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Synthesis of a polysulfated heparin degradation product

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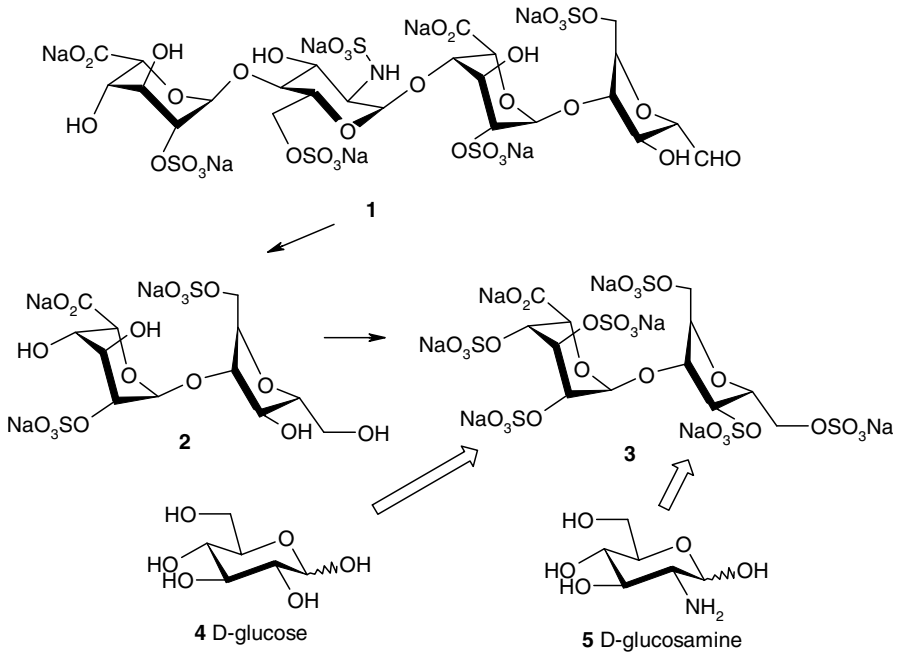
Two suitable methods for the synthesis of the heparin degradation product 2,5-anyhydro-3-*O*-(α-L-idopyranosyluronate)-D-mannitol hexa *O*-sulfate are reported. The synthesis pathways start from D-glucose and D-glucosamine.

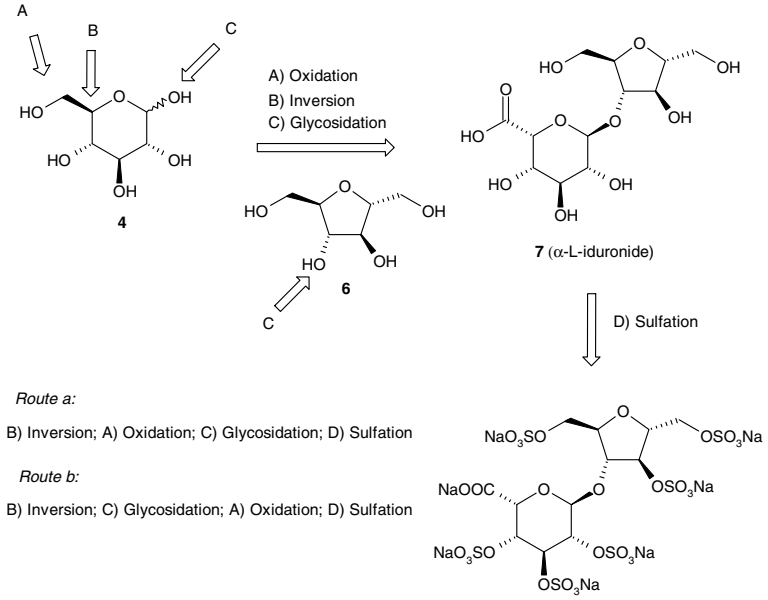
1. Indroduction

The application of glycosaminoglycans (GAGs), especially heparin and related molecules in the treatment of asthma is well documented in the literature (Diamant and Page 2000; Jerzynska et al. 2000; Lever and Page 2001). The mechanism involved in the control of bronchial hyperreactivity by these compounds is poorly understood (Jerzynska et al. 2000). Nevertheless it was proved, that heparin inhibits allergen-induced eosinophil infiltration into guinea-pig lung via a mechanism unrelated to its anticoagulant activity (Seeds and Page 2001). This biological activity is independent of the anticoagulant activity of native heparin, and is displayed by low, and even ultralow molecular weight heparin fragments, obtained by degradation of the original GAGs (Ahmed et al. 1997; Ahmed and Gables 1999). Recently it was claimed (Ahmed and Smith 2002) that hypersulfated disaccharides, obtained by degradation of heparin with HNO_2 and subsequent chemical manipulations (reduction with $NaBH_4$ and sulfation) possess pronounced antiasthmatic activity. It is well known (Jacobson et al. 1979), that the primary degradation product obtained by depolymerisation of GAGs by HNO_2 corresponds to the tetrasaccharide **1** which after further hydrolysis and subsequent reduction with $NaBH_4$ results in a mixture, containing the disaccharide **2** as the major component. Accordingly the structure of the main component obtained

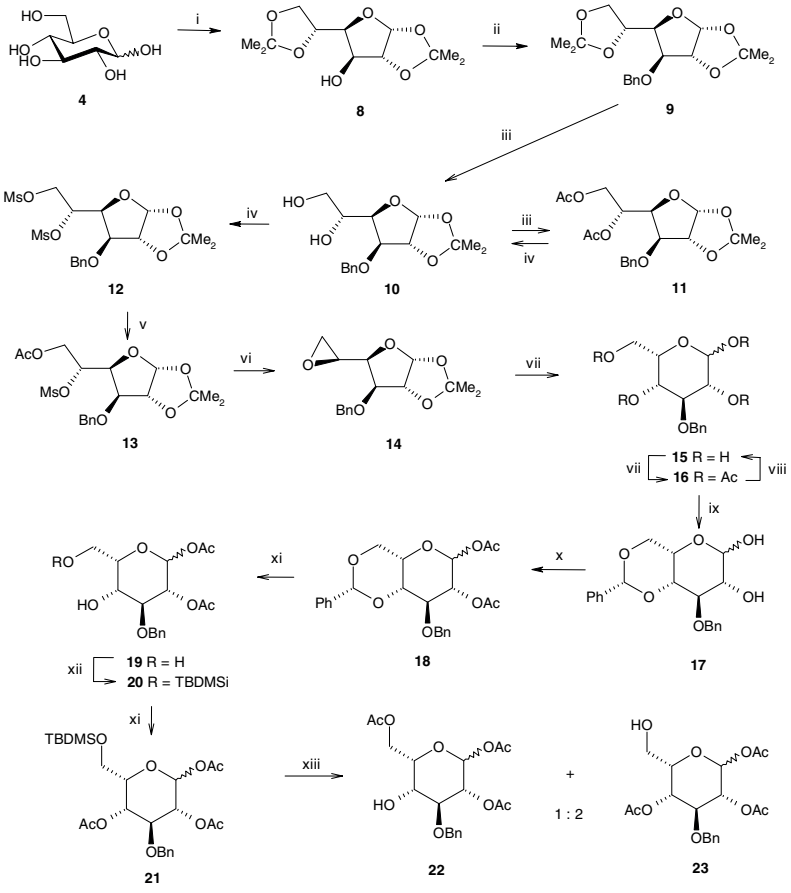
Scheme 1



Scheme 2



Scheme 3



i) acetone/ H_2SO_4, 55%; ii) BnCl/NaOH/DMSO, ~100%; iii) AcOH/H_2O; Ac_2O/Py, 65%; iv) NaOMe/MeOH; MsCl/Py, 91%; v) NaOAc/DMSO, 95%; vi) NaOiPr/iPrOH, 97%; vii) H_2O/H_2SO_4; Ac_2O/Py, 65%; viii) NaOMe/MeOH, 95%; ix) $PhCH(OMe)_2$/pTsOH, 60%, x) Ac_2O/Py, 95%; xi) TFA/H_2O, 61%; xii) TBDMSiCl/Py; Ac_2O/Py, 55%; xiii) MeOH/H_2SO_4, 62%

after sulfation corresponds to the hepta sodium salt of 2,5-anhydro-3-*O*-(α-L-idopyranosyluronate)-D-mannitol hexa-*O*-sulfate (**3**). (Scheme 1) As the purification of the depolymerisation products is a very difficult process, we decided to synthesize **3** by chemical methods, starting from D-glucose (**4**) and D-glucosamine (**5**), respectively.

2. Investigations and results

The following four transformations are necessary to convert D-glucose **4** into the target compound **3** i.e. A) Oxidation of the terminal hydroxymethyl group into a carboxyl group; B) Inversion of configuration at C-5; C) Glycosidation of OH-3 of the proper aglycon (**6**) with α-stereochemistry; and D) Sulfation of all free OH groups of the so obtained α-L-uronide **7** (Scheme 2). As far as the strategy is concerned, there are two possibilities differing in the sequence of these transformations. According to Route a the sequence is B → A → C →D , while Route b differs from it in the order of the oxidation and glycosidation, i.e. the sequence is B → C → A → D.

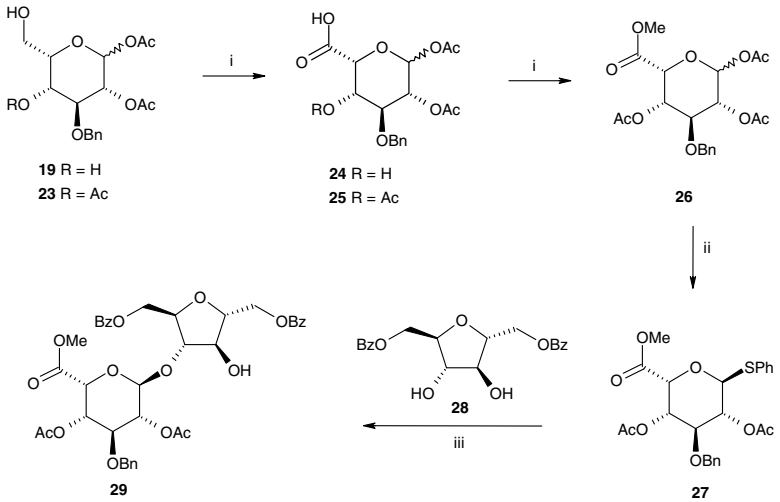
2.1. Conversion of D*-glucose into* L*-idose*

Despite the fact, that many approaches were published in the literature for the transformation of D-glucose into L-idose (Reichstein 1946; Vargha 1954; Meyer; Buss et al. 1965; Blanc-Musser; Defaye 1977; Boeckel et al.1985; Chiba; Sinay 1986; Lee 1988; Marra et al. 1989; Hiebl; Zbiral 1990; Kovensky et al. 1996; Tabeur et al. 1996; Barroca; Jacquinet 2000; Pellissier 2002; Barroca; Jacquinet 2002; Orgueira et al. 2003), we used the method, developed by Boeckel et al. 1985 for the large scale preparation of the 3-*O*-benzyl derivative **15** with some modifications (Scheme 3). Accordingly D-glucose was converted into its 1,2 : 5,6-di-*O*-isopropylidene derivative **8** the free 3-OH group of which was protected by benzylation. The so obtained crude syrupy product **9** was partially hydrolyzed and the obtained 5,6-dihydroxy compound **10** was purified by converting it into it crystalline di-*O*-acetate **11**. Deacetylation of the latter with sodium methoxide in methanol, followed by mesylation led to the crystalline 5,6-di-*O*-mesylate **12** the terminal mesyloxy group of which was exchanged with an acetoxy goup by heating it in DMSO solution with sodium acetate. The formed crystalline mixed ester **13** gave on treatment with sodium-isopropoxide in isopropanol the desired L-ido-epoxide **14** in excellent yield. Acidic hydrolysis of the latter gave a mixture, containing besides 3-*O*-benzyl-L-idopyranose **15** several by-products, therefore it had to be converted into the anomeric mixture of its peracetate **16** which could be purified by column chromatography. This compound was the key intermediate used as starting material in both, Route a and Route b.

2.2. Attempted synthesis of 3 according to Route a

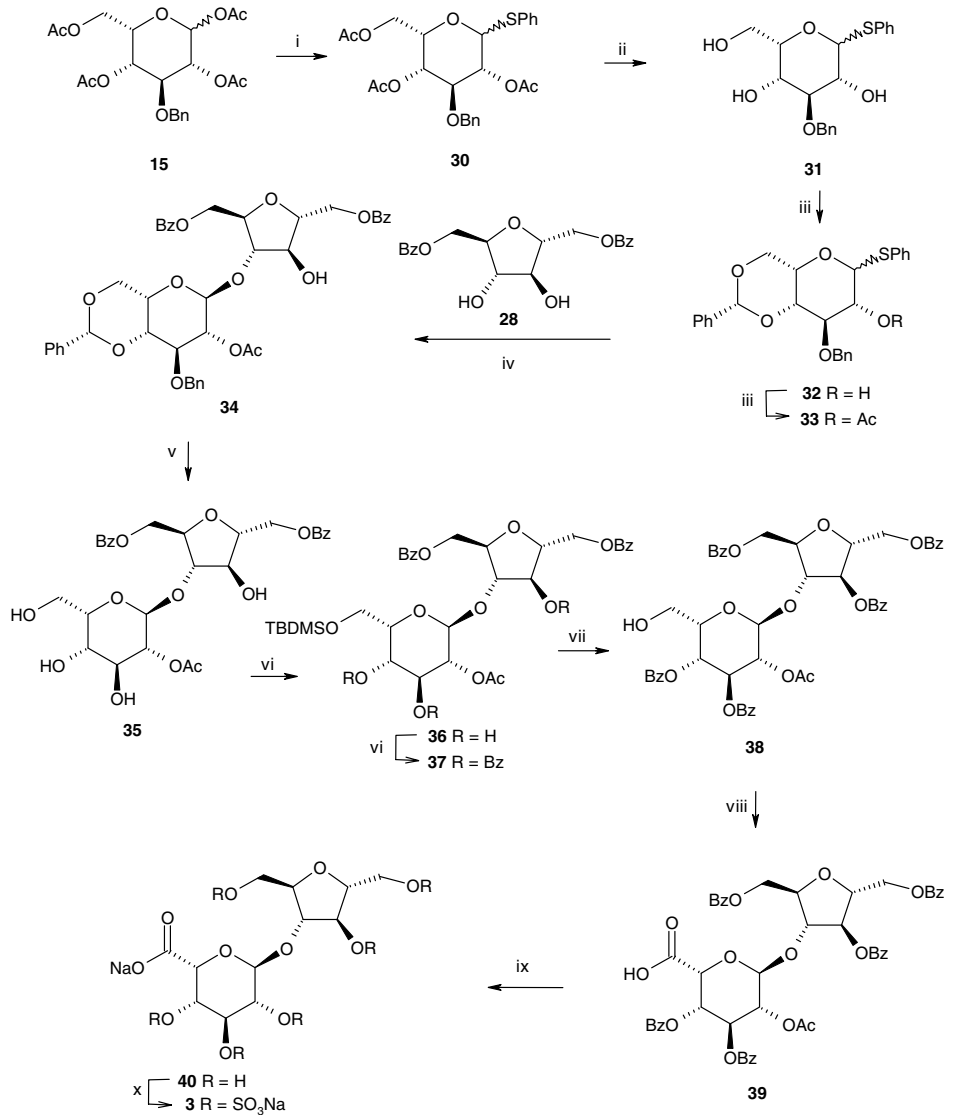
Deacetylation of **16** was carried out with sodium methoxide in methanol and the obtained pyranose **15** was treated with benzaldehyde dimethylacetal for converting it into the 4,6-*O*-benzylidene derivative **17** described first by Tabeur et al. 1996. The benzylidene group was removed after acetylation from the anomeric mixture of the di-*O*-acetates **18** and the terminal OH group of the 4,6-dihydroxy derivative **19** was temporarily protected by silylation with TBDMSiCl. The remaining free 4-OH group of the obtained diacetate **20** was acetylated and the silyl group was removed from the obtained triacetate **21** by treatment with sulfuric acid in methanol. However, under these conditions a partial 4-OAc → 6-OAc acetyl migration took place and the corresponding triacetate isomers **22** and **23** were formed in a ratio of 1 : 2 and had to be separated by column chromatography. The 1,2,4-tri-*O*-acetate **23**, containing a free 6-OH group was submitted to oxidation using NaOCl in the presence of TEMPO as reagent (Davis and Flitsch 1993) (Scheme 4). The obtained carboxylic acid **25** was converted into its methyl ester **26** which on treatment with thiophenol in the presence of boron trifluoride afforded the α-anomer of thioglycoside **27** which was used as donor in the glycosylation reaction of 1,6-di-*O*-benzoyl-2,5-anhydro-D-mannitol **28** (Otero and Simpson 1984). The latter contains two adjacent secondary free OH groups, but because of the C_2 symmetry of the molecule, glycosidation of either of them will lead to the same monoglycoside **29**. For the glycosidation reaction NIS was used as activator in the presence of a catalytic amount of TfOH, but the yield was very low (21%) and could not be increased by changing the conditions. This was not completely unexpected, as Tabeur at al.1996 failed too in glycosylating a secondary OH group using a similar thioglycoside. The overall yield of this approach could be somewhat increased by shortening the synthesis, as the dihydroxy derivative **19** could also be selectively oxidized to the corresponding carboxylic acid **24**, which could be

Scheme 4



i) NaOCl/TEMPO; $KHCO_3$/DMF, 61%; ii) PhSH/BF_3, 52%; iii) NIS/TfOH, 21%

Scheme 5



converted into **26** by acetylation of the free 4-OH group. Nevertheless the difficulties, which arouse during the desilylation procedure as well as the low yield of the glycosidation reaction made this approach disadvantageous, therefore no further attempts were made to convert **29** into the target compound **3**.

2.3. Synthesis of 3 according to Route b

The synthesis of the 1,2,4,6-tetra-*O*-acetyl-3-*O*-benzyl-L-idopyranose intermediate **15** was carried out as described for Route a. This was converted into the corresponding phenyl thioglycoside **30** using boron trifluoride as promoter (Scheme 5). The trihydroxy derivative **31** obtained after deacetylation was converted into its 4,6-*O*-benzylidene derivative **32** which after acetylation (**33**) was used as donor in the condensation with **28** applying the NIS/TfOH system for activation. In this case the reaction proceeded extremely well and the glycoside **34** was obtained in a yield of 84%. The O-benzyl and O-benzylidene groups of **34** were split off simultaneously by reduction with hydrogen over Pd/C and the trihydroxy derivative **35** was isolated as a crystalline compound. In this case the direct oxidation of the primary OH group by NaOCl/TEMPO failed probably because of the presence of four free OH groups. For this reason the primary OH group was temporarily protected by silylation and the remaining free OH groups of the obtained silyl derivative **36** were benzoylated. The silyl group of the obtained penta-*O*-benzoate **37** could be selectively hydrolysed without any benzoyl migration and the formed 6-OH derivative **38** afforded on oxidation with NaOCl/TEMPO the corresponding 6-carboxylate **39** in satisfactory yield (74%). It was not necessary to convert this acid into its ester, as on treatment with sodium methoxide in methanol it gave the sodium salt of the deacylated disaccharide **40** which was directly converted into its polysulfate with SO_3 in DMF. The excess of the reagent was decomposed with aqueous $NaHCO_3$ and the formed sulfate ions were removed with Sr-acetate. Finally Sr ions were removed with a CHELEX

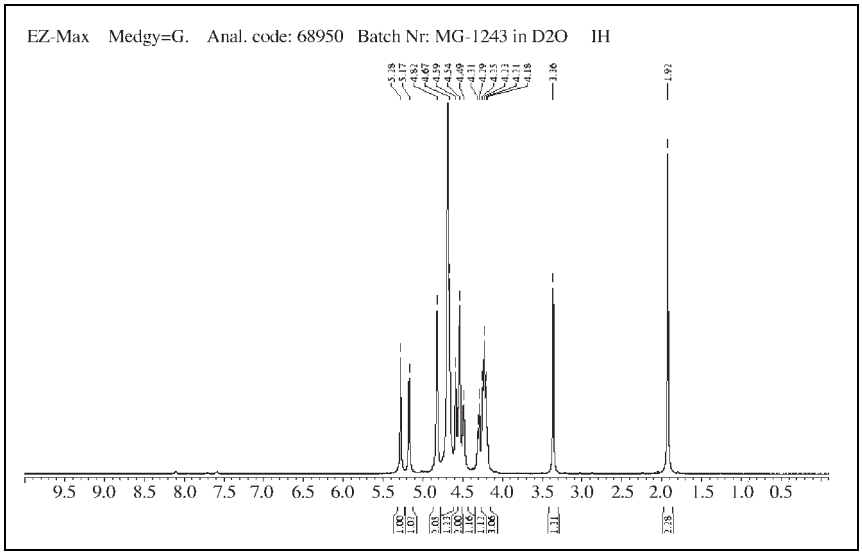


Fig.:
^{1}H NMR (250 MHz) spectrum of **3** in D_2O recorded with a Bruker AC 250 spectrometer

100 (Na form) resin and **3** was precipitated from the concentrated solution with methanol. The so obtained salt contained according to NMR (Fig.) 0.43 eq of MeOH and 0.76 eq of NaOAc but was free from any other contaminants.

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References

Ahmed T, Broder S, Whisnant JK (1997) Method for treating asthma. US Pat. No; 5,690,910

Ahmed T, (1999) Method for treating late phase allergic reactions and inflammatory diseases. US Pat. No: 5,980,865.

Ahmed T, Smith GA (2002) Hypersulfated disaccharides and methods of using the same for the treatment of inflammations. WO Pat 02/083700

Barroca N, Jacquinet J-C (2000) Synthesis of β-D-Gal*p*NAcSO_3-(1→ 4)-L-Ido*p*A2SO_3, a disaccharide fragment of dermatan sulfate, and of its methyl α-L-glycoside derivative. Carbohydr Res 329: 667–679.

Barroca N, Jacquinet J-C (2002) An access to various sulfation patterns in dermatan sulfate: Chemical synthesis of trisaccharide methyl glycosides. Carbohydr Res 337: 673–690.

Blanc-Muesser M, Defaye J (1977) A Simple synthesis of L-idose. Synthesis 568–569.

Boeckel CAA, Beetz T, Vos JN, de Jong AJM, van Aelst SF, van den Bosch RH, Mertens JMR, van der Vlugt FA (1985) Synthesis of a pentasaccharide corresponding to the antithrombin III binding fragment of heparin. J Carbohydr Chem 4, 293–321.

Buss DH, Hall LD, Hough L (1965) Some nucleophilic substitution reactions of primary and secondary sulfonate esters. J Chem Soc 1616–1619.

Chiba T, Sinay P (1986) Application of a radical reaction to the synthesis of L-iduronic acid derivatives from D-glucuronic acid analogues. Carbohydr Res 151: 379–389.

Davis NJ, Flitsch SL (1993) Selective oxidation of monosaccharide derivatives to uronic acids. Tetrahedron Lett 34: 1181–1184.

Diamant Z, Page CP (2000) Heparin and related molecules as a new treatment for asthma. Pulm Pharmacol Ther 13: 1–4.

Hiebl J, Zbiral E (1990) Improved synthesis of 6-deoxy-1,2-*O*-isopropylidene-β-L-talofuranose and 6-deoxy-1,2-*O*-isopropylidene-β-L-idofuranose. Monatsh Chem 121: 691–695.

Jacobson I, Hook M, Pettersson I, Lindhal U, Larm O, Wiren E, von Figura K (1979) Identification of N-sulfated disaccharide units in heparin-like polysaccharides Biochem J 179, 77 (1979).

Jerzynska J, Stelmach I, Kuna P (2000) The role of heparin in allergic inflammation. Pol Merkuriusz Lek 8: 341–346.Kovensky J, Duchaussoy P, Petitou M, Sinay P (1996) Binding of heparan sulfate to fibroblast growth factor-2. Total synthesis of the putative pentasaccharide binding site. Tetrahedron Asymm 7: 3119–3128.

Lee ChK (1988) Synthesis and reactions of chlorodeoxy-L-idofuranoid derivatives. Carbohydr Res 177: 247–252.

Lever R, Page C (2001) Glycosaminoglycans, airways inflammation and bronchial hyperresponsiveness. Pulm Pharmacol Ther 4: 249–254.

Marra A, Dong X, Petitou M, Sinay P (1989) Synthesis of disaccharide fragments of dermatan sulfate. Carbohydr Res 195: 39–50.

Meyer AS, Reichstein T (1946) L-Idose aus D-glucose, sowie ein neuer Weg zur l-idomethylose. Helv Chim Acta 29: 152–162.

Orgueira HA, Bartolozzi A, Schell P, Litjens REJN, Palmacci ER, Seeberger PH (2003) Modular Synthesis of Heparin Oligosaccharides. Chem Eur J 9: 140–169.

Otero DA, Simpson R (1984) 2,5-Anhydro-hexitols: syntheses of 2,5-anhydro-D-altritol and 2,5-anhydro-D-iditol. Carbohydr. Res., 128, 79–86.

Pellissier H (2002) Syntheses of L-iduronyl synthons. A review. Org Prep Proced Int 34: 441–465.

Seeds EA, Page CP (2001) Heparin inhibits allergen-induced eosinophil infiltration into guinea-pig lung via a mechanism unrelated to its anticoagulant activity. Pulm Pharmacol Ther 14: 111–119.

Tabeur C, Machetto F, Mallet J-M, Duchaussoy P, Petitou M, Sinay P (1996) L-iduronic acid derivatives as glycosyl donors. Carbohydr Res 281: 253–276.

Vargha L (1954) Über die Substitution von Tosyloxy-Gruppen durch Acetoxy-Gruppen in Polyoxy-Verbindungen. Eine neue Darstellungsweise von L-Idose-Derivaten aus D-Glucose. Ber. 87: 1351–1356.