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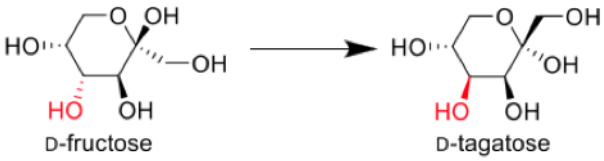

- efficient six-step synthesis
- four FCC purifications, easily scalable
- fully compatible with the preparation of substantial quantities of D-[UL-$^{13}C_6$]tagatose

Synthesis of Natural/^{13}C-Enriched D-Tagatose from Natural/^{13}C-Enriched D-Fructose

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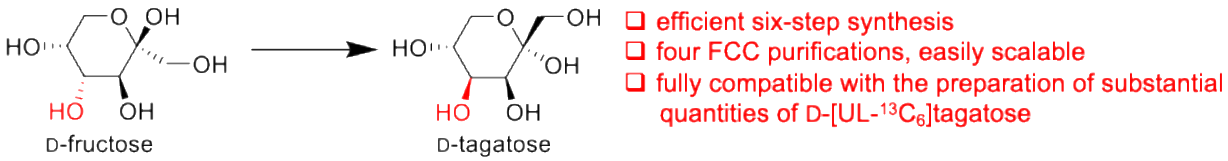
Abstract

A concise, easily scalable synthesis of a rare ketohexose, D-tagatose, was developed, that is compatible with the preparation of D-[UL-$^{13}C_6$]tagatose. Epimerization of the widely available and inexpensive ketohexose D-fructose at the *C*-4 position *via* an oxidation/reduction (Dess-Martin periodinane/$NaBH_4$) was a key step in the synthesis. Overall, fully protected natural D-tagatose (3.21 g) was prepared from D-fructose (9 g) on a 50 mmol scale in 23% overall yield, after five steps and two chromatographic purifications. D-[UL-$^{13}C_6$]Tagatose (92 mg) was prepared from D-[UL-$^{13}C_6$]fructose (465 mg, 2.5 mmol) in 16% overall yield after six steps and four chromatographic purifications.

Graphical abstract



Keywords

D-tagatose, D-fructose, D-[UL-$^{13}C_6$]tagatose, D-[UL-$^{13}C_6$]fructose, isotopic labelling, rare carbohydrates

Introduction

D-Tagatose (**1**, Figure 1) is a naturally occurring rare ketohexose found in small quantities in various dairy products. The sweetness of D-tagatose (92%) is on par with that of the widely used sucrose, however with no cooling effect or aftertaste [1]. The use of D-tagatose is associated with a decreased rate of tooth decay [2], and its low caloric value leads to weight loss at medically desirable rates among healthy individuals [3]. D-Tagatose is now considered a safe energy-reducing healthy lifestyle-compatible artificial sweetener [1].

The chemical structure of D-tagatose (**1**) and its relationship to the corresponding *C*-4 epimer D-fructose (**2**) is depicted in Figure 1. As determined by detailed NMR analysis [4], D-tagatose (**1**) predominantly (> 90%) occurs in pyranose form (79% α-D-tagatopyranose, 14% β-D-tagatopyranose, Figure 1) in aqueous solution. Under identical conditions, the major anomer of D-fructose (**2**) in solution is β-D-fructopyranose (72%), however a substantial proportion of β-D-fructofuranose (20%) is also present [4]. To simplify the discussion, the most predominant forms of D-tagatose (α-D-tagatopyranose **1**, Figure 1) and D-fructose (β-D-fructopyranose **2**, Figure 1) will be used, and the corresponding carbohydrates will be referred to as D-tagatose and D-fructose.

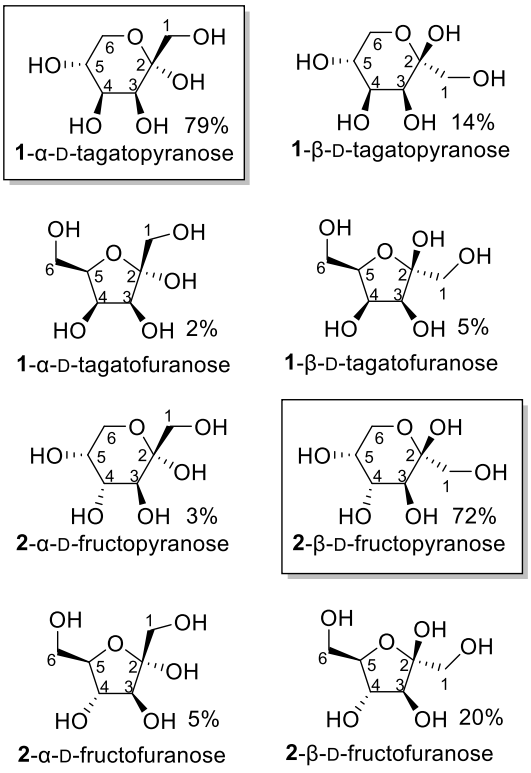


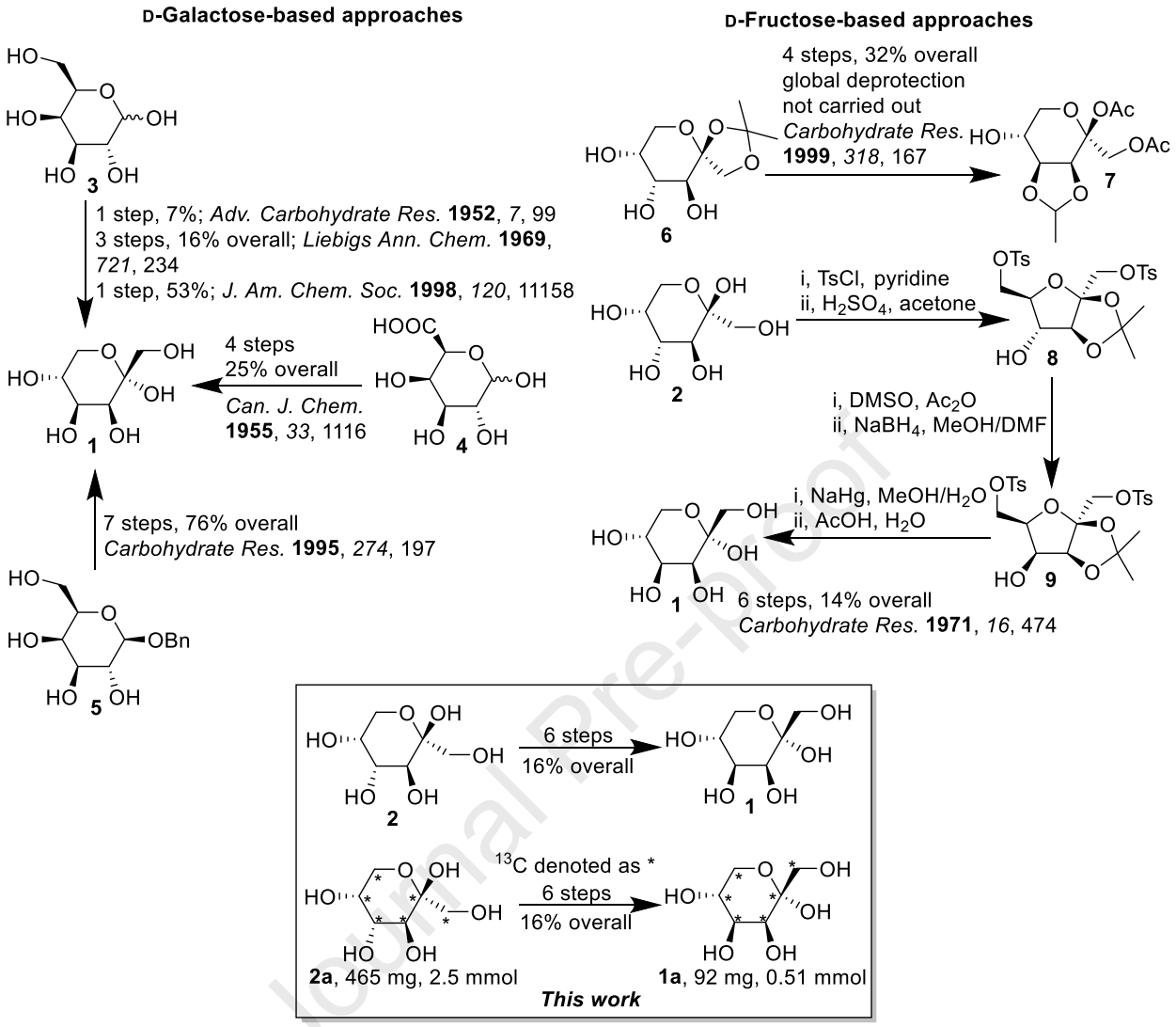
Figure 1. Isomeric forms of D-tagatose (**1**) and D-fructose (**2**) present in aqueous solution. The most prevalent forms, α-D-tagatopyranose and β-D-fructopyranose (boxes), will be used to represent **1** and **2** in all subsequent graphics associated with this work.

The favourable artificial sweetener properties associated with D-tagatose (**1**) have sparked significant interest by the food industry to develop methodologies allowing for the economical production of multi-ton quantities *per* year [1]. Biotransformation-based approaches, primarily based on enzymatic isomerization of readily available and cheaper carbohydrate sources (e. g. whey, milk) are now used to produce D-tagatose (**1**) on an industrial scale [1,5]. Small scale chemical synthesis of D-tagatose (**1**) and its protected derivatives in a laboratory setting appears to be significantly less developed, with no recent examples (since year 2000) describing enzyme-free organic synthesis of D-tagatose (**1**) or its protected derivatives.

The synthetic approaches to D-tagatose (**1**) can be divided into two subgroups based on the carbohydrates utilized as starting materials: D-galactose-based (Scheme 1, left) and D-fructose-based (Scheme 1, right). The first example of the direct isomerisation of D-galactose (**3**) to D-tagatose (**1**) is based on heating **3** in pyridine for an extended period, followed by a tedious

purification to afford a negligible yield (7%) of the desired D-tagatose (**1**, Scheme 1) [6]. Treatment of D-galactose (**3**) with dibenzylamine, followed by catalytic hydrogenation and diazotization/hydrolysis afforded D-tagatose (**1**) in 16% overall yield (Scheme 1) [7]. More recently, D-galactose (**3**) was converted to D-tagatose (**1**) upon alkaline isomerization in the presence of phenylboronic acid. The yield was satisfactory (53%, Scheme 1) and the methodology has also been extended to produce D-[1-^{13}C]tagatose and D-[2-^{13}C]tagatose [8]. In addition to D-galactose (**3**), D-galacturonic acid (**4**) was converted to D-tagatose (**1**) over 4 steps and 25% overall yield, with the reduction of the D-galacturonic acid methyl ester ($LiAlH_4$) being the key step in the synthesis (Scheme 1) [9]. A multistep process involving the formation of bicyclic derivatives has also been utilized to generate D-tagatose (**1**) from 1-Bn-β-D-galactose (**5**, Scheme 1) in seven steps and good overall yield (76%) [10].

To the best of our knowledge, there are only two examples wherein D-tagatose (**1**) or its protected form, was synthesized using D-fructose (**2**) as a starting material. Conversion of 1,3-di-*O*-isopropylidene-β-D-fructopyranoside (**6**, Scheme 1) to 1,2-di-*O*-Ac-3,4-di-*O*-ethylidene-β-D-tagatopyranose (**7**, Scheme 1) in four steps was described in 1999 [11], however, the deprotection to **1** was not carried out. Similarly, the relative stereochemical relationship of C4 in D-tagatose (**1**) and D-fructose (**2**) was previously exploited to generate **1**. In this approach, tosylation of D-fructose (**2**; 1,6 positions) followed by the formation of acetonide (2,3 positions) afforded **8** in 32% yield starting from **2** (Scheme 1) [12]. An oxidation/reduction ($DMSO/Ac_2O$ then $NaBH_4$) resulted in the formation of intermediate **9** bearing the *C*-4-tagato configuration (Scheme 1). Two step deprotection (NaHg in $MeOH/H_2O$ then reflux in aqueous AcOH) furnished the desired D-tagatose (**1**) in 6 steps and 14% overall yield [12]. The toxicity and environmental concerns associated with the use of mercury, even as an amalgam, encouraged the search for alternative methods to generate D-tagatose from D-fructose.



Scheme 1 Previous synthetic approaches to D-tagatose (**1**) based on D-galactose (**3**) or related compounds **4** or **5** (left) and D-fructose (**2**, right) compared to the presented work (bottom).

Recently we have initiated research devoted to understanding the structure-activity relationship of ketohexose analogs through metabolomic-based studies. During this work, we were required to prepare functionalized ketohexose derivatives with ^{13}C-enrichment in order to enable our metabolomics approach. To satisfy this need, we have developed a concise and efficient synthetic methodology allowing for the conversion of inexpensive D-fructose (**2**) to D-tagatose (**1**). Herein we present a synthetic method that works consistently on a small (2.5 mmol) or large

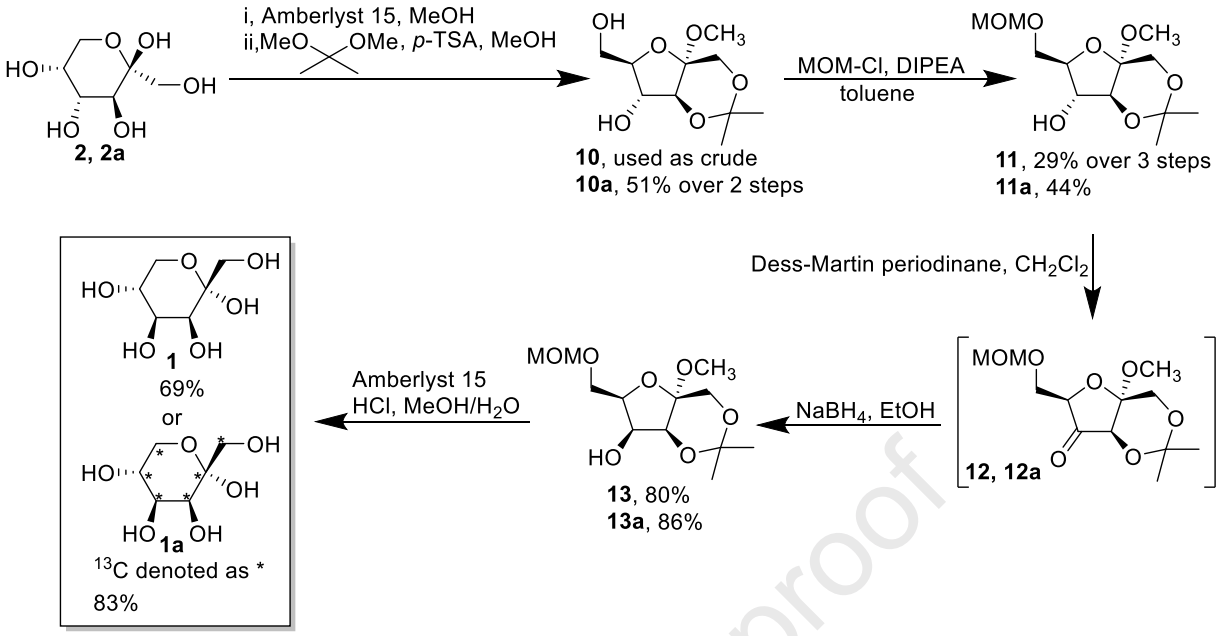
(50 mmol) scales, and is fully compatible with the preparation of multi-milligram quantities of rare and expensive D-[UL-$^{13}C_6$]tagatose (**1a**).

Results and discussion

While a complex mixture of pyranose and furanose forms [4] (Figure 1) exists in solution, it was shown previously [13] that reaction with MeOH in the presence of a catalytic amount of acid leads to the formation of methyl-D-fructofuranoside (anomeric mixture). Subsequent protection of the 1,3-positions (acetonide) then leads to the formation of a single isomer, methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside [14] (**10**, Scheme 2). Among different reagents for the acid catalyzed formation of methyl-D-fructofuranoside {H_2SO_4 [15], *p*-toluenesulfonic acid (*p*-TSA) [14], ion exchange resin Amberlyst 15 [13]} we proceeded with Amberlyst 15. In addition to excellent yields, the ion exchange resin can be filtered off, greatly simplifying workup and purification. The resulting filtrate is concentrated, dissolved in MeOH and 2,2-dimethoxypropane (acetone dimethyl acetal), followed by the addition of a catalytic amount of *p*-TSA [14]. We have first attempted to obtain crude **10** by extraction, however the recovery of the product is not satisfactory (< 40%) on a small scale (2.5 mmol), requiring isolation of **10** [16] by flash column chromatography (FCC, see the Experimental and Supporting Information). On the other hand, when working on 50 mmol scale, *p*-TSA was neutralized with Et_3N, the reaction mixture was concentrated and sufficiently pure crude methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**10**) was isolated upon partitioning the residue between brine and CH_2Cl_2/MeOH (9:1). With intermediate **10** in hand, we proceeded with the selective protection of the *C*-6-hydroxyl group. Since we intended to carry out one-step global deprotection at the end of the synthesis, we have selected the methoxymethyl (MOM) protecting group. The MOM group is readily introduced, is acid sensitive, and possesses reasonably low molecular mass (MW 45) towards atom economy. Treatment of methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**10**) with chloromethyl methyl ether (MOM-Cl) afforded 6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**11**, Scheme 2) in moderate yield. The reaction is somewhat complicated by the presence of two hydroxy groups in **10**, with the best results achieved when toluene (CH_2Cl_2 giving ca. 5% lower yields, and unsatisfactorily slow progress

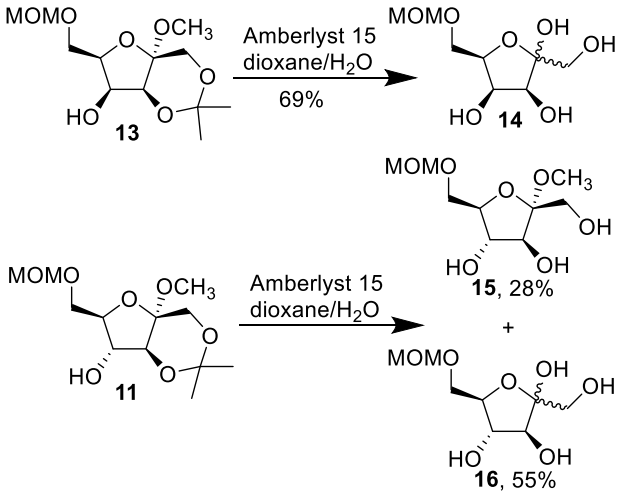
was observed in THF) was used as a solvent and *N*,*N*-diisopropylethylamine (DIPEA or Hünnig's base) was used as a base (see Experimental and Supporting information).

Having prepared 6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**11**), we proceeded with the oxidation/reduction reaction sequence to invert *C*-4-fructo to *C*-4-tagato stereochemistry. Treatment of **11** with Dess-Martin periodinane [17] in CH_2Cl_2 proceeded smoothly with the formation of unstable ketone **12** (Scheme 2). Ketone **12** was isolated by aqueous extraction, however, the attempts to obtain this intermediate in pure form led to extensive decomposition [18]. Crude ketone **12** was dissolved in EtOH, and brief treatment with $NaBH_4$ at low temperature (0 °C) resulted in the formation of 6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-tagatofuranoside (**13**, Scheme 2). While a small amount of the starting material (compound **11**, *C*-4-fructo epimer) was obtained, the *C*-4-tagato inverted product **13** can be consistently obtained in ca. 5:1 molar ratio. The two diastereomers are readily separated by FCC. The stereoselectivity of the reduction is rationalized based on the steric hindrance facilitating the hydride attack from the α-face. Similar outcomes were observed previously when performing the *C*-4-fructo to *C*-4-tagato inversion of **8** to **9** (Scheme 1) [12]. The identity of 6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-tagatofuranoside (**13**) was verified by detailed spectroscopic analysis. ^{1}H NMR spectra associated with **13** (red, Figure 2) and **11** (green, Figure 2; see the full view in the Supporting Information) were compared showing remarkable differences. A similar pattern was also observed when ^{13}C NMR spectra associated with **13** and **11** were compared (Supporting Information). As expected, high resolution mass spectra (HR-MS) of **11** and **13** showed the same value, 301.1 $[M + Na]^+$, further confirming the two molecules are epimers. When performing the reaction sequence on 50 mmol scale, 3.2 g (11.5 mmol) of 6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-tagatofuranoside (**13**) was obtained from 9 g of D-fructose (23% overall yield) after five steps and two chromatographic purifications (Scheme 2).



Scheme 2 Synthesis of D-tagatose and D-[UL-$^{13}C_6$]tagatose from D-fructose and D-[UL-$^{13}C_6$]fructose. All [UL-$^{13}C_6$]-enriched derivatives bear the same number as natural carbohydrate containing molecules containing an "**a**" suffix, e.g. D-tagatose (**1**), D-[UL-$^{13}C_6$]tagatose (**1a**).

To complete the synthesis of D-tagatose, we evaluated several different methods for the acid-mediated hydrolysis of **13**. Since heating in diluted HCl led to the darkening of the reaction mixture and low yields of **1**, we first investigated the use of Amberlyst 15 in dioxane/water. While the acetal-based protecting groups (positions 1, 2 and 3) were completely removed after stirring for 48 hours at room temperature, the MOM group resisted the removal even upon extending the reaction time. The resulting 6-MOM-D-tagatofuranose (**14**, mixture of anomers) was obtained in 69% yield (Scheme 3, Supporting Information) after FCC purification. When 6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**11**) was treated under identical conditions, the corresponding 6-MOM-D-fructofuranose (**16**, mixture of anomers) was isolated as a major product (55%, 2:1 ratio, Scheme 2) along with 6-MOM-methyl-α-D-fructofuranoside (**15**, 28%, Scheme 3). Compound **15** was still present even upon extending the reaction time to 96 h.



Scheme 3 Amberlyst 15-mediated partial deprotection of **11** and **13**

When aqueous HCl (1 M) was used, complete deprotection of **13** took place (after 48 h) with the formation of D-tagatose (**1**) in good yield after FCC purification and lyophilization (69%, Scheme 2). The ^{1}H and ^{13}C NMR spectra of synthetic D-tagatose and corresponding commercially sourced D-tagatose are in excellent agreement (Figure 3, panel A), and remarkable differences are observed when the spectra associated with D-tagatose (**1**) are compared to those associated with D-fructose (**2**, Figure 3, panel A).

The presented synthetic methodology offers several advantages, compared to a previously described method utilizing an oxidation/reduction reaction sequence as a key step in converting D-fructose (**2**) to D-tagatose (**1**) [12] (Scheme 1). The overall yield is slightly higher (16% over six steps, compared to 14% over six steps described previously), it is significantly more atom economical with 35% of the molecular mass lost upon global deprotection as compared to 66% in the previous synthesis, and the use of standard FCC for the fully deprotected product isolation simplifies the procedure and makes it more broadly accessible. Moreover, while we could achieve global deprotection in one step under mild conditions (Amberlyst 15 in dioxane/1 M HCl), two steps requiring global deprotection involving toxic reagents (NaHg) and harsh conditions (reflux in aqueous AcOH) was utilized in the previous synthesis [12].

The newly developed methodology was then applied for the preparation of expensive and scarcely available D-[UL-$^{13}C_6$]tagatose (**1a**) on a 2.5 mmol scale [19]. While the preparation of D-[1-^{13}C]tagatose and D-[2-^{13}C]tagatose has been described previously [8], literature reports describing the preparation of D-[UL-$^{13}C_6$]tagatose (**1a**) were not available. In order to distinguish between the natural and ^{13}C-enriched derivatives, all [UL-$^{13}C_6$]-enriched derivatives bear an "**a**" suffix, e.g. D-tagatose (**1**), D-[UL-$^{13}C_6$]tagatose (**1a**). Treatment of D-[UL-$^{13}C_6$]fructose (**2a**) with Amberlyst 15 in MeOH, followed by protecting the 1,3-positions as acetonide resulted in the formation of [UL-$^{13}C_6$]-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**10a**, Scheme 2) in reasonable yield. All ^{13}C-enriched molecules were characterized by multiple 1- and 2-D NMR techniques (^{1}H NMR, ^{13}C-decoupled ^{1}H NMR, ^{13}C NMR, ^{13}C-^{13}C COSY) as well as HR-MS spectrometry. ^{1}H NMR spectrum of **10a** possessed a complex pattern due to the presence of multiple short- and long-distance heteronuclear (^{1}H-^{13}C) couplings. The ^{13}C-decoupled ^{1}H NMR spectrum associated with **10a** overlayed with ^{1}H NMR spectrum of natural **10** is depicted in the Supporting Information, and excellent agreement between the two spectra is observed although some hyperfine structure in ^{13}C-decoupled ^{1}H NMR spectrum associated with **10a** is lost due to the decoupling. The signals associated with ^{13}C-enriched carbons (splitting pattern consistent with the presence of multiple ^{13}C-^{13}C short- and long-distance couplings) were detected in the ^{13}C NMR spectrum of **10a,** along with signals due to the presence of "natural" carbon (one signal due to the methyl group in position 2, three signals due to the acetonide in positions 1 and 3, see the Supporting Information). Moreover, all expected correlations were detected in the ^{13}C-^{13}C COSY NMR spectrum (Supporting Information). A difference of 6 Da was observed in the HR-MS spectrum between the natural **10**, 257.1 $[M + Na]^+$ and ^{13}C-enriched **10a**, 263.1 $[M + Na]^+$ further confirming the successful preparation of ^{13}C-enriched acetonide **10a**. The MOM protecting group was introduced as described previously for "natural" analog **11**, furnishing [UL-$^{13}C_6$]-6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**11a**) in moderate 44% yield (Scheme 2). All spectral data resulting from **11a** exhibited the features described above, with more detail provided in Figure 2 and the Supporting Information. *C*-4 epimerization of **11a** (Dess-Martin periodinane/$NaBH_4$) proceeded in excellent yield (86%, Scheme 2) furnishing [UL-$^{13}C_6$]-6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-tagatofuranoside (**13a**). Overall, 465 mg (2.5 mmol) of D-[UL-$^{13}C_6$]fructose (**2a**) was converted to 136 mg of **13a** (0.48 mmol, 19% overall after five steps and three FCC

purifications). An overlay of ^{13}C-decoupled ^{1}H NMR spectra associated with **11a** and **13a** with those associated with "natural" carbohydrate-containing derivatives **11** and **13** are depicted in Figure 2.

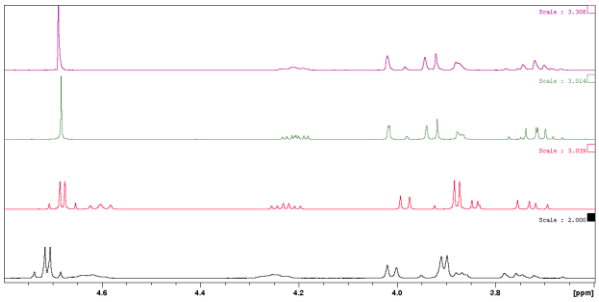


Figure 2 ^{13}C-decoupled ^{1}H NMR spectrum of [UL-$^{13}C_6$]-6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-tagatofuranoside (**13a**, black) overlaid with ^{1}H NMR spectrum of 6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-tagatofuranoside (**13**, red), ^{1}H NMR spectrum of 6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**11**, green) and ^{13}C-decoupled ^{1}H NMR spectrum of [UL-$^{13}C_6$]-6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**11a**, purple). The spectra were acquired in $CDCl_3$ at 300 MHz.

With the fully protected ^{13}C-enriched D-tagatose derivative **13a** in hand, the global acid-mediated deprotection (Amberlyst 15 in dioxane/2 M HCl) was carried out (Scheme 2). D-[UL-$^{13}C_6$]tagatose (**1a**) was purified by FCC and was obtained in good yield (83%) and significant quantity (92 mg, 0.49 mmol from 169 mg, 0.6 mmol of **13a**) as a crystalline solid upon lyophilization. All spectral characteristics associated with the synthetic D-[UL-$^{13}C_6$]tagatose (**1a**) are depicted in Figure 3, identical spectra acquired for D-fructose (**2**) and D-[UL-$^{13}C_6$]fructose (**2a**) are shown for comparison. A commercial sample of D-[UL-$^{13}C_6$]tagatose (**1a**) was also obtained [19], and the corresponding spectra were acquired and compared to those associated with the synthetic D-tagatose (**1**) and D-[UL-$^{13}C_6$]tagatose (**1a**). Notably, ^{1}H NMR spectra of both, commercial and synthetic D-tagatose (**1**) and ^{13}C-decoupled ^{1}H NMR spectra (both commercial and synthetic) of D-[UL-$^{13}C_6$]tagatose (**1a**) are in good agreement, although some hyperfine structure is lost due to the decoupling. An agreement between commercial and synthetic samples of D-[UL-$^{13}C_6$]tagatose (**1a**) was also remarkable, when their ^{13}C NMR spectra and ^{13}C-^{13}C COSY NMR spectra were compared (Figure 3). In HR-MS an expected increase by 6 Da was detected, yielding 209.1 $[M + Na]^+$.

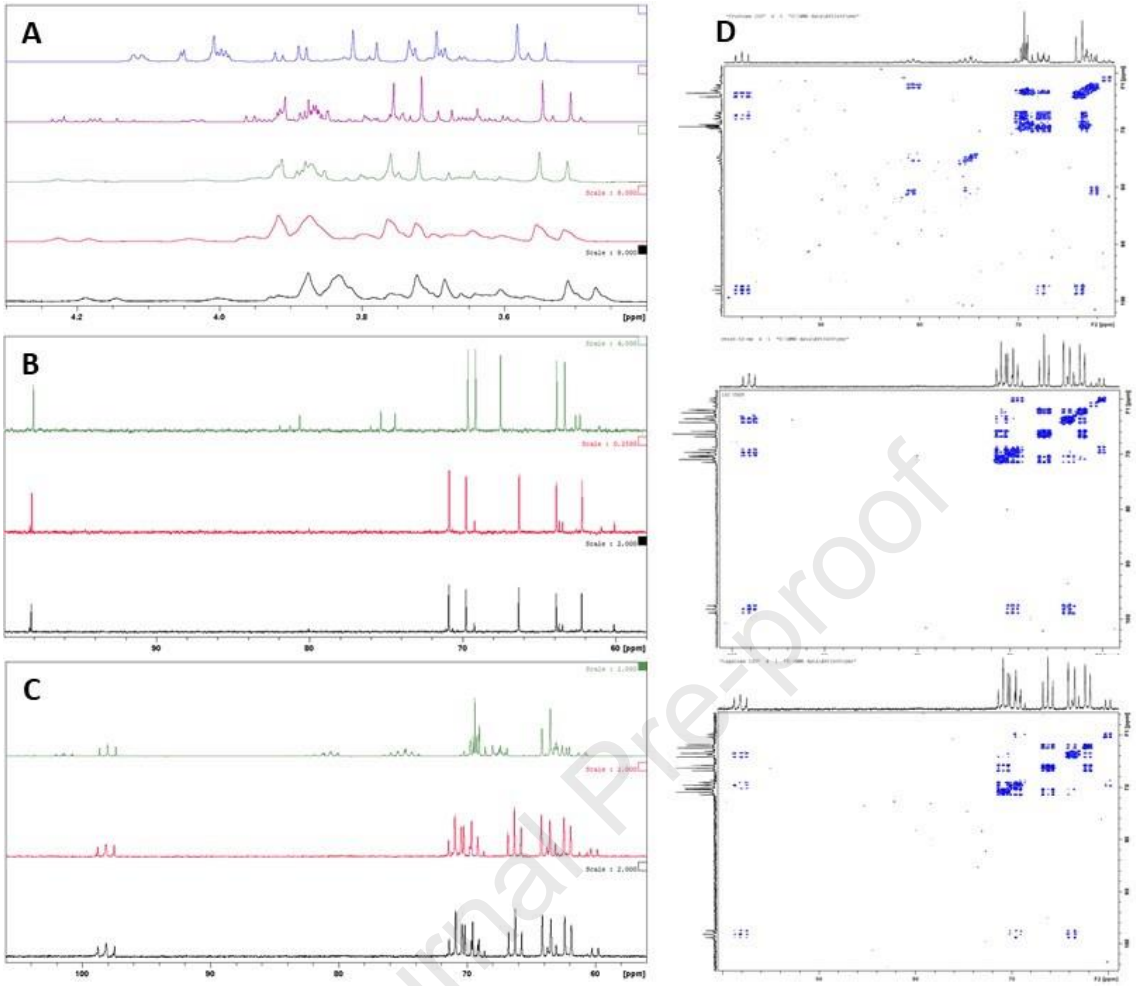


Figure 3 Detailed spectral characterization of synthetic D-tagatose (**1**) and D-[UL-$^{13}C_6$]tagatose (**1a**). Panel A: ^{13}C-decoupled ^{1}H NMR spectrum of the commercial D-[UL-$^{13}C_6$]tagatose (**1a**, black) overlayed with the ^{13}C-decoupled ^{1}H NMR spectrum of the synthetic D-[UL-$^{13}C_6$]tagatose (**1a**, red), ^{1}H NMR spectrum of the synthetic D-tagatose (**1**, green), ^{1}H NMR spectrum of the commercial D-tagatose (**1**, purple) and ^{1}H NMR spectrum of the commercial D-fructose (**2**, blue). Panel B: ^{13}C NMR spectrum of the commercial D-tagatose (**1**, black) overlayed with ^{13}C NMR spectrum of the synthetic D-tagatose (**1**, red) and ^{13}C NMR spectrum of the commercial D-fructose (**2**, green). Panel C: ^{13}C NMR spectrum of the commercial D-[UL-$^{13}C_6$]tagatose (**1a**, black) overlayed with the ^{13}C NMR spectrum of the synthetic D-[UL-$^{13}C_6$]tagatose (**1a**, red) and ^{13}C NMR spectrum of the commercial D-[UL-$^{13}C_6$]fructose (**2a**, green). Panel D: ^{13}C-^{13}C COSY NMR spectrum of the commercial D-[UL-$^{13}C_6$]tagatose (**1a**, bottom), ^{13}C-^{13}C COSY NMR spectrum of the synthetic D-[UL-$^{13}C_6$]tagatose (**1a**, center) and ^{13}C-^{13}C COSY NMR spectrum of

the commercial D-[UL-$^{13}C_6$]fructose (**2a**, top). All spectra were acquired in D_2O at 300 MHz (^{1}H NMR) or 75 MHz (^{13}C NMR).

Conclusions

In conclusion we have developed a new synthetic methodology suitable for the preparation of the rare ketohexose D-tagatose (**1**), along with the ^{13}C-enriched counterpart D-[UL-$^{13}C_6$]tagatose (**1a**). The main advantages associated with our synthetic approach are short time (the whole synthetic sequence can be completed in one week), good reliability (similar yields are obtained upon multiple repetitions of synthetic sequence), reasonable atom economy through the careful selection of protecting groups, the use of standard FCC for the purification of intermediates and final products as well as full compatibility with the preparation of expensive ^{13}C-enriched carbohydrates (similar yields are obtained when altering between "natural" and ^{13}C-enriched carbohydrates). The presented methodology allows for the preparation of scarce and very expensive D-[UL-$^{13}C_6$]tagatose (**1a**) on multi-milligram quantities, providing scientists interested in metabolomic studies with rare carbohydrates with rapid and simplified access to **1a**. Although beyond the scope of the presented work we also believe that the robust and reliable synthetic approach described herein will be compatible with other ^{13}C enrichment patterns and potentially also radioactive isotopes (^{14}C) present in D-fructose and D-tagatose.

Experimental

General information

All reagents were commercially available. In D-[UL-$^{13}C_6$]fructose (**2**), all six carbon atoms were ^{13}C enriched (99% enrichment level). All solvents were HPLC grade and used as such, except for water which was deionized (18.2 MΩ·cm^{-1}) and dichloromethane which was dried over Al_2O_3, in a solvent purification system. Organic extracts were dried with Na_2SO_4 and solvents were removed under reduced pressure in a rotary evaporator. Aqueous solutions were lyophilized. Flash column chromatography (FCC) was carried out using silica gel, mesh size 230 – 400 Å. Thin layer chromatography (TLC) was carried out on Al backed silica gel plates;

compounds were visualized by orcinol stain. Specific rotations $[\alpha]_D$ were determined by polarimeter at ambient temperature using a 2 mL, 1 cm path length cell; the units are 10^{-1} deg cm^2 g^{-1} and the concentrations are reported in g/100 mL. NMR spectra were recorded on 300 MHz spectrometer; for ^1H (300 MHz), δ values were referenced as follows $CDCl_3$ (7.26 ppm); CD_3OD (3.31 ppm); D_2O (4.79 ppm) for ^{13}C (75 MHz) $CDCl_3$ (77.0 ppm), CD_3OD (49.0 ppm). High resolution mass spectra (HR-MS) were obtained by electron spray ionization (ESI) time-of-flight (TOF) method.

Methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (10)

An ion exchange resin, Amberlyst 15 (50 mg) was added to a stirred solution of D-fructose (**2**, 450 mg, 2.5 mmol) in MeOH (25 mL). The mixture was stirred for 18 hours (h) at room temperature (rt), the resin was filtered off, the filter was washed with MeOH and the filtrate was concentrated to leave colorless oil.

This material was dissolved in MeOH (2.5 mL), and 2,2-dimethoxypropane (acetone dimethyl acetal, 2.5 mL), *p*-toluenesulfonic acid (*p*-TSA, 5 mg, 2.6×10^{-5} mol) was added and the mixture was stirred for 1 h at rt. The reaction was quenched by the addition of saturated $NaHCO_3$ solution (2 drops), the solvent was evaporated, and the residue was subjected to FCC on 50 g SiO_2, CH_2Cl_2/MeOH (19:1). Evaporation of the eluate afforded colorless oil, methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**10**, 346 mg, 59% over two steps); $[\alpha]_D$ +29.2 (*c* 1.07, $CHCl_3$), lit. [16d] $[\alpha]_D$ +34.5 (*c* 0.50, $CHCl_3$), lit. [16c] $[\alpha]_D$ +38.6 (*c* 2.00, $CHCl_3$); R_F 0.17 [CH_2Cl_2/MeOH (19:1)]. ^1H NMR ($CDCl_3$) δ 4.24 – 4.12 (m, 1H); 4.04 – 3.90 (m, 4H); 3.88 – 3.71 (m, 2H); 3.36 (s, 0.3H); 3.32 (s, 2.7H); 2.58 (br s, D_2O exch., 2H); 1.52 (s, 0.3H); 1.47 (s, 2.7H); 1.38 (s, 2.7H); 1.35 (s, 0.3H). ^{13}C NMR ($CDCl_3$) δ 101.2, 98.7, 87.9, 79.4, 77.7, 62.8, 61.8, 48.7, 27.9, 19.1. HRMS (ESI) *m/z* found 257.1010 $[M + Na]^+$ (257.1001 calcd for $C_{10}H_{18}O_6Na$). Spectral data matched those reported previously [14, 16c, 16d].

6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (11), large scale procedure

An ion exchange resin, Amberlyst 15 (1 g) was added to a stirred solution of D-fructose (**2**, 9 g, 50 mmol) in MeOH (250 mL). The mixture was stirred for 18 h at rt, the resin was filtered off, the filter was washed with MeOH and the filtrate was concentrated to leave colorless oil.

This material was dissolved in MeOH (50 mL), and 2,2-dimethoxypropane (acetone dimethyl acetal, 50 mL), *p*-TSA (95 mg, 0.5 mmol) was added, and the mixture was stirred for 1 h at rt. The reaction was quenched by the addition of Et_3N (70 µL, 0.5 mmol), the solvent was evaporated. The residue was partitioned between brine (50 mL) and CH_2Cl_2/MeOH (9:1, 2 × 100 + 3 × 50 mL). Combined organic extract was dried and was concentrated; the residue was co-evaporated with toluene (100 mL) to leave methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**10**, 9.27 g) as a colorless oil of sufficient purity to be used for the next step.

Methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**10**, 9.27 g, 39.57 mmol) was dissolved in toluene (200 mL), diisopropylethylamine (DIPEA, 8.62 mL, 49.47 mmol) was added and the mixture was cooled to 0 °C. Freshly distilled chloromethyl methyl ether (MOM-Cl, 3.76 mL, 49.47 mmol) was added drop wise (over 1 min period), the mixture was stirred for 1 h at 0 °C and for 18 h at rt. The reaction was quenched by the addition of saturated NH_4Cl solution and brine (50 mL each), the mixture was stirred vigorously for 10 min at rt, was transferred into a separatory funnel and the organic phase was collected. The aqueous phase was extracted with EtOAc (100 + 2 × 50 mL), combined organic extract was dried and was concentrated. The residue was subjected to FCC on 350 g SiO_2, hexanes/EtOAc (1:1). Evaporation of the eluate afforded pale yellow oil, 6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside [**11**, 4.01 g, 29%, based on D-fructose (**2**)]; $[\alpha]_D$ +21.9 (*c* 0.96, MeOH); R_F 0.42 [hexanes/EtOAc (1:1)], R_F 0.21 [petroleum ether/EtOAc (7:3)], the values were determined upon two developments of the same TLC plate. ^{1}H NMR ($CDCl_3$) δ 4.68 (s, 2H); 4.21 (ddd, *J* = 5.5, 2.5, 1.5 Hz, 1H); 4.02 – 3.87 (m, 4H); 3.75 (dd, *J* = 10.5, 7.0, 1H); 3.69 (dd, *J* = 9.5, 5.5, 1H); 3.38 (s, 3H); 3.32 (s, 3H); 2.52 (br s, D_2O exch., 1H); 1.44 (s, 3H); 1.36 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 101.9, 98.5, 96.6, 86.2, 79.4, 78.4, 68.4, 61.7, 55.2, 48.8, 27.7, 19.5. HRMS (ESI) *m/z* found 301.1259 $[M + Na]^+$ (301.1263 calcd for $C_{12}H_{22}O_7Na$).

6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-tagatofuranoside (13)

Dess-Martin periodinane (6.7 g, 15.81 mmol) was added to a stirred solution of 6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**11**, 4 g, 14.37 mmol) in CH_2Cl_2 (200 mL). The mixture was stirred for 90 min at rt, the reaction was quenched by the addition of $Na_2S_2O_3 \cdot 5H_2O$ solution (10%) and saturated $NaHCO_3$ solution (150 mL each). The mixture was stirred

vigorously for 10 min at rt, was transferred into a separatory funnel and the organic phase was collected. The aqueous phase was extracted with CH_2Cl_2 (2 × 60 mL), combined organic extract was dried and was concentrated to leave crude ketone **12** used for the next step without further purification.

The residue was dissolved in EtOH (450 mL) and the resulting solution was cooled to 0 °C. $NaBH_4$ (652 mg, 17.25 mmol) was added, the mixture was stirred for 15 min at 0 °C, the solvent was evaporated, and the residue was partitioned between brine (250 mL) and CH_2Cl_2 (120 + 2 × 60 mL). Combined organic extract was dried and was concentrated; the residue was subjected to FCC on 250 g SiO_2, petroleum ether/EtOAc (7:3). Evaporation of the eluate afforded pale yellow oil, 6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-tagatofuranoside (**13**, 3.21 g, 80%); $[\alpha]_D$ +24.6 (*c* 1.04, MeOH); R_F 0.32 [petroleum ether/EtOAc (7:3)], the value was determined upon two developments of the same TLC plate. ^{1}H NMR ($CDCl_3$) δ 4.68 (dd, *J* = 9.5, 6.5 Hz, 2H); 4.60 (dd, *J* = 6.5, 5.5 Hz, 1H); 4.22 (m, 1H); 3.98 (d, *J* = 5.5 Hz, 1H); 3.88 (dd, *J* = 15.5, 12.5 Hz, 2H); 3.84 (d, *J* = 3.0 Hz, 1H); 3.72 (dd, *J* = 11.0, 7.0 Hz, 2H); 3.36 (s, 3H); 3.22 (s, 3H); 2.75 (br s, D_2O exch., 1H); 1.45 (s, 3H); 1.41 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 100.3, 99.2, 96.7, 79.5, 73.2, 71.8, 66.6, 62.1, 55.2, 48.7, 27.3, 20.4. HRMS (ESI) *m/z* found 301.1257 $[M + Na]^+$ (301.1263 calcd for $C_{12}H_{22}O_7Na$).

6-MOM-D-tagatofuranose (14)

Amberlyst 15 (30 mg) was added to a stirred solution of 6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-tagatofuranoside (**13**, 140 mg, 0.5 mmol) in dioxane and water (1 mL each). The mixture was stirred for 48 h at rt, the resin was filtered off (Pasteur pipette with a cotton plug), the filter was washed with MeOH and the filtrate was concentrated. The residue was subjected to FCC on 10 g SiO_2, CH_2Cl_2/MeOH (9:1). Evaporation of the eluate afforded colorless oil, 6-MOM-D-tagatofuranose (**14**, 77 mg, 69%); $[\alpha]_D$ -7.0 (*c* 1.32, MeOH); R_F 0.11 [CH_2Cl_2/MeOH (9:1)]. ^{1}H NMR (CD_3OD) δ 4.63 (m, 2H); 4.59 – 4.06 (m, 3H); 3.88 – 3.44 (m, 4H); 3.37 (m, 3H). ^{13}C NMR (CD_3OD) δ 106.5, 104.2, 97.8, 98.7, 80.2, 79.4, 79.3, 73.2, 73.1, 72.1, 70.2, 68.5, 67.8, 64.2, 64.0, 55.5. HRMS (ESI) *m/z* found 247.0797 $[M + Na]^+$ (247.0794 calcd for $C_8H_{16}O_7Na$).

6-MOM-D-fructofuranose (16)

Amberlyst 15 (30 mg) was added to a stirred solution of 6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**11**, 140 mg, 0.5 mmol) in dioxane and water (1 mL each). The mixture was stirred for 96 h at rt, the resin was filtered off (Pasteur pipette with a cotton plug), the filter was washed with MeOH and the filtrate was concentrated. The residue was subjected to FCC on 10 g SiO_2, CH_2Cl_2/MeOH (9:1). Two fractions were obtained upon evaporation of eluate. Less polar fraction, colorless oil, 6-MOM-methyl-α-D-fructofuranoside (**15**, 33 mg, 28%), $[\alpha]_D$ +63.7 (*c* 0.60, MeOH); R_F 0.26 [CH_2Cl_2/MeOH (9:1)]. ^{1}H NMR (CD_3OD) δ 4.66 (s, 2H); 4.02 (d, *J* = 4.0 Hz, 1H); 3.93 (m, 1H); 3.84 (dd, *J* = 6.5, 4.0 Hz, 1H); 3.76 - 3.59 (m, 4H); 3.31 (s, 3H); 3.31 (s, 3H). ^{13}C NMR (CD_3OD) δ 109.3, 97.7, 83.1, 82.7, 79.7, 68.9, 60.2, 55.6, 48.8. HRMS (ESI) *m/z* found 261.0950 $[M + Na]^+$ (261.0950 calcd for $C_9H_{18}O_7Na$). More polar fraction, colorless oil, 6-MOM-D-fructofuranose (**16**, 62 mg, 55%); $[\alpha]_D$ -1.8 (*c* 0.88, MeOH); R_F 0.11 [CH_2Cl_2/MeOH (9:1)]. ^{1}H NMR (CD_3OD) δ 4.67 (s, 1.7H); 4.66 (s, 0.3H); 4.08 - 3.99 (m, 2H); 3.92 - 3.81 (m, 1H); 3.77 - 3.47 (m, 4H); 3.67 (m, 3H). ^{13}C NMR (CD_3OD) δ 106.0, 103.3, 97.7 (2 × C), 84.1, 81.8, 81.5, 78.8, 77.5, 77.2, 70.5, 68.9, 65.1, 64.5, 55.6 (2 × C). HRMS (ESI) *m/z* found 247.0789 $[M + Na]^+$ (247.0794 calcd for $C_8H_{16}O_7Na$).

D-Tagatose (1)

Amberlyst 15 (160 mg) was added to a stirred solution of 6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-tagatofuranoside (**13**, 756 mg, 2.72 mmol) in dioxane and 1 M HCl (5 mL each). The mixture was stirred for 48 h at rt, the resin was filtered off (Pasteur pipette with a cotton plug), the filter was washed with water, the filtrate was cooled to 0 °C. The pH was adjusted to ~ 6.5 – 7 (20% NaOH solution), the liquids were evaporated, and the residue was subjected to FCC on 40 g SiO_2, CH_2Cl_2/MeOH (4:1). Evaporation of the eluate afforded colorless oil, this was dissolved in small amount of water (~ 4 ml), was transferred into a Falcon tube and was lyophilized to afford colorless crystalline solid, D-tagatose (**1**, 338 mg, 69%); $[\alpha]_D$ -5.8 (*c* 0.83, water), lit. [20] $[\alpha]_D$ -5.0 (*c* 5.00, water); R_F 0.07 [CH_2Cl_2/MeOH (4:1)]. ^{1}H NMR (D_2O) δ 4.50 – 3.54 (m, 7H). ^{13}C NMR (D_2O) δ 102.5, 98.3, 98.1, 80.0, 70.9, 70.6, 69.8, 69.2, 66.3, 63.9, 63.7, 63.5, 62.6, 62.2, 60.9, 60.1. Spectral data matched those reported previously [4] and those associated with the commercial sample of D-tagatose (**1**).

[UL-$^{13}C_6$]-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (10a)

Amberlyst 15 (50 mg) was added to a stirred solution of D-[UL-$^{13}C_6$]fructose (**2a**, 465 mg, 2.5 mmol) in MeOH (25 mL). The mixture was stirred for 18 h at rt, the resin was filtered off, the filter was washed with MeOH and the filtrate was concentrated to leave colorless oil.

This material was dissolved in MeOH (2.5 mL), and 2,2-dimethoxypropane (acetone dimethyl acetal, 2.5 mL), *p*-TSA (5 mg, 2.6×10^{-5} mol) was added and the mixture was stirred for 1 h at rt. The reaction was quenched by the addition of saturated $NaHCO_3$ solution (2 drops), the solvent was evaporated, and the residue was subjected to FCC on 50 g SiO_2, CH_2Cl_2/MeOH (95:5). Evaporation of the eluate afforded colorless oil, [UL-$^{13}C_6$]-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**10a**, 308 mg, 51% over two steps); R_F 0.17 [CH_2Cl_2/MeOH (19:1)]. ^{1}H NMR ($CDCl_3$) δ 4.48 – 3.48 (m, 7H); 3.37 (d, *J* = 3.5 Hz, 0.3H); 3.33 (d, *J* = 3.5 Hz, 2.7H); 2.30 (br s, D_2O exch., 2H); 1.53 (s, 0.3H); 1.48 (s, 2.7H); 1.39 (s, 2.7H); 1.36 (s, 0.3H). ^{1}H NMR{^{13}C} ($CDCl_3$) δ 4.23 – 3.72 (m, 7H); 3.37 (s, 0.3H); 3.33 (s, 2.7H); 2.30 (br s, D_2O exch., 2H); 1.53 (s, 0.3H); 1.48 (s, 2.7H); 1.39 (s, 2.7H); 1.36 (s, 0.3H). ^{13}C NMR ($CDCl_3$) δ 101.9 – 100.6 (m); 98.7 – 97.4 (m); 88.3 – 87.3 (m); 80.1 – 79.5 (m); 78.1 – 75.8 (m); 73.5 – 71.7 (m); 63.5 – 59.9 (m); 48.7 (q, *J* = 2.0 Hz); 27.8 (t, *J* = 2.0 Hz); 19.2 (t, *J* = 1.5 Hz). HRMS (ESI) *m/z* found 263.1213 $[M + Na]^+$ (263.1202 calcd for $^{12}C_4{}^{13}C_6H_{18}O_6Na$).

[UL-$^{13}C_6$]-6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (11a)

[UL-$^{13}C_6$]-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**10a**, 308 mg, 1.28 mmol) was dissolved in toluene (13 mL), DIPEA (280 μL, 1.6 mmol) was added and the mixture was cooled to 0 °C. Freshly distilled MOM-Cl (120 μL, 1.6 mmol) was added, the mixture was stirred for 1 h at 0 °C and for 1 h at rt. The reaction was quenched by the addition of saturated NH_4Cl solution and brine (7 mL each), the mixture was stirred vigorously for 10 min at rt, was transferred into a separatory funnel and the organic phase was collected. The aqueous phase was extracted with EtOAc (3 × 20 mL), combined organic extract was dried and was concentrated. The residue was subjected to FCC on 60 g SiO_2, hexanes/EtOAc (1:1). Evaporation of the eluate afforded pale yellow oil, [UL-$^{13}C_6$]-6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**11a**, 161 mg, 44%); R_F 0.42 [hexanes/EtOAc (1:1)], R_F 0.21 [petroleum ether/EtOAc (7:3)], the values were determined upon two developments of the same TLC plate. ^{1}H NMR ($CDCl_3$) δ 4.69 (d, *J* = 6.0 Hz, 2H); 4.49 – 4.12 (m, 3H); 4.00 – 3.92 (m, 1H); 3.78 – 3.43 (m, 3H); 3.39 (d, *J* = 0.5 Hz, 3H); 3.32 (d, *J* = 4.0 Hz, 3H); 2.32 (br s, D_2O exch., 1H); 1.45

(s, 3H); 1.37 (s, 3H). ^{1}H NMR{^{13}C} ($CDCl_3$) δ 4.69 (s, 2H); 4.23 – 4.19 (m, 1H); 4.02 – 3.87 (m, 4H); 3.78 – 3.67 (m, 2H); 3.39 (s, 3H); 3.32 (s, 3H); 2.33 (br s, D_2O exch., 1H); 1.45 (s, 3H); 1.37 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 102.5 – 101.3 (m); 86.9 – 85.8 (m); 80.1 – 77.8 (m); 68.7 – 68.1 (m); 62.1 – 61.4 (m); 61.8 (d, *J* = 2.5 Hz); 55.3 (s); 48.8 (d, *J* = 3.0 Hz); 27.8 (t, *J* = 2.0 Hz); 19.5 (s). HRMS (ESI) *m/z* found 307.1474 $[M + Na]^+$ (307.1465 calcd for $^{12}C_6{}^{13}C_6H_{22}O_7Na$).

[UL-$^{13}C_6$]-6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-tagatofuranoside (13a)

Dess-Martin periodinane (259 mg, 0.61 mmol) was added to a stirred solution of [UL-$^{13}C_6$]-6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-fructofuranoside (**11a**, 158 mg, 0.56 mmol) in CH_2Cl_2 (9 mL). The mixture was stirred for 90 min at rt, the reaction was quenched by the addition of $Na_2S_2O_3 \cdot 5H_2O$ solution (10%) and saturated $NaHCO_3$ solution (10 mL each). The mixture was stirred vigorously for 10 min at rt, was transferred into a separatory funnel and the organic phase was collected. The aqueous phase was extracted with CH_2Cl_2 (20 + 10 mL), combined organic extract was dried and was concentrated to leave crude ketone **12a** used for the next step without further purification.

The residue was dissolved in EtOH (32 mL) and the resulting solution was cooled to 0 °C. $NaBH_4$ (25 mg, 0.67 mmol) was added, the mixture was stirred for 15 min at 0 °C, the solvent was evaporated, and the residue was partitioned between brine (30 mL) and CH_2Cl_2 (2 × 20 + 10 mL). Combined organic extract was dried and was concentrated; the residue was subjected to FCC on 45 g SiO_2, petroleum ether/EtOAc (7:3). Evaporation of the eluate afforded pale yellow oil, [UL-$^{13}C_6$]-6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-tagatofuranoside (**13a**, 136 mg, 86%); R_F 0.32 [petroleum ether/EtOAc (7:3)], the value was determined upon two developments of the same TLC plate. ^{1}H NMR ($CDCl_3$) δ 4.89 – 4.67 (m, 2H); 4.50 – 3.90 (m, 5H); 3.75 – 3.47 (m, 2H); 3.39 (s, 3H); 3.25 (d, *J* = 4.0 Hz, 3H); 2.82 (br d, *J* = 6.0 Hz, D_2O exch., 1H); 1.48 (s, 3H); 1.44 (s, 3H). ^{1}H NMR{^{13}C} ($CDCl_3$) δ 4.71 (dd, *J* = 9.5, 6.5 Hz, 2H); 4.66 – 4.60 (m, 1H); 4.28 – 4.22 (m, 1H); 4.02 – 3.86 (m, 4H); 3.78 – 3.66 (m, 1H); 3.39 (s, 3H); 3.25 (s, 3H); 2.81 (br d, *J* = 8.5 Hz, D_2O exch., 1H); 1.48 (s, 3H); 1.43 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 101.0 – 99.8 (m); 80.2 – 79.0 (m); 73.8 – 71.3 (m); 67.0 – 66.3 (m); 62.5 – 61.8 (m); 60.5 (q, *J* = 2.5 Hz); 59.9 (m); 59.2 (d, *J* = 2.5 Hz); 55.3 (s); 48.8 (q, *J* = 2.0 Hz); 27.4 (t, *J* = 1.5 Hz); 20.5 (s). HRMS (ESI) *m/z* found 307.1455 $[M + Na]^+$ (307.1465 calcd for $^{12}C_6{}^{13}C_6H_{22}O_7Na$).

D-[UL-$^{13}C_6$]tagatose (1a)

Amberlyst 15 (35 mg) was added to a stirred solution of [UL-$^{13}C_6$]-6-MOM-methyl-1,3-di-*O*-isopropylidene-α-D-tagatofuranoside (**13a**, 169 mg, 0.6 mmol) in dioxane and 2 M HCl (1.1 mL each). The mixture was stirred for 24 h at rt, the resin was filtered off (Pasteur pipette with a cotton plug), the filter was washed with water, the filtrate was cooled to 0 °C. The pH was adjusted to ~ 6.5 – 7 (20% NaOH solution), the liquids were evaporated, and the residue was subjected to FCC on 20 g SiO_2, CH_2Cl_2/MeOH (4:1). Evaporation of the eluate afforded colorless oil; this was dissolved in small amount of water (~ 4 ml), was transferred into a Falcon tube and was lyophilized to afford colorless crystalline solid, D-[UL-$^{13}C_6$]tagatose (**1a**, 92 mg, 83%); $[\alpha]_D$ -2.9 (*c* 0.52, water); R_F 0.07 [CH_2Cl_2/MeOH (4:1)] . ^{1}H NMR (D_2O) δ 4.58 - 3.25 (m, 7H). ^{1}H NMR{^{13}C} (D_2O) δ 4.35 - 3.37 (m, 7H). ^{13}C NMR (D_2O) δ 98.8 –97.5 (m); 71.4 – 68.7 (m); 66.8 – 65.8 (m); 64.2 – 61.9 (m); 61.2 – 59.9 (m). HRMS (ESI) *m/z* found 209.0743 $[M + Na]^+$ (209.0733 calcd for $^{13}C_6H_{12}O_6Na$).

Declaration of competing interests

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Detailed spectroscopic characterization associated with compounds **1**, **1a**, **10**, **10a**, **11**, **11a**, **13**, **13a**, **14-16**.

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16. Intermediate **10** has been prepared previously by other routes, see

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18. Decomposition of intermediate ketone when converting **8** to **9** (Scheme 1), like that observed by us (ketone **12**, Scheme 2) was described in ref. 12.

19. While multigram quantities of D-[UL-$^{13}C_6$]fructose (**2a**) are available from multiple commercial sources, we have been able to find only two commercial sources of D-[UL-$^{13}C_6$]tagatose (**1a**). We were interested in purchasing up to 10 mg of **1a** as a spectroscopic standard, the quote requested from one of the commercial suppliers informed us that 10 mg will be US$ 300 and it can be delivered within one month.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: