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PAPER

Synthesis of the complete series of mono acetates of *N*-acetyl-D-neuraminic acid†Paul A. Clarke,<sup>\*a</sup> Nimesh Mistry<sup>a</sup> and Gavin H. Thomas<sup>b</sup>

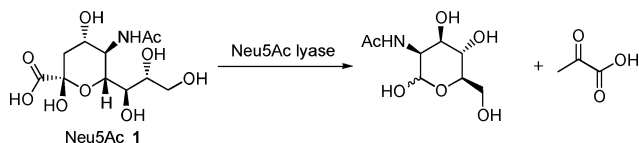
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The short syntheses of each of the mono-acetates of *N*-acetyl-D-neuraminic acid are reported. These are important molecules for studying the mechanism and function of enzymes which utilise Neu5Ac as a substrate. However, until now these molecules were not available as pure compounds and instead had to be studied as mixtures. Neu4,5Ac<sub>2</sub> and Neu5,8Ac<sub>2</sub> were synthesised from a common precursor in 2 and 4 steps respectively, while Neu2,4Ac<sub>2</sub> and Neu5,7Ac<sub>2</sub> were synthesised in 3 and 4 steps respectively from another common precursor. Both precursors could be easily prepared in 3 steps from Neu5Ac itself. Importantly, no scrambling of the anomeric stereochemistry was detected throughout the course of these syntheses.

## Introduction

*N*-Acetyl-D-neuraminic acid **1** (Neu5Ac) is the most common of the sialic acids (Sias) that play important roles in cell biology. Sias are found as the terminal sugar in numerous glycoproteins and glycolipids where they confer a negative charge to the cell surface and play specific roles in recognition of other cell types. The most high-profile function of sialic acid is as a substrate for the sialic acid cleaving neuraminidases of influenza A and B,<sup>1</sup> which are essential enzymes for the infection cycle of the virus. Sias are also important nutrient for many pathogenic bacteria and some bacteria and parasites such as *Trypanosoma cruzi* can utilise sialic acid to decorate their own cells surface.<sup>2</sup> Neu5Ac that has been taken into bacterial cells is catabolised by an *N*-acetylneuraminate lyase (NanA) which cleaves Neu5Ac into *N*-acetylmannosamine and pyruvate (Fig. 1).<sup>2a</sup>



**Fig. 1** Conversion of Neu5Ac to *N*-acetylmannosamine and pyruvate by Neu5Ac lyase.

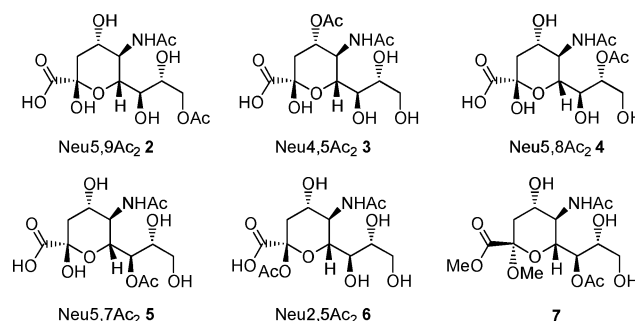
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† Electronic supplementary information (ESI) available: Experimental details and spectroscopic characterisation for all compounds not reported in the experimental section. Copies of <sup>1</sup>H and <sup>13</sup>C NMR spectra of all new compounds. See DOI: 10.1039/c1ob06348k

As well as the common sialic acid Neu5Ac, many organisms contain Neu5Ac derivatives that are *O*-acylated, which have also been linked with distinct biological functions, such as recognition sites for influenza C virus and human coronaviruses that bind to 9-*O*-acetylated Neu5Ac (Neu5,9Ac<sub>2</sub> **2**).<sup>3</sup> Bacteria have also been shown to incorporate acylated Sias into the lipopolysaccharide and capsule, and more recently to use *O*-acylated sialic acids as nutrients. Hence these bacteria require sialate *O*-acetylsterases, at least one of which is known to act on free sialic acids.<sup>4</sup> As there are now a range of eukaryotic, bacterial and viral enzymes that have been identified that act on acylated Sias as substrates, precise chemical tools to understand their mechanisms and specificity are needed.

Since the inception of these studies in the 1980s, the *O*-acylated Sias (Fig. 2) used have generally been from biological sources, and this has complicated and limited the scope of possible investigations. Specifically, the majority of work is carried out on *O*-acylated neuraminic acids isolated from bovine submaxillary mucin or other highly sialylated protein preparation.<sup>5</sup> The isolation procedure generally involves hydrolysis of the glycoconjugates with 0.5 M formic acid at 80 °C, which can



**Fig. 2** Mono-*O*-acetates of Neu5Ac.

lead to hydrolysis of the acetates and acetate migration. The subsequent purification procedure of dialysis, ethereal extraction and ion exchange chromatography also leads to partial (30–60%) hydrolysis of the acetate groups.<sup>5</sup> It should be noted that this procedure only provides a mixture of *O*-acylated neuraminic acids (both mono and per-acylated) and not purified samples of individual *O*-acylated neuraminic acids. The mixture can be assayed by HPLC to give a trace where the peaks are assigned to individual *O*-acylated neuraminic acids without any further authentication of their structure.<sup>6</sup> To our knowledge the only *O*-acylated neuraminic acids to be definitively characterised are the 9-OAc derivative of Neu5Ac (Neu5,9Ac<sub>2</sub> **2**), which is commercially available and the 4-OAc derivative of Neu5Ac (Neu4,5Ac<sub>2</sub> **3**) which, along with **2**, has been synthesised previously.<sup>7</sup> It would seem that the assignment of the other traces in the HPLC of mixed *O*-acylated neuraminic acids is tenuous at best. Therefore, any studies reported on the use of *O*-acylated neuraminic acids as substrates in enzyme assays (with the exception of **2**) use this mixture of *O*-acylated neuraminic acids rather than specific individual molecules and are hence open to possible interpretation errors.

We recently became aware of this limitation when we decided to investigate the effect of mono-*O*-acylated neuraminic acids on a specific *N*-acetylneuraminate lyase. Realising the limitations inherent in this current 'state-of-the-art', and that the lack of authentic characterised standards of individual mono-*O*-acylated neuraminic acids would severely limit our study and complicate the interpretation of results, we embarked upon a program for the unambiguous synthesis and characterisation of each of the mono-*O*-acylated neuraminic acids **2–6** shown in Fig. 2. We reasoned that this would provide a valuable set of standards, not only for ourselves, but also for others investigating the ability of enzymes to process mono-*O*-acylated neuraminic acids.

## Results and discussion

An investigation of the literature showed that there were published routes to both **2** and **3**. While the synthesis of **2** was achieved in 1 step from Neu5Ac **1**,<sup>7</sup> the synthesis of **3** took a not unreasonable 5 steps.<sup>7</sup> In another report the attempted synthesis of **5** was aborted due to acylation at 2-OH, and so the authors settled for the synthesis of the  $\alpha$ -methyl glycoside of methyl *N*, 7-*O*-diacetylneuriminate **7** (Fig. 2).<sup>8a</sup> We were determined to overcome these difficulties and devise syntheses of **3–6** which could be used to produce useful quantities of material for biological evaluation in the most expedient manner possible. One point of concern was that most of the Neu5Ac chemistry literature protects the carboxylic acid function as an ester as the first step in the synthesis and then carries it through to the end. Some reports do not even attempt to deprotect the ester at the end of the synthetic sequence,<sup>8,9</sup> or if they do it involves the simultaneous removal of the acetates on the hydroxyl groups.<sup>10</sup> This generates two problems, firstly that to access our desired molecules the ester will have to be removed at some point and the conditions for its removal will probably not be compatible with *O*-acylation at any of the hydroxyl groups, and secondarily, that scrambling of the  $\alpha$ - and  $\beta$ -anomers has been shown to occur in a number of systems where the carboxylic acid is protected as an ester and the 2-OH 'activated'.<sup>10a,11</sup>

In order to try and avoid these problems we chose two common intermediates which could be used as staging points for our syntheses: **8** which could be used for the synthesis of both **3** and **4**; and **9** which could be used for the synthesis of both **5** and **6** (Fig. 3). While this would, if successful, obviate the potential problem of competing acetate hydrolysis during the late stage removal of the methyl ester, it would also mean carrying an unprotected carboxylic acid group through a number of synthetic transformations, something which had not been attempted previously in Neu5Ac chemistry. We anticipated that this strategy was worth pursuing as it should simplify the synthesis substantially.

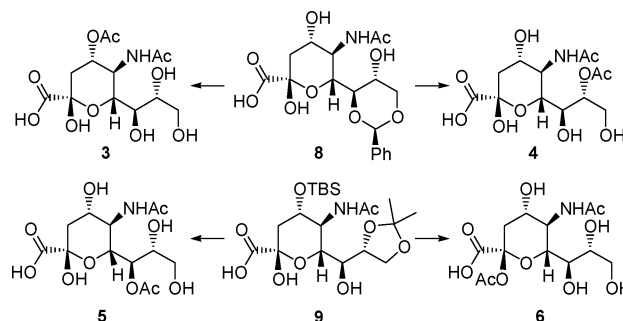
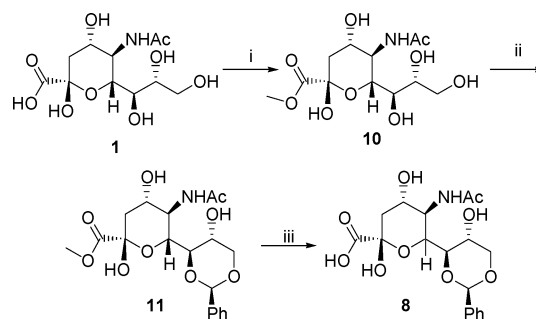


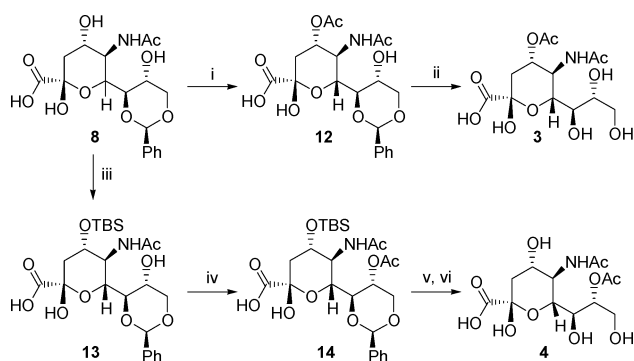
Fig. 3 Common synthetic strategy for the syntheses of **3**, **4**, **5** and **6**.

Our investigations commenced with the synthesis of **8**, the common intermediate *en route* to **3** and **4** (Scheme 1). Neu5Ac **1** was quantitatively converted into its methyl ether **10** by the action of MeOH and acid resin,<sup>11a</sup> which was in turn converted into the benzylidene acetal **11**. Benzylidene acetal **11** was transformed smoothly into **8** by saponification of the methyl ester using the Me<sub>3</sub>SnOH conditions developed by Nicolaou *et al.*,<sup>12</sup> thus removing the difficult problem of the selective saponification of a methyl ester in the presence of an *O*-acetate. With **8** in hand, its conversion into both **3** and **4** could be attempted.



Scheme 1 Reagents and conditions: (i) MeOH, acidic resin, rt, 100%; (ii) PhCHO, TsOH, DMF, rt, 42%; (iii) Me<sub>3</sub>SnOH, CH<sub>2</sub>Cl<sub>2</sub>, rt, 55%.

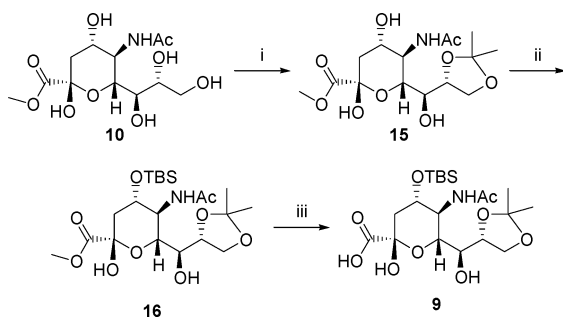
Benzylidene acetal **8** was converted into **3** in two steps (Scheme 2). Treatment of **8** with Ac<sub>2</sub>O, pyridine and DMAP gave **12** in a respectable 64% yield. This demonstrated that the 4-OH can be acylated in the presence of the 8-OH. The previous synthesis of **3** exploited the selective acylation of 4-OH in the presence of the most hindered 7-OH, where the 8-OH and 9-OH were protected as an acetonide.<sup>7</sup> The target, Neu4,5Ac<sub>2</sub> **3**, was revealed by the quantitative hydrogenolysis of benzylidene acetal **12**, using 10% Pd/C in MeOH. This completed the synthesis of **3** in 5 steps.



**Scheme 2** Reagents and conditions: (i)  $\text{Ac}_2\text{O}$ , py, DMAP,  $\text{CH}_2\text{Cl}_2$ , rt, 64%; (ii) 10% Pd/C,  $\text{H}_2$ , MeOH, 100%; (iii) TBSCl, im, DMF, rt, 67%; (iv)  $\text{Ac}_2\text{O}$ , py, DMAP,  $\text{CH}_2\text{Cl}_2$ , rt, 75%; (v) 10% Pd/C,  $\text{H}_2$ , MeOH, 100%; (vi) TBAF, THF/ $\text{H}_2\text{O}$ , rt, 81%.

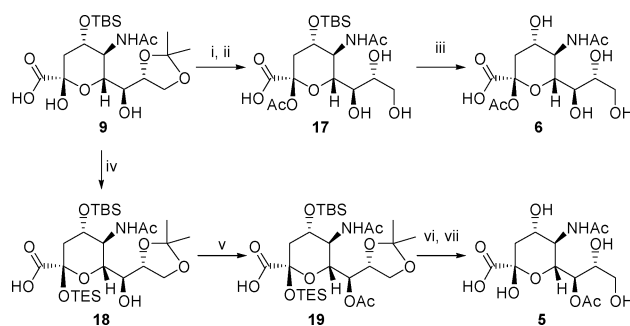
Neu5,8Ac<sub>2</sub> **4** was prepared from the same intermediate **8** by TBS-ether formation at the more reactive 4-OH to give **13** in 67% yield. Acylation of the 8-OH of **13** with  $\text{Ac}_2\text{O}$ , pyridine and DMAP provided **14** in 75% yield. The benzylidene acetal was removed by hydrogenolysis over 10% Pd/C and the resulting compound was treated with TBAF in THF to remove the silyl ether to reveal the desired Neu5,8Ac<sub>2</sub> **4** in 81% yield. This provided previously unknown **4** in 7 steps (Scheme 2).

With the syntheses of Neu4,5Ac<sub>2</sub> **3** and Neu5,8Ac<sub>2</sub> **4** complete, we next turned our attention to the synthesis of **9**, the precursor for our planned syntheses of Neu5,7Ac<sub>2</sub> **5** and Neu2,5Ac<sub>2</sub> **6**. The synthesis of **9** proceeded according to Scheme 3. Methyl ester **10** was converted into acetonide **15** in 71% yield by the action of 2,2-DMP, acetone and TsOH.<sup>7</sup> Acetonide **15** was then silylated at the 4-OH with TBSCl, imidazole in DMF in 82% yield. The desired staging post **9** was revealed by saponification the methyl ester with  $\text{Me}_3\text{SnOH}$  in  $\text{CH}_2\text{Cl}_2$  in a 58% yield.



**Scheme 3** Reagents and conditions: (i); 2,2-DMP, acetone, TsOH, rt, 71%; (ii) TBSCl, im, DMAP, rt, 82%; (iii)  $\text{Me}_3\text{SnOH}$ ,  $\text{CH}_2\text{Cl}_2$ , rt, 58%.

With **9** in hand we could now examine its conversion to Neu2,5Ac<sub>2</sub> **6** and Neu5,7Ac<sub>2</sub> **5** (Scheme 4). Of the two free hydroxyl groups present in **9**, it was difficult to predict which of either the 2-OH or 7-OH would be the most reactive to the acylation conditions. The 7-OH is subject to the steric congestion imposed by the pyranose and acetonide rings, while 2-OH is effectively a tertiary alcohol. When we treated **9** with  $\text{Ac}_2\text{O}$ , pyridine and DMAP we found that the anomeric 2-OH was acylated preferentially in 55% yield, with no sign of acylation of the 7-OH. Remarkably, treatment of this 2-OAc with 60% AcOH gave **17** in 82% yield, with the 2-OAc untouched and still



**Scheme 4** Reagents and conditions: (i)  $\text{Ac}_2\text{O}$ , py, DMAP,  $\text{CH}_2\text{Cl}_2$ , rt, 55%; (ii) 60% AcOH, 82%; (iii) TBAF, THF/ $\text{H}_2\text{O}$ , rt, 87%; (iv) TESOTf,  $\text{Et}_2\text{O}$ , MeCN,  $-20^\circ\text{C}$ , 67%; (v)  $\text{Ac}_2\text{O}$ , py, rt, 75%; (vi) 60% AcOH, 72%; (vii) TBAF, THF/ $\text{H}_2\text{O}$ , rt, 81%.

as the  $\beta$ -anomer. The TBS-ether was removed by the action of TBAF in THF in 87% to give the desired Neu2,5Ac<sub>2</sub> **6**. This completed the synthesis of the previously unreported **6** in only 6 steps. The conversion of **9** into Neu5,7Ac<sub>2</sub> **5** commenced with the TES-protection of the anomeric 2-OH with TESOTf at  $-20^\circ\text{C}$  to give **18** in 67% yield (Scheme 4). Again we were able to effect the derivatisation of the 2-OH, in the presence of the 7-OH with a bulky silylating reagent, without anomerisation. The free 7-OH was then acylated with  $\text{Ac}_2\text{O}$  in pyridine to give **19** in 75% yield. Once again the acetonide was removed in 72% yield by the action of 60% AcOH which remarkably left the 2-OTES hemiketal intact. Removal of the both silyl groups was achieved with TBAF in THF and provided the desired Neu5,7Ac<sub>2</sub> **5** in 81% yield. This completed the synthesis of previously unknown **5** in 7 steps.

With four of the mono-acetates synthesised the series was completed with the synthesis of Neu5,9Ac<sub>2</sub> **2** according the procedure of Ogura *et al.*, which involved stirring **1** with trimethyl orthoacetate and TsOH.<sup>7</sup>

## Conclusions

We have developed the first unambiguous and selective syntheses of each of the previously unreported mono-*O*-acetates of Neu5Ac **1**. Our syntheses utilises common synthetic intermediates which are accessible in only 3 steps from Neu5Ac **1**. These intermediates contain an unprotected carboxylic acid function which allowed us to complete the syntheses Neu2,5Ac<sub>2</sub> **6** (3 steps), Neu5,7Ac<sub>2</sub> **5** (4 steps) and Neu5,8Ac<sub>2</sub> **4** (4 steps) and the previously reported Neu4,5Ac<sub>2</sub> **3** (2 steps) without concern for competing acetate removal or migration, which has been problematic in other studies. We have also shown that in competing acylation and silylation reactions the reactivity of 4-OH > 8-OH > 2-OH and 4-OH > 2-OH > 7-OH, thus enabling future protecting group manipulations to be planned. Importantly, our syntheses do not suffer from epimerisation of the anomeric position, even when the 2-OH is activated as an acetate or masked as a TES-ether. Such anomerisation has been problematic in earlier work on the synthesis of Neu5Ac derivatives. Our syntheses have uncovered some surprising functional group compatibilities, selectivities and reactivities, and the brevity of these syntheses enables the multi-milligram production of **3–6** of a purity suitable to be used as standards in biochemical assays.

## Experimental

### General methods

For general experimental details, including information on solvent purification and the spectrometers used in this research as well as for procedures, spectroscopic and crystallographic data not reported below, see ESI.†

#### Methy 5-acetamido-3,5-dideoxy-7,9-*O*-benzylidene-D-glycero-D-galacto-nonulopyranosonate (11)

To a solution of *N*-acetyl neuraminic acid methyl ester **10** (100 mg, 0.323 mmol) stirring at room temperature under a N<sub>2</sub> atmosphere was added benzaldehyde (37.7 mg, 0.355 mmol) followed by *p*-toluenesulfonic acid (5 mg). The reaction was stirred for 20 h then was concentrated *in vacuo* and purified by flash column chromatography using a 0–5% MeOH:CH<sub>2</sub>Cl<sub>2</sub> gradient to afford a white solid (55.8 mg, 42%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> –24 (*c* 1.0, MeOH); mp: 199–201 °C;  $\nu_{\text{max}}$  (NaCl) 3435–3325 (br), 2954, 1749, 1620, 1559 cm<sup>–1</sup>;  $\delta_{\text{H}}$  (400 MHz, DMSO-*d*<sub>6</sub>): 8.11 (1H, d, *J* = 8.5 Hz, H-10), 7.56–7.54 (2H, m, Ph), 7.39–7.38 (1H, m, Ph), 7.34–7.33 (2H, m, Ph), 6.44 (1H, d, *J* = 2.0 Hz, O-H2), 5.19 (1H, s, H-15), 4.85 (1H, d, *J* = 6.5 Hz, O-H4), 4.35 (1H, d, *J* = 5.5 Hz, O-H8), 4.19–4.15 (1H, m, H-9), 3.84 (1H, ddd, *J* = 11.0, 11.0, 5.5 Hz, H-4), 3.74 (1H, d, *J* = 11.5 Hz, H-6), 3.70 (3H, s, H-14), 3.65–3.55 (2H, m, H-7+H-9), 3.51–3.46 (2H, m, H-5 + H-8), 2.02 (1H, dd, *J* = 12.0, 5.0 Hz, H-3eq), 1.89 (3H, s, H-12), 1.75–1.68 (1H, m, H-3ax) ppm;  $\delta_{\text{C}}$  (100 MHz, DMSO-*d*<sub>6</sub>) 172.0, 170.3, 136.2, 134.4, 129.6, 129.2, 100.6, 95.2, 77.1, 71.5, 70.5, 69.7, 65.6, 53.1, 52.4, 40.0, 22.6 ppm; LRMS (ESI): *m/z* 412 (M<sup>+</sup> + H), 434 (M<sup>+</sup> + Na); HRMS: found (M<sup>+</sup> + H) 412.1532 C<sub>19</sub>H<sub>26</sub>NO<sub>9</sub> requires 412.1529.

#### 5-Acetamido-3,5-dideoxy-7,9-*O*-benzylidene-D-glycero-D-galacto-nonulopyranosic acid (8)

To a solution of **11** (250 mg, 0.608 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) stirring at room temperature under a N<sub>2</sub> atmosphere was added SnMe<sub>3</sub>OH (549 mg, 3.04 mmol). The reaction was stirred for 48 h and was concentrated *in vacuo* to give a yellow oil. The crude material was purified by flash column chromatography using a 9 : 1 CH<sub>2</sub>Cl<sub>2</sub>:MeOH gradient to give a white solid (132 mg, 55%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> –19 (*c* 1.0, MeOH); mp: 191–193 °C;  $\nu_{\text{max}}$  (NaCl) 3442–3307 (br), 2974, 2746, 1744, 1618, 1565 cm<sup>–1</sup>;  $\delta_{\text{H}}$  (400 MHz, DMSO-*d*<sub>6</sub>): 8.11 (1H, d, *J* = 8.5 Hz, H-10), 7.56–7.54 (2H, m, Ph), 7.39–7.38 (1H, m, Ph), 7.34–7.33 (2H, m, Ph), 6.44 (1H, d, *J* = 2.0 Hz, O-H2), 5.19 (1H, s, H-14), 4.84 (1H, d, *J* = 6.5 Hz, O-H4), 4.34 (1H, d, *J* = 5.5 Hz, O-H8), 4.19–4.15 (1H, m, H-9), 3.84 (1H, ddd, *J* = 12.0, 11.0, 5.0 Hz, H-4), 3.74 (1H, d, *J* = 11.5 Hz, H-6), 3.66–3.54 (2H, m, H-7+H-9), 3.51–3.46 (2H, m, H-5 + H-8), 1.98 (1H, dd, *J* = 12.0, 5.0 Hz, H-3eq), 1.89 (3H, s, H-12), 1.70 (1H, dd, *J* = 12.0, 12.0 Hz, H-3ax) ppm;  $\delta_{\text{C}}$  (100 MHz, DMSO-*d*<sub>6</sub>) 172.0, 171.3, 136.2, 134.6, 129.5, 129.2, 100.6, 95.0, 77.2, 71.5, 70.5, 69.7, 65.5, 53.1, 40.0, 22.6 ppm; LRMS (ESI): *m/z* 398 (M<sup>+</sup> + H), 420 (M<sup>+</sup> + Na); HRMS: found (M<sup>+</sup> + H) 398.1379 C<sub>18</sub>H<sub>24</sub>NO<sub>9</sub> requires 398.1373.

#### 5-Acetamido-4-*O*-acetyl-3,5-dideoxy-7,9-*O*-benzylidene-D-glycero-D-galacto-nonulopyranosic acid (12)

To a solution of **8** (40 mg, 0.10 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) stirring at room temperature under a N<sub>2</sub> atmosphere was treated with Ac<sub>2</sub>O (10  $\mu$ L, 0.11 mmol), pyridine (12  $\mu$ L, 0.15 mmol) and DMAP (18 mg, 0.15 mmol). The reaction was stirred for 18 h then partitioned between CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and a saturated aqueous solution of NH<sub>4</sub>Cl (20 mL). The organic layer was washed with brine (20 mL), dried (MgSO<sub>4</sub>) and concentrated *in vacuo* to give an orange oil that was purified by flash column chromatography using a 99 : 1 to 9 : 1 CH<sub>2</sub>Cl<sub>2</sub>:MeOH gradient to give a white solid (28 mg, 64%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> –15 (*c* 1.0, MeOH); mp: 195–197 °C;  $\nu_{\text{max}}$  (NaCl) 3450–3287 (br), 2972, 2722, 1772, 1756, 1623, 1561 cm<sup>–1</sup>;  $\delta_{\text{H}}$  (400 MHz, DMSO-*d*<sub>6</sub>): 8.07 (1H, d, *J* = 8.5 Hz, H-10), 7.56–7.54 (2H, m, Ph), 7.39–7.38 (1H, m, Ph), 7.34–7.33 (2H, m, Ph), 6.44 (1H, d, *J* = 2.0 Hz, O-H2), 5.25 (1H, s, H-14), 4.35 (1H, d, *J* = 5.5 Hz, O-H8), 4.21–4.14 (2H, m, H-4 + H-9), 3.75 (1H, d, *J* = 11.5 Hz, H-6), 3.66–3.58 (2H, m, H-7+H-9), 3.54–3.76 (2H, m, H-5 + H-8), 2.10 (1H, dd, *J* = 12.0, 5.0 Hz, H-3eq), 1.94 (3H, s, H-20), 1.87 (3H, s, H-12), 1.75 (1H, dd, *J* = 12.0, 12.0 Hz, H-3ax) ppm;  $\delta_{\text{C}}$  (100 MHz, DMSO-*d*<sub>6</sub>) 172.0, 171.3, 168.1, 136.1, 134.5, 129.5, 129.2, 100.7, 95.3, 77.2, 71.5, 70.9, 70.5, 69.7, 53.1, 40.0, 22.5, 21.4 ppm; LRMS (ESI): *m/z* 440 (M<sup>+</sup> + H), 462 (M<sup>+</sup> + Na); HRMS: found (M<sup>+</sup> + H) 440.1473 C<sub>20</sub>H<sub>26</sub>NO<sub>10</sub> requires 440.1478.

#### 5-Acetamido-3,5-dideoxy-4-*O*-acetyl-D-glycero-D-galacto-nonulopyranosic acid (3)

To a solution of **12** (20 mg, 0.046 mmol) in MeOH (3 mL) was added 10% Pd/C (10 mg, 50% wt). The reaction was stirred at room temperature and placed under *vacuo* then purged with H<sub>2</sub> (*via* balloon). This procedure was repeated twice before the reaction was stirred for 2 h. The reaction was filtered through Celite and the solids washed with MeOH (20 mL). The combined organics were concentrated *in vacuo* to give a colourless oil (16 mg, quant).  $\delta_{\text{H}}$  (400 MHz, DMSO-*d*<sub>6</sub>): 8.08 (1H, d, *J* = 10.0 Hz, H-10), 6.74 (1H, d, *J* = 2.0 Hz, O-H2), 4.56 (1H, d, *J* = 6.0 Hz, O-H7), 4.34 (1H, d, *J* = 5.5 Hz, O-H8), 4.25 (1H, t, *J* = 6.0 Hz, O-H9), 4.21–4.18 (1H, m, H-4), 3.73 (1H, dd, *J* = 10.5, 1.5 Hz, H-6), 3.60 (1H, br d, *J* = 6.0 Hz, H-9), 3.53–3.46 (1H, m, H-8), 3.50 (1H, ddd, *J* = 10.5, 10.0, 10.0 Hz, H-5), 3.33 (1H, dd, *J* = 11.0, 7.0 Hz, H-9), 3.21 (1H, d, *J* = 8.0 Hz, H-7), 2.10 (1H, dd, *J* = 12.0, 5.0 Hz, H-3eq), 1.95 (3H, s, H-15), 1.89 (3H, s, H-12), 1.75 (1H, dd, *J* = 12.0, 12.0 Hz, H-3ax) ppm;  $\delta_{\text{C}}$  (100 MHz, DMSO-*d*<sub>6</sub>) 171.8, 171.5, 168.1, 94.7, 70.8, 70.4, 69.8, 69.2, 63.6, 53.1, 39.9, 22.7, 21.5 ppm. Other structural data matched that reported in the literature.

#### 5-Acetamido-4-*O*-tert-butylidimethylsilyl-3,5-dideoxy-7,9-*O*-benzylidene-D-glycero-D-galacto-nonulopyranosic acid (13)

Imidazole (170 mg, 2.5 mmol) and *tert*-butyldimethylsilyl chloride (151 mg, 1.0 mmol) were added to a solution of **8** (200 mg, 0.5 mmol) in DMF (5 mL) at 0 °C. The solution was stirred at room temperature for 16 h. The solvent was removed *in vacuo* then was partitioned between CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and H<sub>2</sub>O (50 mL). The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2x 50 mL) and the combined organics were washed with brine (150 mL), dried (MgSO<sub>4</sub>) and concentrated to give a yellow oil. The crude material was purified by flash column chromatography using a 0% to 5%



MeOH : CH<sub>2</sub>Cl<sub>2</sub> gradient to give a white solid (170 mg, 67%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> – 9 (c 1.0, MeOH); mp: 192–194 °C;  $\nu_{\text{max}}$  (NaCl) 3439–3321 (br), 2976, 2740, 1748, 1621, 1570 cm<sup>–1</sup>;  $\delta_{\text{H}}$  (400 MHz, DMSO-*d*<sub>6</sub>): 8.06 (1H, d, *J* = 8.5 Hz, H-10), 7.57–7.54 (2H, m, Ph), 7.39–7.36 (1H, m, Ph), 7.34–7.32 (2H, m, Ph), 6.51 (1H, d, *J* = 2.0 Hz, O-H2), 5.18 (1H, s, H-14), 4.35 (1H, d, *J* = 5.5 Hz, O-H8), 4.19–4.15 (1H, m, H-9), 4.06–4.02 (1H, m, H-4), 3.72 (1H, d, *J* = 12.0 Hz, H-6), 3.67–3.58 (2H, m, H-7 + H-9), 3.54–3.44 (2H, m, H-5 + H-8), 2.09 (1H, dd, *J* = 12.0, 5.0 Hz, H-3eq), 1.88 (3H, s, H-12), 1.75 (1H, dd, *J* = 12.0, 12.0 Hz, H-3ax) 0.81 (9H, s, H-22), 0.04 (3H, s, H-21), 0.03 (3H, s, H-21) ppm;  $\delta_{\text{C}}$  (100 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  171.9, 171.4, 136.0, 134.6, 129.5, 129.2, 100.5, 95.3, 77.2, 71.5, 70.5, 69.7, 69.3, 53.3, 40.0, 25.8, 22.6, 17.8, –4.3, –4.6 ppm; LRMS (ESI): *m/z* 512 (M<sup>+</sup> + H), 534 (M<sup>+</sup> + Na); HRMS: found (M<sup>+</sup> + H) 512.2243 C<sub>24</sub>H<sub>38</sub>NO<sub>9</sub>Si requires 512.2238.

**5-Acetamido-4-*O*-tert-butylidimethylsilyl-8-*O*-acetyl-3,5-dideoxy-7,9-*O*-benzylidene-D-glycero-D-galacto-nonulopyranosic acid (14)**

To a solution of **13** (150 mg, 0.29 mmol) in pyridine (5 mL) stirring at room temperature under a N<sub>2</sub> atmosphere was treated with Ac<sub>2</sub>O (30  $\mu$ L, 0.32 mmol). The reaction was stirred for 24 h then concentrated *in vacuo* to give an orange oil that was purified by flash column chromatography using a 1 : 1 to 3 : 1 EtOAc: petroleum ether gradient to give a white solid (115 mg, 72%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> – 15 (c 1.0, MeOH); mp: 188–190 °C;  $\nu_{\text{max}}$  (NaCl) 3572, 3489–3255 (br), 2966, 2749, 1776, 1750, 1626, 1571 cm<sup>–1</sup>;  $\delta_{\text{H}}$  (400 MHz, DMSO-*d*<sub>6</sub>): 8.10 (1H, d, *J* = 8.5 Hz, H-10), 7.57–7.53 (2H, m, Ph), 7.39–7.35 (1H, m, Ph), 7.36–7.32 (2H, m, Ph), 6.44 (1H, d, *J* = 2.0 Hz, O-H2), 5.19 (1H, s, H-14), 4.21–4.18 (1H, m, H-9), 4.06–4.02 (1H, m, H-4), 3.99–3.95 (1H, m, H-8), 3.76 (1H, d, *J* = 12.0 Hz, H-6), 3.65–3.56 (2H, m, H-7 + H-9), 3.52 (1H, ddd, *J* = 12.0, 12.0, 12.0 Hz, H-5), 2.09 (1H, dd, *J* = 12.0, 5.0 Hz, H-3eq), 1.95 (3H, s, H-20), 1.89 (3H, s, H-12), 1.72 (1H, dd, *J* = 12.0, 12.0 Hz, H-3ax) 0.80 (9H, s, H-22), 0.04 (3H, s, H-21), 0.03 (3H, s, H-21) ppm;  $\delta_{\text{C}}$  (100 MHz, DMSO-*d*<sub>6</sub>) 171.9, 171.3, 169.9, 135.9, 134.6, 129.6, 129.2, 100.5, 95.3, 77.2, 72.4, 71.5, 70.5, 69.6, 53.1, 40.0, 25.8, 22.6, 20.7, 17.7, –4.3, –4.6 ppm; LRMS (ESI): *m/z* 554 (M<sup>+</sup> + H), 576 (M<sup>+</sup> + Na); HRMS: found (M<sup>+</sup> + H) 512.2348 C<sub>26</sub>H<sub>40</sub>NO<sub>10</sub>Si requires 512.2343.

**5-Acetamido-8-*O*-acetyl-3,5-dideoxy-D-glycero-D-galacto-nonulopyranosic acid (4)**

To a solution of **14** (80 mg, 0.144 mmol) in MeOH (5 mL) was added 10% Pd/C (24 mg, 50% wt). The reaction was stirred at room temperature and placed under *vacuo* then purged with H<sub>2</sub> (*via* balloon). This procedure was repeated twice before the reaction was stirred for 5 h. The reaction was filtered through Celite and the solids washed with MeOH (50 mL). The combined organics were concentrated *in vacuo* to give a colourless oil (66 mg, quant). [ $\alpha$ ]<sub>D</sub><sup>25</sup> – 15 (c 1.0, MeOH); mp: 177–179 °C;  $\nu_{\text{max}}$  (NaCl) 3506–3246 (br), 2968, 2752, 1778, 1753, 1623, 1573 cm<sup>–1</sup>;  $\delta_{\text{H}}$  (400 MHz, DMSO-*d*<sub>6</sub>): 8.08 (1H, d, *J* = 10.0 Hz, H-10), 6.74 (1H, d, *J* = 2.0 Hz, O-H2), 4.84 (1H, d, *J* = 6.0 Hz, O-H4), 4.34 (1H, d, *J* = 5.5 Hz, O-H8), 4.25 (1H, t, *J* = 6.0 Hz, O-H9), 4.21–4.18 (1H, m, H-4), 3.73 (1H, dd, *J* = 10.5, 1.5 Hz, H-6), 3.60 (1H, br d, *J* = 6.0 Hz, H-9), 3.53–3.46 (1H, m, H-8), 3.50 (1H, ddd, *J* = 10.5, 10.0, 10.0 Hz, H-5), 3.33 (1H, dd, *J* = 11.0, 7.0 Hz, H-9), 3.21 (1H, d, *J* = 8.0 Hz,

H-7), 2.10 (1H, dd, *J* = 12.0, 5.0 Hz, H-3eq), 1.95 (3H, s, H-15), 1.89 (3H, s, H-12), 1.75 (1H, dd, *J* = 12.0, 12.0 Hz, H-3ax) ppm;  $\delta_{\text{C}}$  (100 MHz, DMSO-*d*<sub>6</sub>) 171.8, 171.5, 168.1, 94.7, 70.8, 70.4, 69.8, 69.2, 63.6, 53.1, 39.9, 22.7, 21.5 ppm; LRMS (ESI): *m/z* 466 (M<sup>+</sup> + H), 488 (M<sup>+</sup> + Na); HRMS: found (M<sup>+</sup> + H) 466.2027 C<sub>19</sub>H<sub>36</sub>NO<sub>10</sub>Si requires 466.2030.

**5-Acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-4-*O*-tert-butylidimethylsilyl-D-glycero-D-galacto-nonulopyranosic acid (9)**

To a solution of **16** (200 mg, 0.45 mmol) in CH<sub>2</sub>Cl<sub>2</sub> stirring at room temperature under a N<sub>2</sub> atmosphere was added SnMe<sub>3</sub>OH (164 mg, 0.91 mmol). The reaction was stirred for 18 h and was concentrated *in vacuo* to give a yellow oil. The crude material was purified by flash column chromatography using a 9 : 1 CH<sub>2</sub>Cl<sub>2</sub>:MeOH gradient to give a white solid (120 mg, 58%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> – 17 (c 1.0, MeOH); mp: 180–182 °C;  $\nu_{\text{max}}$  (NaCl) 3571, 3491–3274 (br), 2731, 1752, 1620, 1575 cm<sup>–1</sup>;  $\delta_{\text{H}}$  (400 MHz, DMSO-*d*<sub>6</sub>): 8.05 (1H, d, *J* = 9.0 Hz, H-10), 6.87 (1H, s, O-H2), 4.53 (1H, br s, O-H7), 4.06–4.02 (1H, m, H-4), 3.98 (1H, dd, *J* = 12.0, 6.0 Hz, H-8), 3.92–3.86 (2H, m, H-6 + H-5), 3.73 (1H, br d, *J* = 10.5 Hz, H-9), 3.64 (1H, br d, *J* = 10.5 Hz, H-9), 3.51 (1H, br d, *J* = 6.0 Hz, H-7), 2.02 (1H, dd, *J* = 12.0, 5.0 Hz, H-3eq), 1.84 (3H, s, H-12), 1.62 (1H, dd, *J* = 12.0, 12.0 Hz, H-3ax), 1.28 (3H, s, H-15), 1.23 (3H, s, H-15), 0.82 (9H, s, H-19), 0.04 (3H, s, H-17), 0.03 (3H, s, H-17) ppm;  $\delta_{\text{C}}$  (100 MHz, DMSO-*d*<sub>6</sub>): 171.8, 171.5, 107.5, 94.7, 76.1, 71.9, 68.4, 65.6, 65.2, 52.9, 39.9, 26.6, 25.8, 25.6, 22.6, 17.5, –4.6, –4.8 ppm; LRMS (ESI): *m/z* 464 (M<sup>+</sup> + H), 486 (M<sup>+</sup> + Na); HRMS: found (M<sup>+</sup> + H) 464.2234 C<sub>20</sub>H<sub>38</sub>NO<sub>9</sub>Si requires 464.2238.

**5-Acetamido-2-*O*-acetyl-3,5-dideoxy-4-*O*-tert-butylidimethylsilyl-D-glycero-D-galacto-nonulopyranosic acid (17)**

To a solution of **9** (40 mg, 0.086 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) stirring at room temperature under a N<sub>2</sub> atmosphere was treated with Ac<sub>2</sub>O (11 mg, 0.10 mmol), pyridine (14 mg, 0.17 mmol) and DMAP (21 mg, 0.17 mmol). The reaction was stirred for 18 h then partitioned between CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and a saturated aqueous solution of NH<sub>4</sub>Cl (20 mL). The organic layer was washed with brine (20 mL), dried (MgSO<sub>4</sub>) and concentrated *in vacuo* to give an orange oil that was purified by flash column chromatography using a 1 : 2 to 3 : 1 EtOAc: petroleum ether gradient to give a white solid (24 mg, 55%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> – 24 (c 1.0, MeOH); mp: 167–169 °C;  $\nu_{\text{max}}$  (NaCl) 3570, 3450–3201 (br), 2738, 1770, 1756, 1617, 1570 cm<sup>–1</sup>;  $\delta_{\text{H}}$  (400 MHz, DMSO-*d*<sub>6</sub>): 8.05 (1H, d, *J* = 9.0 Hz, H-10), 4.53 (1H, br s, O-H7), 4.05–4.01 (1H, m, H-4), 3.96 (1H, dd, *J* = 12.0, 6.0 Hz, H-8), 3.91–3.84 (2H, m, H-6 + H-5), 3.74 (1H, br d, *J* = 10.5 Hz, H-9), 3.62 (1H, br d, *J* = 10.5 Hz, H-9), 3.55 (1H, br d, *J* = 6.0 Hz, H-7), 2.07 (1H, dd, *J* = 12.0, 5.0 Hz, H-3eq), 1.92 (3H, s, H-21), 1.82 (3H, s, H-12), 1.65 (1H, dd, *J* = 12.0, 12.0 Hz, H-3ax), 1.29 (3H, s, H-15), 1.25 (3H, s, H-15), 0.81 (9H, s, H-19), 0.04 (3H, s, H-17), 0.03 (3H, s, H-17) ppm;  $\delta_{\text{C}}$  (100 MHz, DMSO-*d*<sub>6</sub>): 171.8, 171.4, 169.5, 107.3, 97.5, 76.0, 71.7, 68.4, 65.3, 65.2, 52.7, 40.0, 26.6, 25.6, 25.8, 22.5, 21.1, 17.5, –4.6, –4.8 ppm; LRMS (ESI): *m/z* 506 (M<sup>+</sup> + H), 528 (M<sup>+</sup> + Na); HRMS: found (M<sup>+</sup> + H) 464.2234 C<sub>22</sub>H<sub>40</sub>NO<sub>10</sub>Si requires 506.2343.

A solution of the above compound (10 mg, 0.02 mmol) in 60% AcOH (1 mL) was stirred for 3 h at 60 °C. The mixture was concentrated *in vacuo* to yield a brown solid which was purified

by flash column chromatography using a 9:1 CH<sub>2</sub>Cl<sub>2</sub> : MeOH gradient to yield a white solid (7.5 mg, 82%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> –24 (*c* 1.0, MeOH); mp: 151–153 °C;  $\nu_{\text{max}}$  (NaCl) 3462–3247 (br), 2741, 1771, 1758, 1622, 1564 cm<sup>–1</sup>;  $\delta_{\text{H}}$  (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  8.09 (1H, d, *J* = 8.5 Hz, H-10), 4.54 (1H, br s, O-H7), 4.35 (1H, br d, *J* = 5.0 Hz, O-H8), 4.22 (1H, br s, O-H9), 4.05–4.01 (1H, m, H-4), 3.67 (1H, dd, *J* = 10.5, 1.5 Hz, H-6), 3.62 (1H, br d, H-9), 3.55–3.48 (1H, m, H-8), 3.50 (1H, ddd, *J* = 10.0, 10.0, 5.0 Hz, H-5), 3.29 (1H, dd, *J* = 11.0, 7.0 Hz, H-9), 3.16 (1H, d, *J* = 8.0 Hz, H-7), 2.07 (1H, dd, *J* = 12.5, 5.0 Hz, H-3eq), 1.92 (3H, s, H-19), 1.89 (3H, s, H-11), 1.67 (1H, dd, *J* = 13.0, 11.2 Hz, H-3ax), 0.81 (9H, s, H-17), 0.04 (3H, s, H-15), 0.03 (3H, s, H-15) ppm;  $\delta_{\text{C}}$  (100 MHz, DMSO-*d*<sub>6</sub>) 171.8, 170.3, 169.5, 97.5, 70.5, 69.7, 69.1, 65.6, 63.6, 53.1, 40.0, 22.5, 21.1, 17.5, –4.6, –4.8 ppm; LRMS (ESI): *m/z* 466 (M<sup>+</sup> + H), 488 (M<sup>+</sup> + Na); HRMS: found (M<sup>+</sup> + H) 466.2034 C<sub>19</sub>H<sub>36</sub>NO<sub>10</sub>Si requires 466.2030.

#### 5-Acetamido-2-*O*-acetyl-3,5-dideoxy-D-glycero-D-galactononulopyranosic acid (6)

To a solution of **17** (7.5 mg, 0.016 mmol) in THF (1 mL) and H<sub>2</sub>O (1 mL) stirring at 0 °C was added 1.0 M TBAF in THF (80  $\mu$ L, 0.08 mmol). The reaction was stirred for 18 h then was concentrated *in vacuo* to give a yellow oil. The crude material was purified by flash column chromatography using a 3:1 CH<sub>2</sub>Cl<sub>2</sub> : MeOH gradient to give a white solid (4.8 mg, 87%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> –31 (*c* 1.0, MeOH); mp: 189–191 °C;  $\nu_{\text{max}}$  (NaCl) 3435–3311 (br), 2729, 1767, 1750, 1620, 1565 cm<sup>–1</sup>;  $\delta_{\text{H}}$  (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  8.09 (1H, d, *J* = 8.5 Hz, H-10), 4.78 (1H, br s, O-H4), 4.54 (1H, br s, O-H7), 4.35 (1H, br d, *J* = 5.0 Hz, O-H8), 4.24 (1H, br s, O-H9), 3.82 (1H, ddd, *J* = 10.5, 10.5, 5.0 Hz, H-4), 3.74 (1H, dd, *J* = 10.5, 1.5 Hz, H-6), 3.58 (1H, br d, H-9), 3.54–3.45 (1H, m, H-8), 3.50 (1H, ddd, *J* = 10.0, 10.0, 5.0 Hz, H-5), 3.29 (1H, dd, *J* = 11.0, 7.0 Hz, H-9), 3.15 (1H, d, *J* = 8.0 Hz, H-7), 2.07 (1H, dd, *J* = 12.5, 5.0 Hz, H-3eq), 1.92 (3H, s, H-19), 1.89 (3H, s, H-11), 1.70 (1H, dd, *J* = 13.0, 11.2 Hz, H-3ax) ppm;  $\delta_{\text{C}}$  (100 MHz, DMSO-*d*<sub>6</sub>) 172.0, 170.3, 169.5, 97.4, 70.3, 69.6, 69.1, 65.6, 63.6, 53.3, 39.8, 22.6, 21.2 ppm; LRMS (ESI): *m/z* 352 (M<sup>+</sup> + H), 374 (M<sup>+</sup> + Na); HRMS: found (M<sup>+</sup> + H) 352.1160 C<sub>13</sub>H<sub>22</sub>NO<sub>10</sub>Si requires 352.1165.

#### 5-Acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-2-*O*-triethylsilyl-4-*O*-*tert*-butyldimethylsilyl-D-glycero-D-galactononulopyranosic acid (18)

A solution of **9** (200 mg, 0.428 mmol) in Et<sub>2</sub>O (5 mL) and MeCN (5 mL) stirring at –78 °C under a N<sub>2</sub> atmosphere was treated with pyridine (172  $\mu$ L, 2.68 mmol) followed by TESOTf (193  $\mu$ L, 0.856 mmol). The reaction was stirred at –20 °C for 16 h then quenched with a saturated aqueous solution of NaHCO<sub>3</sub> (50 mL) and extracted with EtOAc (3x 50 mL). The combined organics were washed with brine (150 mL), dried (MgSO<sub>4</sub>) and concentrated *in vacuo* to give a colourless oil. Purification by flash column chromatography using a 95:5 to 9:1 to 1:1 petroleum ether : EtOAc gradient afforded the desired compound as a white solid (165 mg, 67%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> –15 (*c* 1.0, MeOH); mp: 163–165 °C;  $\nu_{\text{max}}$  (NaCl) 3427–3227 (br), 2722, 1755, 1630, 1572 cm<sup>–1</sup>;  $\delta_{\text{H}}$  (400 MHz, DMSO-*d*<sub>6</sub>): 7.91 (1H, d, *J* = 9.0 Hz, H-10), 4.55 (1H, br s, O-H7), 4.05–4.01 (1H, m, H-4), 3.99 (1H, dd, *J* = 12.0, 6.0 Hz, H-8), 3.93–3.87 (2H, m, H-6 + H-5), 3.74 (1H, br d, *J* = 10.5 Hz,

H-9), 3.64 (1H, br d, *J* = 10.5 Hz, H-9), 3.49 (1H, br d, *J* = 6.0 Hz, H-7), 2.10 (1H, dd, *J* = 12.0, 5.0 Hz, H-3eq), 1.82 (3H, s, H-12), 1.69 (1H, dd, *J* = 12.0, 12.0 Hz, H-3ax), 1.27 (3H, s, H-15), 1.22 (3H, s, H-15), 0.91 (9H, t, *J* = 8.0 Hz, H-21), 0.81 (9H, s, H-19), 0.50 (6H, q, *J* = 8.0 Hz), 0.04 (3H, s, H-17), 0.03 (3H, s, H-17) ppm;  $\delta_{\text{C}}$  (100 MHz, DMSO-*d*<sub>6</sub>): 171.8, 171.4, 107.3, 95.9, 76.1, 71.9, 68.5, 65.4, 65.0, 53.1, 40.0, 26.7, 25.8, 25.6, 22.6, 17.5, 6.9, 4.9, –4.6, –4.8 ppm; LRMS (ESI): *m/z* 578 (M<sup>+</sup> + H), 600 (M<sup>+</sup> + Na); HRMS: found (M<sup>+</sup> + H) 578.3107 C<sub>26</sub>H<sub>52</sub>NO<sub>9</sub>Si<sub>2</sub> requires 578.3102.

#### 5-Acetamido-7-*O*-acetyl-3,5-dideoxy-8,9-*O*-isopropylidene-2-*O*-triethylsilyl-4-*O*-*tert*-butyldimethylsilyl-D-glycero-D-galactononulopyranosic acid (19)

To a solution of **18** (100 mg, 0.173 mmol) in pyridine (2 mL) stirring at room temperature under a N<sub>2</sub> atmosphere was treated with Ac<sub>2</sub>O (33  $\mu$ L, 0.347 mmol). The reaction was stirred for 18 h then partitioned between CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and a saturated aqueous solution of NH<sub>4</sub>Cl (20 mL). The organic layer was washed with brine (20 mL), dried (MgSO<sub>4</sub>) and concentrated *in vacuo* to give an orange oil that was purified by flash column chromatography using a 1:2 to 3:1 EtOAc : petroleum ether gradient to give a white solid (80 mg, 75%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> –22 (*c* 1.0, MeOH); mp: 174–176 °C;  $\nu_{\text{max}}$  (NaCl) 3564, 2716, 1773, 1750, 1630, 1569 cm<sup>–1</sup>;  $\delta_{\text{H}}$  (400 MHz, DMSO-*d*<sub>6</sub>): 7.91 (1H, d, *J* = 9.0 Hz, H-10), 4.05–4.01 (1H, m, H-4), 3.98 (1H, dd, *J* = 12.0, 6.0 Hz, H-8), 3.91–3.84 (2H, m, H-6 + H-5), 3.74 (1H, br d, *J* = 10.5 Hz, H-9), 3.70 (1H, d, *J* = 6.0 Hz, H-7), 3.64 (1H, br d, *J* = 10.5 Hz, H-9), 2.08 (1H, dd, *J* = 12.0, 5.0 Hz, H-3eq), 1.90 (3H, s, H-15), 1.82 (3H, s, H-12), 1.65 (1H, dd, *J* = 12.0, 12.0 Hz, H-3ax), 1.28 (3H, s, H-17), 1.24 (3H, s, H-17), 0.90 (9H, t, *J* = 8.0 Hz, H-19), 0.81 (9H, s, H-21), 0.50 (6H, q, *J* = 8.0 Hz, H-22), 0.04 (3H, s, H-19), 0.03 (3H, s, H-19) ppm;  $\delta_{\text{C}}$  (100 MHz, DMSO-*d*<sub>6</sub>): 171.8, 171.5, 171.3, 107.3, 97.8, 76.1, 71.7, 70.4, 70.0, 65.6, 53.1, 39.9, 26.6, 25.7, 25.4, 22.5, 17.5, 6.9, 4.9, –4.6, –4.8 ppm; LRMS (ESI): *m/z* 620 (M<sup>+</sup> + H), 642 (M<sup>+</sup> + Na); HRMS: found (M<sup>+</sup> + H) 620.3200 C<sub>28</sub>H<sub>54</sub>NO<sub>9</sub>Si<sub>2</sub> requires 620.3208.

#### 5-Acetamido-7-*O*-acetyl-3,5-dideoxy-D-glycero-D-galactononulopyranosic acid (5)

A solution of **19** (70 mg, 0.11 mmol) in 60% AcOH (2 mL) was stirred for 2 h at 60 °C. The mixture was concentrated *in vacuo* to yield a brown solid which was purified by flash column chromatography using a 9:1 CH<sub>2</sub>Cl<sub>2</sub> : MeOH gradient to yield a white solid (47 mg, 72%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> –13 (*c* 1.0, MeOH); mp: 184–186 °C;  $\nu_{\text{max}}$  (NaCl) 3551, 3418–3265 (br), 2716, 1769, 1751, 1630, 1571 cm<sup>–1</sup>;  $\delta_{\text{H}}$  (400 MHz, DMSO-*d*<sub>6</sub>): 8.04 (1H, d, *J* = 9.0 Hz, H-10), 4.34 (1H, d, *J* = 5.5 Hz, O-H8), 4.24 (1H, t, *J* = 6.0 Hz, O-H9), 4.06–4.01 (1H, m, H-4), 3.90–3.84 (2H, m, H-6 + H-5), 3.63–3.57 (2H, m, H-7 + H-9), 3.51–3.46 (1H, m, H-8), 3.26 (1H, dd, *J* = 17.5, 6.0 Hz, H-9), 2.09 (1H, dd, *J* = 12.0, 5.0 Hz, H-3eq), 1.90 (3H, s, H-15), 1.82 (3H, s, H-12), 1.65 (1H, dd, *J* = 12.0, 12.0 Hz, H-3ax), 0.90 (9H, t, *J* = 8.0 Hz, H-21), 0.78 (9H, s, H-19), 0.52 (6H, q, *J* = 8.0 Hz, H-20), 0.04 (3H, s, H-17), 0.03 (3H, s, H-17) ppm;  $\delta_{\text{C}}$  (100 MHz, DMSO-*d*<sub>6</sub>): 171.9, 171.5, 171.3, 97.7, 71.7, 70.4, 69.8, 65.7, 63.6, 53.1, 40.0, 25.7, 22.6, 17.5, 6.9, 4.9, –4.6, –4.8 ppm;

LRMS (ESI):  $m/z$  580 ( $M^+ + H$ ), 602 ( $M^+ + Na$ ); HRMS: found ( $M^+ + H$ ) 580.2888  $C_{25}H_{50}NO_9Si_2$  requires 580.2895.

To a solution of the above compound (35 mg, 0.06 mmol) in THF (1 mL) and  $H_2O$  (1 mL) stirring at 0 °C was added 1.0 M TBAF in THF (300  $\mu$ L, 0.30 mmol). The reaction was stirred for 20 h then was concentrated *in vacuo* to give a yellow oil. The crude material was purified by flash column chromatography using a 3 : 1  $CH_2Cl_2$  : MeOH gradient to give a white solid (17 mg, 81%).  $[\alpha]_D^{25}$   $-35$  ( $c$  1.0, MeOH); mp: 185–187 °C;  $\nu_{max}$  (NaCl) 3441–3297 (br), 2739, 1775, 1753, 1618, 1561  $cm^{-1}$ ;  $\delta_H$  (400 MHz, DMSO- $d_6$ ): 8.08 (1H, d,  $J$  = 8.0 Hz, H-10), 6.70 (1H, d,  $J$  = 2.0 Hz, O-H2), 4.84 (1H, d,  $J$  = 6.0 Hz, O-H4), 4.34 (1H, d,  $J$  = 5.5 Hz, O-H8), 4.25 (1H, t,  $J$  = 6.0 Hz, O-H9), 3.82 (1H, ddd,  $J$  = 10.5, 10.5, 5.0 Hz, H-4), 3.74 (1H, dd,  $J$  = 10.5, 1.5 Hz, H-6), 3.61–3.55 (2H, m, H-7 + H-9), 3.54–3.45 (1H, m, H-8), 3.53 (1H, ddd,  $J$  = 10.0, 10.0, 10.0 Hz, H-5), 3.30 (1H, dd,  $J$  = 11.0, 7.0 Hz, H-9), 2.07 (1H, dd,  $J$  = 12.5, 5.0 Hz, H-3eq), 1.92 (3H, s, H-15), 1.89 (3H, s, H-12), 1.70 (1H, dd,  $J$  = 13.0, 11.0 Hz, H-3ax) ppm  $\delta_C$  (100 MHz, DMSO- $d_6$ ): 171.8, 171.5, 171.3, 94.7, 70.4, 69.8, 69.2, 65.7, 63.6, 53.1, 22.6, 21.4 ppm; LRMS (ESI):  $m/z$  352 ( $M^+ + H$ ), 374 ( $M^+ + Na$ ); HRMS: found ( $M^+ + H$ ) 352.1160  $C_{13}H_{22}NO_{10}Si$  requires 352.1165.

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