

8. **Marietta Tóth**, László Somsák
Synthesis of *C*-glycosyl-aldehyde-hydrazones and their transformation to *exo*-glycals
20th International Carbohydrate Symposium, Hamburg, Germany, Aug 27-Sep 1 2000, B-364, poster

9. **Tóth Marietta**, Somsák László
C-glikozil-aldehid-hidrazonok szintézise és átalakításuk *exo*-glikálokká
Magyar Kémikusok Egyesülete, Vegyészkonferencia, Hajdúszoboszló, 2001. jún. 27-29., 122. o., poster

10. **Marietta Tóth**, László Somsák
Synthesis of *C*-glycosyl-aldehyde-hydrazone, -semicarbazone and -oxime derivatives
MTA Szénhidrátkémiai Munkabizottság előadóülése, Mátrafüred, 2001. máj. 21-23., lecture