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Zinc(II)-mediated stereoselective construction of 1,2-*cis* 2-azido-2-deoxy glycosidic linkage: assembly of *Acinetobacter baumannii* K48 capsular pentasaccharide derivative†

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The capsular polysaccharide (CPS) is a major virulence factor of the pathogenic *Acinetobacter baumannii* and a promising target for vaccine development. However, the synthesis of the 1,2-*cis*-2-amino-2-deoxyglycoside core of CPS remains challenging to date. Here we develop a highly α-selective ZnI_2-mediated 1,2-*cis* 2-azido-2-deoxy chemical glycosylation strategy using 2-azido-2-deoxy glucosyl donors equipped with various 4,6-*O*-tethered groups. Among them the tetraisopropyldisiloxane (TIPDS)-protected 2-azido-2-deoxy-D-glucosyl donor afforded predominantly α-glycoside (α : β = >20 : 1) in maximum yield. This novel approach applies to a wide acceptor substrate scope, including various aliphatic alcohols, sugar alcohols, and natural products. We demonstrated the versatility and effectiveness of this strategy by the synthesis of *A. baumannii* K48 capsular pentasaccharide repeating fragments, employing the developed reaction as the key step for constructing the 1,2-*cis* 2-azido-2-deoxy glycosidic linkage. The reaction mechanism was explored with combined experimental variable-temperature NMR (VT-NMR) studies and mass spectroscopy (MS) analysis, and theoretical density functional theory calculations, which suggested the formation of covalent α-$C1^{GlcN}$-iodide intermediate in equilibrium with separated oxocarbenium–counter ion pair, followed by an S_N1-like α-nucleophilic attack most likely from separated ion pairs by the ZnI_2-activated acceptor complex under the influence of the 2-azido *gauche* effect.

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Introduction

Nosocomial infections caused by *Acinetobacter baumannii*, a Gram-negative opportunistic pathogenic bacterium, pose a major threat to public health. *A. baumannii* bacteria survive in the host for a long time and colonize the respiratory tract and circulatory system, causing pneumonia and other serious complications. The effectiveness of current antibiotic treatment for *A. baumannii* infections has been increasingly compromised by the emergence of drug resistance.[1] The pathogenicity of *A. baumannii* is mediated by various virulence factors, including capsular polysaccharide (CPS), a complex long-chain glycopolymer anchored in bacterial cell walls by non-covalent interactions.[2] Research indicates that CPS triggers immune responses producing specific antibodies against the pathogen and holds the potential to be developed as a vaccine precursor.[3] *A. baumannii* CPS features pathogen-associated molecular patterns (PAMPs) which interact with pattern recognition receptors (PRRs) on epithelial or immune cells, activating downstream signaling pathways leading to subsequent release of inflammatory cytokines. Currently, CPS molecules are procured from fermentation production as heterogeneous and low-purity materials, falling short of the requirements for vaccine development. The preparation of *A. baumannii*

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polysaccharide repeating unit antigens in pure form with well-defined structures, by either chemical or chemoenzymatic approaches, has attracted significant interest, such as Zhang's[4] work for synthesizing *O*-antigens; Hashimoto,[5] Kosma[6] and Yin's[7] works for lipopolysaccharide (LPS); and Gao,[8] Seeberger,[9] Ragains,[10] Xiao's[11] works for CPS.

The K locus (KL) in *A. baumannii* gene clusters is responsible for CPS biosynthesis. Over 40 types of CPS K-unit structures have been determined in recent years, including the K48 capsule isolated from *A. baumannii* strain *NIPH615*. In 2015, Knirel's group[12] elucidated the structure of the *A. baumannii* CPS K48 capsule type, which comprises two 1,2-*cis* amino glycosyl residues. The 1,2-*cis* 2-amino-2-deoxyglycoside structure occurs widely in various plant metabolites, anticoagulant drugs, and bacteria surface antigens among different serotypes, such as *P. stuartii* O44, *A. baumannii* CPS K47, K48 and K88 polysaccharides (Fig. 1A).[13,14] While chemically constructing the 1,2-*trans* 2-amino-2-deoxyglycosidic bond is readily achieved by exploiting the neighboring group participation (NGP), construction of the 1,2-*cis* linkage remains difficult, and few direct syntheses of 1,2-*cis* 2-amino-2-deoxy glycosides have been reported. Indirect methods include the 2,3-cyclic protection strategies by Kerns[15] and Manabe–Ito[16] groups, which involve the 1,2-*trans* glycosylation with oxazolidinone-fused donors and the following anomerization of the glycosides. Nguyen *et al.*[17] reported the strategy combining a C(2)-*N*-substituted benzylideneamino donor with a nickel triflate catalyst imparting 1,2-*cis* stereoselectivity. In 1978, Paulsen[18] developed a 2-azido-2-deoxy pyranose donor without 2-NGP which undergoes the 1,2-*cis* glycosylation reaction, and the inert azido group was then converted to an amino group. Henceforth multiple new 2-azido-2-deoxy donors have been developed for the synthesis of 1,2-*cis*

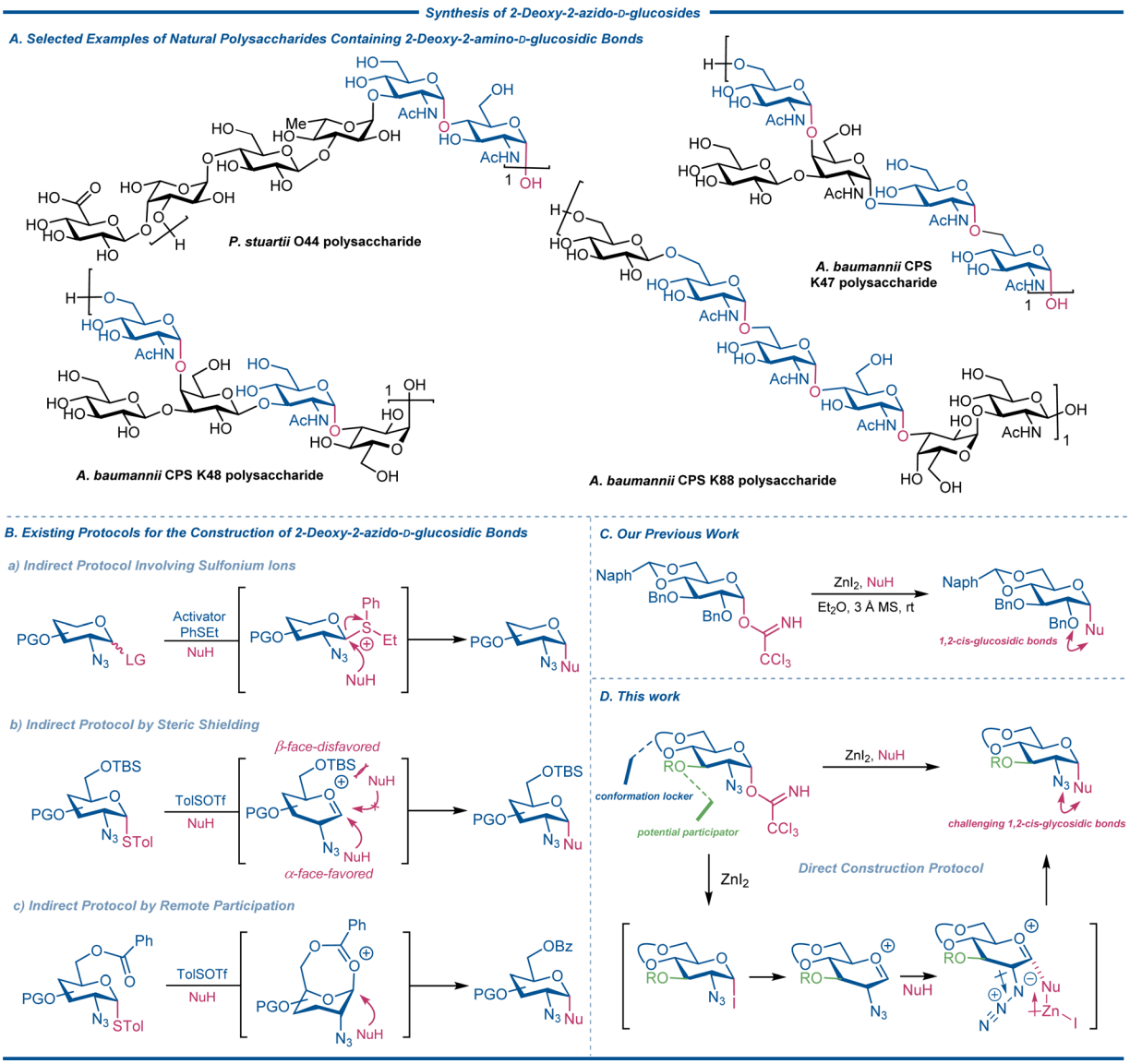


Fig. 1 (A) Selected examples of natural polysaccharides containing 2-deoxy-2-amino-D-glucosidic bonds; (B) indirect approaches to the stereoselective synthesis of 1,2-*cis* 2-deoxy-2-azido glycosides; (C) our previous work for the construction of 1,2-*cis* glucosidic bond mediated by ZnI_2; (D) presenting work about direct approach to 1,2-*cis* 2-deoxy-2-azido glycosides mediated by ZnI_2.

2-amino-2-deoxy glycosides. Boons *et al.*[19] developed an α-selective glycosylation adopting 2-azido-2-deoxyglucosyl trichloroacetimidate donors in the presence of thioether through the formation of β-anomeric sulfonium ion intermediates (Fig. 1B(a)). Gao *et al.*[8] employed 2-azido-2-deoxy-1-thioglucoside donors armed with 6-*O*-TBS and 6-*O*-Bz groups under TolSCl/AgOTf conditions for 1,2-*cis* glycosylation through steric shielding and remote participation tactics (Fig. 1B(b) and (c)). Most of these strategies are restricted to relatively limited substrate scopes, albeit with moderate to excellent yields and selectivities.

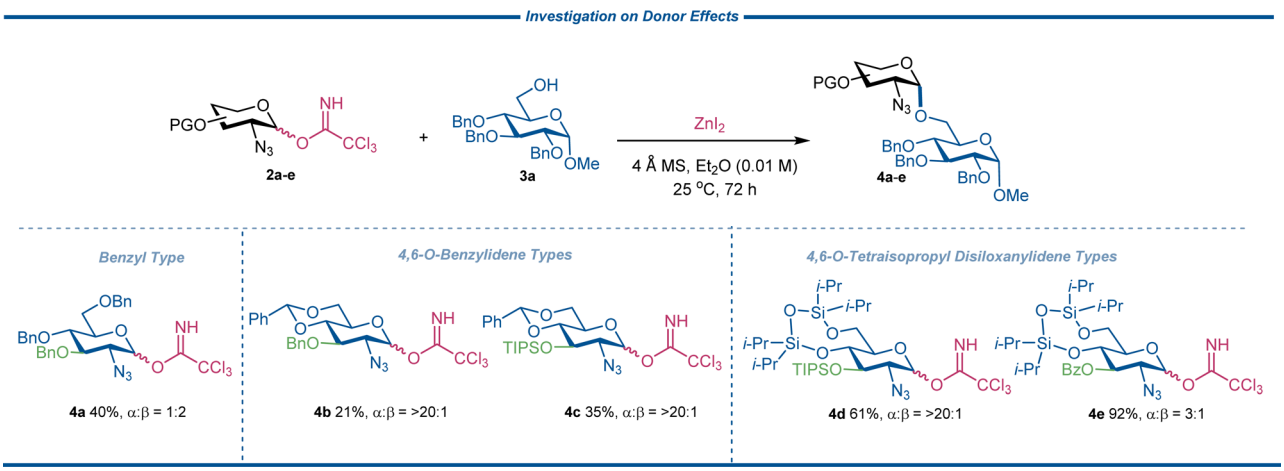
Our previous works have revealed that a mild Lewis acidic salt, namely ZnI_2, effectively promotes *cis* glycosylation such as α-glucosylation,[20] β-mannosylation,[21] and β-rhamnosylation[22] as well as 1,4/6-*cis* β-galactosylation in a selective manner (Fig. 1C).[23] Built upon our established protocols and noticing the biological and medicinal relevance of 1,2-*cis* 2-amino-2-deoxy glucosyl skeleton, we envisioned that the zinc-mediated diastereoselective 1,2-*cis* glycosylation reaction could be extended to the stereoselective synthesis of α-2-deoxy-2-amino-glucoside structures (Fig. 1D). Moreover, Bols' work[24] highlights the stereo-directing effects of "super-armed" silyl ether protecting groups on thioglycoside O-3, enhancing donor reactivities through silyl-assisted conformation shift of the pyranose ring from $^4C_1$ to $^1C_4$. Although there are sporadic reports of the cyclic disiloxane-assisted intramolecular aglycon delivery[25] and arabinofuranosylation,[26] stereoselective glycosylation with 2-amino-2-deoxy type glucosyl donors exploiting stereoelectronic effects of protecting groups remain underexplored by far. Drawing inspiration from previous works, we hypothesized that introducing a ring-conformation-restricting 4,6-*O*-cyclic protecting group and a sterically hindered O-3 protecting group such as silyl ethers can significantly improve α-stereoselectivity through synergistic stereoelectronic effects.

With these in mind, here we report a novel ZnI_2-mediated chemical synthesis of 1,2-*cis* 2-azido-2-deoxyglycosides which employs a rationally designed 4,6-*O*-tethered-*O*-TIPDS-protected 2-azido-2-deoxy-D-glucosyl trichloroacetimidate donor, achieving exclusive α-stereoselectivity with a wide range of acceptor substrate scope. Contrary to the proposed Zn^{2+}-mediated S_N2-like directed nucleophilic attack involving the simultaneous coordination of Zn^{2+} with both benzyl ether on donor and hydroxy group on acceptor in our earlier works,[20–23] results of our mechanistic studies combining experimental variable-temperature nuclear magnetic resonance (VT-NMR) characterization and theoretical density functional theory (DFT) calculations suggest that the new glycosylation reaction proceeds *via* a different mechanism, with the glycosyl oxocarbenium arising from activation of the donor preferentially adopting a conformation with the 3-silyl ether group blocking the β-face, leading to the following S_N1-like nucleophilic attack by the acceptor from α-face exclusively. We showcased the applicability of our method with the synthesis of *A. baumannii* K48 capsular trisaccharide fragment, using this reaction as the key step. We further applied the method to the synthesis of a 2-amino-2-deoxy glucose-containing pentasaccharide repeating unit *via* a convergent [3 + 2] fragment coupling strategy.

Results and discussion

Donors optimization

To identify the most effective donor for the construction of 1,2-*cis* amino glucosyl linkage, we screened a variety of glycosyl donors with different protecting groups under standard conditions (Scheme 1). A 3,4,6-tri-*O*-benzyl 2-azido-2-deoxyglucosyl donor **2a** was examined under optimized conditions (2.0 equiv. of ZnI_2 in Et_2O at 0.01 M of a concentration of the acceptor) for 1,2-*cis* glucosylation[20] and a corresponding disaccharide **4a** was obtained in a 40% yield (α : β = 1 : 2) although the 4,6-*O*-benzylidene-3-*O*-benzyl donor **2b** resulted in 21% yield with a complete α-stereoselectivity. Another 4,6-*O*-benzylidene donor **2c** with bulky TIPS-protected at C3 position gave α-product predominantly in a slightly increased yield of 35%. To enhance the obvious steric effect at C3–O protective



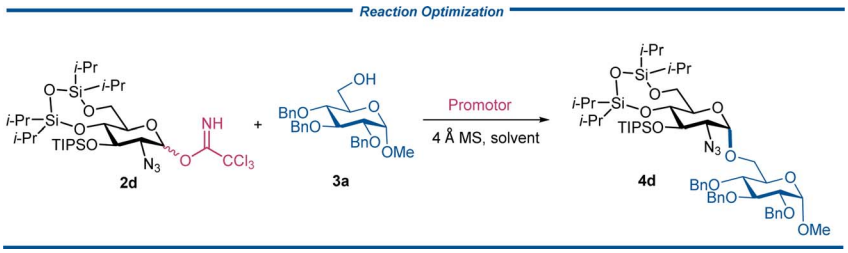
Scheme 1 Optimization of 2-azido-2-deoxy glucosyl donors. Donor **2** (2.0 equiv.), acceptor **3a** (1.0 equiv.), promotor (1.0 equiv.), MS 4 Å (100 mg mL^{-1}) were used unless otherwise specified. Combined yields of the anomeric mixture of corresponding glycosides were shown. Stereoselectivity was determined by the integration ratio obtained from ^{1}H-NMR of crude mixture.

group with 4,6-*O*-cyclic structure, the 4,6-*O*-TIPDS-protected donor **2d** equipped with bulky TIPS group was then examined and gave **4d** in 61% yield. As 3-*O*-benzoyl-4,6-*O*-TIPDS-protected donor **2e** showed relatively low stereoselectivity ($\alpha:\beta = 3:1$), the use of an bulky electron-donating group at C-3 is essential to the desired high 1,2-*cis* selectivity. Therefore, donor **2d** was chosen for further optimization of the reaction conditions.

Reaction conditions

The examination of the effects of different parameters on the stereoselective glycosylation of acceptor **3a** with donor **2d** was further carried out and the results are shown in Table 1. The results of screening of a series of representative Brønsted acids or Lewis acids (entries 1–9) pointed out ZnI_2 to be the most effective promotor among them. Through the solvent screening, Et_2O showed the highest 1,2-*cis* selectivity (entry 9, $\alpha:\beta = >20:1$) and a satisfactory yield of 53% probably because of the optimum solubility of ZnI_2 in Et_2O and ether effect in directing the 1,2-*cis* glycosylation.[20–23] Further optimizations of other various factors, such as temperature, substrate concentration, the equivalent of the promotor, and reaction time, were also conducted. Under the optimum conditions of 2.0 equiv. of ZnI_2 at 0.01 M in Et_2O for 72 h at 25 °C (entry 25), the 2-azido-2-deoxyglucosylation afforded the desired disaccharide **4d** in 82% yield with high α-selectivity ($\alpha:\beta = >20:1$). Notably, the yield of the desired product **4d** decreased at lower temperature (entries 15–18), while the concentration of substrate and equivalent of promotor had no significant impact on stereoselectivity although the promoter loading was optimum at 2.0 equiv. for the yield of the product (entries 19–29).

Table 1 Optimization of reaction conditions[a]

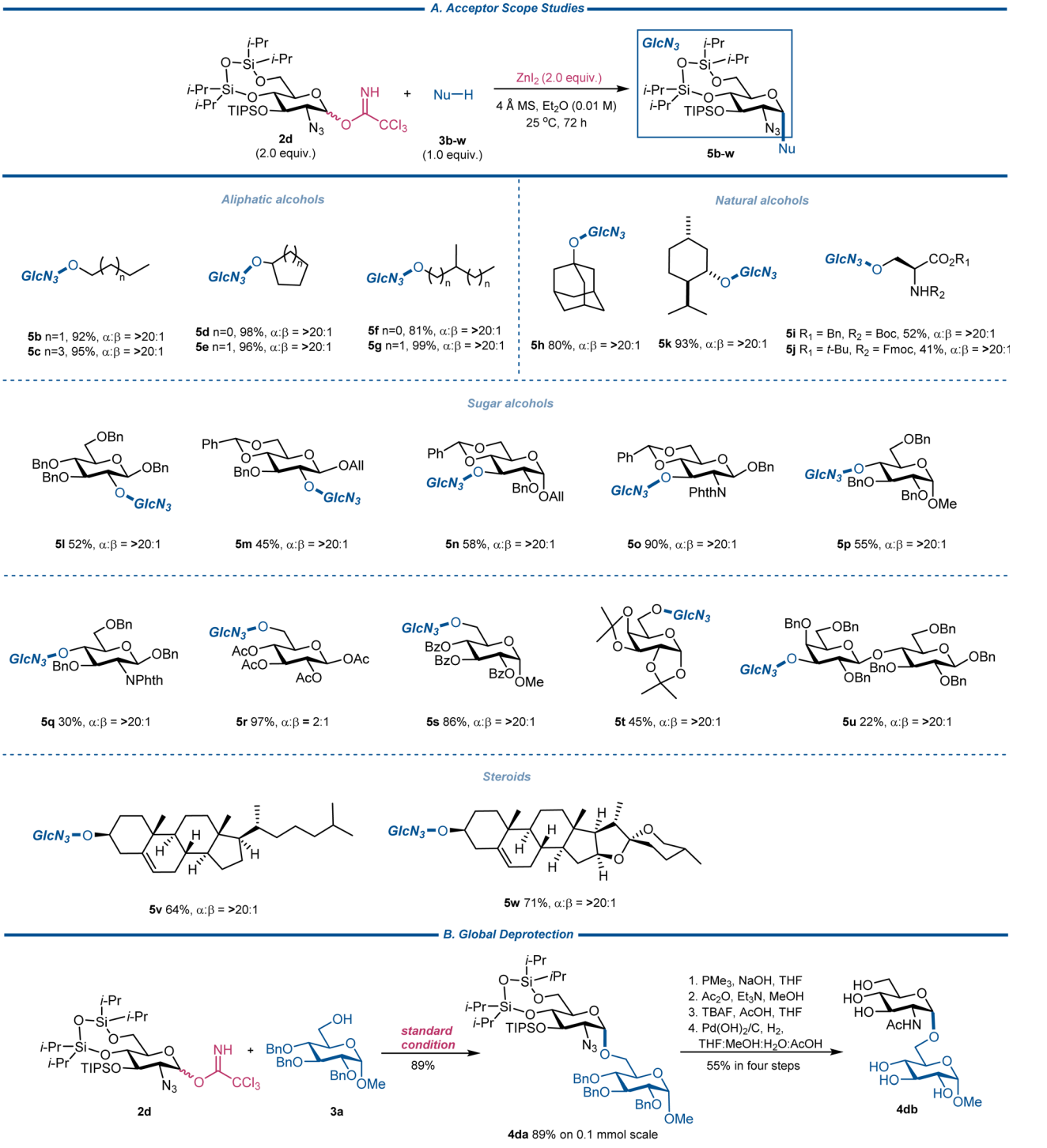


Entry	Promotor	Equiv.	Time	[M]	Solvent	Temp.	Yield[b]	$\alpha:\beta$[c]
1	TMSOTf	1.0	72 h	0.008	Et_2O	25 °C	43%	3 : 1
2	TfOH	1.0	72 h	0.008	Et_2O	25 °C	38%	3 : 1
3	$Cu(OTf)_2$	1.0	72 h	0.008	Et_2O	25 °C	45%	3 : 1
4	$ZnBr_2$	1.0	72 h	0.008	Et_2O	25 °C	Trace	—
5	$Zn(OTf)_2$	1.0	72 h	0.008	Et_2O	25 °C	45%	3 : 1
6	$ZnCl_2$	1.0	72 h	0.008	Et_2O	25 °C	32%	10 : 1
7	$CuBr_2$	1.0	72 h	0.008	Et_2O	25 °C	Trace	—
8	$B(C_6F_5)_3$	1.0	72 h	0.008	Et_2O	25 °C	30%	12 : 1
9	ZnI_2	1.0	72 h	0.008	Et_2O	25 °C	53%	>20 : 1
10	ZnI_2	1.0	72 h	0.008	Toluene	25 °C	54%	1 : 1
11	ZnI_2	1.0	72 h	0.008	DCM	25 °C	56%	3 : 1
12	ZnI_2	1.0	72 h	0.008	MeCN	25 °C	21%	—
13	ZnI_2	1.0	72 h	0.008	THF	25 °C	Trace	—
14	ZnI_2	1.0	72 h	0.008	1,4-Dioxane	25 °C	64%	9 : 1
15	ZnI_2	1.0	72 h	0.008	Et_2O	−78 °C	Trace	—
16	ZnI_2	1.0	72 h	0.008	Et_2O	−40 °C	19%	—
17	ZnI_2	1.0	72 h	0.008	Et_2O	−20 °C	25%	—
18	ZnI_2	1.0	72 h	0.008	Et_2O	0 °C	50%	8 : 1
19	ZnI_2	1.0	72 h	0.01	Et_2O	25 °C	55%	>20 : 1
20	ZnI_2	1.0	72 h	0.005	Et_2O	25 °C	50%	>20 : 1
21	ZnI_2	1.0	72 h	0.003	Et_2O	25 °C	9%	>20 : 1
22	ZnI_2	1.0	72 h	0.001	Et_2O	25 °C	Trace	—
23	ZnI_2	0.5	72 h	0.01	Et_2O	25 °C	34%	>20 : 1
24	ZnI_2	1.0	72 h	0.01	Et_2O	25 °C	61%	>20 : 1
25	**ZnI_2**	**2.0**	**72 h**	**0.01**	**Et_2O**	**25 °C**	**82%**	**>20 : 1**
26	ZnI_2	3.0	72 h	0.01	Et_2O	25 °C	45%	>20 : 1
27	ZnI_2	2.0	12 h	0.01	Et_2O	25 °C	45%	>20 : 1
28	ZnI_2	2.0	24 h	0.01	Et_2O	25 °C	55%	>20 : 1
29	ZnI_2	2.0	48 h	0.01	Et_2O	25 °C	80%	>20 : 1

[a] Reaction conditions: donor **2d** (2.0 equiv.), acceptor **3a** (1.0 equiv.), MS 4 Å (100 mg mL^{-1}). [b] Combined yield of the anomeric mixture of the corresponding glycoside. [c] Determined by the integration ratio obtained from ^{1}H-NMR of crude mixture.

Substrate scope studies

With optimum conditions in hand, the substrate scope of optimized ZnI_2-mediated 2-azido-2-deoxy glycosylation were explored (Scheme 2A). Firstly, with linear (**3b** and **3c**), cyclic (**3d** and **3e**) and branched (**3f** and **3g**) aliphatic alcohols, the desired products **5b–g** were obtained in excellent yields (81–99%) in all cases. Sterically hindered adamantanol (**3h**) and L-menthol (**3k**) were both successfully connected as corresponding 2-azido-2-deoxy glucoside with α-linkages in 80% and 93% yield,



Scheme 2 (A) Substrate scopes of ZnI_2-directed 1,2-*cis* 2-azido-2-deoxy glycosylation.[a] (B) 0.1 mmol scale synthesis and global deprotection. [a]Donor **2d** (2.0 equiv.), acceptor **3** (1.0 equiv.), promotor (2.0 equiv.), MS 4 Å (100 mg mL^{-1}) were used unless otherwise specified. Combined yields of the anomeric mixture of corresponding glycosides were shown. Stereoselectivity was determined by the integration ratio obtained from ^{1}H-NMR of crude mixture.

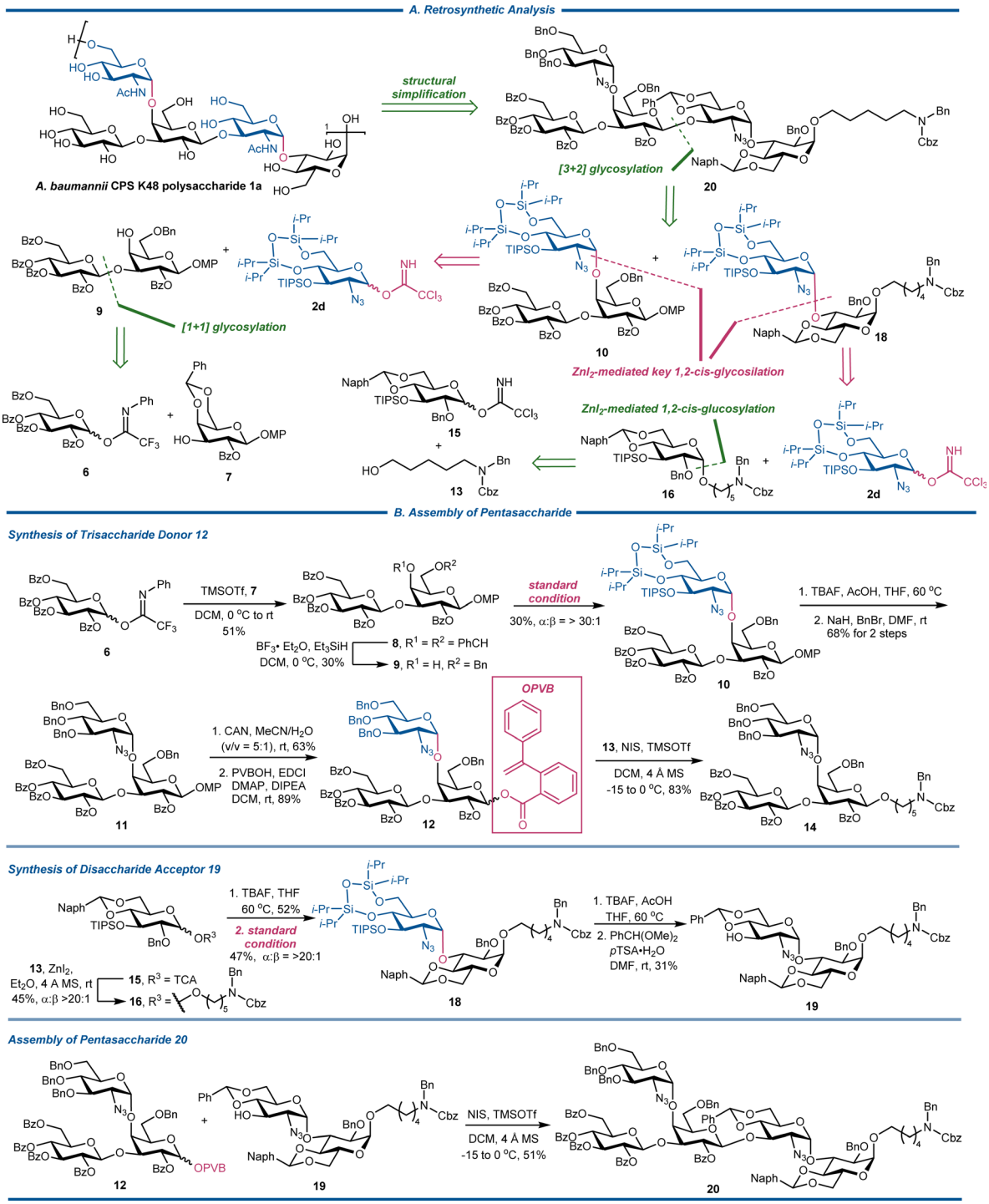
respectively (**5h–k**). ZnI_2-promoted 1,2-*cis* 2-azido-2-deoxyglucosylation tolerated a variety of glycosyl acceptors including $Glc^{O\text{-}2}$ (**3l** and **3m**), $Glc^{O\text{-}3}$ (**3n**), $GlcN^{O\text{-}3}$ (**3o**), $Glc^{O\text{-}4}$ (**3p**), $GlcN^{O\text{-}4}$ (**3q**), $Glc^{O\text{-}6}$ (**3s** and **3t**) and $Gal^{O\text{-}6}$ (**3r**), resulting in good to excellent yields (**5l–t**). Next, the disaccharide acceptor $Gal^{O\text{-}3}$-β-(1→4)-Glc (**3u**) afforded corresponding α-trisaccharide **5u** but only in 22% yield, probably due to the galactoside structure in the acceptor expected to coordinate with ZnI_2 for deactivation.[23] The amino acid such as protected L-serine derivatives **3i–j**, and naturally occurring steroids such as cholesterol (**3v**) and diosgenin (**3w**) could be applied to afford the corresponding α-glycosides (**5i–j** and **5v–w**), predominantly. Most of the acceptors tested resulted in complete α-stereoselectivities (α : β = >20 : 1) under the optimum conditions, except 1,2,3,4-tetra-*O*-acetyl-β-D-glucose **3r** ($Glc^{O\text{-}6}$) as less nucleophilic acceptor (**5r**, α : β = 2 : 1). In addition, to demonstrate the practicality of this methodology, the model glycosylation of **2d** with **3a** under the optimum conditions at a 0.1 mmol scale was performed and afforded GlcN-α-(1→6)-Glc disaccharide (**4da**) in 89% yield and complete stereoselectivity (α : β = >20 : 1). After that, the global deprotection for **4da** was conducted by reduction of azido group, *N*-acetylation, desilylation and hydrogenolysis to afford methyl 2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→6)-α-D-glucopyranoside (**4db**) in 55% yield over four steps (Scheme 2B). These results set a solid foundation for the synthesis of complex oligosaccharides using our methods.

Synthesis of *A. baumannii* CPS K48 pentasaccharide

Inspired by the effective construction of 1,2-*cis* 2-azido-2-deoxy glycosidic linkages, we applied this strategy to the unprecedented synthesis of *A. baumannii* K48 capsular pentasaccharide repeating fragment. Considering the complete and partial fragments' potential to be developed as vaccine precursors, the synthetic target was designed with a 5-aminopentyl spacer at the terminal α-glucose residue of core pentasaccharide, which could be attached to other biological molecules such as carrier protein for further immunological studies of these glycoconjugates. However, the synthesis of target molecules may be relatively tough due to the highly branching nature of the Gal unit, since the hydroxy groups at its C1, C3, and C4 positions are substituted in the meantime. Due to these predictable challenges, currently, there is no synthetic case reported about this CPS K-unit. Retrosynthetically, the desired pentasaccharide could be achieved by glycosylating disaccharide acceptor **19** with the trisaccharide donor **12** through a convergent [3 + 2] glycosylation (Scheme 3A). The 2-azido-2-deoxy α-glucosyl residue occurs in both fragments **12** and **19**, and their key 2-azido-2-deoxy α-glucosidic linkages could be constructed by our developed facile and convenient ZnI_2-mediated stereoselective glycosylation reaction to showcase the versatility and practicability of the methodology.

Our synthetic task commenced with the synthesis of trisaccharide building block **12** (Scheme 3B). The trimethylsilyl trifluoromethanesulfonate (TMSOTf)-mediated glycosylation between the perbenzoylated *N*-phenyl-trifluoroacetimidate (PTFAI) donor **6** and $Gal^{O\text{-}3}$ acceptor **7**, affording the disaccharide with a moderate selectivity (α : β = 1 : 4) mainly *via* neighboring group participation (NGP) effect, and the desired β-linked product **8** was separated in 51% yield. The reductive ring-opening of benzylidene under $BF_3 \cdot Et_2O$–Et_3SiH conditions gave C4–OH of Gal residue in disaccharide acceptor **9**, which was glycosylated with **2d** under standard ZnI_2-promoted 1,2-*cis* 2-azido-2-deoxy glucosylation conditions to afford the branched trisaccharide **10** with satisfactory α-selectivity. The low yield (30%) was mainly attributed to both the significant steric hindrance of O-3 sugar substituent and the weak nucleophilicity of galactose C4–OH due to the electron-withdrawing effect of the axial-oriented hydroxyl group.[27] Subsequent oxidative removal of the MP group of the trisaccharide **10** with the treatment of ceric ammonium nitrate (CAN) afforded the intermediate, which was then ready to be equipped with different leaving groups at C1 position for [3 + 2] glycosylation. The synthesis of $GlcN^{O\text{-}3}$-α-(1→3)-Glc disaccharide building block **19** (Scheme 3B) commenced with the 4,6-*O*-naphthylidene thioglucoside which could be converted to trichloroacetimidate donor **15** and was glycosylated with 5-aminopentyl spacer **13** to afford **16** in 45% yield following our ZnI_2-promoted α-glucosylation standard conditions as reported before (α : β > 20 : 1).[20] After removal of C3–*O*-TIPS by fluoride-mediated desilylation to afford acceptor **17**, the key ZnI_2-promoted 1,2-*cis* 2-azido-2-deoxyglucosylation with 4,6-*O*-TIPDS-protected donor **2d** afforded the desired disaccharide **18** in a complete α-stereoselectivity and 47% yield. Considering the difficulty of [3 + 2] segment ligation, the 4,6-*O*-TIPDS group of **10** was transformed to 4,6-*O*-benzylidene moiety **11** in two steps.

With *A. baumannii* CPS K48 α-$GlcN_3$-linked branched trisaccharide fragment and disaccharide acceptor in hand, we further explored the optimum condition for the key [3 + 2] assembly of pentasaccharide derivative **20** (Table 2). At first, our commonly used trichloroacetimidate (TCA) donor was tried under the strong TMSOTf catalyst but failed mainly because of the instability of the imidate (Table 2, entry 1). Most of the TCA donor was hydrolyzed in the process of silica gel column chromatography. Although *N*-phenyltrifluoroacetimidate (PTFAI) donor could be prepared, its glycosylation turned out to be less effective, affording the desired pentasaccharide in only 21% yield (Table 2, entry 2). Hence, considering the instability of the imidate-type donor, we turned to using stable ester-type glycosyl donors to avoid unpleasant donor hydrolysis.[28] Thus *ortho*-alkynylbenzoyl (ABz)[29] (Table 2, entry 3) and *ortho*-(1-phenylvinyl)benzoyl (PVB)[30] (Table 2, entry 4) groups were equipped to give the corresponding donors, but these two donors gave only trace amounts of the products. In view of the poor yields, the silyl-tethered trisaccharide **10** was converted to donor **12** in four steps (Scheme 3B). While the glycosyl ABz donor was barely effective (Table 2, entry 5), the glycosyl PVB donor **12** was able to be isolated (89%) and resulted in the formation of the corresponding glycoside **20** in 51% yield (Table 2, entry 6). Characterizations of both pentasaccharides with different protection patterns were supported by MALDI-TOF mass spectra, as confirmed by $C_{138}H_{161}N_7O_{32}Si_3Na$ at 2536.038 and $C_{138}H_{133}N_7O_{31}Na$ at 2408.103, respectively. Both



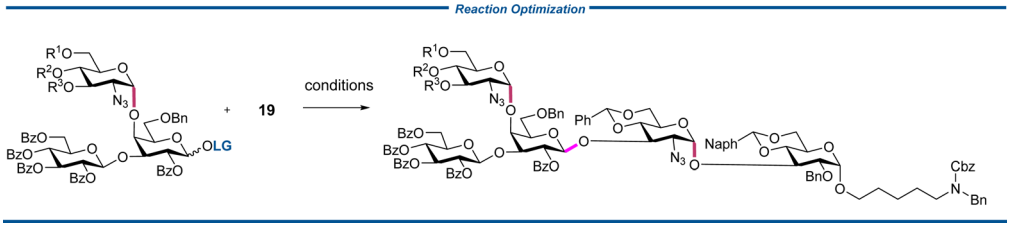
Scheme 3 (A) Retrosynthesis analysis of the *A. baumannii* CPS K48 polysaccharide repeating unit. (B) Stereoselective synthesis of building blocks and assembly of pentasaccharide.

pentasaccharides were obtained with exclusive stereoselectivity under the effect of neighboring group participation (NGP). On the other hand, the coupling of PVB donor **12** with 5-(*N*-benzyl-*N*-benzyloxycarbonylamino)-1-pentanol spacer **13** afforded the desired trisaccharide unit **14** in 83% yield ($\alpha:\beta = 1:>20$, Scheme 3B). These results of glycosylations of **12** suggested that disaccharide acceptor **19** attributed to the low [3 + 2] ligation efficiency because it is a weak and balky nucleophile compared to the spacer alcohol.

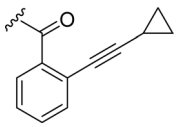
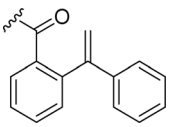
Mechanistic studies

Based on experimental results and controlled model experiments, the variable-temperature nuclear magnetic resonance experiments (VT-NMR)[31] and density functional theory

Table 2 Assembly of pentasaccharide protected fragment **20** [a]

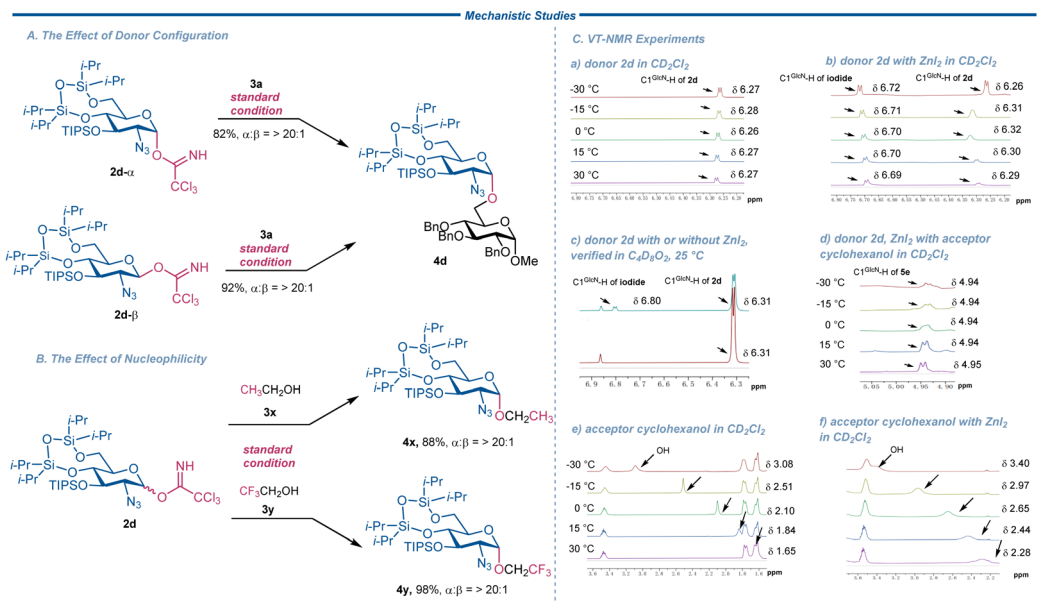


Entry	Leaving group (LG)	R	Promotor	Yield[b]	α : β[c]
1	TCA	R^1, R^2 = TIPDS, R^3 = TIPS	TMSOTf	—	—
2	PTFAI	R^1, R^2 = TIPDS, R^3 = TIPS	TMSOTf	21%	α : β 1 : >20
3	ABz	R^1, R^2 = TIPDS, R^3 = TIPS	PPh_3AuOTf	9%	α : β 1 : >20
4	PVB	R^1, R^2 = TIPDS, R^3 = TIPS	NIS, TMSOTf	12%	α : β 1 : >20
5	ABz	$R^1 = R^2 = R^3$ = Bn	PPh_3AuOTf	—	—
6	PVB	$R^1 = R^2 = R^3$ = Bn	NIS, TMSOTf	51%	α : β 1 : >20

TCA: $C(=NH)CCl_3$, PTFAI: $C(=NPh)CF_3$, ABz:  , PVB: 

[a] Conditions: see ESI and scheme. [b] Combined yield of the anomeric mixture of the corresponding glycoside. [c] Determined by the integration ratio obtained from ^{1}H-NMR of crude mixture.

calculations were conducted for the proposed plausible mechanism of ZnI_2-mediated 1,2-*cis* 2-azido-2-deoxy-glucosylation. As the model experiment, the reaction with both α- and β-isomers of 2-azido-2-deoxy-glucosyl trichloroacetimidate donor (**2d-α** and **2d-β**) favored α-product selectively (Scheme 4A). In addition, the reactions of nucleophilic ethanol (**3x**) and less nucleophilic trifluoroethanol (**3y**) with donor **2d** were performed under the standard conditions (Scheme 4B). The weak nucleophile trifluoroethanol cannot directly attack a covalent glycosyl intermediate in an S_N2-like manner to an appreciable degree.[32] Therefore, if our glycosylation proceeded with an S_N2-like process, the stereoselectivity would be contaminated. Nevertheless, under the optimal conditions, the glycosylation of **3y** invariably delivered the corresponding



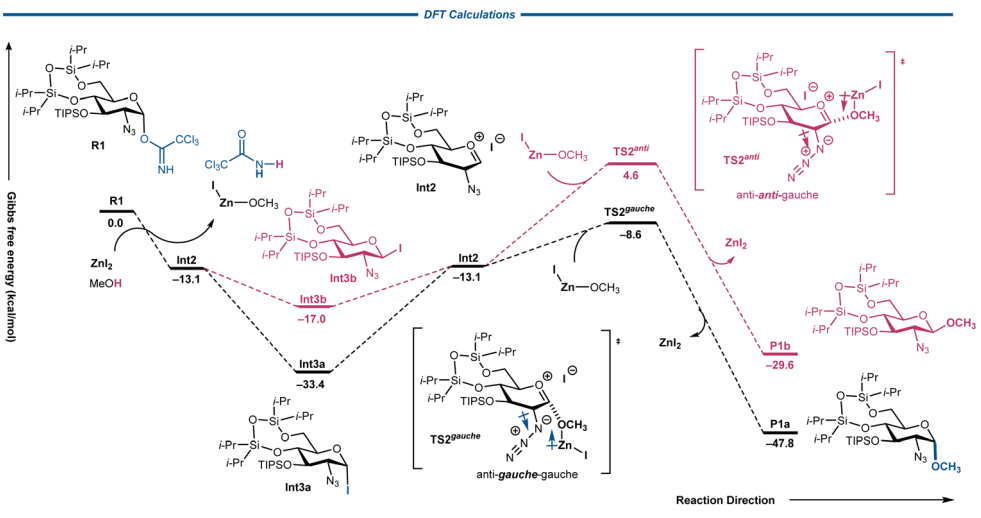
Scheme 4 Mechanistic studies of 1,2-*cis* 2-azido-2-deoxy glycosylation.

product **4y** in excellent yield (98%) and complete α-selectivity. The result implies that the glycosylation reaction is less likely to proceed through an S_N2 mechanism. Based on the literature and our experimental results, we proposed herein a mechanism involving the formation of a key intermediate of glycosyl iodide, which reversibly dissociates into ion pairs of glycosyl oxocarbenium with restricted conformation and close iodide anions. The oxocarbenium was then stereoselectively attacked by the acceptor nucleophile in an S_N1-like manner.

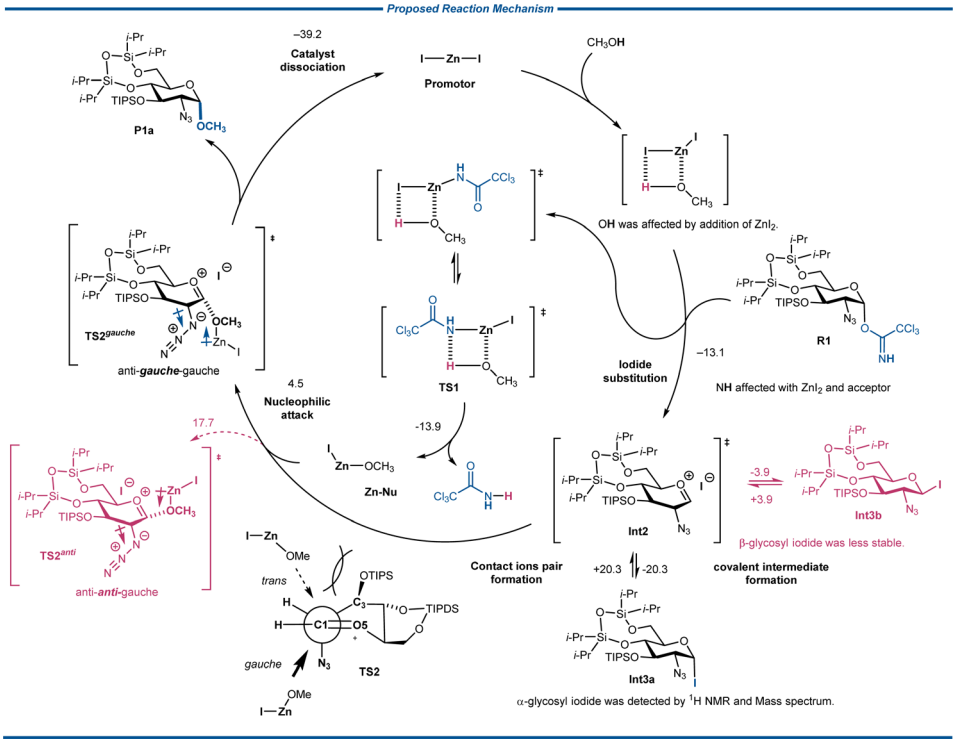
In the previous discussion for the ZnI_2-mediated glycosylation with trichloroacetimidate donor,[20–23] the initial generation of unstable glycosyl iodide intermediate has been considered. Although the formation of $C1^{GlcN}$-iodide from **2d** was expected, the intermediate was too unstable to isolate by silica gel chromatography separation. To confirm the existence of iodide, the VT-NMR studies were therefore performed at five temperature gradients from −30 °C to 30 °C (Scheme 4C). Considering the unavailability of Et_2O-d_{10} and the melting point of dioxane-d_8, dichloromethane-d_2 was selected as the deuterated solvent for VT-NMR study. The equivalent of donor (38 mM), acceptor cyclohexanol (19 mM) and ZnI_2 (38 mM) complied with the standard conditions for ZnI_2-mediated 1,2-*cis* 2-azido-2-deoxyglucosylation. The $\delta(C1^{GlcN}–H)$ of donor **2d** was monitored almost invariably around 6.27 ppm (Scheme 4C(a)) with or without zinc iodide addition. As expected, we observed $\delta(C1^{GlcN}–H)$ of iodide at about 6.70 ppm (Scheme 4C(b)), supported by mass spectra of target $C_{27}H_{56}N_3O_5Si_3I$ at 713.40 and $C_{27}H_{56}N_3O_5Si_3INa$ at 736.80 (Fig. S6 and S7†). A further ^{1}H-NMR experiment in dioxane-d_8 instead of Et_2O at 25 °C also showed $\delta(C1^{GlcN}–H)_{\mathbf{2d}}$ at 6.31 ppm and $\delta(C1^{GlcN}–H)$ of iodide at 6.80 ppm, as verified by VT-NMR experiment (Scheme 4C(c)). The coupling constants ($^3J_{H1–H2}$) of $(C1^{GlcN}–H)$ of iodide were 3.8 Hz in dichloromethane-d_2 and 3.9 Hz in dioxane-d_8, strongly confirming α-iodide formation according to the Karplus equation, while the expected peaks of β-iodide were not detectable in the solution. When the acceptor cyclohexanol was added to the mixture, the $\delta(C1^{GlcN}–H)$ of product **5e** could be observed at 4.94 ppm as α-glycoside ($^3J_{H1–H2}$ = 3.6 Hz, Scheme 4C(d)). Both control and VT-NMR experimental results clearly suggested that the reaction proceeded through α-iodide *via* initial S_N1 reaction followed by subsequent S_N1 reaction to afford the α-glycoside.

For further investigation of the function of ZnI_2 in the reaction process, δ(OH) of acceptor cyclohexanol with or without ZnI_2 addition were compared, and as a result, the δ(OH) shifted downfield from a range of 3.08–1.65 ppm to 3.40–2.28 ppm after addition of ZnI_2 (Scheme 4C(e) and (f)). In contrast, the δ(NH) of donor **2d** remained at 8.62–8.58 ppm after addition of ZnI_2 (Scheme S1†), albeit a chemical shift of $(C1^{GlcN}–H)_{\mathbf{2d}}$ seemed to be slightly affected at the same time (Scheme 4C(b)) and the integrations of ^{1}H peaks related to **2d** decreased from −30 °C to 30 °C with a contrasting increase of those of α-iodide (Scheme S1†). The results indicated that ZnI_2 preferentially coordinated with OH in the acceptor instead of NH in the trichloroacetimidate group, although α-iodide formation also proceeded by the addition of ZnI_2 to **2d** in the absence of acceptor alcohol.

Following the observations from ^{1}H-NMR studies, we proposed a potential mechanism and explained the rationality by density functional theory (DFT) calculation (Schemes 5 and 6). Initially, the zinc cation activated the leaving group of the trichloroacetimidate donor; therefore, C1–O1 bond was weakened and the trichloroacetimidate ion departed from the glycosyl donor. The intermediate **Zn-LG** formed, accompanied by the dissociated iodide anion adding to the anomeric carbon through the oxocarbenium ion **Int2** with $^3H_4$ half-chair[33] and producing α-glycosyl iodide intermediate **Int3a**, as we observed from VT-NMR. Calculations also confirmed that α-glycosyl iodide intermediate **Int3a** was more stable than the corresponding β-iodide intermediate **Int3b** (**Int3a** *vs.* **Int3b** in Scheme 5). Meanwhile, the proton transferred from O–H bond of CH_3OH to the intermediate **Zn-LG** and generated the nucleophilic reagent **Zn-Nu** in an exothermic fashion (Fig. S8†). Although the stable **Int3a** was observed for NMR analysis, **Int2** should be used as the key intermediate to **TS2**. The transition



Scheme 5 DFT calculations.



Scheme 6 Proposed reaction mechanism.

states **TS2**^{gauche}/**TS2**^{anti} were proposed for the suggested second S_N1-like displacement through glycosyl oxocarbenium ion **Int2** by nucleophilic attack of the deprotonated alcohol as zinc methoxide complex to deliver the product. Computational results showed **Zn-Nu** α-nucleophilic attack of **Zn-Nu** took lower energy barrier than that towards oxocarbenium ion from β-face (+4.5 kcal mol^{-1} *vs.* +17.7 kcal mol^{-1}, from α *vs.* β, respectively) (Scheme 6). The conformation-directing nucleophilic attack of **Zn-Nu** in transition state **TS2**^{gauche} might be attributed to the azido *gauche* effect, a preference that orients electronegative substituent to *gauche* form when adjacent azido group exists.[34] Inspection of the structure of the **TS2**^{gauche} pyranose ring also suggested that the bulky C3-OTIPS group effectively shields the β-side and prevents the acceptor from attacking from the β-face. It is also indicated that β-glycoside was +18.2 kcal mol^{-1} higher in energy than the α-selective product (**P1a** *vs.* **P1b**, Scheme 6). According to the calculation results, the α-product was preferred in consistency with the experimental observation.

Conclusions

In summary, we have successfully developed a ZnI_2-mediated strategy for synthesizing 1,2-*cis* 2-azido glucosides with excellent stereoselectivities and wide acceptor substrate scope, employing the rationally designed 4,6-*O*-tethered-tetraisopropyldisiloxanylidene (TIPDS)-protected 2-deoxy-2-azido-D-glucosyl trichloroacetimidate donor **2d**. We demonstrated the usefulness of the novel methodology by synthesizing protected antigenic branched trisaccharide **10** and disaccharide fragment **18** in the *Acinetobacter baumannii* K48 capsular polysaccharide and a pentasaccharide derivative **20** containing the antigenic trisaccharide structure through a convergent [3 + 2] glycosylation reaction. Mechanistic studies combining VT-NMR investigations and DFT calculations delineate a proposed mechanism involving the formation of a key intermediate α-glycosyl iodide **Int2a** upon donor activation with ZnI_2, the reversible conversion of **Int2a** into glycosyl oxocarbenium in the solution, and the preferential α-face attack of the glycosyl oxocarbenium by the acceptor passing through transition state **TS2**^{gauche} along an S_N1-like pathway, under the influences of the stereo-directing azido *gauche* effect. Our lab is carrying out further experimental examinations focusing on the scale-up synthesis of *A. baumannii* K48 capsular pentasaccharide repeating fragment and investigations of the potential biological activity of deprotected moieties with macromolecule conjugates to the terminal residue, paving ways for future vaccine development.

Data availability

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

Author contributions

Conceptualization: F. Q. D. and X. Y. Z.; methodology: X. Y. Z. and X. M. Z.; investigation: X. Y. Z., H. D., A. X. G., Y. H. L. and A. I.; resources: F. Q. D. and X. Y. Z.; original draft: F. Q. D., X. Y. Z. and A. I.; review and editing: all authors; funding acquisition: F.

Q. D. and H. C.; project administration: F. Q. D. and H. C.; supervision: F. Q. D. and H. C.

Conflicts of interest

There are no conflicts to declare.

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