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

Kayo Hasehira [a], Nobumitsu Miyanishi [b], Wataru Sumiyoshi [b], Jun Hirabayashi [b], Shin-ichi Nakakita [a,*]

[a] Department of Functional Glycomics, Life Science Research Center, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan
[b] Department of Glyco-Bioindustry, Life Science Research Center, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan

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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.

* Corresponding author. Tel.: +81 87 891 2409; fax: +81 87 891 2410.
E-mail address: nakakita@med.kagawa-u.ac.jp (S. Nakakita).

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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).

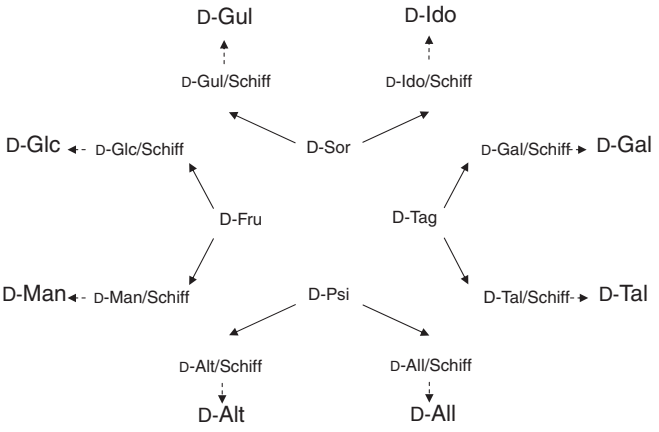
Another critical issue is the efficient generation of free monosaccharides from Schiff-base derivatives. Scheme 2 shows the basic chemistry that we used to prepare aldohexoses with the transformation of D-Fru as an example. In brief, when D-Fru was treated with 2-AP, one Schiff-base derivative product 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.