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An Improved Chemical and Enzymatic Synthesis of New Fructose Derivatives for Import Studies by the Glucose Transporter in Parasites

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Abstract : This paper presents the chemoenzymatic synthesis of D-fructose analogues substitued at position C6. These compounds are the unique products of rabbit muscle aldolase catalyzed aldolisation of D-glyceraldehyde analogues (obtained by stereospecific chemical synthesis) with DHAP, followed by a dephosphorylation step with acid phosphatase.

Rabbit muscle fructose-1,6-diphosphate aldolase (EC 4.1.2.13) reversibly catalyzes the production of D-fructose-1,6-diphosphate (FDP) from D-glyceraldehyde-3-phosphate (G3P) and dihydroxy-acetone-phosphate (DHAP), the equilibrium being in favour of FDP.[1] Whereas the enzyme's selectivity is rather high towards the DHAP structure where only minor changes are possible,[2] more flexibility is accepted with regard to the G3P analogue. Owing to this feature and also the availability of the enzyme, a large number of syntheses leading to carbohydrates of D-*threo* (3*S*, 4*R*) configuration have been developed.[3] Among others, this reaction has been applied to the synthesis of aza-carbohydrates of pharmaceutical interests[4] and of D-fructose analogues substituted at position 6 : 6-deoxy-,[5-7] 6-deoxy-6-fluoro-,[5] 6-deoxy-6-azido-,[4d,8,9] 6-deoxy-6-methoxy-,[5] 6-deoxy-6-chloro-[10] and 6-deoxy-6-vinyl-D-fructoses.[10]

We considered that some of these compounds, particularly 6-deoxy-6-azido-D-fructose might be of interest for the study of the glucose transporter in the trypanosome.[11] It has indeed been shown that, whereas the glucose transporter in the human erythrocyte (Glut-1) only recognizes glucose, that of the trypanosome (THT1) has affinity not only for glucose but also for fructose in its furanose form.[12] It has also been shown that affinity for the transporter is ensured by the intra-cyclic oxygen atom and those at positions 3 and 4.[12]

Therefore, fructose analogues are of large potential interest for the study of this THT1 transporter either for blocking glucose import or for taking advantage of this transporter to internalize drugs. This strategy appears to be highly promising if one considers that the trypanosome uses glucose as its unique source of energy[13] : specific import of glycolytic enzyme inhibitors as fructose derivatives may open the route to new therapies against illnesses such as sleeping sickness and Chagas' disease. We thus

developed an aldolase catalysed synthesis of substituted fructoses, which represents any improvement with respect to the previously described methods. One of these fructoses has been assayed as a substrate for the enzyme glucose isomerase (EC 5.3.1.5).[5]

Results and discussion

Chemical synthesis of D-glyceraldehyde - diethylacetal analogues

The sequence described in Scheme 1 implied the synthesis from D-glyceraldehyde diethylacetal **1** of the key intermediate D-glycidaldehyde diethylacetal **2**.[14] The epoxide ring was then opened by various nucleophiles.[15] After hydrolysis of the acetal group, the corresponding aldehyde was combined with DHAP in an aldolase catalyzed reaction. We have optimised the synthesis of compound **2**. The Mitsunobu reaction was the most practical, giving a 79% yield of **2** (33% from fructose), as compared to 53% for a previously described synthesis.[14] Epoxide **2** has also been prepared in 95% yield from D-3-chloro-2-hydroxypropanal,[18] but the synthesis of the latter required three steps which reduces the overall yield to 21% from the starting material acrolein diethylacetal.

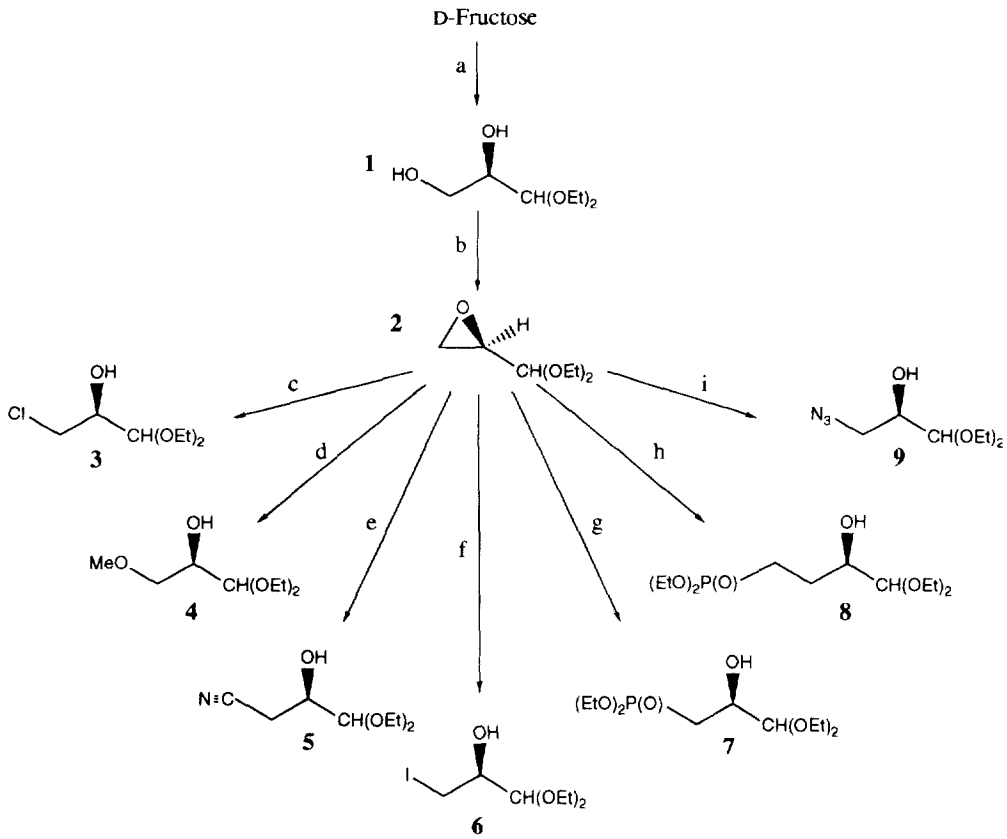
The ring opening reactions of the epoxide **2** allowed the introduction of substituents on the future 6 position of the corresponding fructose. This was effected with a wide variety of nucleophiles to explore the importance of these substitutions on the glucose transporter (compounds **4**, **5**, **7**, **8**, **10** and **11**), and with groups able to undergo a covalent binding with a nucleophile in the transporter (compound **9** which upon photo-activation gives the corresponding nitrene, and compounds **3**, **6**, **12** and **13**).

(S)-3-Chloro-2-hydroxy-propanal diethylacetal **3** and *(R)*-3-methoxy diethylacetal **4** were synthesized by reaction of **2** with dichloro-triphenyl phosphorane[18,19] or sodium methoxide.[18] The *(R)*-3-cyano **5** and *(S)*-3-iodo **6** derivatives were obtained quantitatively by reaction of **2** with diethyl-aluminium cyanide or sodium iodide according to the method of Ko *et al.*[20] Similarly, reaction of diethylphosphite or diethylmethylphosphonate[21,22] on epoxide **2** in the presence of boron trifluoride diethyl etherate yielded compounds **7** and **8**. In the reaction, one observed the formation of a by-product resulting from THF insertion; a similar reaction has already been described.[22]

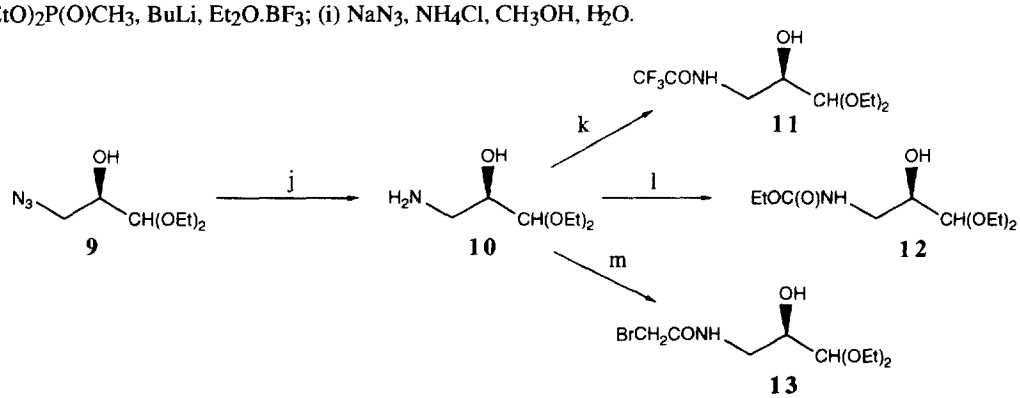
Compounds **10** to **13** were obtained from the azido compound **9**[9] (Scheme 2), by hydrogenation giving the compound **10** followed by acylation to give compounds **11**, **12** and **13**. The reaction of **10** with ethyl-bromoacetate did not go to completion. All compounds, except **10**, were easily purified by flash distillation or flash chromatography on silica gel. It was checked that the reaction of the highly basic nucleophile in the synthesis of **1**, **7** and **8** did not cause racemisation.

Enzymatic synthesis of D-fructose analogues with FDP aldolase.

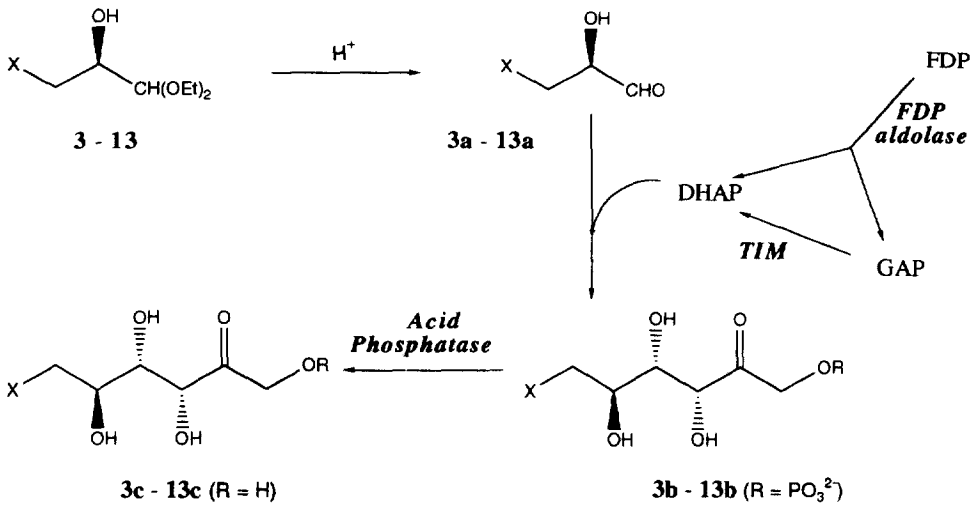
The general procedure, had previously been described[2] (Scheme 3). In the present work, enantiomerically pure aldehydes **3a** to **13a** have been used which simplifies purification procedures, since the resulting D-fructose is not contaminated by the corresponding L-sorbose obtained from the racemic aldehyde. The enantiomerically pure aldehydes were then combined with DHAP in the reaction catalysed by aldolase (1mmol scale). The product was dephosphorylated by acid phosphatase.



Scheme 1 (compounds **1** to **9**). Reagents: (a) 1/ $Pb(OAc)_4$, $(COOH)_2$, AcOH, H_2O 2/ $HC(OEt)_3$, NH_4NO_3, EtOH 3/ $CH_3O^-Na^+$, CH_3OH; (b) DEAD, TPP, C_6H_6; (c) Ph_3PCl_2, CH_2Cl_2 ; (d) $CH_3O^-Na^+$, CH_3OH; (e) Et_2AlCN, Toluene; (f) NaI, NaOAc, AcOH, $EtCO_2H$; (g) $(EtO)_2P(O)H$, BuLi, $Et_2O.BF_3$; (h) $(EtO)_2P(O)CH_3$, BuLi, $Et_2O.BF_3$; (i) NaN_3, NH_4Cl, CH_3OH, H_2O.



Scheme 2 (compounds **10** to **13**). Reagents: (j) H_2, Pd/C, EtOH; (k) CF_3CO_2Et; (l) EtOC(O)Cl, Pyridine, CH_2Cl_2; (m) $BrCH_2CO_2H$, iBuOC(O)Cl, NMM, THF.



Scheme 3. FDP aldolase catalyzed syntheses of fructose analogues.

Deprotection of compounds **3** to **13** was performed using an aqueous hydrochloric acid solution[8] at 40°C. A two fold excess of the resulting aldehydes **3a** to **13a** were directly added to DHAP (formed *in situ* from FDP using aldolase and triose-phosphate isomerase).[2,7] The progress of the aldolase catalyzed condensation was followed by the consumption of DHAP as described by Bednarski *et al.*[2] Relative rates of the different reactions are given in Table 1. Acceptable rates compared to the G3P reference were observed.

Table 1. Relative reactivities of aldehydes ($XCH_2CH(OH)CHO$) for the D stereoisomer with DHAP in rabbit muscle aldolase-catalysed aldol condensations.

X	R_{rel} [a]	substrate class [b]
$O\text{-}PO_3^{2-}$	100	+++
$NHC(O)CH_2Br$	12	++
CN	17	++
NHC(O)OEt	19	++
OCH_3	21	++
NH_2	24	++
$CH_2P(O)(OEt)_2$	25	+++
N_3	36	+++
$NHC(O)CF_3$	38	+++
I	44	+++
Cl	51	+++

[a] Reactivities were measured in 0.1 M triethanolamine buffer (pH 7.0, 25°C) containing both substrates at 50mM concentration, unless otherwise indicated. [b] Substrate class according to the scale previously used by Bednarski et al.[2] (+++) Rrel > 25; (++) 25 > Rrel > 10; (+) 10 > Rrel > 1

The resulting ketose-phosphates **3b** to **13b** were isolated as barium salts, and then hydrolyzed by acid phosphatase[5] to yield the corresponding fructoses **3c** to **13c** (yields 20 to 93 % from DHAP, Table 2). ^{13}C NMR analysis indicates that in each case a single compound was formed. In no case did we observe the formation of the L-sorbose derivative which might have resulted from the racemisation of the aldehyde in the deprotection step. ^{13}C NMR allowed to determine the percentages of the α/β furanose forms. Also, in one case, it showed the presence of a β-pyranose isomer (Table 2). These assignements were based on the chemical shift of the anomeric carbon compared to that of D-fructose (in the range of 110 ppm) and on the fact that, in the furanose form, the hydroxymethyl group at C_2 and the hydroxyl at C_3 are *trans* to each other in the predominent isomer (β form).[23]

Table 2. Proportions of α- and β-furanose forms at equilibrium for 6-deoxy-D-fructose ($XCH_2CH(OH)CH(OH)CH(OH)C(O)CH_2OH$).

Compounds	X	Furanose form (%)		% Yield
		α	β	
Fructose [d]	OH	5	18	-
3c [a]	Cl	17.6	82.4	93
4c [a]	OCH_3	18.9	81.1	93
5c [a]	CN	17.3	82.7	82.7
6c [a]	I	17.3	82.7	86
7c [a,c]	$(EtO)_2P(O)$	4	96	19
8c [a,c]	$(EtO)_2P(O)CH_2$	17.2	82.8	33.5
9c [a]	N_3	17	83	69.1
10c [a,e]	NH_2	<0.05	34	29.6
11c [a,b]	$CF_3C(O)NH$	22	78	66
12c [a]	EtOC(O)NH	19	81	69
13c [a]	$BrCH_2C(O)NH$	16	84	64

Proportions were determined from the intensity of a characteristic shift for C_2-ketose in the ^{13}C NMR spectrum[(a)] or intensity in the ^{31}P[(b)] or ^{19}F[(c)] NMR. [(d)] Pyranose forms α: <0.05%; β: 77%. [(e)] Pyranose forms α: <0.05%; β: 66%.

All compounds exist largely as β-furanoses except for compound **10c** which predominently exists as β-pyranose.

The D-fructose structure was confirmed, in the case of **6c**, by reaction with glucose isomerase (followed by ^{13}C NMR and TLC), an enzyme which only recognizes saccharides of the D-configuration. This treatment resulted in a mixture of **6c** (48%) and 6-deoxy-6-iodo-α,β-D-glucose (52%) as shown from the intensities of characteristic shifts for C_2 ketose and C_1 aldose (Figure 1).

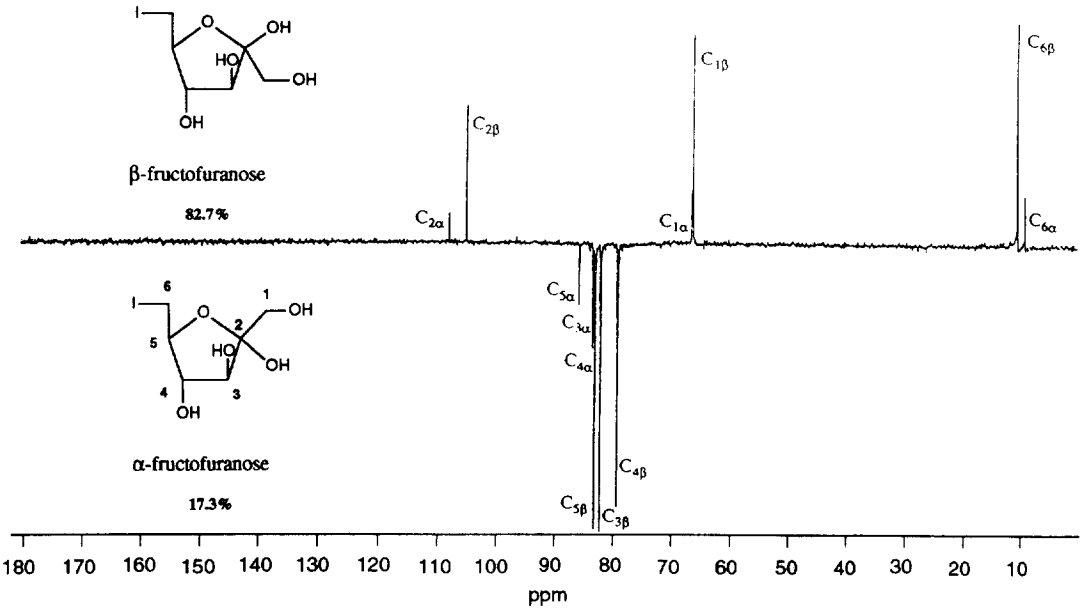


Figure 1a. ^{13}C(DEPT) nuclear magnetic resonance spectrum of 6-deoxy-6-iodo-α,β-D-fructose **6c**. Proportions of α- and β-furanose forms at equilibrium were determined from the intensity of signals at 107 and 104 ppm, characteristic shifts for α and β C_2-ketose respectively.

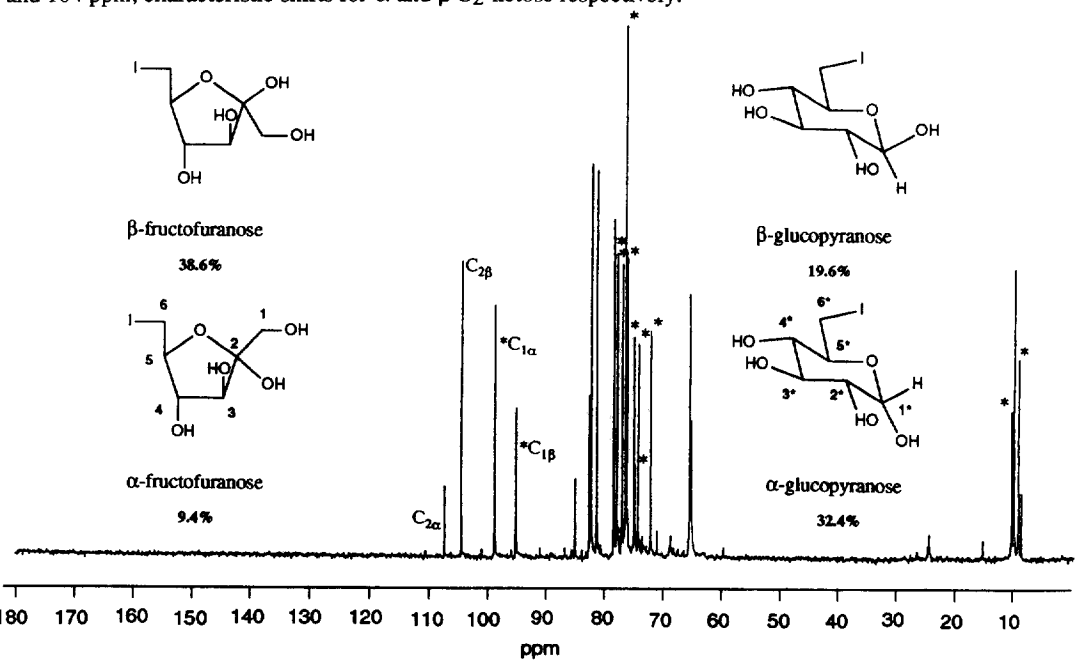


Figure 1b. ^{13}C nuclear magnetic resonance spectrum recorded after addition of glucose isomerase[5] to a solution of 6-deoxy-6-iodo-α,β-D-fructose **6c**. (*) Peaks correspond to α- and β-pyranose forms of 6-deoxy-6-iodo-D-glucose at equilibrium (*$C_{1\alpha}$: 98.5 and *$C_{1\beta}$: 94.9 ppm).

This work illustrates the fact that syntheses are better performed with chiral synthons and not with racemic mixtures : this allows the simplification of the purification procedures and also in some cases makes the synthesis feasible; for instance compound **11c** could not be obtained in previous works[9] from the racemic aldehyde.

The chiral aldehydes are stable enough after deprotection to allow such syntheses and they did not undergo racemisation at the chiral center; such stability had also been observed by other authors.[24] In addition, the ring opening reactions by different nucleophiles, including strong bases of the key intermediate *(R)*-glycidaldehyde diethyl acetal **2** proceeded with good yields and without racemisation. Formation of the latter compound **2** by the Mitsunobu reaction introduced significant improvement to the synthesis of these chiral protected aldehydes. Lastly, the synthesis of compound **10c** from the amino-aldehyde **10a** was a interesting and surprising result, since it could be expected that this compound would polymerize as soon as deprotected.

Preliminary studies on the trypanosomal glucose transporter THT1 indicated that in no case one observed an irreversible binding to the transporter. Also most compounds were recognized, as evidenced by competition experiments with labelled D-fructose.

Experimental section:

Materials and Methods

Fructose-1,6-diphosphate aldolase from rabbit muscle (EC 4.1.2.13), L-glycerol-3-phosphate dehydrogenase (EC 1.1.1.8), triosephosphate isomerase (EC 5.3.1.1), acid phosphatase (EC 3.1.3.2), fructose-1,6-diphosphate trisodium salt (>98%) and NADH were obtained from Boehringer-Mannheim. Maxazyme glucose isomerase liquid (EC 5.3.1.5) was purchased from Gist-Brocades (Delft, The Netherlands). Dihydroxyacetone phosphate lithium salt and Dowex 50WX8(H^+) were supplied by Sigma Chemical Co. D-Fructose (>99% pure) and all other reagents were purchased from Aldrich. The solvents were reagent grade materials. Enzyme activities and concentrations were measured spectrophotometrically.

Assay Method

Enzymatic activities of aldolase (9 Units.mg^{-1} at 25°C) were measured according to standard methods[25] TIM/GDH with a 0.1M solution of TEA.HCl buffer (1mM EDTA, pH 7.6, ionic strength 0.15) using FDP as substrate (1mM). The initial rate (Vi) was calculated by following the conversion NADH to NAD (ε = 6.22 $mM^{-1}.cm^{-1}$) at 340nm with a Perkin Elmer Lambda 15 spectrophotometer.

Chemical Syntheses

The NMR spectra were recorded in $CDCl_3$ or D_2O on either a Bruker AC80, Bruker AC200 or Bruker ARX400-MHz spectrometer. All chemical shifts are reported in parts per million with respect to TMS for ^1H and ^{13}C spectra, H_3PO_4 for ^{31}P and trifluoroacetic acid for ^{19}F as internal standard. IR spectra were recorded

on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter using a 2 cm^3 capacity (1-dm path length) quartz cell. Elemental analyses were performed by the Ecole Nationale Supérieure de Chimie de Toulouse. Flash chromatography were performed on Merck Geduran SI 60 (0.040-0.063mm).

Enzymatic Syntheses

General procedure

- Deprotection of 2-Hydroxypropanal diethylacetal.

a - To 10ml of H_2O, containing 150µl of concentrated HCl 35% was added either 2-hydroxypropanal diethylacetal (1-2mmol) **3**, **4**, **5**, **6**, **9**, or **10**. The solution was warmed to 45°C and monitored by TLC (CH_2Cl_2/MeOH).
b - 2-Hydroxypropanal diethylacetal (1-2mmol) **7**, **8**, **11**, **12**, or **13** was dissolved in demineralized water (10ml), 2 ml of Dowex 50WX8(H^+) was added and the solution was warmed to 45°C and monitored by TLC.

- Enzymatic Aldol Condensation.

After completion of hydrolysis, FDP-Na_3 (0.25 eq) was added directly to the reaction mixture, and the pH was adjusted to 6.5 with NaOH. The solution was degassed with argon, and aldolase (300 Units) and triosephosphate isomerase (500 Units) were added. The reaction mixture was monitored by following the consumption of DHAP as previously described.[2] After 24h, the reaction was stopped by addition of $BaCl_2.2H_2O$ (1.3 eq), the pH was adjusted to 7.8 with NaOH and 150ml of acetone were added. The precipitate was isolated by centrifugation (9000 rpm; 0°C; 30min) and washed with acetone (2x20ml). The white precipitate, was suspended in 25ml of water, and HCl added to a pH of 1. A solution of Na_2SO_4 (1.3 eq) was then added[9], and the pH adjusted to 6.5 with NaOH. The suspension was filtered through Celite 545 to remove barium sulfate. The pH of the filtrate was then adjusted to 4.9 with acetic acid, and acid phosphatase (100 Units) was added. The reaction was monitored by TLC (ethyl acetate-methanol-H_2O; 12:6:2). The reaction was complete after 12h. The solution was neutralised and then freeze-dried, and the residue triturated with ethanol and filtered. After removal of solvent, the resultant oil was dissolved in a minimum of water and acetone was added ($5xV_{H_2O}$) and the precipitate of barium acetate removed by centrifugation (8000 rpm; 0°C; 20min); this operation was carried out twice. After removal of solvent, a pure oil was obtained.

Reactivity of Aldehydes as Substrates

Enzymatic assays and kinetic measurements were carried out by the procedure described previously.[2] To 1ml of TEA.HCl buffer (0.1M; pH 7.0) containing DHAP (50mM) and deprotected aldehydes **3a** to **13a** (50mM) was added 20µl of a solution containing aldolase (~500.U/ml in TEA buffer). At time intervals of 1 min over the course of 5-15 min, 0.1 ml of the assay solution was withdrawn, quenched with 30µl of 7% perchloric

acid solution, neutralized with 20µl of 1 N NaOH, and diluted with 0.5 ml of 0.1 M pH 7 TEA buffer. An aliquot (50µl) of this solution was added to a cuvette containing 925µl of 0.1 M TEA buffer (pH 7.0) and 50µl of a MIX GDH solution (preparation : 100µl of GDH (10mg/ml; 170U/mg) was centrifuged and the supernatant eliminated. The residue was dissolved in 100µl of TEA buffer and 20µl of this solution was added to 1ml of MIX 0.1 M TEA buffer containing 20mM EDTA, 8.4mM NADH and 71.4mM $NaHCO_3$). The oxidation of NADH to NAD was monitored at 340nm. The rate of reaction for aldehydes **3a** to **13a** was calculated by plotting time versus consumption of DHAP. The relative rate (R_{rel}) is defined as the ratio R_{sub}/R_{G3P}.

Determination of the configuration at carbon 5

The reaction of 6-deoxy-D-fructose with glucose isomerase was carried out by the procedure described previously.[5] The reaction (followed by ^{13}C NMR) led to a new compound characterized by the chemical shifts corresponding to the $C_{1\alpha}$ and $C_{1\beta}$ atoms of aldoses. Figure 1b shows the ^{13}C NMR spectrum recorded after addition of glucose isomerase to a solution of compound **6c**. Proportions of α- and β-glucopyranose at equilibrium were 62% and 38% respectively and as shown from the intensity of a characteristic shift for C_1-aldose ($C_{1\alpha}$: 98.5 and $C_{1\beta}$: 94.9ppm).

D-Glyceraldehyde diethyl acetal (1). This product was generated from D-fructose(20g, 111mmol) according the procedure described previously.[14] The compound **1** was purified to yield an oil (7.7g, 47 mmol, 42%). $[\alpha]_D^{25}$ = +31.8° (c = 1.2, EtOH); lit[14] $[\alpha]_D^{28}$ = +30.3° (c = 1.04, EtOH). The ^1H NMR spectrum was consistent with that reported in the literature.[14] ^{13}C NMR (50MHz, $CDCl_3$) δ 15.4(s,CH_3), 62.0(s,CH_2O), 64.4(s,CH_2O acetal), 65.3(s,CH_2O acetal),71.6(s,CH-O),103.4(s,O-C-O). Anal.Calcd for $C_7H_{16}O_4$: C,51.21; H,9.82; O,39.02. Found : C,51.12; H,10.07; O,39.24..

D-Glycidaldehyde diethyl acetal (2). The compound was obtained *via* a Mitsunobu reaction.[16,17] Diethyl azodicarboxylate (DEAD, 2g, 11.5 mmol) was added dropwise to a stirred solution of **1** (1.5g, 9.15 mmol) and TPP (2.59g, 9.85 mmol) in dry benzene (40ml). An exothermic reaction was observed. After the mixture had cooled to room temperature, the benzene was removed under reduced pressure, and the remaining residue was distilled at 82°C (15 mmHg) to give 1.05g (7.2 mmol) of pure **2** (79%). $[\alpha]_D^{25}$ = +8.2° (c = 1.25, EtOH); lit[14] $[\alpha]_D^{28}$ = +7.2° (c = 1, EtOH); lit[18] $[\alpha]_D^{25}$ = +5.3° (c = 1.06, EtOH). ^1H and ^{13}C NMR spectra were consistent with those reported in the literature.[13,17] Anal.Calcd for $C_7H_{14}O_3$: C,57.52; H,9.65; O,32.87. Found : C,57.1; H,9.82; O,33.24.

(2S) 3-Chloro-2-hydroxypropanal diethyl acetal (3). To a 100-ml round-bottomed flask containing 10ml of dry CH_2Cl_2 was added 0.84g (2.52 mmol) of Ph_3PCl_2.[18,19] The solution was cooled to 0°C under N_2. The acetal **2** (0.35g, 2.4 mmol) in 5ml of CH_2Cl_2 was added dropwise, and the mixture was stirred overnight. The resulting solution was added to 10ml of ice/water containing 0.4g (4.7mmol) of $NaHCO_3$, and the mixture was stirred for 90min. The aqueous layer was separated, saturated with NaCl, and extracted with 2x40ml of CH_2Cl_2. The organic fractions were combined, dried over anhydrous Na_2SO_4, and evaporated under reduced

pressure. The remaining residue was purified by flash chromatography (CH_2Cl_2/MeOH, 98:2; silica gel) to yield **3** as a colourless oil (0.21g, 1.15 mmol, 48%). $[\alpha]_D^{25}$ = +25.2° (c = 1.2, $CHCl_3$); lit[18] $[\alpha]_D^{25}$ = +23.6° (c =1.06, $CHCl_3$) ^1H NMR (80MHz, $CDCl_3$) δ 1.20(t,3H,CH_3), 1.22(t,3H,CH_3), 2.55(d,1H,OH,^3J = 4.1Hz), 3.5-3.85(m,7H,CH-O,CH_2O,CH_2Cl), 4.51(d,$^3J_{HH}$ = 5.35Hz,1H,O-CH-O). ^{13}C NMR (50MHz, $CDCl_3$) δ 15.4(s,CH_3), 45.6(s,CH_3Cl), 63.8(s,CH_2O acetal), 64.3(s,CH_2O acetal), 71.6(s,CH-O), 102.5(s,O-C-O). Anal.Calcd for $C_7H_{15}O_3Cl$: C,46.03; H,8.22; O,26.30. Found : C,45.59; H,8.53; O,26.87.

(2R) 3-Methoxy-2-hydroxypropanal diethyl acetal (4). This compound was obtained from **2** (0.35g, 2.4 mmol) according the procedure previously described[14] to yield **4** as a yellow oil (0.32g, 1.8 mmol, 75%). $[\alpha]_D^{25}$ = +32.9° (c = 1.2, EtOH); lit[14] $[\alpha]_D^{25}$ = +31.6° (c = 1, EtOH). ^1H and ^{13}C NMR spectra were consistent with those reported in the literature.[2] Anal.Calcd for $C_8H_{18}O_4$: C,53.92; H,10.18; O,35.95. Found : C,54.29; H,10.47; O,36.41.

(2R) 3-Cyano-2-hydroxypropanal diethyl acetal (5). To a solution of **2** (0.6g, 4.11 mmol) in toluene (5ml) was added Et_2AlCN[20] (1M solution in toluene, 4.6 ml, 4.6 mmol) at room temperature. After 4h, the homogeneous yellow solution was diluted with ether (250ml) and then washed with 10% H_2SO_4, saturated $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and concentrated. The oil was purified by flash chromatography (silica gel, CH_2Cl_2/MeOH, 98:2) to yield **5** as a colourless oil (0.6g, 3.47 mmol, 84%). ^1H NMR(80MHz, $CDCl_3$) δ 1.22(t,3H,CH_3), 1.23(t,3H,CH_3), 2.5-2.8(m,3H,OH, CH_2CN), 3.5-3.9(m,4H,CH_2O), 4.1-4.25(m,1H,CH-O), 4.43(d, $^3J_{HH}$ = 5.59Hz, 1H, O-CH-O). ^{13}C NMR (50MHz, $CDCl_3$) δ 15.3(s,CH_3), 20.9(s,CH_2), 64.3(s,CH_2O acetal), 64.6(s,CH_2O acetal), 66.2(s,CH-O), 103.3(s,O-CH-O), 117.6(s,CN). Anal.Calcd for $C_8H_{15}O_3N$: C,55.49; H,8.67; O,27.74. Found : C,56.02; H,8.92; O,27.98.

(2S) 3-Iodo-2-hydroxypropanal diethyl acetal (6). A mixture of NaI (0.84g, 5.58 mmol), sodium acetate (0.09g, 1.01 mmol) in acetic acid (2.5ml), and propionic acid[21] (5ml) was cooled to -30°C and epoxide **2** (0.49g, 3.36 mmol) was added. The mixture was stirred at -30°C to -20°C for 2h before being warmed to room temperature. Dilution with ether (100ml) was followed by washing with saturated $NaHCO_3$, 5% $NaHSO_3$ and brine. The organic phase was dried (Na_2SO_4) and concentrated. The oil was purified by flash chromatography (silica gel; CH_2Cl_2/MeOH, 96:4) to yield **6** as a colourless oil (0.7g, 2.55 mmol, 76%). $[\alpha]_D^{25}$ = +21.69° (c = 1.2, EtOH). ^1H NMR(80MHz, $CDCl_3$) δ 1.20(t,3H,CH_3), 1.21(t,3H,CH_3), 2.62(m,1H,OH), 3.2-3.85(m,7H,CH-O,CH_2O,CH_2I), 4.41(d,$^3J_{HH}$ = 5.41Hz,1H,O-CH-O). ^{13}C NMR (50MHz, $CDCl_3$) δ 8.7(s,CH_3I), 15.4(s,CH_3), 63.9(s,CH_2O acetal), 64.3(s,CH_2O acetal), 71.1(s,CH-O), 104.4(s,O-CH-O). Anal.Calcd for $C_7H_{15}O_3I$: C,30.65; H,5.47; O,17.52. Found : C,31.22; H,5.84; O,18.01.

(2R) 3-Diethoxyphosphono-2-hydroxypropanal diethyl acetal (7). A 1.6 M solution of *n*-BuLi in hexane (3.84 ml, 6.2 mmol) was added dropwise to a stirred solution of diethylphosphite[20,22] (0.849g, 6.13 mmol) in dry THF (30ml) at -80°C under a nitrogen atmosphere. After 30min of stirring, this mixture was added dropwise to a stirred solution of the epoxide **2** (0.314g, 2.15 mmol) in dry THF (10ml) at -80°C. After 15 min of stirring, $BF_3.OEt_2$ (0.8ml, 6.2 mmol) was slowly introduced, while maintaining the temperature below -80°C. The solution was stirred for 2.5h and quenched with saturated aqueous NH_4Cl. Returning to room

temperature, the solvents were removed under reduced pressure and the residue was dissolved in ethyl acetate (100ml). The organic layer was washed with brine, then dried, concentrated and purified by flash chromatography (CH_2Cl_2/MeOH; 96:4) to yield **7** as a colourless oil (0.23g, 0.81 mmol, 37.6%). $[\alpha]_D^{25}$ = +19.42° (c = 1.55, EtOH). ^{31}P NMR (81 MHz, $CDCl_3$) δ 30.84. ^1H NMR (200MHz, $CDCl_3$) δ 1.18(t,6H,CH_3 acetal), 1.31(t,6H,CH_3 ester), 1.9-2.1(m,2H,CH_2-P), 3.15(s,1H,OH), 3.5-3.8(m,5H,CH-O, OCH_2 acetal), 4.0-4.15(m,4H,CH_2O ester), 4.37(d,1H,CH acetal). ^{13}C NMR (50MHz, $CDCl_3$) δ 15.3(s,CH_3 acetal),16.4(s,CH_3 ester), 28.0(d,CH_2P, $^1J_{CP}$ = 141Hz), 61.8(s,CH_2O,ester), 63.5(s,CH_2O,acetal), 64.0(s,CH_2O,acetal), 67.7(s,CH-O,), 104.1(d,$^3J_{CP}$ = 17.2Hz, CH acetal). IR(film) ν(P=O)cm^{-1} : 1225, ν(P-O)cm^{-1} : 1059, ν(OH)cm^{-1} : 3358. Anal.Calcd. for $C_{11}H_{25}O_6P$: C,46.5; H,8.80; O,33.8. Found : C,46.62; H,8.65; O,33.4.

(2R) 4-Diethoxyphosphono-2-hydroxybutanal diethyl acetal (8) was prepared from **2** (0.5g, 3.33 mmol) in 78% yield (**8**, 0.77g, 2.6 mmol) by following the same procedure described for **7**, using diethylmethylphosphonate (1.55g, 10 mmol) in place of diethyl phosphite. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to yield a colourless oil **8**. $[\alpha]_D^{25}$ = +20.62° (c = 1.1, EtOH). ^{31}P NMR (81 MHz, $CDCl_3$) δ 32.9. ^1H NMR (200MHz, $CDCl_3$) δ 1.15(t,3H,CH_3 acetal), 1.17(t,3H,CH_3 acetal), 1.27(t,6H,CH_3 ester), 1.4-2.1(m,4H,CH_2CH_2-P), 2.5(m,1H,OH), 3.3-3.8(m,5H,CH-O, OCH_2 acetal), 4.0(m,4H,CH_2O ester), 4.21(d,1H,CH acetal). ^{13}C NMR (50MHz, $CDCl_3$) δ 15.4(s,CH_3 acetal),16.5(s,CH_3 ester), 21.8(d,CH_2P, $^1J_{CP}$ = 142Hz), 24.9(d,CH_2), 61.6(s,CH_2O,ester), 63.6(s,CH_2O,acetal), 71.5(d,CH-O,$^3J_{CP}$ = 15.2Hz), 104.8(s,CH, acetal). IR(film) ν(P=O)cm^{-1} : 1242, ν(OH)cm^{-1} : 3412. Anal.Calcd. for $C_{12}H_{27}O_6P$: C,48.3; H,9.0; O,32.2. Found : C,47.85; H,9.02; O,32.4.

(2R) 3-Azido-2-hydroxypropanal diethyl acetal (9). To a 100-ml round-bottomed flask containing **2** (0.75g, 5.14 mmol) in 40ml of CH_3OH and 5ml of demineralized water, were added 3.34g (51.4 mmol) of sodium azide[9] and NH_4Cl (0.61g, 11.31 mmol). The mixture was refluxed with stirring and the solvent removed under reduced pressure. The residue was dissolved in EtOH and the precipitate was eliminated. After removal of solvent, the oil was purified by flash chromatography (silica gel, CH_2Cl_2/MeOH, 98:2) to yield **9** (0.87g, 4.61 mmol, 89.7%). $[\alpha]_D^{25}$ = +32.2° (c = 0.98, EtOH); lit[8] $[\alpha]_D^{25}$ = +45.5° (c = 1.5, chloroform). IR(film) ν(N=N)cm^{-1} : 2102, ν(OH)cm^{-1} : 3446. ^1H and ^{13}C NMR spectra were consistent with those reported in the literature.[8,9] Anal.Calcd for $C_7H_{15}O_3N_3$: C,44.44; H,7.93; O,25.4. Found : C,44.9; H,8.25; O,26.2.

(2R) 3-Amino-2-hydroxypropanal diethyl acetal (10). To a suspension of Pd/C (10%, 0.29g, 0.2 mmol) in 30ml of ethanol, was added azide **9** (0.65g, 3.44mmol). The mixture was degassed and hydrogenated for 12h. The catalyst was filtered off and the ethanol was removed under reduced pressure to yield **10** as a colourless oil (0.55g, 3.37 mmol, 98%). $[\alpha]_D^{25}$ = +30.4° (c = 1.5, EtOH). ^1H and ^{13}C NMR spectra were consistent with those reported in the literature.[8,9] Anal.Calcd for $C_7H_{17}O_3N$: C,51.53; H,10.43; O,29.45. Found: C,50.98; H,10.78; O,29.62.

(2R) 3-Trifluoroacetamido-2-hydroxypropanal diethyl acetal (11). 4.4g (31 mmol) of ethyl trifluoroacetate[9] was added dropwise to amine **10** (0.4g, 2.43 mmol) at -30°C. After 12h of stirring, the

solvent was removed under reduced pressure, and the residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to yield **11** as a colourless oil (0.39g, 1.5 mmol, 62%). $[\alpha]_D^{25}$ = +24.22° (c = 1.2, EtOH). ^{19}F NMR (376MHz, $CDCl_3$) δ 0.77. ^1H and ^{13}C NMR spectrum were consistent with that reported in the literature.[9] Anal.Calcd for $C_9H_{16}O_4F_3N$: C,41.70; H,6.18; N,5.40. Found : C,42.04; H,6.47; N,5.62.

(2R) 3-Ethoxycarbonylamido-2-hydroxypropanal diethyl acetal (12). To a mixture of amine **10** (0.13g, 0.797 mmol) and pyridine (130µl) in dry CH_2Cl_2 (20ml) under a nitrogen atmosphere, was added ethyl choloroformate (85µl, 0.89mmol) at -30°C. The mixture was stirred for 30min at -20°C then allowed to warm to room temperature. 150ml of CH_2Cl_2 was added. The organic layer, after washing with brine, was dried, concentrated and purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to yield **12** as a colourless oil (0.17g, 0.762 mmol, 95.6%). $[\alpha]_D^{25}$ = +19.46° (c = 1.5, CH_2Cl_2). ^1H NMR (200MHz, $CDCl_3$) δ 1.17(t,3H,CH_3), 1.19(t,6H,CH_3 acetal), 2.8(m,1H,OH), 3.35(m,2H,CH_2N), 3.4-3.8(m,5H,CH-O, OCH_2 acetal), 4.06(q,2H,$CH_2OC(O)$,^3J = 7.14Hz), 4.32(d,1H,CH acetal,3J = 5.64Hz), 5.2(m,1H,NH). ^{13}C NMR (50MHz, $CDCl_3$) δ 14.64(s,CH_3), 15.37(s,CH_3 acetal), 42.37(s,CH_2N), 60.91(s,$CH_2OC(O)$), 63.6(s,CH_2O,acetal), 63.9(s,CH_2O,acetal), 71.0(s,CH-O,), 103.29(s, CH acetal), 157.2(s,C=O). IR(film) ν(C=O)cm^{-1} : 1699, ν(NH)cm^{-1} : 3445, ν(OH)cm^{-1} : 3566. Anal.Calcd. for $C_9H_{21}O_5N$: C,48.43; H,9.41; O,35.87. Found : C,48.72; H,9.68; O,35.98.

(2R) 3-Bromoacetamido-2-hydroxypropanal diethyl acetal (13). To a mixture of bromoacetic acid (0.094g, 0.676 mmol) and N-methylmorpholine (101µl, 0.92 mmol) in dry THF (15ml) under a nitrogen atmosphere, was added isobutyl chloroformate (96µl, 0.742 mmol) at -40°C. The mixture was stirred for 15min at -40°C, and 110mg (0.675mmol) of **10** in 10ml of dry THF was added dropwise. The solution was stirred for 15 min at -40°C and then at room temperature for 2h. The solvent was removed under reduced pressure and the residue dissolved in ethyl acetate (200ml). The organic layer, after washing with brine, was dried, concentrated and purified by flash chromatography (CH_2Cl_2/MeOH, 96:4) to yield **13** as a colourless oil (0.15g, 0.528mmol, 78%). $[\alpha]_D^{25}$ = +19.53° (c = 1.5, EtOH). ^1H NMR (200MHz, $CDCl_3$) δ 1.21(t,6H,CH_3 acetal), 2.86(d,1H,OH,^3J = 4.35Hz), 3.35-3.8(m,7H,CH_2N,CH-O,CH_2O), 3.86(s,2H,$BrCH_2$), 4.35(d,1H,CH acetal,3J = 5.37Hz), 7.1(m,1H,NH). ^{13}C NMR (50MHz, $CDCl_3$) δ 15.42(t,CH_3 acetal), 29.17(s,$BrCH_2$), 41.48(s,CH_2N), 63.67(s,CH_2O,acetal), 64.4(s,CH_2O,acetal), 70.3(s,CH-O,), 103.64(s, CH acetal), 166.1(s,C=O). IR(film) ν(C=O)cm^{-1} : 1662. Anal.Calcd. for $C_9H_{18}O_4NBr$: C,38.03; H,6.34; O,22.53. Found : C,38.42; H,6.46; O,22.40.

6-Deoxy-6-Chloro-α,β-D-fructose (3c). This compound was prepared from **3** (0.44g, 2.4 mmol) by following the general procedure (0.222g, 1.12 mmol, 93%). $[\alpha]_D^{26}$ = +8.75° (c = 15.8 mg/ml, EtOH), lit[10] $[\alpha]_D^{23}$ = +1.32 (c = 2.28, MeOH). ^1H NMR (200MHz,D_2O) δ 3.55(d,2H,CH_2Cl, ^3J = 2.8Hz), 3.6-3.8(m,2H,OCH_2), 3.9-4.0(m,1H,CH-O), 4.0-4.2(m,2H,CH-O). ^{13}C NMR (50MHz,D_2O). **3c(α)**: δ 45.25(s,C6), 65.36(s,C1), 79.87(s,C4), 83.07(s,C3), 84.45(s,C5), 107.30(s,C2). **3c(β)**: δ 47.70(s,C6), 65.36(s,C1), 77.87(s,C4), 78.40(s,C3), 82.32(s,C5), 104.38(s,C2). Anal.Calcd. for $C_6H_{11}O_5Cl$: C,36.27; H,5.54; O,40.3. Found : C,35.96; H,5.72; O,40.56.

6-Deoxy-6-Methoxy-α,β-D-fructose (4c). This compound was prepared from **4** (0.26g, 1.46 mmol) by following the general procedure (0.135g, 0.695 mmol, 93%). $[\alpha]_D^{25}$ = -9.2° (c = 1.5, H_2O); lit[14] $[\alpha]_D^{23}$ = -7.9° (c = 1.59, H_2O). ^1H NMR (200MHz,D_2O) δ 3.33(s,3H,CH_3O), 3.45-3.7(d,d,4H,OCH_2), 3.85(m,1H,CH-O), 4.0(m,1H,CH-O), 4.15(m,1H,CH-O). ^{13}C NMR (50MHz,D_2O) **4c(α)**: δ 61.18(s,CH_3O), 65.37(s,C1), 74.67(s,C6), 79.23(s,C4), 82.27(s,C3), 84.35(s,C5), 107.28(s,C2). **4c(β)**: δ 61.18(s,CH_3O), 65.15(s,C1), 76.1(s,C6), 77.38(s,C4), 77.7(s,C3), 81.41(s,C5), 104.27(s,C2). Anal.Calcd. for $C_7H_{14}O_6$: C,43.29; H,7.21; O,49.48. Found : C,42.97; H,7.43; O,49.72.

6-Deoxy-6-Cyano-α,β-D-fructose (5c). This compound was prepared from **5** (0.33g, 1.9 mmol) by following the general procedure (0.15g, 0.794 mmol, 82.7%). $[\alpha]_D^{26}$ = +18.47° (c = 1.36, EtOH). ^1H NMR (200MHz,D_2O) δ 3.04(m,2H,CH_2CN), 3.6-3.8(m,2H,OCH_2), 4.0-4.45(m,3H,CH-O). ^{13}C NMR(50MHz,D_2O) **5c(α)**: δ 23.67(s,C6), 65.40(s,C1), 78.25(s,C4), 81.44(s,C3), 84.40(s,C5), 107.44(s,C2), 121.94(CN). **5c(β)**: δ 24.97(s,C6), 65.16(s,C1), 77.46(s,C4), 77.95(s,C3), 79.92(s,C5), 104.59(s,C2), 121.04(CN). Anal.Calcd. for $C_7H_{11}O_5N$: C,44.44; H,5.82; O,42.32. Found : C,44.21; H,5.87; O,42.71.

6-Deoxy-6-Iodo-α,β-D-fructose (6c). This compound was prepared from **6** (0.67g, 2.4 mmol) by following the general procedure (0.3g, 1.03 mmol, 86%). $[\alpha]_D^{26}$ = +20.12° (c = 1.2, EtOH). ^1H NMR (200MHz,D_2O) δ 3.4-3.6(m,2H,OCH_2), 3.67(d,2H,CH_2I, ^3J = 5.33Hz), 3.85-3.95(m,1H,CH-O), 4.1-4.3(m,2H,CH-O). ^{13}C NMR (50MHz,D_2O) **6c(α)**: δ 8.76(s,C6), 65.62(s,C1), 81.15(s,C4), 82.56(s,C3), 84.80(s,C5), 107.13(s,C2). **6c(β)**: δ 10.05(s,C6), 65.39(s,C1), 78.18(s,C4), 81.12(s,C3), 82.19(s,C5), 104.22(s,C2). Anal.Calcd. for $C_6H_{11}O_5I$: C,24.83; H,3.79; O,27.58. Found : C,24.12; H,3.87; O,27.89.

6-Deoxy-6-Diethoxyphosphono-α,β-D-fructose (7c). This compound was prepared from **7** (0.15g, 0.524 mmol) by following the general procedure (0.032g, 0.107 mmol, 20.3%). $[\alpha]_D^{26}$ = +13.33° (c = 0.765, MeOH). IR (film): ν(P=O)cm^{-1} : 1215; ν(P-O)cm^{-1} : 1031. ^{31}P NMR (81MHz,D_2O) δ(β) = 31.7 (96%), δ(α) = 31.51 (4%). ^1H NMR (400MHz,D_2O) δ 1.27(t,6H,CH_3), 2.15-2.3(m,2H,CH_2P), 3.4-3.55(m,2H,CH_2O), 3.75-4.05(m,2H,CH-O), 4.05-4.3(m,5H,CH-O,CH_2O ester). ^{13}C NMR (100MHz,D_2O) **7c(β)** : δ 16.22(s,CH3), 32.74(d,C6,$^1J_{CP}$ = 139.2Hz), 65.30(s,C1), 66.03(s,CH_2O ester), 66.15(s,CH_2O ester), 77.46(s,C5), 77.59(s,C3), 81.62(s,C4,$^3J_{CP}$ = 14.35Hz), 104.43(s,C2). Anal.Calcd. for $C_{10}H_{21}O_8P$: C,40.0; H,7.0; O,42.67. Found : C,40.14; H,7.09; O,41.87.

6-Deoxy-6-Diethoxymethylphosphono-α,β-D-fructose (8c). This compound was prepared from **8** (0.34g, 1.14 mmol) by following the general procedure (0.06g, 0.191 mmol, 33.5%). $[\alpha]_D^{26}$ = +11.5° (c = 0.78, MeOH). IR (film): ν(P=O)cm-1 : 1254; ν(P-O)cm-1 : 1062. ^{31}P NMR (81MHz,D_2O) δ(β) = 35.97 (83%), δ(α) = 35.84 (17%). ^1H NMR (400MHz,D_2O) δ 1.3(t,6H,CH_3), 1.75-2.15(m,4H,CH_2CH_2P), 3.5-3.65(m,2H,CH_2O), 3.65-4.1(m,2H,CH-O), 4.1-4.3(m,5H,CH-O,CH_2O ester). ^{13}C NMR(100MHz,D_2O) **8c(α)**: δ 18.25(s,CH_3), 22.5(d,CH_2P,$^1J_{CP}$ = 140.5Hz), 28.99(s,C6), 65.25(s,C1), 66.08(s,CH_2O ester), 66.14(s,CH_2O ester), 80.87(s,C4), 82.31(s,C3), 85.01(d,C5, $^3J_{CP}$ = 18.2Hz), 106.82(s,C2). **8c(β)**: δ 18.25(s,CH_3), 22.5(d,CH_2P,$^1J_{CP}$ = 140.5Hz), 28.99(s,C6), 65.33(s,C1), 66.08(s,CH_2O ester), 66.14(s,CH_2O ester),

77.94(s,C4), 80.26(s,C3), 82.18(d,C5,$^3J_{CP}$ = 18.51Hz), 104.05(s,C2). Anal.Calcd. for $C_{11}H_{23}O_8P$: C,42.04; H,7.32; O,40.76. Found. : C,42.28; H,7.52; O,41.02.

6-Deoxy-6-Azido-α,β-D-fructose (9c). This compound was prepared from **9** (0.46g, 2.43 mmol) by following the general procedure (0.17g, 0.83 mmol, 69.1%). $[\alpha]_D^{26}$ = +22.2° (c = 0.6, H_2O); lit[4d] $[\alpha]_D^{20}$ =+52.2° (c = 2.1, H_2O). ^1H NMR (200MHz,D_2O) δ 3.3-3.7(m,4H,CH_2O,CH_2N), 3.7-3.9(m,1H,CH-O), 3.95-4.2(m,2H,CH-O). ^{13}C NMR (50MHz,D_2O) **9c(α)**: δ 54.17(s,C6), 65.97(s,C1), 79.64(s,C4), 82.44(s,C3), 84.46(s,C5), 107.36(s,C2). **9c(β)**: δ 55.12(s,C6), 65.17(s,C1), 77.59(s,C4), 77.69(s,C3), 81.59(s,C5), 104.45(s,C2). Anal.Calcd. for $C_6H_{11}O_5N_3$: C,35.12; H,5.36; O,39.02. Found : C,35.21; H,5.62; O,39.56.

6-Deoxy-6-Amino-β-D-fructose and 6-Deoxy-6-Amino-β-D-fructopyrannose (10c). This compound was prepared from **10** (0.45g, 2.76 mmol) by following the general procedure (0.08g, 0.447 mmol, 29.6%). $[\alpha]_D^{25}$ = +18.45° (c = 1.23, H_2O). ^1H NMR (200MHz,D_2O) δ 3.3-3.9(m,5H,CH_2O,CH_2N,CH-O), 3.9-4.2(m,2H,CH-O). ^{13}C NMR (50MHz,D_2O) **10c(β–pyr)**: δ 66.34(s,C6), 66.56(s,C1), 70.23(s,C4), 71.85(s,C3), 72.34(s,C5), 100.73(s,C2) **10c(β–fur)**: δ 64.93(s,C6), 65.37(s,C1), 77.13(s,C4), 77.92(s,C3), 81.58(s,C5), 104.21(s,C2). Anal.Calcd. for $C_6H_{13}O_5N$: C,40.22; H,7.26; O,44.69. Found : C,39.92; H,7.37; O,44.81.

6-Deoxy-6-Trifluoroacetamido-α,β-D-fructose (11c). This compound was prepared from **11** (0.2g, 0.77 mmol) by following the general procedure (0.07g, 0.254 mmol, 66%). $[\alpha]_D^{26}$ = +19.46° (c = 1.4, H_2O). IR (KBr): ν(C=O)cm-1 : 1661; ν(NH)cm-1 : 3200; ν(OH)cm-1 : 3417. ^{19}F NMR (376MHz,D_2O) δ 3.04(β), 3.20(α). ^1H NMR (200MHz,D_2O) δ 3.5-3.75(m,4H,CH_2O,CH_2N), 3.85-4.0(m,1H,CH-O), 4.0-4.2(m,2H,CH-O). ^{13}C NMR (50MHz,D_2O) **11c(α)**: δ 42.5(s,C6), 65.36(s,C1), 79.83(s,C4), 81.56(s,C3), 84.34(s,C5), 107.15(s,C2), 118.59(q,CF_3, $^1J_{CF}$ = 285.9Hz), 161.97(q,C=O, $^2J_{CF}$ = 37.5Hz). **11c(β)**: δ 44.67(s,C6), 65.14(s,C1), 77.75(s,C4), 78.37(s,C3), 80.71(s,C5), 104.38(s,C2), 118.59(q,CF_3, $^1J_{CF}$ = 285.9Hz), 161.97(q,C=O, $^2J_{CF}$ = 37.5Hz). Anal.Calcd. for $C_8H_{12}O_6NF_3$: C,34.91; H,4.36; O,34.90. Found : C,34.53; H,4.39; O,35.26.

6-Deoxy-6-Ethoxycarbonylamido-α,β-D-fructose (12c). This compound was prepared from **12** (0.17g, 0.723 mmol) by following the general procedure (0.062g, 0.25 mmol, 69%). $[\alpha]_D^{26}$ = +7.06° (c = 1.5, EtOH). IR (KBr): ν(C=O)cm-1 : 1702;. ^1H NMR (200MHz,D_2O) δ 1.18(t,3H,CH_3), 3.3-3.45(m,2H,CH_2N), 3.45-3.65(m,2H,CH_2O), 3.75-3.9(m,1H,CH-O), 3.95-4.2(m,4H,CH-O,CH_2O-C=O). ^{13}C NMR (50MHz,D_2O) **12c(α)**: δ 16.43(s,CH_3), 44.31(s,C6), 64.54(s,CH_2O), 65.43(s,C1), 79.67(s,C4), 82.205(s,C3), 84.6(s,C5), 107.0(s,C2), 161.52(s,C=O). **12c(β)**: δ 16.43(s,CH_3), 45.46(s,C6), 64.54(s,CH_2O), 65.22(s,C1), 77.65(s,C4), 78.18(s,C3), 81.52(s,C5), 104.14(s,C2), 161.52(s,C=O). Anal.Calcd. for $C_9H_{17}O_7N$: C,43.03; H,6.78; O,44.62. Found : C,43.45; H,6.94; O,44.96.

6-Deoxy-6-Bromoacetamido-α,β-D-fructose (13c). This compound was prepared from **13** (0.14g, 0.493 mmol) by following the general procedure (0.05g, O.167 mmol, 64%). $[\alpha]_D^{26}$ = +18.21° (c = 1.2, EtOH). IR (film): ν(C=O)cm-1 : 1669; ν(NH)cm-1 : 3212; ν(OH)cm-1 : 3395. ^1H NMR (200MHz,D_2O) δ 3.65-3.9(m,4H,CH_2O,CH_2N), 4.0-4.15(m,1H,CH-O), 4.17(s,2H,$BrCH_2$) 4.2-4.4(m,2H,CH-O). ^{13}C NMR

(50MHz,D_2O) **13c(α)**: δ 30.6($BrCH_2$), 43.5(s,C6), 65.44(s,C1), 79.6(s,C4), 81.59(s,C3), 84.52(s,C5), 107.0(s,C2), 172.8(s,C=O). **13c(β)**: δ 30.6($BrCH_2$), 44.89(s,C6), 65.14(s,C1), 77.72(s,C4), 78.18(s,C3), 80.99(s,C5), 104.25(s,C2), 172.8(s,C=O). Anal.Calcd. for $C_8H_{14}O_6NBr$: C,32.02; H,4.67; O,32.00. Found : C,31.89; H,4.98; O,32.46.

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