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Substrate-dependent chemoselective aldose–aldose and aldose–ketose isomerizations of carbohydrates promoted by a combination of calcium ion and monoamines

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Abstract

Epimerization of aldoses at C-2 has been extensively investigated by using various metal ions in conjunction with diamines, monoamines, and aminoalcohols. Aldoses are epimerized at C-2 by a combination of alkaline-earth or rare-earth metal ions (Ca^{2+}, Sr^{2+}, Pr^{3+}, or Ce^{3+}) and such monoamines as triethylamine. In particular, the Ca^{2+}–triethylamine system proved effective in promoting aldose–ketose isomerization as well as C-2 epimerization of aldoses. ^{13}C NMR studies using D-(1-^{13}C)glucose and D-(1-^{13}C)galactose with the $CaCl_2$ system in CD_3OD revealed that the C-2 epimerization proceeds via stereospecific rearrangement of the carbon skeleton, or 1,2-carbon shift, and ketose formation proceeds partially through an intramolecular hydrogen migration or 1,2-hydride shift and, in part, via an enediol intermediate. These simultaneous aldose–aldose and aldose–ketose isomerizations showed interesting substrate-dependent chemoselectivity. Whereas the mannose-type aldoses having 2,3-erythro configuration (D-mannose, D-lyxose, and D-ribose) showed considerable resistance to both the C-2 epimerization and the aldose–ketose isomerization, the glucose-type sugars having 2,3-threo and 3,4-threo configurations, D-glucose and D-xylose, are mainly epimerized at C-2 and those having the 2,3-threo and 3,4-erythro configurations, D-galactose and D-arabinose, were mostly isomerized into 2-ketoses. These features are of potential interest in relevance to biomimic sugar transformations by metal ions.

Keywords: C-2 epimerization; Aldose–ketose isomerization; Calcium; Amine; 1,2-Carbon shift; 1,2-Hydride shift

1. Introduction

Studies on biomimic transformations of carbohydrates by metal complexes have been an important subject in the bioinorganic field, since many sugar-metabolizing enzymes have been shown to function with such metal ions as Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+} in the active sites. Mannose-6-phosphate isomerase involves the Zn^{2+} ion in its active site,[1] and xylose isomerases promote the aldose–ketose isomerization by utilizing carboxylate-bridged dimetal center of Mg^{2+}, Mn^{2+}, or Co^{2+} ions in the active site.[2,3] A similar dinuclear array of Mg^{2+} or Mn^{2+} ions also functions in the cleavage of phosphate esters by fructose 1,6-bisphosphatase.[4,5] Polysaccharides in glycoproteins on cell surfaces have been shown to play crucial roles

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with metal ions in cell–cell recognition and adhesion, antigen–antibody interactions, and hormone receptor sites, where sugar–metal–protein interactions have been demonstrated in some cases. Many C-type animal lectins such as mannose-binding proteins require Ca^{2+} ions in their function[6] and selectins also utilize Ca^{2+} ions to bind sialylated polysaccharides.[7,8] Concanavalin A, a lectin which specifically binds saccharides containing mannosyl residues, involves a pair of Ca^{2+} and Mn^{2+} ions bridged by aspartate near the sugar-recognition sites.[9]

We have carried out systematic investigations of transition-metal complexes with carbohydrates anchored onto a metal center by *N*-glycosylamine bond-formation with various diamines and polyamines.[10–23] During our studies, we have found that efficient C-2 epimerization of aldoses is promoted by nickel(II) and cobalt(II) complexes with diamine ligands, *N*,*N*,*N*′-trimethylethylenediamine (*N*,*N*,*N*′-Me_3en) and *N*,*N*,*N*′*N*′-tetramethylethylenediamine (*N*,*N*,*N*′,*N*′-Me_4en), which proceed through a novel stereospecific rearrangement of the carbon skeleton or a pinacol-type 1,2-carbon shift.[24–29] Although similar C-2 epimerizations through a 1,2-carbon shift mediated by molybdate are also known,[30] our reaction requires extremely mild conditions (5 min at 60 °C or 1 h at room temperature), where interactions between metal ions and diamines play crucial roles for the rearrangement. We have further extended the reaction by varying the metal ion and diamine components, aiming for biomimic functional transformations of carbohydrates. In this paper, we report aldose–aldose (C-2 epimerization) and aldose–ketose isomerizations of carbohydrates promoted by combination of Ca^{2+} and triethylamine, in which interesting substrate-dependent chemoselectivity is shown to arise from interaction of sugars with Ca^{2+} ions. Preliminary results have already been reported.[31,32] After our preliminary results, Yanagihara et al. have reported the calcium-catalyzed C-2 epimerization via skeletal rearrangement in basic solutions,[33] an observation originally discovered by Kusin.[34,35]

2. Experimental

Materials.—All reagents were of the best commercially grade and were used as received. D-(1-^{13}C)Glucose and D-(1-^{13}C)galactose were purchased from Aldrich Co., Ltd. and $PrCl_3 \cdot 7\ H_2O$ and $CeCl_3 \cdot 7\ H_2O$ from Nakarai Co., Ltd. Epimaltose, epilactose, epiisomaltose, and epimelibiose were prepared by the reactions presented in this report (see later). The following abbreviations are used: *N*,*N*,*N*′-Me_3en, 1-(*N*,*N*-dimethylamino)-2-(*N*-methylamino)ethane; *N*,*N*,*N*′,*N*′-Me_4en, 1,2-di(*N*,*N*-dimethylamino)ethane.

Measurements.—^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra at 100 MHz on a Jeol GX-400 spectrometer at 35 °C. Chemical shifts are given relative to external Me_4Si. HPLC analysis was performed with a TSK HLC-803D chromatographic system using a column of anion-exchange resin (TSK SA60 or TSK Sugar-AXG) or a reverse-phase resin column (TSK Amido80) maintained at 65 °C. Sugar components were eluted with 0.5 M borate buffer adjusted to pH 8.5 with a flow rate of 0.5 mL/min. Aldoses were fluorimetrically detected by the reaction with 2-cyanoacetamide at 95 °C for 5 min.[36] Ketoses were analyzed with TSK Amido80 and detected by using an RI detector (TSK RI8012).

General reaction promoted by metal ions and amine derivatives.—Aldoses (1.1 mmol) were treated with metal chloride (1.1 mmol) and amine (2.2 mmol) in MeOH for 5–10 min at 60 °C. The mixture was dissolved in 50 mL of water and was kept at pH 6.5 with 0.5 M H_2SO_4 or 1 M HCl for 1 h at rt. The solution was passed through Dowex 50W-X8 (H^+) and Dowex 1-X2 (HCO_3^-) columns prior to HPLC and ^{13}C NMR analyses. *N*,*N*,*N*′-Me_3en, *N*,*N*,*N*′,*N*′-Me_4en, diethylamine, triethylamine, di(*iso*-propyl)amine, 2-(*N*,*N*-dimethylamino)ethanol, and 2-(amino)-ethanol were used as amine components and the metal salts used were as follows; NaCl, KCl, RbCl, CsCl, $MgCl_2 \cdot 6\ H_2O$, $CaCl_2 \cdot 2\ H_2O$, $SrCl_2 \cdot 6\ H_2O$, $BaCl_2 \cdot 2\ H_2O$, $ZnCl_2$, $AlCl_3$, $PrCl_3 \cdot 7\ H_2O$, $LaCl_3 \cdot nH_2O$, $CeCl_3 \cdot 7\ H_2O$, $Y(NO_3)_3 \cdot 6\ H_2O$, $InCl_3 \cdot 3\ H_2O$, and $NdCl_3 \cdot 6\ H_2O$. Aldoses use as substrates were

D-glucose, D-mannose, D-galactose, D-xylose, D-lyxose, D-arabinose, D-ribose, maltose [α-D-glucopyranosyl-(1→4)-D-glucose], lactose [β-D-galactopyranosyl-(1→4)-D-glucose], isomaltose [α-D-glucopyranosyl-(1→6)-D-glucose], melibiose [α-D-galactopyranosyl-(1→6)-D-glucose], epimaltose [α-D-glucopyranosyl-(1→4)-D-mannose], epilactose [β-D-galactopyranosyl-(1→4)-D-mannose], epiisomaltose [α-D-glucopyranosyl-(1→6)-D-mannose], and epimelibiose [α-D-galactopyranosyl-(1→6)-D-mannose].

Isolation of disaccharides having D-*mannose as the reducing terminal.*—A disaccharide (maltose, lactose, isomaltose, or melibiose) (1.0 mmol) was added to a methanolic solution (40–100 mL) containing $CaCl_2 \cdot 2\ H_2O$ (1.0 mmol) and Et_3N (2.0 mmol), and the solution was incubated for 10 min at 60 °C with stirring. The mixture was dissolved in 50 mL of water and kept at pH 7.0 with 1.0 M HCl for 30 min at rt. Subsequently the solution was passed through cation-exchanger (Dowex 50W-X8, H^+) and anion-exchanger (Dowex 1-X2, HCO_3^-) columns prior to isolation by HPLC. The fractionations were carried out on the same HPLC system (column: TSK SA60, 10 × 300 mm) as that used for sugar analysis. The product fractions were collected and adjusted to pH 4.5 with 1 M H_2SO_4, and then deionized with Dowex 50W-X8 (H^+) and 1-X2 (HCO_3^-) columns. Methanol was added to the resultant solution, which was evaporated to remove the remaining boric acid as trimethyl borate (repeated 4–5 times). The remaining syrupy disaccharide was analyzed by ^{13}C NMR spectroscopy[27,37–39] and used as substrate.

Reactions using ^{13}C*-enriched aldoses.*—^{13}C-Enriched aldoses (1 equiv), D-(1-^{13}C)glucose and D-(1-^{13}C)galactose, were treated with $CaCl_2$ (1 equiv) and Et_3N (2 equiv) in CD_3OD for 5 min at 60 °C. The mixture was dissolved in D_2O and kept at pH 6.5 with 1 M DCl for 1 h at rt. The solutions were passed through Dowex 50W-X8 (H^+) and Dowex 1-X2 (HCO_3^-) columns prior to HPLC and ^{13}C NMR analyses. ^{13}C NMR spectra were measured with broad-band decoupled and non-decoupled modes in D_2O.

3. Results and discussion

C-2 Epimerization of aldoses promoted by metal ions and amine derivatives.—Aldoses (D-glucose and D-mannose) (1 equiv) were treated with metal chlorides (1 equiv) and diamines (*N*,*N*,*N*′-Me_3en and *N*,*N*,*N*′,*N*″-Me_4en) (2 equiv) in methanol for 5 min at 60 °C. Among various metal ions used, Ca^{2+}, Sr^{2+}, Pr^{3+}, and Ce^{3+} as well as Ni^{2+} and Co^{2+}[28,29] proved effective for the epimerization with diamines (Table 1, entries 1–8, 23, and 24). These metal ions could be classified into three groups, transition-metal ions (Ni^{2+}, Co^{2+}), alkaline-earth metal ions (Ca^{2+}, Sr^{2+}), and rare-earth metal ions (Pr^{3+}, Ce^{3+}). The alkaline-earth and rare-earth metal ions are known to have stronger affinity to sugars than Ni^{2+} and Co^{2+} ions and have flexible coordination spheres, and they promoted C-2 epimerization, although the reaction proceeded slowly as compared with that by Ni^{2+} or Co^{2+} ions.[28,29]

The C-2 epimerization by alkaline-earth and rare-earth metals was further examined by varying the diamines to include other nucleophilic reagents, and the notable results are listed in Table 2. It may be seen that Ca^{2+}, Sr^{2+}, Pr^{3+}, and Ce^{3+} are effective even with monoamines and aminoalcohols, in interesting contrast to the fact that no C-2 epimerization was promoted by Ni^{2+} and Co^{2+} ions with monoamines and aminoalcohols.[28,29] These results may reflect the characteristics of metal centers; the anchoring effect of the diamine unit may be less important in the Ca^{2+}, Sr^{2+}, Pr^{3+} and Ce^{3+} metal systems, because these metal ions have strong affinity to sugars by themselves.[40]

Aldose–aldose and aldose–ketose isomerizations by a combination of Ca^{2+} *ion and a monoamine.*—The Ca^{2+}–Et_3N system has particularly attracted our attention, since Ca^{2+} ions are of potential importance in biological systems, as described in Section 1, and it promoted both aldose–aldose (C-2 epimerization) and aldose–ketose isomerizations, although similar biological systems with Ca^{2+} have not been reported.

When various aldoses were used as the starting aldose in the reaction with Ca^{2+}–

Et_3N in methanol, a conspicuous substrate-dependent chemoselectivity was observed, as shown in Table 3. The mannose-type aldoses having the 2,3-erythro configuration, D-mannose, D-lyxose, and D-ribose, significantly resisted both C-2 epimerization and ketose formation (entries 2, 5, 7). The mannose-type aldoses are known to form stable complexes with alkaline-earth metal ions through their cis,cis-oriented sequential three hydroxy groups (axial–equatorial–axial coordination) as depicted in Scheme 1,[40,41] and it is presumed that this stable complexation of the mannose-type aldoses with Ca^{2+} prevents their isomerization, resulting in an apparent substrate selectivity. In particular with C-2 epimers of D-xylose and D-lyxose, an almost complete substrate-selective C-2 epimerization (D-xylose → D-lyxose) was established by the Ca^{2+} and Et_3N system.

Among the glucose-type aldoses having the 2,3-threo configuration, D-glucose, D-galactose, D-xylose, and D-arabinose, chemoselectivity between C-2 epimerization and ketose formation was observed, depending on the configurations of the starting aldoses (Table 3). The aldoses with 2,3-threo and 3,4-threo configurations, D-glucose and D-xylose, mainly underwent C-2 epimerization, and those having the 2,3-threo and 3,4-erythro configurations, D-galactose and D-arabinose, were mostly transformed into the corresponding 2-ketoses (Scheme 2). These features are of potential interest in relation to substrate selec-

Table 1
C-2 Epimerization of aldoses promoted by metal ions and diamines [a]

Entry	Metal ions	Diamines	Substrate	Yield of C-2 epimer (%) [b]	Recovery of substrate (%) [b]
1	Ca^{2+}	Me_3en [c]	D-glucose	28	58
2	Ca^{2+}	Me_4en [c]	D-glucose	17	74
3	Ca^{2+}	Me_3en	D-mannose	7	80
4	Ca^{2+}	Me_4en	D-mannose	2	93
5	Sr^{2+}	Me_3en	D-glucose	16	67
6	Sr^{2+}	Me_4en	D-glucose	3	86
7	Sr^{2+}	Me_3en	D-mannose	13	82
8	Sr^{2+}	Me_4en	D-mannose	2	97
9	Mg^{2+}	Me_3en	D-glucose	trace	73
10	Mg^{2+}	Me_3en	D-mannose	0	73
11	Ba^{2+}	Me_3en	D-glucose	2	63
12	Ba^{2+}	Me_3en	D-mannose	2	63
13	Zn^{2+}	Me_3en	D-glucose	4	70
14	Zn^{2+}	Me_3en	D-mannose	0	94
15 [d]	Ni^{2+}	Me_3en	D-glucose	35	56
16 [d]	Ni^{2+}	Me_4en	D-glucose	55	43
17 [d]	Ni^{2+}	Me_3en	D-mannose	36	50
18 [d]	Ni^{2+}	Me_4en	D-mannose	41	53
19 [e]	Co^{2+}	Me_3en	D-glucose	28	47
20 [e]	Co^{2+}	Me_4en	D-glucose	17	67
21 [e]	Co^{2+}	Me_3en	D-mannose	23	49
22 [e]	Co^{2+}	Me_4en	D-mannose	7	88
23	Ce^{3+}	Me_3en	D-glucose	14	86
24	Pr^{3+}	Me_3en	D-glucose	15	85
25	Y^{3+}	Me_3en	D-glucose	4	81
26	In^{3+}	Me_3en	D-glucose	trace	84
27	La^{3+}	Me_3en	D-glucose	4	86
28	Nd^{3+}	Me_3en	D-glucose	3	84

[a] Aldose (substrate) was treated with metal salt (1 equiv) and diamine (2 equiv) in methanol at 60 °C for 5 min.
[b] Determined by HPLC, based on substrate.
[c] Me_3en = *N*,*N*,*N*′-trimethylethylenediamine, Me_4en = *N*,*N*,*N*′,*N*′-tetramethylethylenediamine.
[d] Ref. 28.
[e] Ref. 29.

Table 2
C-2 Epimerization of aldoses promoted by metal ions and monoamine derivatives [a]

Entry	Metal ions	Amines	Substrate	Yield of C-2 epimer (%) [b]	Recovery of substrate (%) [b]
1	Ca^{2+}	Et_3N	D-glucose	38	32
2	Ca^{2+}	Et_2NH	D-glucose	38	25
3	Ca^{2+}	iPr_2NH	D-glucose	37	33
4	Ca^{2+}	$Me_2N(CH_2)_2OH$ [c]	D-glucose	18	66
5	Ca^{2+}	$H_2N(CH_2)_2OH$ [c]	D-glucose	14	63
6	Ca^{2+}	Et_3N	D-mannose	11	70
7	Ca^{2+}	Et_2NH	D-mannose	17	66
8	Ca^{2+}	iPr_2NH	D-mannose	17	62
9	Ca^{2+}	$Me_2N(CH_2)_2OH$ [c]	D-mannose	trace	86
10	Ca^{2+}	$H_2N(CH_2)_2OH$ [c]	D-mannose	6	78
11	Sr^{2+}	Et_3N	D-glucose	15	65
12	Sr^{2+}	Et_3N	D-mannose	12	71
13	Pr^{3+}	Et_3N	D-glucose	7	70
14	Pr^{3+}	$Me_2N(CH_2)_2OH$ [c]	D-glucose	5	78
15	Pr^{3+}	Et_3N	D-mannose	2	78
16	Pr^{3+}	$Me_2N(CH_2)_2OH$ [c]	D-mannose	2	72
17	Ce^{3+}	Et_3N	D-glucose	5	74
18	Ce^{3+}	Et_3N	D-mannose	2	74
19 [d]	Ni^{2+}	Et_3N	D-glucose	trace	99
20 [d]	Ni^{2+}	Et_3N	D-mannose	0	100

[a] Aldose (substrate) was treated with metal salt (1 equiv) and amine derivative (2 equiv) in methanol for 5 min at 60 °C.
[b] Determined by HPLC, based on substrate.
[c] $Me_2N(CH_2)_2OH$ = 2-(*N*,*N*-dimethylamino)ethanol, $H_2N(CH_2)_2OH$ = 2-(amino)ethanol.
[d] Ref. 28.

Table 3
Isomerization of various aldoses promoted by combination of Ca^{2+} and Et_3N [a]

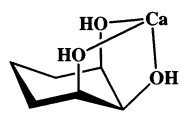
Entry	Substrate	Yield of C-2 epimer (%) [b]	Yield of ketose (%) [a]	Recovery of substrate (%) [b]
1	D-glucose	38	18	32
2	D-mannose	11	8	70
3	D-galactose	3	34	27
4	D-xylose	71	0	29
5	D-lyxose	9	0	75
6	D-arabinose	3	57	31
7	D-ribose	trace	0	85

[a] Aldose (substrate) was treated with $CaCl_2 \cdot 2\ H_2O$ (1 equiv) and Et_3N (2 equiv) in methanol for 10 min at 60 °C.
[b] Determined by HPLC, based on the substrate.

tive isomerizations and transformations of carbohydrates catalyzed by metalloenzymes.

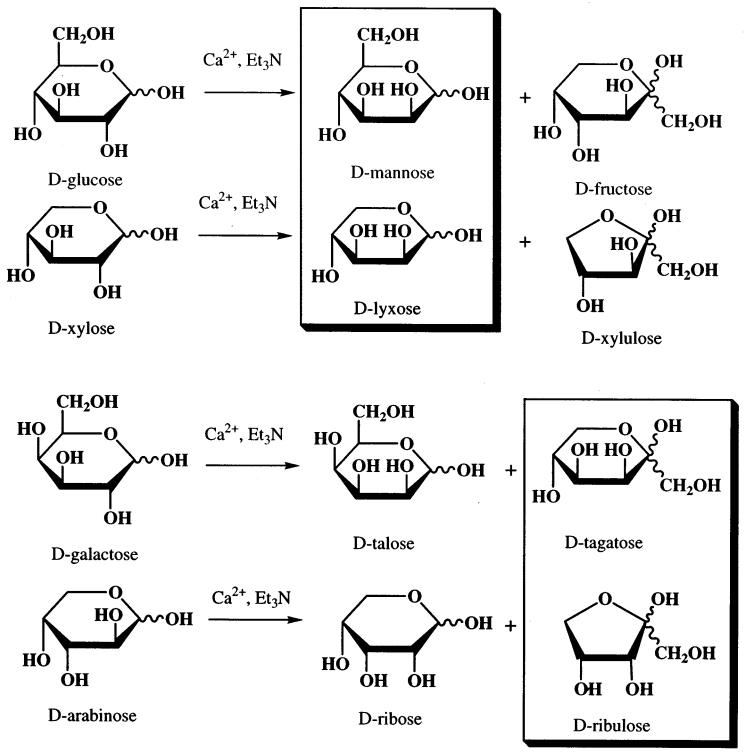
Isomerization mechanisms.—To confirm the detailed paths in the present reactions by Ca^{2+}–monoamine system, D-(1-^{13}C)glucose and D-(1-^{13}C)galactose were used as starting sugars.

D-(1-^{13}C)Glucose was treated with $CaCl_2$ (1 equiv) and Et_3N (2 equiv) for 5 min at 60 °C. The mixtures were quenched by water and HCl, and purified by use of cation- and anion-exchange resins. The ^{13}C NMR spectra of the purified reaction products are given in Fig. 1(a). Besides the peaks at 97.4 and 93.6 ppm, corresponding to (1-^{13}C) of β- and α-D-glucopyranose, four new resonances appeared



ax-eq-ax coordination

Scheme 1.



Scheme 2.

at 72.7, 72.2, 65.4, and 64.4 ppm, and no other significant peak was observed. The two peaks at 72.7 and 72.2 ppm were assigned to those for the (2-^{13}C) carbon of β- and α-D-mannopyranose, and the peaks at 65.4 and 64.4 ppm were assigned to those for the (1-^{13}C) carbon atom of β-D-fructopyranose and β-D-fructofuranose, respectively, which were consistent with the results of HPLC analyses. These observations clearly demonstrated that C-2 epimerization of aldose by inversion of the C-1–C-2 aldose fragment, and ketose formation without the rearrangement of carbon skeleton proceeded simultaneously with the combination of Ca^{2+} and Et_3N. The former is the same type of rearrangement as observed in the reactions by Ni^{2+}–diamine, Co^{2+}–diamine complexes,[28,29] and molybdate.[30]

To clarify further the mechanism of ketose formation, D-(1-^{13}C)glucose was treated with $CaCl_2$ and Et_3N in CD_3OD and quenched with D_2O and DCl. The ^{13}C NMR spectrum of the product mixture is given in Fig. 1(b). Besides the six peaks as observed in Fig. 1(a), a typical triplet caused by coupling with ^{2}H was observed at 65.1 ppm. In the non-decoupled ^{13}C NMR spectrum (Fig. 1(c)), the triplet was split into doublet of triplets due to couplings with ^{1}H, which could be assigned to the C-1 carbon atom of β-D-(1-^{13}C, 1-^{2}H)fructopyranose. The singlet at 65.4 ppm (Fig. 1(b)) was coupled with two ^{1}H atoms, leading to a triplet (Fig. 1(c)), which could be assigned to C-1 of β-D-(1-^{13}C)fructopyranose. Accordingly, there are two reaction paths for ketose formation, via an intramolecular hydrogen migration and via an exchange of C-1 proton with that of the solvent (methanol). For the former reaction path, a 1,2-hydride shift on a Ca^{2+} intermediate complex as depicted in Scheme 3 (**II**) was deduced by analogy with the rearrangement of a carbon skeleton through an intermediate complex (**I**), as discussed in our previous paper on the Ni^{2+}–diamine system.[28] Calcium(II) ion has a much stronger affinity for sugar hydroxyl groups than do Ni^{2+} and Co^{2+} ions and it could fix both the 1,2-cis conformations of the carbinolamine adduct in **I** and **II** (Scheme 3). In these intermediates, the alkyl (R^2) and hydride (H) groups migrated onto the electron deficient C-1 carbon of the sugar to generate D-(2-^{13}C)mannose and D-(1-^{13}C)fructose, respectively. In the intermediate **I**, the C-3 and

C-4 hydroxy groups of the sugar moiety were assumed to coordinate to the Ca^{2+} center, since the configurations at C-3 and C-4 dramatically influenced the C-2 epimerization. As to Kusin's reaction,[34] a related intermediate Ca^{2+} complex with four hydroxy groups of the carbohydrate have been proposed by Angyal.[35] Yanagihara et al. have also proposed a similar mechanism involving an intermediate Ca^{2+} complex ligated by the C-1, C-2, and C-3 hydroxyl groups of the sugar moiety.[33] In the mechanism proposed by Yanagihara et al., nucleophilic attack of a hydroxide ion on the C-1 carbonyl carbon of the aldose was assumed to be a trigger for the epimerization. In the present case, we have proposed that nucleophilic attack of the amine on the C-1 carbon of the sugar is the first step toward the epimerization because of the significant effects of amines on the C-2 epimerization. As for the formation of D-(1-^{13}C, 1-^{2}H)fructose, we speculate at present a mechanism through an enediolate Ca^{2+} intermediate complex (**III**), a typical base-catalyzed isomerization known as the Lobry de Bruyn and Alberda van Ekenstein rearrangement.[42]

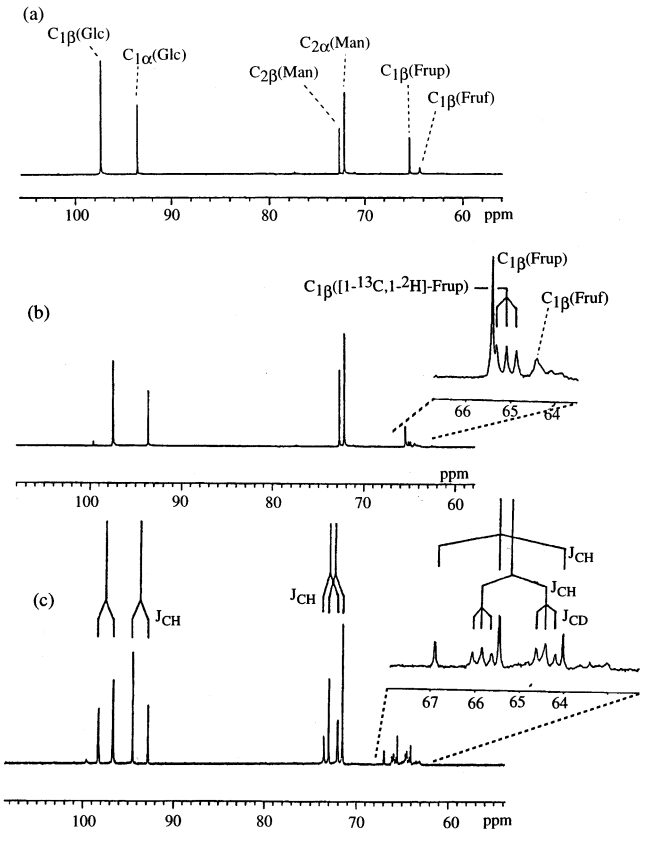


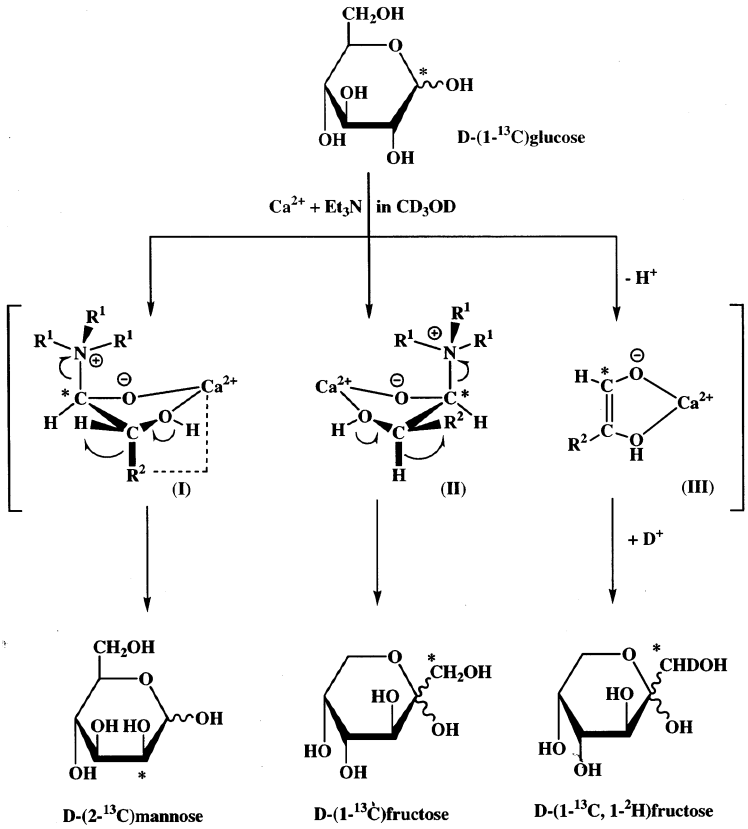
Fig. 1. ^{13}C NMR spectra of the products (a) obtained from the reaction of D-(1-^{13}C)glucose with Ca^{2+} amd Et_3N in CH_3OH; (b) obtained from the reaction of D-(1-^{13}C)glucose with Ca^{2+} and Et_3N in CD_3OD; and (c) obtained from the reaction of D-(1-^{13}C)glucose with Ca^{2+} and Et_3N in CD_3OD (non-decoupled mode).

The ^{13}C NMR spectrum of the reaction products from D-(1-^{13}C)galactose in CD_3OD exhibited a singlet at δ 64.8 corresponding to the C-1 carbon of D-β-(1-^{13}C)tagatose and a triplet near δ 64.4 corresponding to the C-1 carbon of β-D-(1-C^{13}, 1-^{2}H)tagatose, similar to the corresponding part in Fig. 1(b).

Epimerization at C-2 of disaccharides by a combination of Ca^{2+} ion and a monoamine.— Facile syntheses of di- and oligo-saccharide units are extremely important in connection with biorelevant chemistry, because polysaccharide chains of glycoproteins on cell surfaces have been shown to play important roles in cell–cell, antigen–antibody, and hormone–receptor recognition events.[43] As we have already reported, those aldoses that do not have a free hydroxyl group at C-4, such as (1→4)-linked disaccharides, were not epimerized at C-2 by Ni^{2+} or Co^{2+} complexes with *N*-alkylated diamines[27–29] or by molybdate,[30] presumably because the C-4 hydroxy substituent prevented access of the carbohydrate skeleton to the metal center. In the present study, we have examined C-2 epimerization of various disaccharides by using Ca^{2+}–monoamine systems.

The disaccharides, maltose, lactose, isomaltose, melibiose, epimaltose, epilactose, epiisomaltose, and epimelibiose, were treated with $CaCl_2{\cdot}2\ H_2O$ (1 equiv) and such *N*-alkyl amines (2 equiv) as Et_3N and Et_2NH, in methanol for 10 min at 60 °C, and the sugar products were analyzed by HPLC and ^{13}C NMR spectroscopy (Table 4).

(1→4)-Linked disaccharides having D-glucose unit as the reducing terminal, maltose [α-D-glucopyranosyl-(1→4)-D-glucose] and lactose [β-D-galactopyranosyl-(1→4)-D-glucose], epimerized at C-2 of the reducing unit to afford epimaltose [α-D-glucopyranosyl-(1→4)-D-mannose], and epilactose [β-D-galactopyranosyl-(1→4)-D-mannose] (Table 3 and Scheme 4). Epimaltose and epilactose, containing D-mannose at the reducing terminal,



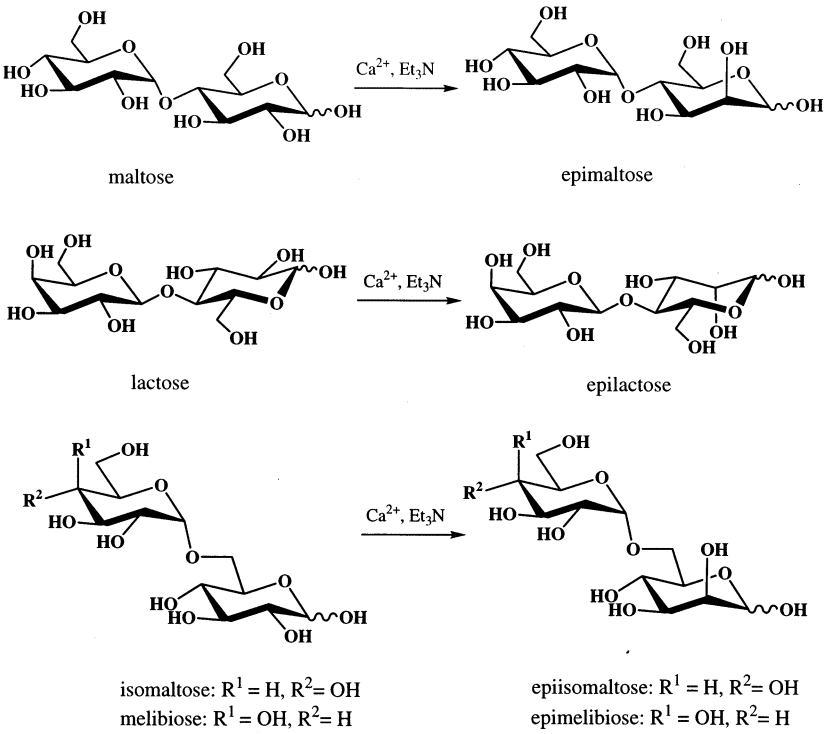
Scheme 3.

Table 4
C-2 Epimerization of disaccharides promoted by combination of Ca^{2+} and amine derivatives [a]

Entry	Substrate	Amine	Yield of C-2 epimer (%) [b]	Recovery of substrate (%) [b]
1	maltose	Et_3N	57	41 [c]
2	maltose	Et_2NH	52	41 [c]
3	maltose	$Me_2N(CH_2)_2OH$	50	48 [c]
4	maltose	Me_4en	37	47 [c]
5	lactose	Et_3N	39	61
6	lactose	Me_4en	4	86
7	isomaltose	Et_3N	31	47
8	melibiose	Et_3N	40	50
9	epimaltose	Et_3N	26	66 [c]
10	epilactose	Et_3N	32	55
11	epiisomaltose	Et_3N	10	62
12	epimelibiose	Et_3N	11	79

[a] Disaccharide (substrate) was treated with $CaCl_2 \cdot 2\ H_2O$ (1 equiv) and Et_3N (2 equiv) in methanol for 10 min at 60 °C.
[b] Determined by HPLC, based on the substrate.
[c] Small amounts of D-glucose and D-mannose were detected (1–5%).

were also transformed into their C-2 epimers, maltose and lactose, respectively. *N*-Alkylated monoamines, Et_3N and Et_2NH, proved more effective than the diamine, *N*,*N*,*N*′,*N*′-Me_4en. The (1 → 6)-linked disaccharide, isomaltose [α-D-glucopyranosyl-(1 → 6)-D-glucose] and melibiose [α-D-galactopyranosyl-(1 → 6)-D-glucose], were epimerized at C-2 to yield epiisomaltose [α-D-glucopyranosyl-(1 → 6)-D-mannose] and epimelibiose [α-D-galactopyranosyl-(1 → 6)-D-mannose], respectively. Epiisomaltose and epimelibiose were also trans-



Scheme 4.

formed into isomaltose and melibiose, respectively, by the action of Ca^{2+} and Et_3N. These reactions proceeded without any significant side reactions such as decomposition into monosaccharide components and formation of ketoses. Only in the case of maltose to epimaltose, a slight decomposition into D-glucose and D-mannose was observed ($< 5\%$). This is the first example of metal-promoted direct C-2 epimerization of $(1 \rightarrow 4)$-linked disaccharides. By analogy with the C-2 epimerization of monosaccharides by the Ca^{2+}–Et_3N system, the present C-2 epimerization of disaccharides could be inferred to involve rearrangement of the carbon skeleton, resulting in inversion of configuration at C-2 of the reducing unit. Unlike the nickel(II) and molybdate(VI) promoted epimerization, the bulky substituents at the C-4 hydroxy group, glycopyranosyl groups, did not hinder the reaction in the Ca^{2+}–monoamine system, due probably to the strong interaction between Ca^{2+} and sugar hydroxy groups, and the flexible coordination sphere around the calcium center. By employing this simple procedure, naturally rare $(1 \rightarrow 4)$-linked heterodisaccharides having a D-mannose unit as the reducing terminal can be easily obtained from disaccharides with D-glucose unit as the reducing terminal, which are abundant in nature.

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