[22] *U. Lindahl & O. Axelsson*, J. biol. Chemistry *246*, 74 (1971).
[23] *A. Studer & G. Zbinden*, Schweizerische Zeitschrift für Allgemeine Pathologie und Bakteriologie *17*, 780 (1954).
[24] *A. Studer, R. Engelberg & L. O. Randall*, «Thrombose und Embolie, I. Internationale Tagung, Basel 1954», Benno Schwabe & Co., Basel 1955, p. 863.
[25] *K. Heyns & H. Paulsen*, Chem. Ber. *88*, 188 (1955).
[26] *S. Takanashi, Y. Hirasaka, M. Kawada & M. Ishidate*, J. Amer. chem. Soc. *84*, 3029 (1962); Chem. pharm. Bull. *14*, 82 (1966).
[27] *R. Lambert & F. Zillikes*, Chem. Ber. *96*, 2350 (1963).
[28] *J. Kiss & F. Burkhardt*, Helv. *52*, 2622 (1969).
[29] *L. Hough & A. C. Richardson*, 'Selective Acylation', in S. Coffey Ed., Rodd's Chemistry of Carbon Compounds, Elsevier Publishing Co., Amsterdam, London & New York 1967, Vol. 1F, p. 387.
[30] *M. W. Horner, L. Hough & A. C. Richardson*, J. chem. Soc. *1970*, 1336.

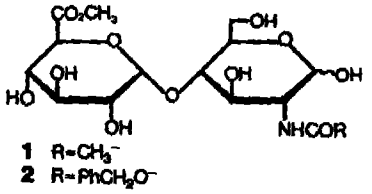
205. Synthesis of Heparin Saccharides[1])
IV. Synthesis of Disaccharides Possessing the Structure of a Repeating Unit of Heparin

by **Pierre C. Wyss, Joseph Kiss** and **Wolf Arnold**
Chemical and Physical Research Departments,
F. Hoffmann-La Roche & Co., Ltd., Basle, Switzerland

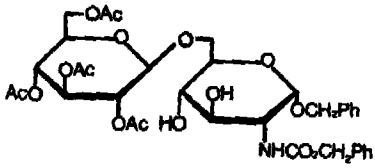
(13. VI. 75)

Summary. The synthesis of disaccharides possessing the structure of a repeating unit of heparin is reported. 2-Acetamido-2-deoxy-4-O-(methyl α-D-glucopyranosyluronate)-D-glucopyranose (**1**) and 2-[1-(benzyloxy)formamido]-2-deoxy-4-O-(methyl α-D-glucopyranosyluronate)-D-glucopyranose (**2**) have been prepared by two routes, (a) from D-glucose and D-glucosamine, and (b) from D-glucuronolactone and D-glucosamine.

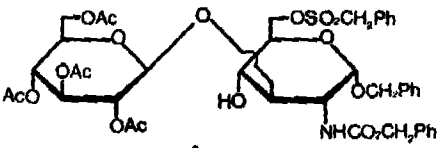
1. Introduction. – One project in this laboratory was concerned with the synthesis of heparinoids having structures closely related to that of heparin [1] [2]. The purpose of this paper is to report the synthesis of disaccharides **1** and **2** as starting materials for heparinoids. Other disaccharides (**3**, **4** and **5**) have also been synthesized as reference substances.



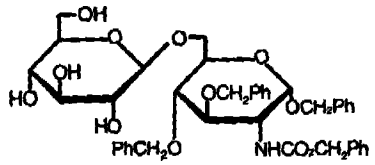
1 R=CH_3–
2 R=$PhCH_2O$–



3



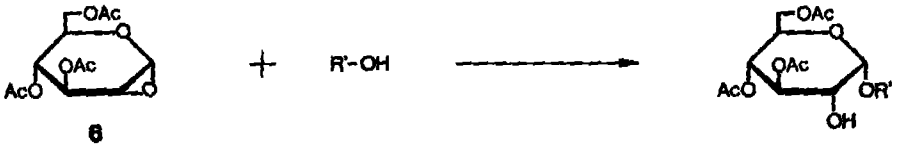
4



5

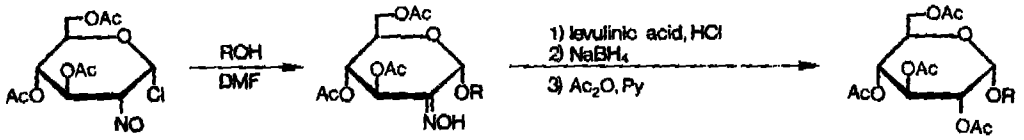
[1]) Part III see [2].

Several approaches to α-D-glucopyranoside synthesis have been developed [3] [4], but most of the methods available have met with only limited success. One of these methods involved the use of "*Brigl*'s anhydride" (**6**) as a glycosylating agent as in the synthesis of the disaccharides maltose [5], maniocose [6], sucrose [7] and trehalose [8].



6

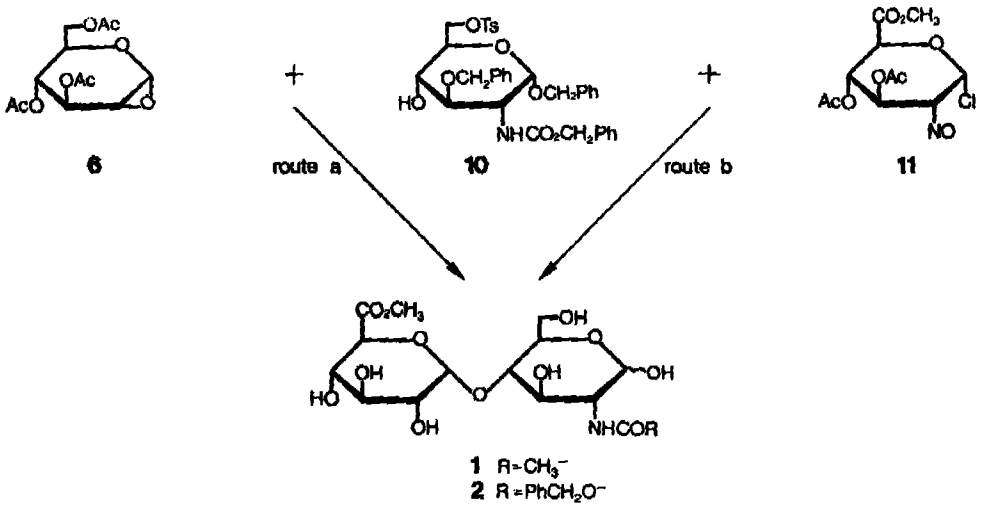
Using 3,4,6-tri-O-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride (**7**), *Lemieux et al.* have developed a promising, new synthesis of α-D-glucopyranosides. This glycosylating agent has been shown to react readily with alcohols in N,N-dimethylformamide at room temperature to give the 3,4,6-tri-O-acetyl-2-oximino-α-D-*arabino*-hexopyranosides **8** in excellent yield [9]. Deoximation of **8** with levulinic acid and hydrochloric acid, followed by borohydride reduction of the liberated ulose, and acetylation of the resulting hydroxyl group, gave the α-glucosides **9** [10]. The glycosidation and reduction reactions have been shown to be highly stereospecific [9–11]. *Lemieux et al.* have applied this new method with success to the synthesis of α-linked disaccharides [12] [13]. However, this method has, to our knowledge, not yet been used for the synthesis of disaccharides of the maltose type.



7 **8** **9**

In contrast with these results, *Miyai* & *Jeanloz* [14] have obtained a mixture of anomers by condensation of benzyl 2,3,4-tri-O-benzyl-β-D-galactopyranoside with **7**. These authors have suggested that the nature of the alcohol might influence the mechanism of the glycosidation reaction.

Scheme 1



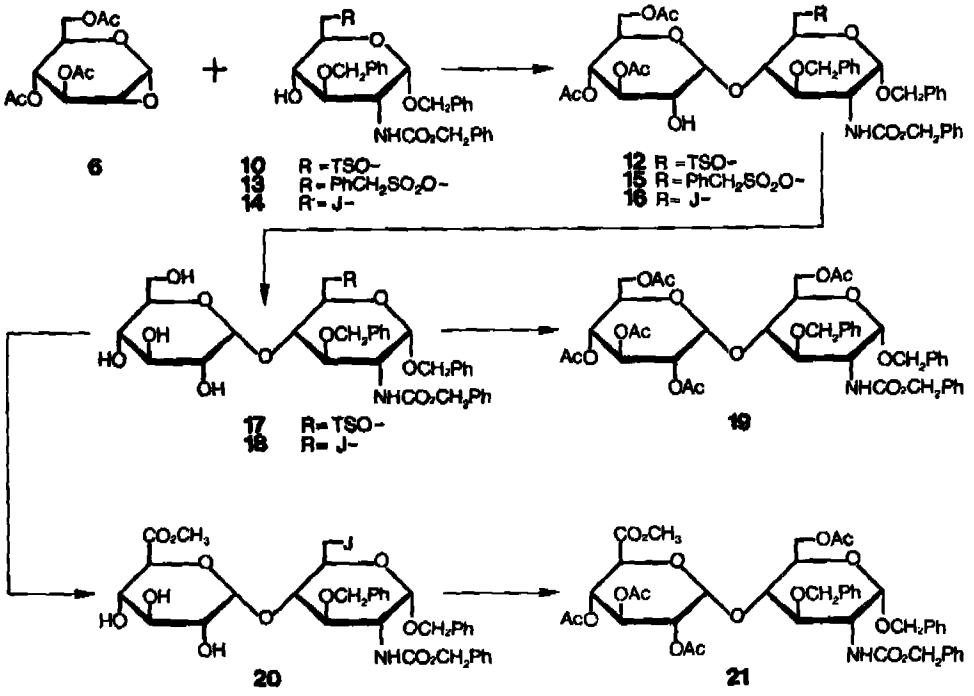
6 **10** **11**

1 R = CH_3^-
2 R = $PhCH_2O^-$

The synthesis of disaccharides **1** and **2** has now been achieved following two different routes: by condensation of the amino sugar **10** (a) with "*Brigl*'s anhydride" (**6**), and (b) with the nitrosyl chloride adduct **11** (*Scheme 1*).

2. Synthesis of benzyl 6-O-acetyl-3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-4-O-(methyl 2,3,4-tri-O-acetyl-α-D-glucopyranosyluronate)-α-D-glucopyranoside (21). – 2.1. *From benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-6-O-p-tolylsulfonyl-α-D-glucopyranoside* (**10**) *and* "Brigl's *anhydride*" (**6**) (*Scheme 2*). Benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-6-O-*p*-tolylsulfonyl-α-D-glucopyranoside (**10**) was allowed to react with "*Brigl*'s anhydride" (**6**) in dry toluene at 130° for 3 days. The reaction mixture was fractionated by column chromatography, to give a 68% yield of starting material **10**, and a 23% yield of benzyl 3-O-benzyl-2-[1-(benzyloxy)-formamido]-2-deoxy-4-O-(3,4,6-tri-O-acetyl-α-D-glucopyranosyl)-6-O-*p*-tolylsulfonyl-α-D-glucopyranoside (**12**) as a foam. The corresponding 6-O-benzylsulfonyl- (**15**; 12% yield) and 6-iodo- (**16**; 11% yield) disaccharides were prepared by the same method from **13** and **14** respectively. The α-configuration of the glycosidic linkage in disaccharides **12**, **15** and **16** was strongly suggested by a comparison of their optical rotations with those of the parent monosaccharide components (Table 1).

Scheme 2



De-O-acetylation of **12** and **16** with methanolic ammonia gave disaccharides **17** and **18** respectively. The crystalline 6-iodo-disaccharide **18** was also prepared in good yield by reaction of its amorphous 6-O-*p*-tolylsulfonyl-analog **17** with sodium iodide in boiling 2-pentanone.

Table 1. *Molecular rotation of disaccharides* **12**, **15** *and* **16** *compared to the sum of the molecular rotations of their monosaccharide components*

	$[M]_D$ (degrees) $\times 10^{-3}$
disaccharide **12**	107
methyl 3,4,6-tri-O-acetyl-β-D-glucopyranoside (**22**)[15] + **10**	57
methyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**23**)[16] + **10**	44
methyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (**24**)[17] + **10**	98
disaccharide **15**	93
22 + **13**	52
23 + **13**	39
24 + **13**	93
disaccharide **16**	81
22 + **14**	53
23 + **14**	40
24 + **14**	94

The α-configuration of the glycosidic linkage in these disaccharides as in their precursors could definitely be established by the analysis of the 360 MHz ^{1}H-NMR. spectrum of the acetylated disaccharide **19** (Fig. 1), obtained by reaction of **18** with silver acetate in a mixture of acetic anhydride and pyridine. The coupling constant $J_{1',2'} \sim 4$ Hz in this compound was consistent with an equatorial-axial arrangement

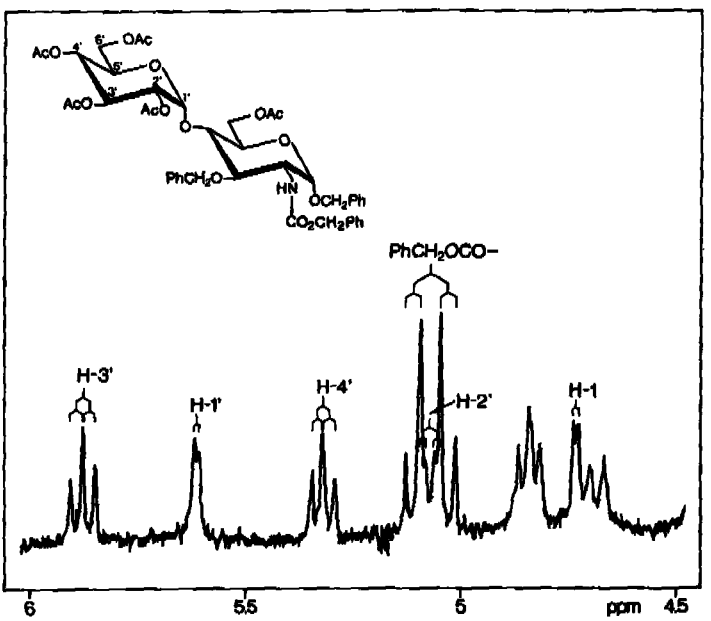
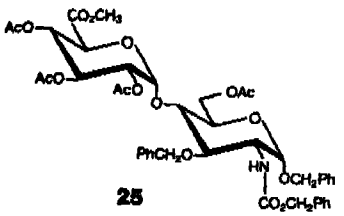


Fig. 1. *Part of the 360 MHz ^{1}H-NMR. spectrum of disaccharide* **19** (*ca.* 30 mg in 0.7 ml C_6D_6)

of protons H–C(1') and H–C(2'), thus confirming the configuration of its glycosidic linkage.

The conversion of disaccharide **17** into uronic acid by catalytic oxidation was difficult to reproduce and gave very poor yields. It might be possible that the *p*-tolylsulfonyl protecting group of **17** acts as a catalyst poison. These difficulties could be avoided by using the iodo-disaccharide **18** as a substrate for oxidation. This disaccharide was oxidized in dioxane/water 1:1 at 65° and pH 8.5 with oxygen in the presence of a 13% Pt/C catalyst. The crude uronic acid was esterified with diazomethane and the resulting ester was purified by column chromatography, to give a 33% overall yield of crystalline methyl uronate **20**. Benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2,6-dideoxy-6-iodo-α-D-glucopyranoside (**14**) was isolated as a by-product of the oxidation reaction. The product was identical (m.p., $[\alpha]_D$, IR. spectrum and TLC.) with an authentic sample of **14**. The lability of the α-glycosidic linkage under the conditions of catalytic oxidation has already been observed [18]. The catalytic oxidation of benzyl maltoside has been reported to give benzyl 4-O-(α-D-glucopyranosyluronic acid)β-D-glucopyranoside and benzyl β-D-glucopyranosiduronic acid as chief products [18].

The iodine atom of **20** was finally substituted by an acetoxy group by treatment with silver acetate in a mixture of pyridine and acetic anhydride at 70° for 12 hours. Purification of the crude product gave a 69% yield of acetylated disaccharide **21**. The α-configuration of the glycosidic linkage in this disaccharide was established by ^{1}H-NMR. spectroscopy. The magnitude of the coupling constants ($J_{1',2'} \sim 3$ Hz, $J_{2',3'} \sim J_{3',4'} \sim J_{4',5'} \sim 8.5$ Hz) is consistent with the equatorial orientation of H–C(1') and axial orientation of H–C(2') to H–C(5') protons in the C 1 conformation (**25**).

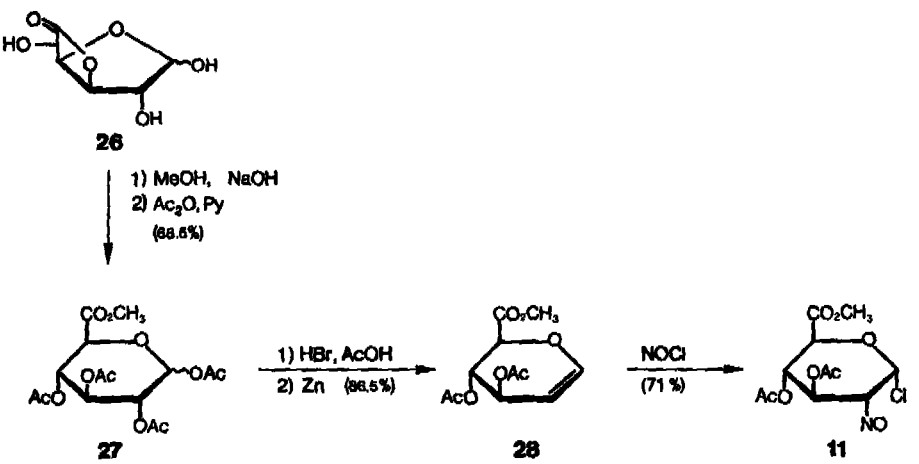


25

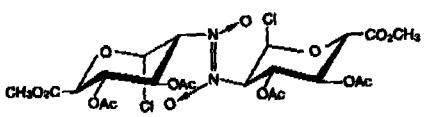
2.2. From benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-6-O-*p*-tolylsulfonyl-α-D-glucopyranoside (10) and methyl 3,4-di-O-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyluronate chloride (11). – 2.2.1. *Synthesis of* **11** (*Scheme 3*). Base-catalyzed esterification of D-glucuronolactone (**26**), followed by acetylation, gave methyl 1,2,3,4-tetra-O-acetyl-D-glucopyranuronate (**27**) as a mixture of the α- and β-anomers [19] [20]. Treatment of **27** with hydrobromic acid in acetic acid [19], followed by reduction of the α-bromide with zinc dust in aqueous acetic acid provided methyl 3,4-di-O-acetyl-1,2-dideoxy-D-*arabino*-hex-1-enopyranuronate (**28**) [21] in 86.5% yield based on **27**.

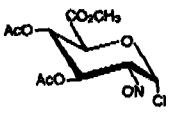
Reaction of **28** with nitrosyl chloride in dichloromethane as in the preparation of 3,4,6-tri-O-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride [22–24] afforded crystalline methyl 3,4-di-O-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyluronate

Scheme 3



chloride (**11**) in 71% yield. This nitroso sugar possesses the characteristic properties shown by C-nitroso compounds. In the solid state, the product is white and exists as a dimer (**29**), dissociating in solution to give a blue color. The ^{1}H-NMR. spectrum of **11** was found to be consistent with formulation as the α-D-gluco-configuration in the C1 conformation (**30**). The first-order coupling constants ($J_{1,2} \sim 3.8$ Hz, $J_{2,3} \sim 10.5$ Hz, $J_{3,4} \sim 9$ Hz and $J_{4,5} \sim 10.2$ Hz) indicated the 1,2-equatorial-axial and 2,3-, 3,4- and 4,5-diaxial arrangement of ring protons.



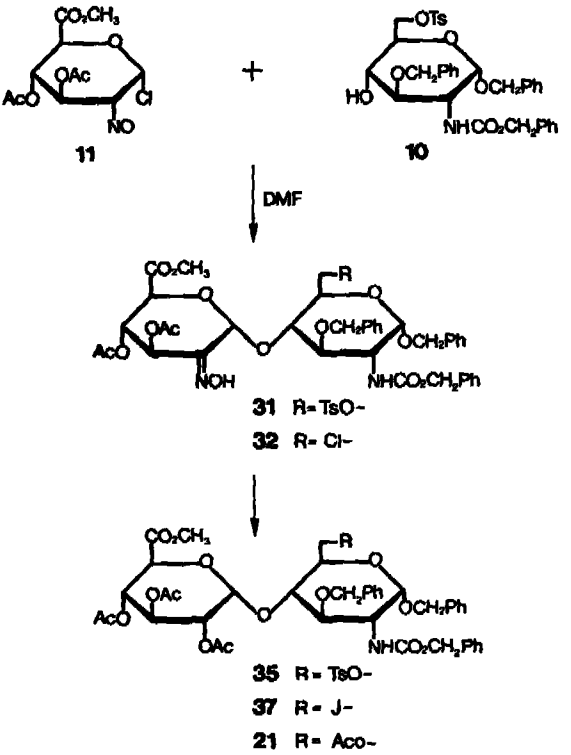


2.2.2. *Condensation of* **10** *with* **11** (*Scheme 4*). The reaction of the amino sugar **10** with the nitrosyl chloride adduct **11** in N,N-dimethylformamide at room temperature was followed by TLC., which indicated the formation of three new products. The intensity of these spots increased very slowly at the expense of the starting materials, and the reaction was practically complete in 7 days. Higher temperatures or use of bases did not affect the reaction rate. Column chromatography on silica gel of the reaction mixture gave a good resolution of products with Rf = 0.39, 0.47 and 0.61, which crystallized easily. However, the product with Rf = 0.72 was still contaminated by components with Rf = 0.61 and 0.39.

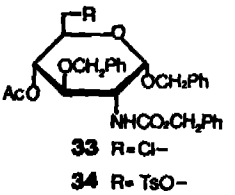
The product with Rf = 0.61 was shown to be starting material **10** (2% yield).

The components with Rf = 0.39 and 0.47 were characterized by elementary analysis, IR. and ^{1}H-NMR. spectroscopy as disaccharides **31** (65% yield) and **32** (17% yield) respectively. Their high molecular rotations supported the α-configuration of their glycosidic linkage.

Scheme 4

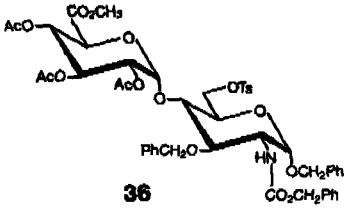


The components with Rf = 0.72 and 0.61 were then easily separated by column chromatography after acetylation of the mixture with acetic anhydride in pyridine. The acetylated products were characterized as benzyl 4-O-acetyl-3-O-benzyl-2-[1-(benzyloxy)formamido]-6-chloro-2,6-dideoxy-α-D-glucopyranoside (**33**) and benzyl 4-O-acetyl-3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-6-O-*p*-tolylsulfonyl-α-D-glucopyranoside (**34**).



Hydrolysis of the hydroxyimino disaccharide **31** with levulinic acid and hydrochloric acid, followed by borohydride reduction and acetylation gave a good yield of amorphous **35**. The structure assigned to disaccharide **35** was fully supported by the analysis of its ^{1}H-NMR. spectrum. The observed coupling constants ($J_{1',2'} \sim 4.2$ Hz,

$J_{2',3'} \sim J_{3',4'} \sim J_{4',5'} \sim 8$ Hz) are consistent with the α-D-gluco configuration in the C1 conformation (**36**).

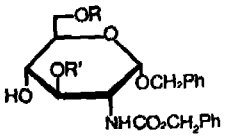


36

Attempts to displace the *p*-toluenesulfonyloxy group of **35** by acyloxy groups were without success. Its substitution had then to be achieved by the sequence of reactions (TsO- → J- → AcO-) previously used for the conversion of disaccharide **17** into **21**. Disaccharide **35** was treated with sodium iodide, followed by reaction of **37** with silver acetate, to give the acetylated disaccharide **21**.

The disaccharides obtained by the two routes were shown to be identical by comparison of their m.p., mixed m.p., optical rotation, IR. and ^{1}H-NMR. spectra, and behaviour in TLC.

Attempts to condense the amino sugars **38**, **39**, **40** and **41** [2] with **11** were undertaken in order to avoid the sequence of substitutions involved in the removal of the *p*-toluenesulfonyl protecting group of **35**, but these efforts remained unsuccessful. As the reactivity of the C(4) hydroxyl group of glycopyranose derivatives is known to be particularly low [25], the above results would then suggest that the C(4) hydroxyl group in the amino sugar **10** might possibly be activated by the *p*-toluenesulfonyl group.

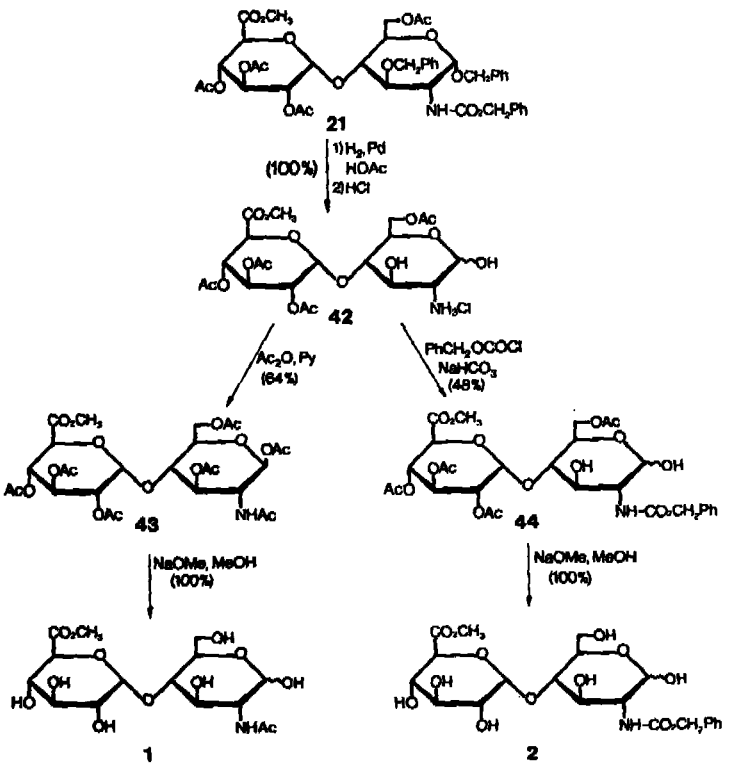


38	R = PhCO⁻	R' = PhCH₂⁻
39	PhCO⁻	PhCO⁻
40	PhNHCO⁻	PhNHCO⁻
41	PhCO⁻	PhNHCO⁻

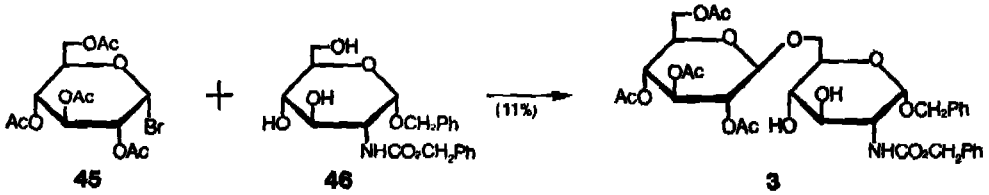
3. Synthesis of 2-acetamido-2-deoxy-4-O-(methyl α-D-glucopyranosyluronate)-D-glucopyranose (1) and 2-[1-(benzyloxy)formamido]-2-deoxy-4-O-(methyl α-D-glucopyranosyluronate)-D-glucopyranose (2) (*Scheme 5*). – Compound **21** was hydrogenated over black palladium and the amino disaccharide **42** was subsequently acetylated with acetic anhydride in pyridine to give a good yield of 2-acetamido-1,3,6-tri-O-acetyl-2-deoxy-4-O-(methyl 2,3,4-tri-O-acetyl-α-D-glucopyranosyluronate)-β-D-glucopyranose (**43**). De-O-acetylation of **43** with sodium methoxide in methanol afforded a quantitative yield of **1**.

N-Acylation of the amino disaccharide **42** with benzyl chloroformate, followed by de-O-acetylation of **44** finally gave **2**.

Scheme 5



4. Synthesis of disaccharides 3, 4 and 5. – 4.1. *Benzyl 2-[1-(benzyloxy)formamido]-2-deoxy-6-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside* (**3**). Benzyl 2-[1-(benzyloxy)formamido]-2-deoxy-α-D-glucopyranoside (**46**) was treated with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (**45**) in chloroform in the presence of silver oxide and a drying agent, giving a low yield (11%) of crystalline **3**. The site of glycosidation and the configuration of the glycosidic linkage in disaccharide **3** were established by ^{1}H-NMR. spectroscopy (Fig. 2). The presence of the hydroxyl signals as doublets established that glycosidation had occurred at C(6). On the other hand, the coupling constant $J_{1',2'} \sim 7.5$ Hz showed that protons H–C(1') and H–C(2') are in axial positions, thus indicating β-anomeric configuration.



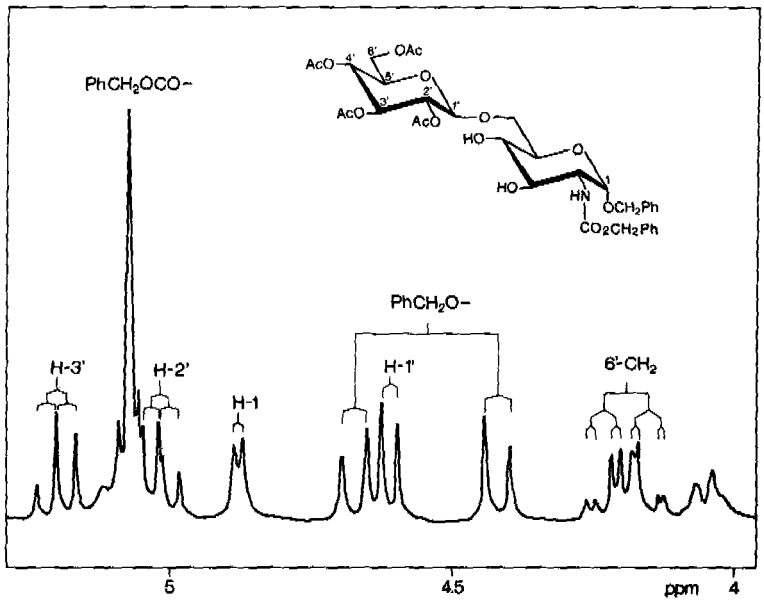
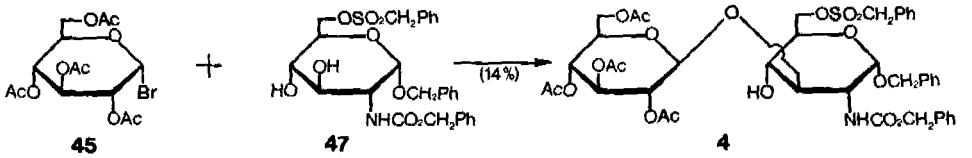
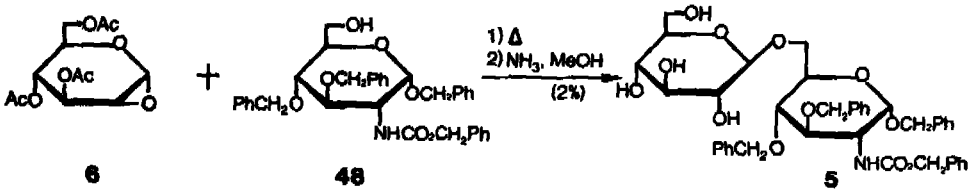


Fig. 2. *Part of the 270 MHz ^{1}H-NMR. spectrum of disaccharide* **3** (*ca.* 30 mg in 0.7 ml $CDCl_3$)

4.2. *Benzyl 2-[1-(benzyloxy)formamido]-6-O-benzylsulfonyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-*D*-glucopyranosyl)-α-*D*-glucopyranoside* (**4**). Condensation of benzyl 2-[1-(benzyloxy)formamido]-6-O-benzylsulfonyl-2-deoxy-α-D-glucopyranoside (**47**) with **45** under the above reaction conditions gave a low yield (14%) of crystalline disaccharide **4**. The coupling site was presumed to be at C(3) on account of the higher reactivity of C(3) compared to C(4) hydroxyl groups. The β-configuration of the glycosidic linkage in this disaccharide was tentatively assigned on the basis of molecular rotation (*Table 2*).



4.3. *Benzyl 3,4-di-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-6-O-(β-*D*-glucopyranosyl)-α-*D*-glucopyranoside* (**5**). Benzyl 3,4-di-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-α-D-glucopyranoside (**48**) was allowed to react with "*Brigl*'s anhydride" (**6**) under the usual conditions, followed by de-O-acetylation with methanolic ammonia. One of the components of the reaction mixture was obtained crystalline in very low yield (2%) and was shown to be **5**. The β-configuration of the glycosidic linkage in **5** was suggested by a comparison of its optical rotation with that of the monosaccharide component **48** (*Table 2*).



6 **48** **5**

Table 2. *Molecular rotation of disaccharides* **4** *and* **5** *compared to the sum of the molecular rotations of their monosaccharide components*

	$[M]_D$ (degrees) $\times 10^{-3}$
disaccharide **4**	+48
methyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**23**) [16] + **47**	+39
methyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (**24**) [17] + **47**	+93
disaccharide **5**	+53
48	+58

The authors wish to express their thanks to Dr. *L. Chopard* and Dr. *M. Grosjean* of our Physical Chemistry Department for the spectroscopic determinations and to Dr. *A. Dirscherl* for the microanalyses. We are grateful for the skillful technical assistance of Mr. *P. Beyer*, Mr. *G. Gébert*, Mr. *G. Humer*, Mr. *K. Lenzin*, Mr. *P. Taschner* and Mr. *W. Schwarz*.

Experimental Part

1. General Methods. – See [2].

2. Synthesis of benzyl 6-O-acetyl-3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-4-O-(methyl 2,3,4-tri-O-acetyl-α-D-glucopyranosyluronate)-α-D-glucopyranoside (21). – 2.1. *From benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-6-O-p-tolylsulfonyl-α-D-glucopyranoside* (**10**) *and "*Brigl's *anhydride"* (**6**). – 2.1.1. *Benzyl 3-O-benzyl-2-[1-(benzyloxy)-formamido]-2-deoxy-4-O-(3,4,6-tri-O-acetyl-α-D-glucopyranosyl)-6-O-p-tolylsulfonyl-α-D-glucopyranoside* (**12**). A solution of **6** (19.5 g, 68 mmol) [26] and **10** (36.8 g, 57 mmol) [27] in toluene (180 ml) was refluxed for 3 days and then evaporated to dryness. TLC. of the syrup obtained, with petroleum ether/acetone 7:3, revealed two major components with Rf = 0.65 and 0.50, and several other minor components. The first two components of the mixture were separated on a silica gel column (1.2 kg), by development with benzene/ethyl acetate 30% and 40% respectively. Crystallization of the component with Rf = 0.65 from isopropyl ether gave starting material **10**, yield 25.0 g (68%), m.p. and mixed m.p. 122–123°. The component, Rf = 0.50, obtained as a glass was shown to be disaccharide **12**, yield 12.0 g (23%), $[\alpha]_D^{25} = +114.4°$ ($c = 0.94$, chloroform). – IR.: 1747 (C=O, ester), 1727 (C=O, carbamate), 1364, 1178 cm^{-1} (SO_2). – UV.: 228 nm (ε 13360).

$C_{47}H_{53}NO_{17}S$ (935.99) Calc. C 60.31 H 5.71 N 1.50% Found C 60.00 H 5.75 N 1.81%

2.1.2. *Benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-6-O-benzylsulfonyl-2-deoxy-4-O-(3,4,6-tri-O-acetyl-α-D-glucopyranosyl)-α-D-glucopyranoside* (**15**). The title compound was prepared from benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-6-O-benzylsulfonyl-2-deoxy-α-D-glucopyranoside (**13**) [2] and **6** according to the general procedure described for the synthesis of disaccharide **12**. The product was obtained as a glass: yield 12%, $[\alpha]_D^{25} = +99.0°$ ($c = 1.00$, chloroform), Rf = 0.50 (petroleum ether/acetone 7:3).

$C_{47}H_{53}NO_{17}S$ (935.99) Calc. C 60.31 H 5.71 S 3.43% Found C 59.97 H 5.84 S 3.18%

2.1.3. *Benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2,6-dideoxy-4-O-(3,4,6-tri-O-acetyl-α-D-glucopyranosyl)-6-iodo-α-D-glucopyranoside* (**16**). The title compound was prepared from benzyl

3-O-benzyl-2-[1-(benzyloxy)formamido]-2,6-dideoxy-6-iodo-α-D-glucopyranoside (**14**) [27] and **6** according to the general procedure described for the synthesis of disaccharide **12**. The product was obtained as a glass: yield 11%, $[\alpha]_D^{25} = +91.0°$ ($c = 1.00$, chloroform), Rf = 0.23 (petroleum ether/acetone 7:3).

$C_{40}H_{46}JNO_{14}$ (891.69) Calc. C 53.87 H 5.20 J 14.24% Found C 53.93 H 5.36 J 13.87%

2.1.4. *Benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-4-O-(α-D-glucopyranosyl)-6-O-p-tolylsulfonyl-α-D-glucopyranoside* (**17**). Compound **12** (12.0 g, 12.8 mmol) was dissolved in 250 ml of dry methanol and the solution was nearly saturated at 0° with ammonia. After standing for 18 h at RT. the solvent was removed under reduced pressure, and the residual syrup was triturated with chloroform to remove acetamide. The crude product was chromatographed on a silica gel column (300 g) by development with ethyl acetate, and the eluate was concentrated to a glass: yield 8.0 g (77%), $[\alpha]_D^{25} = +112.6°$ ($c = 1.53$, chloroform). IR.: 1728 (C=O, carbamate), 1363, 1180 cm^{-1} (SO_2). – UV.: 224 nm (ε 12600).

$C_{41}H_{47}NO_{14}S$	Calc.	C 60.80	H 5.85	N 1.73	S 3.96%
(809.90)	Found	,, 60.68	,, 6.03	,, 1.82	,, 3.64%

2.1.5. *Benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2,6-dideoxy-4-O-(α-D-glucopyranosyl)-6-iodo-α-D-glucopyranoside* (**18**). – 2.1.5.1. *From* **17**. A solution of 27.5 g (34 mmol) of **17** in 375 ml of 2-pentanone was treated with sodium iodide (37.5 g), and the mixture was then refluxed for 8 h under stirring. The reaction mixture was evaporated to dryness, and water (500 ml) was added to the residue. The mixture was extracted with 2× 250 ml of ethyl acetate, and the extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The crystalline residue was recrystallized from ethyl acetate/isopropyl ether: yield 23.7 g (91%), m.p. 132–133°, $[\alpha]_D^{25} = +122.0°$ ($c = 1.00$, chloroform).

$C_{34}H_{40}JNO_{11}$ (765.59) Calc. C 53.34 H 5.27 J 16.58% Found C 53.06 H 5.45 J 17.45%

2.1.5.2. *From* **16**. Compound **16** was deacetylated by the method used for the 6-O-*p*-tolylsulfonyl analog **17**: yield 58%, m.p., mixed m.p. 133–134° (from ethyl acetate/isopropyl ether), $[\alpha]_D^{25} = +122.0°$ ($c = 1.00$, chloroform).

2.1.6. *Benzyl 6-O-acetyl-3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-α-D-glucopyranoside* (**19**). The solution of 5.0 g (6.5 mmol) of **18** in 100 ml of acetic anhydride was heated to 70°. To this solution a warm solution (70°) of 10 g of silver acetate in 100 ml of pyridine was added dropwise under stirring. After the addition was complete, the reaction mixture was heated for three additional hours under stirring. The reaction mixture was evaporated to a dark brown residue from which the last traces of pyridine were removed by codistillation with toluene. The dried residue was extracted with 3× 100 ml of benzene, and the extract was evaporated to dryness. TLC. of the product, with hexane/ethyl acetate 1:1 as developer, revealed a major component, Rf = 0.45, and two minor components, Rf = 0.59 and 0.29. The mixture was fractionated on a column (150 g) of silica gel with hexane/ethyl acetate 2:1. The crystalline product with Rf = 0.45 was recrystallized from ethyl acetate/isopropyl ether: yield 4.0 g (71%), m.p. 91–93°, $[\alpha]_D^{25} = +125.1°$ ($c = 1.00$, chloroform). – IR.: 1758 cm^{-1} (C–O ester, carbamate). – ^{1}H-NMR. (100 MHz, 360 MHz, C_6D_6): 1.53 (*s*, –OAc); 1.69 (*s*, –OAc); 1.72 (*s*, –OAc); 1.74 (*s*, –OAc); 1.79 (*s*, –OAc); ~4.78 (*d*, $J_{1,2} \sim 4$, H–C(1)); 5.06 (*d*×*d*, $J_{1',2'} \sim 4$, $J_{2',3'} \sim 10$, H–C(2')); 5.31 (*d*×*d*, $J_{3',4'} \sim J_{4',5'} \sim 9$, H–C(4')); 5.62 (*d*, H–C(1')); 5.87 (*d*×*d*, H–C(3')).

$C_{44}H_{51}NO_{17}$ (865.88) Calc. C 61.03 H 5.94 N 1.62% Found C 60.86 H 6.07 N 1.50%

2.1.7. *Benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2,6-dideoxy-4-O-(methyl α-D-glucopyranosyluronate)-6-iodo-α-D-glucopyranoside* (**20**). Compound **18** (23.5 g, 30.6 mmol) was dissolved in 50% aqueous dioxane (2.4 l). The solution was treated with platinum (13%)-Darco G-60 catalyst (24 g) [28], and oxygen was bubbled through the solution with vigorous stirring at 65° for 70 h, while the pH was maintained at 8.4 by the addition of a total of 6 g of sodium hydrogen carbonate. Water (1 l) was added to the reaction mixture, and the light brown solution was filtered from catalyst and the latter was washed with hot water. The combined filtrate and washings were concentrated to about 1 l and the solution was adjusted to pH 2 under cooling by the dropwise

addition of conc. hydrochloric acid. The reaction mixture was extracted with 5 × 250 ml of ethyl acetate, and the combined extracts were dried over anhydrous sodium sulfate and evaporated to a white, amorphous solid: yield 15.0 g. The crude acid (4.0 g) was dissolved in dry methanol (80 ml) and the solution was treated with an excess of a solution of diazomethane in ether. After 10 min at RT. the solution was evaporated to a yellow, amorphous foam. TLC., using a mixture of chloroform, methanol and 3N ammonia (40:10:1) revealed a major component with Rf = 0.7 and a minor component with Rf = 0.8. The mixture was fractionated on a silica gel column (150 g), by development with ethyl acetate/benzene 3:1. The crystalline residue (Rf = 0.7) was recrystallized from ethyl acetate/isopropyl ether: yield 2.1 g (33%), m.p. 117–118°, $[\alpha]_D^{25} = +100°$ ($c = 0.40$, chloroform). – IR.: 1730 (C=O, ester), 1700 cm^{-1} (C=O, carbamate). – ^{1}H-NMR. (100 MHz, $CDCl_3$): 3.75 (*s*, $-CO_2CH_3$).

$C_{35}H_{40}JNO_{12}$ (793.60) Calc. C 52.97 H 5.08 J 15.99% Found C 52.76 H 5.26 J 15.62%

The product with Rf = 0.8 was crystallized and recrystallized from ethyl acetate/petroleum ether, and was shown to be benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2,6-dideoxy-6-iodo-α-D-glucopyranoside (**14**) [27]: yield 0.3 g, m.p. 115–116°, $[\alpha]_D^{25} = +83.7°$ ($c = 0.56$, chloroform).

$C_{28}H_{30}JNO_6$	Calc.	C 55.73	H 5.01	N 2.32	J 21.03%
(603.45)	Found	,, 55.49	,, 4.99	,, 2.19	,, 20.82%

2.2. *From benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-6-O-p-tolylsulfonyl-α-D-glucopyranoside* (**10**) *and methyl 3,4-di-O-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyluronate chloride* (**11**). – 2.2.1. *Methyl 1,2,3,4-tetra-O-acetyl-D-glucopyranuronate* (**27**) [19]. D-Glucuronolactone (**26**; 400 g, 2.28 mol) was added portionwise to the solution of 1.1 g of sodium hydroxide in 3 l of methanol and the solution was stirred for 1 h at RT. The yellow solution was concentrated to a syrup which was dried under reduced pressure. The syrup was dissolved in 1 l of pyridine, and 1.5 l of acetic anhydride was added dropwise under ice-cooling and stirring. On standing in the refrigerator overnight crystalline material separated. The crystals were filtered off and washed with cold ethanol. A second crop of crystalline material was obtained after concentration of the reaction mixture to give 331.4 g (38.5%) of methyl 1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronate, m.p. 178–179°, $[\alpha]_D^{25} = +8.1°$ ($c = 1.11$, chloroform) (lit. [19]: m.p. 176.5–178°, $[\alpha]_D^{24} = +7.4°$ ($c = 2$, chloroform)). The dark brown syrup obtained after evaporation of the mother liquor was chromatographed on a silica gel column (2 kg) by elution with ethyl acetate/hexane 1:1. Evaporation of the solvent gave a yellow syrup which was crystallized from ether, to give 257.5 g (30%) of a mixture of methyl 1,2,3,4-tetra-O-acetyl-α-D- and β-D-glucopyranuronate, m.p. 103–115°, $[\alpha]_D^{25} = +75.9°$ ($c = 1.39$, chloroform). TLC. (hexane/ethyl acetate 1:1): spots at Rf = 0.25 (α-anomer) and 0.45 (β-anomer).

2.2.2. *Methyl 3,4-di-O-acetyl-1,2-dideoxy-*D-arabino-*hex-1-enopyranuronate* (**28**) [21]. Compound **27** (200 g, 532 mmol) was dissolved in 750 ml of a 30–33% solution of hydrogen bromide in acetic acid, and this solution was maintained at 5° for 20 h [19]. Zinc dust (400 g) was added to the solution of sodium acetate (500 g) and copper sulfate (40 g) in 1.3 l of 50% aqueous acetic acid. The solution of the bromide was added dropwise to this mixture at −10° under stirring. Stirring was continued for 3 h at −10°. The reaction mixture was filtered, the filter cake was washed with 100 ml of 50% aqueous acetic acid, and the combined filtrates were poured into 3 l of iced water. The reaction mixture was extracted with 3 × 1 l of dichloromethane, and the extract was successively washed with iced water, 10% aqueous sodium hydrogen carbonate, water, and dried over anhydrous sodium sulfate. Evaporation under reduced pressure gave a syrup which crystallized on standing. This crystalline material was triturated with isopropyl ether, stored overnight in a refrigerator, filtered off, washed with isopropyl ether and then with petroleum ether: yield 118.8 g (86.5%), m.p. 88–91°, $[\alpha]_D^{25} = -61.3°$ ($c = 0.87$, chloroform). – IR.: 1762, 1733 (C=O, ester), 1649 cm^{-1} (C=C–O). – ^{1}H-NMR. (100 MHz, C_6D_6): ~4.74 (*d* × *d*, $J_{3,5} = 1.4$, $J_{4,5} = 2.8$, H–C(5)); 4.91 (*d* × *d* × *d*, $J_{1,2} = 6.0$, $J_{2,3} = 5.0$, $J_{2,4} = 1.5$ H–C(2)); 5.09 (*m*, H–C(3)); 5.59 (*d* × *d* × *d*, H–C(4)); 6.42 (*d*, H–C(1)).

$C_{11}H_{14}O_7$ (258.23) Calc. C 51.16 H 5.47% Found C 51.33 H 5.45%

2.2.3. *Methyl 3,4-di-O-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyluronate chloride* (**11**). 51.6 g (200 mmol) of **28** were added to the solution of 15.7 g (240 mmol) of nitrosyl chloride in 250 ml of dichloromethane, and the solution was allowed to stand for 20 h at 5°. TLC. with hexane/ethyl

acetate 1:1 as developer indicated that no starting material, Rf = 0.65, was present, and showed one new substance with Rf = 0.40. The solution was poured into 500 ml of ice-water, and the organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The crystalline residue was recrystallized from acetone/hexane: yield 54.2 g (71%), m.p. 111–112°, $[\alpha]_D^{25} = +145.9°$ ($c = 0.95$, chloroform). – UV. (dioxane): 299 nm (ε 2880). – ^{1}H-NMR. (100 MHz, $CDCl_3$): 2.01 (*s*, –OAc); 2.05 (*s*, –OAc); 3.76 (*s*, $-CO_2CH_3$); 4.62 (*d*, $J_{4,5} \sim 10.2$, H–C(5); 5.27 (*d* × *d*, $J_{3,4} \sim 9$, H–C(4)); 5.43 (*d* × *d*, $J_{1,2} \sim 3.8$, $J_{2,3} \sim 10.5$, H–C(2)); 6.05 (*d* × *d*, H–C(3)); 6.67 (*d*, H–C(1)).

$C_{11}H_{14}ClNO_8 \cdot CH_3COCH_3$	Calc.	C 44.05	H 5.28	N 3.67	Cl 9.29%
(381.76)	Found	,, 43.75	,, 5.27	,, 3.58	,, 9.44%

2.2.4. *Reaction of methyl 3,4-di-O-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyluronate chloride* (**11**) *with benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-6-O-p-tolylsulfonyl-α-D-glucopyranoside* (**10**). 137.5 g (212 mmol) of **10** and 121.4 g (318 mmol) of **11** were dissolved in 500 ml of dry N,N-dimethylformamide, and the solution was allowed to stand in the dark and at RT. for 7 days. The reaction mixture was evaporated under reduced pressure (0.01 Torr) to give a tan-colored syrup. The crude product was dissolved in 500 ml of dichloromethane, and the solution was successively washed with water, 3% aqueous sodium hydrogen carbonate, water, dried over anhydrous sodium sulfate and concentrated to a syrup. TLC., using hexane/ethyl acetate 1:1 as developer, revealed 5 spots with Rf = 0.00, 0.39, 0.47, 0.61 and 0.72. The spot with Rf = 0.61 corresponded with a marker of **10**. The spots with Rf = 0.39 and 0.47 were the most intense spots, whereas the other three were very weak. The mixture was fractionated on a silica gel column (3 kg), and elution was effected with hexane/ethyl acetate 3:2 (fraction A) and 1:1 (fraction B). As shown by TLC., fraction A contained a major component with Rf = 0,61, a minor component with Rf = 0.72 and very little of a third component with Rf = 0.47; fraction B contained two major components with Rf = 0.47 and 0.39, and very small amounts of another component with Rf = 0.61. Solvent removal from these fractions gave a yellow foam (fraction A, 17.2 g) and a crystalline residue (fraction B, 184.4 g).

2.2.4.1. *Benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-4-O-(methyl 3,4-di-O-acetyl-2-hydroxyimino-α-D-*arabino*-hexopyranosyluronate)-6-O-p-tolylsulfonyl-α-D-glucopyranoside* (**31**). The white crystalline solid (fraction B) was triturated in ether/isopropyl ether, filtered off, washed with ether/isopropyl ether 1:1 and dried: yield 107.0 g (54%), m.p. 155–157°. After recrystallization from acetone/ether, the product had m.p. 158–159° and $[\alpha]_D^{25} = +115.1°$ ($c = 0.97$, chloroform). – IR.: 1759 (C=O, ester), 1735 cm^{-1} (C=O, carbamate). – UV.: 225 nm (ε 12800). – ^{1}H-NMR. (90 MHz, $CDCl_3$): 2.05 (*s*, –OAc); 2.07 (*s*, –OAc); 2.41 (*s*, ); 3.75 (*s*, $-CO_2CH_3$); 5.32 (*d* × *d*, $J_{3',4'} \sim J_{4',5'} \sim 9.5$, H–C(4')); 5.77 (*d*, H–C(3')); 6.36 (br. *s*, H–C(1')); ~7.90 (br. *s*, =N–OH).

$C_{46}H_{50}N_2O_{17}S$	Calc.	C 59.09	H 5.39	N 3.00	S 3.43%
(934.97)	Found	,, 58.98	,, 5.23	,, 3.03	,, 3.28%

The mother liquor of the above crystallization was evaporated to dryness, and the residue was fractionated on a silica gel column (2 kg). Elution was effected with hexane/ethyl acetate 2:1 and 3:2. Fractions containing pure materials with Rf = 0.61, 0.47 and 0.39 were evaporated to syrups which were crystallized and recrystallized from ether.

2.2.4.2. *Benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-6-O-p-tolylsulfonyl-α-D-glucopyranoside* (**10**). The product was obtained from fractions with Rf = 0.61: yield 2.5 g (2%), m.p. 121–122°.

2.2.4.3. *Benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-6-chloro-2,6-dideoxy-4-O-(methyl 3,4-di-O-acetyl-2-hydroxyimino-α-D-*arabino*-hexopyranosyluronate)-α-D-glucopyranoside* (**32**). The structure of the material with Rf = 0.47 was proved as shown below: yield 28.7 g (17%), m.p. 138–139°, $[\alpha]_D^{25} = +126.9°$ ($c = 0.69$, chloroform). – IR.: 1757, 1731 (C=O, ester), 1699 cm^{-1} (C=O, carbamate).

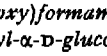
$C_{39}H_{43}ClN_2O_{14}$	Calc.	C 58.61	H 5.42	N 3.50	Cl 4.44%
(799.23)	Found	,, 58.59	,, 5.38	,, 3.53	,, 4.42%

2.2.4.4. *Disaccharide* **31** was obtained from fractions with Rf = 0.39: yield 21.9 g (11%).

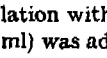
2.2.4.5. Acetylation of the products of fraction A. The products of fraction A (15.5 g) were dissolved in 100 ml of pyridine, the solution was cooled to 0°, and 40 ml of acetic anhydride were added dropwise under stirring. After 24 h standing at RT., the solution was evaporated to dryness. The residue was treated with 200 ml of ice and water and stirred for 1 h. The reaction mixture was extracted with 500 ml of chloroform. The extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to yield 17.2 g of a yellow syrup. TLC., using hexane/ethyl acetate 1:1 as developer, showed a major spot at Rf = 0.72, and a minor one at Rf = 0.82. The mixture was fractionated on a silica gel column (500 g).

2.2.4.6. Benzyl 4-O-acetyl-3-O-benzyl-2-[1-(benzyloxy)formamido]-6-chloro-2,6-dideoxy-α-D-glucopyranoside (**33**). Development with hexane/ethyl acetate 3:1 gave a crystalline product, Rf = 0.82. Recrystallization from ethyl acetate, ether and hexane gave pure product: yield 3.6 g, m.p. 136–138°, $[\alpha]_D^{25} = +97.2°$ ($c = 1.03$, chloroform). – IR.: 1740 (C=O, ester), 1695 cm^{-1} (C=O, carbamate). – ^{1}H-NMR. (100 MHz, C_6D_6): 1.50 (*s*, –OAc); ~3.37 (center of the *AB* part of an *ABX* spectrum, $J_{A,B} \sim 12.5$, $H_2C(6)$); 3.60 (*d*×*d*, $J_{2,3} \sim J_{3,4} \sim 9$, H–C(3); 3.90 (*d*×*d*×*d*, $J_{4,5} \sim 10$, $J_{5,6} \sim 4.5$, $J_{5,6'} \sim 6.5$, H–C(5)); ~4.25 (*m*, $J_{1,2} \sim 3$, H–C(2)); 4.75 (*d*, H–C(1)); ~5.03 (*d*×*d*, H–C(4)).

$C_{30}H_{32}ClNO_7$	Calc.	C 65.04	H 5.82	N 2.53	Cl 6.40%
(554.04)	Found	,, 65.24	,, 5.73	,, 2.46	,, 6.07%

2.2.4.7. Benzyl 4-O-acetyl-3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-6-O-p-tolylsulfonyl-α-D-glucopyranoside (**34**). Development with hexane/ethyl acetate 2:1 gave a syrup, Rf = 0.72; yield 9.6 g. Crystallization and recrystallization from benzene, ether and hexane gave pure product: yield 8.7 g, m.p. 98–100°, $[\alpha]_D^{25} = +83.8°$ ($c = 1.04$, chloroform). – ^{1}H-NMR. (100 MHz, $CDCl_3$): 1.92 (*s*, –OAc); 2.42 (*s*, ); 3.66 (*d*×*d*, $J_{2,3} \sim 10$, $J_{3,4} \sim 9$, H–C(3)); 4.83 (*d*, $J_{1,2} = 3.5$, H–C(1)); ~4.90 (H–C(4), masked by other signals).

$C_{27}H_{39}NO_{10}S$	Calc.	C 64.43	H 5.70	N 2.03	S 4.65%
(689.78)	Found	,, 64.26	,, 5.64	,, 1.98	,, 4.70%

2.2.5. Benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-4-O-(methyl 2,3,4-tri-O-acetyl-α-D-glucopyranosyluronate)-6-O-p-tolylsulfonyl-α-D-glucopyranoside (**35**). 36.0 g (38.5 mmol) of **31** were suspended in 360 ml of levulinic acid and 100 ml of 1N hydrochloric acid, and the suspension was heated for 7 h at 40° (bath) under stirring, whereupon solution occurred. The reaction mixture was extracted with 1 l of ethyl acetate. The extract was successively washed with water, aqueous sodium hydrogen carbonate and water, then dried over anhydrous sodium sulfate and evaporated to dryness. The dried white foam was dissolved in 360 ml of tetrahydrofurane and cooled in ice and water. Sodium borohydride (1.14 g) in water (30 ml) was added dropwise to this solution under stirring. After stirring at 0° for 30 min and at RT. for 1 h, the reaction mixture was neutralized with acetic acid and evaporated under reduced pressure to near dryness. Remaining acids were removed by repeated codistillation with methanol. The dried residue was dissolved in 200 ml of pyridine, acetic anhydride (40 ml) was added with cooling and stirring, and the solution was maintained at RT. overnight. The reaction mixture was evaporated to a syrup. This syrup was treated with 200 ml of ice and water, and the mixture was stirred for 1 h. The reaction mixture was extracted with chloroform (500 ml). The extract was washed with water (100 ml), dried over anhydrous sodium sulfate and evaporated to give an amber syrup; yield 36.4 g. TLC. with hexane/ethyl acetate 1:1 as developer showed one main spot at Rf = 0.40 and minor spots at Rf = 0.50, 0.20 and 0.00. The mixture was separated on a silica gel column (1 kg) by development with hexane/ethyl acetate 1:1. Solvent removal from fractions with Rf = 0.40 left a white foam: yield 25.8 g (70%), $[\alpha]_D^{25} = +114.7°$ ($c = 0.63$, chloroform). – UV.: 224 nm (ε 12175). – ^{1}H-NMR. (100 MHz, $CDCl_3$): 1.81 (*s*, –OAc); 1.92 (*s*, –OAc); 2.06 (*s*, –OAc); 2.42 (*s*, ); 3.73 (*s*, $-CO_2CH_3$); 4.88 (*d*×*d*, $J_{1',2'} \sim 4.2$, $J_{2',3'} \sim 8$, H–C(2')); 5.16 (*d*×*d*, $J_{3',4'} \sim J_{4',5'} \sim 8$, H–C(3') or H–C(4')); 5.38 (*d*, H–C(1')); 5.40 (*d*×*d*, H–C(4') or H–C(3')).

$C_{48}H_{53}NO_{18}S$	Calc.	C 59.81	H 5.54	N 1.45	CH_3O–	3.22%
(964.00)	Found	,, 59.73	,, 5.58	,, 1.41	,,	3.39%

2.2.6. *Benzyl 3-O-benzyl-2-[1-(benzyloxy)formamido]-2,6-dideoxy-4-O-(methyl 2,3,4-tri-O-acetyl-α-D-glucopyranosyluronate)-6-iodo-α-D-glucopyranoside* (**37**). Compound **35** (25.1 g, 26 mmol) was dissolved in 350 ml of 2-pentanone and sodium iodide (33.6 g) was added. The mixture was heated for 7 h under reflux with stirring, and the solvent was removed under reduced pressure. The residue was treated with water (250 ml), and the mixture was extracted with 500 ml of ethyl acetate. The extract was dried over anhydrous sodium sulfate. After solvent removal under reduced pressure a syrup was obtained, which was crystallized from benzene and hexane as small white needles: yield 19.3 g (81%), m.p. 147–148°, $[\alpha]_D^{25} = +118.5°$ ($c = 0.95$, chloroform). – IR.: 1759 (C=O, ester), 1700 cm^{-1} (C=O, carbamate). – ^{1}H-NMR. (100 MHz, $CDCl_3$): 1.83 (*s*, –OAc); 1.94 (*s*, –OAc); 2.05 (*s*, –OAc); 3.75 (*s*, $-CO_2CH_3$); ~4.89 (*d*, $J_{1,2} \sim 3.0$, H–C(1)); 5.19 (*d* × *d*, $J_{2',3'} \sim J_{3',4'} \sim J_{4',5'} \sim 8$, H–C(3') or H–C(4')); 5.43 (*d* × *d*, H–C(3') or H–C(4')); 5.57 (*d*, $J_{1',2'} = 3.5$, H–C(1')).

$C_{41}H_{46}JNO_{15}$	Calc.	C 53.54	H 5.04	N 1.52	J 13.80%
(919.71)	Found	,, 53.72	,, 4.97	,, 1.66	,, 13.81%

2.2.7. *Benzyl 6-O-acetyl-3-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-4-O-(methyl 2,3,4-tri-O-acetyl-α-D-glucopyranosyluronate)-α-D-glucopyranoside* (**21**). – 2.2.7.1. *From* **37**. The solution of 40.0 g (43.5 mmol) of **37** in 1.6 l of acetic anhydride was heated to 70°. To this solution, a warm solution (70°) of 65 g of silver acetate in 2 l of pyridine was added dropwise under stirring over a period of 30 min. After the addition was complete, the reaction mixture was heated 12 additional hours with stirring. The reaction mixture was evaporated under reduced pressure to a dark brown residue from which the last traces of pyridine were removed by repeated evaporation with toluene. The dried residue was triturated with warm benzene (500 ml), the mixture was filtered, and the filter cake was washed with 4 × 100 ml of warm benzene. The brown syrup obtained after solvent removal from the combined benzene extracts was examined by TLC. with hexane/ethyl acetate 1:1 as developer. A major spot with Rf = 0.45 and a minor spot with Rf = 0.65 were observed. The spot with Rf = 0.65 corresponded with a marker of **37**. The mixture was fractionated on a column (2 kg) of silica gel by development with hexane/ethyl acetate 2:1. Solvent removal from fractions with Rf = 0.65 gave crystalline **37**: yield 4.9 g (12%). Fractions with Rf = 0.45 gave, after solvent removal, an amorphous, slightly yellow product, which was crystallized and recrystallized from methanol as small, white needles: yield 20.4 g (55%), m.p. 101–102°, $[\alpha]_D^{25} = +131.8°$ ($c = 0.47$, chloroform). – IR.: 1759 cm^{-1} (C=O ester, carbamate). – ^{1}H-NMR. (100 MHz, $CDCl_3$): 1.86 (*s*, –OAc); 1.94 (*s*, –OAc); 2.03 (*s*, –OAc); 2.08 (*s*, –OAc); 3.72 (*s*, $-CO_2CH_3$); 5.15 (*d* × *d*, $J_{2',3'} \sim J_{3',4'} \sim J_{4',5'} \sim 8.5$, H–C(3') or H–C(4')); 5.43 (*d* × *d*, H–C(3') or H–C(4')); 5.61 (*d*, $J_{1',2'} \sim 3$, H–C(1')).

$C_{43}H_{49}NO_{17}$ (851.85) Calc. C 60.63 H 5.80 N 1.64% Found C 60.35 H 5.83 N 1.57%

2.2.7.1. *From* **20**. Compound **20** was converted into **21** following the above procedure (70% yield).

3. Synthesis of 2-acetamido-2-deoxy-4-O-(methyl α-D-glucopyranosyluronate)-D-glucopyranose (1) and 2-[1-(benzyloxy)formamido]-2-deoxy-4-O-(methyl α-D-glucopyranosyluronate)-D-glucopyranose (2). – 3.1. *2-Acetamido-1,3,6-tri-O-acetyl-2-deoxy-4-O-(methyl 2,3,4-tri-O-acetyl-α-D-glucopyranosyluronate)-β-D-glucopyranose* (**43**). A solution of 7.0 g (8.2 mmol) of **21** in 150 ml of acetic acid was hydrogenated over black palladium (1 g) for 3 days. The catalyst was filtered off and washed with methanol. The combined filtrate and washings were treated with 8.2 ml of 1 N hydrochloric acid and evaporated to dryness to give a quantitative yield of the hydrochloride **42**. TLC. of this glass with chloroform/methanol 1:1 as developer showed a single spot with Rf = 0.65.

A solution of 4.7 g (8.2 mmol) of **42** in 50 ml of dry pyridine was treated dropwise under stirring at 0° with 22 ml of acetic anhydride. After 24 h at RT. the mixture was poured into ice and water (500 ml) and extracted with 2 × 100 ml of dichloromethane. The combined extracts were successively washed with 3 N sulfuric acid, water, and then dried over anhydrous sodium sulfate. Concentration of the solution gave a glass (4.6 g) showing one major spot at Rf = 0.75 and one very small spot at Rf = 0.55 on TLC. with chloroform/methanol 9:1 as developer. The crude material was chromatographed on a column (150 g) of silica gel. Evaporation of the ethyl acetate eluate gave pure product as a white amorphous solid, which was crystallized and recrystal-

lized from acetone and ether: yield 3.5 g (64%), m.p. 184–185°, $[\alpha]_D^{25} = +74.8°$ ($c = 0.90$, chloroform). – ^{1}H-NMR. (100 MHz, 220 MHz, $CDCl_3$): 1.90 (*s*, –NAc); 2.00 (*s*, –OAc); 2.02 (*s*, 2× –OAc); 2.04 (*s*, –OAc); 2.06 (*s*, –OAc); 2.08 (*s*, –OAc); 3.73 (*s*, $-CO_2CH_3$); 4.87 (*d*×*d*, $J_{1',2'} \sim 3.5$, $J_{2',3'} \sim 10$, H–C(2')); 5.41 (*d*×*d*, $J_{3',4'} \sim 9.5$, H–C(3')); 5.54 (*d*, H–C(1')); 5.65 (*d*, $J_{1,2} = 8.5$, H–C(1)).

$C_{27}H_{37}NO_{18}$ (663.58) Calc. C 48.87 H 5.62 N 2.11% Found C 48.62 H 5.44 N 2.02%

3.2. *2-Acetamido-2-deoxy-4-O-(methyl α-D-glucopyranosyluronate)-D-glucopyranose* (**1**). Compound **43** (3.18 g, 4.8 mmol) was dissolved in 30 ml of abs. methanol, and 10 ml of a 0.5% methanolic sodium methoxide solution was added. After stirring at RT. for 20 min the solution was neutralized by stirring with Amberlite IR 120 (H^+) cation-exchange resin and then concentrated under reduced pressure to give 1.97 g (100%) of a white amorphous solid. Crystallization and recrystallization from methanol and acetone gave pure product, m.p. 149–150° (dec.), $[\alpha]_D^{25} = +82 \rightarrow +96°$ ($c = 0.80$, methanol).

$C_{15}H_{25}NO_{12}$ (411.36) Calc. C 43.80 H 6.13 N 3.40% Found C 43.55 H 5.87 N 3.11%

3.3. *6-O-Acetyl-2-[1-(benzyloxy)formamido]-2-deoxy-4-O-(methyl 2,3,4-tri-O-acetyl-α-D-glucopyranosyluronate)-D-glucopyranose* (**44**). Benzyl chloroformate (5.4 g, 31.6 mmol) was added to the solution of **42** (11.0 g, 19.2 mmol) and sodium hydrogen carbonate (3.52 g, 41.8 mmol) in 250 ml water. The reaction mixture was shaken overnight at RT. and then extracted with 2× 250 ml of chloroform. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated to a brown oil (14.6 g). Examination of this oil by TLC., using chloroform/methanol/3N ammonia 40:10:1, revealed a major component with Rf = 0.65 and a minor component with Rf = 0.80. The mixture was fractionated on a column (500 g) of silica gel. Elution with benzene/ethyl acetate 1:1 gave **44** as a yellow, amorphous solid: yield 6.2 g (48%), $[\alpha]_D^{25} = +84.7°$ ($c = 0.47$, chloroform).

$C_{29}H_{37}NO_{17}$ (671.61) Calc. C 51.86 H 5.55 N 2.09% Found C 52.10 H 5.77 N 2.09%

3.4. *2-[1-(Benzyloxy)formamido]-2-deoxy-4-O-(methyl α-D-glucopyranosyluronate)-D-glucopyranose* (**2**). Disaccharide **44** was de-O-acetylated with sodium methoxide as described for **1** (yield 100%). Crystallization and recrystallization from 2-propanol: m.p. 189–190°, $[\alpha]_D^{25} = +97.6 \rightarrow +86.3°$ ($c = 0.89$, water).

$C_{21}H_{29}NO_{13}$ (503.46) Calc. C 50.10 H 5.81 N 2.78% Found C 49.62 H 5.80 N 2.66%

4. Synthesis of disaccharides 3, 4 and 5. – 4.1. *Benzyl 2-[1-(benzyloxy)formamido]-2-deoxy-6-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside* (**3**). Benzyl 2-[1-(benzyloxy)formamido]-2-deoxy-α-D-glucopyranoside (**46**; 4.0 g, 9.9 mmol) [29], Drierite (4 g) and silver oxide (2 g) were stirred in 200 ml of chloroform for 30 min in the dark. 4.1 g (10 mmol) of 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (**45**) [30] were added to this mixture. The reaction mixture was stirred for 16 h at RT. The solids were filtered off, washed with chloroform, and the combined filtrate and washings were concentrated to a syrup. TLC. using ethyl acetate as developer revealed 6 major components with Rf = 0.93, 0.85, 0.78, 0.70, 0.52 and 0.30. The crude mixture was separated on a silica gel column (250 g). Elution with ethyl acetate/benzene 3:1 gave compound **3** with Rf = 0.52. Crystallization and recrystallization from benzene gave pure product: yield 0.8 g (11%), m.p. 137–138°, $[\alpha]_D^{25} = +36.2°$ ($c = 1.01$, chloroform). – ^{1}H-NMR. (100 MHz, 270 MHz, $CDCl_3$): 2.00 (*s*, –OAc); 2.01 (*s*, –OAc); 2.03 (*s*, –OAc); 2.07 (*s*, –OAc); 3.14 (*d*, $J \sim 3.0$, –OH); 3.34 (*d*, $J \sim 3.3$, –OH); ~4.15 (*d*×*d*, $J_{5,6} = 2.2$, $J_{6,6'} \sim 12$, H–C(6)); 4.23 (*d*×*d*, $J_{5,6'} = 4.4$, H–C(6')); 4.61 (*d*, $J_{1',2'} \sim 7.5$, H–C(1')); 4.88 (*d*, $J_{1,2} = 3.6$, H–C(1)); 5.02 (*d*×*d*, $J_{2',3'} = 9.3$, H–C(2')); 5.20 (*d*×*d*, $J_{3',4'} \sim 9.3$, H–C(3')).

$C_{35}H_{43}NO_{16}$ (733.72) Calc. C 57.29 H 5.91 N 1.91% Found C 57.11 H 5.83 N 1.84%

4.2. *Benzyl 2-[1-(benzyloxy)formamido]-6-O-benzylsulfonyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside* (**4**). Benzyl 2-[1-(benzyloxy)formamido]-6-O-benzylsulfonyl-2-deoxy-α-D-glucopyranoside (**47**; 2.7 g, 4.85 mmol), Drierite (4 g) and silver oxide (2 g) were stirred in 300 ml of anhydrous chloroform for 30 min in the dark. 2.1 g (5.1 mmol) of 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (**45**) [30] were added to this mixture. The reaction mixture was refluxed for 6 h with stirring. The solids were filtered off, washed with chloroform, and the combined filtrate and washings were concentrated to a syrup. TLC. using ethyl acetate/dichloromethane 1:1 as developer revealed one major component with Rf = 0.52 and two minor com-

ponents with Rf = 0.80 and 0.65. The crude mixture was fractionated on a silica gel column (100 g), and the component with Rf = 0.52 was eluted with benzene/ethyl acetate 9:1. Crystallization and recrystallization from ethyl acetate and petroleum ether gave pure product: yield 0.6 g (14%), m.p. 162–163°, $[\alpha]_D^{25} = +55°$ ($c = 1.00$, chloroform).

$C_{42}H_{49}NO_{18}S$ (887.89) Calc. C 57.46 H 5.63 S 3.65% Found C 57.08 H 5.89 S 3.32%

4.3. *Benzyl 3,4-di-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-6-O-(β-D-glucopyranosyl)-α-D-glucopyranoside* (**5**). A solution of 3,4,6-tri-O-acetyl-1,2-anhydro-α-D-glucopyranose (**6**; 3.6 g, 12.5 mmol) and benzyl 3,4-di-O-benzyl-2-[1-(benzyloxy)formamido]-2-deoxy-α-D-glucopyranoside (**48**; 6.8 g, 11.6 mmol) [2] in acetonitrile (50 ml) was refluxed for 3 days and then evaporated to dryness. Crystallization from methanol gave starting material **48**: yield 5.1 g (75%), m.p. and mixed m.p. 151–152°. TLC. of the mother liquor, with dichloromethane/ethyl acetate 3:2 as developer, revealed two major components with Rf = 0.55 and 0.45, and several other minor components. The product obtained after solvent removal (2.2 g) was dissolved in dry methanol (100 ml) and the solution was saturated at 0° with ammonia. After standing for 18 h at RT., the reaction mixture was evaporated to a syrup. TLC., using a mixture of chloroform/methanol/3N ammonia 40:10:1 revealed components with Rf = 0.65, 0.55 and 0.45. The crude mixture was chromatographed on a silica gel column (120 g), and development with ethyl acetate gave component **5** with Rf = 0.45. Crystallization and recrystallization from acetone and ether gave pure product: yield 0.15 g (2%), m.p. 174–175°, $[\alpha]_D^{25} = +70.8°$ ($c = 1.02$, chloroform).

$C_{41}H_{47}NO_{12}$ (745.82) Calc. C 66.03 H 6.35 N 1.88% Found C 65.80 H 6.41 N 1.82%

REFERENCES

[1] *J. Kiss & P. C. Wyss*, Tetrahedron Letters *1972*, 3055.
[2] *P. C. Wyss & J. Kiss*, Helv. *58*, 1833 (1975).
[3] *R. J. Ferrier*, Fortschr. chem. Forsch. *14*, 389 (1970).
[4] *G. Wulff & G. Röhle*, Angew. Chem. *86*, 173 (1974).
[5] *R. U. Lemieux*, Canad. J. Chemistry *31*, 949 (1953).
[6] *G. Maghuin-Rogister*, Bull. Soc. chim. Belges *77*, 575 (1968).
[7] *R. U. Lemieux & G. Huber*, J. Amer. chem. Soc. *78*, 4117 (1956).
[8] *R. U. Lemieux & H. F. Bauer*, Canad. J. Chemistry *32*, 340 (1954).
[9] *R. U. Lemieux, T. L. Nagabhushan & S. W. Gunner*, Canad. J. Chemistry *46*, 405 (1968).
[10] *R. U. Lemieux, S. Suemitsu & S. W. Gunner*, Canad. J. Chemistry *46*, 1040 (1968).
[11] *T. L. Nagabhushan*, Canad. J. Chemistry *48*, 257 (1970).
[12] *R. U. Lemieux, K. James & T. L. Nagabhushan*, Canad. J. Chemistry *51*, 42 (1973).
[13] *R. U. Lemieux, K. James & T. L. Nagabhushan*, Canad. J. Chemistry *51*, 48 (1973).
[14] *K. Miyai & R. W. Jeanloz*, Carbohydrate Res. *21*, 45 (1972).
[15] *S. Brennan & P. A. Finan*, J. chem. Soc. *1970*, 1742.
[16] *C. S. Hudson & J. K. Dale*, J. Amer. chem. Soc. *37*, 1264 (1915).
[17] *E. J. Bourne, M. Stacey, J. C. Tatlow & J. M. Tedder*, J. chem. Soc. *1949*, 2976.
[18] *Y. Hirasaka*, Yakugaku Zasshi *83*, 960 (1963).
[19] *G. N. Bollenback, J. W. Long, D. G. Benjamin & J. A. Lindquist*, J. Amer. chem. Soc. *77*, 3310 (1955).
[20] *W. E. Goebel & F. H. Babers*, J. biol. Chemistry *106*, 63 (1934).
[21] *Y. Nitta & H. Morita*, Yakugaku Zasshi *81*, 1214 (1961).
[22] *W. J. Serfontein, J. H. Jordaan & J. White*, Tetrahedron Letters *1964*, 1069.
[23] *R. U. Lemieux, T. L. Nagabhushan & I. K. O'Neill*, Tetrahedron Letters *1964*, 1909.
[24] *R. U. Lemieux, T. L. Nagabhushan & I. K. O'Neill*, Canad. J. Chemistry *46*, 413 (1968).
[25] *K. Heyns, K. Propp, R. Harrison & H. Paulsen*, Chem. Ber. *100*, 2655 (1967).
[26] *R. U. Lemieux & J. Howard*, Methods in Carbohydrate Chemistry *2*, 400 (1963).
[27] *J. Kiss & F. Burkhardt*, Helv. *52*, 2622 (1969).
[28] *C. L. Mehltretter, B. H. Alexander, R. L. Mellies & C. E. Rist*, J. Amer. chem. Soc. *73*, 2424 (1951).
[29] *K. Heyns & H. Paulsen*, Chem. Ber. *88*, 188 (1955).
[30] *R. U. Lemieux*, Methods in Carbohydrate Chemistry *2*, 221 (1963).