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Production of 1,4:3,6-dianhydro- α -D-glucopyranose from methyl 3,6-anhydro- α -D-glucopyranoside and its taste identification†

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1,4:3,6-Dianhydro- α -D-glucopyranose (DGP) is one of important biomass pyrolysis anhydro sugar products that derive from the cellulose and hemicellulose components. There is no reliable method for the preparation of DGP at present, which contributed to its high cost with limited market supply and restricted applied research. In this study, we provided a novel method for the synthesis of DGP from methyl 3,6-anhydro- α -D-glucopyranoside for the first time. A mild and environmentally friendly synthetic approach for 3,6-anhydro glucopyranoside was developed *via* the intramolecular cyclization of 6-O-tosyl glucopyranoside, promoted by a catalytic amount of TBAF. And the preparation of DGP was achieved through the stabilization effect on carbocation intermediates by HFIP in the intramolecular cyclization of 3,6-anhydro glucopyranoside. Further sensory evaluation studies revealed that DGP had a sweetness similar to that of sucrose.

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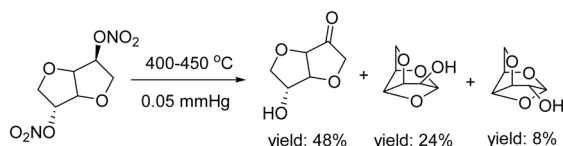
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1 Introduction

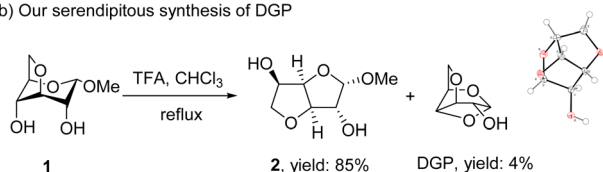
1,4:3,6-Dianhydro- α -D-glucopyranose (DGP) is a dehydration sugar compound with rigid, multi-oxygen-bridged, multi-ring cage-like chiral structure, without C=O or C=C unsaturated

bonds (Scheme 1).¹ This unique polyether cage structure endows DGP with good water solubility and potential applications in pharmaceutical chemistry and food.^{2,3} DGP was first isolated and identified by Tischenko *et al.* in 1948 from the gasification products of wood.^{4,5} And it is one of important biomass pyrolysis anhydro sugar products, such as levoglucosan (LG), 1-hydroxy-3,6-dioxabicyclo[3.2.1]octan-2-one (LAC), levoglucosenone (LGO), and DGP, that derived from the cellulose and hemicellulose components.^{6,7} The selective production and application in organic synthesis of LG, LAC, and LGO has been widely reported.^{8–15} The selective production of DGP from cellulose fast pyrolysis has recently been achieved for the first time by Lu *et al.*, achieving a 14% yield at 400 °C.^{3,16} However, this process remained at the laboratory research stage and has not yet been applied in industry. Additionally, the synthesis of DGP through chemical synthesis method was only discovered by Wiersum *et al.* during the study of the high-temperature rearrangement reaction of isosorbide dinitrate, giving a mixture containing DGP with a yield of approximately 8% (Scheme 1a).¹⁷ Therefore, the current DGP in market is still produced by collecting and separating cellulose pyrolysis gas, contributing to the high cost of DGP with limited market supply (cost 5 mg > \$200, through a comprehensive search on Sci-Finder), and thus its application has been rarely reported.³ While the long-term goal is to achieve large-scale, cost-effective production of DGP through cellulose fast pyrolysis, developing effective conventional chemical synthesis methods suitable for laboratory-scale preparation is crucial for promoting its application in food, pharmaceuticals, and other fields. Previously, our group discovered an unintentional side production of DGP

(a) Only reported chemical synthesis of DGP by Wiersum *et al.*



(b) Our serendipitous synthesis of DGP



Scheme 1 Only reported chemical synthesis and our serendipitous synthesis of DGP.

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with a 4% yield during the investigation of the acid-catalytic rearrangement of methyl 3,6-anhydro- α -D-glucopyranoside **1** for the conversion to its furanose form **2** (Scheme 1b). The structure of DGP was unambiguously confirmed by X-ray crystallographic analysis (ESI, Fig. S1†). Additionally, we have preliminarily determined that DGP possessed a certain degree of sweet taste. Inspired by our previous research on the taste identification of anhydro sugar 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP),^{18,19} this work aimed to further optimize reaction conditions, develop an efficient chemical synthesis method for DGP and investigate the sweetness performance of DGP for the first time.

2 Results and discussions

2.1 Synthesis of methyl 3,6-anhydro- α -D-glucopyranoside **1**

Methyl 3,6-anhydro- α -D-glucopyranoside **1** was usually synthesized by intramolecular cyclization of methyl 6-O-tosyl- α -D-glucopyranoside **3** under strong basic conditions such as sodium hydroxide or sodium methoxide.²⁰ Recently, Nitz *et al.* demonstrated a novel approach to construct various 3,6-anhydrohexosides with excellent yields by treating 6-O-tosylpyranosides with tetrabutylammonium fluoride (TBAF) (ESI, Scheme S1a†).²¹ However, the primary drawback lay in the requirement of 7.5 equivalents of TBAF. Excessive TBAF was neither environmentally friendly nor economically efficient, and presented substantial challenges in product purification, particularly in large-scale synthesis. Water washing resulted in substantial loss of certain water-soluble product **1** in presence

of large amount of TBAF. Additionally, concentrating the solution and employing direct column chromatography necessitated the use of significant quantities of silica gel and eluent. When the amount of TBAF was reduced to 1.2 equivalents, there was a marked increase in reaction time and a noticeable decrease in yield (Table 1, entry 1). Considering the proposed reaction mechanism in the literature,²¹ we hypothesized that an excess of TBAF was unnecessary. This is based on the idea that fluoride ion could be released to promote the reaction with the addition of a small amount of weak base to neutralize the HF generated during the reaction (ESI, Scheme S1b†). Based on the above analysis, we optimized the intramolecular cyclization of 6-O-tosyl-glucopyranoside **3** promoted by a catalytic amount of TBAF, providing a more efficient synthetic approach for 3,6-anhydro glucopyranoside **1**.

6-O-Tosyl-glucopyranoside **3** was synthesized following methods described in the literature.²¹ Subsequently, the reaction conditions for the preparation of **1** were investigated in detail (Table 1). It was discovered that using 0.1 equivalents of TBAF in conjunction with bases like NaHCO₃, Na₂CO₃, or K₂CO₃, efficiently afforded the target product in high yields (entries 2–4). Conversely, the use of organic bases such as DIPEA and pyridine resulted in negligible reaction progress (entries 5–6). Considering both reaction time and yield, Na₂CO₃ emerged as the most effective co-catalyst. Further investigation into the impact of the reaction solvent revealed THF as the optimal choice (entries 7–9). Additional experiments demonstrated that omitting TBAF or replacement by TBAB halted the reaction (entries 10–11), underscoring the crucial role of fluoride ion. TBAF exhibited superior catalytic activity in comparison of NaF (entries 12). The equivalent catalytic efficiency of the more economical TBAF·3H₂O was also confirmed (entry 2c). In summary, the optimal reaction conditions were identified as using TBAF·3H₂O (0.1 eq.), Na₂CO₃ (1.2 eq.), with reflux in THF under nitrogen atmosphere. The high tolerance for scale-up experiment was also further verified with starting material **4** of 50 grams, giving 27.2 grams 3,6-anhydro glucopyranoside **1** with similar yield, thus supporting subsequent research (Scheme 2).

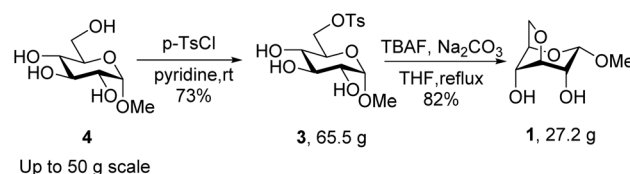
Table 1 Screen of reaction conditions of intramolecular cyclization of **3**^a

Entry	Conditions	Time	1 yield ^b
1	TBAF (1.2 eq.), THF/DMF, 70 °C	64 h	62%
2	TBAF (0.1 eq.), Na ₂ CO ₃ (1.2 eq.), THF, reflux	16 h	86% ^c 85%
3	TBAF (0.1 eq.), K ₂ CO ₃ (1.2 eq.), THF, reflux	12 h	83%
4	TBAF (0.1 eq.), NaHCO ₃ (1.2 eq.), THF, reflux	30 h	75%
5	TBAF (0.1 eq.), DIPEA (1.2 eq.), THF, reflux	48 h	trace ^d
6	TBAF (0.1 eq.), pyridine (1.2 eq.), THF, reflux	48 h	trace ^d
7	TBAF (0.1 eq.), Na ₂ CO ₃ (1.2 eq.), DCE, reflux	40 h	63%
8	TBAF (0.1 eq.), Na ₂ CO ₃ (1.2 eq.), toluene, reflux	40 h	50%
9	TBAF (0.1 eq.), Na ₂ CO ₃ (1.2 eq.), MeCN, reflux	16 h	79%
10	Na ₂ CO ₃ (1.2 equiv.), THF, reflux	48 h	trace ^d
11	TBAB (0.1 eq.), Na ₂ CO ₃ (1.2 eq.), THF, reflux	48 h	trace ^d
12	NaF (0.1 eq.), Na ₂ CO ₃ (1.2 eq.), THF, reflux	48 h	trace ^d

^a All reactions were performed on 0.2 mmol scale and using anhydrous TBAF unless otherwise stated. ^b Isolated yield. ^c Using TBAF·3H₂O. ^d Yield < 5%.

2.2 Synthesis of DGP

Possible reaction mechanisms for the conversion of **1** to DGP and **2** were proposed before optimizing the conditions for DGP generation (Fig. 1). Under acid catalysis, the bridged structure of **1** underwent ring-opening rearrangement to form the less strained fused-ring structure of furanoside **2**, which has been reported in previous literature.²² On the other hand, the acetal



Scheme 2 Synthesis of methyl 3,6-anhydro- α -D-glucopyranoside **1**.



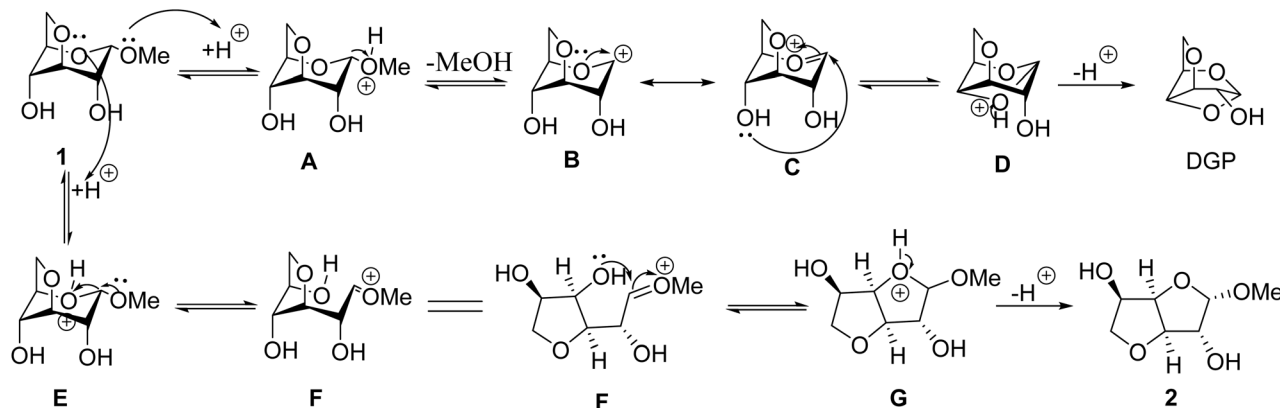


Fig. 1 Proposed mechanisms for the conversion of **1** to DGP and **2**.

moiety in **1** eliminated a molecule of methanol catalyzed by acid to form a glycosyloxonium ion **B** and **C**, which was then attacked by the 4-position hydroxyl to form a new acetal structure,

Table 2 Optimization of reaction conditions for the preparation of DGP^a

Entry	Conditions	Time	Yield ^b : DGP/2	
1	TFA (0.3 eq.), CHCl ₃ , reflux	1 h	4%/85%	
2	TFA (0.3 eq.), CHCl ₃ , 25 °C	48 h	0/87%	
3	TFA (0.3 eq.), MeCN, reflux	1 h	10%/71%	
4	TFA (0.3 eq.), DCE, reflux	1 h	Trace/67%	
5	<i>p</i> -TsOH (0.3 eq.), MeCN, reflux	1 h	9%/47%	
6	<i>p</i> -TsOH (0.3 eq.), THF, reflux	1 h	5%/36%	
7	<i>p</i> -TsOH (0.3 eq.), DCE, reflux	1 h	Trace/65%	
8	<i>p</i> -TsOH (0.3 eq.), THF, reflux	1 h	Trace/44%	
9	AcOH (1.0 eq.), CHCl ₃ , reflux	6 h	0/Trace	
10	HCOOH (0.3 eq.), CHCl ₃ , reflux	3 h	0/55%	
11	FeCl ₃ (0.3 eq.), MeCN, reflux	8 h	12%/49%	
12	FeCl ₃ (0.5 eq.), MeCN, reflux	8 h	9%/52%	
13	FeCl ₃ (0.1 eq.), MeCN, reflux	16 h	4%/66%	
14	FeCl ₃ (0.3 eq.), DCE, reflux	8 h	0/60%	
15	FeCl ₃ (0.3 eq.), THF, reflux	8 h	0/42%	
16	BF ₃ ·Et ₂ O (0.3 eq.), THF, 0 °C-reflux	2 h	0/21%	
17	BF ₃ ·Et ₂ O (0.3 eq.), MeCN, 0 °C-reflux	2 h	0/Trace	
18	NaH (1.2 eq.), THF, reflux	24 h	0/0	
19	<i>p</i> -TsOH (0.3 eq.), TFE, reflux	1 h	9%/38%	
20	<i>p</i>-TsOH (0.3 eq.), HFIP, reflux	1 h	33%/35%	
21	TFA (0.3 eq.), HFIP, reflux	1 h	19%/40%	
22	FeCl ₃ (0.3 eq.), HFIP, reflux	1 h	Trace/trace	
23	H ₂ SO ₄ (0.3 eq.), HFIP, reflux	1 h	10%/25%	
24	CF ₃ SO ₃ H (0.3 eq.), HFIP, reflux	1 h	Trace/22%	
25	<i>p</i> -TsOH (0.1 eq.), HFIP, reflux	1 h	12%/44%	
26	<i>p</i> -TsOH (0.5 eq.), HFIP, reflux	1 h	17%/32%	

^a Reaction conditions: **3** (0.2 mmol), acid and solvent (2 mL) was stirred under argon atmosphere, and monitored by TLC until all **1** was consumed. ^b Isolated yield.

leading to the formation of DGP.^{23,24} Thus, the formation of DGP occurred *via* an acid-catalytic intramolecular transacetalization originating from **1**.

Based on the aforementioned mechanistic analysis, the reaction conditions were further optimized to improve the selectivity of the DGP formation pathway and the results were summarized in Table 2. A series of Brønsted and Lewis acid catalysts in different solvents was investigated, while the yields were generally low disappointingly (entries 1–17). It was found that only product **2** was generated at room temperature, while the formation of DGP required higher temperatures and highly polar solvents. On the other hand, according to the transacetalization of 2-formylpyrrole acetals under strongly basic conditions in the literature,²⁵ NaH was also explored as a potential catalyst. However, this investigation revealed no formation of the desired products DGP and **2** (entry 18).

By analyzing the competition of the formation of **2** and DGP, we hypothesized that the stability of the carbocation intermediate **B** and **C** may be the key factor in enhancing the DGP formation pathway. In addition, strongly hydrogen bond-donating, polar solvents trifluoroethanol (TFE) and hexafluoroisopropanol (HFIP) have found numerous uses in organic synthesis due to its ability to stabilize ionic species, transfer protons, and engage in a range of other intermolecular interactions.^{26,27} For example, in the metal-free Markovnikov-type alkyne hydration reported by Li *et al.*, the carbocation was strongly stabilized in TFE, which facilitated the progression of the reaction.²⁶ Therefore, we investigated the reaction conditions using TFE and HFIP as solvent to stabilize carbocation intermediate **B** and **C**, promoted by different acid catalysts (entries 19–26). Pleasingly, when HFIP was used as the solvent and *p*-TsOH (0.3 eq.) as the acid catalyst, the reaction produced DGP with a 33% yield (entry 20). Although the yield was not high, it sufficed for the rapid preparation of DGP and the study of its taste identification.

To enhance the availability of high-purity materials for the taste evaluation, we have innovatively developed a vacuum sublimation purification technique for DGP, drawing upon previously reported thermogravimetric analysis.⁵ A sample of DGP, isolated through column separation, was subjected to



Table 3 Taste quality and taste threshold

	DGP	1	2
Taste quality ^a	Sweet taste	Tasteless	Tasteless
Relative sweetness ^b (relative to sucrose/times)	1.0	—	—
Sweet taste threshold ^a (% or mmol L ⁻¹ in water)	0.2% or 14 mmol L ⁻¹	—	—

^a Taste quality and detection thresholds of taste compounds were determined in a triangle test. ^b Compare DGP aqueous solutions to a 5% sucrose aqueous solution to determine the concentration at which the sample's sweetness intensity matches that of the sucrose solution and divide this concentration by the sucrose solution concentration (5%).

sublimation using a glass sublimation apparatus under a controlled pressure of 0.15 mm Hg at 120 °C, yielding purified DGP.

2.3 Taste identification

A taste test was carried out on the DGP and 3,6-anhydrohexoside **1** and **2** (Table 3). The results showed that the DGP exhibited a sweet taste at a threshold of 14 mmol L⁻¹ (0.2%) in water, while **1** and **2** were tasteless even at 57 mmol L⁻¹ (1%). The sweetness of DGP relative to sucrose was further determined, and it was found to have a sweetness comparable to that of sucrose. Although DGP did not have exceptionally high sweetness, its moderate thermal volatility may provide it with potential application value in certain specific areas of sweetness adjustment.

3 Experimental section

3.1 General considerations

Acetonitrile (CH₃CN) and Dichloroethane (DCE) was dried with CaH₂ and distilled. Toluene and tetrahydrofuran (THF) were dried with Na and distilled. All other commercial reagents were used as received without additional purification. ¹H NMR and ¹³C NMR spectra were recorded on 400 MHz and 100 MHz spectrometers respectively with TMS as the internal standard, using CDCl₃ or CD₃OD as solvent. Data for ¹H NMR spectra are reported as follows: chemical shift δ (ppm), referenced to TMS; Multiplicities are indicated as the following: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; coupling constants (Hz) and integration. HRMS was recorded on an LC TOF (ES).

3.2 Synthesis

3.2.1 ((2R,3S,4S,5R,6S)-3,4,5-Trihydroxy-6-methoxytetrahydro-2H-pyran-2-yl)methyl 4-methylbenzenesulfonate diethyl 3 (ref. 21). To a stirred mixture of methyl α -D-glucoside (50.0 g, 257.5 mmol) in pyridine (250.0 mL) was added TsCl (61.4 g, 1.25 eq.) in portions under nitrogen atmosphere at 0 °C. The resulting solution was stirred for 3 days at room temperature. Then rotary evaporate the reaction mixture to dryness, and perform azeotropic distillation with toluene three times to remove pyridine, obtaining an oily crude product. Purification of the resulting residue by flash column chromatography (eluting with DCM:MeOH = 50:1 to 20:1, v/v) afforded the product **3**. (65.5 g, 73%). ¹H NMR (400 MHz, CD₃OD) δ 7.77 (d, J

= 8.0 Hz, 2H), 7.42 (d, J = 8.0 Hz, 2H), 4.53 (d, J = 8.0 Hz, 1H), 4.28 (dd, J = 12.0, 4.0 Hz, 1H), 4.13 (dd, J = 12.0, 8.0 Hz, 1H), 3.79–3.66 (m, 1H), 3.66–3.58 (m, 1H), 3.35–3.21 (m, 3H), 3.13–3.18 (m, 1H), 2.43 (s, 3H). ¹³C NMR (100 MHz, CD₃OD) δ 146.5, 134.5, 131.0, 129.1, 101.2, 74.9, 73.2, 71.3, 71.1, 71.0, 55.7, 21.6.

3.2.2 (1R,3S,4R,5S,8R)-3-Methoxy-2,6-dioxabicyclo[3.2.1]octane-4,8-diol **1 (ref. 21).** To a stirred mixture of **3** (65.5 g, 188.0 mmol) and Na₂CO₃ (23.9 g, 1.2 eq.) in THF (500 mL) was added TBAF·3H₂O (6.0 g, 0.1 eq.) under nitrogen atmosphere. The resulting solution was stirred for 16 h under reflux and then cooled to room temperature, quenched with saturated H₂O (1.5 L). The aqueous layer was separated and extracted with DCM (3 $\times$ 500 mL). The combined organic layer was dried over Na₂SO₄, filtered, and concentrated in vacuum. Purification of the resulting residue by flash column chromatography (eluting with DCM:MeOH = 50:1 to 20:1, v/v) afforded the product **1**. (27.2 g, 82%). ¹H NMR (400 MHz, CD₃OD) δ 4.93 (d, J = 3.0 Hz, 1H), 4.23 (t, J = 2.4 Hz, 1H), 4.17–4.12 (m, 2H), 4.12–4.08 (m, 1H), 3.93 (dd, J = 12.0, 3.2 Hz, 1H), 3.78 (s, 1H), 3.56 (s, 3H). ¹³C NMR (100 MHz, CD₃OD) δ 99.78, 76.9, 73.3, 72.7, 71.7, 69.9, 57.5. HRMS (ESI-TOF): calcd. for C₇H₁₂NaO₅ [M + Na]⁺ 199.0582; found 199.0580.

3.2.3 (2S,3R,3aR,6R,6aR)-2-Methoxyhexahydrofuro[3,2-b]furan-3,6-diol **2 (ref. 22).** To a stirred mixture of **1** (1.0 g, 5.7 mmol) in CHCl₃ (30.0 mL) was added TFA (43.0 μ L, 0.1 eq.). The resulting solution was stirred for 1.0 h under reflux and then evaporating the reaction solution to dryness, using a 5:1 mixture of petroleum ether and ethyl acetate (20.0 mL) to slurry wash, then filter to obtain the product **2** (850 mg, 85%). ¹H NMR (400 MHz, CDCl₃) δ 5.08 (d, J = 4.4 Hz, 1H), 4.63 (t, J = 5.4 Hz, 1H), 4.45 (dd, J = 5.3, 2.8 Hz, 1H), 4.22 (dt, J = 6.9, 5.7 Hz, 1H), 4.17 (dd, J = 4.4, 2.8 Hz, 1H), 3.94 (dd, J = 9.3, 5.9 Hz, 1H), 3.61–3.54 (m, 1H), 3.52 (d, J = 3.5 Hz, 3H), 2.69–2.39 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 104.9, 87.8, 79.4, 77.9, 72.0, 71.3, 56.0. HRMS (ESI-TOF): calcd. for C₇H₁₂NaO₅ [M + Na]⁺ 199.0582; found 199.0584.

3.2.4 1,4,3,6-Dianhydro- α -D-glucopyranose DGP.¹ To a stirred mixture of **1** (1.0 g, 5.7 mmol) in HFIP (60.0 mL) was added *p*-TsOH·H₂O (325 mg, 0.3 eq.). The resulting solution was stirred for 1.0 h under reflux and then evaporating the reaction solution to dryness. Purification of the resulting residue by flash column chromatography (eluting with PE:EA = 5:1 to 2:1, v/v) afforded the product DGP. (279 mg, 33%). ¹H NMR (400 MHz, CDCl₃) δ 5.43 (s, 1H), 5.17 (t, J = 4.0 Hz, 1H), 4.22 (s, 1H), 4.12 (d, J = 4.0 Hz, 1H), 4.03 (d, J = 12.0 Hz, 1H), 3.92 (d, J = 12.0 Hz, 1H), 3.76 (s, 1H), 1.86 (s, 1H). ¹³C NMR (100 MHz, CDCl₃)



δ 101.2, 82.9, 79.5, 79.1, 75.9, 71.8. HRMS (ESI-TOF): calcd. for $C_6H_8NaO_4 [M + Na]^+$ 167.0320; found 167.0321. A 1.0 g sample of DGP, isolated through column separation, was subjected to sublimation using a glass sublimation apparatus under a controlled pressure of 0.15 mm Hg and a temperature of 120 °C, yielding 850 mg purified DGP.

3.3 Sensory analyses

Training of the sensory panel and determination of taste followed the methods previously described.^{18,28,29} Assessors were trained to evaluate sweet taste intensity using sucrose (0.5%, 2.5%, 5% in water). Sensory analyses were performed using tap water (pH 6.5) as the solvent in a panel room at 22–25 °C in three different sessions by six panelists (3 women, 3 men) whose data were averaged. The detection thresholds of taste compounds were determined in a triangle test and the samples were presented in order of increasing concentrations (serial 1 : 1 dilutions). Relative sweetness of taste compounds was determined as follows. Prepare a series of aqueous solutions with varying concentrations of the sample. Compare these to a 5% sucrose aqueous solution to determine the concentration at which the sample's sweetness intensity matches that of the sucrose solution. Then, divide this concentration by the sucrose solution concentration (5%) to obtain the relative sweetness of the sample.

4 Conclusions

In conclusion, a reliable method for the preparation of DGP from methyl 3,6-anhydro- α -D-glucopyranoside **1** has been developed for the first time. The 3,6-anhydro glucopyranoside **1** was synthesized *via* an intramolecular cyclization reaction of 6-O-tosyl glucopyranoside promoted by a catalytic amount of TBAF. This new approach for the preparation of 3,6-anhydro glucopyranoside **1** proceeded under mild and environmentally friendly conditions, avoiding the use of strong bases and large excesses of TBAF. And we found that the stabilizing effect of HFIP on carbocation intermediates significantly improved the yield of the intramolecular cyclization of 3,6-anhydro glucopyranoside in the construction of DGP by systematic optimization. Further sensory evaluation studies revealed that DGP had a sweetness similar to that of sucrose. Overall, this work provided an efficient chemical synthesis method for DGP and investigated the sweet performance of DGP for the first time, providing the potential to propel research on the applications of DGP in the fields of pharmaceutical chemistry and the food sector.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. All data supporting the findings of this study are included in the article and its ESI files.† Any additional data related to this paper may be requested from the authors.

Author contributions

Zhifei Chen, Qingfu Wang and Changtong Lu proposed and designed the experiment; Qingfu Wang and Kai Yang wrote the paper; Yibo Ning, Lei Li, Xueying Cao and Gaolei Xi processed the data; Kai Yang and Changtong Lu corrected the language of the paper. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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