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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-2deoxyglycoside core of CPS remains challenging to date. Here we develop a highly α -selective Znl₂mediated 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-p-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-2deoxy glycosidic linkage. The reaction mechanism was explored with combined experimental variabletemperature NMR (VT-NMR) studies and mass spectroscopy (MS) analysis, and theoretical density functional theory calculations, which suggested the formation of covalent $\alpha\text{-C1}^{GlcN}$ -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 ZnI₂-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. 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's⁴ work for synthesizing *O*-antigens; Hashimoto,⁵ Kosma⁶ and Yin's⁷ works for lipopolysaccharide (LPS); and Gao,⁸ Seeberger,⁹ Ragains,¹⁰ Xiao's¹¹ 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 group¹² 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 Kerns¹⁵ and Manabe-Ito¹⁶ 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 benzylideneaming donor with a nickel triflate catalyst imparting 1,2-cis stereoselectivity. In 1978, Paulsen¹⁸ 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

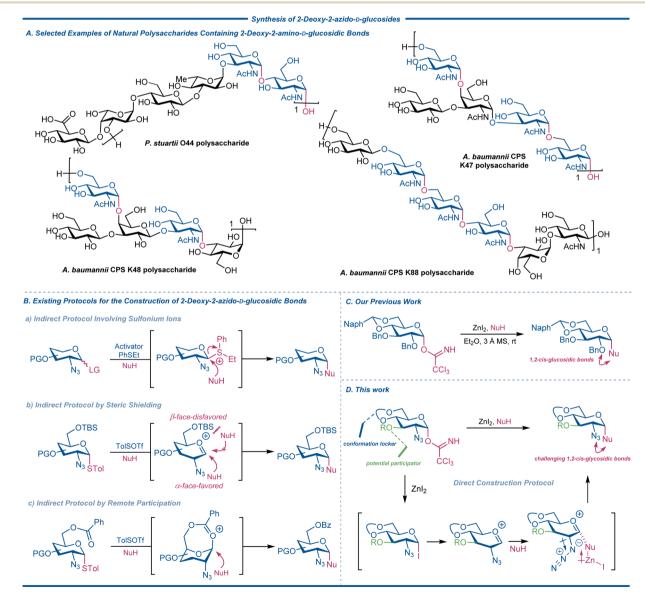


Fig. 1 (A) Selected examples of natural polysaccharides containing 2-deoxy-2-amino-p-glucosidic bonds; (B) indirect approaches to the stereoselective synthesis of 1,2-cis 2-deoxy-2-azido glycosides; (C) our previous work for the construction of 1,2-cis glucosidic bond mediated by Znl₂; (D) presenting work about direct approach to 1,2-cis 2-deoxy-2-azido glycosides mediated by Znl₂.

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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 ZnI₂, effectively promotes cis glycosylation such as α-glucosylation,²⁰ β-mannosylation,²¹ and β-rhamnosylation²² 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-2deoxy 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-aminoglucoside structures (Fig. 1D). Moreover, Bols' work24 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 delivery25 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 silvl ethers can significantly improve α -stereoselectivity through synergistic stereoelectronic effects.

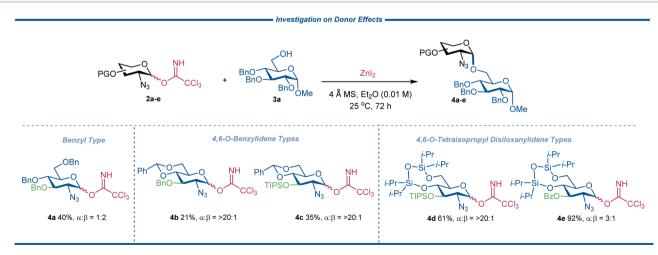
With these in mind, here we report a novel $\rm ZnI_2$ -mediated chemical synthesis of 1,2-cis 2-azido-2-deoxyglycosides which employs a rationally designed 4,6- $\rm O$ -tethered- $\rm O$ -TIPDS-protected

trichloroacetimidate 2-azido-2-deoxy-D-glucosyl donor, achieving exclusive α -stereoselectivity with a wide range of acceptor substrate scope. Contrary to the proposed Zn2+-mediated S_N2-like directed nucleophilic attack involving the simultaneous coordination of Zn2+ with both benzyl ether on donor and hydroxy group on acceptor in our earlier works, 20-23 results of our mechanistic studies combining experimental variabletemperature 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-2deoxy 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 ZnI₂ in Et₂O at 0.01 M of a concentration of the acceptor) for 1,2-cis glucosylation²⁰ 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 ¹H-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 (α : β = 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,

Et₂O showed the highest 1,2-cis selectivity (entry 9, α : β = >20: 1) and a satisfactory yield of 53% probably because of the optimum solubility of ZnI₂ in Et₂O 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 ZnI₂ at 0.01 M in Et₂O 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 (α : β = >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 stereo-selectivity 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

1	TMSOTf						Yield ^b	$\alpha:\beta^c$
		1.0	72 h	0.008	$\mathrm{Et_2O}$	25 °C	43%	3:1
2	TfOH	1.0	72 h	0.008	$\mathrm{Et_2O}$	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	$\mathrm{Et_2O}$	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 ₂ O	25 °C	34%	>20:1
24	ZnI_2	1.0	72 h	0.01	Et ₂ O	25 °C	61%	>20:1
25	ZnI_2	2.0	72 h	0.01	Et ₂ O	25 °C	82%	>20:1
26	ZnI_2	3.0	72 h	0.01	Et ₂ O	25 °C	45%	>20:1
27	ZnI_2	2.0	12 h	0.01	Et ₂ O	25 °C	45%	>20:1
28	ZnI_2	2.0	24 h	0.01	Et ₂ O	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 ¹H-NMR of crude mixture.

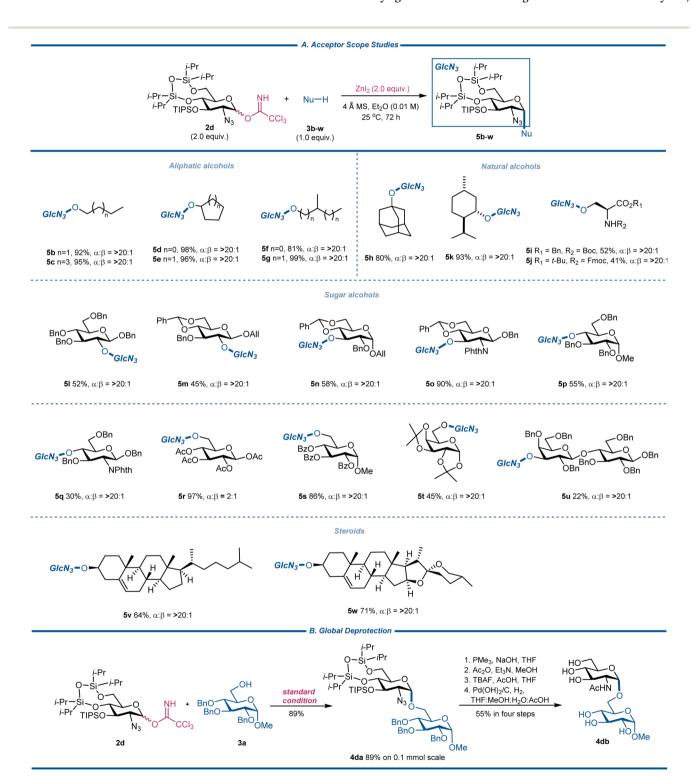
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Substrate scope studies

With optimum conditions in hand, the substrate scope of optimized ZnI2-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-2deoxy glucoside with α-linkages in 80% and 93% yield,



Scheme 2 (A) Substrate scopes of ZnI₂-directed 1,2-cis 2-azido-2-deoxy glycosylation.^a (B) 0.1 mmol scale synthesis and global deprotection. ^aDonor **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 ¹H-NMR of crude mixture.

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respectively 2-azido-2-(5h-k). ZnI₂-promoted 1,2-*cis* deoxyglucosylation tolerated a variety of glycosyl acceptors including Glc^{O-2} (31 and 3m), Glc^{O-3} (3n), GlcN^{O-3} (3o), Glc^{O-4} (3p), GlcN^{O-4} (3q), Glc^{O-6} (3s and 3t) and Gal^{O-6} (3r), resulting in good to excellent yields (51-t). Next, the disaccharide acceptor Gal^{O-3}-β-(1 \rightarrow 4)-Glc (3**u**) afforded corresponding α-trisaccharide 5u but only in 22% yield, probably due to the galactoside structure in the acceptor expected to coordinate with ZnI2 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 (5**i**-**j** and 5**v**-**w**), predominantly. Most of the acceptors tested resulted in complete α-stereoselectivities ($\alpha:\beta = >20:1$) under the optimum conditions, except 1,2,3,4-tetra-O-acetyl-β-p-glucose 3r (Glc^{O-6}) as less nucleophilic acceptor (5r, α : $\beta = 2:1$). 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 \rightarrow 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-Dglucopyranosyl- $(1 \rightarrow 6)$ - 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

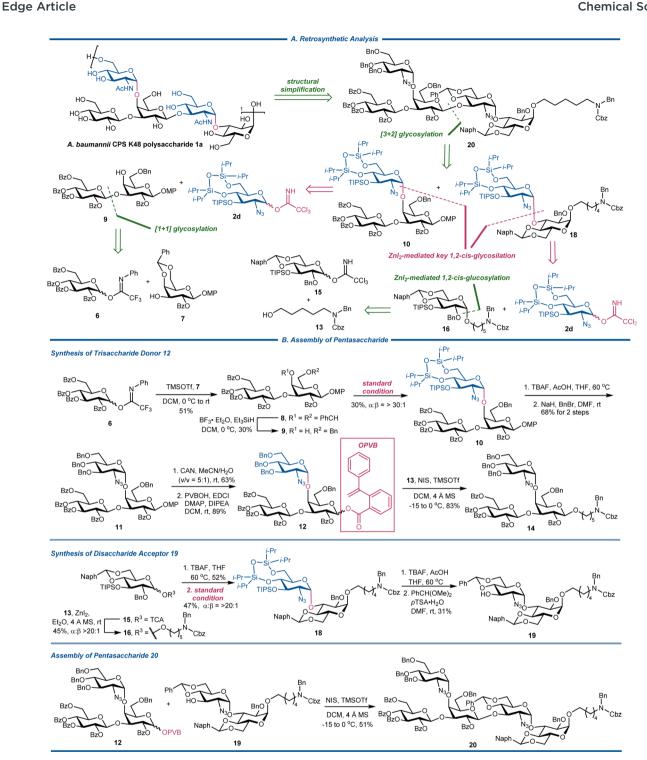
Synthesis of A. baumannii CPS K48 pentasaccharide

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 2azido-2-deoxy α-glucosidic linkages could be constructed by our developed facile and convenient ZnI2-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-3} acceptor 7, affording the disaccharide with a moderate selectivity ($\alpha: \beta = 1:4$) mainly *via* neighboring group participation (NGP) effect, and the desired βlinked product 8 was separated in 51% yield. The reductive ringopening of benzylidene under BF₃·Et₂O-Et₃SiH conditions gave C4-OH of Gal residue in disaccharide acceptor 9, which was glycosylated with 2d under standard ZnI₂-promoted 1,2-cis 2azido-2-deoxy glucosylation conditions to afford the branched trisaccharide 10 with satisfactory \alpha-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-3}- α -(1 \rightarrow 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 ZnI₂-promoted α-glucosylation standard conditions as reported before $(\alpha: \beta > 20: 1)^{20}$ After removal of C3-O-TIPS by fluoride-mediated desilylation to afford acceptor 17, the key ZnI2-promoted 1,2-cis 2-azido-2deoxyglucosylation 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-Obenzylidene moiety 11 in two steps.

With A. baumannii CPS K48 α-GlcN₃-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 orthoalkynylbenzoyl (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 C138H133N7O31Na 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

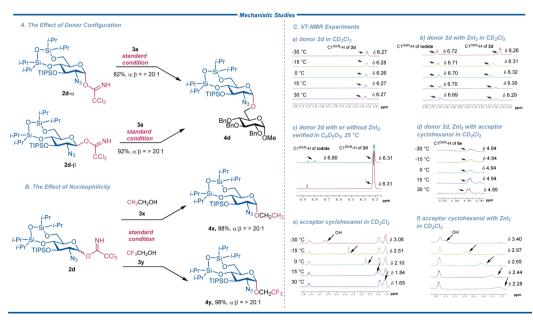
Table 2 Assembly of pentasaccharide protected fragment 20 ^a

Entry	Leaving group (LG)	R	Promotor	Yield ^b	$\alpha:\beta^c$
1 2 3 4 5 6	TCA PTFAI ABz PVB ABz PVB	R^1 , R^2 = TIPDS, R^3 = TIPS R^1 = R^2 = R^3 = Bn R^1 = R^2 = R^3 = Bn	TMSOTf TMSOTf PPh ₃ AuOTf NIS, TMSOTf PPh ₃ AuOTf NIS, TMSOTf	21% 9% 12% — 51%	
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 ¹H-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_N 2-like manner to an appreciable degree. Therefore, if our glycosylation proceeded with an S_N 2-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.

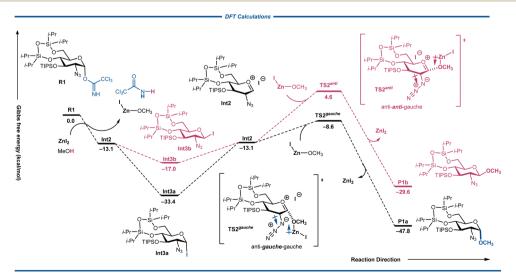
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product 4y in excellent yield (98%) and complete α -selectivity. The result implies that the glycosylation reaction is less likely to proceed through an S_N2 mechanism. Based on the literature and our experimental results, we proposed herein a mechanism 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.

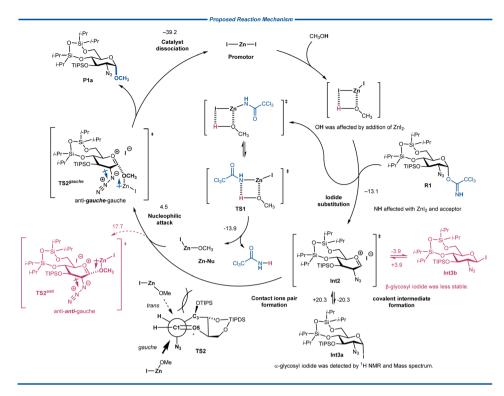
In the previous discussion for the ZnI2-mediated glycosylation with trichloroacetimidate donor, 20-23 the initial generation of unstable glycosyl iodide intermediate has been considered. Although the formation of C1^{GlcN}-iodide from 2d was expected, the intermediate was too unstable to isolate by silica 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 Et₂O- 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 ZnI2 (38 mM) complied with the standard conditions for ZnI2-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 C₂₇H₅₆N₃O₅Si₃I at 713.40 and $C_{27}H_{56}N_3O_5Si_3INa$ at 736.80 (Fig. S6 and S7†). A further ¹H-NMR experiment in dioxane-d₈ instead of Et₂O at 25 °C also showed $\delta(C1^{GlcN}-H)_{2d}$ 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 $(^{3}J_{\text{H1-H2}})$ of $(\text{C1}^{\text{GlcN}}\text{-H})$ 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 (${}^3J_{\rm H1-H2}=3.6$ Hz, Scheme 4C(d)). Both control and VT-NMR experimental results clearly suggested that the reaction proceeded through α -iodide via initial $S_{\rm N}1$ reaction followed by subsequent $S_{\rm N}1$ reaction to afford the α -glycoside.

For further investigation of the function of ZnI_2 in the reaction process, $\delta(OH)$ of acceptor cyclohexanol with or without ZnI_2 addition were compared, and as a result, the $\delta(OH)$ 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 $\delta(NH)$ of donor 2d remained at 8.62–8.58 ppm after addition of ZnI_2 (Scheme S1†), albeit a chemical shift of $(C1^{GlcN}-H)_{2d}$ seemed to be slightly affected at the same time (Scheme 4C(b)) and the integrations of 1H 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 ¹H-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 ${}^{3}H_{4}$ half-chair 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 CH₃OH 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 TS2gauche/TS2anti 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 TS2gauche 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 ZnI₂-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-p-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 ZnI₂, 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 TS2gauche along an SN1-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.

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

Conflicts of interest

There are no conflicts to declare.

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