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MOENURONIC ACID: SYNTHETIC STUDIES AND ABSOLUTE CONFIGURATION

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Abstract - The absolute configuration of the moenuronic acid moiety of the antibiotic moenomycin A has been established by a partial synthesis starting from D-galactose. The relation between the absolute conformation of α-hydroxy lactones and their chiroptical properties is discussed in detail.

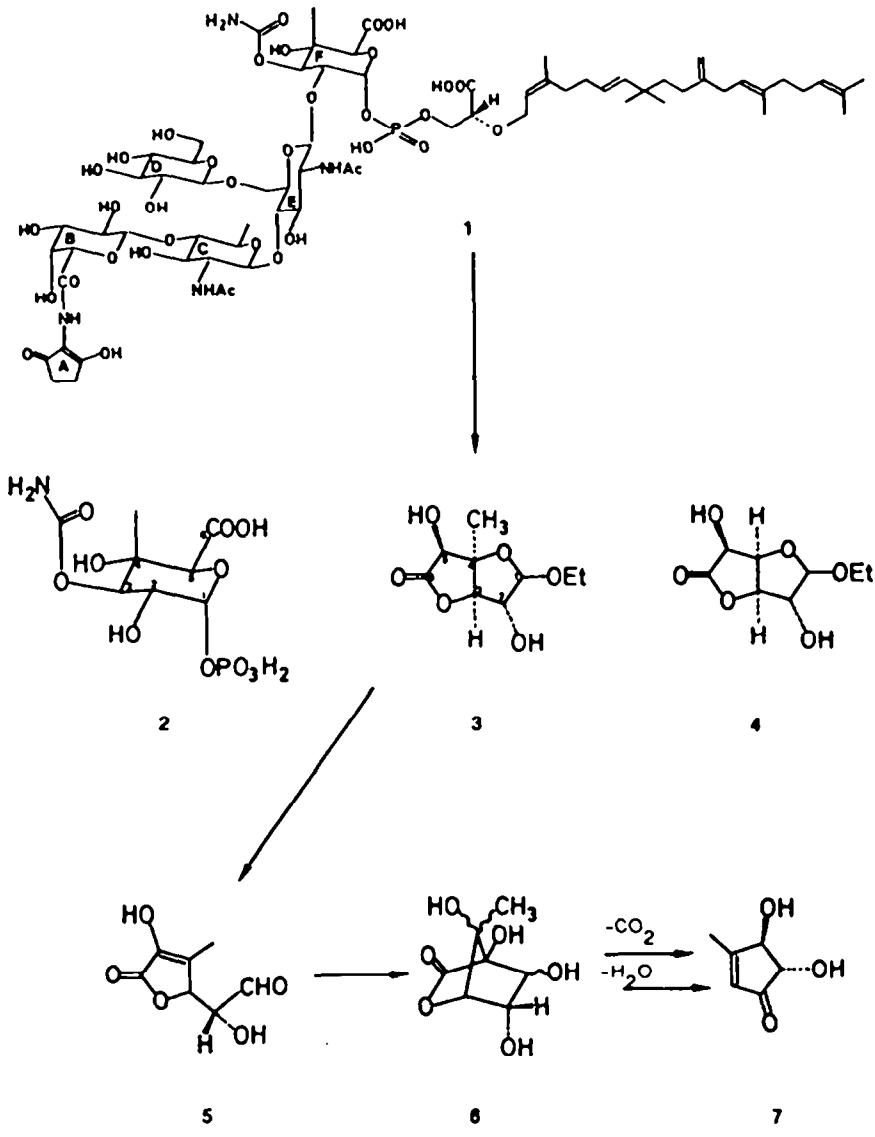
The antibiotic moenomycin A (1) [1] is the main constituent of the trade product flavomycin ® which is utilized in animal nutrition. [2] 1 belongs to the most efficient inhibitors of the biosynthesis of bacterial cell wall peptidoglycans. [3]

Unit F of 1 is the carbamate of a new methyl-branched uronic acid called moenuronic acid. After trifluoroacetic acid degradation of 1 the 3-O-carbamoyl-1-phosphate 2 of moenuronic acid was isolated [4] whereas cleavage of 1 with dilute hydrochloric acid followed by glycoside formation yielded the ethyl furanosidurono-6,3-lactone 3. [5] After treatment of 1 or 3 with 2n HCL (20 h at 100°C) racemic 7 was obtained. [5] Presumably, intermediates of types 5 and 6 are involved in this degradation reaction.

Constitution and relative configuration of moenuronic acid were assigned on the basis of detailed spectroscopic analyses. [5] The proposed structure was recently confirmed by partial synthesis. [6] The absolute configuration depicted in 2 and 3 is based on chiroptical data. 3 and the closely related D-glucuronic acid derivative 4 have almost identical CD spectra [5] (see Table 2). Since it is principally impossible to establish both conformation and absolute configuration by one CD experiment the assumption had to be made that both 3 and model compound 4 exist preferentially in the same conformation. Unfortunately, no information on the conformation of the lactone ring in 3 is accessible by ^{1}H-NMR-spectroscopy. [5] Recently, Kato and coworkers discussed a case where from CD data the wrong absolute configuration was deduced because an incorrect conformation had been assumed. [7] In order to establish the absolute configuration of the moenuronic acid moiety of moenomycin A conclusively, we decided both to correlate moenuronic acid chemically with a compound of known absolute configuration and to study in some detail the CD of uronolactones of type 3.

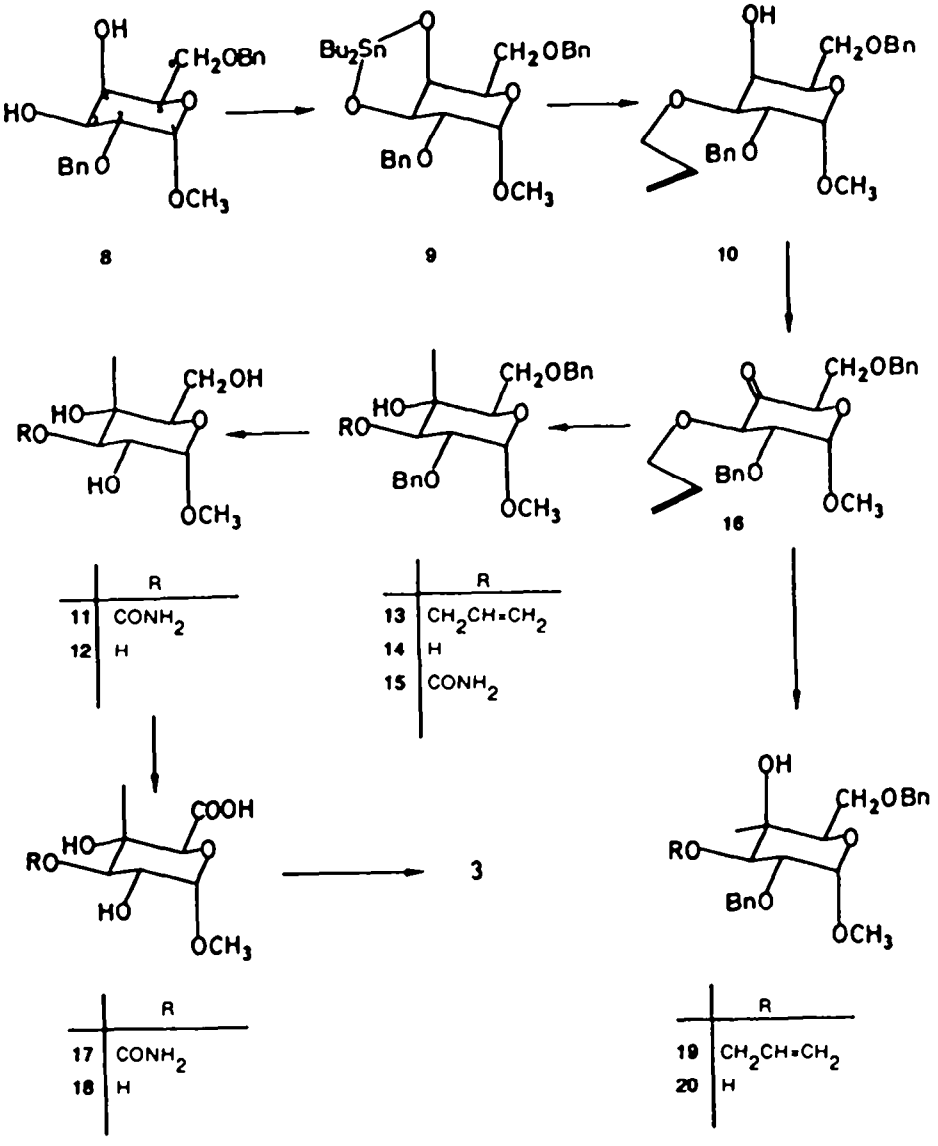
Synthesis of D-moenuronic acid derivatives from D-galactose

D-Galactose was transformed into the known derivative 8. [8] Selective allylation of the equatorial 3-OH group in 8 was achieved under the conditions reported by David and coworkers. [9] Thus, 8 was transformed into the stannylene derivative 9 which in toluene solution (reflux) was treated with allyl bromide in the presence of tetra-n-butylammonium bromide to give 10 in an overall yield of 87%. Oxidation of 10 to ketone 16 turned out to be somewhat difficult, presumably, because 16 is a



rather unstable compound. Best results were obtained using Corey's [10] dimethylsulfide/N-chlorosuccinimide (79% yield) or the Swern procedure [11] (84% yield). Mlijkovic et al. [12] have first reported that under proper conditions methylmagnesium iodide adds stereoselectively to hexapyranosid-4-uloses of type 16 to furnish exclusively addition products such as 19 with an α -(equatorial) methyl group whereas methyllithium yields solely 4β-methyl compounds such as 13 with an axial methyl group. In our hands the reaction of 16 with commercial methyllithium at -78°C led to a mixture (as apparent from the ^{1}H-NMR spectrum) of 13 and 19.

Routinely, separation was not performed at this stage since separation of 14 and 20 turned out to be much simpler. Removal of the allyl group from 13 and 19 was achieved by a well-established two-step procedure. Firstly, the allyl ether was isomerized into the prop-1-enyl ether function (rho-



dium(I) catalysis) [13] which was then efficiently cleaved by oxymercuration [14] to furnish after chromatographic separation 14 and 20 in 61% and 11% yield, respectively, based on 16. The configuration at C-4 in 14 and 20 was established by ^{13}C-NMR spectroscopy. According to Miljkovic et al. [15] in branched-chain sugars of this type an axial methyl group absorbs at higher field (δ = 14.6 for 14) than an equatorial one (δ = 21.8 for 20). Removal of the benzyl protecting groups from 14 by hydrogenolysis furnished 12 which has already been described by Yoshimura et al. [6]. We wished to introduce the 3-O-carbamoyl group present in unit F of 1. This was achieved using the Kishi procedure.[16] Thus, brief treatment of 14 with one equivalent of trichloroacetyl isocyanate followed by hydrolysis yielded 15 in 73% yield. Hydrogenation of 15 occurred smoothly to give 11 in 94% yield. Finally, catalytic oxidation [17] of 11 led to the desired moenuronic acid derivative 17. By treatment of 17 as well as of 18, which was prepared from 12 as described by Yoshimura et al. [6] with 0.1 m HCl in ethanol for 15 h at reflux temperature ethylfuranosiduronolactone 3 was formed in about 27% yield.

Table 1: Positive ion FAB mass spectra of compounds 10-20 (quasi-molecular ion region)

Compound	MATRIX			
	glycerol	glycerol + LiCl	TEA*	TEA* + LiCl
10			584(0.17)M+H+TEA 582(0.25)M-H+TEA 532(1.13)M+H+TEA-32** 413(0.38)M-H 383(0.80)M-OCH_3	835(0.08)2M+Li 584(0.13)M+H+TEA 582(0.24)M-H+TEA 532(1.03)M+H+TEA-32 421(1.82)M+Li 413(0.43)M-H 383(0.95)M-OCH_3
16			562(0.74)M+H+TEA 560(0.32)M-H+TEA	419(1.75)M+Li 411(1.30)M-H
13			578(0.45)M+H+TEA 577(0.38)M-H+TEA 547(1.10)M+H+TEA-32	863(0.10)2M+Li 435(4.55)M+Li
14	778(4.10)2M+H 746(0.50)2M+H-32 387(3.00)M-H 357(43.0)M+H-32		538(1.08)M+H+TEA 536(0.68)M-H+TEA 506(0.48)M+H+TEA-32	784(0.13)2M+Li 401(2.50)M-H+2Li 395(8.30)M+Li
20			538(0.46)M+H+TEA 536(0.46)M-H+TEA 506(0.98)M+H+TEA-OCH_3 387(0.13)M-H 355(0.12)M-OCH_3	
15			581(2.60)M+H+TEA 579(1.46)M-H+TEA 549(1.48)M+H+TEA-32	869(0.36)2M+Li 629(1.26)M+Li+TEA+LiCl 587(2.68)M+Li+TEA 436(8.50)M+Li
11	252(21.0)M+H 220(7.80)M+H-32	300(5.00)M+Li+LiCl 258(28.0)M+Li		
12	301(16.0)M+H+gly. 231(16.0)M+Na 209(11.0)M+H			
17	554(2.03)2M+Na 531(0.78)2M+H 288(19.0)M+Na 266(46.5)M+H 234(16.0)M+H-32	412(2.10)M-H+Li+Li+LiCl+gly. 370(4.80)M-H+Li+Li+gly. 364(5.30)M+Li+gly. 278(15.0)M+Li+Li-H 272(31.0)M+Li	415(0.11)M+H+TEA 413(0.08)M-H+TEA	

*TEA = Triethanolamine ** -32 = $HOCH_3$

This compound proved identical with a sample obtained from 1. [5] Most significantly, both samples showed a positive CD within the lactone absorption band. This proves that the moenuronic acid residue of moenomycin A belongs indeed to the D-series as previously assumed. [5]

Mass spectra of compounds 10 - 20

As may be expected compounds 10 - 20 did not give molecular ions using the EI technique. On the other hand under proper experimental conditions excellent FAB mass spectra were obtained in the positive ion mode fully in accord with the proposed structures. The results are shown in Table 1. When a matrix of triethanolamine (TEA) was used, the characteristic cluster ions $(M+TEA+H)^+$ and $(M+TEA-H)^+$ were formed in most cases. Very intensive quasimolecular ions $(M+Li)^+$ were obtained after addition of LiCl to the sample dissolved in TEA. In some cases $(M+Li)^+$ was the base peak of the spectrum.

Some general remarks on the chiroptical properties of α-hydroxy lactones

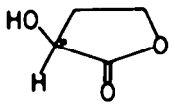
There exist two rules for hydroxy lactones of type 3 predicting the sign of the n→π* CD of the lactone chromophore. The rule of Okuda [18] says that the sign of this CD is determined by the disposition of the OH group in α-position to the CO function. Using projections 21 and 22 the Cotton effect should be positive if the OH group is above the plane of the lactone ring as in 21, and negative if it is below as in 22. Beecham [19] on the other hand stated that it is the chirality of the lactone ring which determines the sign of the n→π* Cotton effect (Wolf-Legrand rule [20,21]). For a negative torsional angle (O-)C-C_α(-C_β) as in 23 a positive CD has to be expected. If the torsional angle is positive (see 24) a negative CD results. Actually, these rules do not differ conceptually from each other. The perturbing influence of a chirally arranged bond connected to the C_α atom of the lactone moiety is mainly through-bond, and it should not matter wether this bond is within the ring or exocyclic to it. The magnitude of the perturbation is approximately the same for C and O as regards the chiroptical properties, that of H is, however, much smaller. It follows then that for a small torsional angle (O-)C-C_α(-C_β) within the ring the influence of the OH group should prevail, whereas ring conformation should determine the Cotton effects when this angle is larger (this torsional angle is not the same as that defined by the C.I.P. rules [22]!). All this is valid, however, only if the carboxylic chromophore is coplanar and, therefore, inherently achiral. X-ray studies have shown that this is approximately the case for most lactones investigated, at least in the solid state. [23]

For D-glucuronic acid 6,3-lactone (25) these relevant torsional angles (in the crystalline state) are -17° (within the ring) and -142° (O-C-C-OH, i.e. +38° in C.I.P. notation), respectively. From molecular models it can be deduced that it is the steric interaction between the 5ß-OH and the atoms of the furane ring which stabilizes this conformation of the lactone ring, and both chiral perturbations give positive contributions to the n→π* - Cotton effect. For the corresponding α-ethyl glycoside 26 we found $\Delta\varepsilon$ = +2.85, and no further steric interaction is introduced by the OEt moiety according to molecular models. This steric repulsion should become more pronounced, however, for the ß-ethyl glycoside 4, stabilizing this same conformation even more. Indeed, a somewhat larger positive CD ($\Delta\varepsilon$ = +3.67) has been measured. For the ß-methyl glycoside 27 [25] this same steric repulsion is obviously much weaker as can be deduced from the $\Delta\varepsilon$ - value of +2.92.

Formation of an additional dioxolane ring as in 28 [26] should also scarcely change this conformational equilibrium, and this view is supported by $\Delta\varepsilon$ = +2.40 for acetonide 28. Epimerization at C-5 to the L-ido compound 29 [27] will release this steric interaction at the ß-side of the lactone ring, but it is difficult to predict from molecular models which conformation of the lactone ring should here be the preferred one. The contribution of the OH group to the Cotton effect is now negative, and as $\Delta\varepsilon$ = -0.96 we can conclude from this small value that the lactone ring is not present solely in the second possible conformation 24, since then we should expect a larger negative CD. Removal of the 5-OH group leads to 30 [28,29] for which a negative CD of $\Delta\varepsilon$ = -1.52 has been recorded. Clearly, for 30 the second lactone ring conformation (c.f. 24) with the positive torsional angle (O-)C-C_α(-C_β) within the ring preponderates here. According to molecular models introduction of the additional methyl group at C-4 present in the moenuronic acid lactone 3 should not drastically change the conformational equilibrium of the lactone ring. Thus, a positive CD is expected for 3, in full agreement with the experimental facts ($\Delta\varepsilon$ = +2.42 was found for a sample of 3 obtained by degradation of 1 [5] and $\Delta\varepsilon$ = +2.91 for synthetic material).

The direct contribution of the 4-methyl group is predicted from the corresponding sector rule to be either negligable or weakly positive, depending on the ring conformation [24]; anyway, it should be of marginal influence only compared to the other through-bond interactions.

In this specific case the determination of the absolute configuration of a sugar lactone from its CD gave the correct result. As can be seen from the other examples one must, however, be very cautious in the interpretation of CD data of α-hydoxy lactones. On the other hand, once the absolute configuration is known, from the CD data very valuable informations about the preferred conformation of the lactone ring in solution can be obtained.



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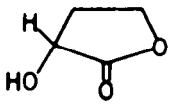

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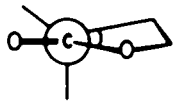

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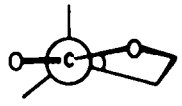

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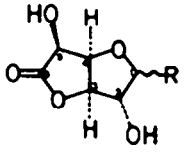
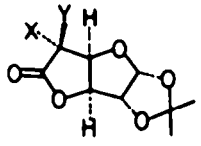


	R
25	ß-OH
26	α-OEt
27	ß-OMe



	X	Y
28	H	OH
29	OH	H
30	H	H

Table 2: CD data for uronolactones 3, 4, and 26 - 30

Compound	λ_{max} (nm)	$\Delta\varepsilon_{max}$
3	223	+ 2.91
4	221	+ 3.67
26	222	+ 2.85
27	220	+ 2.92
28	222	+ 2.40
29	229	- 0.96
30	216	- 1.52

EXPERIMENTAL

All O_2- or moisture-sensitive reactions were performed in oven-dried glassware under a positive pressure of argon. Sensitive liquids and solutions were transferred by syringe, and were introduced into reaction flasks through rubber septa. Usual work-up means partitioning the reaction mixture between water and an organic solvent (given in parenthesis), drying the combined organic layers with Na_2SO_4 and removal of solvent by distillation in vacuo using a rotatory evaporator. The instrumentation used was: ^{1}H-NMR: WP 80 (Bruker), WH-250 (Bruker); ^{13}C-NMR: WH 250 (Bruker); IR: Perkin Elmer 257 and 681; CD: Jobin-Yvon-ISA dichrograph Mark III connected online to a PDP-8/e; LC: Medium pressure chromatography using 31.0 cm x 2.5 cm glass tubes, silica gel Grace (50 μ m), Duramat pump (CfG); UV detector Chromatochord III (Serva).

The FAB mass spectra were obtained using a Finnigan MAT 731 instrument. Samples were dissolved in dimethylsulfoxide, and the matrix (glycerol or triethanolamine [30] or a solution of LiCl in glycerol and triethanolamine, respectively) was added. The solutions were placed on a stainless steel probe tip [31] and bombarded with 6 KeV Xenon from a modified Saddle Field Ion Source.

Methyl 3-O-Allyl-2,6-di-O-benzyl-α-D-galactopyranoside (10).

A mixture of **8** [8] (3.74 g, 10.0 mmol) and di-n-butyltin oxide (2.56 g, 10.3 mmol) in toluene (300 ml) was refluxed (using a Dean-Stark water trap) for 7 h. After removal of 100 ml of the solvent allyl bromide (6.1 ml, 70 mmol) and tetrabutylammonium bromide (1.93 g, 6.0 mmol) were added and the resulting mixture was refluxed for 8 h. The solvent was removed under reduced pressure and the residue partitioned between ether (30 ml) and two 20 ml portions of 5% $NaHCO_3$. The organic layer was dried (Na_2SO_4) and evaporated. LC (EtOAc/petrol 1:4) furnished **10** (3.60 g, 87%) as a colourless oil.- IR (CCl_4): 3590 (OH), 3100-3000, 1650, 1500, 1100, 698 cm^{-1}.- ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 2.60 (s, 1H, OH); 3.37 (s, 3H, OCH_3); 3.50-4.15 (m, 6H, 2-H, 3-H, 4-H, 5-H, CH_2-6); 4.17 and 4.19 (m, 2H, J_{AB} (from decoupling experiments) = 3.0 Hz, -O-C<u>H</u>$_2$-CH=CH_2); 4.45-4.80 (m, 5H, 1-H, benzylic H's);5.07-5.45 (m, 2H, =CH_2);5.70-6.22 (m, 1H, $J_{H\diagdown C=C\diagup H}$ = 10 Hz, $J_{H\diagdown C=C\diagup H}$ = 18 Hz (from decoupling experiments), -C<u>H</u>=CH_2); 7.33 (s, 10H, aromatic H's). - FAB-MS: see Table 1.- $C_{24}H_{30}O_6$ (414.5); Found m/z = 421 (MLi^+, FAB-MS).

Methyl 3-O-Allyl-2,6-di-O-benzyl-α-D-xylo-hexopyranosid-4-ulose (16).

a) To a solution of N-chlorosuccinimide (192 mg, 1.44 mmol) in toluene (3 ml) at 0°C was added dimethylsulfide (0.145 ml, 1.97 mmol). After the solution had stirred for 30 min at 0°C **10** (200 mg, 0.48 mmol) was added at -30°C and the reaction kept at -28°C for 3 h. Then a solution of triethylamine (0.205 ml, 1.47 mmol) in toluene (1 ml) was added and the mixture allowed to warm to ambient temperature. After addition of ether (10 ml) usual work-up (ether) and LC (acetone/petrol 1:6) furnished **16** (156 mg, 79%) as a colourless oil.

b) To a solution of oxalyl chloride (0.38 ml, 4.34 mmol) in CH_2Cl_2 (7 ml) at -60°C was added a solution of DMSO (0.67 ml, 9.40 mmol) in CH_2Cl_2 (1 ml). After slow addition of **10** (1.63 g, 3.93 mmol) dissolved in CH_2Cl_2 (9 ml) at -70°C the reaction mixture was stirred for 25 min at -15°C. At -30°C triethylamine (2.51 ml, 18 mmol) dissolved in CH_2Cl_2 (2 ml) was added and the mixture stirred at -15°C for 5 min. Addition of water (25 ml), usual work-up (CH_2Cl_2), and LC (ethyl acetate/petrol 1:7 then 1:5) furnished **16** (1.352 g, 84%).- IR (CCl_4): 3100-3000, 1745, 1605, 1500, 700 cm^{-1}.- ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 3.48 (s, 3H, OCH_3); 3.55-3.90 (m, 3H); 3.95-4.47 (m, 4H); 4.57 (s, 2H, benzylic H's); 4.63-4.86 (m, 3H, 1-H, benzylic H's); 5.10-5.50 (m, 2H, =CH_2); 5.72-6.24 (m, 1H, -C<u>H</u>=CH_2); 7.32 and 7.35 (10H, aromatic H's).- FAB-MS: see Table 1.- $C_{24}H_{28}O_6$ (412.5); Found m/z = 419 (MLi^+, FAB-MS).

Reaction of **16** with MeLi.

To a cooled (-78°C) solution of **16** (2.91 g, 7.04 mmol) in ether (200 ml) was added 0.90 M MeLi in ether (32 ml, 29 mmol). After stirring at -78°C for 7 h, warming to ambient temperature, and

addition of water the mixture was worked up as usual. LC (acetone-petrol 1:8 and 1:5) furnished a mixture of **13** and **19** (2.50 g, 82%).

Methyl 3-O-Allyl-2,6-di-O-benzyl-4-C-methyl-α-D-glucopyranoside (13).

From the mixture of **13** and **19** a small sample of pure **13** was obtained by LC (ethyl acetate-petrol 1:6).- IR (CCl_4): 3600-3540 (OH), 3100-3000, 1500, 700 cm^{-1}.- ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 1.15 (s, 3H, CH_3); 2.63 (s, 1H, OH); 3.40 (s, 3H, OCH_3); 3.20-4.00 (m, 5H, 2-H, 3-H, 5-H, CH_2-6); 4.24-4.47 (m, 2H, O-CH_2-CH=CH_2); 4.47-4.92 (m, 5H, 1-H, benzylic H's); 5.05-5.48 (m, 2H, =CH_2); 5.75-6.30 (m, 1H, -CH=CH_2); 7.34 (10H, aromatic H's).- FAB-MS: see Table 1.- $C_{25}H_{32}O_6$ (428.5); Found m/z = 435 (MLi^+, FAB-MS).

Removal of the allyl group from **13** and **19**.

A solution of the mixture of **13** and **19** (972 mg, 2.27 mmol) and 1,4-diazabicyclo [2.2.2] octan (113 mg, 1.0 mmol) in 9:1 ethanol/water (36 ml) was refluxed for 5 min. After addition of $Rh(PPh_3)_3Cl$ (243 mg, 0.26 mmol) the reaction mixture was refluxed for 2 h. After this period it was cooled to ambient temperature, filtered to remove the catalyst, diluted with toluene and evaporated in vacuo to dryness. The residue was taken up in 1:5 ethyl acetate-petrol and filtered through a short silica layer (30 g). After evaporation to dryness the reaction products were dissolved in acetone (36 ml) and water (2.7 ml) and treated for 40 min at ambient temperature with $HgCl_2$ (486 mg, 1.79 mmol) and HgO (702 mg, 3.24 mmol). The suspension was then filtered, the clear solution evaporated, and the residue taken up in ether. Washing the solution several times with 30% Klaq, usual work-up (ether) and LC (ethyl acetate-petrol 1:2 then 1:1) furnished **14** (655 mg, 74% based on **16**) and **20** (112 mg, 13% based on **16**).

Methyl 2,6-Di-O-benzyl-4-C-methyl-α-D-glucopyranoside (14).

M.p. 87-89°C (from ethyl acetate).- IR ($CHCl_3$): 3595, 3560-3140 (OH), 3100-3000, 1605, 1500, 1078, 702 cm^{-1}.- ^{1}H-NMR (250 MHz, $CDCl_3$): δ = 1.14 (s, 3H, 4-CH_3); 3.18 (broad s, 2H, 2 OH's); 3.29 (dd, 1H, 2-H); 3.33 (s, 3H, O-CH_3); 3.56 (ABX, 1H, 6-H); 3.75 (ABX, 1H, 6'-H); 3.85 (ABX, 1H, 5-H); 3.94 (d, 1H, 3-H); 4.51 and 4.56 (AB, 2H, $|J_{AB}|$ = 11.9 Hz, benzylic H's); 4.66 and 4.72 (AB, 2H, $|J_{AB}|$ = 12.2 Hz, benzylic H's); 7.32 (10H, aromatic H's). $J_{1,2}$ = 4.0 Hz, $J_{2,3}$ = 10.1 Hz, $J_{5,6}$ = 7.4 Hz, $J_{5,6'}$ = 4.2 Hz, $|J_{6,6'}|$ = 10.1 Hz.- ^{13}C-NMR (62.9 MHz, $CDCl_3$): δ = 14.65 (4-CH_3); 55.04 (OCH_3); 68.81; 71.12; 73.00; 73.39; 73.48; 74.85; 78.28; 97.59 (C-1); 127.56; 127.71; 127.98; 128.10; 128.41; 128.50; 137.87; 138.08 (aromatic C's).- (Found C 66.78, H 6.94. Calc. for $C_{22}H_{28}O_6$ (388.5) x 0.5 H_2O: C 66.48, H 7.35.- FAB-MS: see Table 1.- $C_{22}H_{28}O_6$ (388.5); Found m/z = 395 (MLi^+, FAB-MS).

Methyl 2,6-Di-O-benzyl-4-C-methyl-α-D-galactopyranoside (20).

IR ($CHCl_3$): 3575, 3500 (OH), 3100-3000, 1605, 1500, 1075, 700 cm^{-1}.- ^{1}H-NMR (80 MHz, $CDCl_3$): δ = 1.25 (s, 3H, 4-CH_3); 2.30 (broad s, 3H, OH); 3.15 (broad s, 1H, OH); 3.37 (s, 3H, OCH_3); 3.60-3.90 (m, 5H, 2-H, 5-H, CH_2-6); 4.50-4.80(m, 5H, 1-H, benzylic H's); 7.33 (10H, aromatic H's).- ^{13}C-NMR (62.9 MHz, $CDCl_3$): 21.80 (4-CH_3), 55.34 (OCH_3), 69.62, 71.65, 72.90, 73.08, 73.63, 73.87, 77.26, 98.13 (C-1), 126.66, 126.93, 127.81, 127.91, 127.97, 128.09, 128.51, 129.06, 137.46, 138.25.- FAB-MS: see Table 1.- $C_{22}H_{28}O_6$ (388.5); Found m/z = 536 (M-H+TEA)$^+$, 538 (M+H+TEA)$^+$; FAB-MS).

Methyl 4-C-Methyl-α-D-glucopyranoside (12). [6]

A mixture of **14** (388 mg, 1.0 mmol), ethanol (70 ml) and 10% palladium-on-charcoal (80 mg) was stirred under hydrogen at room temperature and atmospheric pressure. After 4 h, the mixture was filtered and the solvent evaporated. LC (ethyl acetate-methanol 7:1 then 5:1) provided **12** (204 mg, 98%).- M.p. 142°C (from ethyl acetate-methanol).- IR (KBr): 3700-3050 (OH), 2840 (OCH_3); 1050 cm^{-1}.- ^{1}H-NMR (80 MHz, pyridine-d_5): δ = 1.62 (s, 3H, 4-CH_3); 3.49 (s, 3H, OCH_3); 3.90-4.70 (m, 5H, 2-H, 5-H, CH_2-6); 5.14 (d, 1H, $J_{1,2}$ = 4.1 Hz, 1-H); 5.80-6.70 (4H, 4 OH's).- FAB-MS: see Table 1.- $C_8H_{16}O_6$ (208.2); Found m/z = 209 (MH^+, FAB-MS).

Methyl 2,6-Di-O-benzyl-3-O-carbamoyl-4-C-methyl-α-D-glucopyranoside (15).

To a solution of 14 (390 mg, 1.004 mmol) in dry CH_2Cl_2 (15 ml) at -10°C trichloroacetyl isocyanate (125 µl, 1.05 mmol) was added. After warming to ambient temperature (2 h) methanol (0.1 ml) and then 5% K_2CO_3 aq. (10 ml) were added. The resulting suspension was stirred at room temperature for 2 h. Usual work-up (CH_2Cl_2) and LC (ethyl acetate-petrol 1:2 then 1:1) furnished 15 (318 mg, 73%) as a colourless sirup.- IR ($CHCl_3$): 3640-3100 (OH), 3100-3040, 1730 (CO), 1600, 698 cm^{-1}.- ^{1}H-NMR (250 MHz, $CDCl_3$): δ = 1.10 (s, 3H, 4-CH_3); 3.41 (s, 3H, OCH_3); 3.44 (dd, 1H, 2-H); 3.57 (ABX, 1H, 6-H); 3.82 (ABX, 1H, 6'-H); 3.93 (ABX, 1H, 5-H); 4.02 (broad s, 1H, OH); 4.54 and 4.57 (AB, 2H, $|J_{AB}|$ = 12.5 Hz, benzylic H's); 4.59 and 4.66 (AB, 2H, $|J_{AB}|$ = 12.1 Hz, benzylic H's); 4.68 (d, 1H, 1-H); 5.04 (d, 1H, 3-H); 5.15 (2H, NH_2); 7.32 and 7.34 (10H, aromatic H's).- $J_{1,2}$ = 3.9 Hz, $J_{2,3}$ = 10.2 Hz, $J_{5,6}$ = 3.0 Hz, $J_{5,6'}$ = 7.5 Hz, $|J_{6,6'}|$ = 10.5 Hz.- ^{13}C-NMR (62.9 MHz, C_6D_6): δ = 15.8 (4-CH_3); 54.7 (OCH_3); 69.1 (CH_2-6); 72.6, 73.1, 73.2 (C-4 and benzylic C's); 73.7, 77.3, 79.0 (C-2, C-3, C-5); 97.7 (C-1); 158.6 (-$OCONH_2$).- FAB-MS: see Table 1.- $C_{23}H_{29}NO_7$ (431.5); Found m/z = 438 (MLi^+, FAB-MS).

Methyl 3-O-Carbamoyl-4-C-methyl-α-D-glucopyranoside (11).

A mixture of 15 (110 mg, 0.25 mmol), methanol (20 ml) and 10% palladium-on-charcoal (45 mg) was stirred under H_2 at room temperature and atomospheric pressure. After 4 h, the mixture was filtered and the solvent evaporated. LC (ethyl acetate-methanol 10:1) furnished 11 (60 mg, 94%).- M.p. 96-98°C (from ethyl acetate-methanol).- IR (KBr): 3700-3040 (OH, NH), 1700 (CO), 1615 cm^{-1}.- ^{1}H-NMR (80 MHz, pyridine-d_5): δ = 1.50 (s, 3H, 4-CH_3); 3.42 (s, 3H, OCH_3); 3.90-4.70 (m, 4H, 2-H, 5-H, CH_2-6); 5.12 (d, 1H, 1-H); 5.84 (d, 1H, 3-H); 6.05-6.35 (2H, NH_2); $J_{1,2}$ = 4.0 Hz, $J_{2,3}$ = 10.0 Hz.- ^{13}C-NMR (22.6 MHz, DMSO-d_6): δ = 15.0 (4-CH_3); 54.1 (OCH_3); 59.2; 69.2; 71.6; 75.0; 77.1; 99.0 (C-1); 157.3 ($OCONH_2$).- FAB-MS: see Table 1.- $C_9H_{17}NO_7$ (251.2); Found m/z = 258 (MLi^+, FAB-MS), C 40.14, H 7.10, N 5.33. Calc. for $C_9H_{17}O_7N \times H_2O$: C 40.15, H 7.11, N 5.20.

Methyl 3-O-Carbamoyl-4-C-methyl-α-D-glucopyranosiduronic acid (17).

A mixture of 11 (211 mg, 0.84 mmol), water (15 ml), and Adam's catalyst (600 mg) was adjusted to pH 8 with $NaHCO_3$ and then stirred under O_2 at 60°C and atmosperic pressure. After 7 h, the mixture was filtered and the solvent removed by lyophilization. LC, first Dowex 50-H^+ (30 ml), then silica gel ($CHCl_3$-methanol-H_2O-CH_3COOH 10:8:1.5:0.1) gave (after evaporation and lyophilization) 17 (109 mg, 49%) as a colourless solid.- IR (KBr): 3700-3020 (OH, NH), 1715 (CO), 1620 cm^{-1}.- ^{13}C-NMR (62.9 MHz, DMSO-d_6): δ = 17.2 (4-CH_3); 54.8 (OCH_3); 68.8; 71.5; 72.6; 76.3; 99.5 (C-1); 156.9 ($OCONH_2$); 172.4 (C-6).- FAB-MS: see Table 1.- $C_9H_{15}NO_8$ (265.2); Found m/z = 272 (MLi^+, FAB-MS).

Ethyl 4-C-Methyl-ß-D-glucofuranosidurono-6,3-lactone (3).

a) A solution of 17 (50 mg, 0.189 mmol) in 0.1 M ethanolic HCl (10 ml) was refluxed for 15 h. Evaporation to dryness and LC (ethyl acetate-petrol 3:1) furnished 3 (11 mg, 27%) as a colourless sirup, identical with an authentic sample.[5]

b) Similarly, 18 which was obtained from 12 as described by Sato et al.[6], gave 3 in 11% yield. ^{1}H-NMR (250 MHz, $CDCl_3$): δ = 1.19 (t, 3H, J = 7.0 Hz, -O-CH_2-CH_3); 1.67 (s, 3H, 4-CH_3); 2.48 (d, 1H, 2-OH); 2.75 (d, 1H, 5-OH); 3.52 and 3.73 (m, O-CH_2-CH_3); 4.14 (d, 1H, 5-H); 4.42 (d, 1H, 2-H); 4.57 (s, 1H, 3-H); 5.13 (s, 1H, 1-H). $J_{2,OH}$ = 4.2 Hz, $J_{5,OH}$ = 9.2 Hz). CD (ethanol, c = 1.1 mmol/l): λ_{max} (Δε) = 226 nm (+3.10).

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