The Journal of Antibiotics (2019) 72:449–460
https://doi.org/10.1038/s41429-019-0165-0

JARA Japan Antibiotics Research Association
The Society for Actinomycetes Japan

SPECIAL FEATURE: ARTICLE

Step-economy synthesis of β-steryl sialosides using a sialyl iodide donor

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Received: 21 November 2018 / Revised: 12 February 2019 / Accepted: 15 February 2019 / Published online: 19 March 2019

Abstract

Steryl glycosides are prevalent in nature and have unique biological activities dictated by sterol structure, sugar composition, and the stereochemical attachment of the aglycone. A single configurational switch can have profound biological consequences meriting the systematic study of structure and function relationships. Steryl congeners of *N*-acetyl neuraminic acid (NANA) impact neurobiological processes and may also mediate host/microbe interactions. In order to study these processes, a platform for the synthesis of β-steryl sialosides has been established. Promoter-free glycosidations using a novel α-linked sialyl iodide donor efficiently provide unique amphiphilic sialoglycoconjugates for examining bioactivities in various systems.

Introduction

In recent years, we have been increasingly interested in understanding chemical exchange phenomena between bacteria and their hosts. Our attention has focused on the inter-kingdom trade of fatty acids, sterols and sugars, and the subsequent incorporation of these building blocks into glycolipid motifs that modulate host immune function. A recent example from our lab involves glycolipid biosynthesis in *Helicobacter pylori*, which requires that the bacteria receive cholesterol from the host. *H. pylori* then glycosidates the cholesterol to form α-linked cholesteryl-galactoside (α-CG) [1] and may also further decorate the sugar component with fatty acids (α-CAG) [2] or phosphate esters (α-CPG) [3] (Fig. 1). Specific glycolipids resulting from this exchange induce observable changes in lipid raft formation in the host membrane and increase susceptibility to infection [4]. The composition of the sugar head group and the configuration of the lipid attachment at the anomeric center have profound effects upon biological activity. Given that steryl glycosides are prevalent in nature [5], it seems likely that similar mechanisms may be at play in other biological settings. Having access to a large repertoire of chemically characterized glycolipids to systematically probe subtleties of structural and stereochemical variations on the biological activity glycolipids has become a focus of our synthetic efforts [6].

In this report, β-steryl sialosides are targeted as structural variants of CG and in recognition of Professor Samuel J. Danishefsky's contributions to this field of inquiry. He first brought to our attention the importance of making novel glycosides of *N*-acetyl neuraminic acid (NANA) for systematic structural studies [7]. NANA is one member of a larger collection of sialic acids, which are characterized as nine-carbon ketoaldononulosonic acids. This chemical entity is often expressed on cell surfaces and is highly concentrated in the brain where it may be anchored into the membrane through attachment to proteins or lipids [8–10]. Neurobiological studies of cholesteryl NANA indicate that both α- and β-anomers stimulate neurotransmitter release but the mechanisms of action are different [11]. The α-anomer modulates calcium influx, while the β-anomer enhances acetylcholine biosynthesis. The consequence of a single stereochemical change leading to unique biochemistry is a consistent

Dedication: This work is dedicated to Professor Samuel J. Danishefsky in appreciation of his mentorship and recognition of his prophetic and timeless contributions to the chemical sciences.

Supplementary information The online version of this article (https://doi.org/10.1038/s41429-019-0165-0) contains supplementary material, which is available to authorized users.

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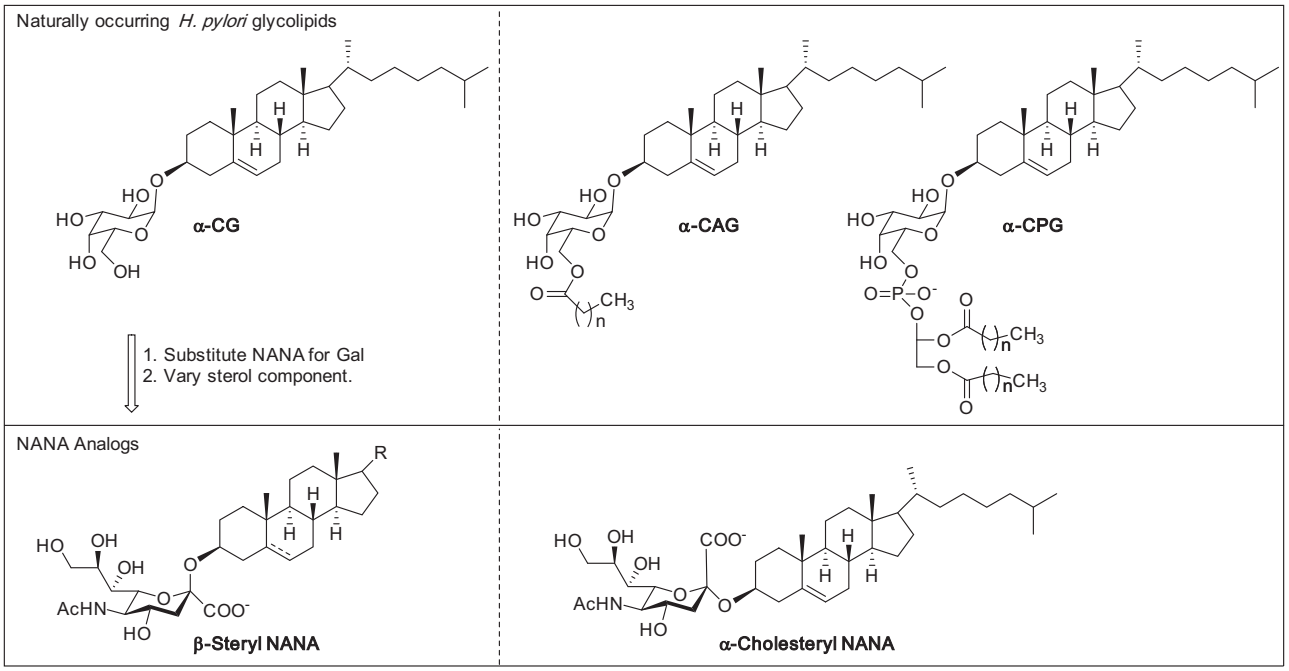


Fig. 1 Structural variants of biologically active steryl glycosides characterized by the sugar composition, sterol composition and the stereochemical attachment between carbohydrate headgroup and sterol

theme in glycobiology that can only be elucidated with access to synthetic analogs, as these compounds have yet to be found in nature.

In the more than 30 years since Danishefsky first reported the total synthesis of NANA, understanding of the biological importance of sialoglycoconjugates has significantly broadened while at the same time the synthetic challenges have been enumerated. Chief among these is achieving stereochemical control at the anomeric position, which is complicated by the C-2 ketosidic linkage and the lack of substitution at C-3, which might otherwise provide control through anchimeric assistance. Here we report a step economical synthesis of a novel sialyl iodide donor and its reactivity toward various acceptors including the stereoselective synthesis of β-steryl glycosides.

Results

A variety of sialosyl donors have been designed and synthesized to achieve stereoselective sialidation of different acceptors. In the case of cholesterol, triflic acid activation of a cyclic phosphite afforded a 60% yield of a 4:1 α:β mixture of anomers [12]. Crich and co-workers also observed 2:1 α-selectivity using thiophenyl donors [13]. Synthetic studies in our lab have centered on the use of pyranosyl iodides to achieve stereoselective glycosidation of cholesterol acceptors. These donors are prepared from the corresponding anomeric acetate or silyl ethers upon treatment with iodotrimethylsilane (TMSI) in a one-step transformation. The iodide is highly reactive and undergoes promoter-free S_N2-like displacement with cholesterol to produce either α- or β-anomers, depending upon the stereochemistry of the iodide when it is intercepted by cholesterol. In moving this strategy to sialic acid, one is faced with the challenge of forming an iodide at a ketoglycosidic linkage and the likelihood of E-2 elimination occurring. Indeed, reaction of the methyl ester of per-*O*-acetylated NANA (**1**) with TMSI gives a complex mixture of products including 2,3-dehydro glycal (Fig. 2). To date, only two reports of iodide-mediated sialylation have been reported. In the 1990s, Linhardt and co-workers prepared *C*-linked sialosides using SmI_2 and suggested that glycosyl iodides may be formed during the reaction [14]. Later, Gregar et al. attempted to prepare sialyl iodides using HI/acetic acid. While the β-iodide was characterized by NMR, it quickly underwent reduction to give the C-2 hydrido analog [15].

Since that time, novel design features have emerged in the synthesis of sialic acid donors. In particular, manipulation of the C-5 *N*-acetyl functionality has proven effective in many applications [16, 17]. Importantly, a report by Boons and co-workers showed that double acylation at the C-5 nitrogen enhanced the glycosylation reaction time when using thiomethyl sialodonors activated with NIS/TfOH [18]. We were delighted to find that compound **1** could be readily *N*-acetylated using isopropenyl acetate and *p*-toluenesulfonic acid with microwave radiation to give **2** and subsequent reaction with

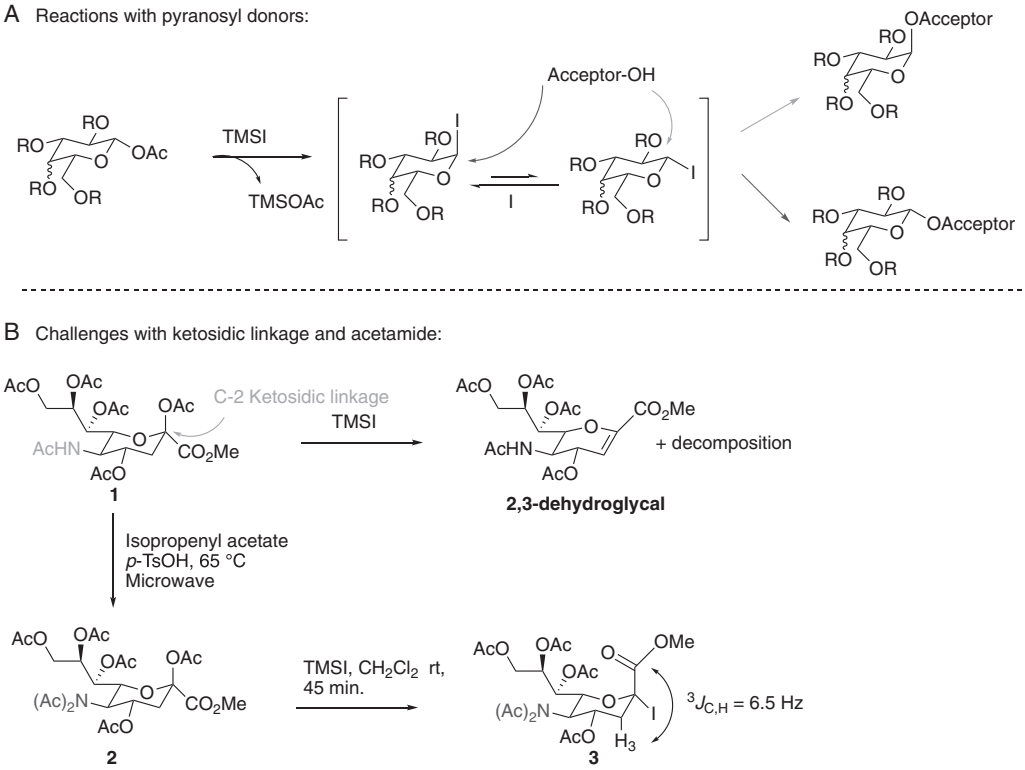


Fig. 2 Challenges and strategy in the synthesis of sialyl iodide **3**

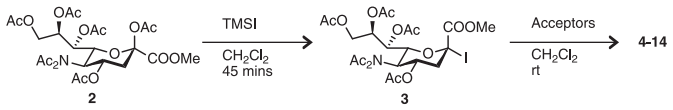
TMSI afforded sialyl iodide **3**. Although purification of the iodide was not attempted, **3** was characterized by NMR in situ (see SI). As is typical of glycosyl iodides, the C-2 anomeric carbon shifted upfield (from δ96.7 for **2** to δ79.5 for **3**). In contrast, C-3 shifted downfield by nearly 10 ppm from δ36.8 in **2** to δ46.1 in **3**. These shifts have been attributed to the heavy atom effect [19]. The α-stereochemistry of the iodide was determined from HMBC experiments showing $^{3}J_{C,H} = 6.5$ Hz coupling between the axial C-1 carbonyl and axial H-3.

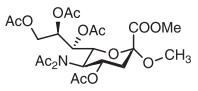
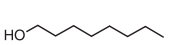
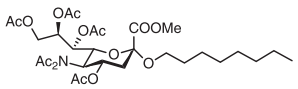
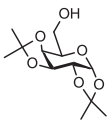
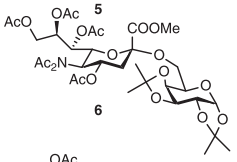
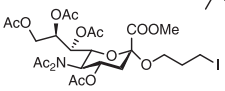
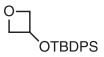
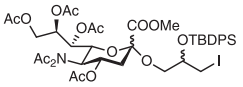
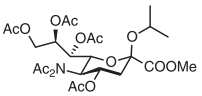
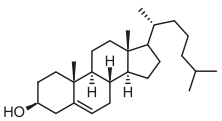
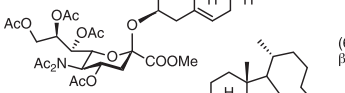
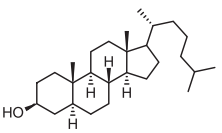
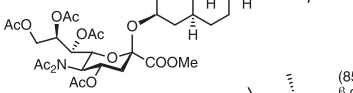
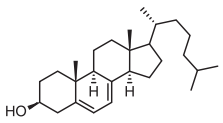
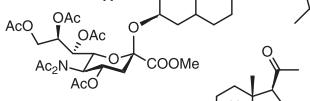
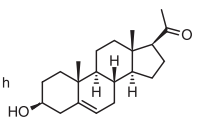
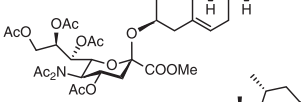
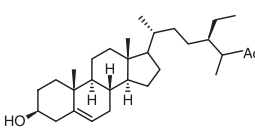
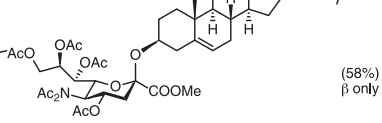
Upon preparation of **3**, glycosidations of various primary alcohols were investigated at room temperature (rt) without exogenous promoter (Table 1). Gratifyingly, methanol and octanol, both primary alcohols, glycosidated in 98% and 89% yields, respectively (Table 1, entries 1 and 2). Previous reactions with methanol using chloro [20] or thiophenyl [13] sialyl donors resulted in α:β mixtures of 3:1 and 1.5:1, respectively. The sialyl iodide afforded slightly more favorable α-selectivity (4:1 α:β). Meanwhile, when Crich and co-workers introduced octanol to a thioadamantyl analog of **3**, a 1:8 ratio of α:β anomers was obtained in 89% yield [21]. These reaction conditions required NIS/TfOH activation and generally were complete in 1 h at −40 °C. In contrast, promoter-free addition of octanol to 3 occurred in 4 h at rt with exclusive formation of the α-sialoside, as evidenced by HMBC NMR studies (see SI). These two methodologies are complimentary giving high yields of either anomer depending upon the donor.

To investigate sugar acceptors, 1,2;3,4-di-*O*-isopropylidene galactose was introduced next (Table 1, entry 3). Previous accounts of this acceptor reacting with thiophenol [22, 23], thiolauryl [24], and thio-benzoxazoyl [25] sialyl donors gave varying mixtures of **6** (α:β 2:1 or 1:1) in 72–96% yields. Gratifyingly, reaction of 1,2;3,4-di-*O*-isopropylidene galactose with **3** gave predominantly α-linkage (22:1). Motivated by these successes, highly reactive cyclic ethers were next explored. Previous studies in our lab indicated that strained cyclic ethers can serve as acceptors giving ring opened products [26, 27]. This also proved to be the case with the sialyl iodide donor (Table 1, entry 4). The utility of having an iodide functionalized handle on glyconjugates for multivalent applications has previously been demonstrated [27]. We were delighted to see that functionalized cyclic ethers could also be glycosidated (Entry 5) providing more sophisticated handles that may eventually lead to glycerol-based fatty acid congeners. Under these circumstances, the stereochemical preference shifted toward the β-linkage (1:3, α:β), giving the first hint that sterics impact sialyl iodide glycosidations (vide infra).

Given the successful glycosidations of primary alcohols and strained cyclic ethers, more hindered secondary alcohols were introduced. Surprisingly, glycosidation with

Table 1 Stereoselective synthesis of sialoglycoconjugates



Entry	Time	Acceptor	Product	Yield
1	4 h		 **4**	(98%) α:β 4:1
2	4 h		 **5**	(89%) α only
3	3 h		 **6**	(66%) α:β 22:1
4	2 h		 **7**	(79%) α only
5	4 h		 **8**	(67%) α:β 1:3
6	6 h		 **9**	(85%) β only
7	16 h		 **10**	(68%) β only
8	16 h		 **11**	(85%) β only
9	16 h		 **12**	(52%) β only
10	24 h		 **13**	(84%) β only
11	16 h		 **14**	(58%) β only

isopropanol gave exclusively β-linked sialoside (Table 1, entry 6). Intrigued by this result, cholesterol was added and it reacted in 6 h to also give the β-cholesteryl sialoside (**10**) in 68% yield (Table 1, entry 7). In a separate account, Crich and co-workers observed a preference for α-glycoside formation (2:1) when employing a sialyl thiophenol donor [13]. β-Sialylation of dihydrocholesterol required 16 h to complete while giving comparable yields to cholesterol (Table 1, entry 8). Reaction of β-pregnenolone containing a ketone motif, which can be further functionalized post-glycosylation, took 24 h to complete giving slightly lower yields compared to cholesterol and dihydrocholesterol (Table 1, entry 10) and β-sitosterol, an abundant sterol found in plants [5], also proved to be a suitable acceptor (Table 1, entry 11). Complications were encountered when 7-dehydrocholesterol (7-DHC) was reacted with **3**, as the conjugated diene rearranged to give a spirocyclic product (Table 1, entry 9). The structure of **12** was evident from the ^{1}H NMR showing no olefinic proton signals while at the same time tetra-substituted carbons were observed in ^{13}C NMR and HMBC NMR experiments. Others have reported that steroidal backbones containing olefins, particularly conjugated dienes, undergo rearrangement in acidic conditions with a notable feature of spirocyclic carbon at approximately δ 67 ppm on ^{13}C NMR [28–32]. Given the production of HI in situ, it is reasonable to conclude that the acidic media facilitates the spirocyclic rearrangement, albeit without jeopardizing the glycosidic linkage.

No problems were encountered in removing both the *N*- and *O*-acetates. The substrates (**10–14**) were first dissolved in methanol and 2 M NaOH was added with vigorous stirring. Upon completion, the reaction media was neutralized with Dowex H^{+} resin, (Fig. 3). Thus starting from commercially available NANA, the iodide donor was prepared in 4 steps (*O*-acetylation, methyl ester formation, *N*-acetylation, and iodide formation). In situ addition of the sterol and subsequent deprotection afforded β-steryl sialosides in a step-efficient process.

Discussion

Mechanistic insight into the reactivity of glycosyl donors enables strategic planning of stereoselective syntheses. As a general rule, glycosyl iodides undergo substitution with inversion of stereochemistry [6, 33, 34]. In the studies reported herein, that trend appears to continue with sterol acceptors, as the α-iodide is converted to β-sialoside. However, the mechanistic details of the reaction are most certainly more complicated, as S_N2-like displacement at a quaternary center is difficult to reconcile. Fukase and co-workers have reported computational studies on the oxocarbenium that would result from the loss of iodide from compound **3** [35]. The minimized structures show the *N*-acetyl groups capable of stabilizing intermediate (**3a**), whereas in the flipped chair (**3b**) no such stabilization is possible. Furthermore, we obtained a crystal structure of compound **2** showing the same preferred trans orientation of the two *N*-acetyl groups as predicted by computations. The proximity of the *N*-acyl group to the anomeric center is also evident in this structure (Fig. 4).

It is reasonable to speculate that the stereochemical outcome of sialyl iodide glycosidation is dictated by equilibria occurring between **3**, **3a**, and **3b** (Fig. 4). The reaction proceeds by initially forming an oxocarbenium ion that favors the $^{5}H_{4}$ conformer (**3a**) where the C_4 *O*-acetyl group and C_5 *N*-diacetyl groups in the pseudoaxial positions participate in stabilizing electrostatic interactions (i.e., stereoelectronic effect) [36, 37]. When reactive and sterically accessible acceptors such as primary alcohols and strained cyclic ethers are added, nucleophilic attack proceeds from the *si*-face of the oxocarbenium giving α-selectivity (Fig. 4). Notably, the displaced iodide resulting from formation of **3a** is also a competing nucleophile. While iodide addition to either **3a** or **3b** is plausible, formation of the β-iodide is not observed even with addition of excess tetrabutylammonium iodide. This finding supports the notion that the equilibrium is biased toward the formation of **3a**, and not **3b** or **3**, both of which would yield the β-sialyl iodide. The conformational bias toward **3a** can also explain the lower yields (in comparison to primary alcohols) and longer reaction times required for secondary acceptors; it is only as **3b** is consumed that the equilibrium shifts toward its formation. Thus the unique reactivity profile of the β-sialyl iodide provides a robust platform for stereochemical control mediated by acceptor sterics.

Experimental procedures

General experimental conditions

All commercially available reagents and solvents were used without further purification and all reactions were conducted under argon atmosphere in an oven dried glassware. ^{1}H NMR, ^{13}C NMR, and 2D NMR experiments were performed on a Bruker 600 or 800 MHz spectrometer. The spectral data are reported relative to deuterated peaks (C_6D_6, δ 7.16 and CD_3OD, δ 3.33) and chemical shifts were reported in parts per million (ppm, δ). ^{1}H NMR splitting patterns are designated as singlet (s), doublet (d), triplet (t), doublet of doublets (dd), apparent triplet (app. t.) or multiplet (m) and coupling constants are reported in Hertz (Hz). Glycosidic linkages were determined by HSQMBC or HMBC NMR experiments. Given the large $^{3}J_{C,H}$ of α-linked sialosides, correlation of the carbon of the methyl ester (C_1) to the axial H-3 was

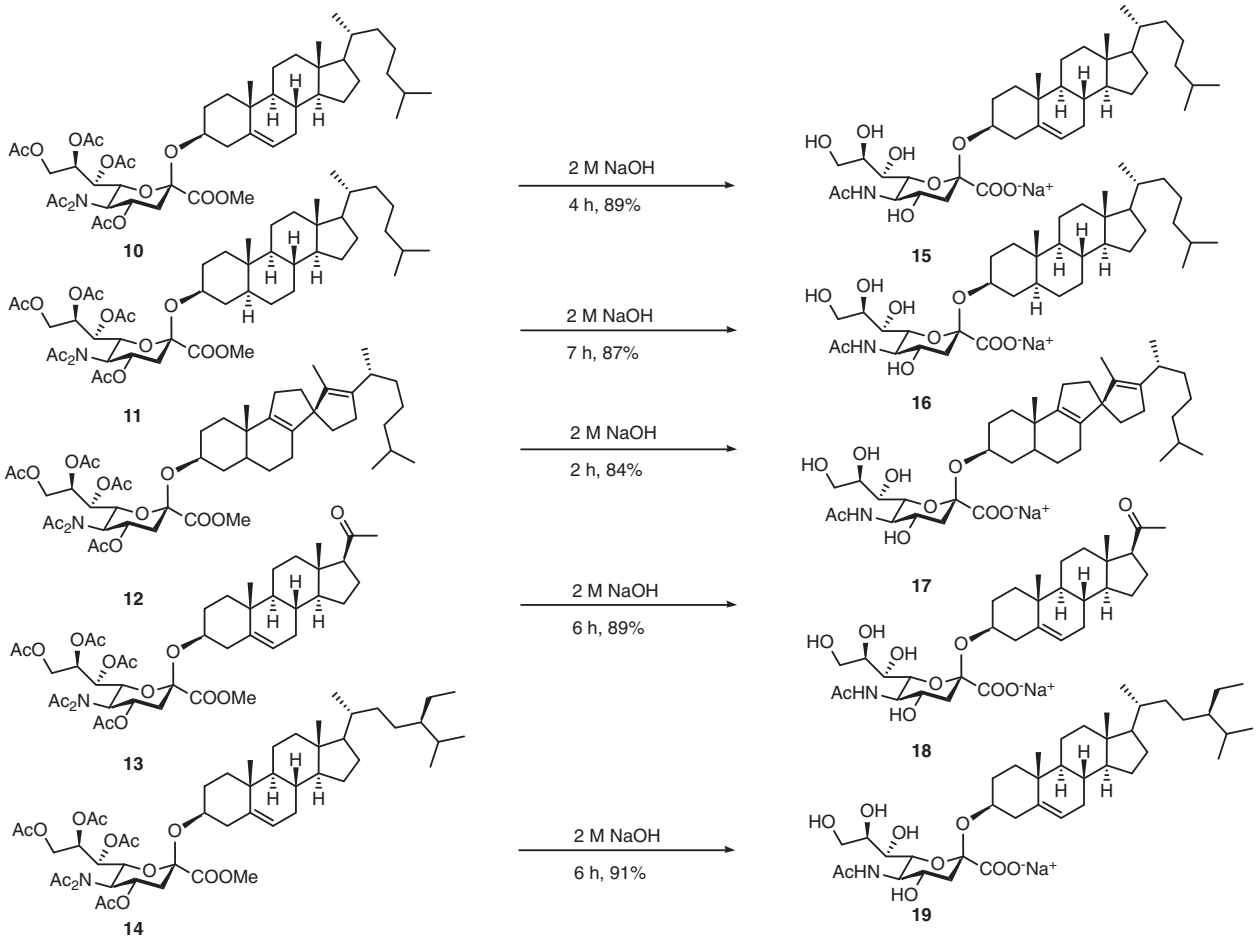


Fig. 3 Global deprotection readily affords β-linked steryl sialosides

observed directly via HMBC. Thin layer chromatography (TLC) was developed on glass-backed TLC plates (silica gel 60 with a 254 nm fluorescent indicator, 250 mm layer thickness) that were stored over drierite in a desiccator. TLC plates were visualized by coating with ammonium molybdate/cerium (IV) sulfate stain heated mildly on a hot plate. Flash column chromatography was performed on silica gel (32–63μ) with reported solvent systems in v/v ratios. Microwave reactions were conducted in a CEM microwave reactor in closed microwave vessels with infrared monitored setting. High resolution mass spectrometry (HRMS) was used to analyze samples by electrospray ionization in positive mode.

General procedure for sialosyl iodide formation and glycosidations

In a 25-mL oven-dried round bottom flask containing **2** was dried via azeotrope using dry benzene thrice and placed under high vacuum overnight. Compound **2** (155 mg, 0.269 mmol, 3 equiv) was stirred and dissolved in dry dichloromethane (2.5 mL) under Argon. TMSI (46 μL, 0.323 mmol, 3.6 equiv) was introduced dropwise and let stir for 45 min. Upon the disappearance of **2** on TLC, the solvent was removed in vacuo then an azeotrope was formed thrice with dry benzene and removed in vacuo, which was then placed under high vacuum for an additional 2 h. Acceptors (1 equiv) were dried in the same manner as prepared by the donor, which was then dissolved in dry dichloromethane in a 25-mL pear-bottom flask. The donor dissolved in dichloromethane was stirred at 0 °C ice bath for 5 min. The acceptor dissolved in dichloromethane was cannulated into the donor dropwise and let stir until the disappearance of the acceptor by TLC. Upon completion, the reaction was diluted with 100% ethyl acetate (50 mL) then washed with saturated sodium thiosulfate (25 mL) in a separatory funnel. The aqueous layer was removed then the organic layer was washed with saturated sodium bicarbonate (10 mL) followed by brine solution (10 mL). The organic layer was dried over sodium sulfate then the solution was removed *in vacuo*, which was loaded directly on to the packed silica gel in

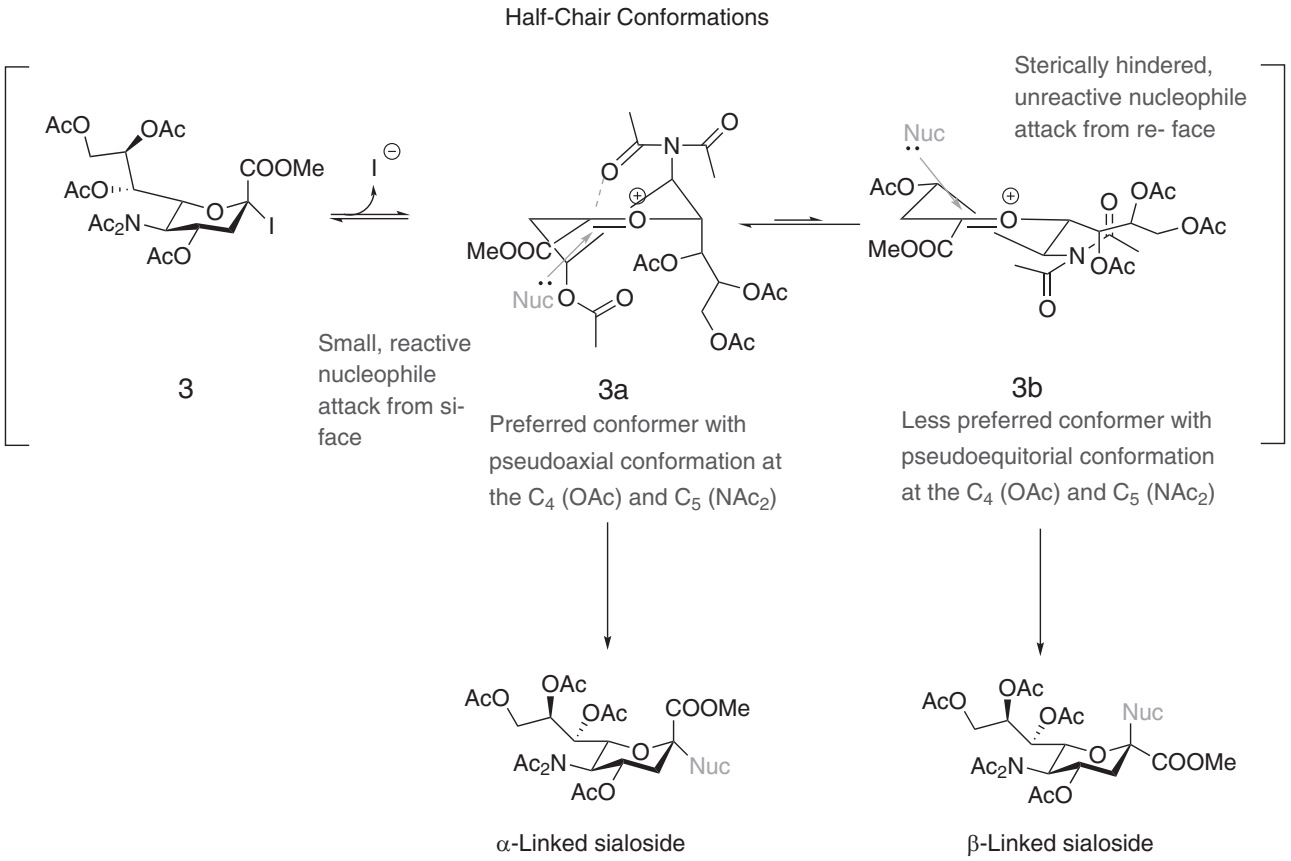


Fig. 4 Proposed mechanism of sialylation using sialyl iodide **3**

preparation for flash column chromatography at respective v/v ratio solvent system.

General procedure for deprotection of *O*-ester protecting groups

In a vigorously stirring 25 mL round-bottom flask, compounds **10–14** were each diluted in methanol and 2 M NaOH solution was added dropwise. The milky white solution became homogeneous after stirring at rt. The reaction was neutralized by Dowex H^+ 50WX8 then the filtrate was removed *in vacuo*. The crude mixture was triturated with cold diethyl ether then the solid was dissolved in minimal amount of DCM/MeOH, which was loaded on to manual flash column chromatography (C_{18}, 230–400 mesh, purchased from Fluka) using 85:15 acetonitrile/water with 0.1% TFA.

Methyl 2α-iodo-5-(*N*-acetyl-acetamido)−4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulpyranosonate (3)

^{1}H NMR (600 MHz, CD_2Cl_2): δ 5.87 (td, $J = 10.4$, 5.0 Hz, 1 H, H_4), 5.28 (dd, $J = 7.7$, 1.8 Hz, H_7), 5.14–5.11 (m, 1 H, H_8), 5.05 (dd, $J = 10.0$, 1.8 Hz, H_6), 4.36–4.29 (m, 2 H, $H_{5,9'}$), 4.08 (dd, $J = 12.7$, 5.2, 1 H, H_9), 3.88 (s, 3 H, COOMe), 3.09 (dd, $J = 14.3$, 5.0 Hz, H_{3eq}), 2.45 (s, 3 H, NAc_2), 2.32 (s, 3 H, NAc_2), 2.12 (s, 3 H), 2.11 (s, 3 H), 2.04 (s, 3 H), 2.01 (s, 3 H), 1.95 (app. t., $J = 14.3$ Hz, 1 H, H_{3ax}). ^{13}C NMR (150 MHz, CD_2Cl_2): δ 174.1, 172.9, 169.9, 169.4, 169.1, 168.9, 167.1, 78.6 ($^3J_{CH} = 6.5$ Hz), 76.0, 68.6, 67.6, 66.3, 61.0, 55.3, 53.2, 45.2, 27.5, 25.5, 20.7, 20.2, 20.06, 20.03.

Methyl [2-methyl 5-(*N*-acetyl-acetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulpyranosonate (4)

$R_f = 0.23$ (ethyl acetate:*n*-hexanes = 3/2); $[\alpha]_D^{24} + 4.26$ (*c* 1.34, CH_2Cl_2). ^{1}H NMR (800 MHz, C_6D_6): δ 6.23 (td, $J = 10.6$, 5.3 Hz, 1 H, $H_{4'}$), δ 5.67–5.65 (m, 2 H, $H_{8',7'}$), δ 5.45 (dd, $J = 10$, 1.4 Hz, 1 H, $H_{6'}$), 4.91 (dd, $J = 13.7$, 2.2 Hz, 1 H, $H_{9'}$), 4.63 (app. t., $J = 10.6$ Hz, 1 H, $H_{5'}$), 4.37 (dd, $J = 13.7$, 2.2 Hz, 1 H, $H_{9''}$), 3.24 (s, 3 H, COOMe), 3.23 (s, 3 H, OMe), 2.72 (dd, $J = 12.9$, 5.3 Hz, 1 H, H_{3eq}), 2.09 (s, 3 H, NAc_2), 1.96 (s, 3 H, NAc_2), 1.79 (m, H_{3ax}), 1.77 (s, 3 H, 8-OAc), 1.77 (s, 3 H, 7-OAc), 1.67 (s, 3 H, 9-OAc), 1.52 (s, 3 H, 4-OAc). ^{13}C NMR (200 MHz, C_6D_6):

δ 175.2, 174.6, 171.4, 171.1, 171.0, 170.1, 168.5, 100.5, 72.8, 70.3, 69.6, 68.1, 63.4, 58.5, 52.9, 52.3, 40.2, 28.9, 27.1, 21.65, 21.59, 21.4, 21.3. HRMS: (ESI-MS *m/z*) calc. mass for $C_{23}H_{33}NO_{14}$ $[M + NH_4^+]$ 565.2239, found 565.2233.

Methyl [2-octanyl 5-(*N*-acetyl-acetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulpyranosonate (5)

$R_f = 0.59$ (ethyl acetate:*n*-hexanes = 3/2); $[\alpha]_D^{25}$ + 11.29 (*c* 1.41, CH_2Cl_2). ^{1}H NMR (800 MHz, C_6D_6): δ 6.28 (td, J = 10.6, 5.2 Hz, 1 H, $H_{4'}$), δ 5.72–5.69 (m, 2 H, $H_{8', 7'}$), δ 5.56 (dd, J = 10.1, 1.7 Hz, 1 H, $H_{6'}$), 5.06 (dd, J = 12.3, 2.1 Hz, 1 H, $H_{9'}$), 4.66 (app. t., J = 10.1 Hz, 1 H, $H_{5'}$), 4.43 (dd, J = 12.3, 6.5 Hz, 1 H, $H_{9''}$), 3.86–3.84 (m, 2 H, CH_2), 3.59–3.56 (m, 2 H, CH_2), 3.31 (s, 3 H, COOMe), 2.80 (dd, J = 10.6, 5.2 Hz, 1 H, H_{3eq}), 2.10 (s, 3 H, NAc_2), 1.96 (s, 3 H, NAc_2), 1.82 (s, 8-OAc), 1.80 (m, 1 H, H_{3ax}), 1.79 (s, 3 H, 7-OAc), 1.68 (s, 3 H, 9-OAc), 1.51 (s, 3 H, 4-OAc), 1.30–1.26 (m, 10 H, CH_2), 0.92–0.90 (m, 4 H, CH_2), 0.29 (s, 3 H, CH_3). ^{13}C NMR (200 MHz, C_6D_6): δ 175.2, 174.6, 171.4, 171.3, 171.1, 170.2, 168.9, 100.1, 73.2, 70.4, 69.9, 68.1, 65.6, 63.6, 58.4, 52.9, 40.3, 33.2, 31.1, 30.9, 30.7, 29.0, 27.6, 27.2, 24.1, 21.7, 21.6, 21.4, 21.3, 15.4, 2.4. HRMS: (ESI-MS *m/z*) calc. mass for $C_{30}H_{47}NO_{14}$ $[M + NH_4^+]$ 663.3335, found 663.3330.

Methyl [5-(*N*-acetyl-acetamido)-2,4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulpyranosonate]-(α-2,6)-1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranoside (6)

$R_f = 0.45$ (ethyl acetate:*n*-hexanes = 3/2); $[\alpha]_D^{24}$ -31.99 (*c* 1.31, CH_2Cl_2). ^{1}H NMR (600 MHz, C_6D_6): δ 5.89 (td, J = 10.4, 5.38 Hz, 1 H, $H_{4'}$), 5.75 (m, 1 H, $H_{8'}$), 5.55 (dd, J = 7.76, 1.58 Hz, 1 H, $H_{7'}$), 5.42 (d, J = 5.03 Hz, 1 H, H_1), 5.38 (dd, J = 10.1, 1.72 Hz, 1 H, $H_{6'}$), 4.56–4.51 (m, 2 H, $H_{5',6}$), 4.46 (dd, J = 7.95, 2.34 Hz, 1 H, H_3), 4.40–4.35 (m, 2 H, $H_{6',9'}$), 4.16 (m, 1 H, H_4), 4.13 (q, J = 2.34, 1 H, H_3), 4.11 (dd, J = 7.83, 1.74, 1 H, H_2), 4.07–4.02 (m, 1 H, $H_{9'}$), 3.36 (s, 3 H, COOMe), 2.94 (dd, J = 13.2, 5.41 Hz, H_{3eq}), 2.14 (s, 3 H, NAc_2), 2.06 (app. t., J = 13.2 Hz, 1 H, H_{3ax}), 2.01 (s, 3 H, NAc_2), 1.99 (s, 3 H, 7-OAc), 1.83 (s, 3 H, 8-OAc), 1.73 (s, 3 H, 9-OAc), 1.54 (s, 3 H, 4-OAc), 1.42 (s, 3 H,), 1.11 (s, 3 H, 1,2-acetal), 1.02 (s, 3 H, 3,4-acetal). ^{13}C NMR (150 MHz, C_6D_6): δ 174.2, 173.2, 169.8, 169.7, 169.3, 169.0, 167.6, 108.9, 108.0, 98.9, 96.4, 71.1, 70.9, 70.8, 70.1, 69.5, 67.5, 67.1, 66.9, 63.5, 61.7, 57.1, 52.0, 38.7, 27.5, 25.9, 25.6, 24.5, 24.2, 20.6, 20.3, 20.1. 19.9. HRMS: (ESI-MS *m/z*) calc. mass for $C_{34}H_{49}NO_{19}$ $[M + NH_4^+]$ 793.3237, found 793.3228.

Methyl [2-(iodo-propyl) 5-(*N*-acetyl-acetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulpyranosonate (7)

$R_f = 0.27$ (ethyl acetate:*n*-hexanes = 3/2); $[\alpha]_D^{24}$ −2.62 (*c* 0.84, CH_2Cl_2). ^{1}H NMR (600 MHz, C_6D_6): δ 5.92 (td, J = 10.7, 5.1 Hz, 1 H, $H_{4'}$). 5.85 (m, 1 H, $H_{8'}$), 5.56 (d, J = 8.4 Hz, 1 H, $H_{7'}$), 5.43 (d, J = 10.4 Hz, 1 H, $H_{6'}$), 4.66–4.59 (m, 2 H, H_5, $H_{9'}$), 4.37 (dd, J = 12.6, 5.5 Hz, 1 H, $H_{9''}$), 3.97 (m, 1 H, OCH), 3.41 (m, 1 H, OCH), 3.33 (s, 3 H, COOMe), 2.99–2.87 (m, 3 H, H_{3eq}, CH_2), 2.07 (s, 3 H, 8-OAc), 2.03 (s, 3 H, NAc_2), 2.00 (m, 1 H, H_{3ax}), 1.90 (s, 3 H, NAc_2), 1.81 (s, 3 H, 7-OAc), 1.74 (s, 3 H, 9-OAc), 1.69 (m, 2 H, CH_2I), 1.53 (s, 3 H, 4-OAc). ^{13}C NMR (150 MHz, C_6D_6): δ 174.2, 173.6, 170.4, 170.2, 169.9, 169.5, 167.9, 99.2, 70.3, 69.3, 67.7, 67.1, 64.4, 62.4, 57.1, 52.5, 39.6, 33.7, 27.9, 26.2, 21.1, 20.7, 20.5, 20.3, 2.62. HRMS: (ESI-MS *m/z*) calc. mass for $C_{25}H_{36}INO_{14}$ $[M + NH_4^+]$ 719.1519, found 719.1530.

Methyl [2-(2-*O*-tertbutyldiphenyl silyl-1-iodo propyl) 5-(*N*-acetyl-acetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulpyranosonate (8)

$R_f = 0.29$ (ethyl acetate:*n*-hexanes = 1/1). ^{1}H NMR (600 MHz, C_6D_6): δ 7.89–7.76 (m, PhH), 7.43–7.19 (m, PhH), 6.18–6.12 (m, 1 H, $H_{4'}$), 5.84 (m, 1 H, $H_{8'}$), 5.72 (m, 1 H, $H_{7'}$), 5.47 (dt, J = 10.1, 1.9 Hz, 1 H, $H_{6'}$), 5.22 (td, J = 13.2, 2.5 Hz, 2 H, $H_{9',9''}$), 4.69 (app. t., J = 10.2 Hz, 1 H, $H_{5'}$), 3.86 (m, 1 H, OCH), 3.67 (m, 1 H, OCH), 3.32 (s, 3 H, COOMe), 3.12 (m, 1 H, OCH), 2.65 (dd, J = 13.0, 5.3 Hz, 1 H, H_{3eq}), 2.08 (s, 3 H, NAc_2), 1.95 (s, 3 H, NAc_2), 1.84 (s, 3 H, 7/8-OAc), 1.81 (s, 3 H, 7/8-OAc), 1.73 (s, 3 H, 9-OAc), 1.71 (s, 3 H, 4-OAc), 1.24 (s, 9 H, *t*Bu). ^{13}C NMR (150 MHz, C_6D_6): δ 174.1, 173.5, 170.5, 170.2, 170.0, 169.2, 167.2, 136.5–136.1, 130.2, 128.3, 99.2, 73.4, 73.0, 70.6, 70.4, 69.9, 69.7, 69.5, 69.1, 68.9, 68.6, 67.7, 67.1, 66.9, 62.9, 56.9, 56.8, 52.5, 52.1, 38.8, 27.9, 27.2, 26.1, 21.1–19.3, 12.9, 11.3. HRMS: (ESI-MS *m/z*) calc. mass for $C_{41}H_{58}IN_2O_{15}Si$ $[M + NH_4^+]$ 973.2646, found 973.2675.

Methyl [2-*iso*-propyl 5-(*N*-acetyl-acetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulpyranosonate (9)

$R_f = 0.31$ (ethyl acetate:*n*-hexanes = 3/2); $[\alpha]_D^{24}$ + 11.48 (*c* 0.27, CH_2Cl_2). ^{1}H NMR (800 MHz, C_6D_6): δ 6.28 (td, J = 10.7, 5.2 Hz, 1 H, $H_{4'}$), δ 5.73–5.71 (m, 2 H, $H_{8', 7'}$), δ 5.66 (dd, J = 10.1, 1.5 Hz, 1 H, $H_{6'}$), 5.17 (dd, J = 12.6, 2.1 Hz, 1 H, $H_{9'}$), 4.65 (app. t., J = 10.2 Hz, 1 H, $H_{5'}$), 4.48–4.45 (m, 1 H, $H_{9''}$), 4.35 (septet, J = 6.48 Hz, 1 H, CH), 3.29 (s, 3 H, COOMe), 2.88 (dd, J = 12.9, 5.2 Hz, 1 H, H_{3eq}), 2.10 (s, 3 H, NAc_2), 1.96 (s, 3 H, NAc_2), 1.81 (s, 8-OAc), 1.80 (s,

3 H, 7-OAc), 1.71 (m, 1 H, H_{3ax}), 1.68 (s, 3 H, 9-OAc), 1.52 (s, 3 H, 4-OAc), 1.39 (d, $J = 6.48$ Hz, 3 H, *i*Pr-CH_3), 1.01 (d, $J = 6.48$ Hz, 3 H, *i*Pr-CH_3). ^{13}C NMR (200 MHz, C_6D_6): δ 175.2, 174.6, 171.4, 171.3, 171.1, 170.2, 169.4, 99.0, 73.6, 70.5, 70.2, 68.2, 68.0, 63.7, 58.3, 52.9, 40.7, 29.0, 27.2, 25.1, 23.5, 21.7, 21.6, 21.4, 21.3. HRMS: (ESI-MS *m/z*) calc. mass for $C_{25}H_{37}NO_{14}$ $[M + H^+]$ 593.2552, found 593.2538.

Methyl [2-(3β-hydroxy-5α-cholestanyl)-5-(*N*-acetyl-acetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulpyranosonate (10)

$R_f = 0.51$ (ethyl acetate:*n*-hexanes = 3/2); $[\alpha]_D^{24}$ -21.55 (*c* 2.83, CH_2Cl_2). ^{1}H NMR (800 MHz, C_6D_6): δ 6.37 (td, $J =$ 10.3, 5.4 Hz, 1 H, $H_{4'}$), δ 5.79 (dd, $J = 10.2$, 1.7 Hz, 1 H, $H_{6'}$), δ 5.76 (app. t., $J = 2.7$ Hz, 1 H, $H_{7'}$), 5.76 (dt., $J = 7.8$, 5.0, 2.7 Hz, 1 H, $H_{8'}$), 5.35 (m, 1 H, C = CH), 5.30 (dd, $J =$ 12.4, 1.8 Hz, 1 H, $H_{9'}$), 4.67 (app. t., $J = 10.2$ Hz, 1 H, $H_{5'}$), 4.54 (dd, $J = 12.4$ Hz, 1 H, $H_{9''}$), 4.06–4.01 (m, 1 H, CH), 3.43 (s, 3 H, COOMe), 2.99 (dd, $J = 13.05$, 5.4 Hz, 1 H, H_{3eq}), 2.51 (t, $J = 12.21$ Hz, 1 H, H_4), 2.34–2.27 (m, 1 H), 2.15 (s, 3 H, NAc_2), 1.97 (s, 3 H, NAc_2), 1.85 (m, 1 H, H_{3ax}), 1.79 (s, 6 H, 7,8-OAc), 1.75 (s, 3 H, 9-OAc), 1.53 (s, 3 H, 4-OAc), 1.50–1.01 (m, 36 H), 1.01 (d, $J = 6.63$ Hz, 3 H, H_{21}), 0.93 (dd, $J = 6.63$, 1.68 Hz, 6 H, $H_{26,27}$), 0.92 (s, 3 H, H_{19}), 0.65 (s, 3 H, H_{18}). ^{13}C NMR (200 MHz, C_6D_6): δ 174.3, 173.5, 170.5, 170.0, 169.2, 168.5, 141.2, 122.0, 98.3, 74.8, 73.8, 69.9, 69.6, 67.2, 63.5, 57.5, 57.1, 56.5, 52.1, 50.6, 42.6, 40.17, 40.13, 39.9, 39.8, 37.8, 36.9, 36.7, 36.2, 32.4, 32.2, 30.6, 30.2, 28.6, 28.5, 28.1, 26.1, 24.6, 24.4, 23.1, 22.8, 21.3, 20.7, 20.54, 20.51, 20.3, 19.4, 19.0, 12.1. HRMS: (ESI-MS *m/z*) calc. mass for $C_{49}H_{75}NO_{14}$ $[M + NH_4^+]$ 919.5526, found 919.5586.

Methyl [2-(3β-hydroxy-20-α, 22-(*R*)-dihydroxycholestanyl)-5-(*N*-acetyl-acetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulpyranosonate (11)

$R_f = 0.49$ (ethyl acetate/*n*-hexanes = 3/2); $[\alpha]_D^{25}$ −4.64 (*c* 1.40, CH_2Cl_2). ^{1}H NMR (800 MHz, C_6D_6): δ 6.36 (td, $J = 5.02$, 10.6 Hz, 1 H, H_4), 5.79–5.76 (m, 2 H, H_7, H_6), 5.71 (dt, $J = 7.9$, 2.5 Hz, 1 H, H_8), 5.35 (dd, $J = 12.3$, .23 Hz, 1 H, $H_{9'}$), 4.68 (app. t., $J = 10.0$ Hz, 1 H, H_5), 4.55 (dd, $J = 12.3$, 7.8 Hz, 1 H, H_9), 4.16 (m, 1 H, CH), 3.39 (s, 3 H, COOMe), 2.97 (dd, $J = 12.8$, 5.0 Hz, 1 H, H_{3eq}), 2.24 (m, 1 H, H_4), 2.13 (s, 3 H, NAc_2), 2.11 (m, 1 H), 2.06 (s, 3 H, NAc_2), 1.96–1.83 (m, 4 H), 1.82 (s, 3 H, 7-OAc), 1.81 (s, 3 H, 8-OAc), 1.80 (m, 1 H, H_{3ax}), 1.75 (s, 3 H, 9-OAc), 1.69–1.54 (m, 3 H), 1.54 (s, 3 H, 4-OAc), 1.3–1.0 (m, 15 H), 1.01 (d, $J = 6.9$ Hz, 3 H, H_{21}), 0.93 (dd, $J = 6.6$, 1.8 Hz, 6 H, $H_{26,27}$), 0.68 (s, 3 H, H_{19}), 0.65 (s, 3 H, H_{18}). ^{13}C NMR (200 MHz, C_6D_6): δ 174.3, 173.6, 170.5, 170.4, 170.0, 169.2, 168.6, 98.0, 74.0, 73.3, 69.7, 69.5, 67.3, 63.1, 57.4, 56.73, 56.71, 54.6, 51.9, 45.1, 42.9, 40.4, 39.9, 39.8, 37.4, 36.7, 36.3, 35.8, 35.7, 35.6, 32.4, 30.4, 29.3, 28.6, 28.4, 28.0, 26.1, 24.5, 24.3, 23.1, 22.8, 21.5, 20.7, 20.6, 20.3, 19.0, 12.35, 12.31. HRMS: (ESI-MS *m/z*) calc. mass for $C_{49}H_{77}NO_{14}$ $[M + H^+]$ 921.5682, found 921.5689.

Methyl [2-(3β-hydroxy-spirocyclicsteryl)–5-(*N*-acetyl-acetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulpyranosonate (12)

$R_f = 0.25$ (ethyl acetate:*n*-hexanes = 3/2); $[\alpha]_D^{24} + 31.14$ (*c* 1.05, CH_2Cl_2). ^{1}H NMR (800 MHz, C_6D_6): δ 6.36 (td, $J =$ 10.7, 5.2 Hz, 1 H, $H_{4'}$), 5.81–5.78 (m, 2 H, H_6, H_7), 5.66 (dt, $J = 8.07$, 3.7, 2.2 Hz, 1 H, H_8), 5.39 (dd, 12.3, 1.9 Hz, 1 H, $H_{9'}$), 4.69 (app. t., $J = 10.25$ Hz, $H_{5'}$), 4.55 (dd, $J = 12.3$ Hz, 1 H, $H_{9''}$), 4.18 (m, 1 H, CH), 3.39 (s, 3 H, COOMe), 2.98 (dd, $J = 13.2$, 5.2 Hz, 1 H, H_{3eq}), 2.53 (m, 1 H, H_4), 2.3–2.1 (m, 5 H), 2.14 (s, 3 H, NAc_2), 1.96 (s, 3 H, NAc_2), 1.84 (app. t., $J = 13.1$ Hz, 1 H, H_{3ax}), 1.80 (s, 6 H, 7-OAc, 8-OAc), 1.70 (s, 3 H, 9-OAc), 1.69–1.54 (m, 6 H), 1.53 (s, 3 H, 4-OAc), 1.46 (t, $J = 1.86$ Hz, 3 H, H_{18}), 1.44–1.06 (m, 10 H), 1.04 (d, $J = 6.94$ Hz, 3 H, H_{21}), 0.91 (dd, $J = 6.62$, 2.02 Hz, 6 H, $H_{26,27}$), 0.83 (s, 3 H, H_{19}). ^{13}C NMR (200 MHz, C_6D_6): δ 174.3, 173.6, 170.5, 170.4, 170.0, 169.3, 168.6, 144.2, 139.6, 137.3, 136.4, 98.1, 74.2, 73.7, 69.8, 69.5, 67.3, 66.9 (spirocyclic), 63.3, 57.4, 52.0, 42.1, 39.7, 39.5, 36.2, 36.1, 35.3, 35.2, 34.99, 34.97, 32.9, 30.2, 29.34, 29.27, 28.5, 28.1, 26.3, 26.18, 26.15, 22.89, 22.85, 22.7, 20.7, 20.5, 20.4, 20.3, 20.1, 17.7, 10.2. HRMS: (ESI-MS *m/z*) calc. mass for $C_{49}H_{73}NO_{14}$ $[M + H^+]$ 917.5369, found 917.5400.

Methyl [2-(3β-hydroxypregn-5-en-20-onyl)-5-(*N*-acetyl-acetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulpyranosonate (13)

$R_f = 0.32$ (ethyl acetate/*n*-hexanes = 3/2); $[\alpha]_D^{23} + 27.17$ (*c* 1.20, CH_2Cl_2). ^{1}H NMR (800 MHz, C_6D_6): δ 6.37 (td, $J =$ 10.7, 5.1 Hz, 1 H, $H_{4'}$), 5.80 (dd, $J = 10.1$, 2.1 Hz, 1 H, $H_{6'}$), 5.76 (dd, $J = 3.1$ Hz, 1 H, $H_{7'}$), 5.64 (dt, $J = 7.9$, 3.1, 2.1 Hz, 1 H, $H_{8'}$), 5.31–5.29 (m, 2 H, C = CH, $H_{9'}$), 4.68 (app. t., $J = 10.7$ Hz, 1 H, H_5), 4.54 (dd, $J = 12.3$ Hz, 1 H, $H_{9'}$), 4.04 (m, 1 H, CH), 3.42 (s, 3 H, COOMe), 2.99 (dd, 13.0, 5.1 Hz, 1 H, H_{3eq}), 2.49 (t, $J = 13.7$ Hz, 1 H, H_3), 2.39–2.27 (m, 3 H), 2.13 (s, 3 H, NAc_2), 2.06 (m, 1 H), 1.97 (s, 3 H, NAc_2), 1.85 (dd, $J = 13.0$, 10.7 Hz, 1 H, H_{3ax}), 1.80 (s, 6 H, 7-OAc, 8-OAc), 1.79 (s, 3 H, 9-OAc), 1.76 (s, 3 H, 4-OAc),

1.53 (s, 3 H, (C = O)CH_3), 0.86 (s, 3 H, H_{19}), 0.56 (s, 3 H, H_{18}). ^{13}C NMR (200 MHz, C_6D_6): δ 208.1, 175.3, 174.6, 171.5, 171.0, 170.3, 169.6, 142.1, 122.7, 99.3, 75.6, 74.7, 70.9, 70.5, 68.2, 64.6, 64.5, 58.5, 57.9, 53.1, 51.3, 44.8, 41.1, 40.7, 39.9, 38.8, 37.8, 33.2, 32.9, 32.2, 31.5, 31.2, 29.0, 27.1, 25.6, 24.1, 22.3, 21.7, 21.5, 21.3, 20.4, 14.3. HRMS: (ESI-MS *m/z*) calc. mass for $C_{43}H_{61}NO_{15}$ [M + NH_4^+] 849.4376, found 849.4379.

Methyl [2-(3β-sitosteranyl)-5-(*N*-acetyl-acetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulpyranosonate (14)

R_f = 0.41 (ethyl acetate/*n*-hexanes = 3/2); $[\alpha]_D^{25}$ -42.38 (*c* 2.1, CH_2Cl_2). ^{1}H NMR (800 MHz, C_6D_6): δ 6.37 (td, *J* = 10.8, 5.16 Hz, 1 H, $H_{4'}$), 5.79 (dd, *J* = 10.1, 2.2 Hz, 1 H, H_6), 5.76 (app. t., *J* = 2.74, 1 H, H_7), 5.62 (dt, *J* = 7.91, 2.2 Hz, 1 H, H_8), 5.36–5.33 (m, 1 H, C = CH), 5.30 (dd, *J* = 12.4, 2.10 Hz, 1 H, H_9), 4.67 (app. t., *J* = 10.17 Hz, 1 H, H_5), 4.54 (dd, *J* = 12.4 Hz, 1 H, $H_{9'}$), 4.07–4.01 (m, 1 H, CH), 3.43 (s, 3 H, COOMe), 2.99 (dd, *J* = 13.1, 5.17 Hz, 1 H, H_{3eq}), 2.54–2.49 (t, *J* = 12.3 Hz, 1 H, H_3), 2.34–2.69 (m, 2 H), 2.14 (s, 3 H, NAc_2), 1.97 (s, 3 H, NAc_2), 1.96–1.78 (m, 6 H), 1.84 (app.t., *J* = 10.8 Hz, 1 H, H_{3ax}), 1.79 (s, 3 H, 7-OAc), 1.78 (s, 3 H, 8-OAc), 1.74 (s, 3 H, 9-OAc), 1.53 (s, 3 H, 4-OAc), 1.5–1.05 (m, 18 H), 1.04 (d, *J* = 7.2 Hz, 6 H, H_{21}), 0.94–0.86 (m, 14 H, $H_{18,19}$), 0.65 (s, 3 H). ^{13}C NMR (200 MHz, C_6D_6): δ 174.9, 173.5, 170.5, 169.9, 169.2, 168.5, 141.2, 128.3, 128.2, 121.9, 98.3, 74.7, 73.8, 69.8, 69.6, 67.2, 63.5, 57.5, 57.1, 56.5, 52.1, 50.1, 46.3, 42.6, 40.16, 40.10, 39.7, 37.8, 36.9, 36.6, 34.5, 32.4, 32.2, 30.6, 29.6, 28.7, 28.0, 26.6, 26.1, 24.6, 23.6, 21.3, 20.7, 20.54, 20.51, 20.3, 20.1, 19.4, 19.3, 19.1, 12.3, 12.1. HRMS: (ESI-MS *m/z*) calc. mass for $C_{51}H_{79}N\ O_{14}$ [M + NH_4^+] 947.5839, found 947.5843.

Sodium 2-(3β-hydroxy-5α-cholestanyl)-5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulpyranosonoate (15)

Data matches previous report [38].

Sodium 2-(3β-hydroxy-20-α, 22-(*R*)-dihydroxycholestanyl)-5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulpyranosonoate (16)

R_f = 0.29 (ethyl acetate/methanol/water = 10/2/1); $[\alpha]_D^{23}$ -7.826 (*c* 0.92, CH_3OH). ^{1}H NMR (600 MHz, CD_3OD): δ 4.04 (m, 1 H, $H_{4'}$), 3.93 (td, *J* = 10.2, 5.1 Hz, 1 H, $H_{8'}$), 3.96–3.87 (m, 4 H, $H_{5',CH,6',7'}$), 3.73 (m, 1 H, $H_{9'}$), 3.531 (m, 1 H, $H_{9''}$), 2.46 (dd, *J* = 13.2, 4.8 Hz, 1 H, H_{3eq}), 2.04 (s, 3 H, NHAc), 2.01–0.96 (m, 45 H), 0.93 (d, *J* = 6.8 Hz, 3 H, H_{21}), 0.88 (dd, *J* = 6.5 Hz, 6 H, $H_{26,27}$), 0.84 (s, 3 H, H_{19}), 0.69 (s, 3 H, H_{18}). ^{13}C NMR (150 MHz, CD_3OD): δ 175.4, 173.4, 100.9, 74.3, 73.3, 71.2, 67.7, 66.2, 56.5, 56.3, 54.3, 53.0, 45.3, 42.23, 40.9, 40.3, 39.2, 37.4, 36.2, 35.8, 35.6, 35.4, 32.3, 29.9, 28.7, 28.2, 28.1, 24.4, 21.53, 21.51, 20.9, 18.1, 17.9, 11.6, 11.05. HRMS: (ESI-MS *m/z*) calc. mass for $C_{38}H_{65}NO_9$ [M + H^+] 680.4732, found 680.4744.

Sodium 2-(3β-hydroxy-spirocyclicsteryl)-5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulpyranosonoate (17)

R_f = 0.21 (ethyl acetate/methanol/water = 10/2/1); $[\alpha]_D^{23}$ -6.517 (*c* 0.72, CH_3OH). ^{1}H NMR (600 MHz, CD_3OD): δ 3.98 (m, 1 H, $H_{4'}$), 3.93–3.86 (m, 2 H, $H_{5',7'}$), 3.84–3.73 (m, 3 H, $H_{8',6',9'}$), 3.81 (m, 1 H, CH), 3.72–3.65 (m, 1 H, $H_{9''}$), 2.57–2.45 (m, 2 H, H_{3eq}), 2.29–2.05 (m, 3 H), 2.01 (s, 3 H, NHAc), 1.95–1.44 (m, 8 H), 1.39 (s, 3 H, H_{18}), 1.36–0.97 (m, 7 H), 0.97 (m, 3 H, H_{21}), 0.85 (dd, *J* = 6.7, 2.4 Hz, H_{19}). ^{13}C NMR (150 MHz, CD_3OD): δ 174.8, 172.6, 150.7, 143.5, 138.6, 135.3, 99.2, 72.6, 70.6, 70.2, 68.2, 66.5, 63.1, 52.1, 41.3, 40.9, 38.4, 34.9, 34.4, 33.7, 33.2, 31.5, 28.5, 28.49, 27.5, 27.1, 24.8, 21.8, 20.9, 20.7, 18.3, 16.2, 8.27. HRMS: (ESI-MS *m/z*) calc. mass for $C_{38}H_{61}NO_9$ [M + H^+] 676.4419, found 676.4430.

Sodium 2-(3β-hydroxypregn-5-en-20-onyl)-5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulpyranosonoate (18)

R_f = 0.15 (ethyl acetate/methanol/water = 10/2/1); $[\alpha]_D^{24}$ -10.294 (*c* 0.61, CH_3OH). ^{1}H NMR (600 MHz, CD_3OD): δ 5.32 (d, *J* = 2.8 Hz, 1 H, C = CH), 3.95 (m, 1 H, H_4), 3.89 (m, 1 H, H_5), 3.81–3.72 (m, 4 H, $H_{7,6,8,9}$), 3.71–3.64 (m, 1 H, $H_{9'}$), 3.63 (m, 1 H, CH), 2.65 (t, *J* = 9 Hz, 1 H), 2.56 (m, 1 H), 2.51 (dd, *J* = 10.5, 3.7 Hz, 1 H, H_{3eq}), 2.26 (m, 1 H), 2.14 (s, 3 H, (C = O)CH_3), 2.03 (s, 3 H, NHAc), 2.04–1.08 (m, 20 H, steryl), 1.04 (s, 3 H, H_{19}), 0.65 (s, 3 H, H_{18}). ^{13}C NMR (150 MHz, CD_3OD): δ 212.3, 176.6, 174.4, 142.6, 121.8, 98.9, 75.6, 72.3, 70.5, 68.5, 64.7, 58.1, 53.9, 51.5, 45.2, 42.9, 40.4, 39.9, 38.7, 37.6, 36.3, 33.2, 32.9, 31.6, 31.1, 30.7, 25.5, 23.7, 22.8, 22.2, 19.8, 13.6. HRMS: (ESI-MS *m/z*) calc. mass for $C_{32}H_{49}NO_{10}$ [M + H^+] 608.3429, found 608.3439.

Sodium 2-(3β-sitosteranyl)-5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulpyrano-sonoate (19)

R_f = 0.26 (ethyl acetate/methanol/water = 10/2/1); $[\alpha]_D^{23}$ -46.117 (*c* 0.41, CH_3OH). ^{1}H NMR (800 MHz, CD_3OD): δ 5.28 (d, *J* = 4.9 Hz, 1 H, C = CH), 4.01 (td, *J* = 10.2, 4.47 Hz, 1 H, H_4), 3.89 (m, 1 H, H_6), 3.83 (t, *J* = 10.2 Hz, 1 H, H_5), 3.78 (m, 1 H, H_7), 3.75–3.71 (m, 2 H, $H_{8,9}$), 3.62 (m, 1 H, CH), 3.54 (app. t., *J* = 7.02 Hz, 1 H, $H_{9'}$), 2.58 (d, *J* = 12.6 Hz, 1 H), 2.49 (dd, *J* = 12.9, 3.98 Hz, 1 H, H_{3eq}),

2.26 (t, $J = 11.6$ Hz, 1 H), 2.02 (s, 3 H, NHAc), 1.98–1.04 (m, 28 H), 1.30 (s, 3 H), 1.03 (s, 3 H), 0.96 (m, 6 H), 0.91–0.78 (m, 10 H), 0.73 (s, 3 H). ^{13}C NMR (200 MHz, CD_3OD): δ 176.9, 174.6, 142.7, 122.2, 101.2, 75.2, 72.2, 70.1, 68.2, 65.1, 58.3, 57.5, 54.2, 51.6, 47.3, 43.5, 41.2, 40.5, 39.3, 37.1, 33.3, 31.1, 30.8, 30.4, 29.4, 27.2, 25.3, 24.2, 23.1, 22.2, 20.2, 19.9, 19.4, 12.5. HRMS: (ESI-MS *m/z*) calc. mass for $C_{40}H_{69}NO_9$ $[M + H^+]$ 706.4889, found 706.4883.

Acknowledgements This work was supported by the National Institutes of Health, under NIH Grant No. R01GM090262. NSF CRIF program (CHE - 9808183), NSF Grant No. OSTI 97–24412, and NIH Grant No. RR11973 provided funding for the NMR spectrometers used on this project, and NSF Grant No. 0840444 for the Dual source X-ray diffractometer. We would like to thank Dr. Bennett Addison for assistance with 2D NMR experiments. We also thank Dr. James Fettinger (University of California, Davis) for his help with X-ray crystallography. JG-H gratefully acknowledges support from the National Science Foundation for independent research development during her tenure as the Director of the Chemistry Division.

Compliance with ethical standards

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

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