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SIALYL ALDOLASE IN ORGANIC SYNTHESIS: FROM THE TROUT EGG ACID, 3-DEOXY-D-*GLYCERO*-D-*GALACTO*-2-NONULOSONIC ACID (KDN), TO BRANCHED-CHAIN HIGHER KETOSES AS POSSIBLE NEW CHIRONS.

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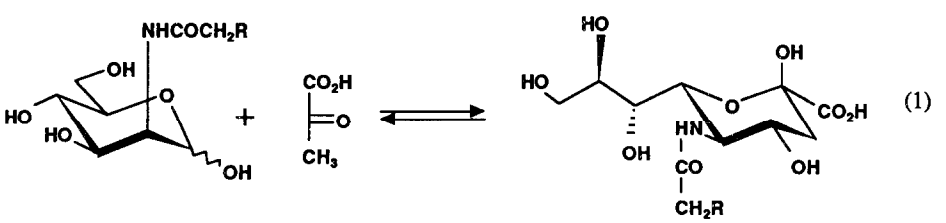
Abstract :N-Acetylneuraminate pyruvate lyase accepts as substrates varied non-nitrogeneous sugars. Condensation of pyruvate with D-mannose yields 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN), a component of rainbow trout egg polysialoglycoprotein. From some easily available monosaccharides were prepared other ulosonic acids : 3-deoxy-D-*galacto*-2-octulosonic, 3,5-dideoxy-D-*gluco*-2-nonulosonic, and 3,7-dideoxy-D-*galacto*-2-nonulosonic acids. Glucose was not a good substrate, and D-arabinose gave a mixture of the expected 3-deoxy-D-*gluco*-2-octulosonic acid (19%) with 3-deoxy-D-*manno*-2-octulosonic acid (16%). The latter is the well-known sugar acid KDO from bacterial polysaccharides, and results from an anomalous orientation at the condensation step. The relative indifference of the enzyme to the nature of the substituent at C(2) of the sugar, provided the D-*manno* configuration is retained, is demonstrated by the conversion in 76% yield of 2-deoxy-2-*C*-phenyl-D-mannose into 3,5-dideoxy-5-*C*-phenyl-D-*gluco*-2-nonulosonic acid. All these preparations utilized immobilized lyase, and can be scaled up *ad libitum*.

Introduction

One of the major goals of organic chemistry during the last decade has been the construction of precursor molecules suitable for an enantioselective aldol condensation.[1] Obviously the enzymatic aldol condensation might be convenient for such purpose. The corresponding enzymes, the *aldolases* are a subgroup of the general category of enzymes called *lyases*. They are found in many types of living cells, and show varied specificities. Being present in plants and microorganisms, they are fairly easily available. The isolation and description of a number of them may be found in the collection *Methods in Enzymology*.[2] Their utilization in preparative organic chemistry has been already considered several times.[3] Although Nature elaborated each aldolases for the synthesis or catabolism of a specific substrate, a sugar in most cases, these enzymes generally exhibit broader specificity. Thus, knowledge of this specificity is of prime importance to chemists who want to use them as general reagents.

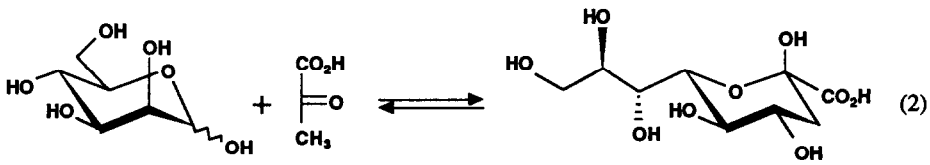
We describe here synthetic methods using as catalyst N-acetylneuraminate pyruvate lyase (E.C.4.1.3.3) henceforth abbreviated as "sialyl aldolase". This enzyme was studied in detail by Comb and Roseman.[4] It was quickly recognized that it catalysed an equilibrium between N-acetylneuraminic acid (**2**, R=H) or N-glycolylneuraminic acid (**2**, R=OH) and their products of aldol cleavage, that is N-acetylmannosamine (**1**, R=H) or N-glycolylmannosamine (**1**, R=OH) and pyruvate (eq. 1) :



(1)

1 **2**

The numerous, natural functionnal derivatives of neuraminic acid present in cells are cleaved by sialyl aldolase with comparable efficiency, among them the 9-O-acetate of acid **2** (R = H).[5] Of course, derivatization of the hydroxyl function at position 4 precluded aldol cleavage. We have reported already the syntheses with immobilized sialyl aldolase of a number of sialic acids and congeners, on the multigram scale whenever necessary for our studies.[6,7] However, the most significant observation for organic synthesis was perhaps the discovery that 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN), a naturally-occuring deaminated sialic acid, was cleaved by sialyl aldolase[8] to a mixture of mannose and pyruvate (eq. 2) :



(2)

3

This suggested that we could extend work with sialyl aldolase to non nitrogeneous compounds, and this extension is the subject of the present paper. Part of this work has been already reported in preliminary forms.[9,10]

Discussion

There are several commercial sources for sialyl aldolase, and the price of the unit * seems to be decreasing. We use it immobilized on agarose by the cyanogen bromide method.[11] This preparation contains 4 U of enzymatic activity per mL of gel. We lost up to 40% of the activity in immobilization. In spite of this, this technique is more economical in the long run than the direct use of the soluble enzyme, for the aldolase-agarose conjugate can be recovered by filtration and utilized again at least ten times, and kept for six months at 4°C in a refrigerator without noticeable loss of activity. After removal of the gel, the sugar acids are separated from the aqueous solution by ion-exchange chromatography. Repeated utilization of the same gel leads to yields at least 2 mmol per unit of enzyme.

The natural occurrence of our first target, KDN, **3**, was reported in 1986.[8] KDN was isolated on the mg scale from rainbow trout egg polysialoglycoprotein; it is exclusively located at the non-reducing end of the sialyl chains and therefore may be involved in egg activation of salmonid fishes by protecting them against sialidases. We found that KDN may be synthesized in a very straightforward manner, by the enzymatic condensation of mannose and pyruvate in the presence of sialyl aldolase. From analytical data of our first samples, it seemed that KDN had picked up inorganic cations present in the solutions which were retained in the purification procedure. Conversion to the ammonium salt gave a product with the expected composition.

This preparation of KDN commends itself for its simplicity and the cheapness of reagents, and this acid thus becomes available on the multigram scale. Mannose was our best substrate from the preparative point of view,[9] a fact which has been substantiated by a recent estimation of its kinetic parameters.[11] Thus, the replacement of NHAc by OH, keeping the D-*manno* configuration, makes no great difference to the enzyme. This

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*We adopt the common definition of a unit of enzyme (U) as the activity able to catalyse the conversion of one micromole of substrate in one minute, in the best possible conditions.

is in sharp contrast to the fact that N-acylglucosamines are not substrates, a fact which in our hands allowed inexpensive syntheses of the two most common sialic acids.[6a,6c,7]

This observation led us to examine other sugar derivatives as substrates. Experiments which, in our view, have preparative significance, are summarized in Table I. We shall deal later on with the preparation of 2-deoxy-2-*C*-phenyl-D-mannose. Proton-proton coupling constants, with our assignment, for acids **3** - **7** are given in Table II. We have joined the already reported[12] azido derivative **8** for comparison. These coupling constants are very similar, and agree with the configurations and conformations drawn in Table I, including the axial orientation of the anomeric hydroxyl. (cf. Ref. 13)

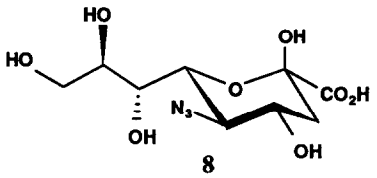

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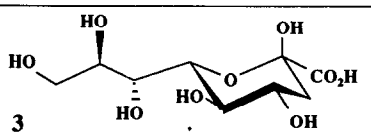
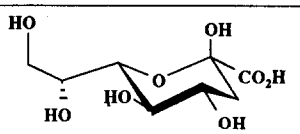
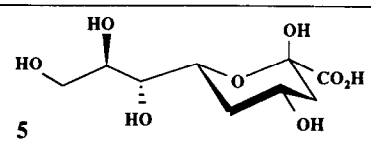
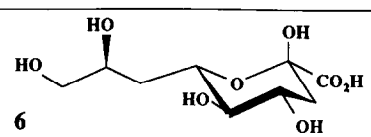
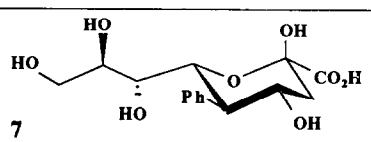
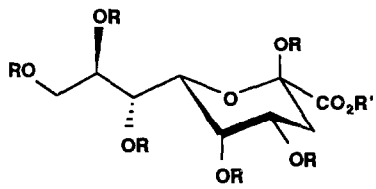
Table I-Preparation of nonulo- and octulosonic acids

Substrate	U/mmol of substrate	Reaction time(days)	Product	Isolated yield (%)
D-Mannose	15	1	 **3**	84
D-Lyxose	14	2	 **4**	66
2-deoxy-D-*arabino* -hexose	6	5	 **5**	36
4-deoxy-D-*lyxo* -hexose[a]	12	2	 **6**	67
2-deoxy-2-*C*-phenyl D-mannose	8	8	 **7**	76

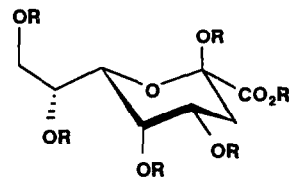
a:Prepared as in Ref. 35

Although N-acetylglucosamine is not a substrate of sialyl aldolase, D-glucose was transformed in our system at a slow rate. The complexity of the ^1H NMR spectrum of the product indicated that it was a mixture. This was partially resolved by peracetylation and esterification, followed by silica gel chromatography. A derivative which still retained 10% impurities was obtained in 28% overall yield. The proton n.m.r. spectrum was in agreement with configuration and conformation **9**. The product is a C(5) epimer of KDN. D-Xylose, which has the same ring constitution as D-glucose, behaved in the same way, giving **11** in 18% yield. In these two aldol condensations the complexity of the ^1H n.m.r. spectra before derivatization may be due to 1-5 lactone formation, as it has been suggested for 3-deoxy-D-*manno*-2-octulosonic acid.[14]



9 R=Ac R'=Me

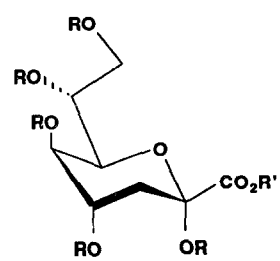
10 R=R'=H



11 R=Ac R'=Me

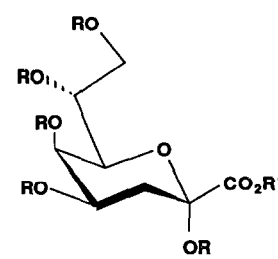
12 R=R'=H

D-Arabinose also reacted sluggishly to give a mixture of diastereoisomers, **14** and **16** in a 56:44 ratio (HPLC analysis). This was partially resolved by flash chromatography of the derived peracetate methyl esters. Structures **13** and **15** were ascribed to these diastereoisomers (each obtained in about 17% yield) from their ^1H n.m.r. spectra. Compound **13** is the one expected in the enzymic condensation, whereas **15** is the anomalous one. Compound **15** is the derivative of the well-known 3-deoxy-D-*manno*-2-octulosonic acid (KDO), and the ascribed structure was unambiguously confirmed by comparison with an authentic sample of peracetylated methyl ester of KDO, m.p. 157-158°. Epimerization at C(4) could not arise from the work up.



13 R=Ac R'=Me

14 R=R'=H



15 R=Ac R'=Me

16 R=R'=H

Table II-Proton-proton coupling constants in Hz for sugar-acids 3-8[a]

Protons involved	3	4	5	6	7	8[b]
3a, 3e	12	12.5	12	13	13	13
3a, 4	12	12.5	12	12	11	11.5
3e, 4	5	5	4	5	5	5
4, 5a	9	9.5	12	9	10	10
4, 5e			nd			
5a, 5e			12			
5a, 6	9	9.5	12	9	11	10
5e, 6			nd			
6, 7	1	0	1.5	2[c]	1	1
6, 7'				10		
7, 7'				15		
7, 8	8.5	6	8	10	10	10
7, 8'		6				
7', 8				3[c]		
8, 8'		nd				
8, 9	5.5		6	7	7	7
8, 9'	2		2.5	4	3	3
9, 9'	11		11	12	12	12

a:In D_2O; for measurements and chemical shifts, see Experimental; nd=not determined.
b:From ref. 12. c:These assignments may be exchanged.

Moreover, the opical rotation we measured from the analytically pure mixture of **14** and **16** corresponded to an intermediate value (+27°) between the optical rotations of KDO (+40,3°)[15] and 3-deoxy-D-*gluco*-2-octulosonic acid (+12,5°).[16] Thus we observe for the first time lack of specificity in sialylaldolase. The results of this series of unsatisfactory aldol condensation are summarized in Table III, and the assignments of the proton-proton coupling constants of the products are given in Table IV. The proton-proton coupling constants of the peracetylated methyl pyranosides derived from ulosonic acid **3** and **4**, respectively methyl(hexa-*O*-acetyl-3-deoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**42**) and methyl (penta-*O*-acetyl-3-deoxy-α-D-*galacto*-2-octulopyranosid)onate (**43**), have been added in this Table for convenience.

Table III-Other condensations catalyzed by Sialylaldolase

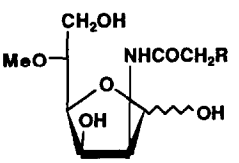
Sugar	U/mmol of substrate	Reaction time(days)	Product	Yield (%)
D-glucose	16	5	**10**	28
D-xylose	20	5	**12**	18
D-arabinose	12	5	**14** **16**	19 16

Table IV-Proton-proton coupling constants in Hz for peracetylated methyl esters 9,11,13,15,42 and 43[a]

Protons involved	9	11	13	15	42	43
3a,3e	12	1	15		13	13.5
3a,4		11.5	3.5	11	11.5	11
3e,4	5.5	6	2.5	7	5	5
4,5	3	3	2.5	3	10	10
5,6	1	1	1.5	1	10	10
6,7	8	8	10	10	2.5	2.5
7,8	3	3	2.5	2	5.5	7.5
7,8'		7	5	5		4.5
8,8'		12	12	12		11.5
8,9	5.5				2.5	
8,9'	6				6.5	
9,9'	12				12.5	

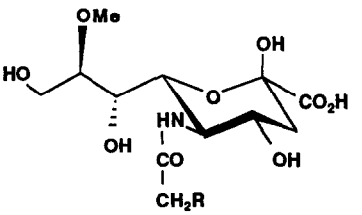
a: in C_6D_6; for measurements and chemical shifts, see Experimental

This work, together with some previous observations in our laboratory, allows to assess some features of the specificity of sialyl aldolase. We have noted already that, keeping the D-*manno* configuration, we could replace NHAc in the "normal" substrate, N-acetylmannosamine, by $NHCOCH_2OAc$, N_3, OH, and H. The hydroxyl at C(4) may be methylated, acetylated, or, in the mannose series, eliminated by reduction. The group CH_2OH may be methylated, acetylated, lactoylated, or deleted. On the other hand, while the enzyme appeared tolerant to modifications at position 2,4 and 6, we found[6c] that the obligatory furanose, 2-acetamido-2-deoxy-5-*O*-methyl-D-mannose (**17**) was a very poor substrate, giving only traces of **19**. Furthermore, it has been known for a long time[17] that the starfish sialic acid **20** was not cleaved to mannosamine **18**.



17 R=H

18 R=OH



19 R=H

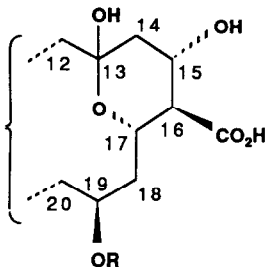
20 R=OH

Thus it is tempting to speculate that sialyl aldolase does not recognize the furanose tautomer. The corresponding non enzymic aldolisation would require the *aldehydo* sugar as partner to pyruvic acid enolate, and

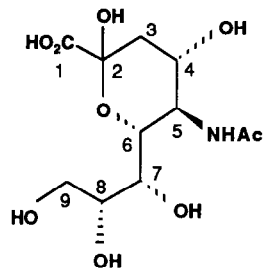
we expect that the tautomeric configuration of the starting material would not be of prime importance. Thus, with sialyl aldolase, ring opening might occur on the enzyme, or the mechanism be different.

We also observed a highly anomalous enzymic condensation with D-arabinose. Now, all the sialic acids and deaminated sialic acids we have prepared so far, except acid **14**, exist in the D-$^1C_4$ conformation. On the other hand, because of the different configuration of carbon 3, D-arabinose cannot be converted to an octulosonic with a stable D-$^1C_4$ conformation, for this would involve a highly unfavorable axial orientation of the side chain on C(6), with severe 1,3-diaxial interactions whatever may be the anomeric equilibrium. The low yield of the normal product **14** together with an almost equal yield of a product **16** corresponding to an anomalous orientation of the newly built hydroxyl, may be taken as indications of the highly perturbed state of the enzyme in front of substrate D-arabinose. In the same way, qualitative tests with the D-altrose which has the same ring constitution as D-arabinose indicated, at best, a sluggish conversion. Thus, sialyl aldolase may also have been adapted to recognize the usual D-$^1C_4$ configuration of sialic acids, and thus equilibrate two pyranoses, rather than two carbonyl compounds.

The good yields achieved with D-*manno* sugars substituted with nitrogen or oxygen, or even reduced at C(2), suggested to examine as substrates branched-chain sugars with carbon at that position. This would give a deaminated sialic acid branched at C(5), a structure similar in several respects to sequence 12-20 of Amphotericin B (**21**).[18] For the sake of comparison, N-acetylneuraminic acid **22** has been shown with the same conventions.

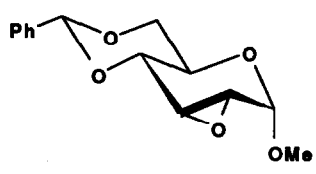


21

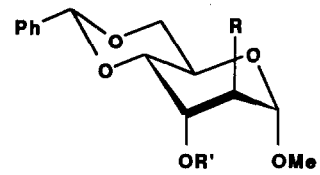


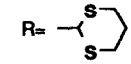
22

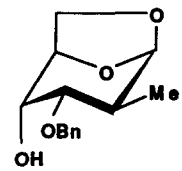
For the preparation of such tentative substrates we first explored the ring-opening reactions of epoxide **23**. Compounds **24**[19], **25**[20], and **26**[21] have been prepared with suitable nucleophiles but we could not achieve the configuration inversion at C(3) on **25** as a model compound. Attempted displacement of the imidazylate or triflate with benzoate led to 3-4 elimination, which is indeed favored by the *anti* configuration of H-4 and leaving group. We could not achieve Pfittzner-Moffatt oxidation to the ketone with the Swern technique without migration of the methyl to the equatorial position. Then we prepared the benzyl ether **27**, with the hope that it would move to the equatorial position by alkaline equilibration of a ketone, generated on C(4). However, even the mild acidic conditions (60% acetic acid in water) necessary to remove the benzylidene ketal were sufficient to give the anhydro sugar **28**, as the only product identified, in more than 56% yield. This very easy methoxyl cleavage should be, at least in part, the result of a participation of the C(6) oxygen.



23

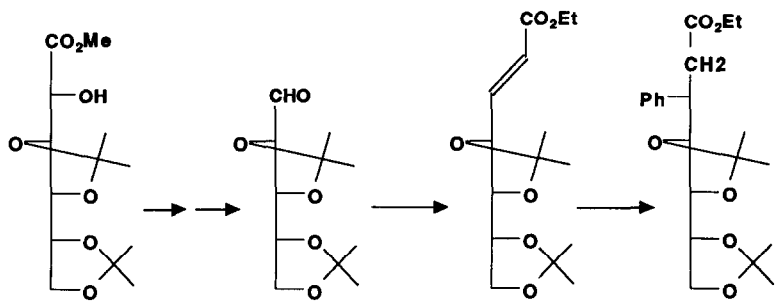


24 R=$CH(CO_2Et)_2$ R'=H
25 R=Me R'=H
26 R=  R'=H
27 R=Me R'=Bn



28

Because of these difficulties, we explored an alternative route, that is 1-4 addition to an enoate. Indeed, addition of lithium tris(methylthio)methane to an α,β unsaturated lactone has been already utilized[22] to introduce a precursor to the carboxyl in a synthesis of sequence 13-19 of **21**. Lawston and Inch[23] prepared enoate **31** by condensation of the D-*arabino* aldehyde **30** with triethylphosphonoacetate in the presence of sodium hydride, and found that conjugate addition of phenylmagnesium bromide (and other aryl Grignard reagents), in the presence of 5 mol% cuprous iodide gave an excellent yield (89%) of the D-*manno* branched-chain heptonate **32** as a single, crystalline isomer. They also observe a neat reaction with t-butylmagnesium chloride, giving again the D-*manno* product, while simple aliphatic Grignard reagents gave erratic results. Vinyl organometallics were not considered.

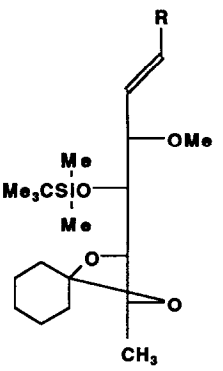


29 30 31 32

In a very recent paper,[24] Roush *et al.* described 1-4 addition of vinyl cuprate to **33** and **34**, which may be viewed as D-*galacto* analogues of **31**. In the best case, a single diastereoisomer **35** (Fischer projection) was formed in very good yield. Thus, the orientations of the addition to both the D-*arabino* and the D-*galacto* enoates seem dictated by the configuration at C(4). According to Roush *et al.*, this reflects the absence of chelation in the reaction transition state, a behaviour contrasting with that of allylic cuprates.

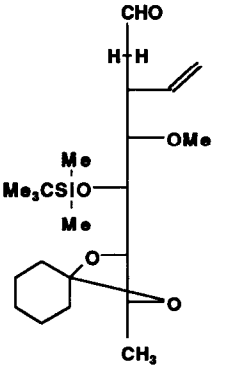
Route **29-32** looked attractive, as aldehyde **30** may be prepared in two nearly quantitative steps[25] from gluconate **29**, which is available on the half kilogram scale.[26] Addition of vinyl cuprate to the same enoate **31**

gave us the corresponding vinyl-branched heptonate **36**, to which we ascribe the D-*manno* configuration by analogy with precedents.

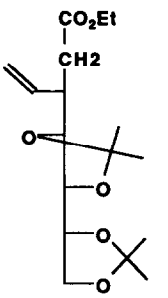


33 R=CO_2Et

34 R=CHO

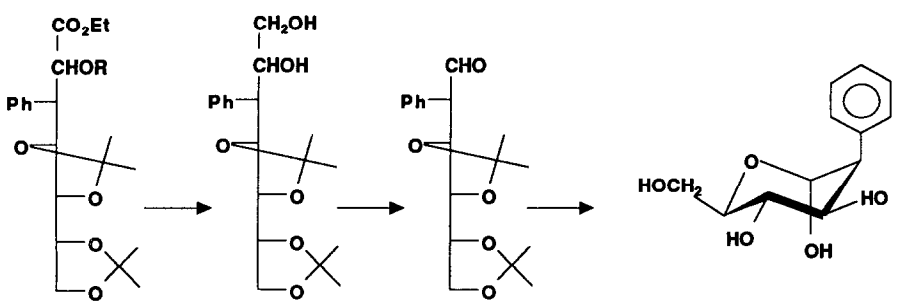


35



36

Now coming back to the *C*-phenyl ester **32**, conversion to a branched-chain mannose involves the shortening of the seven carbon chain. Oxidation of the lithium enolate of **31** with the complex of molybdenum pentoxide with pyridine and hexamethylphosphorotriamide (HMPA),[27] following the Vedejs procedure[28] gave in 52% yield a mixture of epimeric hydroxy derivatives **37**. We recovered 42% starting ester, this corresponding to a 90% conversion yield. The hydroxylation is stereoselective, the ratio of epimers being 85:15, as determined by ^1H n.m.r., after acetylation of the mixture. This preference may be steric in origin, as it is markedly smaller (61:39) with the vinyl analogue **36**. We have not determined the configuration of the major isomer. The mixture was reduced with $LiAlH_4$ and oxidized with periodate, and the *aldehydo* mannose de-ketalized in water-acetic acid (Sequence **37**, **39**, **40**, **41**).



37 R=H

38 R=Ac

39 **40** **41**

The ^1H n.m.r. spectrum of 2-deoxy-2-*C*-phenyl-D-mannose **41** in D_2O solution shows three anomeric protons signals, with intensities adding to one proton, an indication that there are three tautomers in these

conditions, and no more. While it is difficult to analyse in detail such a spectrum, we may venture conformational considerations : the conformational free-energy of a phenyl substituent in a cyclohexane ring varies from 2.0 to 3.1 kcal/mol according to the solvent.[29] Thus it is inferior, or at best nearly equal to the enhanced conformational free energy of a hydroxymethyl group at C(5) of a pyranose,[30,31] that is,[30] 2.89 kcal/mol at -100°C. Thus, if the molecule adapt a chair conformation, we see no reason for a reversal of the usual D-$^1C_4$ chair. Other factors will be more important. The main isomer, $J_{1,2}$ 1.5 Hz (48%) could be the α-pyranoside, and the second one in importance, $J_{1,2}$ 3 Hz (29%) the β-pyranoside. The third anomeric proton $J_{1,2}$ 7.5 Hz (23%) then would correspond to a furanose. The percentage composition of an equilibrated aqueous solution of D-mannose itself is α-pyranose : 67; β-pyranose : 33; furanose < 1.[32]

Whatever its tautomeric composition, 2-deoxy-2-*C*-phenyl-D-mannose was converted to the *C*-phenyl nonulosonic acid 7 in the presence of sialylaldolase, in a very good 76% yield. There are no conformational problem with this pyranose, since the phenyl group is equatorial. Surely, this enzymatic conversion is an extreme case, and its success suggests that most other substitutions at C(2), provided the D-*manno* configuration is retained, will give substrates for sialyl aldolase.

EXPERIMENTAL PART

General Methods.- The progress of reactions and purity of samples were checked by t.l.c. on silica gel plates, with mixtures of ethyl acetate and hexane as eluent for protected sugars and ethyl acetate - 2-propanol - water, 3:3:2, as eluent for free sugars. Proton n.m.r. spectra were recorded at 250 MHz, with Me_4Si as internal reference in $CDCl_3$ or C_6H_6 for protected sugars, and the HOD peak (4,80 ppm) as reference for D_2O solutions of free sugars. Assignments of *J* values already reported in Tables II and IV are not repeated below.

Ethyl 3-deoxy-4,5:6,7-di-O-isopropylidene-3-C-phenyl-D-glycero-D-galacto and *D-glycero-D-talo-heptonate* (**37**).- A solution of heptonate **32** (627 mg; 1.66 mmol) in oxolane (15 mL) is added dropwise to a 0.4 M solution of $LiNEt_2$ in oxolane (4.25 mL) cooled to -70°C, and the mixture is stirred at that temperature for 20 min. Then, the complex MoO_5-pyridine-HMPA (809 mg, 1.87 mmol) is added, and the mixture stirred for 1.5 h at -70°C. Sodium sulfite (saturated aqueous solution ; 8 mL), and then water are added, the mixture is stirred for 30 min, and extracted with ether. The ether layer is washed with water, separated, volatiles are removed. Silica gel chromatography of the residue (hexane - ethyl acetate, 4:1) first separated the starting heptonate **32** (253 mg, 42%) and afterwards the epimeric mixture **37** (326 mg, 52%) as a gum : max 3480 (OH), 1740 (CO) cm^{-1}; ^1H NMR ($CDCl_3$), major epimer (85%) : δ 1.10, 1.17, 1.39, 1.42 (4 s, 4x3 H, 2 CMe_2), 1.24 (t, 3H, *J* 7Hz, CH2-*Me*), 3.36 (dd, 1H, $J_{2,3}$ 3, $J_{3,4}$ 10Hz, H-3), 3.49 (d, 1H, $J_{2,OH}$ 5Hz, OH), 3.51 (ddd, 1H, $J_{5,6} = J_{6,7} = J_{6,7'}$ = 6.5Hz, H-6), 3.73 (dd, 1H, $J_{7,7'}$ 8Hz, H-7), 3.85 (dd, 1H, H-7'), 3.92 (dd, 1H, $J_{4,5}$ 5.5Hz, H-5), 4.13, 4.20 (two q, 2H, CH_2Me), 4.46 (dd, 1H, H-4), 4.47 (dd, 1H, H-2), 7.30 (5H, Ph); minor epimer (15%) : δ 1.16 (t, CH_2-*Me*), 1.10, 1.17, 1.46, 1.56 (4 s, 2 CMe_2), 3.24 (dd, $J_{2,3}$ 3, $J_{3,4}$ 10.5Hz, H-3), 4.08 (q, *J* 7.5Hz, C*H*Me), 4.27 (q, *J* 7.5 Hz, C*H*Me), 4.56 (dd, $J_{4,5}$ 5Hz, H-4), 4.63 (dd, H-2).

Anal. Calc. for $C_{21}H_{30}O_7$: C, 63.94; H, 7.66; O, 28.40. Found : C, 63.97; H, 7.57; O, 28.32.

The mixture of alcohols **37** was acetylated in the usual way with acetic anhydride (0.1 mL) and pyridine (1 mL) to give the O-acetates **38**. NMR : major epimer (85%) δ 1.0-1.7 (15H, two CMe_2, CH_2-CH_3), 2.21 (s, 3H, Ac), 3.53 (dd, 1H, $J_{2,3}$ 5, $J_{3,4}$ 8Hz, H-3), 3.70 - 4.12 (6H, H-5, H-6, H-7, H-7', CH_2Me), 4.45 (dd, 1H,

$J_{4,5}$ 5Hz, H-4), 5.39 (d, 1H, H-2), 7.30 (5H, Ph); minor epimer (15%) δ 2.23 (s, Ac), 3.42 (dd, $J_{2,3}$ 3.5, $J_{3,4}$ 10.5Hz, H-3), 4.46 (1H, H-4), 5.63 (d, 1H, H-2).

2-Deoxy-2-C-phenyl-D-mannose (**41**).- A solution of esters **37** (534 mg; 1.35 mmol) and $LiAlH_4$ (102 mg; 2.7 mmol) in oxolane (10 mL) is kept for one hour at room temperature. Water (0.1 mL), 15% aqueous NaOH (0.1 mL) and again water (0.3 mL) are added consecutively. Filtration of the solution followed by evaporation to dryness left as residue diol **39** (419 mg; 88%). This was dissolved in a mixture of alcohol (10 mL) and water (5 mL) and sodium metaperiodate was added (0.4 g). The mixture was kept for 0.5 h at room temperature and overnight at 4°C, and then filtered. After removal of alcohol by evaporation, extraction with dichloromethane of the aqueous solution gave 2-deoxy-3,4:5,6-di-*O*-isopropylidene-2-*C*-phenyl-*aldehydo*-D-mannose (**40**), as a syrup (0.3 g). NMR : δ 1.21, 1.25, 1.40, 1.42 (4 s, 4x3H, 4 CMe), 3.80-3.90 (m, 4H), 4.50 (m, 1H), 4.54 (dd, *J* 5 and 5Hz, 1H), 7.35 (m, 5H, Ph), 9.83 (d, 1H, $J_{1,2}$ 3Hz, H-1).

A solution of this syrup (0.3 g) in water and acetic acid (1:1, 10 mL) is kept for 0.5 h on a boiling water-bath, then evaporated to dryness and coevaporated with toluene. Silica gel column chromatography of the residue (ethyl acetate) gave the free sugar **41** (201 mg; 65%). NMR (D_2O) :δ 3.50 (dd, 0.48H, $J_{1,2}$ 1.5, $J_{2,3}$ 6Hz, H-2, α-pyranose), 5.23 (d, 0.29H, $J_{1,2}$ 3Hz, H-1, β-pyranose), 5.40 (d, 0.48H, H-1, α-pyranose), 5.86 (d, 0.23H, $J_{1,2}$ 7.5Hz, H-1, α-furanose), 7.50 (5H, Ph).

Anal. Calc. for $C_{12}H_{16}O_5$, 0.5 H_2O : C, 57.83; H, 6.82; O, 35.34. Found : C, 58.38; H, 6.87; O, 35.01.

1,6-Anhydro-2-deoxy-3-O-benzyl-2-C-methyl-β-D-altropyranose (**28**).- A solution of alcohol **25** (560 mg; 2 mmol) and benzyl bromide (0.62 mL) in N,N-dimethylformamide (3.3 mL), in the presence of sodium hydride (60% dispersion in oil; 180 mg) is heated at 80°C for 16 h. Methanol (2 mL) was added, and the mixture was refluxed for 0.5 h. After a two-phase separation in a water-ether system, the organic layer was evaporated to dryness, the residue was dissolved in acetic acid (30 mL) and water (20 mL), and kept at 100°C for 1 h. Then, the solution was evaporated to dryness. Chromatography of the residue (hexane-ethyl acetate, 2:1) separated the anhydro sugar, as a syrup (305 mg, 56%); ^1H NMR data : δ 1.10 (d, 3H, *J* 7Hz, Me), 1.92 (dq, 1H, $J_{2,3}$ 10Hz, H-2), 3.00 (br., OH), 3.28 (dd, 1H, $J_{3,4}$ 4Hz, H-3), 3.59 (d, 1H, $J_{6,6'}$ 8Hz, H-6), 3.69 (dd, 1H, $J_{5,6}$ 6Hz, H-6'), 3.82 (dd, 1H, $J_{4,5}$ 3Hz, H-4), 4.40-4.70 (2H, PhCH_2), 4.66 (d, 1H, H-5), 5.20 (s, 1H, H-1), 7.35 (5H, Ph).

Ethyl 2,3-dideoxy-4,5:6,7-di-O-isopropylidene-3-C-vinyl-D-manno-*heptanoate* (**36**).-A one molar solution of vinylmagnesium bromide in oxolane (60 mL) was added in the course of two hours to a suspension of CuI (5.7 g ; 30 mmol) in oxolane (20 mL) , cooled to - 20°C. To the new black suspension was added a solution of enoate **32** (6 g ; 20 mmol) in oxolane (20 mL) in 0.5 h., at -20°C. A saturated aqueous NH_4Cl solution was added, the mixture was extracted with dichloromethane, which was washed with a NH_4Cl solution and evaporated. Flash chromatography of the residue (dichloromethane) gave the vinyl derivative **36** (5.55 g ; 85%) ^1H NMR ($CDCl_3$): δ 1.24 (t, 3H, *J* 7Hz, CH_2*Me*), 1.34, 1.36 (2s, 2x3H, 2 CMe), 1.41 (s, 6H, 2 CMe), 2.36 (dd,1H,$J_{2a,2b}$ 15, $J_{2b,3}$ 10Hz, H-2b), 2.68 (dd, 1H, $J_{2a,3}$ 4Hz, H-2a), 2.87 (m, 29Hz, 1H, H-3), 3.825 (dd, 1H, *J* 5.5 and 7Hz, H-5), 4.11 (q, 1H, -OCH_2Me), 3.9-4.1 (m, 4H, H-4,H-6,H-7a and H-7b), 5.12 (dd, 1H, $^3J_{cis}$ 10, ^2J 3Hz, -CH=CH*H*), 5.17 (dd, 1H, $^3J_{trans}$ 18.5Hz, -CH=C*H*H), 5.72 (ddd, 1H, $J_{3,CH=}$ 8Hz, -C*H*=CH2).

Anal. Calc. for $C_{17}H_{28}O_6$: C, 62.18; H, 8.59; O, 29.23. Found : C, 62.23; H, 8.41; O, 29.14.

General methods for enzymatic aldolisations.- The details of enzyme immobilization have been reported already.[6c] In a typical experiment a 0.1 M solution of sugar (**2-8**) (1 mmol) in 0.05 M potassium phosphate buffer, pH 7.2, containing 0.01 M dithiothreitol and 0.02% sodium azide was treated with 10 equiv. of sodium pyruvate. The mixture was gently stirred in the presence of acylneuraminate pyruvate lyase covalently bound to 4% agarose, at 37°C under nitrogen for 1 to 5 days. 39.After filtration of the gel the pmroducts were isolated by anion exchange chromatography according to method a or b; method a : the products were eluted from Dowex-1 ($HCOO^-$) with a 0-2 M HCOOH gradient and freeze-dried; method b : the products were eluted from Dowex-1 (HCO_3^-) with a 0-0.2 M HCO_3NH_4 gradient, freeze-dried, deionized with Dowex-50 (H^+) and again freeze-dried. The gel which retained good enzymatic activity was reused in the next run.

Ammonium salts were obtained by neutralization to pH 7 with dilute ammonia, the solution obtained after de-ionization with Dowex-50 (H^+), and freeze-drying.

We give below additional characterization not yet reported in the Table for the osonic acids,

3-Deoxy-D-glycero-*D*-galacto-*2-nonulosonic acid* (KDN, **3**).- $[\alpha]_D^{20}$ -41° (H_2O). ^1H NMR (D_2O) : δ 1.80 (t, 1H, H-3a), 2.23 (dd, 1H, H-3e), 3.56 (t, 1H, H-5), 3.63 (dd, 1H, H-9), 3.73 (m, 1H, H-8), 3.88 (dd, 2H, H-7, 9'), 4.01 (dd, 1H, H-6), 4.02 (m, 1H, H-4); ^{13}C NMR (62.9 MHz, internal 1,4-dioxane reference, δ 67.40) δ 39.19 (C-3), 63.86 (C-9), 68.51 (C-4), 69.27 (C-7), 70.67 (C-5), 71.06 (C-6), 72.29 (C-8), 96.02 (C-2), 174.29 (C-1). For analysis, acid **3** was converted to the ammonium salt.

Anal..Calc. for $C_9H_{19}NO_9$: C, 37.87; H, 6.71; N, 4.91; O, 52.48.Found : C, 37.93; H, 6.72; N, 4.80; O, 52.32.

3-Deoxy-D-galacto-*2-octulosonic acid* (**4**).- ^1H NMR (D_2O, HOD = 4.80 ppm) : δ 1.78 (dd, 1H, H-3a), 2.16 (dd, 1H, H-3e), 3.52 (dd, 1H, H-5), 3.60 (d, 2H, H-8,8'), 3.70 (d, 1H, H-6), 3.89 (m, 1H, H-4), 4.00 (dd, 1H, H-7); for analysis, acid **4** was converted to its ammonium salt.

Anal. Calc. for $C_8H_{17}NO_8$: C, 37.64; H, 6.71; N, 5.49; O, 50.15. Found : C, 37.20; H, 6.83; N, 5.17; O, 50.71.

3,5-Dideoxy-D-gluco-*2-nonulosonic acid* (**5**).- ^1H NMR (D_2O; HOD = 4.80 ppm) : δ 1.57 (dd, 1H, H-3a), 1.62 (dd, 1H, H-5a), 1.88 (m, 1H, H-5e), 2.17 (dd, 1H, H-3e), 3.48 (dd, 1H, H-7), 3.62 (dd, 1H, H-9), 3.74 (m, 1H, H-8), 3.82 (dd, 1H, H-9'), 4.20 (m, 2H, H-4,6); for analysis acid **5** was converted to its ammonium salt.

Anal. Calc. for $C_9H_{19}NO_8$: C, 40.14; H, 7.11; N, 5.20; O, 47.54. Found : C, 39.52; H, 6.97; N, 4.63; O, 48.42.

3,7-Dideoxy-D-galacto-*2-nonulosonic acid* (**6**).- 1H NMR (D_2O, HOD = 4.80 ppm) δ : 1.58 (ddd, 1H, H-7), 1.82 (dd, 1H, H-3a), 1.92 (O, 1H, H-7'), 2.23 (dd, 1H, H-3e), 3.18 (dd, 1H, H-5), 3.45 (dd, 1H, H-9), 3.57 (dd, 1H, H-9'), 3.87 (m, 3H, H-4, 6, 8).

Mixture of *3-deoxy-D*-gluco *and D*-manno-*2-octulosonic acids*(**14**) and **16**).- This mixture, $[\alpha]_D^{20}$ +27° (H_2O) was converted to the mixture of ammonium salts for analysis.

Anal. Calc. for $C_8H_{17}NO_8$: C, 37.64; H, 6.71; N, 5.49; O, 50.15. Found : C, 38.08; H, 6.67; N, 4.87; O, 50.02.

3,5-Dideoxy-5-C-phenyl-α-D-glycero-D-galacto-2-nonulosonic acid (**7**).- ^{1}H NMR (D_2O, HOD = 4.80 ppm) : δ 1.84 (dd, 1H, H-3a), 2.31 (dd, 1H, H-3e); 2.90 (dd, 1H, H-5), 2.98 (dd, 1H, H-7), 3.34 (dd, 1H, H-9), 3.69 (ddd, 1H, H-8), 3.69 (dd, 1H, H-9'), 4.38 (dd, 1H, H-6), 4.39 (ddd, 1H, H-4), 7.70 (m, 5H, Ph).

General procedure for the preparation of peracetylated methyl esters (**9, 11, 13** and **15**).- After ion exchange separation, acetylation of 3 or 4, or of mixtures containing respectively **10, 12,** and **13** and **15** was achieved according to the procedure of Unger *et al.* [33] This was followed by esterfication by treatment with iodomethan,e, in N,N-dimethylformamide for 3 h at room temperature.34 The derivatized sugars were purified by silica gel column chromatography (hexane-ethyl acetate).

Methyl (hexa-O-acetyl-3-deoxy-α-D-glycero-D-gulo-2-nonulopyra-nosid)onate (**9**).- ^{1}H NMR (C_6D_6) : δ 1.63, 1.68, 1.71, 1.77, 1.85, 1.98 (6 s, 6x3H, 6 Ac), 2.47 (m, 2H, H-3a, 3e), 3.33 (s, 3H, OMe), 4.13 (dd, 1H, H-9), 4.36 (dd, 1H, H-6), 4.42 (dd, 1H, H-9'), 5.22 (ddd, 1H, H-8), 5.46 (ddd, 1H, H-4), 5.90 (dd, 1H, H-5), 5.95 (dd, 1H, H-7).

Methyl (penta-O-acetyl-3-deoxy-α-D-gulo-2-octulopyranosid)onate (**11**).- ^{1}H NMR (C_6D_6) : δ 1.56, 1.60, 1.67, 1.70, 1.72 (5 s, 5x3H, 5 Ac), 2.50 (m, 2H, H-3a, 3e), 3.31 (s, 3H, OMe), 3.90 (dd, 1H, H-8), 4.22 (dd, 1H, H-6), 4.46 (dd, 1H, H-8), 5.42 (ddd, 1H, H-4), 5.65 (m, 2H, H-5, 7).

Methyl(penta-O-acetyl-3-deoxy-α-D-gluco-2-octulopyranosid(onate (**13**).- HPLC analysis (silica gel column, hexane-ethyl acetate, 2:1) of the derivatized osonic acids from D-arabinose indicated the presence of a 56:44 mixture of **13** and **15**. Ester **13** was partially separated by flash chromatography (silica gel, hexane-ethyl acetate, 1:1); ^{1}H NMR (C_6D_6) : δ 1.54, 1.61, 1.66, 1.68, 1.75 (5 s, 5x3H, 5 Ac), 2.27 (dd, 1H, H-3a), 2.62 (dd, 1H, H-3e), 3.33 (s, 3H, OMe), 4.34 (dd, 1H, H-8'), 4.63 (dd, 1H, H-6), 4.68 (dd, 1H, H-8), 5.10 (ddd, 1H, H-4), 5.30 (dd, 1H, H-5), 5.52 (ddd, 1H, H-7).

Methyl (penta-O-acetyl-3-deoxy-α-D-manno-2-octulopyranosid)onate (**15**).- This was partially separated from ester **13** by flash chromatography; m.p. 157-159° ( ) alone or mixed with a sample with the same melting point prepared according to the literature;[15,33,34] ^{1}H NMR (C_6D_6) : δ 1.52, 1.58, 1.64, 1.70, 1.76 (5 s, 5x3H, 5 Ac), 2.37 (m, 2H, H-3a, 3e), 3.34 (s, 3H, OMe), 4.06 (dd, 1H, H-6), 4.23 (dd, 1H, H-8'), 4.56 (dd, 1H, H-8), 5.41 (ddd, 1H, H-4), 5.51 (ddd, 1H, H-7), 5.64 (dd, 1H, H-5).

Methyl (hexa-O-acetyl-3-deoxy-α-D-glycero-D-galacto-2-nonulo-pyranosid)onate (**42**).- ^{1}H NMR(C_6D_6) : δ 1.47, 1.60, 1.70, 1.74, 1.80 (6 s, 6x3H, 6 Ac),2.01 (dd, 1H, H-3a), 2.66 (dd, 1H, H-3e), 3.40 (s, 3H, OMe), 4.35 (dd, 1H, H-9), 4.40 (dd, 10Hz, H-6), 4.85 (dd, 1H, H-9'), 5.35 (dd,1H,H-5), 5.52 (m, 2H, H-4, 8), 5.83 (dd, 1H, H-7).

Methyl(penta-O-acetyl-3-deoxy-α-D-galacto-octulopyranoside)onate (**43**).- ^{1}H NMR (C_6D_6) : δ 1.48, 1.58, 1.60, 1.76, 1.80 (5 s, 5x3 H, 5 Ac), 1.95 (dd, 1H, H-3a), 2.65 (dd, 1H, H-3e), 3.30 (s, 3H, OMe), 4.16 (dd, 1H, H-6), 4.37 (dd, 1H, H-8), 4.54 (dd, 1H, H-8'), 5.44 (dd, 1H, H-5), 5.51 (m, 1H, H-4), 5.67 (m, 1H, H-7).

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