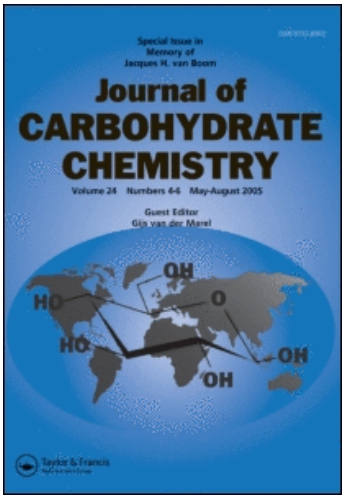


Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Synthesis and High-Field NMR of α-D-Arabinofuranosyl-(1⟶6)-2-AcetamidO-2-Deoxy-O-D-Glucopyranosyl-(1⟶3)-6-Deoxy-L-Talose, the Repeating Unit of an O-Specific Lipopolysaccharide from Pseudomonas Maltophilia N.C.I.B. 9204

András Lipták[a]; János Kerékgyártó[a]; Velimir Popsavin[b]; Maria Kajtár-Peredy[c]; Lajos Radies[c]
[a] L. Kossuth University, Institute of Biochemistry, Debrecen, Hungary [b] Institute of Chemistry, Faculty of Sciences, University of Novi Sac., V. Vlahovica 2, Yugoslavia [c] NMR Laboratory, Central Research Institute of Chemistry, Budapest, Hungary

To cite this Article Lipták, András , Kerékgyártó, János , Popsavin, Velimir , Kajtár-Peredy, Maria and Radies, Lajos(1988) 'Synthesis and High-Field NMR of α-D-Arabinofuranosyl-(1⟶6)-2-AcetamidO-2-Deoxy-O-D-Glucopyranosyl-(1⟶3)-6-Deoxy-L-Talose, the Repeating Unit of an O-Specific Lipopolysaccharide from Pseudomonas Maltophilia N.C.I.B. 9204', Journal of Carbohydrate Chemistry, 7: 2, 337 — 357
To link to this Article: DOI: 10.1080/07328308808058929
URL: http://dx.doi.org/10.1080/07328308808058929

J. CARBOHYDRATE CHEMISTRY, 7(2), 337-357 (1988)

SYNTHESIS AND HIGH-FIELD NMR OF α-D-ARABINOFURANOSYL-(1→6)-2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL-(1→3)-6-DEOXY-L-TALOSE, THE REPEATING UNIT OF AN O-SPECIFIC LIPOPOLYSACCHARIDE FROM PSEUDOMONAS MALTOPHILIA N.C.I.B. 9204

András Lipták*, János Kerékgyártó

L. Kossuth University, Institute of Biochemistry,
P.O.Box 55, H-4010 Debrecen, Hungary

Velimir Popsavin

Institute of Chemistry, Faculty of Sciences, University of Novi Sad,
2100 Novi Sad, V. Vlahovica 2, Yugoslavia

Maria Kajtár-Peredy, and Lajos Radics

NMR Laboratory, Central Research Institute of Chemistry,
P.O.Box 17, H-1525 Budapest, Hungary

Received September 18, 1987 - Final Form November 12, 1987

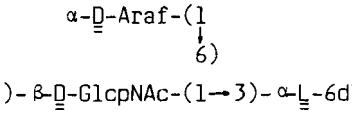
ABSTRACT

Starting from L-rhamnose, benzyl 2,4-di-O-benzyl-6-deoxy-α-L-talopyranoside (7) was prepared by hydrogenolysis of a dioxolane-type benzylidene acetal and used as the aglycon to prepare 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-deoxy-L-talose (13) and the title trisaccharide (20). Due to fast interconversion between the α-, β-pyranose/furanose forms at the reducing end of the molecule in aqueous solutions, the di- and trisaccharides occur as mixtures of four isomers all in significant concentration. By two-dimensional (2D) methods, the proton (400) and carbon (100 MHz) NMR spectra of the individual trisaccharide isomers were completely assigned and interpreted in terms of stereochemistry of the 6-deoxy-L-talose residue.

Dedicated to Professor Rezső Bognár in celebration of his 75th birthday.

INTRODUCTION

Recently Wilkinson et al.[1] proposed a trisaccharide repeating unit for the O-specific polysaccharide of a lipopolysaccharide isolated from cell-walls of Pseudomonas maltophilia N.C.I.B. 9204. This tentative repeating unit is composed of three different monosaccharide building blocks and can be depicted by the following structure:



The synthesis of the trisaccharide attracted our attention for two different reasons: we expected i) to find thereby a suitable method for the preparation of a 6-deoxy-L-talopyranose derivative with a free HO-3 group and ii) to create an α-D-arabinofuranosyl bond in a medium-size oligosaccharide.

RESULTS AND DISCUSSION

The sensitivity to acid hydrolysis of pentofuranosyl residues of oligo- and polysaccharides is well documented.[2] In order to avoid the splitting-off of the constructed α-arabinofuranosyl unit during deprotection steps of the planned synthesis, we followed a synthetic route that made use of readily removable protecting groups, such as acyl- and benzyl groups. For this reason, we choose benzyl 2,4-di-O-benzyl-6-deoxy-α-L-talopyranoside (7) as the aglycon. This compound recently was prepared from benzyl 2,3-O-isopropylidene-α-L-rhamnopyranoside[3] by Aspinall and Takeo.[4] We used the same starting material as did the authors of reference 4; most of the steps, however were modified to improve the yields and work-up procedures.

Of the numerous methods proposed for the preparation of alkyl 6-deoxy-2,3-O-isopropylidene-α-L-lyxo-hexopyranosid-4-ulose,[4,5] we choose pyridinium dichromate[6] in the presence of molecular sieves to obtain benzyl 6-deoxy-2,3-O-isopropylidene-α-L-lyxo-hexopyranosid-4-ulose (1). This procedure was successfully applied by Defaye, Gadelle and Angyal[7] for the oxidation of methyl 2,3-O-isopropylidene-α-L-rhamnopyranoside. Reduction of 1 was achieved by $LiAlH_4$ using benzene-ether (1:1) as the solvent. The reduction proceeded with excellent stereoselectivity and the ratio of the two C-4 epimers was 2:98 (GLC) with benzyl 6-deoxy-2,3-O-isopropylidene-α-L-talopyranoside (2) being the predominant com-

ponent. The main advantage of this method is the ease of the work-up procedures; the excess of the reagent was decomposed by adding ethyl acetate and water to the reaction mixture; the easily separated organic phase (ether) contained compound 2. Compound 2 was benzylated to give 3; subsequent acid hydrolysis of the isopropylidene group resulted in compound 4. This substance was then treated with α,α-dimethoxytoluene in the presence of **p-toluenesulfonic** acid to obtain exo- (5) and endo-benzylidene (6) derivatives of 4. Using kinetic conditions (60 min, 50 °C), the endo-isomer 6 was isolated in a yield of 88%, while only 1.4% of compound 5 was found to be present. To the best of our knowledge, this is the first reported case in which the kinetic isomer of a dioxolane-type benzylidene acetal could be isolated in such a high yield. Hydrogenolysis of the dioxolane ring of 6 followed the well-documented regularity[8] of the ring-opening reactions of dioxolane-type benzylidene acetals and afforded the benzyl 2,4-di-O-benzyl-6-deoxy-α-L-talopyranoside (7).

Nucleophile 7 was glycosylated with 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide using Lemieux's conditions.[9] After column chromatographic purification, the syrupy disaccharide derivative 8 was isolated in a yield of 84%. The β-anomeric configuration of the newly formed glycosidic bond was attested to by the three-bond proton proton coupling constant ($^3J_{1',2'}$ = 9.6 Hz). Zemplén deacetylation of 8 gave 9 from which the phthalimido group was removed by hydrazine hydrate treatment. The resulting crude product was then acetylated to give the crystalline disaccharide derivative (10). Subsequently, the benzyl groups were cleaved *via* catalytic hydrogenolysis and the product thus obtained was acetylated. Two anomers (11 and 12) were formed; these were separated and characterized through their ^{13}C NMR spectra. Saponification of 11 and 12 gave the unique free disaccharide 13.

In preparing the aglycon of the planned trisaccharide, conventional steps, such as tritylation/acetylation (9→14) and detritylation of 14 gave the partially protected disaccharide derivative 15 containing a free HO-6' group. Using the Helferich conditions,[10] aglycon 15 was subsequently treated with 2,3,5-tri-O-benzoyl-α-D-arabinofuranosyl bromide[11] to afford, in excellent yields, the crystalline, fully protected trisaccharide 16.

The acyl groups of 16 were removed by catalytic transesterification, while the phthalimido group was split-off *via* hydrazine hydrate treatment. The resulting crude product was reacetylated to give

the completely protected trisaccharide 17. The benzyl groups of 17 were hydrogenolyzed and the product was acetylated. Two anomers (18 and 19) were formed which could be separated by column chromatography. Trisaccharide peracetates 18 and 19 proved to be anomers at the reducing end, both having a 6-deoxy-L-talose unit in the pyranose form.

Deacetylation of either 18 or 19 resulted in the same, chromatographycally homogeneous, trisaccharide 20. The carbon-13 NMR spectrum of 20 run at 100 MHz exhibited over 60 well-separated resonances of distinctly unequal intensity. While separation of some of the signals was clearly due to the employed high resonance frequency, this finding suggested that, in aqueous solutions, trisaccharide 20 occurs as a mixture of four isomers of significant abundance and, most likely, the isomerism is associated with the α-, β-pyranoside and α-, β-furanoside forms of the reducing end group. A similar spectral behaviour has been reported earlier for a branched trisaccharide with the L-arabinose residue at the reducing end; however, the relative concentration of the furanose forms in the equilibrium mixture was substantially lower than in the present study and, accordingly, quadruplication of individual carbon-13 signals could not be detected.[12]

A detailed two-dimensional (2D) ^{1}H and ^{13}C NMR study was set up in order to select the 'sub-spectra' of individual isomeric trisaccharides and assign the pertinent spectral parameters in terms of stereochemistry at the reducing end. To facilitate spectral assignments, 6-deoxy-3-O--methyl-L-talose (21) was prepared *via* methylation of 7 followed by direct hydrogenolysis of the product into 21 and subjected to high-field NMR analysis.

In a similar manner to the case of trisaccharide (20), the ^{13}C NMR spectrum of 21, displaying 28 separate resonances, indicating the presence in comparable amounts of all four isomeric forms in the solution. Proton chemical shift correlation (COSY) experiment[13] afforded the sequential assignment of protons within individual isomeric monosaccharides. These pieces of information, combined with the results from the ^{13}C, ^{1}H chemical shift correlation (C,H-COSY) experiment[13] have led to the complete assignment of the carbon-13 spectrum while proton-proton couplings were determined from a conventional (1D) spectrum run under high resolution conditions. Distinction between individual isomeric forms (labelled in the sequel for brevity as F^{α},F^{β}, P^{α} and P^{β}) was made by comparison of the ^{1}H and ^{13}C chemical shift data of the anomeric C-H groups with values published on related monosaccharides.[14] The assigned

resonances for 21 are collected in Table 2. Integration of the anomeric proton signals afforded the relative abundances of individual forms as 41.3% (P^{α}), 26.0% (P^{β}), 21.3% (F^{α}) and 11.4% (F^{β}), in good overall agreement with the values reported for 6-deoxy talose by Angyal's group.[7]

An analogous approach has been adopted to assign resonances in the spectra of the trisaccharide mixture 20. The pertinent spectral data are reported in Table 1. With the relative chemical shifts and $^{3}J_{1,2}$ couplings of the C-1H protons of the talose moiety already determined, assignment of the anomeric proton signals (4.55 to 5.33 ppm) proved straightforward. Identified by their characteristic (and constant) value of $^{3}J_{1,2}$ (8.5 Hz), the C-1H signals of the glucosamine residues appeared at four distinct chemical shift values with integrated intensities identical to those measured for the C-1H resonances of the reducing unit, supporting thereby the expectation that isomerism is, in fact, associated with the four forms of the talose residue. Integration afforded the relative concentration of the isomeric trisaccharides as 36% (P^{α}), 24% (P^{β}), 31% (F^{α}) and 9% (F^{β}). Inspection of the chemical shift values of the former resonances also disclosed that effects of isomerism at the reducing end are reflected to a different extent in the chemical shifts of the anomeric protons of the non-reducing residues. Larger relative shifts are found for the transition pyranose - furanose while the change in the anomeric state of the reducing end affects these chemical shifts to a much lesser extent. Accordingly, with the isomerisation--induced chemical shifts further attenuated, the anomeric protons of the arabinose residue display (at the frequency employed in this study) but two distinguishable resonances mirroring the relative abundance of the P^{α}+ P^{β} and F^{α} + F^{β} forms. Subsequent assignment procedures disclosed that this tendency was a general trend for most ^{13}C and ^{1}H sites of the trisaccharide.

Due to heavy overlap of correlation peaks in the standard COSY maps obtained from 20, additional 2D experiments were set up to enable the required sequential assignment of protons to be performed. First, a series of relayed COSY experiments[15] were run in which the number of relay steps (1,2 and 3) and the relevant delay times were varied in order to optimize magnetization transfer between ring protons and anomeric H's. (For isomeric talose residues, the sequential information thus derived received further corroboration from the occurrence of relay peaks transferred stepwise from the C-6 methyl group to the ring protons.) Second, COSY experiments were performed under conditions in which chemical

TABLE 1. NMR Spectral Data for Isomeric Trisaccharides (20)[a]

		6dTal				GlcpNAc				Araf			
Isomer		δ_C	δ_H	$^3J_{H,H}$		δ_C	δ_H	$^3J_{H,H}$		δ_C	δ_H	$^3J_{H,H}$	
	1	97.08	5.203	$J_{1,2}$: 1.9	1'	102.88	4.741	$J_{1',2'}$: 8.4	1''	110.20	5.074	$J_{1'',2''}$:	1.7
	2	72.80	3.997	$J_{2,3}$: 3.3	2'	58.43	3.793	$J_{2',3'}$: 8.8	2''	83.80	4.147	$J_{2'',3''}$:	3.4
	3	76.46	4.024	$J_{3,4}$: 3.2	3'	76.54	3.600	$J_{3',4'}$:10.5	3''	79.34	3.962	$J_{3'',4''}$:	6.1
P^{α}	4	72.86	3.813	$J_{4,5}$: 2.1	4'	72.59	3.564	$J_{4',5'}$: 8.7	4''	86.69	4.091	$J_{4'',5''}$:	5.8; 3.2
	5	69.88	4.152	$J_{5,6}$: 6.6	5'	77.15	3.607	$J_{5',6'}$: 5.7;2.3	5''	64.01	3.719 3.837		
	6	18.54	1.250		6'	68.87	3.874 4.000						
					CH_3-CONH	25.06	2.054						
					CO	177.75	-						
	1	96.51	4.780	$J_{1,2}$: 1.2	1'	102.67	4.744		1''	110.16	5.074		
	2	73.46	4.028	$J_{2,3}$: 3.3	2'	58.43	3.793		2''	83.78	4.143		
	3	78.96	3.896	$J_{3,4}$: 3.6	3'	76.50	3.600		3''	79.37	3.964		
P^{β}	4	71.81	3.740	$J_{4,5}$: 1.0	4'	72.64	3.553		4''	86.74	4.087		
	5	74.06	3.653	$J_{5,6}$: 6.5	5'	77.18	3.602		5''	64.03	3.720 3.875		
	6	18.45	1.280		6'	68.87	3.874 4.000						
					CH_3-CONH	25.06	2.054						
					CO	177.75	-						

	1	103.50	5.245	$J_{1,2}$: 0.8	1'	104.64	4.612		1''	110.20	5.096
	2	78.01	4.173	$J_{2,3}$: 4.2	2'	58.15	3.781		2''	83.88	4.148
	3	83.74	4.169	$J_{3,4}$: 7.5	3'	76.32	3.580		3''	79.30	3.972
F^{α}	4	86.93	3.727	$J_{4,5}$: 6.7	4'	72.54	3.570		4''	86.67	4.099
	5	72.54	3.786	$J_{5,6}$: 6.3	5'	77.11	3.616		5''	63.99	3.725 3.836
	6	20.63	1.168		6'	68.87	3.874 4.000				
					CH_3-CONH	25.00	2.033				
					CO	177.41	-				
	1	99.20	5.325	$J_{1,2}$: 3.9	1'	104.05	4.580		1''	110.20	5.096
	2	73.61	4.188	$J_{2,3}$: 5.9	2'	58.20	3.777		2''	83.84	4.146
	3	82.12	4.167	$J_{3,4}$: 4.8	3'	76.24	3.580		3''	79.30	3.972
F^{β}	4	87.33	3.907	$J_{4,5}$: 3.3	4'	72.54	3.570		4''	86.69	4.091
	5	70.18	3.770	$J_{5,6}$: 6.7	5'	77.18	3.602		5''	64.01	3.719 3.837
	6	21.05	1.195		6'	68.79	3.866 3.974				
					CH_3-CONH	25.00	2.033				
					CO	177.41	-				

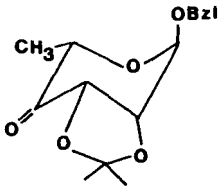
[a] In D_2O at 30°. Chemical shifts are with respect to internal DSS. Mutual interproton couplings are given only once at their first occurrence in the Table. Proton-proton couplings for G and A units remain unaffected by isomerism. Individual isomers are labelled according to the form of the talose unit.

TABLE 2. NMR Spectral Data for 6-Deoxy-3-O-methyl-L-talose (21) Isomers[a]

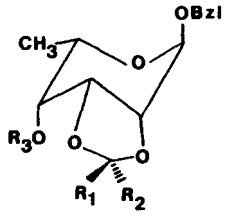
Position	P^{α}		P^{β}		F^{α}		F^{β}	
	δC	δH	δC	δH	δC	δH	δC	δH
1	97.46	5.215	96.77	4.761	103.85	5.248	99.01	5.322
2	70.63	3.980	71.43	4.032	75.47	4.203	72.88	4.198
3	77.05	3.590	80.23	3.450	83.45	3.898	82.74	3.767
4	72.23	3.931	71.25	3.854	87.01	3.730	87.37	3.972
5	69.80	4.160	74.12	3.673	72.04	3.820	70.18	3.830
6	18.56	1.244	18.43	1.272	20.70	1.196	21.06	1.205
OMe	57.95	3.451	58.08	3.456	60.43	3.425	60.61	3.416

$^{n}J_{i,j}$				
$^{3}J_{1,2}$	1.8	1.2	1.3	4.1
$^{3}J_{2,3}$	3.2	3.0	4.4	5.4
$^{3}J_{3,4}$	3.1	3.2	6.6	4.4
$^{3}J_{4,5}$	1.2	1.1	6.2	4.3
$^{3}J_{5,6}$	6.6	6.6	6.5	6.5
$^{4}J_{1,3}$	≤ 0.5	-	≤ 0.5	-
$^{4}J_{2,4}$	1.5	1.4	≤ 0.5	-
$^{4}J_{1,5}$	~ 0.6	-	-	-
$^{5}J_{1,4}$	≤ 0.5	-	-	-

[a] In D_2O at 30^{o}. Chemical shifts are with respect to internal DSS.



1

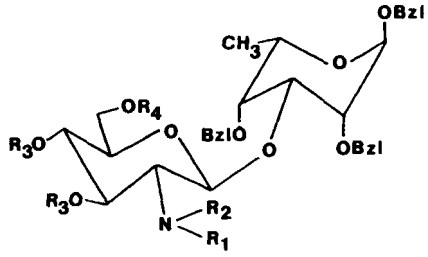


2 $R_1=R_2=CH_3$; $R_3=H$

3 $R_1=R_2=CH_3$; $R_3=Bzl$

5 $R_1=Ph$; $R_2=H$; $R_3=Bzl$

6 $R_1=H$; $R_2=Ph$; $R_3=Bzl$



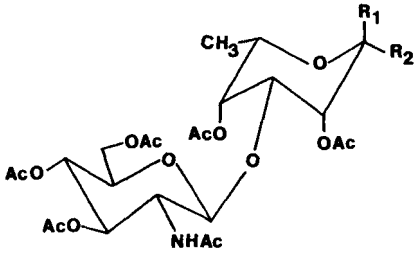
8 $R_1=R_2=Phth$; $R_3=R_4=Ac$

9 $R_1=R_2=Phth$; $R_3=R_4=H$

10 $R_1=H$; $R_2=R_3=R_4=Ac$

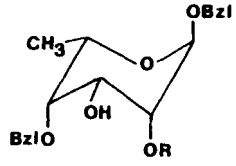
14 $R_1=R_2=Phth$; $R_3=Ac$; $R_4=Tr$

15 $R_1=R_2=Phth$; $R_3=Ac$; $R_4=H$



11 $R_1=OAc$; $R_2=H$

12 $R_1=H$; $R_2=OAc$

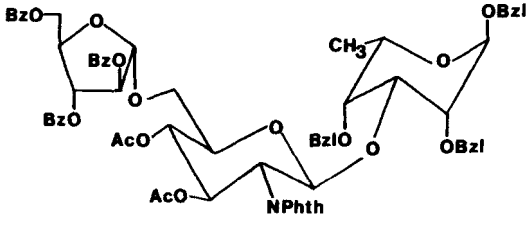


4 R = H

7 R = Bzl

β-D-GlcpNAc-(1→3)-6d-L-Tal

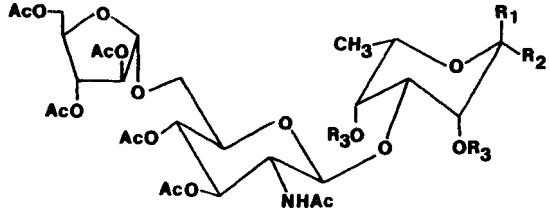
13



16

α-D-Araf-(1→6)-β-D-GlcpNAc-(1→3)-6d-L-Tal

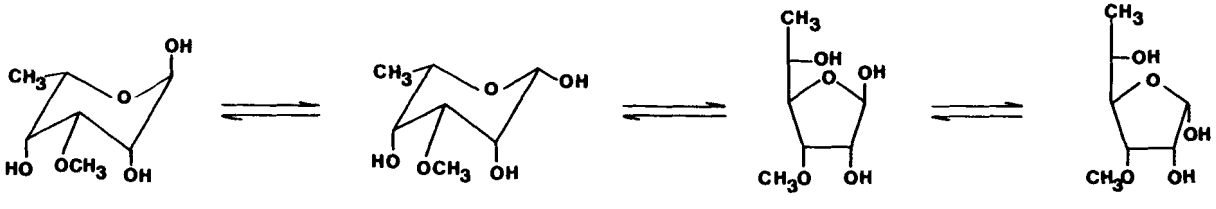
20



17 R_1 = OBzl; R_2 = H; R_3 = Bzl

18 R_1 = OAc; R_2 = H; R_3 = Ac

19 R_1 = H; R_2 = OAc; R_3 = Ac



21

shift correlations mediated by small (0.5 to 2 Hz) vicinal and long-range interproton couplings became substantially enhanced,[16] and these have led to a full set of correlations between ring and anomeric protons for residues featuring small vicinal couplings, such as the arabinose and talo-furanose units. It may be noted that, in this experiment, the anomeric proton resonances of the glucosaminyl residue exhibited correlation peaks due to inter-residual coupling ($^4J_{H\text{-}C\text{-}O\text{-}C\text{-}H}$) to 3-H proton of the talose unit, providing further supporting evidence for the proposed sequence of sugar residues in 20.

Heteronuclear, ^{13}C-^1H, chemical shift correlation experiments run at high digital resolution in both frequency domains afforded the complete assignment of carbon resonances both in terms of position within the molecule and isomerism at the reducing end.

Proton-proton coupling constants related to the stereochemistry of isomeric 20 were, in part, evaluated by plotting individual traces of phase-sensitive 2D correlation spectra. This method, however, failed in highly crowded regions of the 2D map even at high digital resolution. A more convenient approach was offered by observing the coupling patterns of individual protons through the resonances of directly bonded carbon--13 atoms. To this end, the experiment proposed by Morris[17] was carried out at high digital resolution. The quality of data thus obtained is illustrated on the partial 2D map of Figure 1. The possibility to distinguish closely spaced carbon signals through the coupling pattern of directly bonded protons, on the other hand, provided an ultimate verification of the assignment of carbon resonances in cases where the pertinent proton shifts proved practically indistinguishable.

EXPERIMENTAL

General Procedures. Melting points (uncorrected) were determined on a Kofler apparatus. Optical rotations were measured with a PERKIN--ELMER 241 polarimeter. Reactions were monitored by TLC on Kieselgel 60 F_{254} (Merck) with detection by charring with sulfuric acid. Both Kieselgel G and Kieselgel H (Reanal) were used for short-column chromatography. All NMR spectra were run at 30 °C on 15 mg of 21 and 50 mg 20 dissolved in 0.65 mL D_2O in the presence of DSS internal standard using a VARIAN XLA-400 instrument and standard pulse sequences. Proton 2D experiments were performed with an 1800 Hz spectral window, 2K data points in F_2 and 512 (absolute value mode) or 1024 (phase-sensitive mode

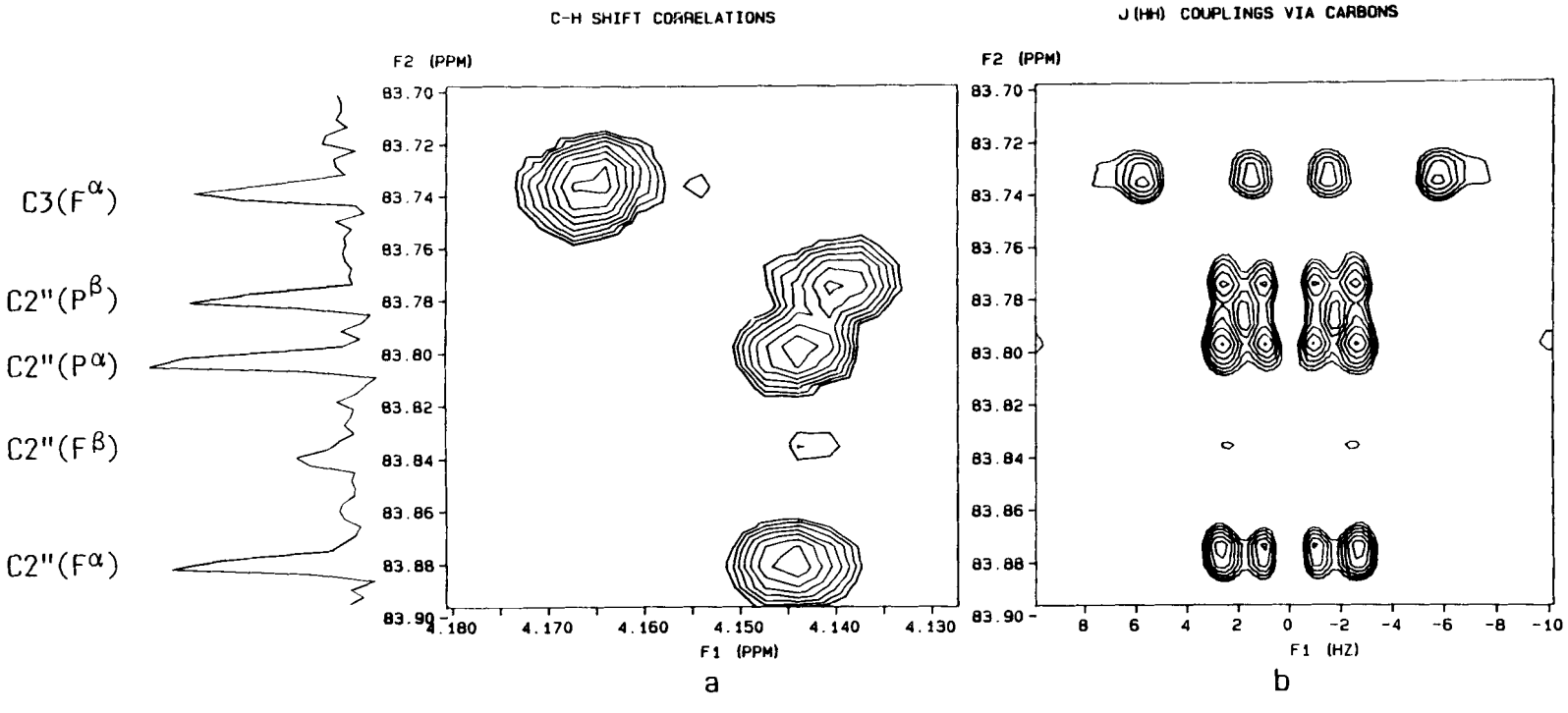


Figure 1. Partial 2D Maps of the C2"(P$^{\alpha}$), C2"(P$^{\beta}$), C2"(F$^{\alpha}$), C2"(F$^{\beta}$) and C3(F$^{\alpha}$) Carbon Resonances in a Typical Crowded Region of the Carbon-13 Spectrum of 20 : a) Chemical Shift Correlations with Directly Bonded Ring Protons, and b) J_{HH} Multiplets of the Same Protons as Detected via Carbon-13 Resonances.

according to States et al.[18]) increments in F_1. The acquired time-domain data matrices, after filtering, were transformed into a 2K by 2K data table using real (non-phased mode) or complex (phased mode) arithmetics. Relayed COSY experiments with one, two and three relay steps were made either with fixed, but not necessarily equal, delay times, τ_1, τ_2 and τ_3, or were run such that experiments with incremented τ_1 values were coadded in time-domain to achieve a uniform coherence transfer for a wider range of couplings. In these experiments the τ_1 values were varied typically from 30 to 100 milisec in 4 to 8 steps, or incremented regularly with t_1 as $\tau_1 = \tau_1^o + \kappa t_1$. Heteronuclear correlation experiments were performed with 8K by 512 time-domain matrices which were transformed into an 8K by 2K data table. High resolution conditions were attained by reducing the spectral window to 5000 Hz in F_2 and 700 Hz in F_1. Indirect proton J spectra were run in pure phase mode with digital resolution of 0.5 Hz in F_1.

Benzyl 6-Deoxy-2,3-O-isopropylidene-α-L-lyxo-hexopyranosid-4-ulose (1). To a stirred solution of benzyl 2,3-O-isopropylidene-α-L-rhamnopyranoside (8.18 g, 27.8 mmol) in dry dichloromethane (120 mL) were added pyridinium dichromate (28.88 g, 139 mmol) and sodium acetate (3 g). The reaction mixture was stirred for 2 h at room temperature and then diluted with ether (120 mL), filtered and washed with ether (50 mL). The filtrate was concentrated and toluene (30 mL) was evaporated from the residue to give a crude syrup. This was then purified on a column of silica gel (250 g) and resulted in compound 1 (6.5 g, 80%) as an amorphous mass: $[\alpha]_D$ -109.8° (c 0.69, chloroform) lit.[4] $[\alpha]_D$ -115.9° (c 2.2, chloroform), R_f 0.67 (dichloromethane-ethyl acetate, 95:5); IR (KBr) 1740 cm^{-1} (C=O).

Anal. Calcd for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. Found: C, 66.01; H, 6.88.

Benzyl 6-Deoxy-2,3-O-isopropylidene-α-L-talopyranoside (2). Compound 1 (6.21 g, 21.3 mmol) was dissolved in a 1:1 mixture of benzene and ether (150 mL), and then 2.83 g (74.6 mmol) of $LiAlH_4$ was added. The reaction mixture was boiled for 30 min under reflux. After cooling, ethyl acetate (5 mL) was added to decompose the excess of the reagent and then water added to precipitate the aluminium hydroxide. The organic layer was decanted and then was washed with water, dried (sodium sulfate) and evaporated. GLC investigation showed that 2 and benzyl 2,3-O-isopropylidene-α-L-rhamnopyranoside were produced in a ratio of 98:2. The residue from the evaporation was crystallized from n-hexane to obtain com-

pound 2 (5.5 g, 88%), mp 40-41 °C, $[\alpha]_D$ -63.2° (c 1.32, chloroform). Lit.[4] mp 41-42 °C, $[\alpha]_D$ -68.2° (c 2.0, chloroform).

Benzyl 4-O-Benzyl-2,3-O-isopropylidene-α-L-talopyranoside (3). Compound 2 (5.05 g, 12.2 mmol) was dissolved in dry N,N-dimethyl formamide (20 mL) and then 9.6 g of powdered potassium hydroxide and 5.6 mL (47.1 mmol) benzyl bromide were added. The reaction mixture was stirred for 15 min at room temperature, then diluted with dichloromethane (100 mL), and filtered. The filtrate was washed with water (5 x 50 mL), dried (sodium sulfate) and evaporated. The syrupy residue was crystallized from methanol to give 3 (5.45 g, 83%), mp 94 °C, $[\alpha]_D$ -23.7° (c 0.87, chloroform), R_f 0.55 (dichloromethane-ethyl acetate), 97:3). Lit.[4] mp 92-93 °C, $[\alpha]_D$ -25.2° (c 1.6, chloroform).

Anal. Calcd for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 71.81; H, 7.36.

Benzyl 4-O-Benzyl-6-deoxy-α-L-talopyranoside (4). To a stirred solution of 3 (4.22 g, 10.9 mmol) in dichloromethane (100 mL) was added 10 mL of trifluoroacetic acid containing 1% of water. After 25 min stirring TLC showed the complete conversion of the starting material. The reaction mixture was diluted with dichloromethane (200 mL) and washed with water (4 x 50 mL). The organic layer was dried (sodium sulfate) and evaporated. The syrupy residue was crystallized from a mixture of ethyl acetate-hexane (1:9) to give 4 (3.25 g, 86%), mp 89 °C, $[\alpha]_{D_4}$ -100.5° (c 0.25, chloroform), R_f 0.60 (dichloromethane-acetone, 9:1). Lit.[4] mp 86-87 °C, $[\alpha]_D$ -99.6° (c 2.2, chloroform).

Anal. Calcd for $C_{20}H_{24}O_5$: C, 69.75; H, 7.02. Found: C, 69.81; H, 7.00.

Benzyl 4-O-Benzyl-exo- (5) and -endo-2,3-O-benzylidene-6-deoxy-α-L-talopyranoside (6). Compound 4 (3.12 g, 9.06 mmol) in dry N,N-dimethylformamide (12 mL) was treated with α,α-dimethoxytoluene (8.2 mL) in the presence of p-toluenesulfonic acid (200 mg) at 50 °C for 60 min under vacuum. Then sodium hydrogen carbonate was added. The mixture was diluted with dichloromethane (150 mL), washed with water (3 x 50 mL), dried, and concentrated to a syrup. The residue was separated on a silica gel (200 g) column using light petroleum-ethyl acetate (8:2) as eluent. The faster moving component proved to be compound 5 (40 mg, 1.4%), mp 107 °C, $[\alpha]_D$ -21.1° (c 0.29, chloroform), R_f 0.56. ^{1}H NMR ($CDCl_3$):δ 1.16 (d, 3H, $J_{5,6}$ = 6.4 Hz, Me), 3.57 (dd, 1H, $J_{3,4}$ = 5.3 Hz, H-4), 3.86 (m, 1H, $J_{4,5}$ = 1.8 Hz, H-5), 4.01 (d, 1H, $J_{1,2}$ = 0.7 Hz, H-2), 4.57 (Abq, 2H, $PhCH_2$), 4.59 (dd, 1H, $J_{2,3}$ = 5.5 Hz, H-3), 4.80 (ABq, 2H, $PhCH_2$), 5.20

(d, 1H, H-1), 6.35 (s, 1H, PhCH). ^{13}C NMR ($CDCl_3$): 104.34 (C_a), 96.90 (C-1), 76.00 (C-2), 74.18 (C-3), 73.57 ($PhCH_2$-4), 72.52 (C-6), 69.57 ($PhCH_2$-1), 66.21 (C-5), 16.88 (C-6).

Anal. Calcd for $C_{27}H_{28}O_5$: C, 74.98; H, 6.52. Found: C, 74.89; H, 6.54.

Compound 6 (3.45 g, 88%), eluted as second, had mp 75-76 °C, $[\alpha]_D$ -47.6° (c 0.78, chloroform), R_f 0.48 ^{1}H NMR ($CDCl_3$): δ 1.25 (d, 3H, $J_{5,6}$ = 6.5 Hz, Me), 3.69 (dd, 1H, $J_{3,4}$ = 4.3 Hz), 3.97 (m, 1H, $J_{4,5}$ = 3.7 Hz, H-5), 4.12 (dd, 1H, $J_{1,2}$ = 1.5 Hz, H-2), 4.44 (dd, 1H, $J_{3,4}$ = 7.2 Hz, H-3), 4.45 (ABq, 2H, $PhCH_2$), 4.62 (ABq, 2H, $PhCH_2$), 5.11 (d, 1H, H-1), 5.78 (s, 1H, PhCH). ^{13}C NMR ($CDCl_3$): 104.72 (C_a), 96.62 (C-1), 75.18 (C-2), 74.61 (C-3), 74.47 ($PhCH_2$-4), 72.58 (C-4), 69.36 ($PhCH_2$-1), 64.31 (C-5), 16.75 (C-6).

Anal. Calcd for $C_{27}H_{28}O_5$: C, 74.98; H, 6.52. Found: C, 75.00; H, 6.50.

Benzyl 2,4-Di-O-benzyl-6-deoxy-α-L-talopyranoside (7). To a stirred solution of 6 (3.28 g, 7.58 mmol) in 1:1 dichloromethane-ether (100 mL) 1.15 g (30.3 mmol) of $LiAlH_4$ and 4.03 g (30.3 mmol) of $AlCl_3$ were added. The solution was heated under reflux for 15 min. During this period the starting 6 disappeared (TLC). After cooling, the excess of the reagents was decomposed by adding ethyl acetate (5 mL) and water (8 mL). The reaction mixture was diluted with ether (100 mL) and the organic layer was decanted, dried and concentrated. The residue was crystallized from a mixture of ethyl acetate-n-hexane to afford 7 (3.1 g, 94%), mp 84-85 °C, $[\alpha]_D$ -58.5° (c 0.84, chloroform), R_f 0.64 (dichloromethane-ethyl acetate, 95:5). Lit.[4] mp 83-84 °C, $[\alpha]_D$ -62.4° (c 1.7, chloroform). ^{1}H NMR ($CDCl_3$): δ 1.30 (d, 3H, $J_{5,6}$ = 6.5 Hz, Me), 3.48 (dd, 1H, $J_{3,4}$ = 4.0 Hz, H-4), 3.56 (dd, 1H, $J_{1,2}$ = 1.6 Hz, H-2), 3.88 (dd, 1H, $J_{2,3}$ = 4.4 Hz, H-3), 3.93 (m, 1H, $J_{4,5}$ = 1.7 Hz, H-5), 5.02 (d, 1H, H-1), 7.20-7.42 (m, 15H, aromatic).

Anal. Calcd for $C_{27}H_{30}O_5$: C, 74.63; H, 6.96. Found: C, 74.65; H, 7.00.

Benzyl 2,4-Di-O-benzyl-6-deoxy-3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-L-talopyranoside (8). A mixture of 7 (2.12 g, 4.88 mmol) and 1.85 g (7.32 mmol) of $Hg(CN)_2$ was dissolved in 1:1 benzene-nitromethane (160 mL) and concentrated until 80 mL had been distilled off. The solution was then cooled to room temperature, 4 g of 4 Å molecular sieves was added and the mixture was stirred for 30 min. This mixture was treated with 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-

- β-D-glucopyranosyl bromide (3.65 g, 7.32 mmol) for 15 min. During this time the aglycon 7 completely disappeared. The reaction mixture was diluted with dichloromethane (50 mL) and filtered and the solvent was evaporated to dryness. The residue was dissolved in dichloromethane (100 mL) and the inorganic salts were removed by filtration. The filtrate was washed with 5% potassium iodide solution (3 x 50 mL) and finally with water (3 x 50 mL). After being dried, the solvent was evaporated and the residue was purified using silica gel (250 g) column chromatography to give syrupy 8 (3.5 g, 84%). $[\alpha]_D$ -62.1° (c 0.75, chloroform). R_f 0.51 (dichloromethane-ethyl acetate, 9:1). ^{1}H NMR ($CDCl_3$): δ 4.92 (d, 1H, $J_{1',2'}$ = 9.5 Hz, H-1'); 5.02 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1).

Anal. Calcd for $C_{47}H_{49}NO_{14}$: C, 66.27; H, 5.80; N, 1.64. Found: C, 66.21; H, 5.76; N, 1.66.

Benzyl 2,4-Di-O-benzyl-6-deoxy-3-O-(2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-L-talopyranoside (9). A catalytic amount of sodium methoxide was added to a solution of 8 (3.34 g, 3.92 mmol) in methanol (80 mL). The solution was left at room temperature overnight and then neutralized with Amberlite IR-120 (H^+) resin, filtered and evaporated to give 9 (2.7 g, 95%), amorphous solid, $[\alpha]_D$ -72.7° (c 0.55, chloroform), R_f 0.45 (dichloromethane-methanol, 9:1).

Anal. Calcd for $C_{41}H_{43}NO_{11}$: C, 67.85; H, 5.97; N, 1.93. Found: C, 67.90; H, 5.95; N, 1.97.

Benzyl 2,4-Di-O-benzyl-6-deoxy-3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-α-L-talopyranoside (10). Compound 9 (600 mg, 0.83 mmol) was dissolved in ethanol (15 mL) and treated with 3 mL of 87% hydrazine hydrate at reflux temperature for 2 hours. The reaction mixture was concentrated to dryness and the residue was acetylated in pyridine (5 mL) with acetic anhydride (5 mL) at room temperature overnight. The usual work-up procedure resulted in a solid substance, crystallized from ethanol to give 10 (390 mg, 61.8%), mp 197 °C, $[\alpha]_D$ -64° (c 0.42, chloroform), R_f 0.44 (dichloromethane-acetone, 9:1).

Anal. Calcd for $C_{41}H_{49}NO_{13}$: C, 64.47; H, 6.47; N, 1.83. Found: C, 64.50; H, 6.50; N, 1.86.

1,2,4-Tri-O-acetyl-6-deoxy-3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-β- (11) and -α-L-talopyranose (12). The disaccharide derivative 10 (350 mg, 0.46 mmol) was hydrogenated in ethanol (15 mL) with Pd-on-carbon catalyst (50 mg) in the presence of acetic acid (0.3 mL). After 24 hours complete reaction occurred. The catalyst was removed by filtration and the filtrate was evaporated. The syrupy residue

in pyridine (4 mL) was acetylated with acetic anhydride (4 mL) at room temperature overnight. The syrupy product obtained after work-up showed two components by TLC. These were separated on a silica gel (40 g) column. The faster moving component 11 (73 mg, 26%) gave the following data: amorphous solid, $[\alpha]_D$ +26.3° (c 0.41, chloroform), R_f 0.61 (dichloromethane-acetone, 65:35). ^{13}C NMR ($CDCl_3$): 102.01 ($J_{C1'-H1'}$ = 158 Hz, C-1'), 97.81 (J_{C1-H1} = 161 Hz, C-1).

Anal. Calcd for $C_{26}H_{34}NO_{16}$: C, 50.65; H, 5.56. Found: C, 50.58; H, 5.51.

The second component 12 (147 mg, 52%) showed the following data: mp 238 °C, $[\alpha]_D$ -42.8° (c 0.51, chloroform), R_f 0.52 (dichloromethane-acetone, 65:35). ^{13}C NMR ($CDCl_3$): 98.64 ($J_{C1'-H1'}$ = 159.5 Hz, C-1'), 91.68 (J_{C1-H1} = 172 Hz, C-1).

Anal. Calcd for $C_{26}H_{34}NO_{16}$: C, 50.65; H, 5.56. Found: C, 50.69; H, 5.59.

6-Deoxy-3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-talose (13). A solution of 12 (100 mg) in dry methanol (5 mL) was saponified with a catalytic amount of sodium methylate. After 24 hours the reaction mixture was neutralized by Amberlite IR-120 (H^+) resin, filtrated and the solvent evaporated. The residue was purified on a silica gel (10 g) column using n-butanol-methanol-water (2:1:1) eluant. The fractions were collected and concentrated. The residue was dissolved in water and freeze-dried to obtain 13 (50 mg, 84%), amorphous solid, $[\alpha]_D$ -18.9° (c 0.51, water), R_f 0.58.

Anal. Calcd for $C_{14}H_{25}NO_{10}$: C, 45.77; H, 6.86; N, 3.81. Found: C, 45.81; H, 6.90; N, 3.86.

Benzyl 2,4-Di-O-benzyl-6-deoxy-3-O-(3,4-di-O-acetyl-2-deoxy-2-phthalimido-6-O-trityl-β-D-glucopyranosyl)-α-L-talopyranoside (14). Compound 9 (2.0 g, 2.76 mmol) was treated in pyridine (30 mL) with trityl chloride (1.51 g, 5.23 mmol) for 16 hours. This reaction mixture was directly acetylated with acetic anhydride (20 mL) for 2 hours. The solution was evaporated to a syrup, and the residue was treated with ice (200 g) and extracted with dichloromethane (2 x 100 mL). The organic layer was washed with a solution of 0.5 M H_2SO_4 (2 x 50 mL), then with a solution of saturated $NaHCO_3$ and finally with water (3 x 100 mL), dried and concentrated. The product was purified on a silica gel (150 g) column to give 14 (1.92 g, 68%), syrup, $[\alpha]_D$ -55.7° (c 0.36, chloroform), R_f 0.45 (light petroleum-ethyl acetate, 65:35).

Anal. Calcd for $C_{64}H_{61}NO_{13}$: C, 73.06; H, 5.84. Found: C, 73.15; H, 5.94.

Benzyl 2,4-Di-O-benzyl-6-deoxy-3-O-(3,4-di-O-acetyl-2-deoxy-2--phthalimido- β-D-glucopyranosyl)- α -L-talopyranoside (15). Compound 14 (1.74 g, 1.65 mmol) was dissolved in 80% acetic acid (100 mL) and stirred for 60 min at 80 °C. The reaction mixture was cooled to room temperature, diluted with water (100 mL) and extracted with dichloromethane (3 x 50 mL). The combined organic phase was washed with saturated $NaHCO_3$ solution (5 x 100 mL) and then with water (3 x 50 mL), dried and the solvent evaporated. The residual syrup 14 (950 mg, 71%) was pure enough for the next step. 100 Mg were purified for analytical purposes using silica gel (10 g) as adsorbent and dichloromethane-acetone (9:1) as eluant $[\alpha]_D$ -60.9° (c 0.45, chloroform), R_f 0.81.

Anal. Calcd for $C_{45}H_{47}NO_{13}$: C, 66.74; H, 5.85. Found: C, 66.89; H, 5.94.

Benzyl 2,4-Di-O-benzyl-6-deoxy-3-O- 3,4-di-O-acetyl-2-deoxy-2--phthalimido-6-O-(2,3,5-tri-O-benzoyl- α -D-arabinofuranosyl)- β -D-glucopyranosyl - α-L-talopyranoside (16). The mixture of 15 (930 mg, 1.15 mmol), 436 mg (1.73 mmol) of $Hg(CN)_2$, and 80 mL of 1:1 benzene-nitromethane was concentrated until 40 mL had distilled. The solution was then cooled to room temperature, 1 g 4 Å molecular sieves was added and the mixture was stirred for 30 min. Afterwards 909 mg (1.73 mmol) of 2,3,5-tri-O-benzoyl- - α-D-arabinofuranosyl bromide was added. After 30 min the reaction mixture was worked up. The resulting product was purified on a silica gel (130 g) column to give 1.28 g (89%) of 16, crystallized from ethanol, mp 162-163 °C, $[\alpha]_D$ -44.1° (c 0.63, chloroform), R_f 0.74 (dichloromethane--acetone, 97:3). ^{13}C NMR ($CDCl_3$): 105.50 (C-1''), 98.55 (C-1'), 98.40 (C-1).

Anal. Calcd for $C_{71}H_{67}NO_{20}$: C, 67.99; H, 5.38. Found: C, 68.10; H, 5.42.

Benzyl 2,4-Di-O-benzyl-6-deoxy-3-O- 2-acetamido-3,4-di-O-acetyl-2--deoxy-6-O-(2,3,5-tri-O-acetyl- α -D-arabinofuranosyl)- β -D-glucopyranosyl - - α -L-talopyranoside (17). A catalytic amount of sodium methylate was added to a solution of 16 (1.0 g, 0.8 mmol) in methanol (40 mL) and left overnight at room temperature. Then the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated. The residue was dissolved in ethanol (20 mL), hydrazine hydrate (3 mL) was added and the mixture was refluxed for 2 hours. After cooling, the solution was concentrated to dryness and the residue was acetylated in pyridine (10 mL) with acetic anhydride (10 mL) at room temperature overnight. After concentration, the product was purified on a silica gel (100 g) column to

yield 17 (690 mg, 88%), $[\alpha]_D$ -19.4° (c 0.26, chloroform), R_f 0.54 (dichloromethane-acetone, 85:15).

Anal. Calcd for $C_{50}H_{61}NO_{19}$: C, 61.28; H, 6.27. Found: C, 61.41; H, 6.31.

1,2,4-Tri-O-acetyl-6-deoxy-3-O-[2-acetamido-3,4-di-O-acetyl-2-deoxy-6-O-(2,3,5-tri-O-acetyl-α-D-arabinofuranosyl)-β-D-glucopyranosyl]-β-L- (18) and -α-L-talopyranose (19). Compound 17 (600 mg, 0.61 mmol) was hydrogenated in ethanol (20 mL) over 10% Pd-on-carbon (100 mg) catalyst in the presence of acetic acid (0.5 mL) for 24 hours. After filtration and concentration the residue was acetylated in pyridine (7 mL) with acetic anhydride (7 mL) at room temperature overnight. After work-up, TLC showed the presence of two compounds which were separated on a silica gel (80 g) column to give, first, 18 (142 mg, 27.7%), mp 174 °C, $[\alpha]_D$ +27.7° (c 0.88, chloroform), R_f 0.59 (dichloromethane-acetone, 65:35). ^{13}C NMR ($CDCl_3$): 105.48 (C-1''), 102.20 (C-1'), 97.81 (C-1).

Anal. Calcd for $C_{35}H_{49}NO_{22}$: C, 50.30; H, 5.91. Found: C, 50.34; H, 5.96.

The second component proved to be 19 (283 mg, 55.3%), a foam, $[\alpha]_D$ +4.4° (c 0.57, chloroform), R_f 0.53 (dichloromethane-acetone, 65:35). ^{13}C NMR ($CDCl_3$): 105.32 (C-1''), 102.22 (C-1'), 91.46 (C-1).

Anal. Calcd for $C_{35}H_{49}NO_{22}$: C, 50.30; H, 5.91. Found: C, 50.26; H, 5.88.

6-Deoxy-3-O-[2-acetamido-2-deoxy-6-O-(α-D-arabinofuranosyl)-β-D-glucopyranosyl]-L-talose (20). Compound 19 (250 mg) was saponified in methanol (10 mL) with a catalytic amount of sodium methylate. After 24 hours the solution was neutralized with resin, filtered and concentrated. The residue was purified on a silica gel (15 g) column using n-butanol-methanol-water (2:1:1) as eluant. The syrup, obtained after evaporation, was dissolved in water (2 mL) and freeze-dried to give 20 (130 mg, 87%) as amorphous powder, $[\alpha]_D$ +11.3° (c 0.13, water), R_f 0.54. ^1H and ^{13}C NMR data are given in Table 1.

Anal. Calcd for $C_{19}H_{33}NO_{14}$: C, 45.69; H, 6.66. Found: C, 45.75; H, 6.70.

6-Deoxy-3-O-methyl-L-talose (21). Compound 7 (250 mg) was treated in N,N-dimethylformamide (5 mL) with powdered KOH (0.5 g) followed by methyl iodide (0.5 mL). The usual work-up procedure resulted in a syrupy product which was directly hydrogenated in ethanol (10 mL) in the pres-

ence of 10% Pd-on-carbon (50 mg) catalyst for 24 hours. After filtration and evaporation, the residue was purified on silica gel (12 g) to give 21 (80 mg, 78%), syrup, $[\alpha]_D$ -28.6° (c 0.65, water), R_f 0.62 (dichloromethane-methanol, 85:15). Lit.[3] $[\alpha]_D$ -29.6° (c 1.1, water). ^1H and ^{13}C NMR data are summarized in Table 2.

ACKNOWLEDGMENT

The authors express their thanks to Professor Pál Nánási for his interest. Financial support from the National Research Foundation (OTKA 234), the Institute for Scientific Coordination and Information, and the Hungarian Ministry of Education is greatfully acknowledged.

REFERENCES

1. S. G. Wilkinson, L. Galbraith, and W. J. Anderton, Carbohydr. Res., 112, 241 (1983).

2. G. A. Adams, Meth. Carbohydr. Chem., 5, 269 (1965).

3. G. O. Aspinall and K. Takeo, Carbohydr. Res., 121, 61 (1983).

4. A. Lipták, J. Imre, and P. Nánási, Carbohydr. Res., 92, 154 (1981).

5. P. M. Collins and W. G. Overend, J. Chem. Soc., 1912 (1965).

6. E. J. Corey and G. Schmidt, Tetrahedron Lett., 399 (1979).

7. J. Defaye, A. Gadelle, and S. J. Angyal, Carbohydr. Res., 126, 165 (1984).

8. A. Lipták, Tetrahedron Lett., 3551 (1976).

9. R. U. Lemieux, T. Takeda, and B. Y. Chung, ACS Symp. Ser., 39, 90 (1976).

10. B. Helferich and K. F. Wedemeyer, Liebigs Ann. Chem., 563, 139 (1949).

11. H. G. Fletcher, Jr., Meth. Carbohydr. Chem., 2, 228 (1963).

12. A. Lipták, Z. Szurmai, P. Nánási, and A. Neszmélyi, Tetrahedron, 38, 3489 (1982).

13. A. Bax, Two-Dimensional Nuclear Magnetic Resonance in Liquids, Delft University Press, Delft, (1982).

14. P. E. Pfeffer, K. M. Valentine, and F. W. Parrish, J. Am. Chem. Soc., 101, 1265 (1979).

15. G. W. Eich, G. Bodenhausen, and R. R. Ernst, J. Am. Chem. Soc., 104, 3731 (1982).

16. A. Bax and R. Freeman, J. Magn. Reson., 44, 542 (1981).

17. G. A. Morris, J. Magn. Reson., 39, 277 (1981).

18. D. J. States, R. A. Haberkorn, and D. J. Ruben, J. Magn. Reson., 48, 286 (1982).