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SPECIAL FEATURE: ARTICLE

De novo synthesis of novel bacterial monosaccharide fusaminic acid

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Abstract

Fusobacterium nucleatum is an oral bacteria related to various types of diseases. As Gram-negative bacteria, lipopolysaccharide (LPS) of *Fusobacterium nucleatum* could be a potential virulence factor. Recently, the structure of O-antigen in LPS of *Fusobacterium nucleatum* strain 25586 was elucidated to contain a trisaccharide repeating unit -(4-β-Non*p*5Am-4-α-L-6dAlt*p*NAc3PCho-3-β-D-QuipNAc)-. The nonulosonic acid characterized as 5-acetamidino-3,5,9-trideoxy-L-*glycero*-L-*gluco*-non-2-ulosonic acid (named as fusaminic acid), and 2-acetamido-2,6-dideoxy-L-altrose are the novel monosaccharides isolated. Herein we report the de novo synthesis of 5-*N*-acetyl fusaminic acid and the thioglycoside derivative in order to further investigate the biological significance of nonulosonic acids for bacterial pathogenesis.

Introduction

Fusobacterium nucleatum is a Gram-negative anaerobic bacterium found in human mouth [1]. It is one of the most prevalent species in oral environment and regarded as a dental pathogen to cause serious periodontal diseases including periodontitis [2–6], gingivitis [2, 5, 7–9], and endodontic infections [10–12]. Besides periodontal disease, *F. nucleatum* is notorious for dissemination to cause a wide spectrum of diseases, some of which are lethal [1, 13]. It was reported that *F. nucleatum* causes diseases including adverse pregnancy outcomes [14–17], GI disorders [18–20], rheumatoid arthritis [21], respiratory tract infections [22], Lemierre's syndrome [22, 23], and Alzheimer's diseases [24]. For instances, it was reported that the bacteria originating from mother's subgingival plague could translocate to fetus thus causes term stillbirth in human pregnancies [14].

Although *F. nucleatum* infection results in numerous serious diseases, little is known about the virulence factor. So far, only one protein, FadA, was identified to be crucial in colonization and infection of the bacteria [1, 13, 20]. FadA is not only an adhesin but also an invasin that binds to cadhesin, which is widely distributed in tissues and cells, and this might be the reason of migration of *F. Nucleatum* [20, 25, 26]. LPS of the bacteria is also thought to be a virulence factor participating in the infection. It was shown that serum antibody levels to *F. nucleatum* LPS did correlate to poor oral health and the degree of periodontitis [27]. Recently, the structure of O-antigen of *F. nucleatum* strain 25586 was identified and two novel sugars, 5-acetamidino-3,5,9-trideoxy-L-*glycero*-L-*gluco*-non-2-ulosonic acid (fusaminic acid), and 2-acetamido-2,6-dideoxy-L-altrose (6dAlt2NAc), were isolated and found as components of the O-antigen (structures are shown in Fig. 1) [28]. Fusaminic acid attracts our interests due to its structural similarity to pseudaminic acid and legionaminic acid. These nonulosonic acids are unique to bacteria species, as the congener of sialic acids, and considered as an important virulence factor, although their precise roles in evasion of the bacteria have not been elucidated yet [29]. Moreover, being of crucial importance to Gram-negative bacteria, LPS containing these molecules could be candidate targets for developing new antimicrobial agents. However, the major difficulty in investigating the biological significance of the related nonulosonic acid is the inaccessibility of the target molecules in homogeneous form as well as sufficient

Dedication: This is dedicated to Professor Samuel. J. Danishefsky for his great scientific contribution to total synthesis of highly complex and biologically important natural products.

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amount. Herein we report a de novo total synthesis of fusaminic acid based on the strategies applied in our pseudaminic acid total synthesis reported recently [30].

Results and discussion

Although the isolated fusaminic acid contains an amidine group, we planned to synthesize the general *N*-acetyl derivative, Fus5Ac **1**. The retrosynthesis of Fus5Ac is shown in Scheme 1. The α-keto acid structure can be obtained by ozonolysis of the acrylate **6**, which can be prepared from the corresponding aldehyde by indium-mediated Barbier type allylation. The aldehyde can be derived via the Fukuyama reduction from thioester **7**, which can be constructed via the aldol-type reaction of thioester derived isonitrile **8** [31] and aldehyde **9**. Aldehyde **9** can be prepared from commercial available (*S*)-(-)-ethyl lactate via Weinreb-Nahm ketone synthesis followed by 1,2-*anti* selective reduction and ozonolysis. Our planned synthesis involves 3 chain elongation steps with four new chiral centers formed during this process. The absolute configuration of the chiral centers generated has to be carefully determined. Armed with the experience from our former synthesis of pseudaminic acid, we anticipated that the stereoselectivity of the key transformations could be modulated through utilization of different protecting groups with different electronic and steric hindrance effect and optimization of reaction parameters.

To choose suitable protecting groups for the intermediates, we first analyzed the stereoselectivity model of the addition of aldehyde **9**. In our recent total synthesis of pseudaminic acid, the selectivity in the aldol-type addition was achieved by lithium chelation of the protected β-hydroxyl group and the aldehyde carbonyl group [30]. Less-hindered protection of β-hydroxy group and bulky protection of α-hydroxyl group of aldehyde **9** were expected to attribute to the diastereoselectivity. Taking these into consideration, we chose *p*-methoxybenzyl (PMB) and *t*-butyldiphenylsilyl (TBDPS) ether, respectively (shown in Scheme 2). The synthesis commenced with (*S*)-(-)-ethyl lactate PMB ether protection, followed by saponification and coupling with *N*, *O*-dimethylhydroxylamine to obtain the Weinreb amide **13** in good yield. The latter was transformed to vinyl ketone **15** via vinylmagnesium bromide addition, and the corresponding diol **17** was obtained by stereoselective reduction of the ketone via zinc borohydride [32, 33]. Chelation of zinc ion resulted in 1,2-*anti* selectivity and the ratio of the diastereomers **17a/17b** was around 10:1. The resultant diastereomeric mixture was inseparable and then directly silylated with TBDPSCl followed by

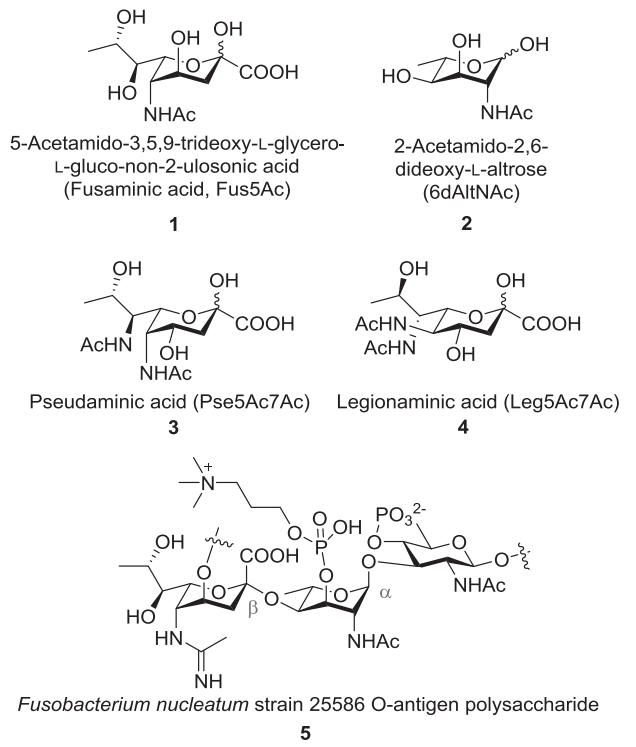
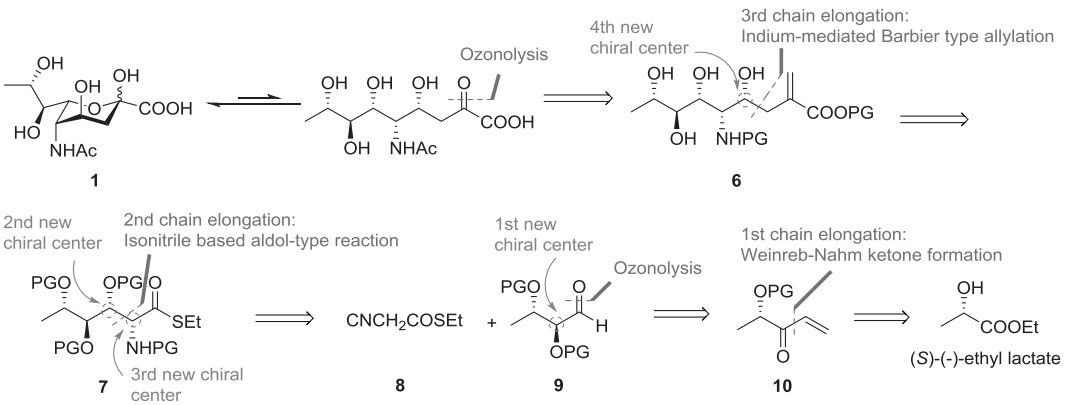
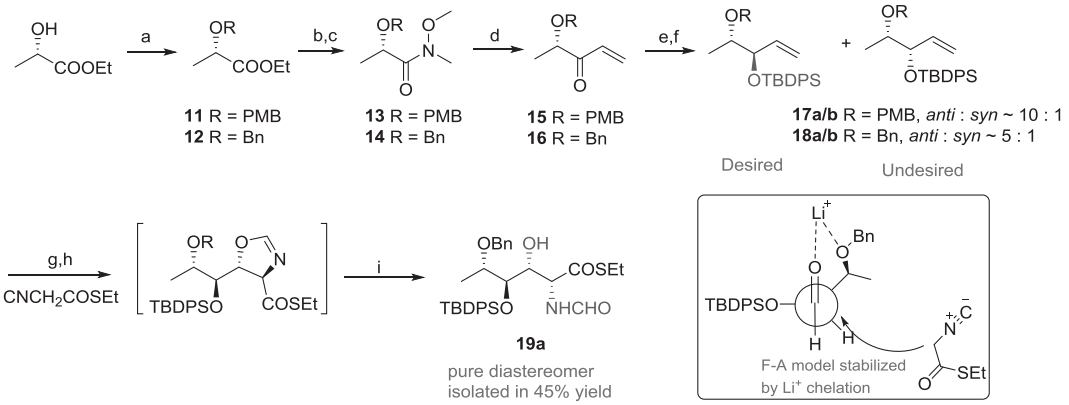


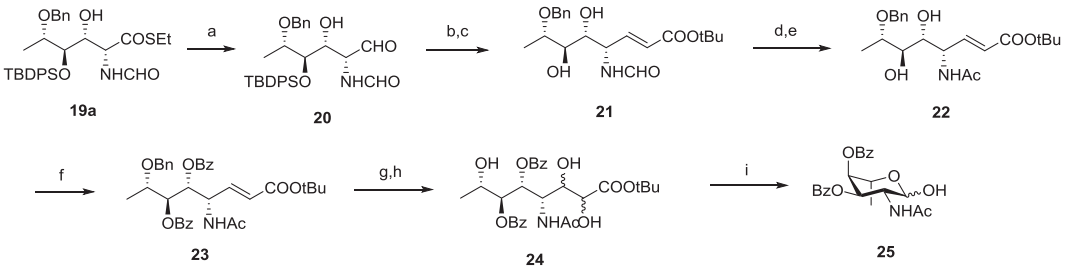
Fig. 1 Structures of Fus5Ac, 6dAltNAc, Pse5Ac7Ac, Leg5Ac7Ac, and polysaccharide in *Fusobacterium nucleatum* 25586 O-antigen



Scheme 1 Retrosynthesis of fusaminic acid **1**



Scheme 2 Synthesis of key intermediate thioester **19**[a]. [a]Reagents and conditions: **a** NaH, PMBCl for **11**, BnBr for **12**, DMF/THF, 0 °C to rt, 2 h, 81% for **11**, 83% for **12**. **b** LiOH, THF-MeOH-H_2O, 2 h, quant. **c** PivCl, Et_3N, 0 °C, 1 h, then *N*,*O*-dimethylhydroxyamine, DCM, 0 °C to rt, 3 h, 92% for **13**, 93% for **14**. **d** vinylmagnesium bromide, THF, −10 °C, 1 h, 87% for **15** and **16**. **e** $Zn(BH_4)_2$, diethyl ether, −20 °C, 1 h.; **f** TBDPSCl, imidazole, DMF, ovn, 89% for **17**, dr 10 : 1, 87% for **18**, dr 5 : 1, over 2 steps. **g** O_3, DCM, −78 °C, 0.5 h, then Ph_3P. **h** $CNCH_2COSEt$ (**8**), LiOTf, DIPEA, DCE-DMF, 2 h. **i** 80% HOAc (aq), 8 h, 45% over three steps



Scheme 3 Synthesis of hexosamine derivative[a]. [a]Reagents and condition: **a**Pd/C, Et_3SiH, THF, 1 h. **b** $Ph_3P{=}COOtBu$, DCM, 0.5 h. **c** TBAF, HOAc, THF, 2 h, 62% over three steps. **d** 3% HCl in MeOH, 0 °C to rt, 6 h. **e** Ac_2O, Et_3N, 86% over two steps. **f** BzCl, pyridine, DMAP, DCM, ovn, 92%. **g** OsO_4, NMO, THF-H_2O, ovn. **h** Pd/C, H_2, MeOH, 3 h. **i** $NaIO_4$, DCM-H_2O, 6 h, 67% over three steps

ozonolysis to give aldehyde **9**. With the key aldehyde in hand, we tried to convert it to the thioester **19a** by treating the aldehyde with the isonitrile thioester in the presence of lithium triflate and diisopropylethylamine, followed by acidolysis of the 4,5-*trans* oxazoline intermediate in aqueous acetic acid [30, 34–40]. Fortunately, we succeeded in getting target diastereomers in good to moderate yield, but the difficult separation remained a problem. Considering the significance of confirming the chiral centers generated, and the plausible complexity in the generation of the new chiral center in the following Barbier allylation step, it would be necessary to obtain the uniform diastereomer. To our delight, we found that changing PMB ether to benzyl ether simplified the purification without seriously impacting the stereoselectivity, and more importantly, prevented partially decomposition of PMB ether in ozonolysis and acidolysis. We planned to convert the thioester to the corresponding hexosamine derivative, 2-acetamido-2,6-dideoxy-L-altrose, to confirm the absolute configuration. It is noted that the altrose derivative, 6dAlt2NAc, is also the novel rare monosaccharide found in the LPS of *Fusobacterium nucleatum* strain 25586 [28].

Our initial attempts to directly convert the thioester to the altrose hemiacetal failed due to the difficulty in removing the benzyl ether. Catalytic hydrogenation or oxidation with sodium bromate combined with sodium dithionite [41] could not free the hydroxyl group without affecting the thioester. While deprotecting the benzyl ether after the Fukuyama reduction [42] was also challenging due to the difficulty in manipulating the corresponding α-N-formyl aldehyde. Thus, we firstly converted the thioester to the aldehyde and trapped the resultant aldehyde with ylide to give the α,β-unsaturated ester (shown in Scheme 3). With this stable olefin as the surrogate to the aldehyde, we had the chance to manipulate the protecting groups. Different solvents such as DCM, acetone, THF were tried to prevent partially triethylsilyl (TES) installation in the Fukuyama reduction, while the silylation seemed to be inevitable [43]. Therefore, we directly removed all silyl ethers by tetrabutylammonium fluoride (TBAF) to give product **20**. The formamide **20** was converted to acetamide **21** by acidolysis with methanolic hydrochloride followed by acetylation with acetic anhydride and trimethylamine in 86% yield [44]. The hydroxyl groups were masked by benzoyl chloride in order

to prevent side reactions in the following dihydroxylation and vicinal diol cleavage steps. Osmium tetraoxide was used for dihydroxylation, and benzyl ether could be removed by catalytic hydrogenation without affecting any other functional groups at this step. Finally, the hemiacetal **25** was formed by converting the diol **24** to aldehyde via the Malaprade reaction. With the altrose derivative **25** in hand, we carefully examined the configuration of the chiral centers newly generated.

As the 1,2-*anti* selective reduction of α-hydroxyl ketone via zinc borohydride was an established method, the absolute configuration of C-4 obtained from enantiopure starting material (*S*)-(-)-ethyl lactate could be settled [33]. Moreover, the isonitrile addition to the aldehyde would form 4,5-*trans* oxazoline in the presence of base and the following acidolysis did not affect the chiral center, thus the relative configuration of C-2 was also determined [34–40]. Although starting with a 5:1 mixture of the diastereomers of the vinyl alcohol, we could get the major diastereomer of the thioester in 45% yield in pure form, which excluded the contribution of the minor *syn* diol from the reduction step to the isolated thioester. Therefore, the thioester could only be **19a** or **19b**. Compound **19a** is the precursor of 2-acetamido-2,6-dideoxy-L-altrose while **19b** derives to 2-acetamido-2,6-dideoxy-L-glucose, also known as *N*-acetyl-L-quinovosamine (shown in Fig. 2). Peaks of this *N*-acetyl quinovosamine in ^{1}H-NMR spectrum should fit well to *N*-acetyl glucosamine pattern, of which peaks have large vicinal coupling constant as all protons in have axial orientation. On the contrary, for the altrose derivative in which protons orient both axially and equatorially, small vicinal coupling constants should be. We assigned protons of **24** and found several small coupling constant values such as $J_{H4,H5}$ (3 Hz, see Experimental section). Although some of the small coupling constants may come from the deviation from the typical chair-like conformation, the observed coupling patterns significantly different from the quinovosamine derivatives excluded the possibility of the structure **19b**. We decided to use the major diastereomer **19a** to continue the synthesis of fusaminic acid and planned to further confirm the configurations by comparing the splitting pattern of the synthetic final product with the isolated one.

The indium-mediated Barbier type allylation is the key step for carbon chain elongation due to its compatibility with various functional groups [45]. Successful conversion of the aldehyde to the desired acrylate was achieved by mixing the aldehyde generated via the Fukuyama reduction with organoindium species generated beforehand in situ in the mixture of ethanol and aqueous ammonium chloride [46]. After simple aqueous work-up, the reaction mixture was subjected to TBAF in THF solution buffered with acetic acid to remove the TBDPS group. Two diastereomers were formed with ratio of 5.5:1 as shown in ^{1}H NMR spectrum, which were readily separated by silica gel column chromatography to give the major diastereomer **26a** in 59 yield%. Regarding the previous experience in pseudaminic acid synthesis and similar compounds in the Seeberger's [46] and Ito's [47] syntheses, we proposed that the major diastereomer **26a** should possess the desired configuration at the newly formed chiral center [30, 40, 41]. The formamide was converted to acetamide as described above with partially intramolecular lactonization (**27/27a**), and the five-membered ring lactone could be hydrolyzed in 1 M NaOH solution to provide free acid **28** after careful neutralization. However, direct ozonolysis of the acid **28** gave unsuccessful result, while numerous byproducts including glycal were generated. To our delight, we found that protection of the carboxylic acid as benzyl ester **27** could significantly suppress side reactions, and the following ozonolysis gave hemiketal **29** in good yield. After final hydrogenation to remove benzyl groups, we successfully obtained the target molecule, Fus5Ac **1** (Shown in Scheme 4).

In order to further confirm the configuration of Fus5Ac synthesized, we derived **29** to thioglycoside **31** via thioglycosylation of acetate **30**. Relatively large $J_{C1,\ H3a}$ value (~6.4 Hz) indicates the equatorial orientation of the thiol aglycone (β). The small vicinal proton coupling constants of $J_{H3a,H4}$, $J_{H3e,H4}$, $J_{H4,H5}$, and $J_{H5,H6}$ (3.5, 2.0, < 0.1, 2.0 Hz, respectively) indicate the equatorial orientation of both H-4 and H-5. Combining with relatively large $J_{H6,\ H7}$ (7.5 Hz), we identified C4-C7 as *gluco* configuration [48]. Moreover, the spin system of the synthetic fusaminic acid **1** was highly consistent with the isolated sample as well as 5,7-

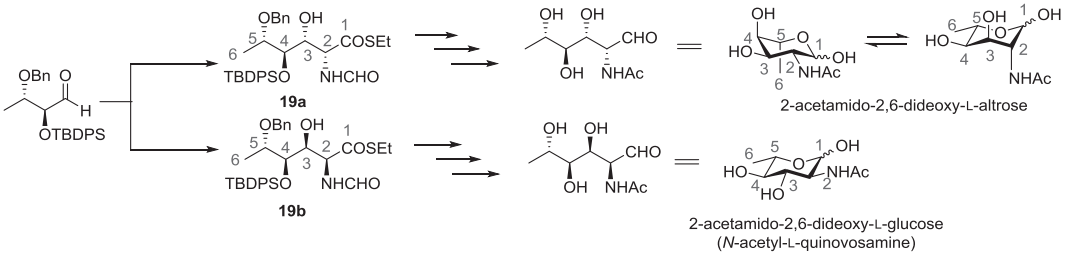
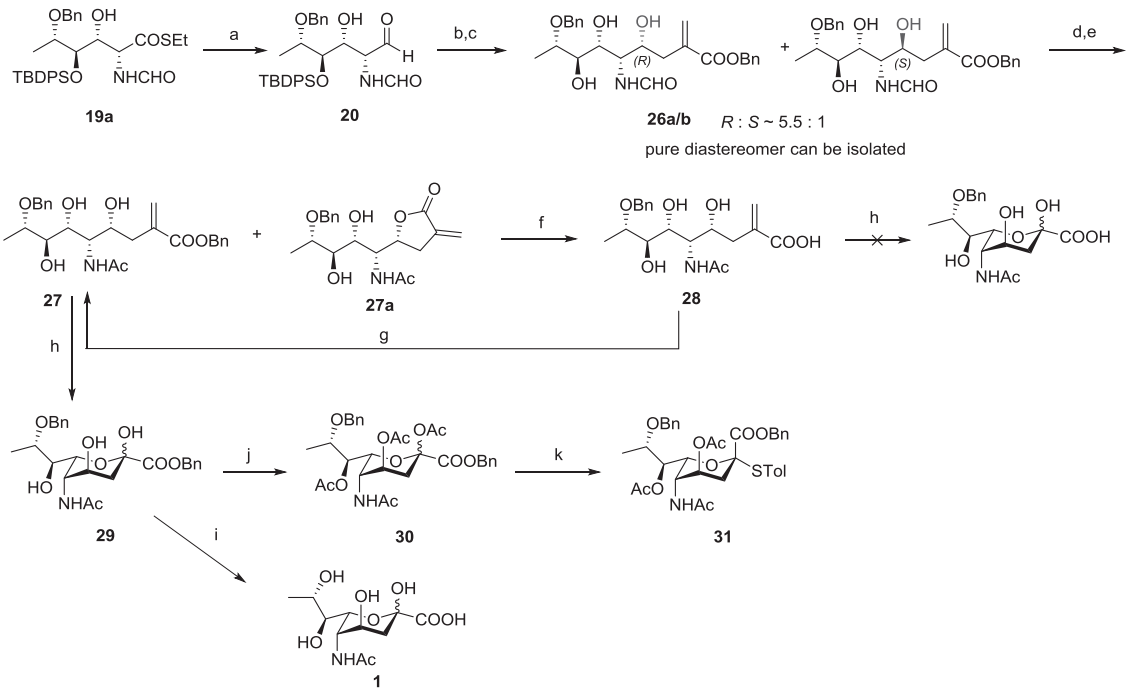


Fig. 2 Elucidation of the configurations of isonitrile adducts via derivatization to hexosamine



Scheme 4 De novo synthesis of fusaminic acid[a]. [a]Reagents and conditions: **a** Pd/C, Et_3SiH, THF, 1h. **b** Benzyl bromomethacrylate, indium powder, NH_4Cl, EtOH, 2 h. **c** TBAF, HOAc, THF, 2 h, 59% over three steps. **d** 3% HCl in MeOH, 0 °C to rt, 6 h. **e** Ac_2O, Et_3N, 89% over two steps; **f** 1M NaOH aqueous solution, 1 h, 87%.; **g** K_2CO_3, BnBr, DMF, 5 h, 79%.; **h** O_3, −78 °C, DCM-MeOH, 0.5 h, then Me_2S, 75%.; **i** Pd $(OH)_2$/C, H_2, MeOH-H_2O, 12 h, 89%.; **j** Ac_2O, pyridine, DMAP, 3 h, 83%.; **k** *p*-TolSH, $BF_3{\cdot}Et_2O$, dry DCM, ovn, 62%

diacetamido-3,5,7,9-tetradeoxy-L-*glycero*-L-*gluco*-non-2-ulosonic acid (compound 42 in ref. [48]) [28].

In summary, we have developed the de novo total synthesis of a newly discovered nonulosonic acid unique to bacteria, fusaminic acid. The synthesis started with commercial available (*S*)-(-)-ethyl lactate, involving 18 steps to give Fus5Ac in 8% overall yield. The key steps include the diastereoselective addition of the thioester derived isonitrile to aldehyde and indium-mediated diastereoselective Barbier-type allylation. We derived the key thioester intermediate to 6dAltNAc and synthesized fusaminic acid thioglycoside donor for structure confirmation. The success in generating the thioglycoside broadens the application of the synthesis as it provides the opportunity to assembly complex fusaminylated glycan and glycoconjugates.

Experimental section

General

Commercially available reagents were used without further purification, unless otherwise stated. The anhydrous solvents were either prepared from AR grade solvents via standard methods (DCM, THF, etc.), or purchased in anhydrous form (DMF, pyridine, etc.). The analytical TLC was performed on silica gel 60-F254 precoated on glass plate (E. Merck), with detection by fluorescence and/or or by staining with acidic ceric ammonium molybdate. The normal phase column chromatography was performed on silica gel (230–400 mesh, Merck), while the reverse phase chromatography was performed on C18 silica gel (Davisil 633NC18E, Grace Materials Technologies). The ^1H and ^{13}C NMR spectra were recorded on Advance DRX Bruker 400/500 MHz spectrometers at 25 °C. The 2D NMR spectra were recorded on Advance DRX Bruker 500 MHz spectrometers at 25 °C. The high-resolution mass spectrometry was performed on a Waters Micromass Q-TOF Premier Mass Spectrometer.

Ethyl (*S*)-2-(benzyloxy)propanoate (12)

To a mixture of anhydrous THF (20 mL) and DMF (20 mL) in round bottle flask was added sodium hydride (1.76 g, 44 mmol, 1.1 equiv, 60% suspension in mineral oil) followed by (*S*)-(−)-ethyl lactate (4.63 mL, 40 mmol, 1.0 equiv) at 0 °C. After strring for 10 min, benzyl bromide (7.13 mL, 60 mmol, 1.5 equiv) was added. The reaction mixture was warmed to room temperature and monitored by TLC. After full conversion (about 2 h) of the starting material, the reaction was quenched by sat. NH_4Cl (aq), then diluted with ethyl acetate (500 mL) and water (100 mL). The organic

layer was separated and washed sequentially with 1 M HCl (aq, 100 mL), sat. $NaHCO_3$ (aq, 100 mL), and brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel flash chromatography using *n*-hexane: ethyl acetate 12: 1 *v/v* as eluent to obtain compound **12** as colorless oil (6.90 g, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28–7.40 (5 H, m), 4.72 (1 H, d, $J = 11.5$ Hz), 4.47 (1 H, d, $J = 11.5$ Hz), 4.19–4.28 (2 H, m), 4.07 (1 H q, $J = 7.0$ Hz), 1.46 (3 H, d, $J = 6.5$ Hz), 1.31 (3 H, t, $J = 7.0$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.2, 137.6, 128.4, 14.2, 128.0, 127.8, 74.1, 72.0, 60.8, 18.7; HR-ESI-MS (*m/z*): calcd for $C_{12}H_{16}O_3Na^+$ ($M + Na^+$): 231.0992, found 231. 0998.

(*S*)-2-(benzyloxy)-N-methoxy-N-methylpropanamide (14)

To a stirred solution of **12** (6.90 g, 33.2 mmol, 1.0 equiv) in MeOH (15 mL) and THF (60 mL), the solution of LiOH (2.0 eq in 15 mL H_2O) was added. The mixture was stirred at r.t. for 2 h. After the full conversion was achieved, the mixture was acidified by 1 M HCl (aq). The mixture was diluted with ethyl acetate (500 mL), and the organic layer was separated. The aqueous layer was extract with ethyl acetate (200 mL) again, and the organic layer was combined. The organic layer was washed with brine (2 × 100 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was further dried under oil pump for 3 h. The residue above was used in the next step without purification.

The saponification product was dissolved in anhydrous DCM (200 mL) and cooled to 0 °C. Triethylamine (11.6 mL, 82.9 mmol, 2.5 equiv) was added to the solution above followed by PivCl (4.49 mL, 36.5 mmol, 1.1 eq) was added dropwise and the solution was kept stirring at 0 °C for 1 h. Then *N*, *O*-dimethyl hydroxylamine hydrochloride (3.88 g, 39.8 mmol, 1.2 equiv) was added and the reaction mixture was warmed to r.t. and kept stirring for another 3 h. After full conversion (monitored by TLC), the mixture was diluted with ethyl acetate (500 mL) and washed with 1 M HCl (aq, 100 mL), sat. $NaHCO_3$ (aq, 100 mL) and brine (100 mL) subsequently. The solvent was removed under vacuum and the residue obtained was purified by silica gel flash chromatography using *n*-hexane: ethyl acetate 3:1 *v/v* as eluent to obtain compound **14** as slightly yellow oil (7.20 g, 93%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24–7.36 (5 H, m), 4.64 (1 H, d, $J = 12.0$ Hz), 4.36–4.41 (2 H, m), 4.07 (1 H, q, $J = 7.0$ Hz,), 3.55 (3 H, s), 3.18 (3 H, s), 1.38 (3 H, d, $J = 6.5$ Hz,); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.8, 128.3, 127.9, 127.7, 71.1, 61.2, 17.9; HR-ESI-MS (*m/z*): calcd for $C_{12}H_{17}NO_3Na^+$ ($M + Na^+$): 246.1101, found 246. 1109.

(S)-4-(benzyloxy)pent-1-en-3-one (16)

To a solution of Weinreb amide **14** (7.20 g, 30.9 mmol) in anhydrous THF (100 mL) was added vinylmagnesium bromide (1.0 M solution in THF, 46.3 mL, 1.5 equiv) dropwise at −20 °C. The reaction mixture was gradually warmed to r.t. and kept stirring for 2 h. After full conversion achieved, the reaction mixture was poured into a cold 1 M HCl aqueous solution. The mixture was extracted with ethyl acetate (500 mL) and washed with sat. $NaHCO_3$ (aq, 100 mL) and brine (100 mL). The solvent was removed under vacuum and the residue obtained was purified by silica gel flash chromatography using *n*-hexane: ethyl acetate 15: 1 *v/v* as eluent to obtain compound **14** as colorless oil (5.10 g, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.38 (4 H, m), 7.28–7.33 (1 H, m), 6.81 (1 H, dd, $J =$ 17.5, 11.0 Hz), 6.45 (1 H, dd, $J = 17.5$, 1.5 Hz), 5.80 (1 H, dd, $J = 10.5$, 1.5 Hz), 4.58 (1 H, d, $J = 12.0$ Hz), 4.46 (1 H, d, $J = 11.5$ Hz), 4.13 (1 H, q, $J = 7.0$ Hz), 1.39 (3 H, d, $J =$ 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.0, 137.5, 130.93, 129.68, 128.39, 127.81, 127.76, 79.7, 71.7, 17.6; HR-ESI-MS (*m/z*): calcd for $C_{12}H_{14}O_2Na^+$ ($M + Na^+$): 213.0886, found 246. 0894.

(((*R*)-4-(benzyloxy)pent-1-en-3-yl)oxy)(tert-butyl) diphenylsilane (18a/b)

To a solution of the vinyl ketone **16** (5.10 g, 26.8 mmol, 1.0 equiv) in anhydrous diethyl ether (200 mL) was added zinc borohydride (0.2 M solution in diethyl ether, 135 mL, 1.0 equiv) dropwise at −20 °C. The reaction was kept stirring at the temperature for 3 h. When full conversion was achieved, the reaction was quenched by carefully adding 1 M HCl (aq), then diluted with ethyl acetate (200 mL) and water (100 mL). The organic phase was separated and washed with sat. $NaHCO_3$ (aq, 100 mL) and brine (100 mL). The solvent was removed under vacuum and the residue was purified by silica gel flash chromatography using *n*-hexane: ethyl acetate 5: 1 *v/v* as eluent to obtain (4 *S*)-4-(benzyloxy) pent-1-en-3-ol as colorless oil (5 (*anti*): 1 (*syn*) mixture of diastereomers, 4.75 g, 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.41 (4 H, m), 7.30–7.35 (1 H. m), 5.90 (1 H, ddd, $J = 17.5$, 11.0, 6.0 Hz), 5.36 (1 H, dt, $J = 17.0$, 1.5 Hz), 5.25 (1 H, dt, $J = 11.0$, 1.5 Hz), 4.66 (1 H, d, $J = 12.0$ Hz), 4.57 (1 H, d, $J = 12.0$ Hz), 4.25–4.29 (1 H, m), 3.64 (1 H, ddd, $J = 12.5$, 6.5, 3.5 Hz), 2.61 (1 H, d, $J = 4.0$ Hz), 1.20 (3 H, d, $J = 6.5$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4, 136.7, 128.5, 127.7, 116.4, 77.5, 74.6, 70.8, 14.0; HR-ESI-MS (*m/z*): calcd for $C_{12}H_{16}O_2Na^+$ ($M + Na^+$): 215.1043, found 245. 1050.

To a solution of the alcohol above (4.75 g, 24.7 mmol) in DMF solution (30 mL) was added imidazole (3.36 g, 43.4 mmol, 2.0 equiv) and TBDPSCl (9.63 mL, 37.0 mmol,

1.5 equiv). The mixture was kept stirring for 8 h and monitored by TLC. After full conversion, the mixture was diluted with ethyl acetate (500 mL) and washed with 1 M HCl (aq, 100 mL), sat. $NaHCO_3$ (aq, 100 mL) and brine (100 mL) subsequently. The solvent was removed under vacuum and the residue obtained was purified by silica gel flash chromatography using *n*-hexane: ethyl acetate 40: 1 *v/v* as eluent to obtain compound **18a/b** as colorless oil (5 (*anti*): 1 (*syn*) mixture of diastereomers, 10.63 g, 97%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85–7.88 (2 H, m), 7.79–7.84 (2 H, m), 7.35–7.55 (11 H, m), 6.03 (1 H, ddd, $J = 17.0$, 10.0, 7.0 Hz), 5.16 (1 H, dt, $J = 10.5$, 1.0 Hz), 5.07 (1 H, dt, $J = 17.0$, 1.0 Hz), 4.73 (1 H, d, $J = 12.0$ Hz), 4.66 (1 H, d, $J = 12.0$ Hz), 4.33–4.36 (1 H, m), 3.66 (1 H, ddd, $J = 13.0$, 6.5, 3.0 Hz), 1.23–1.28 (12 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.2, 137.5, 136.25, 136.21, 134.23, 134.13, 129.65, 129.59, 128.3, 127.61, 127.53, 127.42, 127.34, 116.7, 78.8, 78.2, 71.5, 27.2, 19.6, 15.8; HR-ESI-MS (*m/z*): calcd for $C_{28}H_{34}O_2SiNa^+$ ($M + Na^+$): 453.2220, found 453.2231.

S-Ethyl (2 R,3 R,4 R,5 S)-5-(benzyloxy)-4-((tert-butyldiphenylsilyl)oxy)-2-formamido-3-hydroxyhexanethioate (19a)

The solution of **18a/b** (10.6 g, 24.7 mmol) in DCM (150 mL) was cooled to −78 °C. The O_3 (generated from O_2 and carried by the flow of O_2) was bubbled through this solution. The color of the solution truned purple, which indicated the saturation of O_3 in DCM. The excess amount of O_3 was blown off by the flow of O_2 and the purple color disappeared. To this solution, Me_2S (2 mL) was added to reduce the peroxide intermediate. After 1 h reduction at r.t., the solution was diluted with DCM (500 mL) and washed with water (100 mL) to remove DMSO (generated from Me_2S). The solvent was removed under vacuum and the residue was used in the next step without further purification.

To the solution of the above aldehyde in DCE (125 mL), isonitrile **8** (3.83 g, 29.6 mmol, 1.2 equiv) was added, followed by the solution of LiOTf (4.62 g, 29.6 mmol, 1.2 equiv) in anhydrous DMF (25 ml). The final concentration of the aldehyde was controlled at 0.2 M. To this mixture, DIPEA (0.86 ml, 4.94 mmol, 0.2 equiv) was added to initiate the reaction. The mixture was stirred at r.t. for 2 h, then was diluted with DCM (500 ml) and thoroughly washed with water and brine. The organic phase was concentrated under vacuum. The residue was dissolved in 80% acetic acid aqueous solution (30 mL) and kept stirring for 8 h. The acetic acid was removed under vacuum and the residue was diluted with ethyl acetate (500 mL). The mixture was washed with sat. $NaHCO_3$ (aq, 3 × 100 mL) and brine (100 mL), and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by silica gel flash chromatography using *n*-hexane: ethyl acetate 3: 1 *v/v* as eluent to obtain **19a** as white solid. (6.44 g, 45%). ^{1}H NMR (500 MHz, $CDCl_3$, 3: 1 mixture of two rotamers) δ 7.66–7.71 (4 H, m, major), 7.60–7.65 (1.33 H, m, minor), 7.19–7.47 (14.6 H, m, major and minor), 6.27 (1 H, d, $J = 8.5$ Hz, major), 6.05 (0.33 H, t, $J = 11.0$ Hz, minor), 4.98 (1 H, d, $J = 9.0$ Hz, major), 4.51–4.56 (1.66 H, m, major and minor), 4.44 (1 H, d, $J = 11.5$ Hz, major), 4.42 (0.33 H, d, $J = 12.5$ Hz, minor), 4.30 (1 H, d, $J = 12.5$ Hz, major), 4.19 (0.33 H, d, $J = 10.0$ Hz, minor), 3.72 (1 H, qd, $J = 6.5$, 2.0 Hz, major), 3.81 (0.33 H, qd, $J = 6.5$, 1.5 Hz, minor), 3.67 (0.33 H, dd, $J = 8.5$, 1.5 Hz, minor), 3.58 (1 H, dd, $J = 8.0$, 1.5 Hz, major), 3.21 (1 H, d, $J = 3.5$ Hz, major), 3.05 (0.33 H, d, $J = 4.0$ Hz, minor), 2.88–2.95 (2.66 H, m, major and minor), 1.26 (3 H, t, $J = 7.5$ Hz, major), 1.25 (1 H, t, $J = 7.5$ Hz, minor), 1.06 (9 H, s, major), 1.05 (3 H, s, minor), 1.02 (1 H, d, $J = 7.0$ Hz, minor), 0.92 (3 H, d, $J = 7.0$ Hz, major); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.7 (major), 164.3 (minor), 161.3 (major), 138.2 (major), 138.1(minor), 136.3 (major), 136.14 (major), 136.09 (minor), 133.9 (major), 133.1 (minor), 132.9 (minor), 132.5 (major), 130.5 (minor), 130.3 (minor), 130.2 (major), 129.8 (major), 128.64 (minor), 128.58 (major), 128.2 (minor), 128.0 (major), 127.9 (minor), 127.8 (major), 127.7 (major and minor), 127.5 (major and minor), 79.3 (minor), 78.9 (major), 76.3 (major), 75.5 (minor), 74.0 (major), 73.9 (minor), 71.7 (minor), 71.4 (major), 62.5 (minor), 59.4 (major), 24.0 (minor), 23.7 (major), 19.6 (major and minor), 17.5 (minor), 17.2 (major), 14,37 (major), 14.35 (minor); HR-ESI-MS (*m/z*): calcd for $C_{32}H_{41}NO_5SiSNa^+$ ($M + Na^+$): 602.2367, found 602.2376.

tert-Butyl (4 S,5 R,6 R,7 S,E)-7-(benzyloxy)-4-formamido-5,6-dihydroxyoct-2-enoate (21)

To a 50 mL round bottle flask, thioester **19a** (579 mg, 1.0 mmol, 1.0 equiv) and Pd/C (10% Pd on activated carbon, 100 mg, 0.1 equiv based on Pd) were added. After agron protection of the flask, anhydrous DCM (5 mL) was added, and the mixture was stirred mildly. Et_3SiH (0.60 mL, 3.8 mmol, 3.8 equiv) was added dropwise during 20 min, then the mixture was mildly stirred at r.t. for 2 h. When full conversion achieved, the mixture was filtered through celite. To the filtrate was added (*tert*-butoxycarbonylmethylene) triphenylphosphorane (451 mg, 1.2 mmol, 1.2 eq), and the mixture was kept stirring for 1 h. After full conversion as indicated by TLC, the solvent was removed under vacuum. The residue was re-dissolved in THF (10 mL) and a mixture of TBAF (1.0 M solution in THF, 3.0 mL, 3.0 equiv) and acetic acid (90 μL, 1.5 mmol, 1.5 equiv) were added. The mixture was then kept stirring for 2 h and monitored by TLC. After full conversion, the reaction mixture was diluted

with ethyl acetate (200 mL), washed with brine (2 × 50 mL), and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by by silica gel flash chromatography using DCM: ethyl acetate 3:1 *v/v* as eluent to obtain **21** as white foam. (235 mg, 62%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26–8.28 (1 H, m), 7.29–7.37 (5 H, m), 6.89 (1 H, dd, $J = 15.5$, 4.5 Hz), 6.25 (1 H, d, $J = 9.0$ Hz), 5.94 (1 H, dd, $J = 16.0$, 2.0 Hz), 5.09 (1 H, q, $J = 4.5$ Hz), 4.70 (1 H, d, $J = 11.0$ Hz), 4.43 (1 H, d, $J = 11.5$ Hz), 3.77 (1 H, dd, $J = 8.5$, 1.0 Hz), 3.71 (1 H, dd, $J = 8.0$, 6.0 Hz), 3.17 (1 H, t, $J = 8.5$ Hz), 1.48 (9 H, s), 1.38 (3 H, d, $J = 6.0$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2, 162.4, 143.7, 137.3, 128.85, 128.35, 128.19, 124.8, 81.0, 79.6, 72.3, 71.0, 50.0, 28.3, 16.8; HR-ESI-MS (*m/z*): calcd for $C_{20}H_{29}NO_6Na^+$ ($M + Na^+$): 402.1887, found 402.1896.

tert-Butyl (4 S,5 R,6 R,7 S,E)-4-acetamido-7-(benzyloxy)-5,6-dihydroxyoct-2-enoate (22)

To a 50 mL round bottle flask containing compound **21** (235 mg, 0.62 mmol, 1.0 equiv), a solution of HCl (aq) in MeOH (3%, 12.0 mL prepared from conc. HCl 1.0 mL and MeOH 11.0 mL) cooled to 0 °C was added. The reaction mixture was gradually warmed to r.t. and kept stirring for 6 h. After full conversion as indicated by TLC, the mixture was cooled to 0 °C again and triethylamine was added until the solution turned basic. Acetic anhydride (120 µL, 1.24 mmol, 2.0 equiv) was added and the solution was kept stirring for 1 h in basic environment. After full conversion, the reaction was diluted with ethyl acetate (200 mL), washed with1 M HCl (aq, 50 mL), sat. $NaHCO_3$ (aq, 50 mL) and brine (50 mL), and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by silica gel flash chromatography using DCM: ethyl acetate 3:1 *v/v* as eluent to obtain **22** as white foam (209.6 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27–7.37 (5 H, m), 6.88 (1 H, dd, $J = 16.0$, 4.5 Hz), 6.01 (1 H, d, $J = 9.0$ Hz), 5.91 (1 H, d, $J = 16.0$ Hz), 4.96–5.01 (1 H, m), 4.71 (1 H, d, $J = 11.0$ Hz), 4.42 (1 H, d, $J = 11.0$ Hz), 3.75 (1 H, d, $J = 8.5$ Hz), 3.66–3.73 (1 H, m), 3.08 (1 H, t, $J = 8.5$ Hz), 2.10 (3 H, s), 1.48 (9 H, s), 1.39 (3 H, d, $J = 6.0$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 165.2, 144.4, 137.3, 128.8, 128.3, 128.2, 124.5, 81.0, 80.0, 72.4, 71.1, 51.2, 28.2, 23.2, 17.1; HR-ESI-MS (*m/z*): calcd for $C_{21}H_{31}NO_6Na^+$ ($M + Na^+$): 416.2044, found 416.2058.

(2 S,3 S,4 R,5 S,E)-5-acetamido-2-(benzyloxy)-8-(tert-butoxy)-8-oxooct-6-ene-3,4-diyl dibenzoate (23)

To a solution of compound **22** (235 mg, 0.53 mmol, 1.0 eq) in anhydrous pyridine (10 mL) was added benzoyl chloride (173 µL, 1.32 mmol, 2.5 equiv) dropwise, followed by DMAP (4 mg, 0.03 mmol, 0.05 eq). The reaction was kept stirring for 16 h. When full conversion was achieved, the mixture was diluted with ethyl acetate (200 mL), and washed with 1 M HCl (aq, 3 × 50 mL), sat. $NaHCO_3$ (aq, 50 mL) and brine (50 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by silica gel flash chromatography using *n*-hexane: ethyl acetate 3: 1 *v/v* as eluent to obtain **23** as white foam (318.2 mg, 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (2 H, d, $J = 7.5$ Hz), 7.93 (2 H, d, $J = 7.5$ Hz), 7.59 (2 H, t, $J = 7.0$ Hz), 7.40–7.47 (4 H, m), 7.25–7.36 (10 H, m), 6.84 (1 H, dd, $J = 16.0$, 5.0 Hz), 6.20 (1 H, d, $J = 9.0$ Hz), 5.78 (1 H, d, $J = 15.5$ Hz), 5.69 (1 H, t, $J = 5.0$ Hz), 5.60 (1 H, t, $J = 5.0$ Hz), 5.21–5.28 (1 H, m), 4.63 (1 H, d, $J = 11.0$ Hz), 4.58 (1 H, d, $J = 11.0$ Hz), 3.92–3.99 (1 H, m), 1.86 (3 H, s), 1.42 (9 H, s), 1.34 (3 H, d, $J = 6.0$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.4, 165.53, 165.51, 165.1, 142.8, 137.7, 133.68, 133.53, 129.90, 129.65, 129.32, 128.71, 128.70, 128.63, 128.22, 128.02, 124.6, 80.8, 74.00, 73.92, 73.0, 71.4, 50.5, 28.2, 23.3, 16.1; HR-ESI-MS (*m/z*): calcd for $C_{35}H_{39}NO_8Na^+$ ($M + Na^+$): 624.2568, found 624.2580.

2-Acetamido-3,4-di-O-benzoyl-2,6-dideoxy-L-altrose (25)

To a mixture of compound **23** (318 mg, 0.53 mmol, 1.0equiv) in THF (6 mL) and H_2O (2 mL) was added OsO_4 (0.04 M solution in H_2O, 265 µL, 0.02 equiv) and 4-methylmorpholine 4-oxide (NMO, 4.8 M solution in H_2O, 550 µL, 5.0 equiv). The mixture was kept stirring for 8 h. When full conversion was achieved, the mixture was quenched with sat. $Na_2S_2O_3$ (aq), then diluted with ethyl acetate (200 mL), and washed with brine (50 mL). The organic solvent was dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel flash chromatography using *n*-hexane: ethyl acetate 2: 3 *v/v* to obtain **24** as white foam. (286.4 mg, 85%)

To a round bottom flask containing compound **24** (143.2 mg, 0.225 mmol), Pd/C (10% Pd on activated carbon, 50 mg) was added. The flask was filled with argon and methanol (10 mL) was added. The mixture was kept stirring under 1 atm H_2 atmosphere and monitored by TLC. After full conversion, the mixture was filtered through celite to remove the catalyst. The filtrate was concentrated under vacuum and used in the next step without further purification.

To the solution of the above residue in DCM (10 mL) was added a solution of $NaIO_4$ (241 mg, 1.13 mmol, 5.0 equiv) in H_2O (5 mL). The reaction mixture was kept vigorous stirring for 6 h. After full conversion, the mixture was diluted with ethyl acetate (200 mL), and washed with brine (50 mL). The organic solvent was dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by

silica gel flash chromatography using DCM: methanol 40: 1 *v/v* as eluent to obtain **25** as white foam (76.2 mg, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (2 H, t, $J = 7.5$ Hz, ArH), 7.52 (2 H, t, $J = 7.5$ Hz, ArH), 7.47 (3 H, t, $J = 7.5$ Hz, ArH), 7.37 (3 H, t, $J = 7.5$ Hz, ArH), 6.60 (1 H, d, $J = 8.0$ Hz, NH), 5.66 (1 H, qd, $J = 6.5$, 3.0 Hz, H-5), 5.50 (1 H, dd, $J = 9.5$, 3.0 Hz, H-4), 4.65 (1 H, d, $J = 9.0$ Hz, H-3), 4.44 (1 H, dd, $J = 8.0$, 2.0 Hz, H-2), 4.10 (1 H, s, OH), 2.05 (3 H, s, CH_3CO), 1.52 (3 H, d, $J = 6.5$ Hz, H-6); HR-ESI-MS (*m/z*): calcd for $C_{22}H_{23}NO_7Na^+$ ($M + Na^+$): 436.1367, found 436.1375.

Benzyl (4 R/S,5 S,6 R,7 R,8 S)-8-(benzyloxy)-5-formamido-4,6,7-trihydroxy-2-methylenenonanoate (26a/b)

To a 100 mL round bottom flask, thioester **19a** (2.89 g, 5.0 mmol, 1.0 equiv) and Pd/C (10% Pd on activated carbon, 500 mg, 0.1 equiv based on Pd) were added. After agron protection of the flask, anhydrous DCM (25 mL) was added, and the mixture was stirred mildly. Et_3SiH (3.0 mL, 19 mmol, 3.8 equiv) was added dropwise during 20 min, then the mixture was mildly stirred at r.t. for 2 h. When full conversion was achieved, the mixture was filtered through celite and the filtrate was concentrated under vacuum to give crude aldehyde **20**. The aldehyde was used in the next step without further purification.

To a 50 mL round bottom flask, indium power (1.72 g, 15 mmol, 3.0 equiv) was added, followed by EtOH (20 mL), benzyl bromomethylacrylate (5.15 g, 22.5 mmol, 4.5 equiv) and sat. NH_4Cl solution (3 mL). The mixture was sonicated for 20 min at 50 °C to generate the corresponding indium reagent. This so-obtained solution of indium reagent was added into the solution of the above crude aldehyde **20** in EtOH (10 mL) in one portion, and the mixture was stirred at r.t. for 2 h. The reaction was quenched by sat. $NaHCO_3$, then dilute with ethyl acetate (500 mL). The organic layer was separated and the aqueous layer was extract with another 500 mL ethyl acetate. The organic layer was combined and washed with 1 M HCl (aq, 200 mL), sat. $NaHCO_3$ (aq, 200 mL), and brine (200 mL), and was dried over anhydrous Na_2SO_4. The organic solvent was concentrated under vacuum and the residue was directly used in the next step.

To a solution of the above residue in THF (100 mL) was added a mixture of TBAF (1.0 M solution in THF, 15.0 mL, 3.0 equiv) and acetic acid (0.44 mL, 15.0 mmol, 1.5 equiv). The reaction mixture was kept stirring for 2 h and monitored by TLC. When full conversion was achieved, the reaction mixture was diluted with ethyl acetate (500 mL), then was thoroughly washed with brine (200 mL) and dried over Na_2SO_4. The organic solvent was concentrated under vacuum and the residue was purified by flash column chromatography using DCM: ethyl acetate 3: 2 *v/v* as eluent to obtain compound **26a/b** as white foam. (6 (4 *R*): 1 (4 *S*) mixture of diastereomers, 1.35 g for the major diastereomer, 59% over 3 steps). For major diastereomer **26a**: [α] + 27.25 (c 0.6, DCM); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.32 (1 H, s), 7.28–7.37 (10 H, m), 6.54 (1 H, d, $J = 9.0$ Hz), 6.31 (1 H, s), 5.66 (1 H, s), 5.20 (1 H, d, $J = 12.5$ Hz), 5.18 (1 H, d, $J = 12.5$ Hz), 4.94 (1 H, s), 4.74 (1 H, s), 4.70 (1 H, d, $J = 11.5$ Hz), 4.41 (1 H, d, $J = 11.5$ Hz), 4.27 (1 H, t, $J = 8.5$ Hz), 4.15 (1 H, d, $J = 9.0$ Hz), 3.88 (1 H, s), 3.81 (1 H, d, $J = 9.0$ Hz), 3.63–3.70 (1 H, m), 3.10 (1 H, t, $J = 8.0$ Hz), 2.56 (1 H, dd, $J = 14.0$, 7.5 Hz), 2.41 (1 H, dd, $J = 14.0$, 6.0 Hz), 1.38 (3 H, d, $J = 6.0$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.93, 163.34, 137.21, 136.38, 136.01, 128.88, 128.82, 128.73, 128.40, 128.39, 128.28, 128.19, 80.34, 80.28, 72.7, 71.8, 71.0, 66.8, 49.9, 37.0, 17.2; HR-ESI-MS (m/z): calcd for $C_{25}H_{31}NO_7Na^+$ ($M + Na^+$): 480.1993, found 480.2002. For minor diastereomer **26b:** [α] + 17.3 (c 0.3, DCM); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (1 H, s), 7.27–7.42 (10 H, m), 6.66 (1 H, d, $J = 8.5$ Hz), 6.34 (1 H, s), 5.73 (1 H, s), 5.20 (2 H, s), 4.84 (1 H, s), 4.71 (1 H, d, $J = 11.5$ Hz), 4.64 (1 H, s), 4.42 (1 H, d, $J = 11.0$ Hz), 4.15 (1 H, dd, $J = 9.0$, 4.5 Hz), 3.99 (1 H, d, $J = 9.0$ Hz), 3.85–3.92 (1 H, m), 3.65–3.73 (1 H, m), 3.46 (1 H, d, $J = 9.0$ Hz), 3.15 (1 H, t, $J = 8.5$ Hz), 2.65 (1 H, dd, $J_1 = 14.0$ Hz, $J_2 = 3.5$ Hz), 2.54 (1 H, dd, $J_1 = 14.0$ Hz, $J_2 = 9.0$ Hz), 1.39 (3 H, d, $J = 6.0$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.12, 162.69, 137.36, 136.74, 135.91, 128.84, 128.75, 128.58, 128.44, 128.35, 128.28, 128.19, 80.1, 75.1, 72.8, 71.6, 70.9, 66.9, 50.9, 37.7, 17.0; HR-ESI-MS (*m/z*): calcd for $C_{25}H_{31}NO_7Na^+$ ($M + Na^+$): 480.1993, found 480.2003.

Benzyl (4 R,5 S,6 R,7 R,8 S)-5-acetamido-8-(benzyloxy)-4,6,7-trihydroxy-2-methylenenonanoate (27)

To a 50 mL round bottom flask containing compound **26a** (1.35 g, 2.95 mmol, 1.0 equiv), a cold solution of HCl (aq) in MeOH (3%, 12.0 mL prepared from conc. HCl 1.0 mL and MeOH 11.0 mL) was added. The reaction mixture was gradually warmed from 0 °C to r.t. and kept stirring for 6 h. When full conversion was achieved as indicated by TLC, the mixture was cooled to 0 °C again and solid sodium bicarbonate was carefully added until the solution turned neutral. Acetic anhydride (120 μL, 1.24 mmol, 2.0 equiv) was added and the solution was kept stirring for 1 h. After full conversion as indicated by TLC, the reaction was diluted with ethyl acetate (500 mL), washed with1 M HCl (aq, 50 mL), sat. $NaHCO_3$ (aq, 50 mL), and brine (50 mL), and dried over anhydrous Na_2SO_4. The organic solvent was removed under vacuum and the residue was purified by flash column chromatography using DCM: ethyl acetate 3:

2 *v/v* as eluent to obtain compound **27** as white foam (347 mg, 24.5%) and DCM: ethyl acetate 1: 1 *v/v* as eluent to obtain lactone **27a** as white foam (686 mg, 64%). For lactone **27a**: [α] + 12.3 (c 0.5, DCM); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27–7.40 (5 H, m), 6.25 (1 H, t, $J = 2.0$ Hz), 6.09 (1 H, d, $J = 9.0$ Hz), 5.67 (1 H, s), 4.69–4.75 (1 H, m), 4.70 (1 H, d, $J = 11.0$ Hz), 4.43 (1 H, d, $J = 11.0$ Hz), 4.29 (1 H, t, $J = 7.0$ Hz), 3.67–3.75 (1 H. m), 3.68 (1 H, d, $J = 9.0$ Hz), 3.21 (1 H, t, $J = 8.0$ Hz), 3.06 (1 H, dt, $_J = 17.0$, 2.0 Hz), 2.76 (1 H, dt, $J = 17.0$, 3.0 Hz), 2.08 (3 H, s), 1.36 (3 H, d, $J = 6.0$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 169.8, 137.5, 133.6, 128.8, 128.29, 128.15, 123.0, 79.2, 74.8, 72.1, 71.0, 54.1, 31.4, 23.3, 16.4; HR-ESI-MS (*m/z*): calcd for $C_{19}H_{25}NO_6Na^+$ (M + Na^+): 386.1574, found 386.1581.

To a 50 mL round bottom flask containing lactone **27a** (686 mg, 1.88 mmol, 1.0 equiv) was added 1 M NaOH (aq, 5 mL). The reaction was kept vigorous stirring for 2 h and monitored by TLC. When full conversion was achieved, ion exchange resin Dowex 50 W X8 (H^+) was carefully added to neutralize the reaction. The mixture was filtered through celite to remove the resin and a small portion of the acid **28** (10 mg) was purified by HPLC for characterization. To the aqueous solution of acid **28** was added K_2CO_3 (259 mg, 1.88 mmol, 1.0 equiv). Water was removed by oil pump and the residue obtained was re-dissolved in DMF (5 mL). Benzyl bromide (446 μL, 3.76 mmol, 2.0 equiv) was added and the reaction mixture was kept stirring for 6 h. When full conversion was achieved as indicated by TLC, the mixture was diluted with ethyl acetate (300 mL), washed with brine (50 mL), and dried over anhydrous Na_2SO_4. The organic solvent was removed under vacuum and the residue was purified by flash column chromatography using DCM: ethyl acetate 3: 2 *v/v* as eluent to obtain compound **27** as white foam (761.5 mg, 86%, together with previous potion, 1.10 g, 79% from **26a**). For acid **28**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28–7.38 (5 H, m), 6.76 (1 H, d, $J = 10.5$ Hz), 6.38 (1 H, s), 5.73 (1 H, s), 4.70 (1 H, d, $J = 11.0$ Hz), 4.41 (1 H, d, $J = 11.0$ Hz), 4.30 (1 H, t, $J = 7.0$ Hz), 4.12 (1 H, d, $J = 9.0$ Hz), 3.88 (1 H, dd, $J = 9.0$, 1.0 Hz), 3.68–3.73 (1 H, m), 3.10 (1 H, t, $J = 9.0$ Hz), 2.55 (1 H, dd, $J = 14.0$, 7.5 Hz), 2.43 (1 H, dd, $J = 14.0$, 6.0 Hz), 2.17 (3 H, s), 1.40 (3 H, d, $J = 5.5$ Hz). No carbon spectrum recorded due to the unstability of the acid.

For Benzyl ester **27**: [α] + 19.96 (c 0.3, DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.40 (10 H, m), 6.38 (1 H, d, $J =$ 9.2 Hz), 6.30 (1 H, d, $J = 0.8$ Hz), 5.65 (1 H, s), 5.27 (1 H, br), 5.21 (1 H, d, $J = 12.4$ Hz), 5.17 (1 H, d, $J = 12.4$ Hz), 4.78 (1 H, s), 4.70 (1 H, d, $J = 11.2$ Hz), 4.40 (1 H, d, $J =$ 11.2 Hz), 4.25 (1 H, t, $J = 6.8$ Hz), 4.06 (1 H, d, $J = 9.2$ Hz), 3.09 (1 H, s), 3.79 (1 H, dd, $J = 8.8$, 0.8 Hz), 3.62–3,71 (1 H, m), 3.06 (1 H, t, $J = 8.8$ Hz), 2.55 (1 H, dd, $J = 14.0$, 7.6 Hz), 2.41 (1 H, dd, $J = 14.0$, 6.0 Hz), 2.11 (3 H, s), 1.39 (3 H, d, $J = 6.0$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 166.9, 137.23, 136.5, 136.1, 128.87, 128.72, 128.61, 128.39, 128.35, 128.24, 128.20, 80.75, 80.64, 71.98, 71.89, 71.0, 66.8, 51.1, 37.0, 23.2, 17.3; HR-ESI-MS (*m/z*): calcd for $C_{26}H_{33}NO_7Na^+$ (M + Na^+): 494.2149, found 494.2160.

5-Acetamido-3,5,9-trideoxy-L-glycero-L-gluco-non-2-ulosonic acid (1)

The solution of compound **27** (235 mg, 0.5 mmol) in DCM (10 mL) and methanol (5 mL) was cooled to −78 °C. The O_3 (generated from O_2 and carried by the flow of O_2) was bubbled through this solution. The color of the solution turned purple, which indicated the saturation of O_3. The excess amount of O_3 was blown off by the flow of O_2 and the purple color disappeared. To this solution, Me_2S (2 mL) was added to reduce the peroxide intermediate. After 1 h reduction at r.t., the solvent was removed under vacuum and the residue was purified by flash column chromatography using DCM: methanol 30: 1 *v/v* as eluent to obtain compound **29** as white foam, which was the mixture of linear and cyclic tautomers (177.4 mg, 75%).To a round bottom flask containing compound **29** (109.4 mg, 0.375 mmol), Pd $(OH)_2$/C (20% Pd on activated carbon, 50 mg) was added. methanol (10 mL) and water (3 mL) were added to the flask. The mixture was kept stirring under 1 atm H_2 atmosphere for 12 h. After filtration, the solvent was removed under vacuum and the residue was further purified by BioGel column using H_2O as eluent. The product **1** was obtained after lyophilization as white foam (92.2 mg, 87%). ^{1}H NMR (500 MHz, D_2O) δ 4.28 (1 H, dd, $J = 9.5$, 2.0 Hz, H-6), 4.00–4.08 (3 H, m, H-4, H-5, H-8), 3.69 (1 H, dd, $J = 9.5$, 7.0 Hz, H-7), 2.05–2.11 (1 H, m, H-3e), 2.05 (3 H, s, CH_3CO), 1.83–1.88 (1 H, m, H-3a), 1.15 (3 H, d, $J =$ 6.5 Hz, H-9); ^{13}C NMR (125 MHz, D_2O) δ 176.7 (CH_3CO), 174.3 (COOH), 96.0 (C-1), 71.5 (C-7), 67.6 (C-8), 66.3 (C-6), 65.9 (C-4), 47.9 (C-5), 32.3 (C-3), 21.7 (CH_3CO), 14.6 (C-9); HR-ESI-MS (*m/z*): calcd for $C_{11}H_{19}NO_8Na^+$ (M + Na^+): 316.1003, found 316.1009.

Benzyl 5-acetamido-8-O-benzyl-2,4,7-tri-O-acetyl-3,5,9-trideoxy-L-glycero-L-gluco-2-nonulopyranosonate (30)

To the solution of compound **29** (112 mg, 0.378 mmol) in pyridine (5 mL), Ac_2O (2 mL) and DMAP (3 mg, 0.02 mmol, 0.06 equiv) were added sequentially. The mixture was kept stirring for 3 h. The mixture was concentrated under vacuum and the residue was dissolved in ethyl acetate (100 mL). The solution was washed with 1 M HCl (aq, 20 mL), sat. $NaHCO_3$ (aq, 20 mL), and brine (20 mL) and was dried over anhydrous Na_2SO_4. The organic solvent was removed under vacuum and the residue was purified by silica gel flash chromatography using *n*-hexane: ethyl

acetate 2.5: 1 *v/v* as eluent to obtain acetate **30** as white foam (183 mg, 83%). ^{1}H NMR (0.9: 1 mixture of two anomers, peaks selected for the major anomer, 500 MHz, $CDCl_3$) δ 7.27–7.39 (10 H, m), 6.20 (1 H, d, $J = 9.0$ Hz), 5.26 (1 H, dd, $J = 9.0$, 2.5 Hz), 5.20 (1 H, d, $J = 12.0$ Hz), 5.13 (1 H, d, $J = 12.5$ Hz), 5.02 (1 H, q, $J = 3.5$ Hz), 4.80 (1 H, dd, $J = 9.0$, 2.0 Hz), 4.56 (1 H, d, $J = 11.5$ Hz), 4.45 (1 H, d, $J = 11.5$ Hz), 4.16 (1 H, dt, $J = 9.0$, 2.0 Hz), 3.87 (1 H, qd, $J = 6.5$, 3.5 Hz), 2.59 (1 H, dd, $J = 15.5$, 2.5 Hz), 2.32 (2 H, dd, $J = 15.0$, 3.5 Hz), 2.09 (3 H, s), 2.03 (3 H, s), 1.96 (3 H, s), 1.83 (3 H, s), 1.17 (3 H, d, $J = 6.5$ Hz); ^{1}H NMR (0.9: 1 mixture of two anomers, peaks selected for the minor anomer, 500 MHz, $CDCl_3$) δ 7.27–7.39 (9 H, m), 6.20 (0.9 H, d, $J = 9.0$ Hz), 5.22 (0.9 H, d, $J = 12.5$ Hz,), 5.18 (0.9 H, dd, $J = 9.0$, 2.5 Hz), 5.13 (0.9 H, d, $J = 12.5$ Hz), 4.92–4.94 (0.9 H, m), 4.63 (0.9 H, dd, $J = 9.0$, 2.0 Hz), 4.57 (0.9 H, d, $J = 11.5$ Hz), 4.49 (0.9 H, d, $J = 11.5$ Hz), 4.20 (1 H, dt, $J = 9.0$, 2.0 Hz), 3.78 (0.9 H, qd, $J = 6.5$, 2.5 Hz), 2.40 (0.9 H, dd, $J = 16.0$, 1.5 Hz), 2.11 (2.7 H, s), 2.04 (2.7 H, s), 1.99 (0.9 H, dd, $J = 16.0$, 4.0 Hz), 1.93 (2.7 H, s), 1.92 (2.7 H, s), 1.24 (d, $J = 6.5$ Hz, 2.7 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.46, 170.19, 170.06, 169.91, 169.57, 169.16, 168.55, 167.65, 168.42, 167.36, 138.53, 138.46, 134.87, 134.81, 128.89, 128.84, 128.80, 128.70, 128.54, 128.45, 128.44, 128.08, 128.03, 127.79, 127.67, 96.3, 95.8, 75.0, 74.1, 71.5, 71.23, 71.05, 70.7, 70.2, 68.20, 68.16, 67.8, 67.3, 45.05, 44.60, 30.88, 29.89, 23.32, 23.11, 21.49, 21.25, 21.00, 20.97, 20.73, 20.58, 15.5, 14.3; HR-ESI-MS (*m/z*): calcd for $C_{31}H_{37}NO_{11}Na^+$ ($M + Na^+$): 622.2259, found 622.2271.

4-Methylphenyl 7-acetamido-8-benzyl-4,7-di-O-acetyl-3,5,9-trideoxy-β-thiofusaminoside (31)

To a 25 mL round bottom flask, acetate **30** (183 mg, 0.31 mmol, 1.0 equiv) and 4-toluenethiol (234 mg, 1.88 mmol, 6.0 equiv) were added. Anhydrous DCM (6.0 mL) was added under argon, and the concentration of **30** was kept at 50 mM. The mixture was cooled to 0 °C, then $BF_3{\cdot}Et_2O$ (77 μL, 0.62 mol, 2.0 equiv) was added dropwise. The mixture was then kept stirring at 0 °C to r.t. for 16 h. The reaction was quenched by sat. $NaHCO_3$ (aq), and the organic phase was dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by silica gel flash chromatography using *n*-hexane: ethyl acetate 4:1 *v/v* as eluent to obtain thioglycoside **31** as yellow foam (127.4 mg, 62%) and characterized as β anomer ($J_{C1,\ H3a} = 6.4$ Hz). [α] + 10.85 (c 0.7, DCM); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26–7.35 (10 H, m, ArH), 7.15–7.20 (ArH, m, 2 H), 7.02 (2.0 H, d, $J = 8.0$ Hz, ArH), 6.31 (1 H, d, $J = 8.0$ Hz, NH), 5.23 (1 H, dd, $J = 7.5$, 4.5 Hz, H-7), 5.11 (1 H, dd, $J = 7.0$, 2.0 Hz, H-6), 4.98–5.02 (1 H, m, H-4), 4.86 (1 H, d, $J = 12.0$ Hz, $PhCH_2O$), 4.83 (1 H, d, $J = 12.0$ Hz, $PhCH_2O$), 4.57 (1 H, d, $J = 11.0$ Hz, $PhCH_2O$), 4.48 (1 H, d, $J = 11.0$ Hz, $PhCH_2O$), 4.14 (1 H, dt, $J = 7.5$, 1.0 Hz, H-5), 3.89 (1 H, qd, $J = 6.5$, 4.5 Hz, H-8), 2.68 (1 H, dd, $J = 16.0$, 2.0 Hz, H-3e), 2.32 (3 H, s, $SC_6H_4CH_3$), 2.20 (1 H, dd, $J = 16.0$, 3.5 Hz, H-3a), 2.18 (3 H, s, CH_3CO), 2.08 (3 H, s, CH_3CO), 1.71 (3 H, s, CH_3CO), 1.30 (3 H, d, $J = 6.5$ Hz, H-9); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 170.0, 169.8, 167.9, 139.6, 138.1, 135.3, 134.9, 129.7, 128.7, 128.6, 128.4, 127.9, 127.5, 89.3, 74.1, 71.9, 71.3, 68.5, 67.6, 46.1, 32.9, 27.2, 22.9, 21.7, 21.4, 21.0, 15.8; HR-ESI-MS (*m/z*): calcd for $C_{36}H_{41}NO_9SNa^+$ ($M + Na^+$): 686.2394, found 686.2407.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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