An unexpected elimination product leads to 4-alkyl-4-deoxy-4-*epi*-sialic acid derivatives

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Abstract: A useful, unexpected β,γ-unsaturated-α-keto ester (ethyl (*E*)-5-acetamido-3,4,5-trideoxy-6,7:8,9-di-*O*-isopropylidene-D-*manno*-non-3-en-2-ulosonate **5**) was isolated in 91% yield following ozonolysis and chromatographic purification of its enoate ester precursor ethyl 5-acetamido-2,3,4,5-tetradeoxy-6,7:8,9-di-*O*-isopropylidene-2-methylene-4-nitro-D-*glycero*-D-*galacto*-nononate (**6**). When the 4*R* enoate ester (ethyl 5-acetamido-2,3,4,5-tetradeoxy-6,7:8,9-di-*O*-isopropylidene-2-methylene-4-nitro-D-*glycero*-D-*talo*-nononate, **7**) was subjected to the same conditions, enone **5** was a minor product (18%) while the major product did not eliminate HNO_2 but instead cyclized to form a five-membered ring containing a hemiaminal linkage between C-2 and the amide nitrogen on C-5 (**9**, 70%). Conjugate addition to enone **5** opens up the potential to generate 4-substituted sialic acid derivatives, a general route to such compounds that has not been previously reported. In a preliminary investigation of such a route, diethylzinc and dimethylzinc were added to enone **5** resulting in generation of 4-alkyl-substituted cyclic hemiaminal structures **11** and **13**, which could be deprotected to form 2,7-anhydrosialic acid analogues **14** and **15**. These products could then be converted to peracetylated glycals **16** and **17**, the 4-methyl-substituted compound **17** being finally deprotected to give a 4-methyl-substituted analogue of the glycal of sialic acid (5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-methyl-D-*glycero*-D-*talo*-non-2-enonic acid **18**).

Key words: conjugate addition, dialkylzinc reagent, sialic acid, ozonolysis, inhibitors.

Résumé : À la suite de l'ozonolyse et la purification de son précurseur, l'ester énoate 5-acétamido-2,3,4,5-tétradésoxy-6,7:89-di-*O*-isopropylidène-2-méthylène-4-nitro-D-*glycéro*-D-*galacto*nononate d'éthyle (**6**), on a isolé avec un rendement de 91% le (*E*)-5-acétamino-3,4,5-tridésoxy-6,7:8,9-di-*O*-isopropylidène-D-*manno*-non-3-én-2-ulosonate d'éthyle (**5**), un α-cétoester β,γ-insaturé utile et inattendu. Lorsqu'on soumet l'ester énoate 4*R* [5-acétamino-2,3,4,5-tétradésoxy-6,7:8,9-di-*O*-isopropylidène-2-méthylène-4-nitro-D-*glycéro*-D-*tallo*-nononate d'éthyle (**7**)], aux même conditions, l'énone **5** n'est qu'un produit mineur (18%) alors que le produit majeur n'élimine pas de HNO_2, mais conduit plutôt à un produit de cyclisation comportant un cycle à cinq chaînons et une liaison hémiaminale entre le C-2 et l'azote de l'amide en C-5 (**9**, 70%). Une addition conjuguée sur l'énone **5** donne la possibilité de généré des dérivés de l'acide sialique substitués en position 4 pour lesquels aucune voie de synthèse générale n'avait été proposée antérieurement. Dans une étude préliminaire de cette route, l'addition de diéthylzinc et de diméthylzinc à l'énone **5** conduit à la formation des structures hémiaminales cycliques **11** et **13** portant des substituants alkyles en position 4 qui peuvent être déprotégées pour conduire aux analogues **14** et **15** de l'acide 2,7-anhydrosialique. Ces produits peuvent ensuite être convertis en glycals peracétylés **16** et **17**, le composé 17 portant un groupe méthyle en position 4 étant finalement déprotégé pour fournir un analogue substitué en position 4 de l'acide sialique, l'acide 5-acétamido-2,6-anhydro-3,4,5-didésoxy-4-méthyl-D-*glycéro*-D-*talo*-non-2-énonique, **18**.

Mots-clés : addition conjuguée, réactif dialkylzinc, acide sialique, ozonolyse, inhibiteurs.

[Traduit par la Rédaction]

Introduction

Sialic acid (*N*-acetylneuraminic acid, **1**) is a nine-carbon sugar that is often found α-ketosidically linked to the termini of oligosaccharides on glycoconjugates (1). These linkages can be hydrolyzed by sialidases (neuraminidases, EC 3.2.1.18). Because of their terminal locations on glycoconjugates, sialic acid and the proteins that recognize it are important in many biological events, including cell recognition and pathogen infectivity (1, 2). For instance, influenza virus sialidase activity is critical for viral proliferation (3). The virus first recognizes and binds to sialic acid residues on the surfaces of host-cell glycoconjugates in the upper respiratory tract, an event that initiates the infection cycle. After replication, the newly formed virus particles emerge from the infected cell and become coated in cellular sialic acids that cause the virus particles to clump together. The influenza sialidase then cleaves bound sialic acid residues and thus allows the virus particles to move freely to infect further cells. As a consequence, inhibitors of this enzyme, which include the glycal of sialic acid, 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid (DANA, **2**) and its 4-guanidinyl derivative

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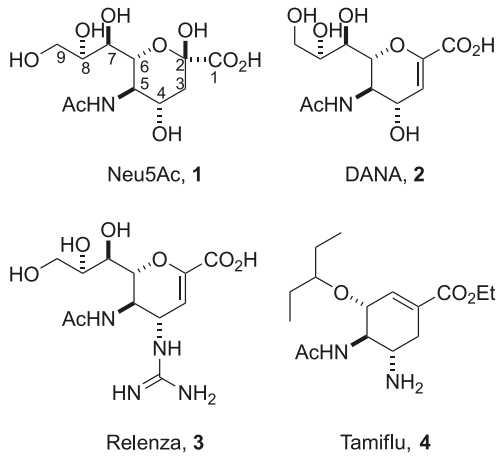
Fig. 1. Sialic acid (Neu5Ac, **1**) and structurally similar sialidase inhibitors (**2**–**4**).



that is marketed as Relenza™ (**3**), are useful biological tools (Fig. 1) (3).

It is important to be able to synthesize analogues of sialic acid to generate selective inhibitors of sialic acid-metabolizing enzymes, which can also be used as probes of the biological pathways. It is therefore advantageous to have access to general synthetic routes that can lead to a number of derivatives for the generation of compound libraries. While there are several positions that can be varied on sialic acid and many derivatives have been made (3, 4), carbon 4 remains a target for derivatization. This is partly due to the fact that variation at this position is tolerated by influenza sialidase as shown by the successful development of 4-substituted sialic acid analogues as influenza sialidase inhibitors, such as Relenza™ (**3**) and Tamiflu™ (**4**) bearing 4-guanidinyl and 4-amino substituents, respectively (Fig. 1) (5, 6).

Design of a general route to 4-modified sialic acid analogues is synthetically challenging. Sialic acid analogues can be made chemoenzymatically using sialic acid aldolase (*N*-acylneuraminate pyruvate lyase, EC 4.1.3.3), which catalyzes the nucleophilic attack of pyruvate on the aldehyde carbon of *N*-acetylmannosamine to form sialic acid. This enzyme is fairly promiscuous with respect to its specificity toward various aldose units, and it can thus be used to make many sialic acid analogues; however, a hydroxyl group is mandatorily installed at the 4-position in the resulting addition product (7). As well, many chemical synthetic routes involve nucleophilic attack of a three-carbon moiety on an aldose carbonyl group, also installing a 4-hydroxyl group (8–10).

While several 4-substituted sialic acid analogues have been synthesized (4), many of these substituents had to be incorporated early in the synthetic route giving intermediate compounds with limited synthetic versatility. Several 4-substituted sialic acid analogues had been synthesized from a common 4-oxo intermediate by Zbiral and co-workers in which the 4-hydroxyl group had been oxidized to a ketone. This could be reduced to give 4-*epi*-sialic acid (11), undergo addition of a nucleophilic methyl group to generate both diastereomers of the 4-methyl addition product (12) and be transformed into the 4-*C*-methylene derivative (12). The 4-*C*-methylene derivative could be reduced to give two 4-epimers of 4-deoxy-4-methylsialic acid (12) and was converted into an epoxide by von Itzstein and co-workers that could be opened with various nucleophiles to generate 4-disubstituted sialic acids (13). As well, several modifications at the 4-position of DANA (**2**) have been made from a common oxazoline intermediate resulting from the $BF_3{\cdot}OEt_2$-promoted displacement of a 4-acetyl group by the carbonyl oxygen of the neighbouring 5-acetamido group. This oxazoline intermediate has been hydrogenated to give 4-deoxy-DANA, hydrolyzed to give 4-*epi*-DANA, and opened with azide to give 4-azido-4-deoxy-DANA that was reduced to 4-amino-4-deoxy-DANA and then transformed into 4-formamido- and 4-acetamido-substituted 4-deoxy-DANA analogues (14). The oxazoline intermediate has also been opened with halogens to generate allylic halides (15), which have been used in palladium(0)-mediated couplings with organotin reagents to install unsaturated functional groups at the 4-position (16). Although these intermediates are useful for synthesizing 4-modified sialic acid analogues, the number of such analogues that can be generated is limited.

Recently, the synthesis of 4-deoxy-4-nitrosialic acid was reported (17), and during the course of that study, an unexpected β,γ-unsaturated-α-keto ester (**5**) was isolated following the ozonolysis of enoate ester **6**. The results described herein report the investigation into this elimination reaction and the use of this enone in conjugate addition reactions for the installation of different substituents at the 4-position in sialic acid analogues.

Results and discussion

Isolation of β,γ-unsaturated α-keto ester 5

A diastereomeric mixture of isopropylidene-protected enoate esters **6** and **7** was synthesized in six steps from D-arabinose as previously reported (17). The intention was to cleave the methylene group of **6** through ozonolysis to install the α-keto ester moiety (**8**), which would allow the molecule to cyclize to the pyranose structure of sialic acid upon removal of the isopropylidene protecting groups. The initial ozonolysis was performed solely on the 4*S* enoate ester **6** rather than on the diastereomeric mixture of **6** and **7** to reduce the complexity of product identification. However, the only product isolated from the reaction, following chromatographic purification, was the β,γ-unsaturated-α-keto ester **5** in 91% yield resulting from oxidative cleavage of the methylene group and elimination of HNO_2 across carbons 3 and 4 (Scheme 1). IR, ^{1}H NMR, and ^{13}C NMR spectra supported the structure of enone **5** because of the presence of signals in the vinylic regions of the NMR spectra, a ketone in the ^{13}C NMR spectrum, and the absence of nitro stretching bands in the IR spectrum. The *trans*-geometry of the double bond was assigned based on the large H3/H4 coupling constant ($J_{3,4}$ = 15.9 Hz).

Isolation of enone **5** was an unexpected result, as no base is present during the ozonolysis of **6** that could effect such an elimination. It was reasoned that the elimination must be occurring post-ozonolysis because if the elimination occurred during ozonolysis, the newly-formed double bond would also be cleaved. The ozonolysis was repeated in the presence of acetic acid to neutralize any possible base generated during the reaction, but enone **5** was again the sole product isolated. Carrying out the ozonolysis in a 1:1

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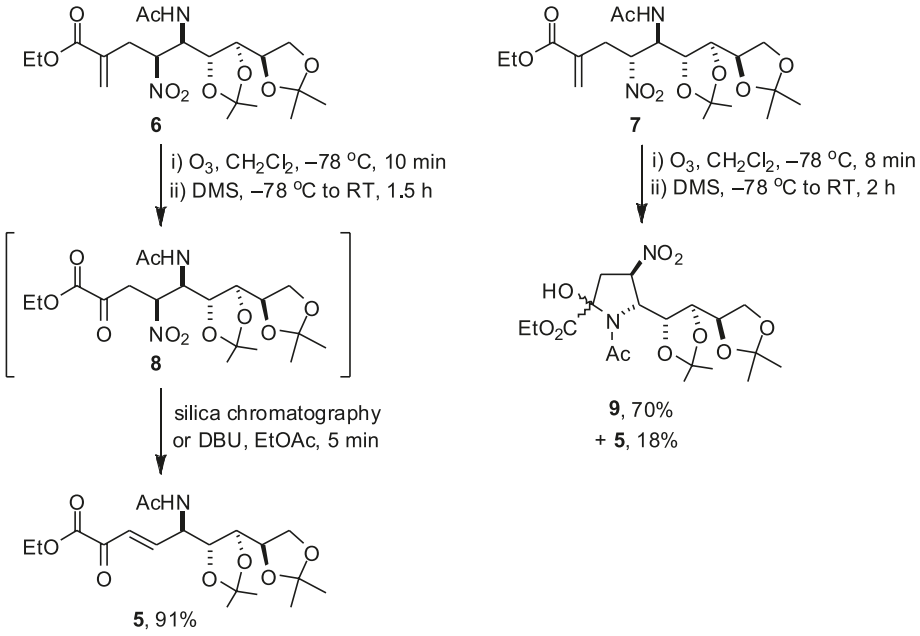
Scheme 1. Ozonolysis of 4*S* enoate ester **6** leads exclusively to unstable α-keto ester **8** that eliminates to enone **5** upon silica chromatography or treatment with DBU, while ozonolysis of 4*R* enoate ester **7** leads predominantly to hemiaminal **9**.



dichloromethane/methanol solvent mixture or using triphenylphosphine as a reducing agent in place of methyl sulfide also had no effect on the reaction outcome.

It was noticed that very little enone was present in the crude ozonolysis product mixture as judged by ^{1}H NMR spectroscopy. In fact, the major compound present in the crude product mixture appeared to be the α-keto ester **8** that had been originally desired (Scheme 1). No elimination had occurred owing to the absence of vinylic signals in the ^{1}H and ^{13}C NMR spectra, and the IR spectrum showed the nitro group was intact. Upon chromatographic purification on silica gel, however, complete elimination to enone **5** was observed, indicating that the elimination of HNO_2 was silica-induced (Scheme 1). Indeed, this silica-induced elimination was not efficient enough to convert α-keto ester **8** into enone **5** completely on larger scales (**5** and **8** are co-polar); thus, the elimination could be induced by treating the crude α-keto ester **8** with DBU (Scheme 1). It was crucial not to allow this elimination to proceed for extended periods, since complete product decomposition was observed after 15 min. Of note, Yao and co-workers noticed a similar silica-induced elimination when synthesizing an α-keto ester similar to **8** that contained a 4-azido group en route to 4-azido-4-deoxysialic acid; this ester eliminated HN_3 upon chromatographic purification (18). These researchers circumvented the problem by using the 4-azido α-keto ester without purification.

It was later noticed that ozonolysis of diastereomeric mixtures of the enoate esters **6** and **7** resulted in isolation of varying yields of enone **5** that reflected the **6/7** ratio, indicating that only one diastereomer may have eliminated HNO_2. When the 4*R* enoate ester **7** was subjected to ozonolysis by itself, enone **5** was isolated in 18% yield, but the major product, isolated in 70% yield, was a saturated product in which the 5-acetamido nitrogen cyclized onto the newly generated 2-keto group to form two five-membered ring hemiaminal anomers (**9**, Scheme 1). These anomers were observed to interconvert in solution by ^{1}H NMR spectroscopy, but only the major anomer could be isolated in sufficient quantities for adequate characterization. The hemiaminal structure was supported by the disappearance of the amide-proton signal around 6.0 ppm from the ^{1}H NMR spectrum and by the appearance of a singlet at 4.8 ppm that disappeared when a drop of D_2O was added to the $CDCl_3$ solution, corresponding to the new 2-hydroxyl group. In addition, an HMBC spectrum showed a correlation between H-5 and C-2, which would not be possible unless the amide nitrogen was bonded to C-2. As well, the IR spectrum indicated the nitro group was intact.

It therefore appears as though the ozonolysis product of the 4*S* enoate ester **6** prefers to eliminate HNO_2 to give enone **5**, while ozonolysis of the 4*R* enoate ester **7** gives rise to a product that cyclizes to a five-membered ring hemiaminal **9** (Scheme 1). Fortunately, the 4*R* enoate ester **7** could be epimerized to a 3:2 mixture of **6**/**7** that was separable by chromatography by treatment with DBU in THF for 40 min, allowing the conversion of **7** to the synthetically useful 4*S* enoate ester **6**. Interestingly, when enoate ester **6** does not have isopropylidene protecting groups, no elimination occurs after ozonolysis; the product spontaneously cyclizes to the sialic acid pyranose (17). This, in addition to the fact that the ozonolysis product of the 4*R* enoate ester **7** prefers to cyclize to a hemiaminal, suggests the possibility that cyclization is faster than elimination for these compounds and elimination is prohibited following cyclization. While the reasons for these differences in reactivity are not apparent, it appears as though something prevents or dramatically slows down cyclization of **8** to a hemiaminal structure, allowing it to undergo facile HNO_2 elimination.

Conjugate addition reactions with enone 5

Although isolation of enone **5** was initially disappointing,

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it was quickly realized that this enone could be a useful synthetic intermediate, since a conjugate addition would afford a sialic acid analogue bearing a new substituent in the 4-position. Indeed, these types of enones have been deliberately synthesized for various purposes. Vasella and co-workers synthesized a similar enone from a protected enoate ester similar to **6**/**7** bearing a 4-*O*-acetyl group, eliminating HOAc with sodium bicarbonate during ozonolysis. They then hydrogenated the resulting double bond, leading to 4-deoxysialic acid (19). Shing also synthesized a related 4-deoxysugar by hydrogenation of such an enone resulting from a Wittig reaction between an aldose and a phosphorus ylide containing an α-keto ester moiety (20). As well, this enone was used in alkoxymercuration reactions resulting in installation of alkoxy groups in the 4-position after reduction of the organomercury intermediate (21). However, literature precedent could not be found for conjugate additions being attempted on such enones.

Introduction of a methyl group was attempted via well-known addition reactions using alkylcopper reagents (22). Several conditions were tried for the conjugate addition of a methyl group to enone **5**, including the following: (*i*) Me_2CuLi from 5 equiv. CuI and 10 equiv. MeLi, warming from –78 °C to room temperature while stirring overnight; (*ii*) Me_2CuLi with TMSCl and $BF_3{\cdot}OEt_2$ as promoters; (*iii*) $Me_2CuMgBr$ from MeMgBr instead of MeLi; and (*iv*) $MeCuBF_3$ prepared from 1 equiv. each of CuI, MeLi, and $BF_3{\cdot}OEt_2$. None of these conditions afforded appreciable quantities of conjugate addition products. Also, 1 equiv. of MeLi was added to enone **5** on one occasion, but no 1,2- or 1,4-addition products were observed, and most of the starting material was recovered, indicating the low reactivity of **5** toward these nucleophiles.

Since the conditions for conjugate addition of alkylcopper reagents to enone **5** seemed to be too harsh, conjugate additions of alkyl halides under radical conditions were attempted. These reactions are normally performed with Bu_3SnH/AIBN in refluxing benzene to generate radicals thermally that are added to α,β-unsaturated carbonyl compounds containing electron-withdrawing groups (23, 24). Initially, benzyl bromide was used as an alkylating agent with Bu_3SnH and AIBN in refluxing toluene. This yielded a complex mixture of products, none of which seemed to have incorporated a benzyl group. The same reagents were tried using a 100 W incandescent light source to generate radicals at room temperature but also gave no addition products. These conditions using a 100 W lamp at room temperature were tried with isopropyl iodide and allyl iodide as alkylating agents to try to generate a stable alkyl radical but resulted in decomposition of the enone. Slow addition of a solution containing Bu_3SnH and AIBN over 1 h via syringe pump to a solution of allyl iodide and enone **5** in toluene at 60 °C was attempted to generate radicals more slowly in hopes that they would react with the enone, but this reaction also failed.

Milder conjugate additions were attempted using a dialkylzinc reagent with a copper catalyst, as alkylzinc reagents are less reactive than their alkylcopper counterparts (25). Alexakis and co-workers have screened a number of copper catalysts for use in conjugate addition reactions with dialkylzinc reagents and found that $Cu(OTf)_2$ was one of the most active, and these reactions were greatly accelerated by the addition of a phosphorus ligand, usually PBu_3 or $P(OEt)_3$ (26, 27). Thus, $Cu(OTf)_2$ and $P(OEt)_3$ were mixed with diethylzinc and enone **5** to give a 53% yield of conjugate addition products (Scheme 2). The initial product in the crude reaction mixture appeared to be a single diastereomer of the enol tautomer **10** as observed by ^1H NMR spectroscopy. It was clear that the ethyl group had added due to the presence of two new signals in the ^1H and ^{13}C NMR spectra, and although H-4 had shifted upfield to 2.8 ppm in the ^1H NMR spectrum, H-3 appeared as a doublet integrating to one proton at 5.5 ppm, indicating that it was a vinylic proton. As well, a singlet was observed at 6.5 ppm that disappeared upon addition of a drop of D_2O to the $CDCl_3$ solution, indicating that this was the enol hydroxyl proton. However, the enol could not be purified for proper characterization, since upon silica chromatography, the product tautomerized to form an anomeric mixture of five-membered ring hemiaminal structures, giving **11** in 53% yield (Scheme 2). As with the 4-nitro hemiaminal **9**, the structure of 4-ethyl hemiaminal **11** was assigned based on the disappearance of the amide proton signal in the ^1H NMR and the appearance of a signal for the new hydroxyl group proton around 4.9 ppm that disappeared when a drop of D_2O was added to the $CDCl_3$ solution. An HMBC spectrum showed a correlation between H-5 and C-2, which further supported the five-membered ring structure. As well, the IR spectrum showed absence of an NH stretching band but did show an OH stretch. As with the 4-nitro hemiaminal **9**, only the major anomer of **11** could be purified for adequate characterization, but the two anomers were observed to interconvert when left for extended periods in $CDCl_3$ solution, indicating that they were indeed anomers and not C4-epimers. Since **11** was a five-membered ring, the stereochemistry of the ethyl addition could be probed through a series of 1D NOE difference ^1H NMR spectra. Upon irradiation of the methylene protons of the newly-installed ethyl group, enhancement of the signals for H-5 and H-3a (on the same face) was observed, while irradiation of H-3b (on the opposite face) only enhanced the signal for H-4. These spectra indicate that H-5 and the new ethyl group are on the same face of the ring, which possesses the 4*R*-configuration. The exclusive formation of the 4*R*-diastereomer is unfortunate, since natural sialosides possess the 4*S*-configuration; however, 4-*epi*-sialic acid derivatives can still be useful inhibitors/probes for various sialic acid processing enzymes (28). While there are several possible explanations for this diastereoselectivity, it is likely that the alkylzinc/alkylcopper complex is coordinating to the acetamido group, thus directing nucleophilic attack from the same face to give *cis*-addition.

Several variables were manipulated to try to increase the yield of the 4-ethyl hemiaminal **11**. Specifically, no reaction was observed when THF was used as a coordinating solvent, and when $BF_3{\cdot}OEt_2$ was used to replace $P(OEt)_3$, no reaction products were afforded in either THF or toluene. Moreover, longer reaction times resulted in the formation of increased amounts of other unidentified products, and the use of larger amounts of diethylzinc did not result in increased product yields. Alexakis and co-workers have observed that α,β-unsaturated esters are less reactive toward copper-catalyzed dialkylzinc conjugate additions (26); thus, the 53% yield of 4-ethyl hemiaminal **11** was considered to be satisfactory.

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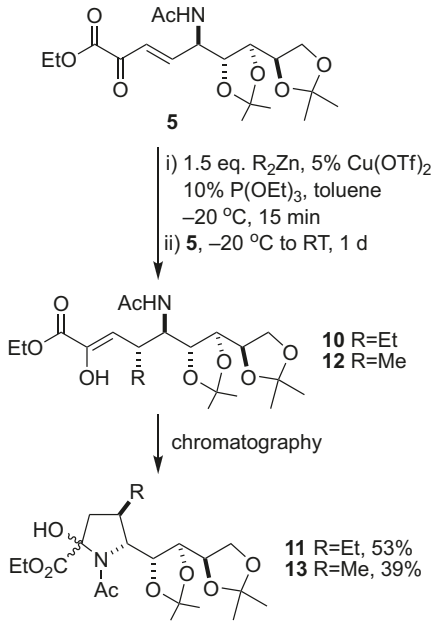
Scheme 2. Conjugate addition of diethylzinc and dimethylzinc to enone **5**, initially affording enol products **10** and **12** that rearrange to five-membered ring hemiaminals **11** and **13** upon chromatography.



The scope of the copper-catalyzed conjugate addition to enone **5** was examined by testing other dialkylzinc reagents. Commercially available dimethylzinc was added, which also afforded a single diastereomer of the conjugate addition product as its enol tautomer **12** that rearranged to the five-membered ring hemiaminal structure during silica chromatography to give **13** in 39% yield (Scheme 2). Lower product yields were expected, since dimethylzinc is known to be less reactive than diethylzinc (29). The 4-methyl hemiaminal **13** exhibited similar spectral characteristics to its ethyl homologue **11**; notably, a series of 1D NOE difference ^{1}H NMR spectra showed that **13** also had the 4*R*-configuration. Also similarly, the 4-methyl hemiaminal product was obtained as an interconverting mixture of anomers of which only the major anomer could be purified for adequate characterization although NMR spectra were obtained on the minor anomer.

Attempts to perform conjugate addition reactions were made using other alkylzinc reagents that had been synthesized. Benzyl zinc bromide was generated by stirring benzyl bromide with zinc dust in toluene at room temperature for 6 h (30, 31), but it afforded no conjugate addition products when reacted with enone **5** under the standard conditions despite giving a 26% yield of 3-benzylcyclohexanone when reacted with a model enone (2-cyclohexen-1-one). Dibenzylzinc was synthesized by zinc-halogen exchange from the reaction of 1 equiv. dimethylzinc with 2 equiv. benzyl bromide in toluene at –30 °C for 45 min (25, 32). When this was added to 2-cyclohexen-1-one, a 22% yield of 3-benzyl-2-methylcyclohexanone was isolated, as the enolate resulting from the dibenzylzinc conjugate addition was trapped by the in situ generated methyl bromide (33). Despite the generation of electrophilic methyl bromide during the synthesis of dibenzylzinc, these conditions were used with enone **5** but only afforded a very small amount of 4-methyl hemiaminal **13**, likely resulting from addition of unreacted dimethylzinc. The synthesis of diphenylzinc through transmetallation was accomplished by reacting 2 equiv. phenyl magnesium bromide with 1 equiv. $ZnCl_2$ in toluene at –30 °C for 2 h and gave a 68% yield of 3-phenylcyclohexanone when reacted with 2-cyclohexen-1-one. However, no conjugate addition products were obtained when diphenylzinc was added to enone **5**. The failure of benzyl and phenyl groups to add to enone **5** in a conjugate manner suggests that such groups may be too bulky to add to the sterically congested 4-position of this enone. It may also be that impurities generated during the synthesis of the alkylzinc reagents impeded the conjugate addition to enone **5**; thus, it may be useful to purify these alkylzinc reagents prior to performing the addition reactions.

Conversion of 4-alkylhemiaminals to sialic acid analogues

To convert the conjugate addition products **11** and **13** into sialic acid analogues, removal of the isopropylidene protecting groups was necessary to allow rearrangement to the six-membered ring pyranose form of sialic acid. This was achieved by stirring the 4-alkyl hemiaminals **11** and **13** in 90% aq. trifluoroacetic acid. These conditions resulted in removal of the isopropylidene groups, allowing the compounds to cyclize to the pyranose form, but also induced the formation of a 2,7-anhydro linkage to give the 2,7-anhydro-4-ethylsialoside **14** and the 2,7-anhydro-4-methylsialoside **15** (Scheme 3). These 2,7-anhydrosugars were moderately unstable to silica gel chromatography and were normally used in subsequent reactions without purification. The 2,7-anhydro linkage in **15** was assigned based on NMR spectroscopic evidence, as H-5 and H-6 showed very little coupling to any other protons, indicating they were not trans-diaxial, and the frequencies of H-6 and C-2 were shifted downfield from 4.2 to 4.6 ppm and from 94 to 105 ppm, respectively. Zbiral and co-workers noticed similar spectral differences when they made a 4-deoxy-4-*epi*-4-methylsialic acid by deprotecting its precursor under acidic conditions, giving a product that contained a 2,7-anhydro linkage (12). HMBC spectra of **14** and **15** showed correlations between H-7 and C-2, which would only be possible if a 2,7-anhydro linkage existed. Incidentally, these spectra also showed correlations between H-6 and C-2, indicating that the compounds were indeed in their pyranose forms. It therefore seems as though 4-alkyl-4-deoxy-4-*epi*-sialic acid analogues preferentially form such 2,7-anhydro linkages under acidic conditions. Since 2,7-anhydrosialic acids are of interest themselves as conformationally restrained derivatives (34), this synthetic route has the potential to lead to a number of such derivatives.

It was decided to convert the 2,7-anhydro-4-alkylsialosides **14** and **15** into glycals so as to develop a route to give compounds that could be tested as sialidase inhibitors against benchmark compounds such as DANA (**2**). This was accomplished by stirring the crude 2,7-anhydrosugars in acetic anhydride/acetic acid with a catalytic amount of H_2SO_4 followed by stirring in saturated aq. $NaHCO_3$, a reaction sequence that opened the 2,7-anhydro linkage, peracetylated all hydroxyl groups, and eliminated HOAc in one pot (35).

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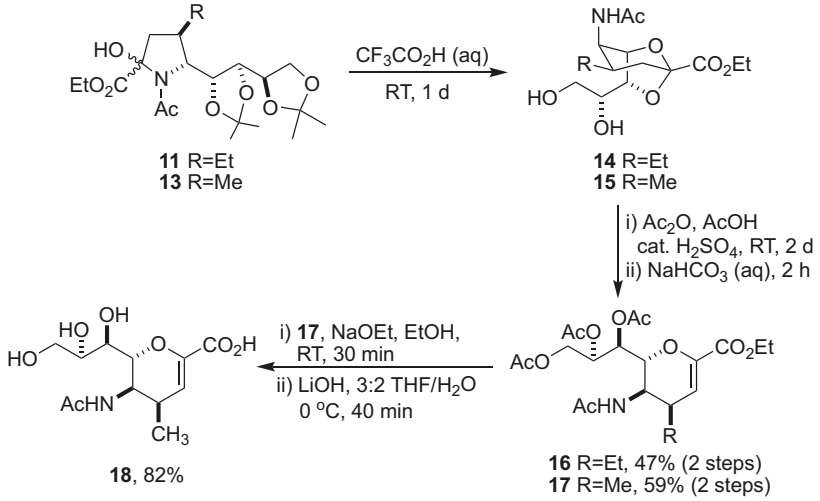
Scheme 3. Conversion of 4-alkyl hemiaminals **11** and **13** into glycals **16** and **17** via their 2,7-anhydrosugars **14** and **15**.



This afforded the peracetylated glycals **16** and **17**, the 4-ethyl-DANA analogue **16** being obtained in 47% yield over two steps from 4-ethyl hemiaminal **11**, and the 4-methyl-DANA analogue being obtained in 59% yield over two steps from 4-methyl hemiaminal **13** (Scheme 3). As with the 2,7-anhydrosialoside precursors, these glycals could be purified by silica chromatography, but their yields decreased significantly despite the crude product mixtures being composed mainly of the glycal products. It was attempted to convert the 4-methyl hemiaminal **13** directly to glycal **17** by performing the deprotection, acetylation, and elimination reactions in one pot, but this did not result in the isolation of any useful products.

The deprotection of 4-deoxy-4-*epi*-4-methyl-DANA **17** was achieved first by removal of the acetyl groups using NaOEt in ethanol, followed by hydrolysis of the ethyl ester using LiOH in 3:2 THF/water to afford the deprotected 4-deoxy-4-*epi*-4-methyl glycal of sialic acid **18** in 82% yield over the two deprotection steps (Scheme 3). A series of 1D NOE difference ^{1}H NMR spectra again confirmed the 4*R*-stereochemical assignment, as irradiation of the 4-methyl group showed a strong contact to H-6 and a weak contact to H-5, indicating that the 4-methyl group and H-6 were on the same face of the six-membered ring. As well, H-4 showed a strong contact to H-5 and virtually no contact to H-6, indicating that H-4 and H-5 are on the same face.

Conclusions

Isolation of an unexpected β,γ-unsaturated α-keto ester **5** led to the development of a synthetic route leading to 4-substituted sialic acid analogues. Ozonolysis of the 4*S* enoate ester **6** led to enone **5** following elimination of HNO_2 during silica chromatography or upon treatment of the crude ozonolysis product with DBU, while ozonolysis of the 4*R* enoate ester **7** led mainly to a five-membered ring hemiaminal structure **9**. Conditions were developed to add alkyl groups to enone **5** through copper-catalyzed conjugate additions of diethylzinc and dimethylzinc. The resulting 4-alkyl-substituted conjugate addition products could be deprotected to generate 2,7-anhydro-4-alkylsialosides **14** and **15** that could then be converted into 4-modified DANA analogues **16** and **17**. Given the functional-group-tolerant nature of dialkylzinc additions, this synthetic route should allow the synthesis of a number of 4-modified sialic acid analogues, including 2,7-anhydrosugars and DANA analogues. Unfortunately, these conjugate addition reactions exclusively afforded the less desirable 4*R*-diastereomer. As well, addition of small nucleophiles from commercially available diethylzinc and dimethylzinc proceeded relatively smoothly, while the addition of bulkier dibenzylzinc and diphenylzinc did not proceed at all. Current work is focusing on attempts to change the diastereoselectivity of the conjugate addition to afford the more desirable 4*S*-diastereomer and on the search for nucleophiles that will participate in conjugate addition reactions with enone **5** to afford more interesting 4-modified sialic acid analogues.

Experimental

Thin-layer chromatography (TLC) was performed on aluminum-backed TLC plates pre-coated with Merck silica gel 60 F_{254}. Compounds were visualized with UV light and (or) staining with phosphomolybdic acid (5% solution in EtOH). Flash chromatography was performed using Avanco silica gel 60 (230–400 mesh). Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. Solvents used for anhydrous reactions were dried and distilled immediately prior to use. Ethanol was dried and distilled over magnesium ethoxide. Dichloromethane and toluene were dried and distilled over calcium hydride. Toluene was degassed by repeated freezing, then thawing while under vacuum. Glassware for anhydrous reactions was flame-dried and cooled under a nitrogen atmosphere immediately prior to use. The majority of the NMR spectra were recorded on a Varian Unity 500 MHz spectrometer, while some were recorded on Bruker AMX-400 MHz or Bruker TCI-600 MHz spectrometers. Chemical shifts (δ) are listed in ppm downfield from TMS using the residual solvent peak as an internal reference. ^{1}H and ^{13}C NMR peak assignments are made based on ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMQC experiments. IR spectra were recorded on a Bomem IR spectrometer, and samples were prepared as cast evaporative films on NaCl plates from CH_2Cl_2. Optical rotations were measured

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using a PerkinElmer 341 polarimeter and are reported in units of deg cm^2 g^{-1} (concentrations reported in units of g/100 cm^3). Shown in the supporting information are the ^1H NMR and ^{13}C NMR spectra for compounds **14**–**18**.[2]

Ethyl 5-acetamido-3,4,5-trideoxy-6,7:8,9-di-*O*-isopropylidene-4-nitro-D-*glycero*-D-*galacto*-non-2-ulosonate (8)

The 4*S* enoate ester **6** (494 mg, 1.11 mmol) was dissolved in dry CH_2Cl_2 (50 mL) and cooled in a dry ice/acetone bath to –78 °C. The flask was fitted with a $CaCl_2$ drying tube and ozone was bubbled through the solution until a blue color persisted (10 min). The solution was purged with oxygen, and methyl sulfide (1 mL) was added. The colorless solution was allowed to warm to room temperature while stirring for 1.5 h, after which time it was concentrated under reduced pressure to yield a colorless syrup (588 mg) that consisted mainly of DMSO and α-keto ester **8**. The crude material was unstable and could not be fully characterized. IR (cm^{-1}): 1373 (NO_2), 1557 (NO_2), 1673 (amide C=O), 1733 (α-keto ester C=O, one band), 3267 (NH). ^1H NMR ($CDCl_3$, 500 MHz) δ: 1.35 (s, 3H, CMe_2), 1.377 (t, 3H, J = 7.1 Hz, C*H*$_3$CH$_2$O), 1.382 (s, 6H, CMe_2), 1.43 (s, 3H, CMe_2), 2.01 (s, 3H, NHCOC*H*$_3$), 3.46 (dd, 1H, $J_{3a,3b}$ = 19.2 Hz, $J_{3a,4}$ = 6.8 Hz, H-3a), 3.63 (dd, 1H, $J_{3a,3b}$ = 19.2 Hz, $J_{3b,4}$ = 7.3 Hz, H-3b), 3.76 (dd, 1H, $J_{6,7}$ = 5.2 Hz, $J_{7,8}$ = 8.8 Hz, H-7), 3.81 (dd, 1H, $J_{8,9a}$ = 6.7 Hz, $J_{9a,9b}$ = 8.7 Hz, H-9a), 3.98 (dt, 1H, $J_{7,8}$ = 8.8 Hz, $J_{8,9a}$ + $J_{8,9b}$ = 12.9 Hz, H-8), 4.13 (dd, 1H, $J_{5,6}$ = 9.8 Hz, $J_{6,7}$ = 5.2 Hz, H-6), 4.15 (dd, 1H, $J_{8,9b}$ = 6.2 Hz, $J_{9a,9b}$ = 8.7 Hz, H-9b), 4.35 (q, 2H, J = 7.1 Hz, CH$_3$C*H*$_2$O), 4.43 (dt, 1H, $J_{4,5}$ = 2.3 Hz, $J_{5,6}$ + $J_{NH,5}$ = 18.5 Hz, H-5), 5.49 (dt, 1H, $J_{3a,4}$ + $J_{3b,4}$ = 14.1 Hz, $J_{4,5}$ = 2.3 Hz, H-4), 5.96 (d, 1H, $J_{NH,5}$ = 8.7 Hz, N*H*COCH$_3$). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 13.2 (*C*H$_3$CH$_2$O), 22.2 (NHCO*C*H$_3$), 24.7, 25.8, 26.4, 26.9 (CMe_2 × 2), 38.4 (C-3), 52.3 (C-5), 62.1 (CH$_3$*C*H$_2$O), 66.4 (C-9), 75.9 (C-8), 77.5 (C-6), 80.5 (C-7), 80.7 (C-4), 109.0, 110.2 (*C*Me$_2$ × 2), 158.9 (C-1), 170.3 (NH*C*OCH$_3$), 188.7 (C-2).

Ethyl (*E*)-5-acetamido-3,4,5-trideoxy-6,7:8,9-di-*O*-isopropylidene-D-*manno*-non-3-en-2-ulosonate (5)

The crude α-keto ester **8** from ozonolysis of enoate ester **6** was subjected to flash chromatography (hexanes–EtOAc gradient solvent system from 1:1 *v*/*v* to 100% EtOAc), inducing the elimination reaction to afford enone **5** as a colorless syrup (403 mg, 1.01 mmol, 91%). The elimination could also be effected by stirring **8** with 1 equiv. DBU in EtOAc for 2 min, followed by quenching with saturated aq. ammonium chloride and extraction with Et_2O to afford crude enone **5** that could be purified as described earlier. $[\alpha]^{20}_D$ +18.8 (*c* 1.10, $CHCl_3$). IR (cm^{-1}): 1073 (aliphatic ether C–O–C), 1654 (amide C=O), 1733 (α-keto ester C=O, one band), 3292 (NH). ^1H NMR ($CDCl_3$, 500 MHz) δ: 1.36–1.38 (m, 12H, C*H*$_3$CH$_2$O, CMe_2 × 2), 1.44 (s, 3H, CMe_2), 2.04 (s, 3H, NHCOC*H*$_3$), 3.70 (dd, 1H, $J_{6,7}$ = 7.2 Hz, $J_{7,8}$ = 8.7 Hz, H-7), 3.89 (dd, 1H, $J_{8,9a}$ = 5.8 Hz, $J_{9a,9b}$ = 8.7 Hz, H-9a), 3.99–4.04 (m, 2H, H-6, H-8), 4.17 (dd, 1H, $J_{8,9b}$ = 6.2 Hz, $J_{9a,9b}$ = 8.7 Hz, H-9b), 4.34 (q, 2H, J = 7.1 Hz, CH$_3$C*H*$_2$O), 4.80–4.85 (m, 1H, H-5), 6.16 (d, 1H, $J_{NH,5}$ = 7.9 Hz, N*H*COCH$_3$), 6.85 (dd, 1H, $J_{3,4}$ = 15.9 Hz, $^4J_{3,5}$ = 1.2 Hz, H-3), 7.15 (dd, 1H, $J_{3,4}$ = 15.9 Hz, $J_{4,5}$ = 6.4 Hz, H-4). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 13.8 (*C*H$_3$CH$_2$O), 23.0 (NHCO*C*H$_3$), 24.9, 26.5, 26.7, 27.0 (CMe_2 × 2), 52.5 (C-5), 62.3 (CH$_3$*C*H$_2$O), 67.7 (C-9), 76.8 (C-8), 79.0 (C-7), 81.3 (C-6), 109.8, 110.3 (*C*Me$_2$ × 2), 126.1 (C-3), 147.9 (C-4), 161.5 (C-1), 169.4 (NH*C*OCH$_3$), 182.6 (C-2). Anal. calcd. for $C_{19}H_{29}NO_8$: C 57.13, H 7.32, N 3.51; found: C 56.82, H 7.17, N 3.76.

Ethyl *N*-acetyl-5-amino-3,4,5-trideoxy-6,7:8,9-di-*O*-isopropylidene-4-nitro-D-*glycero*-D-*talo*-non-2-ulofuranosonate (9)

The 4*R* enoate ester **7** (168 mg, 0.377 mmol) was converted to the 4-nitro hemiaminal **9** (colorless syrup, 118 mg, 0.265 mmol, 70%) under the same conditions used to generate α-keto ester **8** as described earlier. A quantity of enone **5** was also obtained (26.5 mg, 0.066 mmol, 18%). IR (cm^{-1}): 1372 (NO_2), 1562 (NO_2), 1658 (amide C=O), 1753 (ester C=O), 3492 (OH). ^1H NMR ($CDCl_3$, 500 MHz) δ: 1.25 (t, 3H, J = 7.1 Hz, C*H*$_3$CH$_2$O), 1.33 (s, 3H, CMe_2), 1.35 (s, 3H, CMe_2), 1.39 (s, 3H, CMe_2), 1.45 (s, 3H, CMe_2), 2.20 (s, 3H, NHCOC*H*$_3$), 2.97–2.98 (m, 2H, H-3a, H-3b), 3.58 (t, 1H, $J_{6,7}$ = $J_{7,8}$ = 8.6 Hz, H-7), 3.99 (dd, 1H, $J_{8,9a}$ = 4.5 Hz, $J_{9a,9b}$ = 8.9 Hz, H-9a), 4.06–4.11 (m, 1H, H-8), 4.14–4.26 (m, 3H, CH$_3$C*H*$_2$O, H-9b), 4.29 (d, 1H, $J_{6,7}$ = 8.3 Hz, H-6), 4.81–4.83 (m, 2H, H-4, 2-OH), 5.40 (s, 1H, H-5). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 13.9 (*C*H$_3$CH$_2$O), 22.5 (NHCO*C*H$_3$), 25.0, 26.5, 26.6, 26.9 (CMe_2 × 2), 41.5 (C-3), 62.2 (C-5), 62.3 (CH$_3$*C*H$_2$O), 68.0 (C-9), 77.3 (C-8), 78.4 (C-7), 81.8 (C-6), 84.1 (C-4), 89.2 (C-2), 110.2, 110.7 (*C*Me$_2$ × 2), 169.9 (C=O), 171.1 (C=O). Anal. calcd. for $C_{19}H_{30}N_2O_{10}$: C 51.12, H 6.77, N 6.27; found: C 51.24, H 6.81, N 6.01.

Ethyl 5-acetamido-3,4,5-trideoxy-4-ethyl-6,7:8,9-di-*O*-isopropylidene-D-*glycero*-D-*talo*-non-3-enonate (10)

Copper(II) trifluoromethanesulfonate (10 mg, 0.028 mmol) was suspended in dry, degassed toluene (7 mL), and triethylphosphite (10 μL, 0.058 mmol) was added slowly. The resulting colorless solution was stirred under N_2 at room temperature for 15 min, after which it was cooled to –20 °C, and a solution of diethylzinc in hexanes (1.0 mol/L, 0.90 mL, 0.90 mmol) was added dropwise. After stirring under N_2 for 10 min, a solution of enone **5** (236 mg, 0.59 mmol) in toluene (3 mL) was injected dropwise. The solution was stirred under N_2 overnight as it warmed to room temperature. After 18 h, the reaction was quenched by the addition of saturated aq. NH_4Cl (5 mL). The mixture was diluted with water (15 mL) and extracted with Et_2O (2 × 50 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a mixture of compounds that contained mainly the enol tautomer of the ethyl addition product **10** as colorless syrup (217 mg). The enol addition product could not be purified for characterization as it rearranged to the five-membered

[2] Supplementary data for this article are available on the journal Web site (canjchem.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0R6, Canada. DUD 3711. For more information on obtaining material, refer to cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.

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ring hemiaminal **11** upon chromatography. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 0.86 (t, 3H, J = 7.3 Hz, CH_2CH_3), 1.29–1.41 (m, 16H, CMe_2 × 2, OCH_2CH_3, $CH_aH_bCH_3$), 1.51–1.58 (m, 1H, $CH_aCH_bCH_3$), 1.95 (s, 3H, $COCH_3$), 2.82–2.88 (m, 1H, H-4), 3.78–3.83 (m, 3H, H-6, H-7, H-9a), 3.89–3.95 (m, 1H, H-8), 4.06–4.09 (m, 1H, H-9b), 4.23–4.28 (m, 3H, OCH_2CH_3, H-5), 5.50 (d, 1H, $J_{3,4}$ = 10.2 Hz, H-3), 5.73 (d, 1H, $J_{5,NH}$ = 9.4 Hz, NH), 6.54 (s, 1H, OH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 12.0 (CH_2CH_3), 14.1 (OCH_2CH_3), 23.4 ($COCH_3$), 24.6 (CH_2CH_3), 25.3, 26.5, 27.2, 27.5 ($C(CH_3)_2$ × 2), 39.1 (C-4), 54.1 (OCH_2CH_3/C-5), 61.8 (OCH_2CH_3/C-5), 67.8 (C-9), 77.0 (C-8), 80.5 (C-6/C-7), 80.7 (C-6/C-7), 109.6, 110.3 ($C(CH_3)_2$ × 2), 113.1 (C-3), 142.3 (C-2), 164.4, 170.1 (C=O × 2).

Ethyl *N*-acetyl-5-amino-3,4,5-trideoxy-4-ethyl-6,7:8,9-di-*O*-isopropylidene-D-*glycero*-D-*talo*-non-2-ulofuranosonate (11)

The crude enol **10** was purified via flash chromatography (hexanes–EtOAc gradient solvent system from 2:1 to 1:2 *v*/*v*) to afford the hemiaminal **11** as a colorless syrup (134 mg, 0.31 mmol, 53%). IR (cm^{-1}): 1648 (amide C=O), 1747 (ester C=O), 3490 (OH). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 0.97 (t, 3H, J = 7.4 Hz, CH_2CH_3), 1.25 (t, 3H, J = 7.1 Hz OCH_2CH_3), 1.34, 1.35, 1.40, 1.41 (s, 12H, $C(CH_3)_2$ × 2), 1.65–1.71 (m, 2H, CH_2CH_3), 1.89 (d, 1H, $J_{3a,3b}$ = 13.2 Hz, H-3a), 2.14–2.19 (m, 4H, $COCH_3$, H-4), 2.62 (ddd, 1H, $J_{3a,3b}$ = 13.2 Hz, $J_{3b,4}$ = 7.6 Hz, $J_{3b,OH}$ = 1.6 Hz, H-3b), 3.47 (t, 1H, $J_{6,7}$ = $J_{7,8}$ = 8.5 Hz, H-7), 3.93 (dd, 1H, $J_{8,9a}$ = 5.8 Hz, $J_{9a,9b}$ = 8.7 Hz, H-9a), 4.04 (dt, 1H, $J_{7,8}$ + $J_{8,9a}$ + $J_{8,9b}$ = 20.8 Hz, H-8), 4.12 (s, 1H, H-5), 4.14–4.26 (m, 4H, H-9b, H-6, OCH_2CH_3), 4.87 (d, 1H, $J_{3b,OH}$ = 1.5 Hz, OH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.6 (CH_2CH_3), 14.0 (OCH_2CH_3), 22.5 ($COCH_3$), 25.1, 26.6, 26.8, 27.0 ($C(CH_3)_2$ × 2), 27.3 (CH_2CH_3), 40.5 (C-4), 41.1 (C-3), 61.7 (OCH_2CH_3), 65.3 (C-5), 68.5 (C-9), 77.5 (C-8), 78.9 (C-7), 83.4 (C-6), 90.6 (C-2), 109.8, 110.2 ($C(CH_3)_2$ × 2), 171.4, 171.8 (C=O × 2).

Ethyl *N*-acetyl-5-amino-3,4,5-trideoxy-6,7:8,9-di-*O*-isopropylidene-4-methyl-D-*glycero*-D-*talo*-non-2-ulofuranosonate (13)

Enone **5** (1.02 g, 2.56 mmol) was reacted with dimethylzinc (2.0 mol/L in toluene, 1.9 mL, 3.8 mmol) to generate the 4-methyl hemiaminal **13** (1.4:1.0 anomeric ratio, 373 mg, 0.898 mmol, 35%) using the methods reported earlier for the synthesis of the ethyl-addition product **10**. The major anomer of **13** was purified to analytical standards for complete characterization.

Major anomer

$[\alpha]^{20}_D$ +16.5 (*c* 1.95, $CHCl_3$). IR (cm^{-1}): 1648 (amide C=O), 1746 (ester C=O), 3482 (OH). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.25 (t, 3H, J = 7.1 Hz, CH_3CH_2O), 1.32 (d, 3H, $J_{CH3,4}$ = 7.3 Hz, 4-CH_3), 1.33, 1.34, 1.39, 1.42 (s, 12H, $C(CH_3)_2$ × 2), 1.78 (d, 1H, $J_{3a,3b}$ = 13.1 Hz, H-3a), 2.16 (s, 3H, $COCH_3$), 2.44–2.53 (m, 1H, H-4), 2.66 (dd, 1H, $J_{3a,3b}$ = 13.0 Hz, $J_{3b,4}$ = 7.6 Hz, H-3b), 3.49 (t, 1H, $J_{6,7}$ + $J_{7,8}$ = 17.2 Hz, H-7), 3.99 (dd, 1H, $J_{8,9a}$ = 4.9 Hz, $J_{9a,9b}$ = 8.7 Hz, H-9a), 4.03 (s, 1H, H-5), 4.03–4.07 (m, 1H, H-8), 4.12–4.28 (m, 4H, H-6, H-9b, CH_3CH_2O), 4.91 (s, 1H, OH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 14.0 (CH_3CH_2O), 21.3 (4-CH_3), 22.6 ($COCH_3$), 25.1, 26.8 (× 2), 27.0 ($C(CH_3)_2$ × 2), 33.0 (C-4), 43.0 (C-3), 61.8 (CH_3CH_2O), 67.6 (C-5), 68.2 (C-9), 77.5 (C-8), 78.8 (C-7), 83.0 (C-6), 90.8 (C-2), 109.8, 110.1 ($C(CH_3)_2$ × 2), 171.4, 171.7 (C=O × 2). Anal. calcd. for $C_{20}H_{33}NO_8$: C 57.82, H 8.01, N 3.37; found: C 57.52, H 7.90, N 3.59.

Minor anomer

^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.23 (d, 3H, $J_{CH3,4}$ = 7.5 Hz, 4-CH_3), 1.30 (t, 3H, J = 7.1 Hz, CH_3CH_2O), 1.34, 1.37, 1.42, 1.44 (s, 12H, $C(CH_3)_2$ × 2), 1.97 (d, 1H, $J_{3a,3b}$ = 12.5 Hz, H-3a), 2.14 (s, 3H, $COCH_3$), 2.50–2.60 (m, 2H, H-3b, H-4), 3.57 (t, 1H, $J_{6,7}$ + $J_{7,8}$ = 17.0 Hz, H-7), 3.95 (s, 1H, H-5), 3.98 (dd, 1H, $J_{8,9a}$ = 4.9 Hz, $J_{9a,9b}$ = 8.8 Hz, H-9a), 4.04–4.08 (m, 1H, H-8), 4.17–4.30 (m, 4H, H-6, H-9b, CH_3CH_2O), 4.47 (s, 1H, OH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 14.0 (CH_3CH_2O), 21.4 (4-CH_3), 22.1 ($COCH_3$), 25.0, 26.8, 26.90, 26.93 ($C(CH_3)_2$ × 2), 31.9 (C-4), 45.0 (C-3), 62.0 (CH_3CH_2O), 67.6 (C-5), 68.1 (C-9), 77.6 (C-8), 78.8 (C-7), 83.1 (C-6), 90.0 (C-2), 109.8, 110.6 ($C(CH_3)_2$ × 2), 170.5 ($COCH_3$), 171.8 (C-1).

Ethyl 5-acetamido-2,7-anhydro-3,4,5-trideoxy-4-ethyl-D-*glycero*-D-*talo*-non-2-ulopyranosonate (14)

4-Ethyl hemiaminal **11** (239 mg, 0.557 mmol) was dissolved in trifluoroacetic acid (4.0 mL) to which water (0.4 mL) was added. The resulting solution was stirred at room temperature for 15 h and was then concentrated under reduced pressure to give a dark brown syrup (249 mg) that was used directly in the next reaction. A sample for characterization could be purified by flash chromatography (EtOAc–MeOH–H_2O gradient solvent system from 20:3:1 to 10:3:1 *v*/*v*/*v*), giving the 2,7-anhydro-4-ethylsialoside **14** as a light brown foamy syrup. IR (cm^{-1}): 1645 (amide C=O), 1746 (ester C=O), 3328 (OH). ^{1}H NMR (D_2O, 600 MHz) δ: 0.82 (t, 3H, J = 7.4 Hz, CH_2CH_3), 1.24–1.33 (m, 5 H, CH_2CH_3, OCH_2CH_3), 1.65 (t, 1H, $J_{3a,3b}$ = $J_{3a,4}$ = 13.1 Hz, H-3a), 2.02–2.06 (m, 4H, H-3b, $COCH_3$), 2.12–2.17 (m, 1H, H-4), 3.52–3.58 (m, 2H, H-8, H-9a), 3.71 (br d, 1H, $J_{9a,9b}$ = 11.5 Hz, H-9b), 4.05 (dd, 1H, $J_{4,5}$ = 2.0 Hz, $J_{5,6}$ = 4.7 Hz, H-5), 4.21 (d, 1H, $J_{7,8}$ = 7.4 Hz, H-7), 4.29 (q, 2H, J = 7.1 Hz, OCH_2CH_3), 4.59 (d, 1H, $J_{5,6}$ = 1.9 Hz, H-6). ^{13}C NMR (D_2O, 150 MHz) δ: 10.2 (CH_2CH_3), 13.1 (OCH_2CH_3), 21.7 ($COCH_3$), 23.5 (CH_2CH_3), 32.3 (C-4), 34.3 (C-3), 47.9 (C-5), 61.9 (C-9), 63.5 (OCH_2CH_3), 71.3 (C-8), 77.9 (C-7), 79.9 (C-6), 105.0 (C-2), 168.2 (C-1), 173.9 ($COCH_3$). HR–MS (ESI) for $C_{15}H_{25}NO_7$: (MNa^+) calcd: 354.1528; found: 354.1532.

Ethyl 5-acetamido-2,7-anhydro-3,4,5-trideoxy-4-methyl-D-*glycero*-D-*talo*-non-2-ulopyranosonate (15)

4-Methyl hemiaminal **13** (228 mg, 0.548 mmol) was converted into the 2,7-anhydro-4-methylsialoside **15** (263 mg) using the method described earlier for synthesis of the ethyl analogue **14**. The light brown foamy syrup was of adequate purity to be used directly in the next reaction. An analytical sample was purified via flash chromatography (20:3:1 EtOAc–methanol–water *v*/*v*/*v*) to afford the product as a tan solid; mp 117–120 °C (dec). $[\alpha]^{20}_D$ +48 (*c* 0.13, H_2O). IR (cm^{-1}): 1647 (amide C=O), 1741 (ester C=O), 3333 (OH).

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^{1}H NMR (D_2O, 500 MHz) δ: 0.87 (d, 3H, $J_{Me,4}$ = 6.8 Hz, 4-CH_3), 1.28 (t, 3H, J = 7.1 Hz, CH_3CH$_2$O), 1.65 (dd, 1H, $J_{3a,3b}$ = 14.0 Hz, $J_{3a,4}$ = 12.3 Hz, H-3a), 1.97 (dd, 1H, $J_{3a,3b}$ = 14.1 Hz, $J_{3b,4}$ = 5.6 Hz, H-3b), 2.06 (s, 3H, COCH_3), 2.34–2.43 (m, 1H, H-4), 3.52–3.59 (m, 2H, H-8, H-9a), 3.71 (dd, 1H, $J_{8,9b}$ = 2.4 Hz, $J_{9a,9b}$ = 11.5 Hz, H-9b), 3.99 (dd, 1H, $J_{4,5}$ = 4.7 Hz, $J_{5,6}$ = 2.0 Hz, H-5), 4.22 (d, 1H, $J_{7,8}$ = 7.4 Hz, H-7), 4.29 (q, 2H, J = 7.1 Hz, CH$_3$CH_2O), 4.60 (d, 1H, $J_{5,6}$ = 1.8 Hz, H-6). ^{13}C NMR (D_2O, 125 MHz) δ: 13.2 (CH_3CH$_2$O), 15.5 (4-CH_3), 21.9 (COCH_3), 26.0 (C-4), 36.0 (C-3), 49.7 (C-5), 62.1 (C-9), 63.7 (CH$_3$CH_2O), 71.5 (C-8), 78.2 (C-7), 80.0 (C-6), 105.1 (C-2), 168.4 (C-1), 174.2 (COCH$_3$). HR–MS (ESI) for $C_{14}H_{23}NO_7$: (MH^+) calcd: 318.1552; found: 318.1564.

Ethyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,4,5-trideoxy-4-ethyl-D-*glycero*-D-*talo*-non-2-enonate (16)

The crude preparation of 2,7-anhydro-4-ethylsialoside **14** from the previous reaction (249 mg) was dissolved in acetic anhydride (2.0 mL) and glacial acetic acid (2.0 mL). Concentrated H_2SO_4 (15 drops) was added, and the resulting solution was stirred at room temperature. After 3 d, the reaction was poured into saturated aq. $NaHCO_3$ (100 mL), which bubbled vigorously, and the resulting basic mixture was stirred for 2 h. The mixture was then extracted with EtOAc (2 × 100 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give the 4-ethyl-substituted glycal **16** as a light brown syrup (119 mg, 0.261 mmol, 47%). The glycal could be further purified via flash chromatography (hexanes–EtOAc gradient solvent system from 1:2 *v*/*v* to 100% EtOAc) to afford a sample for characterization. $[\alpha]^{20}_D$ +3 (*c* 0.24, $CHCl_3$). IR (cm^{-1}): 1656 (amide C=O), 1747 (ester C=O). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.04 (t, 3H, J = 7.4 Hz, CH$_2$CH_3), 1.31 (t, 3H, J = 7.1 Hz, OCH$_2$CH_3), 1.34–1.39 (m, 1H, CH_aH$_b$CH$_3$), 1.48–1.56 (m, 1H, CH$_a$$H_bCH_3$), 1.97 (s, 3H, COC$H_3$), 2.06 (s, 3H, COC$H_3$), 2.07 (s, 3H, COC$H_3$), 2.11 (s, 3H, COC$H_3$), 2.56–2.60 (m, 1H, H-4), 4.20–4.31 (m, 4H, H-6, H-9a, OCH_2CH$_3$), 4.40 (dd, 1H, $J_{8,9b}$ = 4.4 Hz, $J_{9a,9b}$ = 12.0 Hz, H-9b), 4.55–4.58 (m, 1H, H-5), 5.19 (ddd, 1H, $J_{7,8}$ + $J_{8,9b}$ = 11.4 Hz, $J_{8,9a}$ = 3.2 Hz, H-8), 5.40 (dd, 1H, $J_{6,7}$ = 8.5 Hz, $J_{7,8}$ = 3.1 Hz, H-7), 5.54 (d, 1H, $J_{5,NH}$ = 9.4 Hz, NH), 6.03 (br d, 1H, $J_{3,4}$ = 2.6 Hz, H-3). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 11.4 (CH$_2$CH_3), 14.1 (OCH$_2$CH_3), 20.66 (COCH_3), 20.72 (COCH_3), 20.9 (COCH_3), 23.2 (CH_2CH$_3$), 23.3 (COCH_3), 33.9 (C-4), 43.7 (C-5), 61.1 (C-9), 61.5 (OCH_2CH$_3$), 68.7 (C-7), 69.3 (C-8), 76.7 (C-6), 112.8 (C-3), 141.1 (C-2), 161.8, 169.7, 170.0, 170.3, 170.6 (C=O × 5). HR–MS (ESI) for $C_{21}H_{31}NO_{10}$: (MH^+) calcd: 458.2026; found: 458.2020.

Ethyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,4,5-trideoxy-4-methyl-D-*glycero*-D-*talo*-non-2-enonate (17)

The crude 2,7-anhydro-4-methylsialoside **15** from the previous reaction (263 mg) was converted into glycal 17 (light brown syrup, 0.144 mg, 0.324 mmol, 59% over 2 steps) using the same conditions described earlier for synthesis of the ethyl analogue **16**. This product could be further purified via flash chromatography (hexanes–EtOAc gradient solvent system from 1:2 *v*/*v* to 100% EtOAc) giving the pure glycal as a colorless syrup (58 mg, 0.13 mmol, 24% over 2 steps). $[\alpha]^{20}_D$ +1.2 (*c* 0.995, $CHCl_3$). IR (cm^{-1}): 1653 (amide C=O), 1683 (trisubstituted C=C), 1748 (ester C=O), 3291 (NH). ^{1}H NMR ($CDCl_3$) δ: 1.08 (d, 3H, $J_{Me,4}$ = 7.1 Hz, 4-CH_3), 1.30 (t, 3H, J = 7.1 Hz, CH_3CH$_2$O), 1.98 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.77–2.83 (m, 1H, H-4), 4.20 (dd, 1H, $J_{5,6}$ = 5.0 Hz, $J_{6,7}$ = 7.1 Hz, H-6), 4.20–4.27 (m, 2H, CH$_3$CH_2O), 4.28 (dd, 1H, $J_{8,9a}$ = 7.1 Hz, $J_{9a,9b}$ = 12.1 Hz, H-9a), 4.48 (dd, 1H, $J_{8,9b}$ = 4.3 Hz, $J_{9a,9b}$ = 12.0 Hz, H-9b), 4.48–4.51 (m, 1H, H-5), 5.21 (br quintet, 1H, $J_{7,8}$ + $J_{8,9a}$ + $J_{8,9b}$ = 14.5 Hz, H-8), 5.43 (dd, 1H, $J_{6,7}$ = 7.1 Hz, $J_{7,8}$ = 3.3 Hz, H-7), 5.55 (d, 1H, $J_{NH,5}$ = 9.6 Hz, NHCOCH$_3$), 5.96 (d, 1H, $J_{3,4}$ = 3.2 Hz, H-3). ^{13}C NMR ($CDCl_3$) δ: 14.1 (CH_3CH$_2$O), 15.6 (4-CH_3), 20.61, 20.67, 20.8, 23.2 (COCH_3 × 4), 27.8 (C-4), 45.1 (C-5), 61.36 (CH$_3$CH_2O), 61.38 (C-9), 68.6 (C-7), 70.0 (C-8), 75.8 (C-6), 114.7 (C-3), 141.3 (C-2), 161.8, 169.7, 170.0, 170.3, 170.5 (C=O × 5). HR–MS (ESI) for $C_{20}H_{29}NO_{10}$: (MH^+) calcd: 444.1869; found: 444.1870.

5-Acetamido-2,6-anhydro-3,4,5-trideoxy-4-methyl-D-*glycero*-D-*talo*-non-2-enonic acid (18)

A 1.2 mol/L solution of sodium ethoxide in ethanol (0.080 mL, 0.096 mmol) was added to a solution of peracetylated 4-methyl-substituted glycal **17** (29 mg, 0.065 mmol) in dry ethanol (3.0 mL). This solution was stirred at room temperature for 30 min, after which time TLC analysis indicated the absence of starting material. The reaction was neutralized with Amberlite™ H^+ resin and filtered. The resin was washed with methanol (30 mL), and the filtrate was concentrated under reduced pressure to give a light yellow syrup (21.1 mg, 0.065 mmol). The de-acetylated product was then dissolved in THF/water (3:2 *v*/*v*, 5 mL) and cooled in an ice bath. Lithium hydroxide monohydrate was added (23.7 mg, 0.565 mmol), and the solution stirred in ice for 30 min, after which time TLC analysis indicated complete de-esterification. The reaction was removed from the cooling bath, diluted with water (5 mL), and neutralized with Amberlite™ H^+ resin to pH 5, which was then filtered and washed with water (30 mL). The combined filtrates were concentrated under reduced pressure to afford the lithium salt of the deprotected glycal **18** as a colorless syrup (16.0 mg, 0.0542 mmol, 82%). $[\alpha]^{20}_D$ –100 (*c* 0.48, H_2O). IR (cm^{-1}): 1645 (amide C=O), 1712 (acid C=O), 3287 (NH), 3340 (OH). ^{1}H NMR (D_2O) δ: 1.04 (d, 3H, $J_{Me,4}$ = 7.2 Hz, 4-CH_3), 2.02 (s, 3H, NHCOCH_3), 2.70–2.76 (m, 1H, H-4), 3.64 (dd, 1H, $J_{8,9a}$ = 6.3 Hz, $J_{9a,9b}$ = 11.9 Hz, H-9a), 3.67 (dd, 1H, $J_{6,7}$ = 2.2 Hz, $J_{7,8}$ = 8.8 Hz, H-7), 3.86 (dd, 1H, $J_{8,9b}$ = 2.8 Hz, $J_{9a,9b}$ = 11.9 Hz, H-9b), 3.90 (ddd, 1H, $J_{7,8}$ = 8.9 Hz, $J_{8,9a}$ = 6.3 Hz, $J_{8,9b}$ = 2.8 Hz, H-8), 4.20 (dd, 1H, $J_{5,6}$ = 8.8 Hz, $J_{6,7}$ = 2.2 Hz, H-6), 4.30 (dd, 1H, $J_{4,5}$ = 5.8 Hz, $J_{5,6}$ = 8.8 Hz, H-5), 6.14 (d, 1H, $J_{3,4}$ = 5.0 Hz, H-3). ^{13}C NMR (D_2O) δ: 15.1 (4-CH_3), 21.9 (NHCOCH_3), 28.9 (C-4), 46.4 (C-5), 63.0 (C-9), 69.0 (C-7), 70.5 (C-8), 72.6 (C-6), 116.7 (C-3), 141.8 (C-2), 166.6 (C=O), 174.1 (C=O). HR–MS (ESI) for $C_{12}H_{18}NO_7$: (M^-) calcd: 288.1083; found: 288.1091.

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