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Development of a chemical strategy to produce rare aldohexoses from ketohexoses using 2-aminopyridine

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ABSTRACT

Rare sugars are monosaccharides that are found in relatively low abundance in nature. Herein, we describe a strategy for producing rare aldohexoses from ketohexoses using the classical Lobry de Bruyn–Alberda van Ekenstein transformation. Upon Schiff-base formation of keto sugars, a fluorescence-labeling reagent, 2-aminopyridine (2-AP), was used. While acting as a base catalyst, 2-AP efficiently promoted the ketose-to-aldose transformation, and acting as a Schiff-base reagent, it effectively froze the ketose–aldose equilibrium. We could also separate a mixture of Sor, Gul, and Ido in their Schiff-base forms using a normal-phase HPLC separation system. Although Gul and Ido represent the most unstable aldohexoses, our method provides a practical way to rapidly obtain these rare aldohexoses as needed.

1. Introduction

Rare sugars are monosaccharides that exist in nature in extremely small amounts. They include the aldohexoses, D-/L-allose (All), D-/L-altrose (Alt), L-galactose (Gal), L-glucose (Glc), D-/L-gulose (Gul), D-/L-idose (Ido), L-mannose (Man), and D-/L-talose (Tal), and the ketohexoses, L-fructose (Fru), D-/L-psicose (Psi), D-/L-sorbose (Sor), and D-/L-tagatose (Tag). Recently, rare sugars have attracted attention because some can be used as low calorie sweeteners,[1,2] can act as inhibitors of cancer cell growth[3] and microbial proliferation,[4] and may also be memory enhancers.[5] For the preparation of rare aldohexoses, the cyanohydrin (Kiliani–Fischer) synthesis has long been the classic chemistry enabling a systematic synthesis of aldoses starting from the smallest glyceraldehyde.[6] However, this procedure uses the highly toxic reagent hydrogen cyanide, and most of the precursor aldopentoses are not available in nature.

During the last 25 years, enzyme catalysis has become a viable, alternative approach for the production of rare sugars in many cases. Izumori and his co-workers have extensively studied the converting enzyme, D-tagatose 3-epimerase, originally identified in *Pseudomonas cichorii* ST-24.[7,8] Because the enzyme also catalyzes the 3-epimerization of D-Fru, a common ketohexose, it is used for the mass production of D-Psi.[9,10] L-Rhamnose isomerase from *Escherichia coli*[11] and many other bacteria[12] are also used to produce rare sugars. L-Rhamnose isomerase from *Pseudomonas stutzeri* has been used to produce D-All, an aldose, from D-Psi, a ketose, probably via the Lobry de Bruyn–Alberda van Ekenstein rearrangement,[13] another classic chemistry that is base catalyzed.[14] In general, enzymatic reactions are preferable to chemical ones as the former are stereospecific and less dangerous. From a practical standpoint, however, a complete set of 'specific' enzymes for the production of all aldoses does not currently (and may not) exist. To date, D-Tag and L-Tag have been produced from galactitol by *Arthrobacter globiformis* ST48 cells.[15] L-Psi was also produced from allitol by *Gluconobacter frateurii* IFO 3254 cells.[16] There are also reports of enzymatic production of D-Sor from D-Tag and L-Gal from L-Sor.[17,18] Nevertheless, a practical and general strategy for the preparation of aldohexoses, many of which are not found in [large quantities in] nature, does not exist.

Recently, we developed a general analytical method for the synthesis and purification of D-hexoses using the monoamine-coupling reagent 2-aminobenzamide (2-AB) and subsequent HPLC purification.[19] Although we attempted to use the fluorescence tag 2-aminopyridine (2-AP) in the aforementioned study, the attempt was unsuccessful, because 2-AP seemed to specifically catalyze ketose–aldose transformation via the Lobry de Bruyn–Alberda van Ekenstein rearrangement (hereafter simply denoted the Lobry

Abbreviations: All, allose; Alt, altrose; 2-AP, 2-aminopyridine; Fru, fructose; Gal, galactose; Glc, glucose; HPLC, high performance liquid chromatography; Man, mannose; PA-, pyridylaminated; Psi, psicose; RI, refractive index; Tag, tagtose; Tal, talose; TFA, trifluoroacetic acid.

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rearrangement;[14]), while other labeling reagents, including 2-aminobenzamide, had no such effect. Since it was first introduced by Hase et al.,[20] 2-AP has been widely used as a fluorescence-labeling reagent for glycoproteins,[21,22] glycolipids,[23,24] and free oligosaccharides.[25,26] The reaction uses a monoamine-coupling mechanism to produce a Schiff-base derivative, which can be converted into a stable, reduced pyridylaminated (PA) saccharide by dimethylamine · borane treatment. As far as we know, however, 2-AP has never been shown to catalyze a Lobry rearrangement. Therefore, we first investigated the reaction conditions for the transformation of a ketose to the two aldose products to establish a general procedure for the chemical synthesis of rare aldoses (Scheme 1).

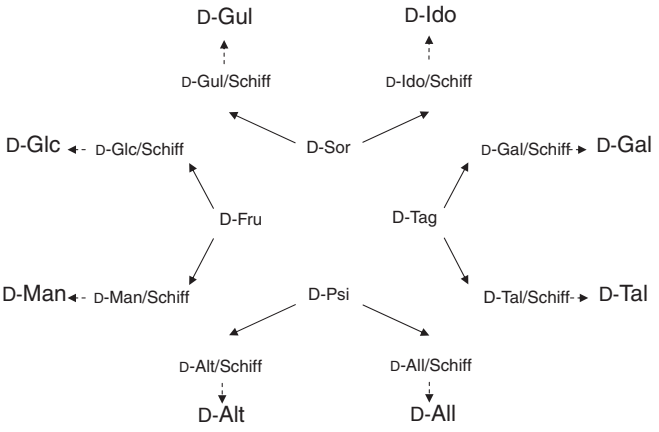
Another critical issue is the efficient generation of free monosaccharides from Schiff-base derivatives. Scheme 2 shows the basic chemistry that we used to prepare aldohexoses with the transformation of D-Fru as an example. In brief, when D-Fru was treated with 2-AP, one Schiff-base derivative product was that of D-Fru itself, and the two others were those of Glc and Man. The pyridylamino groups were subsequently removed by trifluoroacetic acid (TFA) treatment, which generated the free monosaccharides (Fru, Glc, and Man). After removing TFA by evaporation, the monosaccharides were separated by normal-phase HPLC with refractive index (RI) detection. For this report, we show that this scheme is applicable to the other ketohexoses, D-Psi, D-Tag, and D-Sor, and that rare aldohexoses can be systematically produced.

Notably, certain rare aldohexoses, especially Gul and Ido, are not very stable, and therefore, easily undergo undesirable transformation reaction(s) when stored. To circumvent the need to store free Gul and Ido, D-Sor, D-Gul, and D-Ido were separated by HPLC as Schiff-base derivatives. These aldohexoses can be stored as stable Schiff-base derivatives with the pyridylamino group removed only when a free aldohexose is needed, which makes the method valuable from an industrial standpoint.

2. Materials and methods

2.1. Materials

D-All and D-Tal were purchased from Tokyo Chemical Industry (Tokyo, Japan). D-Alt, D-Gul, and D-Ido were purchased from Funakoshi (Tokyo, Japan). D-Fru, 2-AP, and dimethylamine · borane were from Wako Pure Chemical Industries (Osaka, Japan). D-Gal, D-Man, and COSMOSIL Sugar-D columns (4.6 × 150 mm, 4.6 × 250 mm, and 10 × 250 mm) were obtained from Nacalai tesque Inc. (Kyoto, Japan). D-Psi, D-Tag, and D-Sor were obtained from Fushimi Pharmaceutical Co., Ltd (Kagawa, Japan). PA-Glc was purchased from Takara Bio Inc. (Otsu, Japan). A TSKgel sugar AXI column (4.6 × 150 mm) and TSKgel HW-40F resin were purchased from Tosoh (Tokyo, Japan).



Scheme 1. Preparation of D-aldohexoses from D-ketohexoses via the Lobry rearrangement and Schiff-base formation using 2-AP (solid arrows) followed by TFA treatment (dotted arrows). The Schiff-base derivative of each monosaccharide is designated with /'Schiff'.

2.2. Schiff-base formation between 2-AP and monosaccharides

Each ketohexose (10 μmol) was put into a conical square-cap type glass tube, and it was dissolved in the coupling reagent (20 μL, prepared by mixing 552 mg of 2-AP and 200 μL of AcOH[20,27]), and the reaction mixture was sealed and heated at 90 °C for 60 min. Next, 20 μL of MeOH and 40 μL of toluene were added into each mixture, over which a stream of N_2 was blown at 60 °C for 10 min in vacuo in a Palstation model 4000 apparatus (Takara Biomedicals, Kyoto, Japan)[20,28] to remove the 2-AP. By repeating this procedure three times, most of the unreacted 2-AP was removed. Residual amounts of unwanted materials including 2-AP were removed by gel filtration through a TSK gel HW-40F column (7 × 55 mm) equilibrated with 10 mM ammonium acetate (pH 6.0).[21] The fluorescence of each chromatographic fraction was measured using excitation and emission wavelengths of 320 nm and 400 nm, respectively. Fractions that fluoresced were pooled and concentrated.

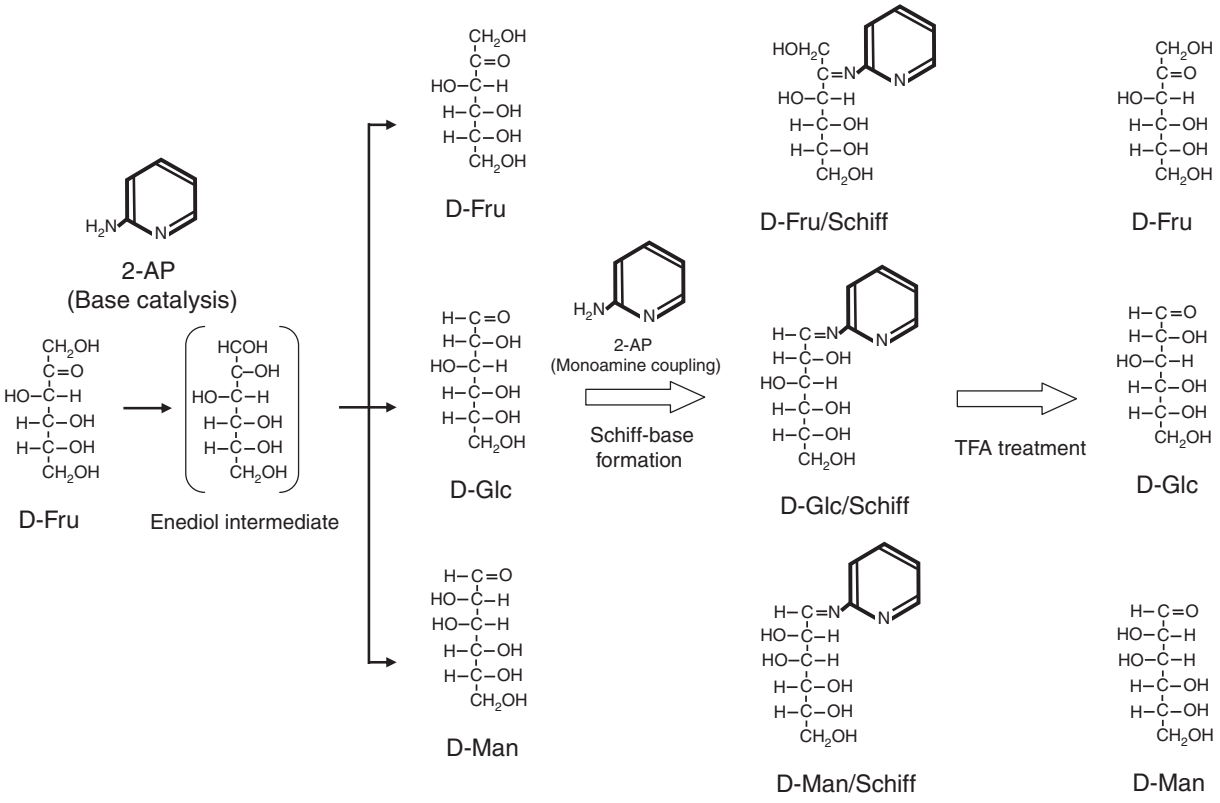
2.3. Calculation of Schiff-base formation efficiency

To measure the yields of the Schiff-base derivatized monosaccharides, a sample of each purified product was reduced to generate a stable PA-saccharide as follows. To an aliquot of each chromatographic fraction containing a Schiff-base derivative, 70 μL of freshly prepared reducing reagent (100 mg of dimethylamine · borane, 40 μL of AcOH, and 25 μL of water) was added. Each reaction mixture was heated at 80 °C for 35 min and then was applied to a TSKgel Sugar AX-I column (4.6 × 150 mm) for anion-exchange HPLC.[29] The elution buffer was 0.8 M boric acid–KOH, pH 9.0, 10% (v/v) acetonitrile. The flow rate was 0.3 mL/min, and the column temperature was 72 °C. The fluorescence of the PA-monosaccharides was detected using an excitation wavelength of 310 nm and an emission wavelength of 380 nm.

Each PA-monosaccharide was quantified by normalizing the chromatographic peak areas of its *R*/*S* C2 isomers[19] to that of the PA-Glc standard for which a chromatogram had been obtained under the same conditions.

2.4. Generation of free monosaccharides by TFA hydrolysis

To each mixture containing the Schiff-base derivatives Glc/Man/Fru, Alt/All/Psi, Tal/Gal/Tag, and Sor/Gul/Ido) was added 50 μL of 2 M TFA. After each reaction mixture had been heated at its optimized temperatures (see below) for 1 h, the TFA was evaporated. To confirm that the PA groups had been completely removed from the monosaccharides, an aliquot of each reaction mixture was subjected to size-fractionation HPLC over a Shodex Asahipak NH2P-50 column (4.6 × 50 mm, Showa Denko, Tokyo) at 25 °C and at a flow rate of 0.6 mL/min. The fluorescence of the eluate was monitored using an excitation wavelength of 310 nm and an emission wavelength of 380 nm. The gradient system consisted of eluents A and B. Eluent A was 970:70:3 acetonitrile–water–AcOH titrated to pH 7.0 with 7 M aqueous ammonia, and eluent B was 200:800:3 acetonitrile–water–AcOH titrated to pH 7.0 with 7 M aqueous ammonia. The column was equilibrated with 97% eluent A/3% eluent B. After loading a sample onto the column, eluent B was [immediately] changed linearly from 3% to 33% (v) in 3 min and then to 71% (v) in 35 min.



Scheme 2. The reaction scheme for the production of D-Glc and D-Man from D-Fru using 2-AP. The transformation precedes via 2-AP base-catalyzed Lobry rearrangement and 2-AP monoamine coupling. The Schiff-base derivatives are converted to the monosaccharides by TFA hydrolysis.

2.5. Separation of free monosaccharides

The Glc/Man/Fru, Alt/All/Psi, Tal/Gal/Tag, and Sor/Gul/Ido mixtures were each separated through a COSMOSIL Sugar-D column (10 × 250 mm) using an 80% acetonitrile isocratic eluent with a flow rate of 1.0 mL/min at 25 °C. Elution of the monosaccharides was detected using a Shimadzu RID-6A RI detector. Fractions containing a purified monosaccharide were pooled. To assess the degree of purification, an aliquot of each fraction was chromatographed through a smaller COSMOSIL Sugar-D column (4.6 × 150 mm) under the aforementioned conditions. Each monosaccharide was quantified on the basis of its peak area in comparison with that of standard D-Glc, which had been chromatographed under the same HPLC conditions.

2.6. Separation of the Schiff-base-derivatized monosaccharides

The Schiff-base derivatives of the D-Sor-series of hexoses, that is, Sor, Gul, and Ido, were separated by isocratic elution (95% (v/v) acetonitrile, 2 mM ammonium acetate, pH 4.0) through a COSMOSIL Sugar-D column (4.6 × 250 mm) with a flow rate of 1.0 mL/min at 25 °C. The fluorescence of the Schiff-base-derivatized monosaccharides was detected using an excitation wavelength of 320 nm and an emission wavelength of 400 nm. Fractions that fluoresced were pooled and then treated with TFA to generate free monosaccharides.

3. Results

3.1. Ketose–aldose transformation catalyzed by 2-AP

The basic strategy for the preparation of rare aldohexoses is summarized in Scheme 1. The starting materials, the ketohexoses, were treated with 2-AP, to produce the Schiff-base derivative of the ketose and the two aldose derivatives (see Scheme 2 with D-Fru as the reactant). After excess reagents were removed, the mixtures of Schiff-base derivatives were treated with TFA to generate the free monosaccharides. The TFA was then removed by evaporation. The three hexoses in each mixture were separated by normal-phase HPLC with RI detection.

To obtain good yields of the aldoses, the experimental conditions used for Schiff-base formation were first investigated for D-Fru. D-Fru (10 μmol) was dissolved in 20 μL of the coupling reagent, and the mixture was then heated at 90 °C for 60 min.[20,27] Next, excess reagent was removed first by solvent evaporation and then by TSKgel HW-40F chromatography,[21] which completely separated the Schiff-base derivatives from residual 2-AP and other lower molecular weight compounds (Fig. 1). Fractions containing the Schiff-base derivatives were pooled (shown in Fig. 1 as the horizontal bar), but before doing so, an aliquot of each sample was reduced to generate the PA-monosaccharides. Next, the reaction mixture was subjected to anion-exchange HPLC TSKgel Sugar AX-I chromatography (chromatogram not shown). The yields of the Schiff-base derivatives were 35% (Fru), 12% (Man), and 8% (Glc). Total product recovery was 53% and the keto–aldose conversion ratio was 36%.

Next, the reaction temperature and time period were varied. After the coupling reagent was added to samples of D-Fru (10 μmol each), the following conditions were assessed: 90 °C, 60 min; 90 °C, 120 min; 100 °C, 60 min; and 100 °C, 120 min. But, yield did not increase even if the temperature and time were changed. The yield was also not substantially improved by increasing the reaction time. Therefore, 90 °C and 60 min (standard conditions) were used for the other three ketose–aldose transformations.

Using the standard conditions D-Psi, D-Tag, and D-Sor (each 10 μmol) were individually reacted with 2-AP. Yields for the D-Psi sample were 10% (Psi), 11% (Alt), and 32% (All), for a total yield of 53%. Yields for the D-Tag sample were 28% (Tag), 16%

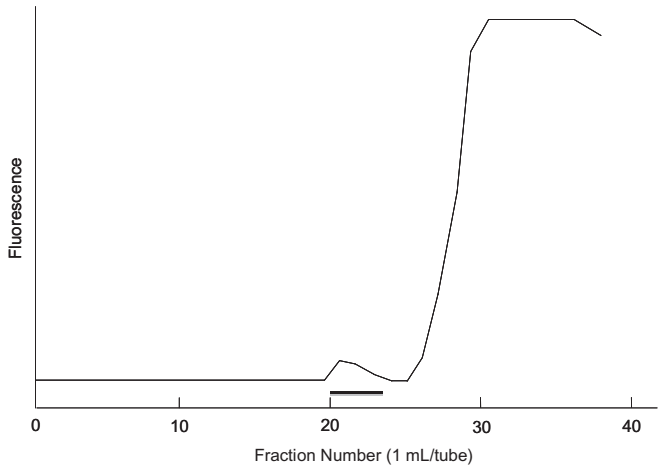


Figure 1. Size-fractionation chromatography for the mixture produced by reaction of 2-AP and D-Fru. Separation conditions: 7 × 500 mm HW-40F column equilibrated and developed with 10 mM ammonium acetate (pH 6.0) at a flow rate of 0.1 mL/min with fluorescence detection, and 1-mL fractions. The fractions marked by the bar contain the Schiff-base products.

(Tal), and 11% (Gal), for a total yield of 55%, and those for the D-Sor sample were 29% (Sor), 24% (Gul), and 10% (Ido), for a total yield of 63%. The ketose–aldose conversion ratio was the greatest for the D-Psi sample, (81%) followed by those for the D-Sor sample (54%) and the D-Tag sample (49%).

3.2. Liberation of the PA moieties to generate free monosaccharides by TFA treatment

Next, we searched for the best conditions to use for the conversion of the Schiff-base derivatives to free monosaccharides. To each of the Schiff-base-derivative mixture was added 50 μL of 2 M TFA (final concentration, 1 M). The mixtures were heated at 100 °C for 3 h, after which TFA was removed by evaporation. An aliquot of each reaction mixture was subjected to size-fractionation HPLC with fluorescence detection to confirm that no Schiff-base derivatives remained. The fractions corresponding to the retention times of the Schiff-base derivatives did not fluoresce (data not shown).

The remainder of each mixture containing the free monosaccharides was chromatographed through the 10 × 250 mm COSMOSIL Sugar-D column with RI detection. Contrary to our expectation, we could identify peaks for only D-Glc and D-Man. Peaks for the other monosaccharides were absent. Moreover, the yields for D-Glc and D-Man were low, 8% and 12%, respectively.

Considering that the molar amounts of D-Glc and D-Man were almost the same as those of their Schiff-bases, it seemed that the acid treatment (1 M TFA, 100 °C, 3 h) destroyed the other Schiff-base derivatives. Therefore, to produce the rare aldohexoses efficiently, for each Schiff-base derivative mixture, the TFA-treatment conditions needed to be optimized.

First, the conditions for the D-Psi derivatives were examined. To four mixtures of the D-Psi Schiff-base derivatives was added 50 μL of 2 M TFA (final concentration, 1 M). Each sample was incubated at 55, 60, 65, or 70 °C for 1 h. An aliquot of each mixture was chromatographed by size-fractionation HPLC with fluorescence detection (Fig. 2). For the reactions run at 55 and 60 °C fluorescent peaks were observed. For the reactions performed at the higher temperatures, essentially no fluorescence materials were chromographically retarded. Then, portions of the reaction mixture were subjected to TFA hydrolysis at 65, 70, 75, or 80 °C and then chromatographed through the 10 × 250 mm COSMOSIL Sugar-D column with RI detection. TFA treatment at 65 °C gave the largest yield, which substantially decreased the higher temperatures (data not shown). Therefore the reaction temperature for TFA treatment of the D-Psi derivatives was set to 65 °C. Figure 3A shows a typical chromatogram for the products of the reaction at 65 °C.

The optimum temperatures for TFA treatment of the D-Tag and D-Sor mixtures were also assessed and found to be 75 °C and 70 °C, respectively (Fig. 3). Table 1 summarizes the yields of the free monosaccharides for the four ketoses and the ketose–aldose conversion ratios. The monosaccharides, including the five rare aldohexoses, D-All, D-Alt, D-Tal, D-Gul, and D-Ido, were each separated to apparent homogeneity by COSMOSIL Sugar-D chromatography (Fig. 4).

3.3. Separation of the D-Sor/D-Gul/D-Ido Schiff-base derivatives

We also explored the possibility that the Schiff-base derivatives of D-Sor, D-Gul, D-Ido could be separated chromatographically because, although the Schiff-base derivatives are stable, the corresponding free monosaccharides are not. A mixture of the Schiff-base derivatives was chromatographed through the 4.6 × 250 mm COSMOSIL Sugar-D column with fluorescence detection. The three Schiff-base derivatives had retention times of 21.6 min (D-Sor), 23.7 min (D-Glu), and 11.1 min (D-Ido), and were well separated (Fig. 5). To confirm that the separated Schiff-base derivatives could each be converted into the corresponding free monosaccharide, after pooling the chromatography fractions of each Schiff-base derivative, the PA groups were removed using 1 M TFA at 70 °C for 1 h. After removing the TFA by evaporation, a sample of the reaction mixture was subjected to size-fractionation HPLC to confirm that the PA groups had been removed. Finally, the mixture was chromatographed through the 4.6 × 150 mm

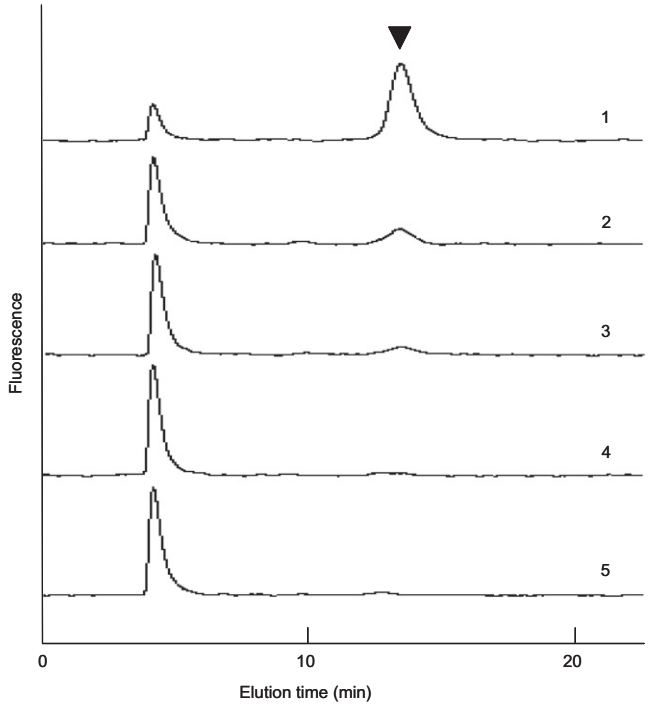


Figure 2. Size-fractionation HPLC chromatography of the D-Fru-derived Schiff-base derivatives after trifluoroacetic acid treatment. To samples of the Schiff-base D-Fru/Glc/Man mixture, 50 μL of 2 M TFA (final concentration, 1 M) was added. The samples were held for 1 h at (2) 55 °C, (3) 60 °C, (4) 65 °C, or (5) 70 °C before chromatography. Chromatogram 1 shows the elution profile of a sample that was held at room temperature for 1 h. The arrowhead marks the elution position of the Schiff-base derivatives. The size of the peaks indicates the extent to which the PA group had been removed.

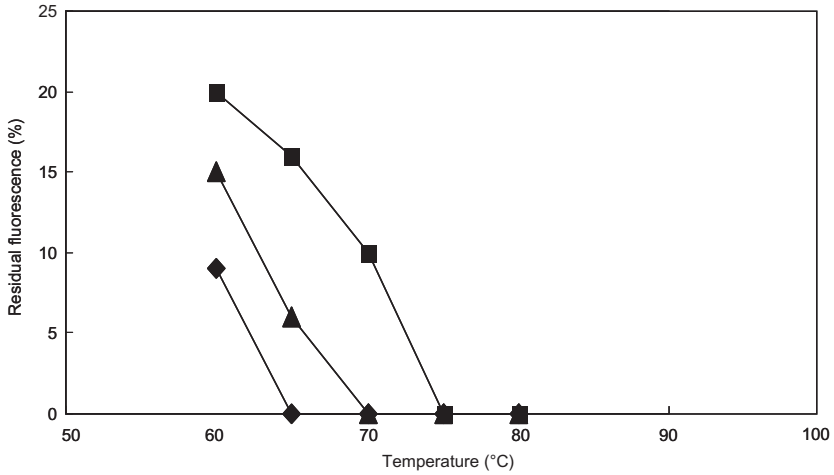


Figure 3. Effect of temperature on the yield of free monosaccharides. The residual fluorescence correlates with the amounts of Schiff-base derivatives that remained after TFA treatment for 1 h at the indicated temperatures. D-Tag (squares), D-Sor (triangles), and D-Psi (diamonds).

Table 1
Yields of monosaccharides derived from the Lobry de Bruyn–Alberda van Ekenstein rearrangement that used 2-AP[a]

	Sor					Fru				
	Sor	Gul	Ido	Total	Conversion ratio	Fru	Man	Glc	Total	Conversion ratio
Yield[b] (%)	29 (93)	24 (97)	10 (95)	63	54	35 (0)[c]	12 (95)	8 (93)	53	36
	Psi					Tag				
	Psi	Alt	All	Total	Conversion ratio	Tag	Tal	Gal	Total	Conversion ratio
Yield (%)	10 (91)	11 (100)	32 (92)	53	82	28 (97)	16 (94)	11 (80)	55	49

[a] Yields were normalized to the amounts of the ketose reactants (10 μmol = 100%). The value (%) for the ratio of each keto–aldose conversion is the yield of the two aldohexoses, for example, D-Alt + D-All, relative to the total yield, for example, D-Psi + D-Alt + D-All.
[b] Numbers in parentheses denote the yield for each monosaccharide after TFA hydrolysis.
[c] D-Fru had not recovered probably because its Schiff base was destroyed during TFA hydrolysis (see text).

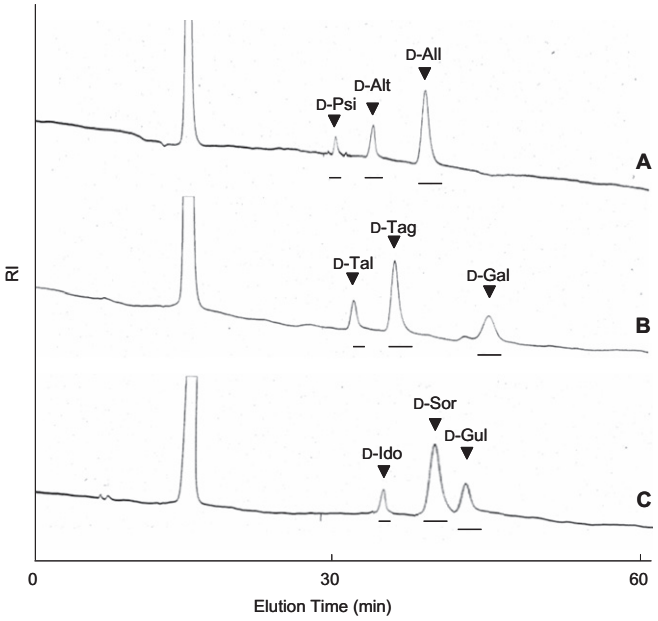


Figure 4. Separation of monosaccharides after TFA hydrolysis by a COSMOSIL Sugar-D HPLC column. Schiff-base derivatives of monosaccharides produced from (A) D-Psi, (B) D-Tag, and (C) D-Sor were treated with TFA under their optimized conditions, and the free monosaccharide products were separated by COSMOSIL Sugar-D column (10 × 250 mm) chromatography with RI detection. The arrowheads indicate the elution positions of authentic samples of the monosaccharides.

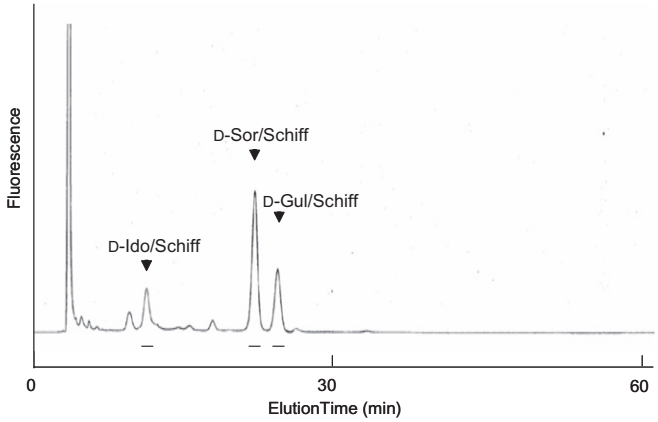


Figure 5. Separation of the D-Sor-series Schiff-base derivatives. After Schiff-base formation and removal of excess reagents, the Schiff-base derivatives of D-Sor/D-Gul/D-Ido were separated by COSMOSIL Sugar-D column (4.6 × 250 mm) chromatography with fluorescence detection. The fractions indicated by the bars were pooled and treated with TFA.

COSMOSIL Sugar-D column. The free monosaccharides had been purified to apparent homogeneity. We therefore developed a practical procedure for the preparation of D-Gul and D-Ido, which are the most unstable of the aldohexoses, by Schiff-base derivatization with 2-AP and subsequent generation by acid treatment that can be used only when needed.

4. Discussion

For the study reported herein, we developed a chemical procedure enabling systematic preparation of aldohexoses from ketohexoses, which uses 2-AP as the base catalyst for the Lobry rearrangement[14] and for Schiff-base formation with the reactant ketohexose and product aldohexoses. The conditions used for the ketose–aldose transformation and monoamine coupling were 20 μL of coupling reagent (552 mg of 2-AP and 200 μL of acetic acid) and 10 μmol ketohexose, at 90 °C for 60 min. Total yields of the monosaccharide Schiff-base derivatives of D-Fru, D-Psi, D-Tag, and D-Sor were 53%, 53%, 55%, and 63%, respectively (Table 1). The ketose–aldose conversion ratio was much greater for D-Psi (82%) than for D-Fru (36%), D-Tag (49%), and D-Sor (54%). Usually, it has been found that the transformation precedes easily via base catalysis when starting with ketoses and 2-AP. However, when starting with aldoses, the transformation does not occur readily, because aldoses are pyridylaminated much more readily than are ketoses.[30,31] Thus, the conversion ratio should depend, at least in part, on the reaction rate of Schiff-base formation in comparison with the forward isomerization rate. The detailed mechanism for the 2-AP-catalyzed Lobry rearrangement is largely unknown, and its elucidation is an important subject for future studies.

The optimum temperatures and reaction times for the TFA treatment, which liberates the PA groups of the Schiff-base derivatives are: 65 °C, 1 h (D-Psi); 75 °C, 1 h (D-Tag), and 70 °C, 1 h (D-Sor). At less than optimum temperatures, the PA group was not completely removed, whereas at higher temperatures side reactions occurred destroying the monosaccharides. After HPLC separation, 1.1 μmol of D-Alt (11%) and 2.9 μmol of D-All (32%) were obtained from 10 μmol of D-Psi; 1.5 μmol of D-Tal (16%) and 0.9 μmol of D-Gal (11%) were obtained from 10 μmol of D-Tag; and 2.3 μmol of D-Gul (16%) and 1.0 μmol of D-Ido (10%) were obtained from 10 μmol of D-Sor.

The Lobry rearrangement has often been used to prepare ketoses from aldoses, but rarely aldoses from ketoses.[14,32,33] H.O.L. Fischer et al. reported that boiling pyridine was useful as both the solvent and the base catalyst for ketose preparation[34] because it has a lesser tendency to induce side reactions than do aqueous alkaline solutions, thereby allowing the aldose–ketose transformation to dominate with epimerization occurring mainly at the C2 position.[34–37] Conversely, ketoses have rarely been used to produce aldoses with boiling pyridine as the solvent. Moreover, as an extension of the classic Lobry rearrangement, 3-epimerization of the ketoses occurs.[35–37] Landis reported the transformation of D-Fru to D-Psi in boiling pyridine (48 h reaction period[35]). However, for that reaction, the ketose–aldose conversion ratio was only 23%, and the reaction was not very specific because D-Glc (13%) and D-Man (10%) were present in addition to the expected D-Psi (25%) and D-Fru (52%). More recently, Ekeberg and co-workers reported the transformation of D-Fru in boiling pyridine with aluminum oxide as a catalyst to improve the reaction yield.[37] They obtained D-Glc (22%), D-Man (13%), D-Psi (10%), and D-Fru (55%) after a 9-h reaction period. Unlike the two aforementioned reports, we found that under our conditions 3-epimerization of D-Fru to D-Psi was undetectable. Although classic Lobry rearrangement is considered to proceed to equilibrium that depends on the relative thermodynamic stabilities of the monosaccharide reactants and products, for example, Glc:Fru:Man (5:4:1),[38] our method produced more D-Fru (35%) and less D-Glc (8%) than expected. A major difference between the transformations catalyzed by 2-AP and pyridine is that 2-AP exclusively catalyzes the ketose–aldose conversion with no concurrent 3-epimerization, because no significant peak corresponding to D-Psi was detectable when 2-AP catalyzed the Lobry rearrangement. Reaction mechanisms in the presence of 2-AP and pyridine remain to be clarified.

From a practical viewpoint, it is advantageous that the Schiff base stabilizes the monosaccharide, especially thermodynamically unstable D-Gul and D-Ido. In a similar attempt, an *O*-isopropylidene group has previously been shown to be effective to stabilize these aldoses.[36,37] When we use a larger column (e.g., 20 × 250 mm), a g-order of preparation of rare sugars is possible in theory by repeating the simple separation procedure. Now that these rare sugars are readily available, their potential industrial and biological applications, as well as their bioactivities, can be characterized more readily. These monosaccharides can now also be used as lead compounds with their multiple chiral centers being targeted for the first time for drug development.

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