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Highly stereoselective α-glycosylation with $GalN_3$ donors enabled collective synthesis of mucin-related tumor associated carbohydrate antigens†

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Mucin-related tumor-associated carbohydrate antigens (TACAs) are important and interesting targets for cancer vaccine therapy. However, efficient access to a library of mucin-related TACAs remains a challenging task. One of the key issues is the challenging construction of α-GalNAc linkages. Here, we report highly stereoselective α-glycosylation with $GalN_3$ *N*-phenyl trifluoroacetimidate donors, which features excellent yields, outstanding stereoselectivities, broad substrate scope and mild reaction conditions. This method is successfully applied to highly stereoselective synthesis of $GalN_3$-α-*O*-Ser, which served as the common intermediate for collective synthesis of a wide range of TACAs including T_N antigen, ST_N antigen, 2,6 STF antigen, 2,3 STF antigen, glycophorin and cores 1–8 mucin-type *O*-glycans. In particular, the rationale for this highly stereoselective α-glycosylation is provided for the first time using DFT calculations and mechanistic studies, highlighting the crucial roles of reagent combinations (TMSI and Ph_3PO) and the H-bonding directing effect of the N_3 group.

Introduction

O-Linked and *N*-linked glycans are two principal classes of glycans obtained from post-translational modifications of proteins *via* glycosylation.[1,2] Compared with the unique trimannosyl chitobiosyl pentasaccharide core structure $Man_3GlcNAc_2$-α-*N*-Asn in all *N*-linked chains, *O*-linked chains are more diverse with such various monosaccharides connected to hydroxyl amino acids as GalNAc-α-*O*-Ser/Thr,[3] Fuc-α-*O*-Ser/Thr,[4] Xyl-β-*O*-Ser,[5] GlcNAc-β-*O*-Ser,[6] Man-α-*O*-Ser/Thr,[7] Gal-β-*O*-hydroxyLys[8] and Ara-β-*O*-hydroxyPro.[9] Among these, GalNAc-α-*O*-Ser/Thr is the most ubiquitous form of protein *O*-linked glycosylation, namely, mucin-type *O*-glycosylation, which occurs widely in higher plants and the whole animal kingdom.[3] Mucin-type *O*-glycans play vital roles in a variety of biological processes, such as mediating cell–cell interactions, influencing the stability, conformation and structure of proteins, serving as receptor-binding ligands, and being involved in cell adhesion and immune responses.[10] Interestingly, aberrant expression of mucin-type *O*-glycans on the surface of cancer cells usually correlates with different stages of tumor progression and various human disorders, which can differentiate them from normal tissues.[11] Therefore, TACAs are highly important and desirable targets for cancer immunotherapy.[12]

T_N antigen referred to the core structure GalNAc-α-*O*-Ser/Thr, which was often associated with colon and prostate carcinoma and T_N syndrome.[13] Substitutions at C3 and/or C6 hydroxyl groups of the core structure GalNAc-α-*O*-Ser **1** with Gal, GlcNAc, GalNAc and Neu5NAc sugar motifs could give rise to large collections of mucin-type *O*-glycans, including cores 1–8 *O*-glycans **2–9**, ST_N antigen **10**, 2,6 STF antigen **11**, 2,3 STF antigen **12**, and glycophorin **13** (Scheme 1A). Cores 1–2 *O*-glycans **2–3** are the most abundant, while cores 3–8 *O*-glycans **4–9** are more restricted and organ-characteristic in mucin expression, such as in human bronchial and colonic mucins (cores 3–4),[14] fetal mucins in meconium and rectal adenocarcinomas (core 5),[15] fetal mucins from gastric carcinomas (core 6),[16] bovine submaxillary mucin (core 7),[17] and human bronchial mucin (core 8).[18] ST_N antigen is abundantly expressed in breast, colon, stomach and ovary epithelial tumors,[19] while 2,3 STF antigen[20] and 2,6 STF antigen[21] are found in breast tumors and myelogenous leukemia cells, respectively. Glycophorin is observed as a motif in the erythrocyte membrane glycoprotein[22] and acute myelogenous leukemia cells.[21] These molecules, especially tumor associated carbohydrate antigens are interesting targets for the development of new therapeutic agents, diagnostic tools and cancer vaccine therapy, as well as structural and functional studies.[11–13] However, it is time-consuming and extremely difficult to isolate these TACAs from tumor tissues in pure and well-defined forms. Therefore,

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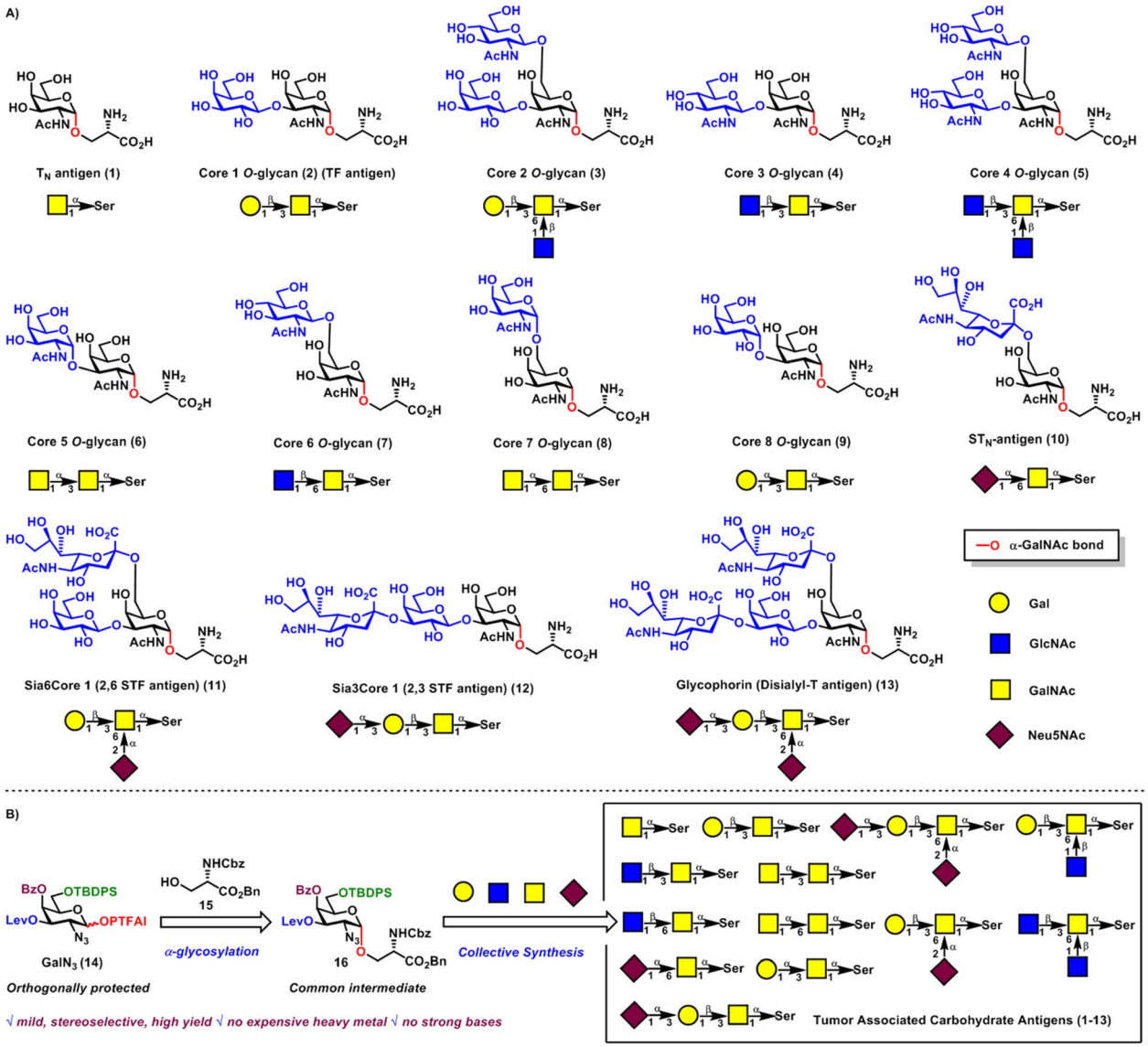


Scheme 1 (A) Mucin-related tumor associated carbohydrate antigens **1–13**; (B) collective synthesis of tumor associated carbohydrate antigens **1–13** from the common intermediate **16** *via* a new α-glycosylation strategy.

development of strategies for the efficient synthesis of a library of TACAs is highly desirable and remains a challenging task in chemical synthesis.

The stereoselective construction of α-GalNAc linkages is one of the key issues to be addressed for efficient access to a large library of TACAs. During the past several decades, different methods have been developed to tackle these challenges, such as glycosylation of donors with non-assisting C2-aza groups, such as azide[23] and oxazolidinone,[24] O-Michael addition to C2-nitro-galactals,[25] 4,6-di-*tert*-butylsilylene directed galactosylation,[26] ring-opening of aziridine-2-carboxamides with O-nucleophiles of C(1)-hemiacetals,[27] and nickel-catalyzed glycosylation of donors incorporated with C(2)-*N*-substituted benzylidene groups.[28] Despite these remarkable advances, due to merits and demerits of the current state-of-the-art strategies, it remains challenging to achieve efficient, stereoselective and practical formations of α-GalNAc linkages.

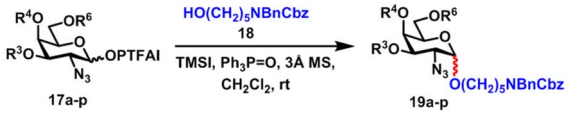
Previous efforts toward syntheses of TACAs usually adopt the traditional "single-target" approach, which is not flexible enough to build large collections of these antigens. Inspired by biosynthesis of molecules in nature *via* the assembly of a common intermediate, a general strategy for the collective synthesis of these molecules would be ideal to expediently produce these antigens.[29]

Here, we report the collective synthesis of TACAs including T$_N$ antigen, ST$_N$ antigen, 2,6 STF antigen, 2,3 STF antigen, glycophorin and cores 1–8 mucin-type O-glycans **1–13** from the common intermediate GalN$_3$-α-*O*-Ser **16** with levulinoyl (Lev), benzoyl (Bz) and *tert*-butyldiphenylsilyl (TBDPS) orthogonal protecting groups at C3, C4 and C6 positions, respectively (Scheme 1B). The C6-TBDPS group could serve as a temporary protecting group for (1 → 6) branching, while the C3-Lev group could serve not only as a temporary protecting group for (1 → 3) branching, but also as a remote participating group for α-glycosylation. The

C4-Bz group could serve as both a permanent protecting group and a remote participating group for stereoselective constructions of α-$GalN_3$ linkages. The α-$GalN_3$ linkage of the common intermediate **16** can be highly stereoselectively constructed *via* the newly developed merging reagent modulation and remote participation α-glycosylation[30] between strategically protected $GalN_3$ *N*-phenyl trifluoroacetimidate[31] (PTFAI) donor **14** and Cbz-protected serine amino acid **15**. This α-glycosylation method features mild reaction conditions (TMSI, Ph_3PO, rt), broad substrate scope, and excellent stereoselectivities and yields. DFT calculations and mechanistic studies provided rationales for this highly stereoselective α-glycosylation for the first time, uncovering important roles of TMSI and Ph_3PO and the H-bonding directing effect of the N_3 group.

Results and discussion

We embarked on the investigation of α-glycosylation with $GalN_3$ PTFAI as donors **17** and $HO(CH_2)_5NBnCbz$ as a strong nucleophile acceptor **18** (Scheme 2). After extensive optimization studies, when $GalN_3$ donor **17a** with both C3 and C4 Bz groups was coupled with **18** using TMSI and Ph_3PO reagent combination at room temperature, glycoside **19a** was obtained in excellent yield (95%) and excellent α-stereoselectivity (α/β > 20 : 1) (entry 1). In comparison, when donors **17b–d** with no acyl groups at either the C3 or C4 position were glycosylated with **18**, stereoselectivities of glycosides **19b–d** were rather low (α/β = 1.3 : 1 to 1.7 : 1) (entries 2–4). Interestingly, the moderate stereoselectivity (α/β = 4 : 1) of glycoside **19f** and good stereoselectivity (α/β = 9 : 1) of glycoside **19e** were achieved when donor **17f** with the Bz group at the C3 position and donor **17e** with the Bz group at the C4 position were coupled with **18**, respectively (entries 5 and 6), underlining the importance of remote participation effects of acyl groups at C3 and C4 positions.[30,32] Furthermore, glycoside **19a** was produced in low yield (21%) and decreased stereoselectivity (α/β = 10 : 1) with TMSI only (entry 7), while the low stereoselectivity (α/β = 2.8 : 1) of glycoside **19a** was obtained with TMSOTf instead of TMSI and Ph_3PO (entry 8), emphasizing the crucial role of reagent combination (TMSI and Ph_3PO).[30,33]

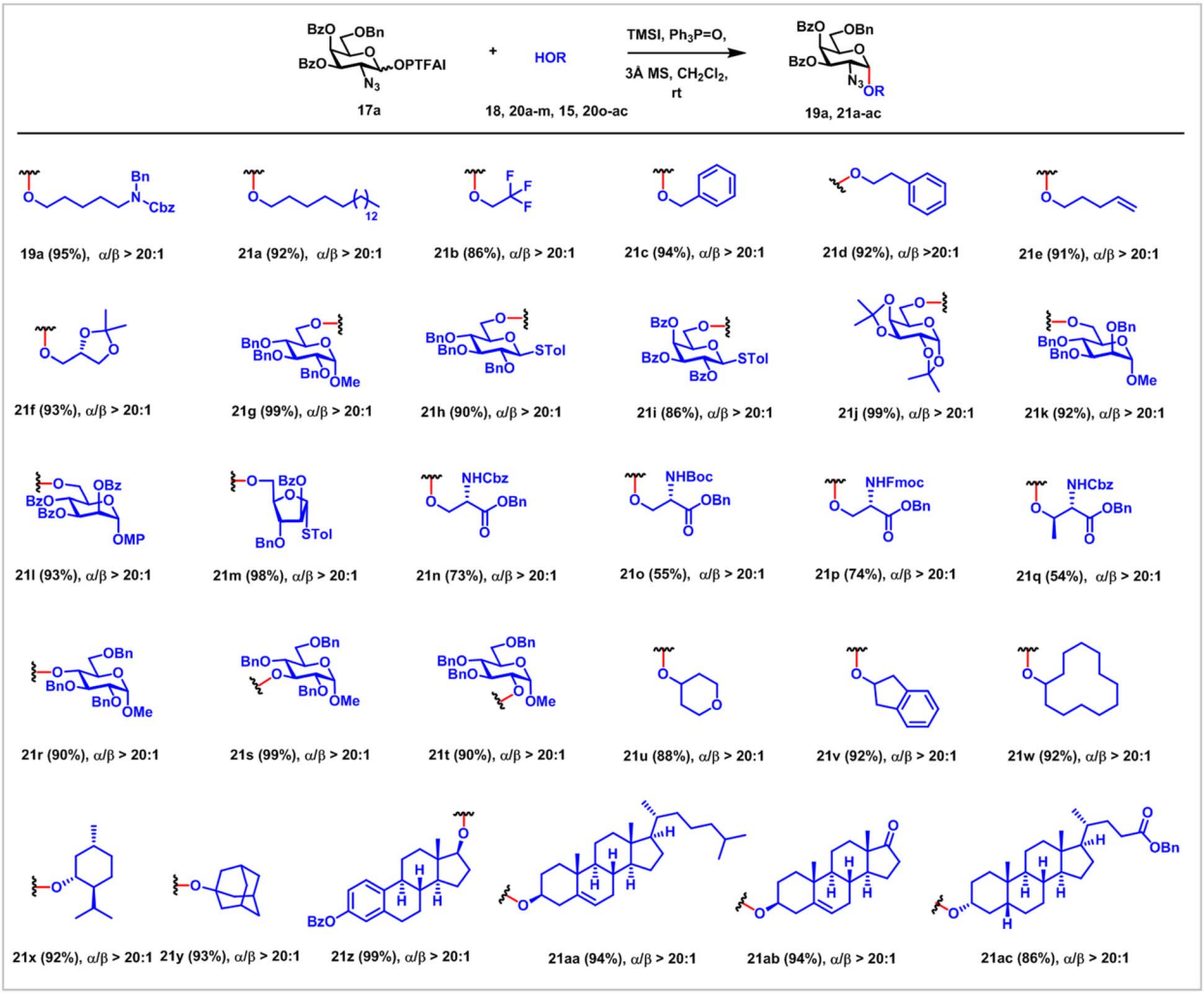


Entry	Donor	R^6	R^4	R^3	Product	Yield (%)	α/β[a]
1	17a	Bn	Bz	Bz	19a	95	> 20:1
2	17b	Bn	Bn	Bn	19b	89	1.7:1
3	17c	Lev	Bn	Bn	19c	85	1.3:1
4	17d	TBDPS	Bn	Bn	19d	95	1.5:1
5	17e	Bn	Bz	Bn	19e	90	9:1
6	17f	Bn	Bn	Bz	19f	85	4:1
7[b]	17a	Bn	Bz	Bz	19a	21	10:1
8[c]	17a	Bn	Bz	Bz	19a	93	2.8:1
9	17g	Lev	Bz	Bz	19g	76	> 20:1
10	17h	TBDPS	Bz	Bz	19h	90	> 20:1
11	17i	Ac	Bz	Bz	19i	78	> 20:1
12	17j	Bz	Bz	Bz	19j	83	> 20:1
13	17k	Bn	Ac	Ac	19k	82	15:1
14	17l	TBDPS	Ac	Ac	19l	76	12:1
15	17m	Ac	Ac	Ac	19m	68	10:1
16	17n	Bn	Bz	Lev	19n	85	> 20:1
17	17o	Bn	Bz	Ac	19o	84	> 20:1
18	17p	Bn	Lev	Lev	19p	86	14:1

Scheme 2 Investigations of α-glycosylation with $GalN_3$ PTFAI as donors and $HO(CH_2)_5NBnCbz$ as a strong nucleophile acceptor.

Next, the scope of $GalN_3$ PTFAI donors was investigated, using **18** as a strong nucleophile and TMSI and Ph_3PO as the reagent combination (entries 9–18). C3 and C4 Bz protected $GalN_3$ PTFAI with different C6 functional groups including Lev, TBDPS, Ac and Bz were also suitable donors **17g–j**, providing the corresponding glycosides **19g–j** in satisfactory yields (76–90%) and outstanding α-stereoselectivities. When donors **17k–p** equipped with different acyl groups at C3 and C4 were used, glycosides **19k–p** were also obtained in good yields (68–85%) and excellent α-stereoselectivities (α/β = 10 : 1 to > 20 : 1).

We next examined the scope of this new α-glycosylation method using $GalN_3$ **17a** as the donor with a large number of alcoholic nucleophiles (**20a–m**, **15** and **20o–ac**), including primary, secondary, and tertiary alcohols and bioactive and complex natural products (Scheme 3). When reactive primary alcohols such as 1-octadecanol, benzyl alcohol, 4-penten-1-ol, 2-phenylethanol and 2,3-isopropylideneglycerol are employed, the desired glycosides **21a** and **21c–f** were uniformly obtained in excellent yields (91–94%) and excellent α-stereoselectivity (α/β > 20 : 1). It was noted that stereoselective α-glycosylation often fell short when strong nucleophilic alcohols were employed as the acceptors.[30] Furthermore, coupling of primary alcohols of carbohydrate acceptors such as glucosides **20g–h**, galactosides **20i–j**, mannosides **20k–l** and arabinofuranoside **20m** with **17a** proceeded smoothly, providing the desired α-glycosides **21g–m** in great yields and stereoselectivities (α/β > 20 : 1). Of note, both higher and lower nucleophilic alcoholic acceptors (*e.g.* **21k** and **21l**) are amenable to this glycosylation protocol. It was worth noting that the obtained thioglycosides **21h–i**, **21m** and disaccharide **21l** with an anomeric *para*-methoxyphenyl (MP) group could be readily used as building blocks for subsequent sugar chain enlongation. Successful and stereoselective installation of α-$GalN_3$ unit **17a** to primary alcohols of L-serine **15** and **20o–p** and the secondary alcohol of L-threonine **20q**, including the use of different *N*-protecting groups, particularly, the Fmoc group (**20p**), indicates the promising applications of this α-glycosylation method for solid-phase synthesis of mucin glycoproteins. Besides primary alcohols, secondary alcohols of carbohydrates were also suitable acceptors, providing α-(1 → 4)-, (1 → 3)-, and (1 → 2)-glycosides **21r–t** in outstanding yields and selectivities. The late stage installation of the α-$GlaN_3$ unit on diverse bioactive natural products and drugs such as epiandrosterone **20ab**, menthol **20x**, cholesterol **20aa**, lithocholic benzoate **21ac** and estradiol benzoate **20z** also successfully produced the desired

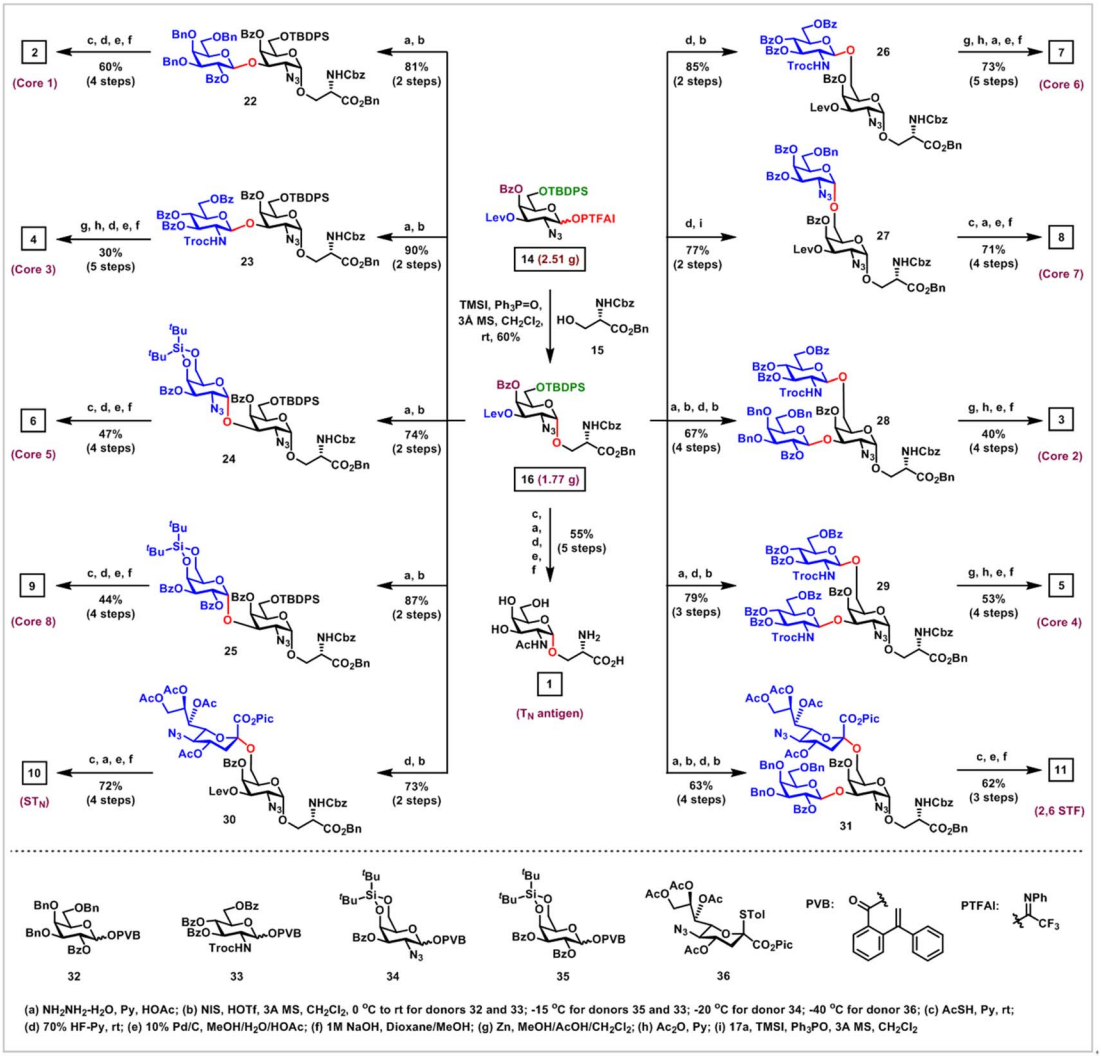


Scheme 3 Scope of stereoselective α-glycosylation using $GalN_3$ **17a** as the donor.

glycoconjugates **21x** and **21z–ac** in excellent yields and stereoselectivities. Diverse functional groups such as alkene (**21e** and **21aa–ab**), thioacetal (**21h–i** and **21m**), acetal (**21f** and **21j**), carbamate (**21n–q**) and ketone (**21ab**) were untouched in this α-glycosylation method, clearly demonstrating the mildness of the current protocol.

Next, application of this α-glycosylation method to the collective synthesis of mucin-type O-glycans was investigated (Scheme 4). Coupling of the strategically protected $GalN_3$ PTFAI donor **14** with serine **15** in the presence of TMSI and Ph_3PO at room temperature proceeded smoothly, furnishing the desired $GalN_3$-α-*O*-Ser **16** with acceptable 60% yield and excellent stereoselectivity (α/β > 20 : 1) on a gram scale, which served as the common intermediate for the synthesis of all mucin-type O-glycans **1–13**.

As for the synthesis of core 1, 3, 5 and 8 mucin-type *O*-glycans, selective removal of the Lev group at the C3 position of **16** afforded the C3–OH acceptor, which was glycosylated with superarmed galactosyl *ortho*-(1-phenylvinyl)benzoate[34] (PVB) donor **32**, GlcNTroc PVB donor **33**, 4,6-di-*tert*-butylsilyl (DTBS) protected $GalN_3$ PVB donor **34** and DTBS protected Gal PVB donor **35** in the activation of NIS and HOTf, efficiently and stereoselectively furnishing the desired glycosides **22–25** in 81%, 90%, 74% and 87% overall yields over two steps, respectively. It was noted that when superarmed PVB donor **32** was replaced with the perbenzoyl protected disarmed Gal PVB donor, the yield of the desired glycoside was low, due to the formation of significant amounts of side-products. The DTBS group ensures the highly stereoselective formation of α-(1 → 3)-$GalN_3$ linkage in **24** and α-(1 → 3)-Gal linkage in **25**.[35] Interestingly, when the current method was used to install α-(1 → 3)-$GalN_3$ linkage with $GalN_3$ PTFAI **17a** as a donor, the conversion of the reaction was low, probably due to the low reactivity of C3–OH in this $GalN_3$ acceptor with the C4-Bz electron withdrawing group. Reductive acetylation of N_3 groups to AcHN groups with thioacetic acid, followed by the first removal of silyl groups with 70% HF-Py, the second hydrogenolysis of Bn and Cbz groups, and the final saponifications of Bz groups in **22** and **24–25** successfully generated the core 1, 5 and 8 mucin-type O-glycans **2**, **6** and **9** in 60%, 47% and 44% overall yields, respectively. The core 3 mucin-type O-glycan **4** was obtained in 30% overall yield over five steps *via* zinc-mediated deprotection of the Troc group



Scheme 4 Collective synthesis of TACAs including T_N antigen, ST_N antigen, 2,6 STF antigen and cores 1–8 mucin-type *O*-glycans from the common intermediate $GalN_3$-α-*O*-Ser 16 *via* this α-glycosylation method.

and concomitant reduction of the N_3 group in **23** to the amine groups, followed by acetylation and global deprotection.

As for the preparation of core 6 *O*-glycan **7**, core 7 *O*-glycan **8** and ST_N antigen **10**, selective removal of the TBDPS group at the C6 position in **16** afforded the C6–OH acceptor, which was coupled with GlcNTroc PVB donor **33**, $GalN_3$ PTFAI donor **17a**, and 1-picolinyl-5-azido thiosialoside donor[36] **36**, affording the desired glycosides **26–27** and **30** in 85%, 77% and 73% overall yields over two steps, respectively. It was worthy of note that sialylation using Sun and Schmidt's protocol with donor **36** constructed α-(2 → 6)-sialyl linkage in **30** with excellent α-stereoselectivity (α/β > 20 : 1),[36] while this α-glycosylation method using donor **17a** highly stereoselectively assembled α-(1 → 6)-$GalN_3$ linkage in **27**. Similarly, functional group transformations and global deprotection of disaccharides **26** and **27** afforded the core 6 *O*-glycan **7** and core 7 *O*-glycan **8** in 73% and 71% overall yields, respectively. ST_N antigen **10** was obtained in 72% yield from **30** over the following four steps, including: (1) reductive acetylation of N_3 groups to AcHN groups with thioacetic acid; (2) removal of the Lev group with NH_2NH_2–H_2O; (3) hydrogenolysis of Bn, Cbz and Pic groups; (4) saponifications of Ac and Bz groups.

As for the synthesis of core 2 *O*-glycan **3** and 2,6 STF antigen **11**, disaccharide **22** served as a common intermediate. Removal of the TBDPS group in **22** provided the C6–OH alcoholic acceptor, which was coupled with **33** and **36** in the presence of NIS and HOTf, successfully and stereoselectively producing the branched trisaccharides **28** and **31** in 67% and 63% overall yields *via* two steps, respectively. Following the above similar functional group transformation and deprotection protocol, core 2 *O*-glycan **3** was obtained in 40% overall yield over four steps, while 2,6 STF antigen **11** was obtained in 62% overall yield over three steps.

As for the core 4 *O*-glycan **5** synthesis, sequential removal of Lev and TBDPS groups in **16** gave the free C3–OH and C6–OH acceptor, which underwent double glycosylation with GlcNTroc PVB donor **33** in the activation of NIS and HOTf at −15 °C, efficiently affording the desired trisaccharide **29** in 79% overall

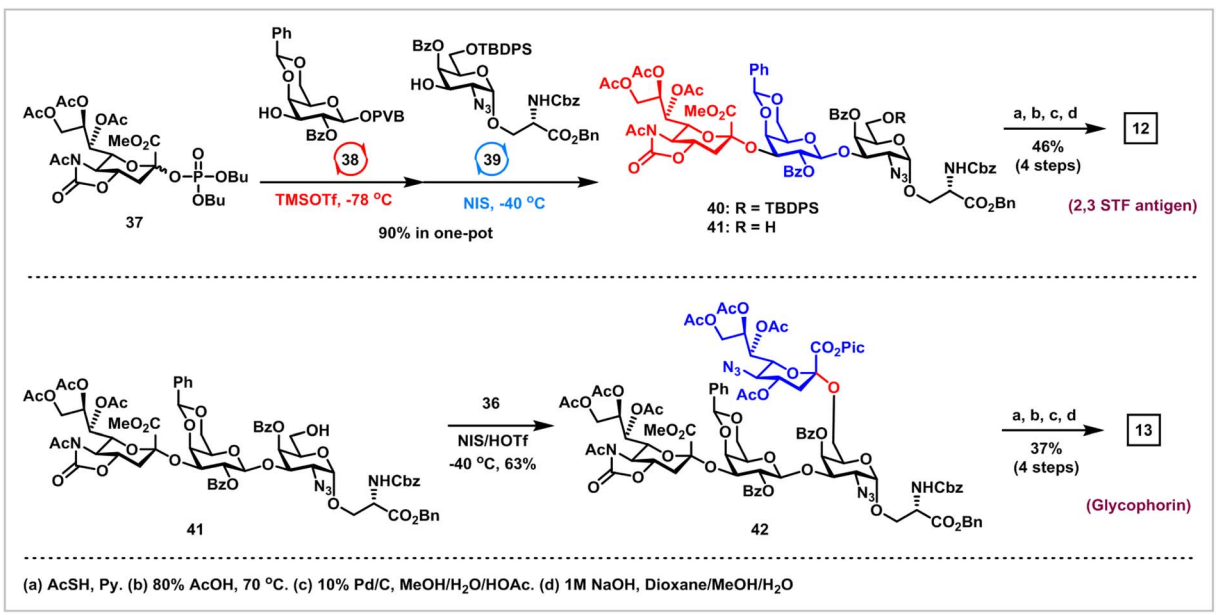
yield over three steps. The core 4 *O*-glycan **5** was readily prepared in 53% overall yield over four steps *via* conversion of two TrocHN and azido groups to acetamido groups and global deprotection of all protecting groups.

As for T_N antigen synthesis, reductive acetylation of the azido group to the acetamido group in **16**, followed by global deprotection, successfully generated T_N antigen **1** in 55% overall yield, which is commercially available, but very expensive to purchase ($587 per mg from Sigma-Aldrich).

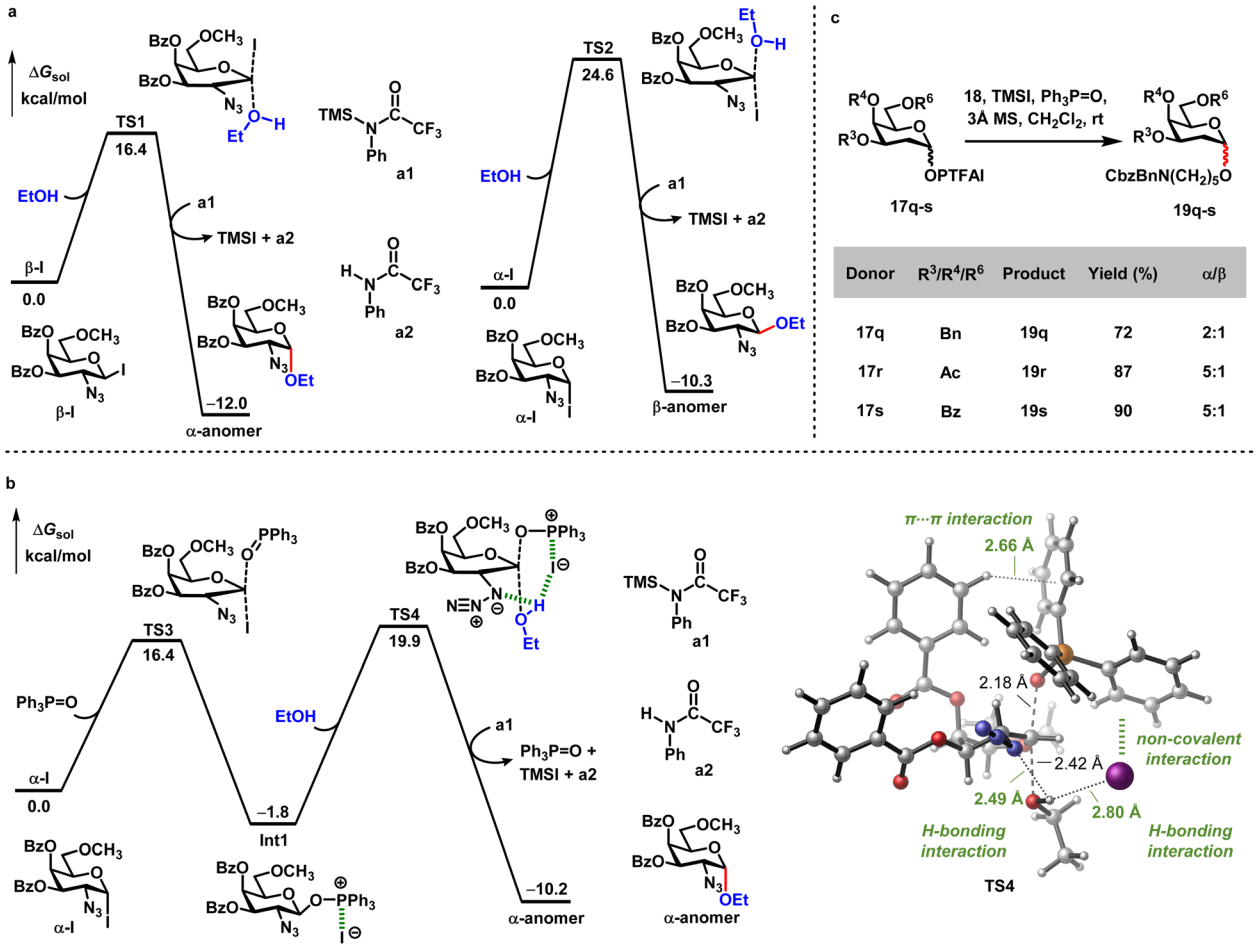
Finally, we embarked on the synthesis of 2,3 STF antigen **12** and glycophorin **13** *via* the orthogonal one-pot glycosylation strategy (Scheme 5).[37] Glycosylation of sialyl phosphate donor **37** (2.5 equiv.) with 3-OH of Gal PVB **38** (1.0 equiv.) using Wong's protocol (TMSOTf, −78 °C) afforded α-NeuNAc-(2 → 3)-Gal disaccharide as a single α-isomer, which was further coupled with the poorly reactive 3-OH in **39** (0.8 equiv.) derived from **16** *via* Lev group removal in the activation of NIS and TMSOTf at −40 °C, successfully providing the desired α-NeuNAc-(2 → 3)-β-Gal-(1 → 3)-α-$GalN_3$-*O*-Ser **40** with 90% overall yield in one pot. Deprotection of the TBDPS group in **40** with 70% HF-Py afforded the trisaccharide acceptor **41**, which was coupled with 1-picolinyl-5-azido thiosialoside donor **36** under the activation of NIS and HOTf at −40 °C, furnishing the desired α-NeuNAc-(2 → 3)-β-Gal-(1 → 3)-[α-NeuNAc-(2 → 6)]-α-$GalN_3$-*O*-Ser **42** in satisfactory 63% yield. The final task is the removal of all protecting groups in trisaccharide **41** and tetrasaccharide **42** to generate 2,3 STF antigen **12** and glycophorin **13**, which was found to be challenging due to the presence of many diverse polar groups. After extensive optimizations, the following sequences were used: (1) reductive acetylation of N_3 to AcNH with thioacetic acid; (2) removal of the benzylidene group with 80% HOAc; (3) hydrogenolysis of all Bn, Pic and Cbz groups with 10% Pd/C; (4) saponifications of all esters and 5-*N*,4-*O*-carbonyl groups. 2,3 STF antigen **12** was obtained in 46% overall yield using the above optimized protocols, while glycophorin **13** was prepared in 37% overall yield using a similar protocol. It was worth noting that orthogonal one-pot glycosylation based on the pair of phosphates and PVB donors for the synthesis of 2,3 STF antigen **12** and glycophorin **13** avoided issues such as aglycone transfer inherent to orthogonal one-pot glycosylation on the basis of thioglycosides.[34,38]

DFT calculations were further performed to understand how exogenous reagents (TMSI and $Ph_3P{=}O$) modulate the process of stereoselective α-glycosylation. Based on the experimental results shown in Scheme 2, the combination of donor **17a** with TMSI gave relatively low yield (21%) and decreased stereoselectivity (α/β = 10 : 1) (entry 7). The control experiments indicated that donor **17a** can be quickly activated by TMSI, generating a mixture of α-iodide and β-iodide species (α-iodide/β-iodide = 4 : 1) (see Fig. S1†). This is supported by the computational results, showing that α-iodide is 1.8 kcal mol^{-1} more stable than β-iodide (see Fig. S3†). We thus calculated the energetics for the reaction of these iodides with the alcoholic nucleophile. As shown in Scheme 6a, the S_N2 transition state (**TS1**) with β-iodide requires a barrier of 16.4 kcal mol^{-1}, which is much lower than that with α-iodide (**TS2**, $\Delta G^{\ddagger} = 24.6$ kcal mol^{-1}). This indicates that while all *in situ* formed β-iodide species are transformed into the desired α-anomer, only a small amount of α-iodide actually undergoes the nucleophilic attack by alcohol to afford the β-anomer. This is consistent with the low efficiency only in the presence of TMSI.

By contrast, the treatment of $Ph_3P{=}O$ with the mixture of donor **17a** and TMSI significantly enhanced both yield and stereoselectivity (95% yield, α/β > 20 : 1, entry 1 in Scheme 2). Since β-iodide is highly reactive towards the alcoholic acceptor (Scheme 6a), the promotion effect of $Ph_3P{=}O$ could be attributed to its capacity for converting α-iodide into a more reactive glycosylating species. The computations indeed find that $Ph_3P{=}O$ can replace the iodine atom of α-iodide in the S_N2



Scheme 5 Collective synthesis of 2,3 STF antigen **12** and glycophorin **13** *via* orthogonal one-pot glycosylation.



Donor	$R^3/R^4/R^6$	Product	Yield (%)	α/β
17q	Bn	19q	72	2:1
17r	Ac	19r	87	5:1
17s	Bz	19s	90	5:1

Scheme 6 (a) Energy profiles for the S_N2 reaction of the alcoholic acceptor with α-iodide and β-iodide; (b) energy profile for the $Ph_3P{=}O$ promoted S_N2 nucleophilic reaction of α-iodide with alcoholic acceptors; (c) glycosylation between 2-deoxy glycosyl PTFAI donors **17q–s** and **18** with TMSI and $Ph_3P{=}O$.

fashion with a relatively low barrier (**TS3**, $\Delta G^{\ddagger} = 16.4$ kcal mol^{-1}, Scheme 6b), generating the β-anomeric phosphonium iodide intermediate (**Int1**). The species of phosphonium iodide intermediate can be detected by ESI-MS experiment monitoring (see Fig. S2†). In **Int1**, there is no covalent interaction between iodine and phosphine. Instead, this intermediate is stabilized by electrostatic interactions and non-covalent interactions between I and $Ph_3P{=}O$ moieties (see Fig. S4†). Subsequently, **Int1** is attacked by the alcoholic acceptor *via* **TS4**, delivering the α-selective glycosidic linkage. Compared with the α-iodide (**TS2**, $\Delta G^{\ddagger} = 24.6$ kcal mol^{-1}), **Int1** derived from α-iodide exhibits a greater reactivity to the α-face S_N2 displacement (**TS4**, $\Delta G^{\ddagger} = 21.7$ kcal mol^{-1}). The lower barrier of **TS4** is mostly due to a series of stabilizing interactions (Scheme 6b), including the H-bonding interactions of alcohol with both N_3 and I moieties, the non-covalent interactions between I and $Ph_3P{=}O$ (Fig. S4†), and the T-shaped π···π interaction between C4 Bz and $Ph_3P{=}O$. Except for enhancing the reactivity of α-iodide, more importantly, $Ph_3P{=}O$ also plays a critical role in transforming α-iodide into the desired α-anomer mediated by the β-anomeric phosphonium iodide species. In addition, we also studied the reaction of β-iodide with $Ph_3P{=}O$, which is less favorable than the direct α-face S_N2 displacement by the alcoholic acceptor *via* **TS1** (see Fig. S5†). Taken together, the role of TMSI is to generate α-iodide and β-iodide species, and $Ph_3P{=}O$ can further ensure the formation of α-selective glycosidic linkage. The combination of these exogenous reagents facilitates excellent yield and α/β selectivity. To further support H-bonding interactions between alcohol and N_3 moieties, different 2-deoxy glycosyl PTFAI donors **17q–s** were coupled with **18** in the presence of TMSI and $Ph_3P{=}O$. Low stereoselectivity (α/β = 5 : 1) of products **19r–s** was obtained (Scheme 6c), highlighting the H-bonding directing role of the N_3 group for this α-glycosylation.

Conclusions

In summary, highly stereoselective α-glycosylation with $GalN_3$ PTFAI donors has been achieved, which features mild reaction conditions, broad substrate scope, and excellent stereoselectivities and yields. Furthermore, this synergistic α-glycosylation strategy was successfully applied to efficient and highly stereoselective synthesis of the common intermediate $GalN_3$-α-

O-Ser **16** with orthogonal protecting groups for collective synthesis of tumor associated carbohydrate antigens including T_N antigen, ST_N antigen, 2,6 STF antigen, 2,3 STF antigen, glycophorin, and cores 1–8 mucin-type *O*-glycans **1–13**. Mechanistic studies and DFT calculations uncovered the crucial roles of reagent combinations (TMSI and $Ph_3P{=}O$) and the H-bonding directing effect of the N_3 group for achieving highly stereoselective α-glycosylation for the first time. This library of synthetic mucin-related TACAs can be used to investigate the structure–reactivity relationships of mucin-type *O*-glycans. We believe that the present work could facilitate and inspire advances in complex carbohydrate synthesis for the development of new carbohydrate-based therapeutics and understandings of their functions.[39]

Data availability

Experimental procedures, characterisation data, and NMR spectra for new compounds can be found in the ESI.†

Author contributions

G. X. conceived the idea and designed the research. K. S. investigated this α-glycosylation, explored the scope, and synthesized the T_N antigen. Y. Z. achieved synthesis of ST_N antigen, 2,6 STF antigen, 2,3 STF antigen, glycophorin, and cores 1–8 mucin-type *O*-glycans. Y. J. and G. L. performed DFT calculations. B. L., Q. Z., Q. T. and F. L. conducted preparation of some building blocks. G. X. G. L. and X. W. wrote the manuscript with feedback from all authors.

Conflicts of interest

The authors declare no competing financial interests.

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Notes and references

1 H. Herzner, T. Reipen, M. Schultz and H. Kunz, *Chem. Rev.*, 2000, **100**, 4495–4538.
2 M. J. Grogan, M. R. Pratt, L. A. Marcaurelle and C. R. Bertozzi, *Annu. Rev. Biochem.*, 2002, **71**, 593–634.
3 (*a*) N. Martínez-Sáez, J. M. Peregrina and F. Corzana, *Chem. Soc. Rev.*, 2017, **46**, 7154–7175; (*b*) L. A. Marcaurelle and C. R. Bertozzi, *Glycobiology*, 2002, **12**, 69R–77R.
4 R. J. Harris and M. W. Spellman, *Glycobiology*, 1993, **3**, 219–224.
5 (*a*) J. D. Esko and L. Zhang, *Curr. Opin. Struct. Biol.*, 1996, **6**, 663–670; (*b*) I. B. H. Wilson, *Cell. Mol. Life Sci.*, 2004, **61**, 794–809; (*c*) B. Yang, K. Yoshida, Z. Yin, H. Dai, H. Kavunja, M. H. El-Dakdouki, S. Sungsuwan, S. B. Dulaney and X. Huang, *Angew. Chem., Int. Ed.*, 2012, **51**, 10185–10189.
6 (*a*) G. W. Hart, R. S. Haltiwanger, G. D. Holt and W. G. Kelly, *Annu. Rev. Biochem.*, 1989, **58**, 841–874; (*b*) T. Pohl and H. Waldmann, *J. Am. Chem. Soc.*, 1997, **119**, 6702–6710.
7 (*a*) N. R. Smalheiser, S. M. Haslam, M. Sutton-Smith, H. R. Morris and A. Dell, *J. Biol. Chem.*, 1998, **273**, 23698–23703; (*b*) J. Seifert, T. Ogawa and Y. Ito, *Tetrahedron Lett.*, 1999, **40**, 6803–6807.
8 J. Broddefalk, J. Bäcklund, F. Almqvist, M. Johansson, R. Holmdahl and J. Kihlberg, *J. Am. Chem. Soc.*, 1998, **120**, 7676–7683.
9 M. J. Kieliszewski, M. O'Neill, J. Leykam and R. Orlando, *J. Biol. Chem.*, 1995, **270**, 2541–2549.
10 F.-G. Hanisch, *Biol. Chem.*, 2001, **382**, 143–149.
11 S. J. Danishefsky and J. R. Allen, *Angew. Chem., Int. Ed.*, 2000, **39**, 836–863.
12 (*a*) W.-H. Li and Y.-M. Li, *Chem. Rev.*, 2020, **120**, 11420–11478; (*b*) R. Mettu, C.-Y. Chen and C.-Y. Wu, *J. Biomed. Sci.*, 2020, **27**, 9; (*c*) M.-M. Wei, Y.-S. Wang and X.-S. Ye, *Med. Res. Rev.*, 2018, **38**, 1003–1026; (*d*) D. Feng, A. S. Shaikh and F. Wang, *ACS Chem. Biol.*, 2016, **11**, 850–863; (*e*) N. Gaidzik, U. Westerlind and H. Kunz, *Chem. Soc. Rev.*, 2013, **42**, 4421–4442; (*f*) C. Sorieul, F. Papi, F. Carboni, S. Pecetta, S. Phogat and R. Adamo, *Pharmacol. Ther.*, 2022, **235**, 108158.
13 (*a*) T. Ju, V. I. Otto and R. D. Cummings, *Angew. Chem., Int. Ed.*, 2011, **50**, 1770–1791; (*b*) C. Pifferi, A. Ruiz-de-Angulo, D. Goyard, C. Tiertant, N. Sacristán, D. Barriales, N. Berthet, J. Anguita, O. Renaudet and A. Fernández-Tejada, *Chem. Sci.*, 2020, **11**, 4488–4498.
14 (*a*) J. Breg, H. van Halbeek, J. F. G. Vliegenthart, A. Klein, G. Lamblin and P. Roussel, *Eur. J. Biochem.*, 1988, **171**, 643–654; (*b*) D. K. Podolski, *J. Biol. Chem.*, 1985, **260**, 8262–8271; (*c*) D. K. Podolsky, *J. Biol. Chem.*, 1985, **260**, 15510–15515.
15 (*a*) E. F. Hounsell, A. M. Lawson, J. Feeney, H. C. Gooi, N. J. Pickering, M. S. Stoll, S. C. Lui and T. Feizi, *Eur. J. Biochem.*, 1985, **148**, 367–377; (*b*) A. Kurosaka, H. Nakajima, I. Funakoshi, M. Matsuyama, T. Nagayo and I. Yamashina, *J. Biol. Chem.*, 1983, **258**, 11594–11598.
16 Y. Yamashita, Y. S. Chung, T. Sawada, Y. Kondo, K. Hirayama, A. Inui, B. Nakata, M. Okuno, R. Horie, T. Saito, K. Murayama, R. Kannagi and M. Sowa, *Int. J. Cancer*, 1994, **58**, 349–355.
17 W. Chai, E. F. Hounsell, G. C. Cashmore, J. R. Rosankiewicz, C. J. Bauer, J. Feeney, T. Feizi and A. M. Lawson, *Eur. J. Biochem.*, 1992, **203**, 257–268.

18 H. van Halbeek, A.-M. Strang, M. Lhermitte, H. Rahmoune, G. Lamblin and P. Roussel, *Glycobiology*, 1994, **4**, 203–209.
19 G. D. MacLean, M. A. Reddish, R. R. Koganty and B. M. Longenecker, *J. Immunother.*, 1996, **19**, 59–68.
20 K. O. Lloyd, J. Burchell, V. Kudryashov, B. W. T. Yin and J. Taylor-Papadimitriou, *J. Biol. Chem.*, 1996, **271**, 33325–33334.
21 M. Fukuda, S. R. Carlsson, J. C. Klock and A. Dell, *J. Biol. Chem.*, 1986, **261**, 12796–12806.
22 D. B. Thomas and R. J. Winzler, *J. Biol. Chem.*, 1969, **244**, 5943–5946.
23 (*a*) R. Kaifu and T. Osawa, *Carbohydr. Res.*, 1977, **58**, 235–239; (*b*) R. M. Ratcliffe, D. A. Baker and R. U. Lemieux, *Carbohydr. Res.*, 1981, **93**, 35–41; (*c*) H. Paulsen and J.-P. Hölck, *Carbohydr. Res.*, 1982, **109**, 89–107; (*d*) H. Kunz and S. Birnbach, *Angew. Chem., Int. Ed.*, 1986, **25**, 360–362; (*e*) S. D. Kuduk, J. B. Schwarz, X.-T. Chen, P. W. Glunz, D. Sames, G. Ragupathi, P. O. Livingston and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1998, **120**, 12474–12485; (*f*) L. Wang, Y. Zhang, H. S. Overkleeft, G. A. van der Marel and J. D. C. Codee, *J. Org. Chem.*, 2020, **85**, 15872–15884; (*g*) Y. Zhang, X. Ma and L. Zhang, *CCS Chem.*, 2023, **5**, 2799–2807.
24 (*a*) K. Benakli, C. Zha and R. J. Kerns, *J. Am. Chem. Soc.*, 2001, **123**, 9461–9462; (*b*) S. Manabe, K. Ishii and Y. Ito, *J. Am. Chem. Soc.*, 2006, **128**, 10666–10667.
25 (*a*) K. B. Pal, A. Guo, M. Das, G. Báti and X.-W. Liu, *ACS Catal.*, 2020, **10**, 6707–6715; (*b*) J.-L. Liu, Y.-T. Zhang, H.-F. Liu, L. Zhou and J. Chen, *Org. Lett.*, 2017, **19**, 5272–5275; (*c*) S. Medina, M. J. Harper, E. I. Balmond, S. Miranda, G. E. M. Crisenza, D. M. Coe, E. M. McGarrigle and M. C. Galan, *Org. Lett.*, 2016, **18**, 4222–4225; (*d*) R. R. Schmidt and Y. D. Vankar, *Acc. Chem. Res.*, 2008, **41**, 1059–1073; (*e*) G. A. Winterfeld and R. R. Schmidt, *Angew. Chem., Int. Ed.*, 2001, **40**, 2654–2657.
26 A. Imamura, A. Kimura, H. Ando, H. Ishida and M. Kiso, *Chem.–Eur. J.*, 2006, **12**, 8862–8870.
27 D. A. Ryan and D. Y. Gin, *J. Am. Chem. Soc.*, 2008, **130**, 15228–15229.
28 (*a*) E. A. Mensah and H. M. Nguyen, *J. Am. Chem. Soc.*, 2009, **131**, 8778–8780; (*b*) E. A. Mensah, F. Yu and H. M. Nguyen, *J. Am. Chem. Soc.*, 2010, **132**, 14288–14302; (*c*) F. Yu and H. M. Nguyen, *J. Org. Chem.*, 2012, **77**, 7330–7343; (*d*) M. S. McConnell, F. Yu and H. M. Nguyen, *Chem. Commun.*, 2013, **49**, 4313–4315; (*e*) F. Yu, M. S. McConnell and H. M. Nguyen, *Org. Lett.*, 2015, **17**, 2018–2021.
29 (*a*) M. R. Gadi, C. Chen, S. Bao, S. Wang, Y. Guo, J. Han, W. Xiao and L. Li, *Chem. Sci.*, 2023, **14**, 1837–1843; (*b*) S. Wang, C. Chen, M. R. Gadi, V. Saikam, D. Liu, H. Zhu, R. Bollag, K. Liu, X. Chen, F. Wang, P. G. Wang, P. Ling, W. Guan and L. Li, *Nat. Commun.*, 2021, **12**, 3573; (*c*) M. Guberman, M. Bräutigam and P. H. Seeberger, *Chem. Sci.*, 2019, **10**, 5634–5640.
30 (*a*) Y. Zhang, H. He, Z. Chen, Y. Huang, G. Xiang, P. Li, X. Yang, G. Lu and G. Xiao, *Angew. Chem., Int. Ed.*, 2021, **60**, 12597–12606; (*b*) Y. Zhang, Y. Hu, S. Liu, H. He, R. Sun, G. Lu and G. Xiao, *Chem. Sci.*, 2022, **13**, 7755–7764; (*c*) P. Li, H. Fan, Q. Tan and G. Xiao, *Org. Lett.*, 2023, **25**, 2788–2792; (*d*) K. Shou, S. Liu, Y. Zhang and G. Xiao, *Chin. J. Chem.*, 2024, **42**, 1593–1598.
31 (*a*) B. Yu and J. Sun, *Chem. Commun.*, 2010, **46**, 4668–4679; (*b*) B. Yu and H. Tao, *Tetrahedron Lett.*, 2001, **42**, 2405–2407.
32 (*a*) A. A. Hettikankanamalage, R. Lassfolk, F. S. Ekholm, R. Leino and D. Crich, *Chem. Rev.*, 2020, **120**, 7104–7151; (*b*) B. S. Komarova, Y. E. Tsvetkov and N. E. Nifantiev, *Chem. Rec.*, 2016, **16**, 488–506.
33 (*a*) L. Wang, H. S. Overkleeft, G. A. van der Marel and J. D. C. Codée, *J. Am. Chem. Soc.*, 2018, **140**, 4632–4638; (*b*) S.-R. Lu, Y.-H. Lai, J.-H. Chen, C.-Y. Liu and K.-K. T. Mong, *Angew. Chem., Int. Ed.*, 2011, **50**, 7315–7320; (*c*) S. K. Mulani, W.-C. Hung, A. B. Ingle, K.-S. Shiau and K.-K. T. Mong, *Org. Biomol. Chem.*, 2014, **12**, 1184–1197; (*d*) J. Zeng, R. Wang, S. Zhang, J. Fang, S. Liu, G. Sun, B. Xu, Y. Xiao, D. Fu, W. Zhang, Y. Hu and Q. Wan, *J. Am. Chem. Soc.*, 2019, **141**, 8509–8515.
34 (*a*) P. Li, H. He, Y. Zhang, R. Yang, L. Xu, Z. Chen, Y. Huang, L. Bao and G. Xiao, *Nat. Commun.*, 2020, **11**, 405; (*b*) H. He, L. Xu, R. Sun, Y. Zhang, Y. Huang, Z. Chen, P. Li, R. Yang and G. Xiao, *Chem. Sci.*, 2021, **12**, 5143–5151.
35 (*a*) A. Imamura, H. Ando, S. Korogi, G. Tanabe, O. Muraoka, H. Ishida and M. Kiso, *Tetrahedron Lett.*, 2003, **44**, 6725–6728; (*b*) A. Imamura, N. Matsuzawa, S. Sakai, T. Udagawa, S. Nakashima, H. Ando, H. Ishida and M. Kiso, *J. Org. Chem.*, 2016, **81**, 9086–9104.
36 J. Chen, T. Hansen, Q.-J. Zhang, D.-Y. Liu, Y. Sun, H. Yan, J. D. C. Codée, R. R. Schmidt and J.-S. Sun, *Angew. Chem., Int. Ed.*, 2019, **58**, 17000–17008.
37 (*a*) Y. Ma, Y. Zhang, Y. Huang, Z. Chen, Q. Xian, R. Su, Q. Jiang, X. Wang and G. Xiao, *J. Am. Chem. Soc.*, 2024, **146**, 4112–4122; (*b*) Y. Zhang, L. Wang, Q. Zhou, Z. Li, D. Li, C. Yin, X. Wang and G. Xiao, *Angew. Chem., Int. Ed.*, 2023, **62**, e202301351; (*c*) Y. Zhang, Z. Chen, Y. Huang, S. He, X. Yang, Z. Wu, X. Wang and G. Xiao, *Angew. Chem., Int. Ed.*, 2020, **59**, 7576–7584; (*d*) Z. Chen and G. Xiao, *Org. Lett.*, 2023, **25**, 7395–7399; (*e*) X. Sun, Z. Chen, R. Yang, M. Wang, X. Wang, Q. Zhang and G. Xiao, *Org. Lett.*, 2023, **25**, 7364–7368; (*f*) Y. Ma, Q. Jiang, X. Wang and G. Xiao, *Org. Lett.*, 2022, **24**, 7950–7954; (*g*) R. Yang, X. Sun, Y. Zhang and G. Xiao, *Org. Biomol. Chem.*, 2022, **20**, 6755–6758.
38 (*a*) Y. Hu, K. Yu, L.-L. Shi, L. Liu, J.-J. Sui, D.-Y. Liu, B. Xiong and J.-S. Sun, *J. Am. Chem. Soc.*, 2017, **139**, 12736–12744; (*b*) X. Xiao, J. Zeng, J. Fang, J. Sun, T. Li, Z. Song, L. Cai and Q. Wan, *J. Am. Chem. Soc.*, 2020, **142**, 5498–5503.
39 For selective reviews, see: (*a*) X. Wang and G. Xiao, *Curr. Opin. Chem. Biol.*, 2023, **77**, 102387; (*b*) S. Wang, Y. Yang, Q. Zhu, G. Q. Lin and B. Yu, *Curr. Opin. Chem. Biol.*, 2022, **69**, 102154; (*c*) L. Del Bino, K. E. Østerlid, D.-Y. Wu, F. Nonne, M. R. Romano, J. Codée and R. Adamo, *Chem. Rev.*, 2022, **122**, 15672–15716; (*d*) P. H. Seeberger, *Chem. Rev.*, 2021, **121**, 3598–3626; (*e*) D. Crich, *J. Am. Chem. Soc.*, 2021, **143**, 17–34; (*f*) L. Krasnova and C.-H. Wong, *J. Am. Chem. Soc.*, 2019, **141**, 3735–3754; (*g*) S. S. Kulkarni, C.-C. Wang, N. M. Sabbavarapu, A. R. Podilapu, P.-H. Liao

and S.-C. Hung, *Chem. Rev.*, 2018, **118**, 8025–8104; (*h*) C. S. Bennett and M. C. Galan, *Chem. Rev.*, 2018, **118**, 7931–7985; (*i*) M. Panza, S. G. Pistorio, K. J. Stine and A. V. Demchenko, *Chem. Rev.*, 2018, **118**, 8105–8150; (*j*) W.-L. Leng, H. Yao, J.-X. He and X.-W. Liu, *Acc. Chem. Res.*, 2018, **51**, 628–639; (*k*) P. Peng and R. R. Schmidt, *Acc. Chem. Res.*, 2017, **50**, 1171–1183; (*l*) S. J. Danishefsky, Y.-K. Shue, M. N. Chang and C.-H. Wong, *Acc. Chem. Res.*, 2015, **48**, 643–652; (*m*) T. J. Boltje, T. Buskas and G.-J. Boons, *Nat. Chem.*, 2009, **1**, 611–622; (*n*) X. Zhu and R. R. Schmidt, *Angew. Chem., Int. Ed.*, 2009, **48**, 1900–1934.