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Chemical Synthesis and Antigenic Evaluation of Oligosaccharides of *Bordetella hinzii* O-Antigen Containing Unique Amidated 2,3-Diacetamido-2,3-dideoxy-alduronic Acids

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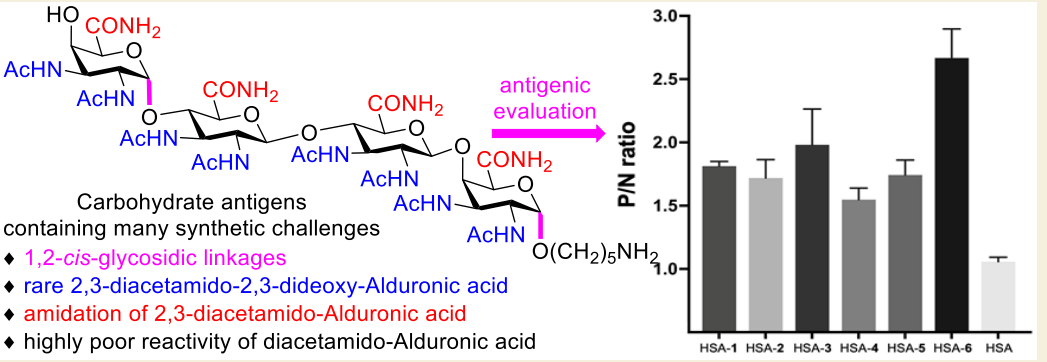

ABSTRACT: *Bordetella hinzii* is a zoonotic pathogen, which can cause brain abscess, pneumonia, bacteremia, and urinary tract infection. Vaccines are economical and effective means for combating infectious diseases. Herein, we present the first total synthesis of the highly functionalized mono- and oligosaccharides of *B. hinzii* O-antigen for vaccine development. The rare 2,3-diacetamidopyranoses were generated from 3-*O*-acetyl-2-nitroglycals via an organocatalyzed one-pot relay glycosylation method. The postglycosylation oxidation strategy was used to overcome the poor reactivity of 2,3-diacetamido-aldouronic acid building blocks in glycosylation reactions. Direct amidation of alduronic acid with NH_3 in the late stage reduced the protecting group operation and increased the synthetic efficiency. Di-*tert*-butylsilylidene-directed α-galactosylation method was used to construct challenging 1,2-*cis*-glycosidic bond. Six oligosaccharides of *B. hinzii* O-antigen were obtained and further conjugated to human serum albumin for antigenicity evaluation (the sera antibodies were obtained from vaccinated mouse via inactivated *B. hinzii*). The terminal tetrasaccharide of *B. hinzii* O-antigen has been identified as a potential glycol-epitope and might be useful for vaccine development against *B. hinzii*.

KEYWORDS: *Bordetella hinzii, carbohydrate-based vaccines, rare oligosaccharide, 2,3-diacetamido-alduronic acids, stereoselective glycosylation*

1. INTRODUCTION

Bordetella hinzii (*B. hinzii*) is a Gram-negative zoonotic bacterial pathogen, which can cause many diseases including brain abscess, pneumonia, bacteremia, and urinary tract infection.[1−5] To avoid the problem of antibiotic resistance, vaccination is a good option against *B. hinzii* infection. Cell surface carbohydrates have been proven as effective antigens, which can be conjugated to carrier protein for generating long-lasting glycoconjugate vaccines with broad application.[6−9] To date, there are some commercially available glycoconjugate vaccines to prevent *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, and *Salmonella typhi* infections.[6,10,11] With the advances of oligosaccharide synthesis and glycoimmunology, development of synthetic carbohydrate-based vaccines with rational, well-defined epitopes and suitable carriers has aroused great interests.[8,12−27] The O-specific polysaccharide chain of *B. hinzii* consists of a [→4(-*β*-GlcpNAc3NAcAN-(1→4)-*β*-GlcpNAc3NAcAN-(1→4)-*α*-GalpNAc3NAcAN-)1-] trisaccharide repeating unit with a 4-*O*-methylated GalNAc3NAcAN residue as the terminal sugar (Figure 1).[28,29] Up to now, synthesis of *B. hinzii* O-antigen has not been reported, owing to its structure complexity and

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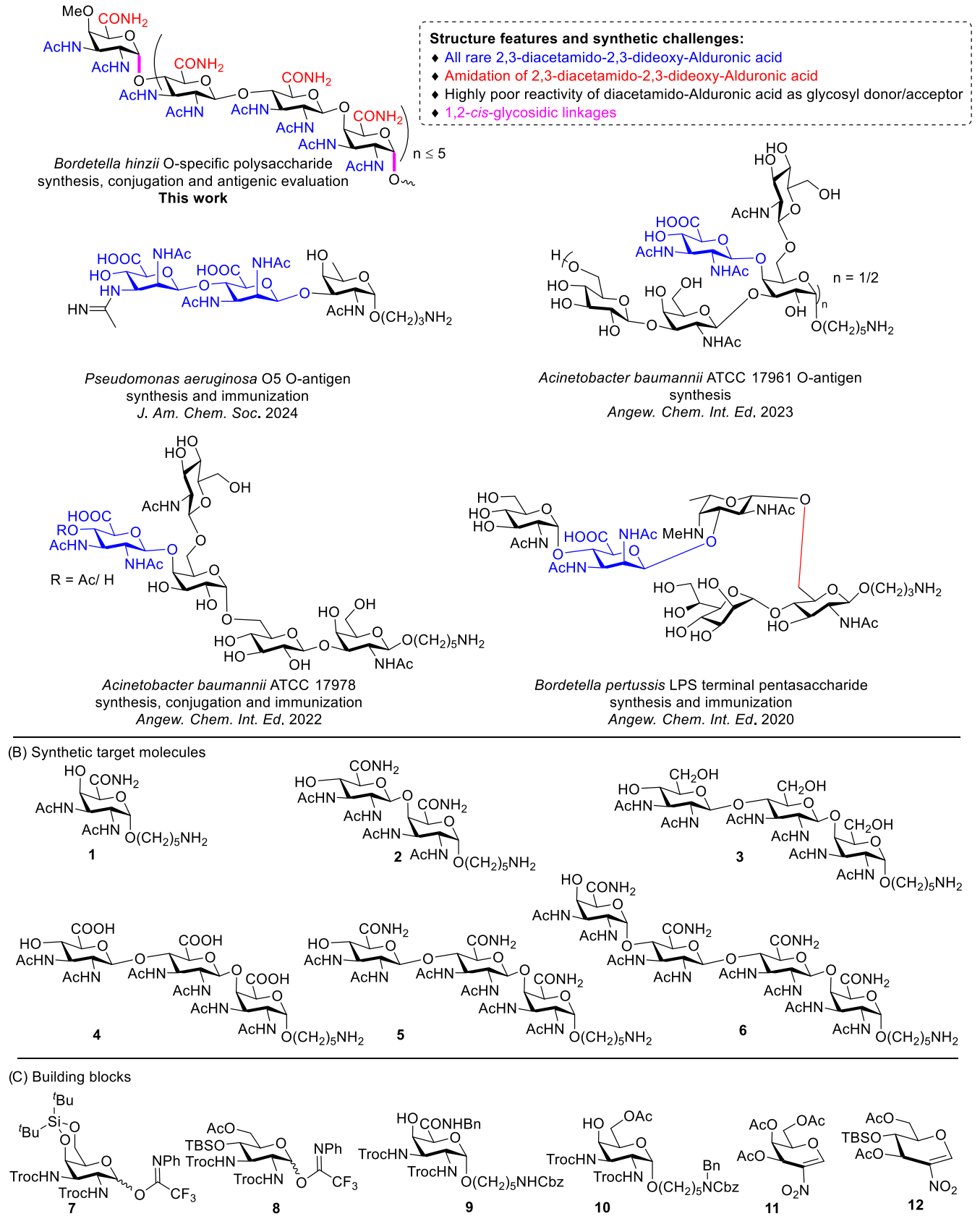

Figure 1. (A) Representative structure of bacteria glycan containing 2,3-diacetamido-galacturonic acid. (B) Synthetic target molecules. (C) Synthetic building blocks.

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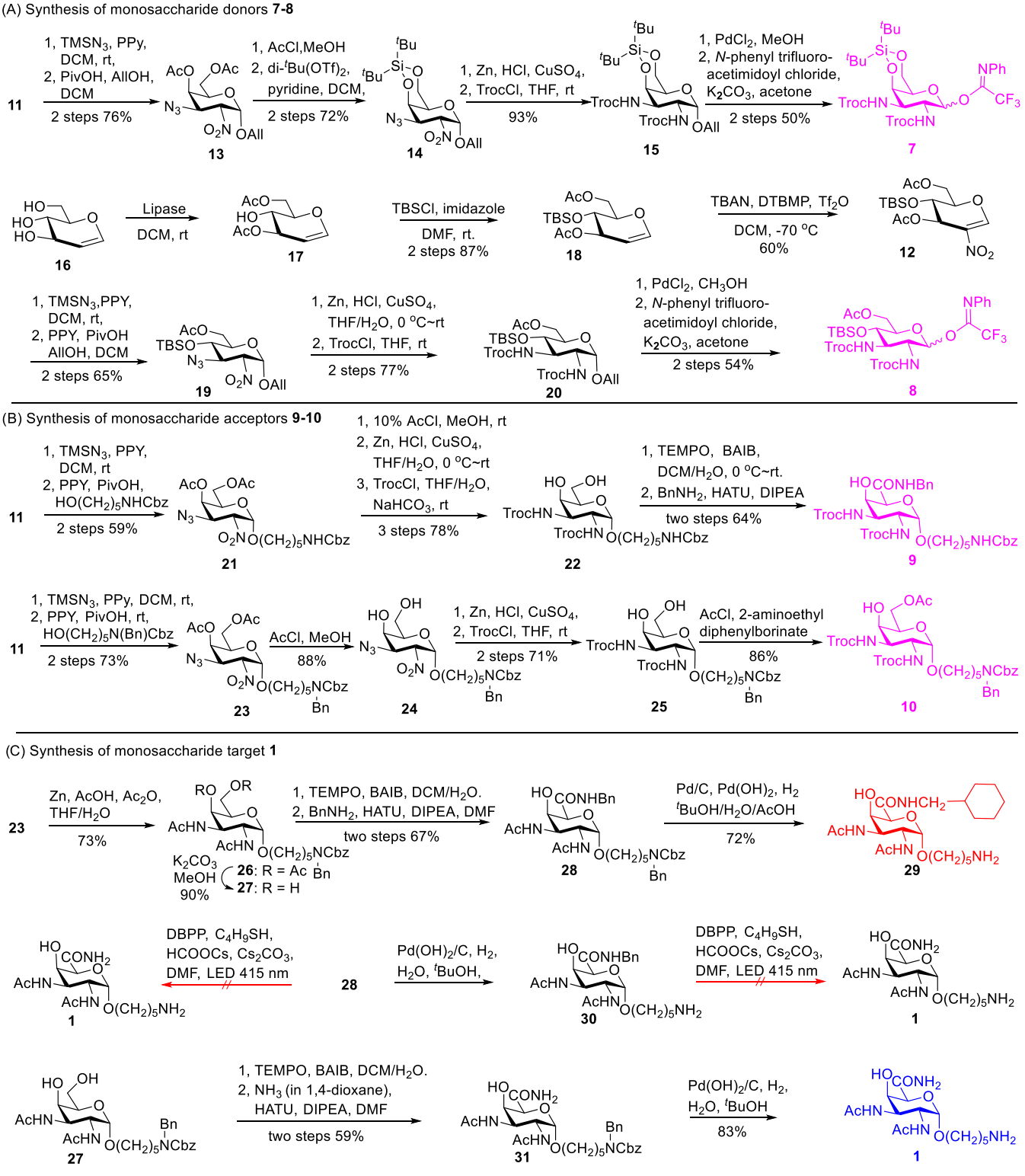
Scheme 1. (A) Synthesis of Monosaccharide Donors 7-8. (B) Synthesis of Monosaccharide Acceptors 9-10. (C) Synthesis of Monosaccharide Target 1



synthetic challenges. First, all sugar fragments are rare 2,3-diacetamido-2,3-dideoxy-alduronic acids which are difficult to obtain and their reactivities are very poor as both glycosyl donor and acceptor.[30−32] It is noting that the rare sugar 2,3-diacetamido-2,3-dideoxy-alduronic acids, which plays an important role in the antigenicity, is also present in many other strains such as *Pseudomonas aeruginosa* O5,[26] *Acinetobacter baumannii* ATCC 17961/17978[27,33], and *Bordetella pertussis*.[23] Second, the amidation of 2,3-diacetamido-2,3-dideoxy-alduronic acid increases synthetic difficulty.[34,35] Third, the nucleophilicity of the 4-hydroxyl group of the diacetamido-galacturonic acid residue is extremely poor.[36,37] In addition, the construction of 1,2-*cis*-glycosidic bonds of

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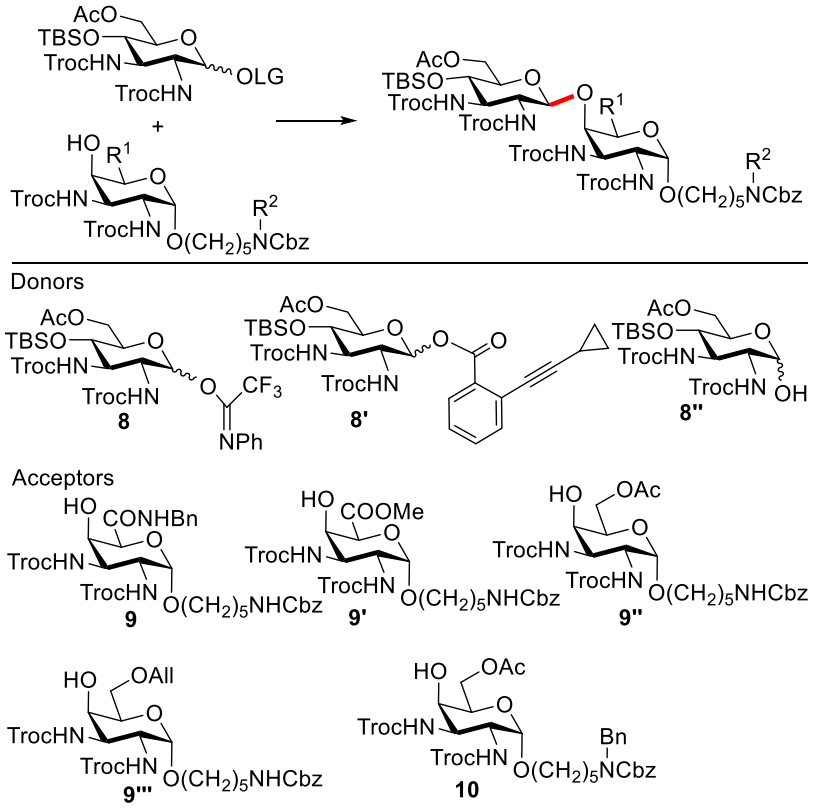
Table 1. Screening of Conditions for the Glycosylation of Monosaccharide Donors and Acceptors



entry	donor	acceptor	activator	product	yield
1	**8**	**9**	TMSOTf	**32**	trace
2	**8**	**9**	TBSOTf	**32**	trace
3	**8**	**9**	TfOH	**32**	trace
4	**8′**	**9**	$Ph_3PAuNTf_2$	**32**	trace
5	**8″**	**9**	Tf_2O, Ph_2SO, TTBP	**32**	trace
6	**8**	**9′**	TBSOTf	**33**	20%
7	**8**	**9″**	TBSOTf	**34**	27%
8	**8**	**9‴**	TMSOTf	**35**	21%
9	**8**	**9**	TBSOTf	**36**	62%

aminosugars remains challenging, especially in highly functional complex oligosaccharides.[38−42]

Herein, we report the total synthesis and antigenic evaluation of the *B. hinzii* O-antigen terminal tetrasaccharide **6**, trisaccharide **5** (one repeating unit), trisaccharide derivatives **3**−**4** and fragments **1**−**2**. To overcome the poor reactivity of 2,3-diacetamido-galacturonic acid building blocks in glycosylation reactions when used either as a donor or as an acceptor, we chose the post-glycosylation oxidation strategy for glycan assembly (oxidation stage adjustment): The introduction of the carboxylic acids was postponed after the glycan backbone was fabricated. This strategy allowed the use of a 4,6-di-*tert*-butylsilylidene-protected 2,3-diacylamidogalactose donor to stereoselectively construct the 1,2-*cis*-glycosidic bonds, even though a neighboring participation group existed at the C2 position of the donor;[20,36,43−45] meanwhile the silyl protecting group could increase the reactivity of the donor.[46−48] Furthermore, the amidation of the uronic acids could be arranged at a late stage of the synthesis so as to minimize the steps of protecting group manipulations on advanced intermediates. The rare 2,3-diamino-2,3-dideoxy-sugars could be obtained from the corresponding 3-*O*-acetyl-2-nitroglycals using organocatalyzed one-pot relay glycosylation method.[27,49] Thus, the target compounds **1**−**6** could be synthesized from building blocks **7**−**12**, which were further conjugated to carrier protein for antigenic evaluation (Figure 1).

2. RESULTS AND DISCUSSION

2.1. Synthesis of Monosaccharide Building Blocks 7−10 and Target Monosaccharide 1

The synthesis of 4,6-*O*-silylidene-protected 2,3-diaminogalactose donor **7** started with 3,4,6-tri-*O*-acetyl-D-2-nitrogalacal **11** (Scheme 1A). The desired intermediate allyl 2-nitro-3-azide-galactoside **13** was obtained in 76% yield with excellent stereoselectivity from 3-*O*-acetyl-2-nitrogalacal **11** using 4-pyrrolidinopyridine (PPY) mediated one-pot relay glycosylation.[27,49] Deacetylation in **13** with acetyl chloride/methanol afforded the corresponding 4,6-diol, which was followed by di-*tert*-butylsilylene protection to furnish **14** in 72% yield over two steps. Reduction of the nitro and azide groups in **14** with Zn/HCl/$CuSO_4$ gave free amines, which was subjected to *N*-acylation using 2,2,2-trichloroethoxycarbonyl chloride (TrocCl)/Et_3N to generate compound **15** in 93% yield over

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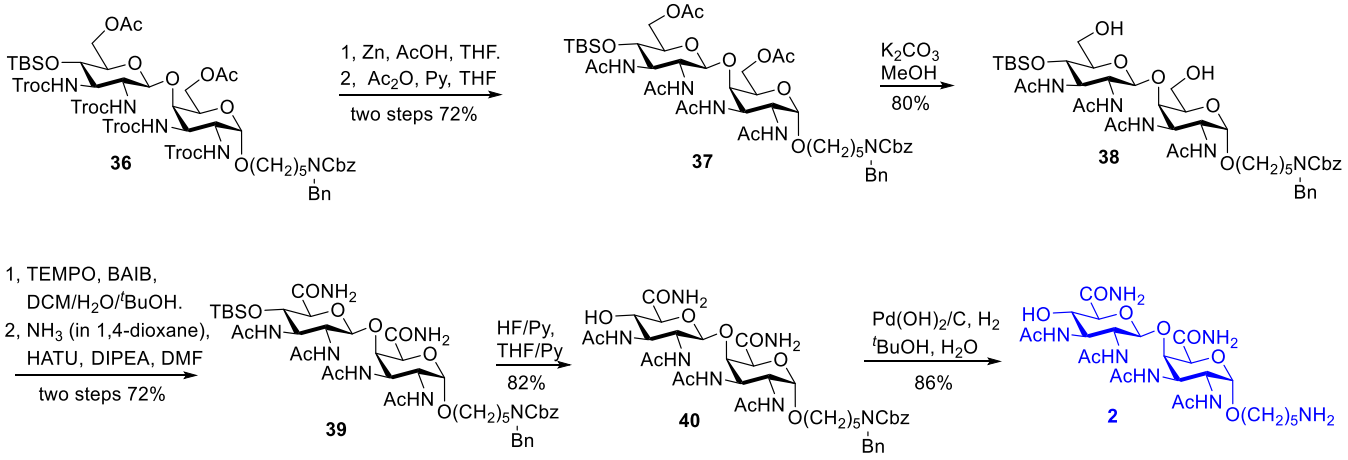
Scheme 2. Synthesis of Target Disaccharide 2



two steps.[27] Removal of the allyl group in **15** using $PdCl_2$/MeOH gave the corresponding hemiacetal, which was subsequently transformed into the desired *N*-phenyl trifluoroacetimidate donor **7** in 50% yield over two steps.[50] The monosaccharide donor **8** was generated from glucal **16** in nine steps. Glucal **16** was efficiently transformed into 3,6-di-*O*-acetyl-glucal under lipase-catalyzed reaction,[51] and the C4−OH was then masked by the *tert*-butyldimethylsilyl group to give protected glucal **18** in 87% yield over two steps. Treatment of **18** with $^{n}Bu_4NNO_3/Tf_2O$/DTBMP in dichloromethane at −70 °C afforded **12** in 60% yield, which was smoothly transformed into the desiredintermediate 2-nitro-3-azide-glucose **19** in 65% yield using PPY-mediated one-pot relay glycosylation.[49] Following the similar procedures from **14** to donor **7**, the desired monosaccharide donor **8** was smoothly obtained from compound **19** in four steps. The acceptor **9** was synthesized from 2-nitroglactal **11** in seven steps (Scheme 1B). Stereoselective installation of the spacer on the anomeric position and azide group on the C3 position in **11** afforded desired **21** in 59% yield via one-pot relay glycosylation. Removal of the acetyl group, reduction of the nitro and azide groups, and selective protection of free amines furnished **22** in 78% over three steps. The diol **22** was subjected to selective oxidation at C6 hydroxy group using 2,2,6,6-tetramethylpiperidinyl-1-oxide (TEMPO)/diacetoxyiodobenzene (BAIB) oxidation method to furnish the corresponding carboxylic acid, which was amidated using $BnNH_2$/HATU/DIPEA to afford acceptor **9** in 64% yield over two steps. Following the similar procedures from **11** to **22**, compound **25** was obtained from **11** in 5 steps with good yield, which was selectively protected at the C6 position with acetyl group to give acceptor **10** under the catalysis of 2-aminoethyl diphenylborinate in 86% yield.[52] To synthesize target monosaccharide **1**, reduction-acetylation in **23** produced **26** in 73% yield (Scheme 1C). Removal of the *O*-acetyl group using K_2CO_3/MeOH furnished diol **27** in a 90% yield. Subsequent oxidation of the C6 hydroxy group of **27** via TEMPO/BAIB oxidation generated corresponding carboxylic acid, which was amidated using BnNH2/HATU/DIPEA to afford **28** in 67% yield over two steps.[53] Unfortunately, the deprotection of the benzyl group on amide **28** failed. Hydrogenation of **28** gave byproduct **29** in 72% yield rather than the target **1**. The newly developed mild debenzylation method via visible-light-induced mesolytic fragmentation also did not work in this case.[54] To our delight, amide **31** without any protecting group on the amide, which was obtained from diol **27** in 59% yield via selective oxidation and direct coupling with NH_3, was successfully transformed into the desired monosaccharide **1** through $Pd(OH)_2$/C-catalyzed hydrogenolysis of benzyl ether and benzyl carbamate in 83% yield (δ $H1_{\alpha\text{-diacetamidoGalAN}}$ = 4.94 ppm (s), $C1_{\alpha\text{-diacetamidoGalAN}}$ = 96.6 ppm).

2.2. Synthesis of Target Disaccharide 2

With the monosaccharide donor **7** and **8**, as well as monosaccharide acceptor **9** and **10** in hand, we started the synthesis of target disaccharide **2**. The glycosylation of *N*-phenyl trifluoroacetimidate donor **7** with acceptor **9** under many activated conditions did not generate the desired disaccharide product (Table 1, entries 1−3). Gold(I)-catalyzed glycosylation with glycosyl *o*-alkynylbenzoate **8′** as donor and preactivation glycosylation with glycosyl hemiacetal **8″** as donor could not work as well (Table 1, entries 4−5).[55,56] The possible reason was that the nucleophilicity of the C4 axial hydroxyl group of 2,3-diacylamidogalacturonic amide **9** was highly poor. To increase the nucleophilicity, we adjusted the group at the C5 position of acceptor **9** to −COOMe (**9′**), $-CH_2OAc$ (**9″**), and $-CH_2OAll$ (**9‴**). The glycosylation of donor **8** with acceptor **9′**/**9″**/**9‴** gave disaccharide products **33**−**35** in 20−27% yields (Table 1 entries 6−8). To our delight, acceptor **10** with a fully masked spacer was coupled with donor **8** catalyzed by TBSOTf to furnish disaccharide **36** in 62% yield (Table 1 entry 9). Removal of the Troc groups in **36** followed by in situ acetylation by Ac_2O afforded **37** in 72% yield over two steps (Scheme 2). Selective removal of the *O*-acetyl group in **37** using K_2CO_3/MeOH furnished diol **38** in 80% yield, which was smoothly transformed into amidated disaccharide **39** via TEMPO/BAIB oxidation and amidation in 72% yield over two steps. Desilylation in **39** with HF/pyridine gave disaccharide **40** in 82% yield, which was then converted into target disaccharide **2** via $Pd(OH)_2$/C-catalyzed hydrogenolysis in 86% yield (δ $H1_{\alpha\text{-diacetamidoGalAN}}$ = 4.94 ppm (d, J = 3.3 Hz), $C1_{\alpha\text{-diacetamidoGalAN}}$ = 96.6 ppm; $H1_{\beta\text{-diacetamidoGluAN}}$ = 4.40 ppm (d, J = 7.5 Hz), $C1_{\beta\text{-diacetamidoGluAN}}$ = 100.7 ppm).

2.3. Synthesis of Target Trisaccharides 3−5 and Tetrasaccharide 6

The target trisaccharides **3**−**5** could be obtained from the key trisaccharide intermediate **44** through a diversity-oriented synthesis (Scheme 3A). Removal of the TBS protecting group in **36** with HF/pyridine afforded disaccharide acceptor **41** in 92% yield, which was glycosylated with monosaccharide donor

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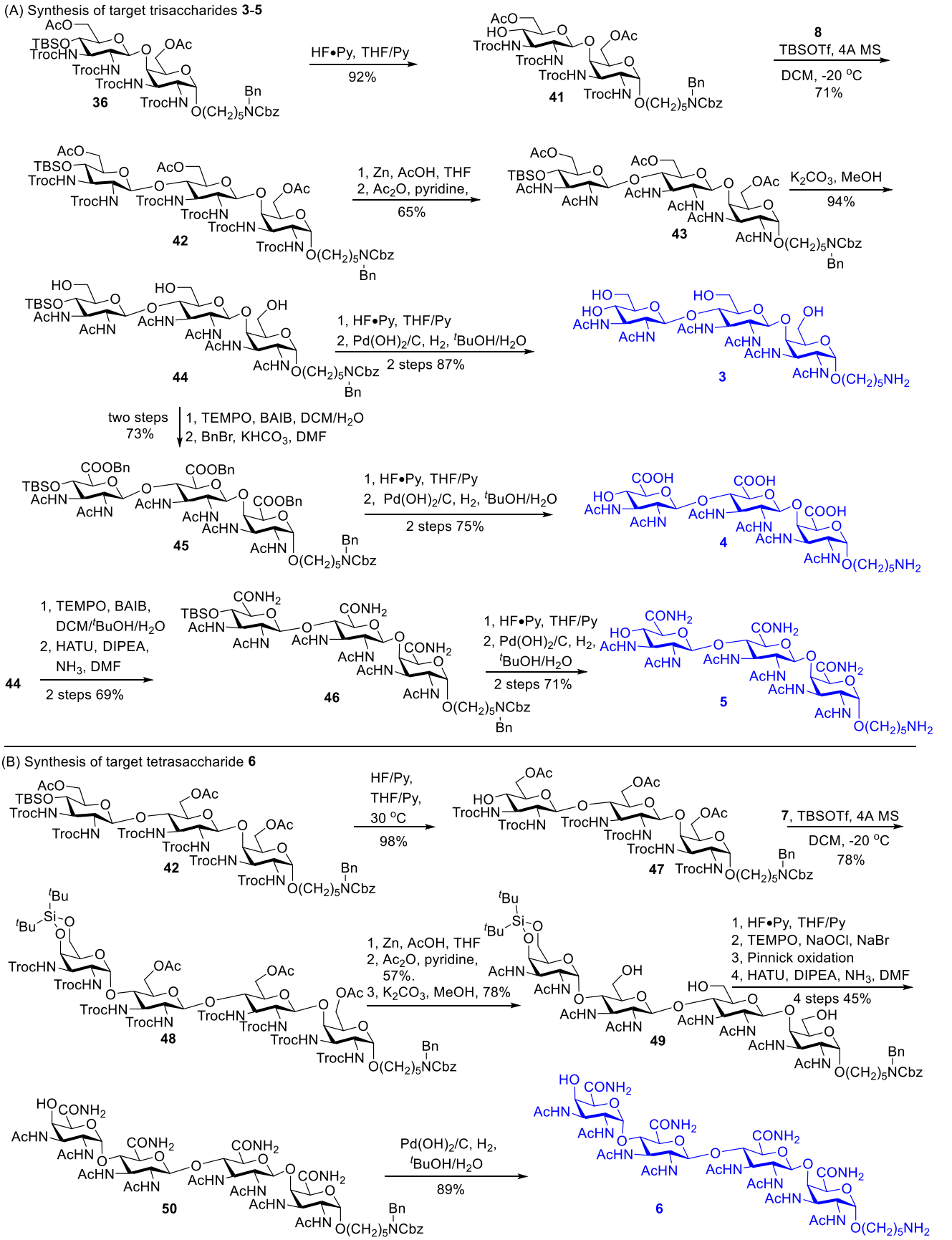
Scheme 3. (A) Synthesis of Target Trisaccharides 3-5. (B) Synthesis of Targettetrasaccharide 6



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8 catalyzed by TBSOTf to furnish trisaccharide **42** in 71% yield. Removal of the Troc group in **42** using Zn/AcOH, followed by in situ acetylation with Ac_2O generated **43**. Then selective deacetylation of *O*-Ac with K_2CO_3/MeOH produced **44** in 94% yield. The trisaccharide **44** was smoothly transformed into trisaccharide **3** through desilylation and hydrogenation in 87% yield. The trisaccharide derivative **4** was generated from trisaccharide **44** via selective TEMPO/BAIB oxidation, benzylation with BnBr/$KHCO_3$, desilylation with HF/pyridine, and Pd(OH)$_2$/C-catalyzed hydrogenolysis in 75% yield over four steps. The trisaccharide **5** with one repeating unit of *B. hinzii* O-antigen was synthesized from trisaccharide **44** through selective TEMPO/BAIB oxidation, amidation with HATU/NH_3/DIPEA, desilylation and Pd(OH)$_2$/C-catalyzed hydrogenolysis in 49% yield over four steps (δ H1$_{\alpha\text{-diacetamidoGalAN}}$ = 4.93 (d, J = 3.2 Hz), C1$_{\alpha\text{-diacetamidoGalAN}}$ = 96.6 ppm; H1$_{\beta\text{-diacetamidoGluAN}}$ = 4.67 ppm, C1$_{\beta\text{-diacetamidoGluAN}}$ = 101.1 ppm; H1′ $_{\beta\text{-diacetamidoGluAN}}$ = 4.55 ppm (d, J = 8.3 Hz), C1′$_{\beta\text{-diacetamidoGluAN}}$ = 101.6 ppm). The synthesis of target tetrasaccharide **6** started from trisaccharide **42** (Scheme 3B). Desilylation in **42** using HF/pyridine furnished trisaccharide acceptor **47** in 98% yield, which was coupled with monosaccharide donor **7** activated by TBSOTf to give the desired tetrasaccharide **48** in 78% yield as a single anomer. Removal of eight NHTroc groups in **48** via Zn/AcOH/THF reduction, followed by in situ acetylation afforded desired per-*O*,*N*-acetylated product in 57% yield over two steps, which was selectively de-*O*-acetylated with K_2CO_3/MeOH to provide **49** in 78% yield. Another challenging point is selective oxidation of the multiple hydroxyl groups in the complex tetrasaccharide. After numerous attempts, the tetrasaccharide **49** was successfully converted to amidated tetrasaccharide **50** through desilylation, selective oxidation of the primary hydroxyl groups using the two-step sequence (TEMPO/NaOCl/NaBr,[57] then Pinnick oxidation[58]), and amidation with HATU/NH_3/DIPEA in 45% yield over four steps. Finally, removal of the benzyl and benzyl carbamate groups on the spacer of **50** with Pd(OH)$_2$/C-catalyzed hydrogenolysis afforded the desired tetrasaccharide **6** in 89% yield (δ H1$_{\alpha\text{-diacetamidoGalAN}}$ = 4.95 ppm (d, J = 3.4 Hz), C1$_{\alpha\text{-diacetamidoGalAN}}$ = 96.6 ppm; H1$_{\beta\text{-diacetamidoGluAN}}$ = 4.68 ppm, C1$_{\beta\text{-diacetamidoGluAN}}$ = 101.1 ppm; H1′$_{\beta\text{-diacetamidoGluAN}}$ = 4.60 ppm (d, J = 8.3 Hz), C1′$_{\beta\text{-diacetamidoGluAN}}$ = 101.4 ppm; H1′$_{\alpha\text{-diacetamidoGalAN}}$ = 5.21 ppm (d, J = 3.5 Hz), C1′$_{\alpha\text{-diacetamidoGalAN}}$ = 96.8 ppm).

2.4. Preparation and Antigenic Evaluation of Conjugates HSA-1−HSA-6

To screen out the optimal epitope of the synthetic *B. hinzii* O-antigens 1−6, synthesis of the corresponding conjugates HSA-1−HSA-6 was carried out (Figure 2A). By employing the bifunctional di(*N*-succinimidyl) glutarate (DSG) as the linker, the six *B. hinzii* O-antigens were first reacted with the DSG to obtain the desired activated monoesters, followed by covalent coupling with human serum albumin (HSA) to produce conjugates HSA-1−HSA-6, respectively (Figures S1−S6 and Table S1; see the Supporting Information for details).[59,60] Although the carbohydrate loadings of HSA-4 and HSA-6 were relatively low, presumably due to the structural complexity of the oligosaccharides, analysis of the SDS-PAGE and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) results indicated that the glycoconjugates were indeed formed. To evaluate the antigenicity of the mono- and

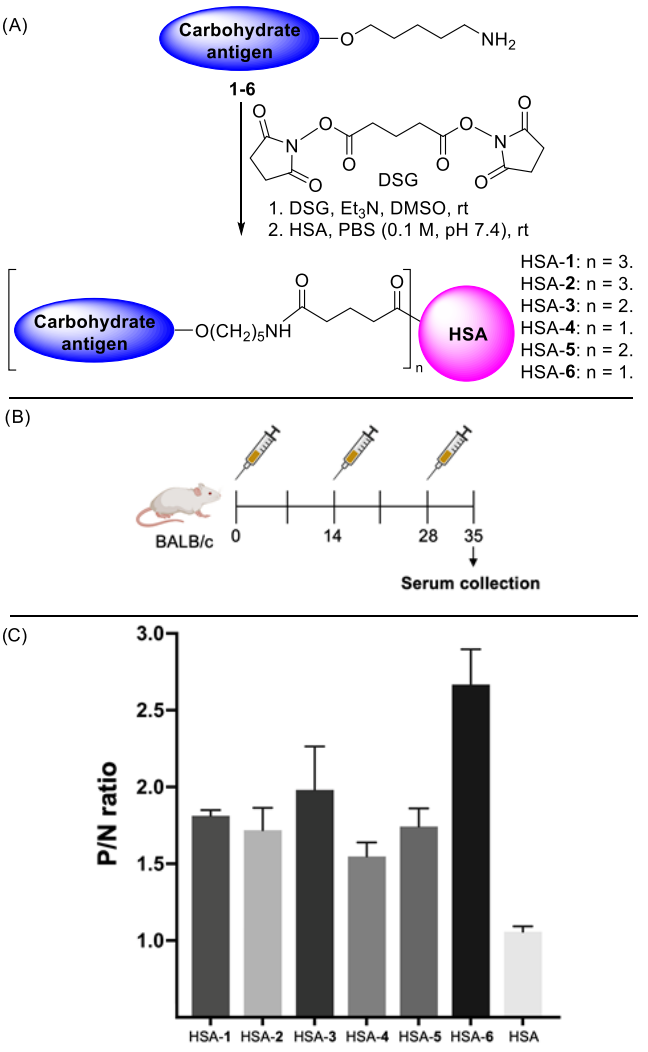

Figure 2. Synthesis of the glycoconjugates and their antigenicity evaluation. (A) Preparation of the six conjugates HSA-**1**−HSA-**6**. (B, C) Immunization schedule and recognition of the six glycoconjugates by pooled sera (1:4000 dilution) from mice immunized with inactivated *B. hinzii* formulated with Freund's adjuvant (FA) on day 35 as determined by an ELISA. The P/N ratio means the ratio of the OD450 nm value of positive immune mice sera to the OD450 nm value of negative nonimmune mice sera. Which was calculated to account for plate-to-plate variability in optical density (OD).[59,61,62] The P/N ratios were measured in triplicate and are plotted as mean ± the standard deviation.

oligosaccharides, HSA was used as a negative control and the synthetic conjugates HSA-1−HSA-**6** were used as the coating antigens for enzyme-linked immunosorbent assays (ELISAs). The BALB/c mice were subcutaneously immunized with a mixture of inactivated *B. hinzii* and Freund's adjuvant (FA) as the positive immune group. The mice in the negative nonimmune group received only PBS. The positive immune group received one priming vaccine on day 0 and received booster dose on days 14 and 28 (Figure 2B). On day 35, the sera of mice in the positive immune group and the negative nonimmune group were extracted for ELISAs.

As shown in Figure 2C, the day 35 sera from mice immunized with inactivated *B. hinzii* barely bound to the HSA. Among the six synthetic glycoconjugates, HSA-**6** bearing the tetrasaccharide was best recognized by day 35 sera from the

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positive immune group. It was interesting that the conjugates HSA-**1**−HSA-**4** of monosaccharide and disaccharide fragments **1**−**2**, as well as trisaccharide derivatives **3**−**4**, had a similar binding affinity with the conjugate HSA-**5** containing one repeating unit of *B. hinzii* O-antigen. Owing to HSA could not be recognized by the serum antibodies, the terminal amidated 2,3-diacetamido-galacturonic acid and the 2,3-diacetamido-pyranoses may play an important role for their antigenicity, and the tetrasaccharide **6** was found to be a key epitope for further vaccine development against *B. hinzii*. It should be noted that the carbohydrate loading and the glycan orientation on the protein might affect recognition by the serum antibodies in the positive immune group. Thus, further immunization studies to confirm the immunogenicity and establish the epitope profile are underway.[59,60]

3. CONCLUSIONS

We have achieved the first total synthesis of highly functionalized mono- and oligosaccharides **1**−**6** from *B. hinzii* the O-antigen. All of the rare 2,3-diacetamidopyranoside building blocks were obtained from 2-nitroglycals through a novel organocatalyzed one-pot relay glycosylation method. The combination of postglycosylation oxidation strategy and di-*tert*-butylsilylidene directed α-galactosylation method has proven effective to assemble these complex oligosaccharides. During the synthetic process, installation and functionalization of multiple amines, selective oxidation of multiple hydroxyl groups, and direct amidation in the late stage were achieved. These synthetic methods and strategies may find further applications in the assembly of other complex oligosaccharides and glycoconjugates. In addition, through screening of the synthetic *B. hinzii* O-antigen glycans with the mouse sera antibodies, the terminal tetrasaccharide of *B. hinzii* O-antigen was identified as a potential glycol-epitope for the development of vaccine against *B. hinzii*.

4. METHODS

4.1. General Procedure for Stereoselective Synthesis of 2-Nitro-3-azido-2,3-Dideoxyglycosides by Using 3-*O*-acetyl-2-nitroglycals as Donors

The 2-nitroglycal (1.0 equiv), PPY (0.1 equiv), and $TMSN_3$ (1.2 equiv) were dissolved in dry DCM (0.1 M). The reaction mixture was stirred for 30 min at room temperature and monitored by TLC analysis. When the reaction was finished, the next step reaction was carried out without workup and purification. PivOH (0.2 equiv) and acceptor (1.4 equiv) was added to the reaction mixture. The reaction mixture was stirred for 2 days at room temperature and monitored by TLC analysis. When the reaction was complete, the mixture was concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (petroleum ether/ethyl acetate) to give the corresponding 2-nitro-3-azido-2,3-dideoxyglycoside.

4.2. General Procedure for Transformation of the Nitro Group and Azide Group to NHTroc

To a suspension of substrate, zinc powder and copper sulfate in THF/H_2O were added HCl (12 M) at ice bath. The reaction mixture was warmed to room temperature and monitored by a TLC analysis. When the reaction was finished, it was filtered with Celite and washed with THF. The resulting organic solution was concentrated in vacuo and was directly used for the next step without further purification. The obtained residue in THF was added $NaHCO_3$ and TrocCl at ice bath and stirred at room temperature. When the reaction was finished, it was filtered by Celite, washed with THF, and then concentrated in vacuo. The obtained residue was purified by silica gel column chromatography to afford the corresponding product.

4.3. General Procedure for the Transformation of the NHTroc Groups to NHAc by Reduction and Acetylation

To a suspension of substrate and zinc powder in THF were added acetic acid at ice bath. The reaction mixture was warmed to room temperature and monitored by TLC analysis. When the reaction was finished, it was filtered by Celite and washed with THF. The resulting organic solution was concentrated in vacuo, which was directly used for the next step without further purification. The obtained residue in THF was added to pyridine and acetic anhydride and stirred at room temperature. When the reaction was finished, the reaction was quenched with MeOH and concentrated in vacuo. The obtained residue was purified by silica gel column chromatography to afford the corresponding product.

4.4. General Procedure for TEMPO/BAIB Oxidation and Amidation

To a suspension of substrate in DCM/^{t}BuOH/H_2O was added TEMPO and BAIB, stirred at room temperature, and monitored by TLC analysis. When the reaction was finished, the mixture was concentrated in vacuo. The obtained residue was roughly purified by gel column chromatography (Sephadex LH-20, eluent: 1:1, MeOH-H_2O) to give corresponding crude product. To the solution of the resulting residue in DMF was added HATU, DIPEA, and NH_3 (in 1,4-dioxane), and then stirred at room temperature. When the reaction was finished, it was concentrated in vacuo. The obtained residue was purified by silica gel column chromatography to afford the corresponding product.

4.5. General Procedure for Hydrogenation

To a solution of substrate in ^{t}BuOH/H_2O (7:3, v/v, 10 mL) was added $Pd(OH)_2/C$ (20% on carbon (wetted with ca.50% water)) and stirred under an atmosphere of H_2. When the reaction was finished, the reaction mixture was filtered by Celite and 0.45 μm filtration membrane, and the filtrate was concentrated in vacuo. The obtained residue was purified by gel column chromatography (Sephadex LH-20, eluent: 1:1, MeOH-H_2O) to afford the corresponding target.

4.6. General Procedure for Conjugation of the Glycan to HSA

To a solution of di(*N*-succinimidyl) glutarate (DSG; 9.06 mg, 27.8 μmol; 5.28 mg, 16.2 μmol; 3.72 mg, 11.4 μmol; 3.72 mg, 11.4 μmol; 3.91 mg, 12 μmol; 2.87 mg, 8.8 μmol) and triethylamine (9; 6; 4; 4; 4; 3 μL) in anhydrous DMSO (160 μL) was added dropwise a solution of glycans (1:1 mg, 2.78 μmol; 2:1 mg, 1.62 μmol; 3:1 mg, 1.2 μmol; 4:1 mg, 1.14 μmol; 5:1 mg, 1.14 μmol; 6:1 mg, 0.88 μmol) in anhydrous DMSO (160 μL) at room temperature. After being stirred at room temperature for 2 h, the solution was treated with phosphate buffer saline (PBS; 100 mM, pH = 7.4, 1 mL). The mixture was then extracted with chloroform (5 mL) and separated by centrifugation (2 min, 1800*g*). The aqueous layer was separated by centrifugation (1 min, 14,500*g*) in a 1.5 mL Eppendorf tube and added to a solution of HSA (0.73 mg, 11 nmol; 0.4 mg, 6 nmol; 0.33 mg, 5 nmol; 0.33 mg, 5 nmol; 0.33 mg, 5 nmol; 0.27 mg, 4 nmol, MKBio) in PBS (100 mM, pH = 7.4, 1 mL). The mixture was stirred at room temperature for 18 h and dialyzed against water and PBS three times using a centrifugal filter (30 kDa MWCO, Millipore).

4.7. Materials and Methods for Biology

Bacterial strains: *B. hinzii* (LMG14052) bacteria were purchased from BeNa Culture Collection (BNCC). Animals: six to 8 weeks old female BALB/c mice were purchased from Yuxiu, Shanghai, China. The in vivo experiments were conducted with mice under the ethics certificate ECUST-2022−032 of East China University of Science and Technology. All mice were kept under specific pathogen-free conditions.

4.8. Immunization of Mice with the Inactivated *B. hinzii*

Immunization of 6 week old female BALB/c mice (n = 4) was carried out subcutaneously (s.c.) with the inactivated *B. hinzii* (LMG14052). Each mouse was immunized with the inactivated bacteria (80 μg) emulsified with 1:1 (v/v) complete Freund's adjuvant (CFA, Sigma)

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on day 0 and boosted twice with the inactivated bacteria (80 μg) emulsified with 1:1 (v/v) incomplete Freund's adjuvant (IFA, Sigma) on days 14 and 28. The control mice received only PBS. Mouse blood samples were collected via the mice eye socket vein on days 35. Antisera were prepared from the clotted blood samples

4.9. ELISA Assays

Costar high-binding polystyrene 96-well plates (Corning) were coated with the HSA-**1**, HSA-**2**, HSA-**3**, HSA-**4**, HSA-**5**, HSA-**6**, and HSA at a concentration of 10 μg/mL in sodium carbonate-sodium hydrogencarbonate buffer solution (0.05 M, pH = 9.6) at 4 °C for 20 h. The plates were washed three times with PBS containing 0.1% Tween-20 (PBS-T) and blocked with 2% BSA-PBS at 37 °C for 1 h. After washing with PBS-T for three times, primary antiserum dilutions (1:4000 dilution) in 1% BSA-PBS were added, and the plates were incubated at 37 °C for 2 h. The plates were washed with PBS-T three times and incubated with 1:10,000 diluted solution of horseradish peroxidase (HRP) conjugated goat antimouse IgG antibody in 1% BSA-PBS at 37 °C for one h in dark. The plates were washed with PBS-T three times, developed with TMB substrate at 37 °C for 20 min, and stopped with 2% sulfuric acid. The absorbance values were recorded at a wavelength of 450 nm with an ELISA reader.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacsau.5c00113.

Experimental procedures; analytical data; NMR and MS spectra of all synthetic compounds (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Collercandy, N.; Petillon, C.; Abid, M.; Descours, C.; Carvalho-Schneider, C.; Mereghetti, L.; Lartigue, M.-F.; Fenwick, B. *Bordetella hinzii*: an Unusual Pathogen in Human Urinary Tract Infection. *J. Clin. Microbiol.* **2021**, *59*, No. e02748-20.

(2) Launay, A.; Wu, C. J.; Dulanto Chiang, A.; Youn, J. H.; Khil, P. P.; Dekker, J. P. In vivo evolution of an emerging zoonotic bacterial pathogen in an immunocompromised human host. *Nat. Commun.* **2021**, *12*, No. 4495.

(3) Maison-Fomotar, M.; Sivasubramanian, G. *Bordetella hinzii* Pneumonia and *Bacteremia* in a Patient with SARS-CoV-2 Infection. *Emerging Infect. Dis.* **2021**, *27*, 2904−2907.

(4) Mehta, N.; Gerdts, J.; Fung, M.; Guterman, E. L. Brain Abscess Caused by *Bordetella hinzii*. *Neurol. Clin. Pract.* **2021**, *11*, e787−e789.

(5) Ben Lakhal, H.; Cachinho, J. B.; Kalfon, P.; Naas, T.; Benseddik, Z. *Bordetella hinzii* Pneumonia in Patient with SARS-CoV-2 Infection. *Emerging Infect. Dis.* **2022**, *28*, 844−847.

(6) Myers, M. G. *The History of Vaccine Development and the Diseases Vaccines Prevent.*; John Wiley & Sons, Ltd, 2015; pp 1−31.

(7) Rappuoli, R. Glycoconjugate vaccines Principles and mechanisms. *Sci. Transl. Med.* **2018**, *10*, No. eaat4615.

(8) Micoli, F.; Del Bino, L.; Alfini, R.; Carboni, F.; Romano, M. R.; Adamo, R. Glycoconjugate vaccines: current approaches towards faster vaccine design. *Expert Rev. Vaccines* **2019**, *18*, 881−895.

(9) Saleh, A.; Qamar, S.; Tekin, A.; Singh, R.; Kashyap, R. Vaccine Development Throughout History. *Cureus* **2021**, *13* (7), No. e16635.

(10) Decker, M. D.; Edwards, K. M. *Haemophilus influenzae* type b vaccines: history, choice and comparisons. *Pediatr. Infect. Dis. J.* **1998**, *17*, S113−S116.

(11) Geno, K. A.; Gilbert, G. L.; Song, J. Y.; Skovsted, I. C.; Klugman, K. P.; Jones, C.; Konradsen, H. B.; Nahm, M. H. *Pneumococcal* Capsules and Their Types: Past, Present, and Future. *Clin. Microbiol. Rev.* **2015**, *28*, 871−899.

(12) Adamo, R. Advancing Homogeneous Antimicrobial Glycoconjugate Vaccines. *Acc. Chem. Res.* **2017**, *50*, 1270−1279.

(13) Micoli, F.; Costantino, P.; Adamo, R. Potential targets for next generation antimicrobial glycoconjugate vaccines. *FEMS Microbiol. Rev.* **2018**, *42*, 388−423.
(14) Seeberger, P. H. Discovery of Semi- and Fully-Synthetic Carbohydrate Vaccines Against Bacterial Infections Using a Medicinal Chemistry Approach. *Chem. Rev.* **2021**, *121*, 3598−3626.
(15) Rohokale, R.; Guo, Z. Development in the Concept of Bacterial Polysaccharide Repeating Unit-Based Antibacterial Conjugate Vaccines. *ACS Infect. Dis.* **2023**, *9*, 178−212.
(16) Del Bino, L.; Østerlid, K. E.; Wu, D.-Y.; Nonne, F.; Romano, M. R.; Codée, J.; Adamo, R. Synthetic Glycans to Improve Current Glycoconjugate Vaccines and Fight Antimicrobial Resistance. *Chem. Rev.* **2022**, *122*, 15672−15716.
(17) Liao, G.; Zhou, Z.; Suryawanshi, S.; Mondal, M. A.; Guo, Z. Fully Synthetic Self-Adjuvanting α-2,9-Oligosialic Acid Based Conjugate Vaccines against Group C Meningitis. *ACS Cent. Sci.* **2016**, *2*, 210−218.
(18) Nishat, S.; Andreana, P. R. Entirely Carbohydrate-Based Vaccines: An Emerging Field for Specific and Selective Immune Responses. *Vaccines* **2016**, *4*, No. 19.
(19) Wei, M. M.; Wang, Y. S.; Ye, X. S. Carbohydrate-based vaccines for oncotherapy. *Med. Res. Rev.* **2018**, *38*, 1003−1026.
(20) Keith, D. J.; Townsend, S. D. Total Synthesis of the Congested, Bisphosphorylated *Morganella morganii* Zwitterionic Trisaccharide Repeating Unit. *J. Am. Chem. Soc.* **2019**, *141*, 12939−12945.
(21) Behera, A.; Rai, D.; Kulkarni, S. S. Total Syntheses of Conjugation-Ready Trisaccharide Repeating Units of *Pseudomonas aeruginosa* O11 and *Staphylococcus aureus* Type 5 Capsular Polysaccharide for Vaccine Development. *J. Am. Chem. Soc.* **2020**, *142*, 456−467.
(22) Shivatare, S. S.; Wong, C. H. Synthetic Carbohydrate Chemistry and Translational Medicine. *J. Org. Chem.* **2020**, *85*, 15780−15800.
(23) Wang, P.; Huo, Cx.; Lang, S.; Caution, K.; Nick, S. T.; Dubey, P.; Deora, R.; Huang, X. Chemical Synthesis and Immunological Evaluation of a Pentasaccharide Bearing Multiple Rare Sugars as a Potential Anti-pertussis Vaccine. *Angew. Chem., Int. Ed.* **2020**, *59*, 6451−6458.
(24) Zhu, Q.; Shen, Z.; Chiodo, F.; Nicolardi, S.; Molinaro, A.; Silipo, A.; Yu, B. Chemical synthesis of glycans up to a 128-mer relevant to the O-antigen of Bacteroides vulgatus. *Nat. Commun.* **2020**, *11*, No. 4142.
(25) Wei, R.; Yang, X.; Liu, H.; Wei, T.; Chen, S.; Li, X. Synthetic Pseudaminic-Acid-Based Antibacterial Vaccine Confers Effective Protection against *Acinetobacter baumannii* Infection. *ACS Cent. Sci.* **2021**, *7*, 1535−1542.
(26) Tian, G.; Hu, J.; Qin, C.; Li, L.; Ning, Y.; Zhu, S.; Xie, S.; Zou, X.; Seeberger, P. H.; Yin, J. Chemical Synthesis and Antigenicity Evaluation of an Aminoglycoside Trisaccharide Repeating Unit of *Pseudomonas aeruginosa* Serotype O5 O-Antigen Containing a Rare Dimeric-ManpN3NA. *J. Am. Chem. Soc.* **2024**, *146*, 18427−18439.
(27) Duan, L.; Nie, Q.; Hu, Y.; Wang, L.; Guo, K.; Zhou, Z.; Song, X.; Tu, Y.; Liu, H.; Hansen, T.; Sun, J. S.; Zhang, Q. Stereoselective Synthesis of the O-antigen of *A. baumannii* ATCC 17961 Using Long-Range Levulinoyl Group Participation. *Angew. Chem., Int. Ed.* **2023**, *62*, No. e202306971.
(28) Vinogradov, E. Structure of the O-specific polysaccharide chain of the LPS of *Bordetella hinzii*. *Carbohydr. Res.* **2007**, *342*, 961−963.
(29) Novikov, A.; Marr, N.; Caroff, M. A comparative study of the complete lipopolysaccharide structures and biosynthesis loci of *Bordetella avium*, *B. hinzii*, and *B. trematum*. *Biochimie* **2019**, *159*, 81−92.
(30) Walvoort, M. T.; Moggre, G. J.; Lodder, G.; Overkleeft, H. S.; Codee, J. D.; van der Marel, G. A. Stereoselective synthesis of 2,3-diamino-2,3-dideoxy-beta-D-mannopyranosyl uronates. *J. Org. Chem.* **2011**, *76*, 7301−7315.
(31) Emmadi, M.; Kulkarni, S. S. Synthesis of Rare Deoxy Amino Sugar Building Blocks Enabled the Total Synthesis of a Polysaccharide Repeating Unit Analogue from the LPS of *Psychrobacter cryohalolentis* K5(T). *J. Org. Chem.* **2018**, *83*, 14323−14337.
(32) Qin, C.; Schumann, B.; Zou, X.; Pereira, C. L.; Tian, G.; Hu, J.; Seeberger, P. H.; Yin, J. Total Synthesis of a Densely Functionalized *Plesiomonas shigelloides* Serotype 51 Aminoglycoside Trisaccharide Antigen. *J. Am. Chem. Soc.* **2018**, *140*, 3120−3127.
(33) Sianturi, J.; Priegue, P.; Hu, J.; Yin, J.; Seeberger, P. H. Semi-Synthetic Glycoconjugate Vaccine Lead Against *Acinetobacter baumannii* 17978. *Angew. Chem., Int. Ed.* **2022**, *61*, No. e202209556.
(34) Taylor, J. G.; Li, X.; Oberthür, M.; Zhu, W.; Kahne, D. E. The Total Synthesis of Moenomycin A. *J. Am. Chem. Soc.* **2006**, *128*, 15084−15085.
(35) Ghosh, B.; Bhattacharjee, N.; Podilapu, A. R.; Puri, K.; Kulkarni, S. S. Total Synthesis of the Repeating Units of O-Specific Polysaccharide of *Pseudomonas chlororaphis* subsp. aureofaciens UCM B-306 via One-Pot Glycosylation. *Org. Lett.* **2022**, *24*, 3696−3701.
(36) Zhang, Q.; Gimeno, A.; Santana, D.; Wang, Z.; Valdes-Balbin, Y.; Rodriguez-Noda, L. M.; Hansen, T.; Kong, L.; Shen, M.; Overkleeft, H. S.; Verez-Bencomo, V.; van der Marel, G. A.; Jimenez-Barbero, J.; Chiodo, F.; Codee, J. D. C. Synthetic, Zwitterionic Sp1 Oligosaccharides Adopt a Helical Structure Crucial for Antibody Interaction. *ACS. Cent.Sci.* **2019**, *5*, 1407−1416.
(37) Wang, Z.; Poveda, A.; Zhang, Q.; Unione, L.; Overkleeft, H. S.; van der Marel, G. A.; Jesus, J. B.; Codee, J. D. C. Total Synthesis and Structural Studies of Zwitterionic Bacteroides fragilis Polysaccharide A1 Fragments. *J. Am. Chem. Soc.* **2023**, *145*, 14052−14063.
(38) Demchenko, A. 1,2-cis O-Glycosylation: Methods, Strategies, Principles. *Curr. Org. Chem.* **2003**, *7*, 35−79.
(39) Nigudkar, S. S.; Demchenko, A. V. Stereocontrolled 1,2-cis glycosylation as the driving force of progress in synthetic carbohydrate chemistry. *Chem. Sci.* **2015**, *6*, 2687−2704.
(40) Wang, L.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. Reagent Controlled Stereoselective Synthesis of α-Glucans. *J. Am. Chem. Soc.* **2018**, *140*, 4632−4638.
(41) Báti, G.; He, J.-X.; Pal, K. B.; Liu, X.-W. Stereo- and regioselective glycosylation with protection-less sugar derivatives: an alluring strategy to access glycans and natural products. *Chem. Soc. Rev.* **2019**, *48*, 4006−4018.
(42) Ling, J.; Bennett, C. S. Recent Developments in Stereoselective Chemical Glycosylation. *Asian. J. Org. Chem.* **2019**, *8*, 802−813.
(43) Imamura, A.; Ando, H.; Korogi, S.; Tanabe, G.; Muraoka, O.; Ishida, H.; Kiso, M. Di-*tert*-butylsilylene (DTBS) group-directed α-selective galactosylation unaffected by C-2 participating functionalities. *Tetrahedron Lett.* **2003**, *44*, 6725−6728.
(44) Zhang, Y.; Gomez-Redondo, M.; Jimenez-Oses, G.; Arda, A.; Overkleeft, H. S.; van der Marel, G. A.; Jimenez-Barbero, J.; Codee, J. D. C. Synthesis and Structural Analysis of Aspergillus fumigatus Galactosaminogalactans Featuring alpha-Galactose, alpha-Galactosamine and alpha-N-Acetyl Galactosamine Linkages. *Angew. Chem., Int. Ed.* **2020**, *59*, 12746−12750.
(45) Kazakova, E. D.; Yashunsky, D. V.; Krylov, V. B.; Bouchara, J. P.; Cornet, M.; Valsecchi, I.; Fontaine, T.; Latge, J. P.; Nifantiev, N. E. Biotinylated Oligo-α-(1 → 4)-D-galactosamines and Their N-Acetylated Derivatives: α-Stereoselective Synthesis and Immunology Application. *J. Am. Chem. Soc.* **2020**, *142*, 1175−1179.
(46) Pedersen, C. M.; Nordstrøm, L. U.; Bols, M. "Super Armed" Glycosyl Donors: Conformational Arming of Thioglycosides by Silylation. *J. Am. Chem. Soc.* **2007**, *129*, 9222−9235.
(47) Crich, D. Mechanism of a Chemical Glycosylation Reaction. *Acc. Chem. Res.* **2010**, *43*, 1144−1153.
(48) Frihed, T. G.; Bols, M.; Pedersen, C. M. Mechanisms of Glycosylation Reactions Studied by Low-Temperature Nuclear Magnetic Resonance. *Chem. Rev.* **2015**, *115*, 4963−5013.
(49) Wu, X.; Zheng, Z.; Wang, L.; Xue, Y.; Liao, J.; Liu, H.; Liu, D.; Sun, J. S.; Zhang, Q. Stereoselective Synthesis of 2,3-Diamino-2,3-dideoxyglycosides from 3-*O*-Acetyl-2-nitroglycals. *Eur. J. Org. Chem.* **2022**, *2022*, No. e202200519.
(50) Yu, B.; Sun, J. Glycosylation with glycosyl *N*-phenyltrifluoroacetimidates (PTFAI) and a perspective of the future

https://doi.org/10.1021/jacsau.5c00113
JACS Au 2025, 5, 1903−1913

development of new glycosylation methods. *Chem. Commun.* **2010**, *46*, 4668−4679.
(51) Sugai, T.; Okazaki, H.; Ueda, Y.; Hanaya, K.; Shoji, M. Synthesis of Benzyl Tetra-*O*-acetyl-*α*-L-glucopyranoside from Benzyl 2,3-Dideoxy-*β*-D-erythro-hex-2-enopyranoside. *Heterocycles* **2017**, *95*, 862−871.
(52) Taylor, M. S. Catalysis based on reversible covalent interactions of organoboron compounds. *Acc. Chem. Res.* **2015**, *48*, 295−305.
(53) Carpino, L. A. l-Hydroxy-7-azabenzotriazole. An Efficient Peptide Coupling Additive. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.
(54) Tao, Y.; Jin, C.; Liu, C.; Bu, J.; Yue, L.; Li, X.; Liang, K.; Xia, C. Deuteration of arenes in pharmaceuticals via photoinduced solvated electrons. *Chem* **2024**, *10*, 3374−3384.
(55) Toshima, K.; Tatsuta, K. Recent Progress In *O*-Glycosylation Methods and Its Application to Natural Products Synthesis. *Chem. Rev.* **1993**, *93*, 1503−1531.
(56) Yu, B. Gold(I)-Catalyzed Glycosylation with Glycosyl o-Alkynylbenzoates as Donors. *Acc. Chem. Res.* **2018**, *51*, 507−516.
(57) Huang, L.; Teumelsan, N.; Huang, X. A facile method for oxidation of primary alcohols to carboxylic acids and its application in glycosaminoglycan syntheses. *Chem. - Eur. J.* **2006**, *12*, 5246−5252.
(58) Hagen, B.; van Dijk, J. H. M.; Zhang, Q.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. Synthesis of the *Staphylococcus aureus* Strain M Capsular Polysaccharide Repeating Unit. *Org. Lett.* **2017**, *19*, 2514−2517.
(59) Zhang, L.; Zhang, Y.; Hua, Q.; Xu, T.; Liu, J.; Zhu, Y.; Yang, Y. Promoter-Controlled Synthesis and Antigenic Evaluation of Mannuronic Acid Alginate Glycans of *Pseudomonas aeruginosa*. *Org. Lett.* **2022**, *24*, 8381−8386.
(60) Zhang, Y.; Wang, X.; Liang, Y.; Zhang, L.; Fan, J.; Yang, Y. A Semisynthetic Oligomannuronic Acid-Based Glycoconjugate Vaccine against *Pseudomonas aeruginosa*. *ACS Cent. Sci.* **2024**, *10*, 1515−1523.
(61) Nehring, M.; Pugh, S.; Dihle, T.; Gallichotte, E.; Nett, T.; Weber, E.; Mayo, C.; Lynn, L.; Ebel, G.; Fosdick, B. K.; VandeWoude, S. Laboratory-Based SARS-CoV-2 Receptor Binding Domain Serologic Assays Perform with Equivalent Sensitivity and Specificity to Commercial FDA-EUA Approved Tests. *Viruses* **2023**, *15*, No. 106.
(62) Ciccone, E. J.; Zhu, D. R.; Gunderson, A. K.; Hawke, S.; Ajeen, R.; Lodge, E. K.; Shook-Sa, B. E.; Abernathy, H.; Garrett, H. E.; King, E.; Alavian, N.; Reyes, R.; Taylor, J. L.; Beatty, C.; Chung, C.; Mendoza, C. E.; Weber, D. J.; Markmann, A. J.; Premkumar, L.; Juliano, J. J.; Boyce, R. M.; Aiello, A. E. Magnitude and Durability of the Antibody Response to mRNA-Based Vaccination Among SARS-CoV-2 Seronegative and Seropositive Health Care Personnel. *Open Forum Infect. Dis.* **2024**, *11*, No. ofae009.

https://doi.org/10.1021/jacsau.5c00113
JACS Au 2025, 5, 1903−1913