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Synthesis of α-D-fructofuranosides and difructose dianhydride IV using glycosyl fluorides as donors

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ABSTRACT

α-D-Fructofuranoside derivatives are prevalent in natural products, exhibiting diverse bioactivities. Herein, a stereoselective glycosylation method for synthesizing α-D-fructofuranosides using fructofuranosyl fluorides as donors is described. Glycosylation of 1,3,4,6-tetra-*O*-benzoyl-2-D-fructofuranosyl fluoride, promoted by tris(pentafluorophenyl)borane, proved highly effective, yielding glycosylation products in high yields (up to 98%) with primary alcohols, thiols, *N*- and *C*-nucleophiles. Furthermore, β-(2→6) cyclodisaccharide di-D-fructose-2,6′:6,2′-dianhydride (DFA IV), a disaccharide enzymatically derived from levan, was chemically synthesized through orthogonal glycosylation reactions and hydrogen-bond-mediated aglycone delivery involving thioglycosides and glycosyl fluorides for the first time.

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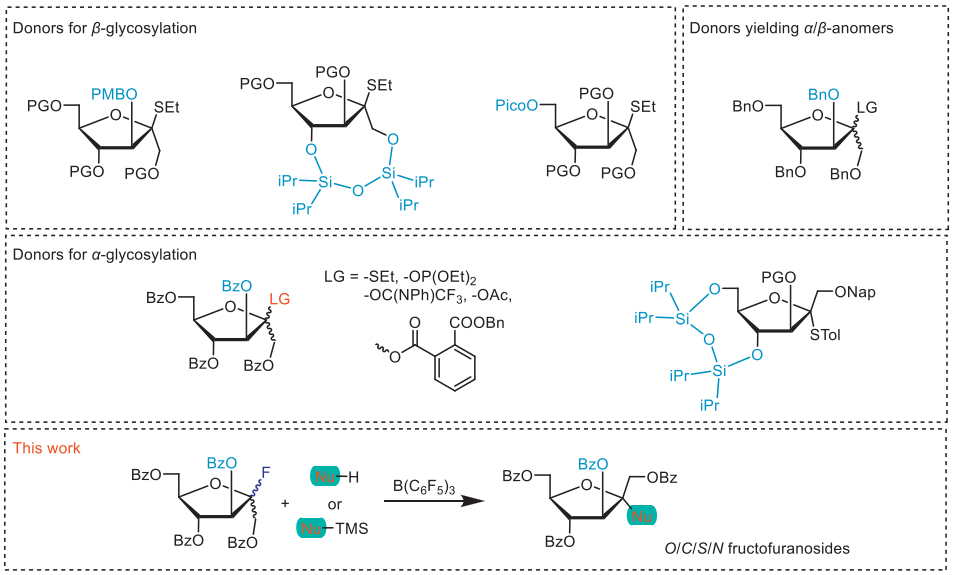
Fructofuranosides are widely distributed in nature and they play crucial roles in biological processes. These compounds are found in plants and bacteria, as constituents of sucrose, β-(2→1)-linked inulins, β-(2→6)-linked levans, and α- or β-fructofuranosides [1–3]. In addition to their biological functions, such as energy storage and structural roles in cells, fructofuranosides also exhibit pharmacological properties, including antiviral, antioxidant, antitumor, and anti-inflammatory activities [4,5]. Compared with enzymatic synthesis, chemical glycosylation methods offer easier product isolation, broader substrate compatibility, and greater suitability for synthesizing structurally defined fructosides and derivatives. Despite their importance, the chemical synthesis of fructofuranosides, especially with high stereoselectivity and yield, remains a significant challenge in carbohydrate chemistry.

One major obstacle to synthesizing fructofuranosides is the difficulty in controlling the stereochemistry during the glycosylation process. Employing donors with nonparticipating groups like benzyl often results in a mixture of α- and β-anomers, complicating their separation. Several methods have been developed for synthesizing β-D-fructofuranosides. The internal aglycone delivery (IAD) method developed by Oscarson and colleagues involves the use of thiofructoside donors and is notable for its β-stereoselectivity (Scheme 1). However, the intermediate acetal formed by the protocol is unstable, and this strategy fails in the synthesis of complex bacterial oligosaccharides [6,7]. The other strategy uses conformationally restricted donors that possess a siloxane spanning *O*1 and *O*4 in fructofuranose rings to block α-face attack and promote the formation of β-D-fructofuranosides. However, the drawback of this method is the high cost and complexity involved in preparing the fructosyl donors, limiting its broader application in synthetic chemistry [8–10]. Our previous work developed an elegant hydrogen-bond-mediated aglycone delivery (HAD) method for constructing β-D-fructofuranosidic linkages with 6-*O*-picoloyl-protected fructofuranosyl thioglycoside as the glycosyl donor [11]. On the other hand, α-selective fructosylation could be achieved through neighboring group participation by an acyl group at C3. Thio-fructofuranosides [6], fructofuranosyl phosphites [12], acetates [13], and trifluoroacetimidates [14] have been employed as donors in the synthesis of α-D-fructofuranosides. Currently, there are limited options for leaving groups available for fructose glycosylation, especially for application in orthogonal glycosylation strategies. Moreover, we were surprised to find that fructosyl fluorides have not been explored, despite their ease of synthesis and relative stability.

Glycosyl fluorides are widely and effectively utilized in both *O*- and *C*-glycosylation reactions. One key advantage of glycosyl fluorides as glycosyl donors is their remarkably high thermal and chemical stability. With these favorable synthetic properties, glycosyl fluorides were first developed in practical glycosylation by Mukaiyama and co-workers in 1981 with a fluorophilic activator, $SnCl_2$-$AgClO_4$ [15]. Since their significant development, numerous effective chemical methods for *O*-glycosylations and

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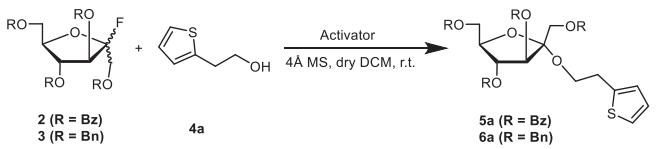
Scheme 1. Glycosyl donors developed in previous studies and the present study for constructing fructofuranosidic bonds.

C-glycosylations using glycosyl fluorides have been established. Different reagents and cooperative systems for the activation of glycosyl fluorides as glycosyl donors including tin-based activation systems ($SnCl_2$ or $SnCl_4$ in combination with AgOTf or $AgClO_4$ or $TrClO_4$ or $AgB(C_6F_5)_3$, SnF_4) [15–20], group IVB metal-based activators (TiF_4, Cp_2MCl_2 in combination with AgOTf or $AgClO_4$, M = Ti or Zr or Hf) [21,22] and other Lewis acids (SiF_4, TMSOTf, $BF_3{\cdot}OEt_2$, $TrB(C_6F_5)_4$, $HB(C_6F_5)_4$, $B(C_6F_5)_3$, $LiClO_4$, Tf_2O, $Ca(OTf)_2$, $Cu(OTf)_2$, $Yb(OTf)_3$, $La(ClO_4)_3{\cdot}nH_2O$) have been developed [23–36], providing a diverse and versatile synthetic toolbox for oligosaccharide synthesis, particularly for the development of orthogonal glycosylation strategies. Furthermore, glycosyl fluorides exhibit versatile synthetic accessibility as they can be readily synthesized from diverse precursors including anomeric acetates, glycosyl hemiacetals, and thioglycosides, and are efficiently interconvertible with thioglycosides. Notably, this reactivity has enabled the development of a novel orthogonal activation strategy in oligosaccharide synthesis based on the independent activation of glycosyl fluoride donors and thioglycoside acceptors under distinct reaction conditions [37]. Since no applications of glycosyl fluoride donors in the synthesis of fructofuranosides have been reported, in pursuit of advancing glycosylation methodologies, herein we synthesized a fructofuranosyl fluoride donor and investigated its reactivity in corresponding glycosylation processes. Additionally, this work explored the application potential of fructofuranosyl fluoride in the orthogonal synthesis of fructooligosaccharides, offering a novel approach for the synthesis of complex oligosaccharides.

The fructofuranosyl fluoride donor **2** was prepared by direct fluorination of fructofuranosyl hydroxy sugar **1** in a satisfactory yield of 98 % (Scheme S1 in Supporting information), which maintained stability at room temperature. Separately, the armed donor **3** was prepared from thio-fructofuranosides by treatment with NIS/DAST in 88 % yield (Scheme S2 in Supporting information).

Under different conditions for the activation of glycosyl fluoride, the glycosylation of donor **2** (1.2 equiv.) was conducted with 2-thiopheneethanol **4a** (1.0 equiv.) in the presence of 4 Å molecular sieves (MS) at room temperature in anhydrous CH_2Cl_2 (Table 1). Systematic screening of Lewis acids revealed reactivity trends. Strikingly, $Ca(OTf)_2$, $Cu(OTf)_2$, and camphorsulfonic acid (CSA) failed to activate the fluoride donor (Table 1, entry 1–3). $BF_3{\cdot}OEt_2$ exhibited partial activation to yield glycoside products, along with significant leftover of donor and acceptor (entry 5).

Table 1
Effect of activator on fructofuranosyl fluoride donor glycosylation.[a]



2 (R = Bz)
3 (R = Bn)

4a

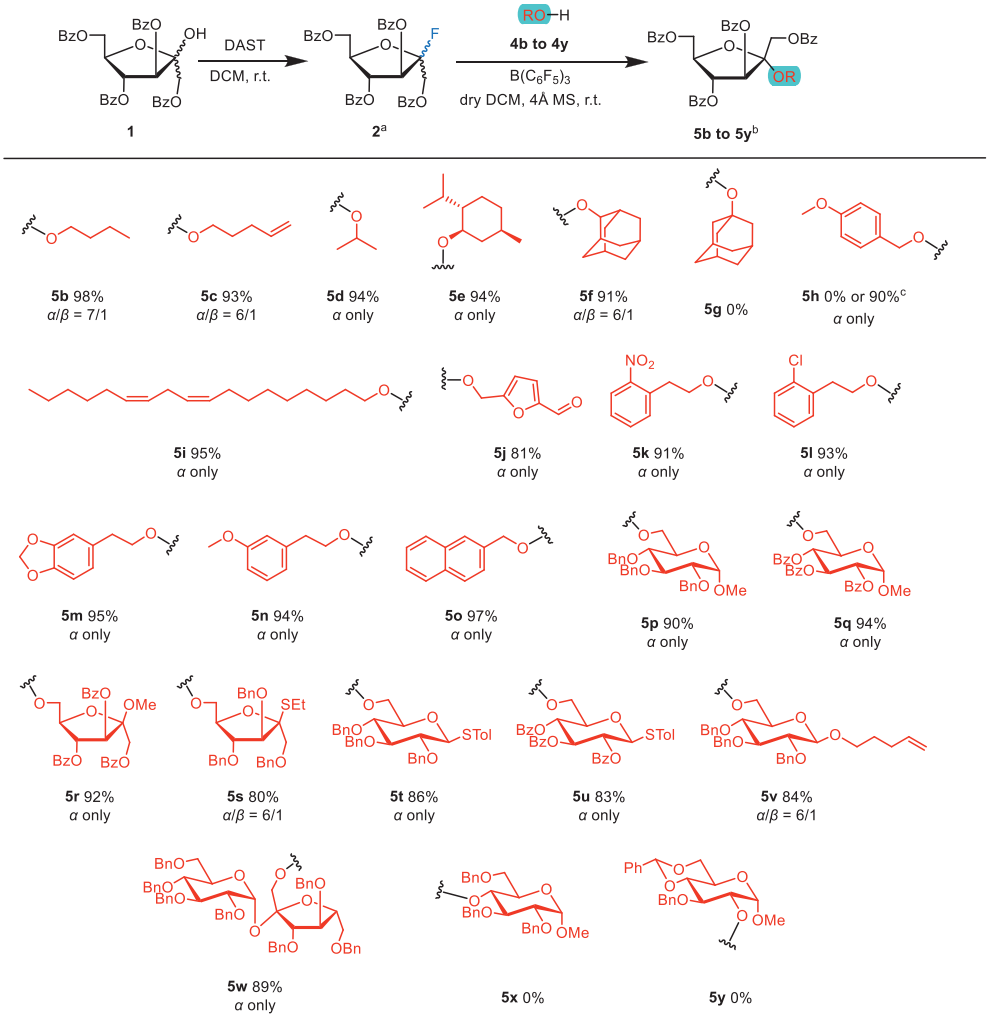
5a (R = Bz)
6a (R = Bn)

Entry	Donor	Activator (equiv.)	Yield (%)[b]	Ratio of α/β[c]
1	**2**	$Cu(OTf)_2$ (1.2)	NR	–
2	**2**	$Ca(OTf)_2$ (1.2)	NR	–
3	**2**	CSA (1.2)	NR	–
4	**2**	BBr_3 (1.2)	0	–
5	**2**	$BF_3{\cdot}OEt_2$ (1.2)	27	α only
6	**2**	$SnCl_4$ (1.2)	97	α only
7	**2**	$B(C_6F_5)_3$ (0.4)	95	α only
8	**2**	Cp_2ZrCl_2/AgOTf (0.6/1.2)	94	α only
9	**2**	$SnCl_2$/AgOTf (0.6/0.6)	93	α only
10	**3**	$B(C_6F_5)_3$ (0.4)	93	1.5/1

[a] Glycosylations were conducted with fructofuranosyl fluoride donor **2** or **3** (1.2 equiv.) in anhydrous CH_2Cl_2 (0.025 mol/L, calculated based on donor) in the presence of 4 Å MS (80 mg/mL, calculated based on solvent) at room temperature for 3 h.
[b] Isolated yield. NR = No reaction.
[c] The α/β ratio was determined by NMR analysis.

The stronger acid BBr_3 caused donor degradation without glycoside formation (entry 4). In comparison, $B(C_6F_5)_3$, with intermediate acidity, enabled efficient donor activation and quantitative coupling with the acceptor. Additionally, activation systems including $SnCl_4$, Cp_2ZrCl_2 in combination with AgOTf, and $SnCl_2$ in combination with AgOTf have also demonstrated remarkable efficiency and stereospecificity in α-glycoside formation (entries 6–10). In contrast, the reaction of armed donor **3** resulted in the formation of inseparable α/β-anomeric glycosides, which were identified by NMR analysis to have an α/β ratio of 1.5/1. This outcome suggests that the α-stereoselectivity in donor **2** was likely controlled by the neighboring group participation of the C-3 benzoyl group.

Subsequently, we investigated the *O*-glycosylation scope of donor **2** (1.2 equiv.) with various acceptors (**4b**–**4y**) under the optimal conditions (Scheme 2, donor: 1.2 equiv., acceptor: 1.0 equiv., CH_2Cl_2: 0.025 mol/L calculated based on donor, 4 Å MS: 80 mg/mL calculated based on solvent, r.t.). Except for the acid-sensitive PMB



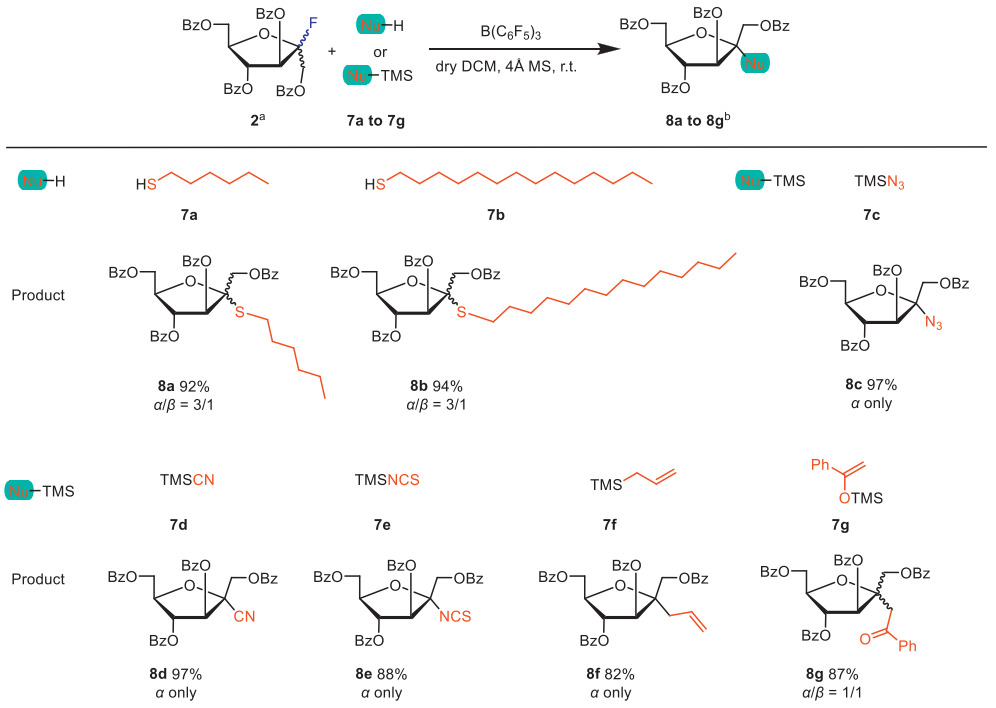
Scheme 2. Substrate scope of the fructofuranosyl *O*-glycosylation. [a] For **5b**–**5y**, glycosylations were conducted with fructofuranosyl fluoride donor **2** (1.2 equiv.) and acceptor **4b**–**4y** (1.0 equiv.) under the activation of $B(C_6F_5)_3$ (0.4 equiv.) in anhydrous CH_2Cl_2 (0.025 mol/L, calculated based on donor) for 3 h. [b] Isolated yield, and the α/β ratio was determined by analysis of NMR for **5b**–**5y**. [c] The activation system for **5h** was replaced with Cp_2ZrCl_2/AgOTf (0.6 equiv./1.2 equiv.).

group, which would be cleaved under $SnCl_4$ or $B(C_6F_5)_3$ conditions and required switching to milder Cp_2ZrCl_2/AgOTf activation, all three activation systems afforded α-fructofuranosides with good stereoselectivity (α/β ratio $\geq$ 6/1) and high yields (80%–98%) in glycosylation of donor **2** with primary alcohols. For the reactions with thioglycoside acceptors, the glycosylation process between donor **2** and acceptor **4s**–**4u** produced α-fructofuranosyl disaccharides **5s**–**5u** with yields ranging from 80% to 86%, demonstrating the orthogonal reactivity of thioglycosides and fluorides and their potential utility in complex oligosaccharide synthesis. Notably, glycosylation reactions involving secondary hydroxyl–containing glycosyl acceptors or tertiary hydroxyl acceptors failed to yield detectable products, an outcome that may be attributed to steric hindrance effects at these nucleophilic oxygen centers, consistent with prior findings [11].

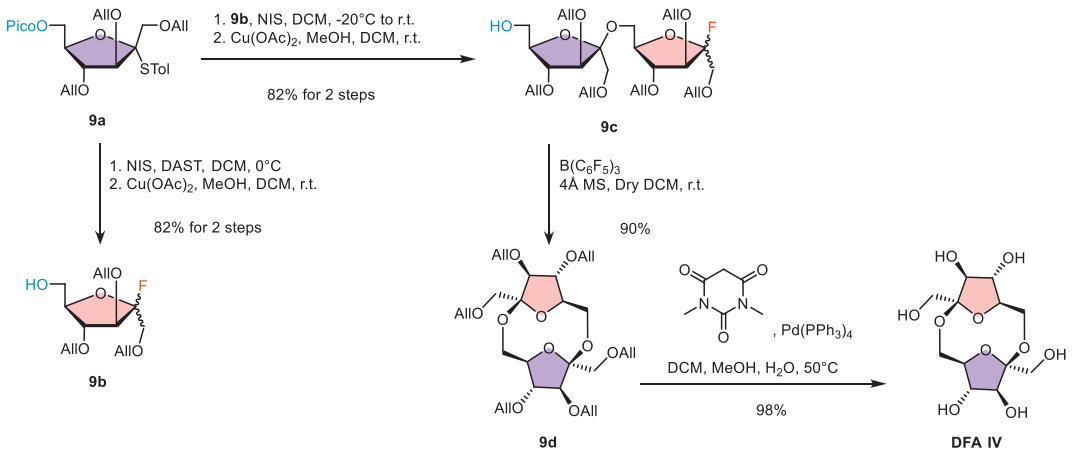
The biosynthesis of amino sugars proceeds through the hexosamine biosynthetic pathway (HBP), with the rate-limiting step being the conversion of fructose-6-phosphate to glucosamine-6-phosphate. Therefore, fructose derivatives hold promise as molecular probes for investigating glycobiological processes and developing novel enzymatic inhibitors. Furthermore, *C*-, *S*-, and *N*-glycosides exhibit unique characteristics compared to *O*-glycosides, demonstrating resistance to enzymatic hydrolysis [38]. However, as a crucial metabolic molecule, fructose has rarely been reported in the synthesis of its *C*- and *N*-glycosides. Thus, we attempted to broaden the structural diversity of fructose derivatives by coupling fructofuranosyl fluoride donor **2** with common nucleophilic reagents **7a**–**7g** (Scheme 3).

Under standard glycosylation conditions, donor **2** was coupled with thiol **7a** or **7b**, resulting in over 90% yield of thiofructorufanoside **8a** or **8b** with a ratio of α/β at 3/1. Donor **2** reacted with nitro or carbo nucleophilic reagents **7c**–**7g** to afford the corresponding glycoside derivatives **8c**–**8g** in high yields (82%–97%), with **8c**–**8f** exhibiting complete α-stereoselectivity. The results demonstrate the significant potential of fructosyl fluoride in the synthesis of fructose derivatives.

Di-D-fructose-2,6′:6,2′-dianhydride, also known as difructose anhydride IV (DFA IV), is a centrally symmetric cyclic disaccharide composed of two fructose units linked by β-glycosidic bonds. Similar to cyclodextrins and inulin-type cyclofructans [39,40], DFA IV is derived from the conversion of levan by levan fructotransferase (LFTase) [41] and is rarely found in nature, making it a promising low-calorie sugar substitute. Several beneficial effects associated with the consumption of DFA IV have been reported [42,43]. To date, the preparation of DFA IV has been achieved solely through enzymatic conversion using LFTase [44–47], whereas there are no known reports of its chemical synthesis, which may be attributed to the lack of orthogonal glycosylation methods for fructose. To better serve human health, basic research and the practical applications of DFA IV deserve attention.



Scheme 3. Substrate scope of the fructofuranosyl *S*-, *N*-, and *C*-glycosylation. [a] For **8a**–**8g**, glycosylations were conducted with fructofuranosyl fluoride donor **2** (1.0 equiv.) and nucleophiles **7a**–**7g** under the activation of $B(C_6F_5)_3$ in anhydrous CH_2Cl_2 (0.025 mol/L, calculated based on donor) for 3 h. [b] Isolated yield, and the α/β ratio was determined by analysis of NMR for **8a**–**8g**. TMS = trimethylsilyl.



Scheme 4. Synthesis of DFA IV.

Given the intriguing structural properties of DFA IV, its chemical synthesis inherently demands the implementation of two orthogonal glycosylation strategies, exemplified by thioglycoside and glycosyl fluoride methodologies, to address the unique challenges posed by its molecular architecture. The HAD methodology enables the stereoselective construction of β-glycosidic bonds, while the facile interconversion and orthogonal reactivity between thioglycosides and glycosyl fluorides strategically define the reactive sites for subsequent cycloglycosylation. As shown in Scheme 4, thioglycoside donor **9a** was first converted to glycosyl fluoride using DAST, followed by deprotection of the picoloyl group *via* $Cu(OAc)_2$ to afford glycosyl acceptor **9b**. The initial β-glycosidic linkage was constructed *via* the HAD method employing thioglycoside donor **9a**. Subsequently, glycosyl fluoride **9c** was subjected to cycloglycosylation under high dilution conditions (2×10^{-3} mol/L), consistent with the concentrations required in other reports on cyclic sugar synthesis [48,49]. The reaction afforded DFA IV as allylated derivative **9d** in 90% yield with complete β-selectivity, presumably due to the symmetric nature of the intermediate. Finally, deprotection of the allyl group *via* $Pd(PPh_3)_4$ afforded the target molecule DFA IV in 98% yield.

We further explored the reaction mechanism of cycloglycosylation by density functional theory (DFT) calculations and evaluated the configurational stereoselectivity of the reaction. As shown in Fig. 1, the donor **9c** undergoes C–F bond cleavage under the activation of $B(C_6F_5)_3$, forming an oxonium ion intermediate **9c-INT-1**, with its ΔG set to 0 kcal/mol. Subsequently, the hydroxyl group of the acceptor attacks the anomeric carbon of the donor from the α-side to form intermediate **9c-INT-2**, or from the β-side to form intermediate **9c-INT-3**. However, due to the higher ΔG required for the formation of **9c-INT-3** (67.70 kcal/mol) compared to **9c-INT-2** (4.55 kcal/mol), this intermediate is difficult to generate. As a result, only the symmetric β,β-product **9d** is formed *via* **9c-INT-2**, and the computational results align with experimental observations. Scheme 5 shows the proposed reaction mechanism of cycloglycosylation with **9c**. Tris(pentafluorophenyl)borane activates the

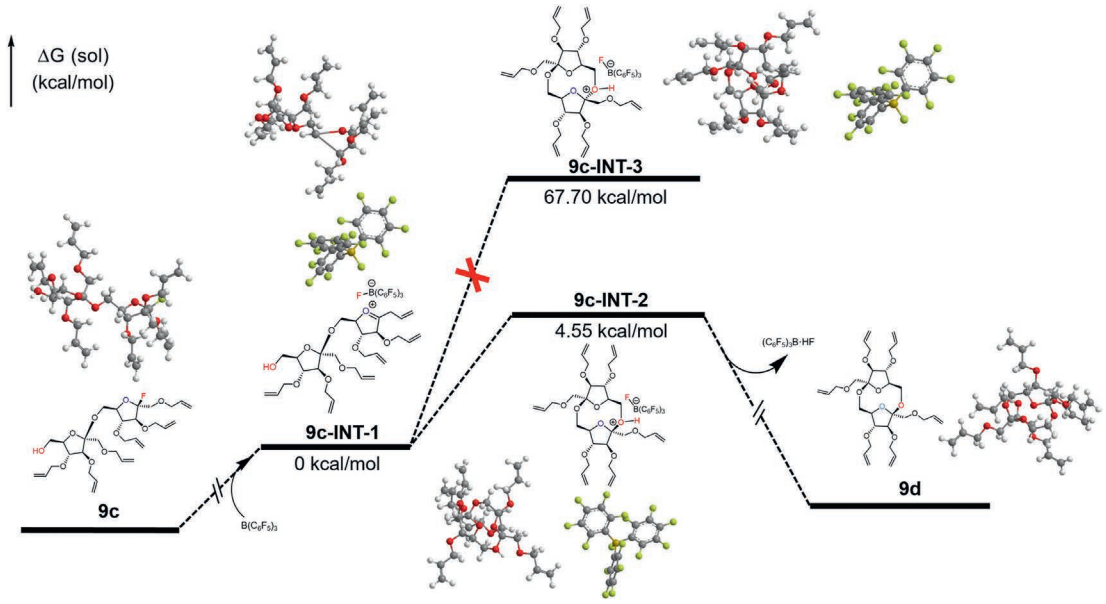
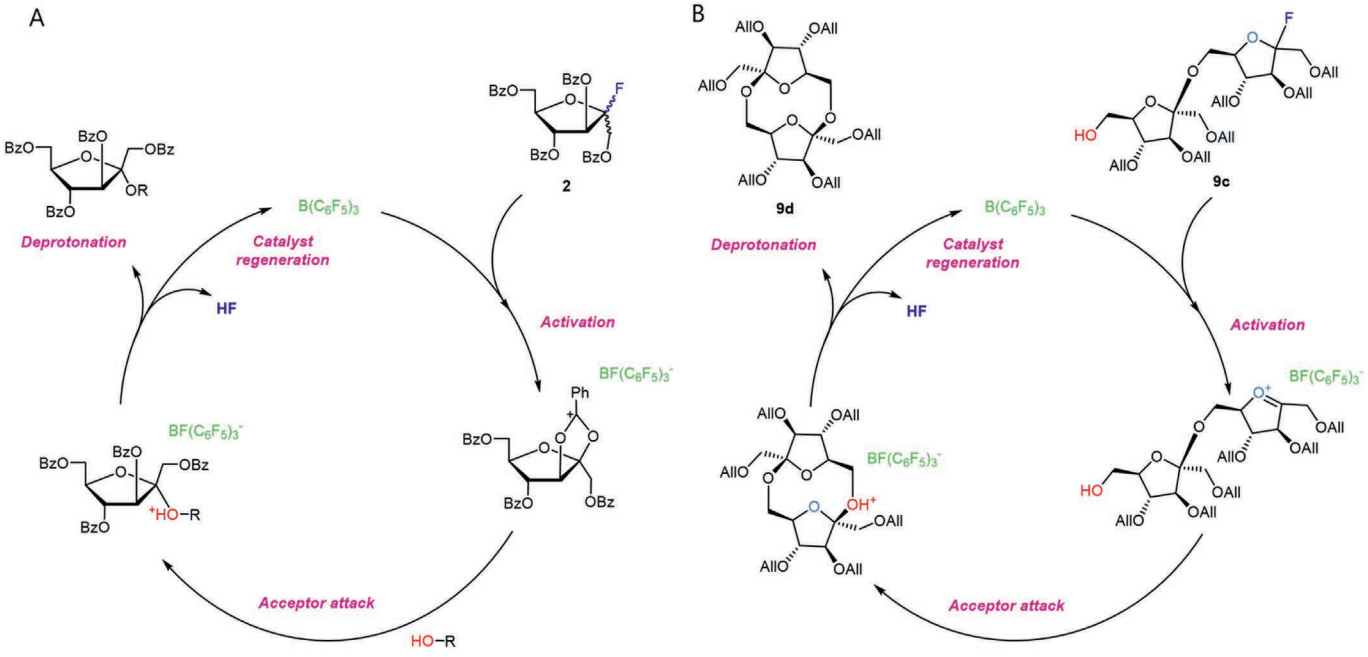


Fig. 1. The DFT calculation results of the intermediates in cycloglycosylation from **9c** to **9d.**



Scheme 5. Proposed mechanism of tris(pentafluorophenyl)borane promoted glycosylation with fluoride **2** or **9c**.

fluoride donor, breaking the C–F bond and forming an oxonium ion intermediate. The hydroxyl group of the acceptor attacks from the α-side because of the lower energy barrier for forming the symmetric β,β-cyclodisaccharide. After proton dissociation, DFA IV is formed with complete β-stereoselectivity.

In summary, we developed fructofuranosyl fluorides as donors for synthesizing α-D-fructofuranosides. Promoted by tris(pentafluorophenyl)borane, this method proved highly effective, yielding the glycosylation products in almost quantitative yields with primary alcohols, thiols and *N*- and *C*-nucleophiles. These fructofuranosyl derivatives represent new structural types for bioactive molecules or molecular probes for investigating HBP processes. Furthermore, the fluoride donor can undergo facile interconversion with thioglycoside and enable orthogonal glycosylation, based on which we achieved the first chemical synthesis of DFA IV through orthogonal glycosylation reactions involving thioglycosides and glycosyl fluorides.

Declaration of competing interest

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.

CRediT authorship contribution statement

Yidian Mo: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ao Sun:** Writing – review & editing, Methodology, Investigation. **Hang Dong:** Writing – review

& editing, Investigation. **Zhongtang Li:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis. **Zhongjun Li:** Writing – review & editing, Project administration, Funding acquisition.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2025.111922.

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