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# Observations on chemical and enzymatic approaches to α-2,3-sialylated octyl β-lactoside

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**Abstract**—A comparison of chemical and chemo-enzymatic syntheses of α-2,3-sialylated octyl lactoside is reported. The chemical approach, starting from lactose and sialic acid, required 14 steps and proceeded in 5% overall yield; poor α-selectivity in the sialylation step necessitated a difficult and low yielding separation of anomers. A chemoenzymatic approach, employing recombinant *Trypanosoma cruzi trans*-sialidase to effect the key sialylation reaction, required 10 steps and gave a similar overall yield. Whereas the chemo-enzymatic synthesis required only three chromatographic purification steps overall, the chemical synthesis required at least nine. 

## 1. Background

### 1.1. Introduction

Sialylated oligosaccharides are widespread in nature and fulfill a host of biological roles, particularly relating to cellular adhesion and recognition events.[1] There is increasing interest in therapeutics based on glycobiology targets,[2] and at a more fundamental level, chemical biologists have begun to address the manipulation of cellular glycosylation.[3] It is widely accepted that whilst there are a plethora of literature procedures for the chemical synthesis of such molecules,[4] few methods are general, or routinely reliable in inexperienced hands. The major drawback of a chemical approach to oligosaccharide synthesis is the necessity for extensive (and tedious) protecting group chemistry, which is often essential to control both the regio- and stereoselectivity of glycosylation reactions. This invariably leads to multi-step reaction sequences and low overall yields often result. Recent advances in 'programmable' one-pot synthesis[5] and automated solid-phase synthesis[6] of oligosaccharides have begun to make synthesis of this complex class of natural products tractable. The alternative is to use the enzymatic procedures on which Nature relies for constructing and remodelling complex glycans in vivo.[7–9] Increasingly these highly precise bio-catalysts are becoming available for use as synthetic tools, especially the glycosidases,[10] and so-called 'glycosynthase' variants thereof,[11,12] which do not rely on expensive sugar-nucleotide donor substrates. Of late, this last point has also been addressed.[13] The efficient production of sugar nucleotides by recombinant proteins is becoming more routine, earlier problems with heterologous expression of active glycosyltransferase have seemingly been overcome, multi-enzyme and fusion protein-based syntheses are effective in vitro, and metabolic engineering has also proved effective in kilogram scale oligosaccharide production.[14–17]

### 1.2. Objective

In connection with studies on sialylated glycoconjugates we had a need to prepare α-2,3-sialylated octyl β-lactoside, **1**, along with the de-*N*-acetyl derivative thereof. With only limited prior experience of preparative sialylation procedures, we embarked on a comparison of chemical and chemo-enzymatic approaches to our desired targets.

### 1.3. Previous chemical and enzymatic syntheses of α-2,3-sialyl lactosides

Trisaccharide **1** is based on the ganglioside $GM_3$ structure, several syntheses of which have been reported previously.[18] Both chemical and enzymatic approaches have been employed. More generally, chemical and enzymatic methods for *O*-sialylation have been extensively reviewed by Boons and Demchenko.[19]

The hydrolytic neuraminidases have not proved overly effective in reverse synthetic mode,[20a] although recent

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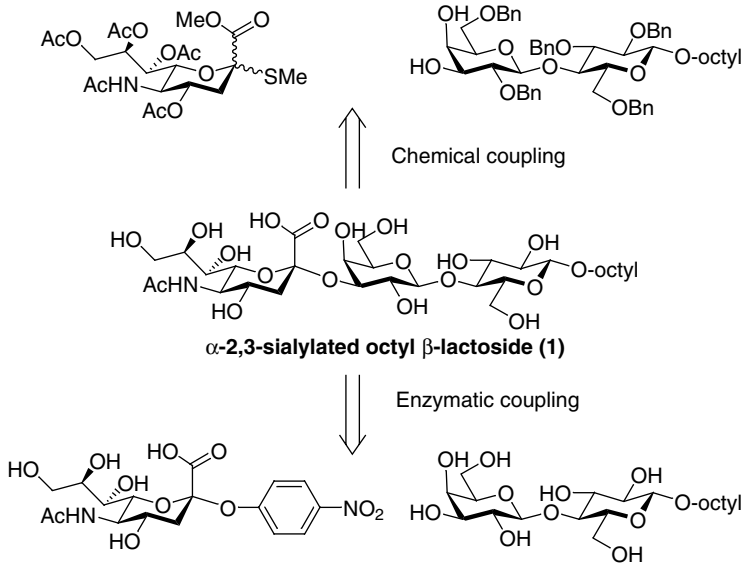
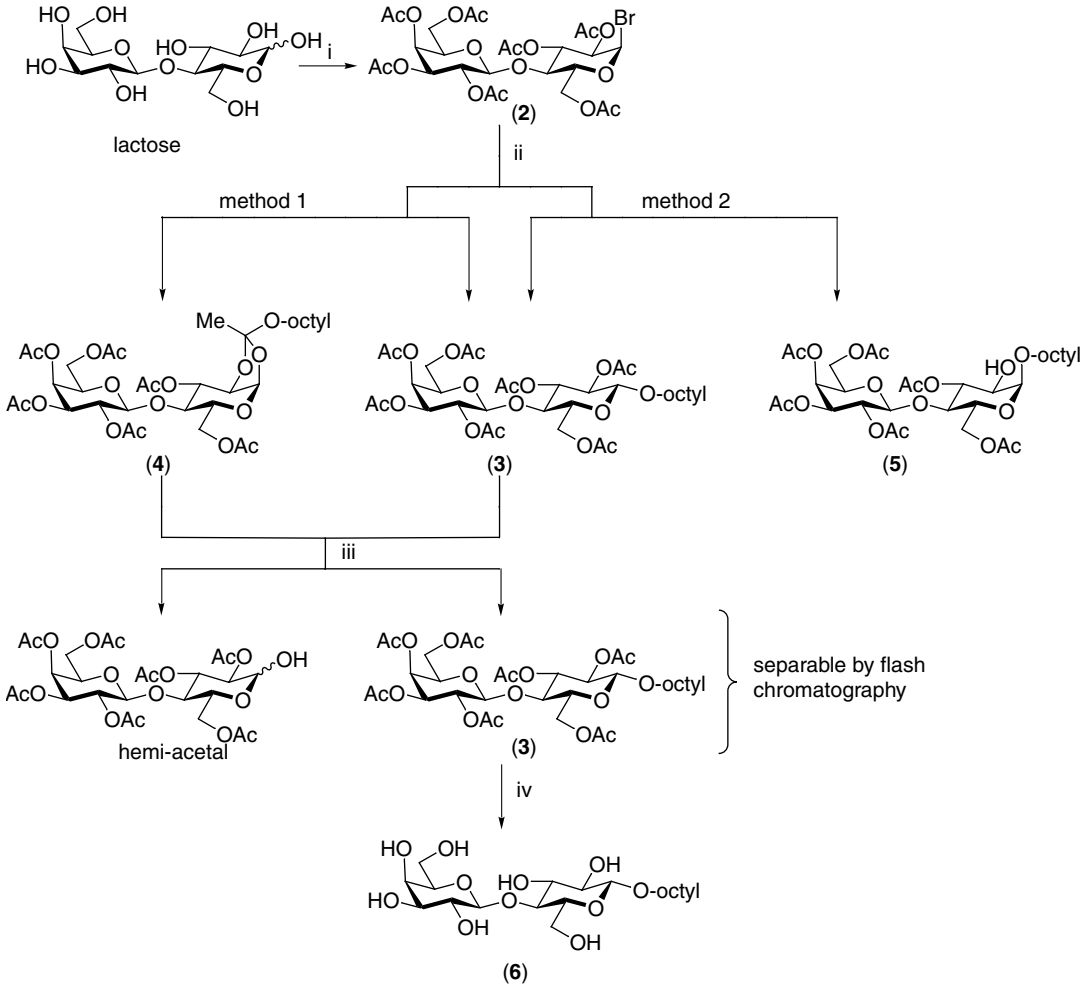
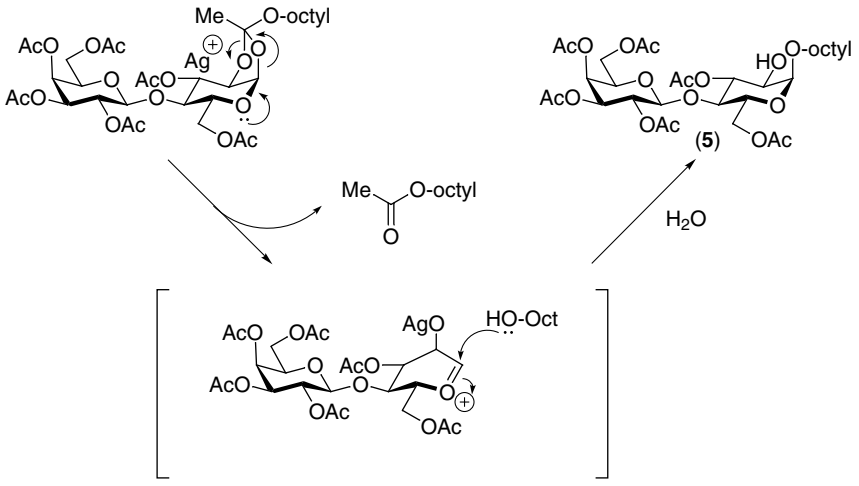



**Scheme 1.** Key disconnections for chemical and chemo-enzymatic syntheses of α-2,3-sialylated octyl lactoside **1**.




**Scheme 2.** Synthesis of octyl β-lactoside **6**. *Reagents*: (i) $Ac_2O$, HBr–AcOH (95%); (ii) $AgClO_4$, $Ag_2CO_3$, octanol (see Section 4); (iii) TFA, $H_2O$, $CH_2Cl_2$; (iv) NaOMe, MeOH (99%).




**Scheme 3.** Mechanism for the formation of α-glycoside **5**.

work from Thiem, in particular, highlights their potential.[20b,c] The *trans*-sialidase from the South American parasite *Trypanosoma cruzi* was the enzyme of choice for our particular study. This α-2,3-specific enzyme is used by these parasites to 'steal' sialic acid from infected host glycoconjugates—the parasite does not produce sialic acid. Acquisition of a negatively charged surface coat is thought to be key to host cell adhesion and hence the cell invasion process by this obligate intracellular parasite.[21] Whilst *trans*-sialidase usually employs sialylated oligosaccharides as donor substrates, it can also transfer sialic acid from simple glycosides such as the 4-methylumbelliferyl and the readily available *p*-nitrophenyl sialosides.[22c] There have been several reports of the use of this enzyme in synthesis.[22] A 70 kDa recombinant form of the catalytic domain of *trans*-sialidase is under investigation in our laboratory[23] and was employed in studies reported herein.

## 2. Results and discussion

### 2.1. Disconnections

In the current study, two routes were considered for the synthesis of α-2,3-sialylated octyl β-D-lactoside **1**, one purely chemical, the other using a combination of chemical and enzymatic steps (Scheme 1).

### 2.2. Octyl β-lactoside synthesis (Scheme 2)[‡]

Lactose, being inexpensive and readily available, was chosen as the starting material for the preparation of octyl β-lactoside, a key intermediate for both chemical and enzymatic elaboration. Lactose was converted in a one-pot reaction[24] to hepta-*O*-acetyllactosyl bromide **2** in 95% yield with acetic anhydride and 30% HBr/AcOH. However, subsequent glycosylation of octanol with this glycosyl halide in the presence of silver perchlorate/silver carbonate gave variable results. A 10% excess of $AgClO_4$ monohydrate and 2.5 equiv. of octanol, dried together overnight with 4 Å molecular sieves prior to addition of $Ag_2CO_3$ and glycosyl bromide **2** gave a 56% yield of the desired product, **3** [$\delta_H$ 4.40, 4.50 (2×1H, 2d, $J_{1a,2a}=J_{1b,2b}=7.7$, 8.0 Hz, 1a-H, 1b-H)], but also a considerable quantity of the orthoester **4** [$\delta_H$ 4.62 (1H, d, $J_{1b,2b}=8.0$ Hz, 1b-H), 5.64 (1H, d, $J_{1a,2a}=5.2$ Hz, 1a-H)]. Although formation of the orthoester creates a new stereogenic centre only one diastereomer was observed, presumably the *exo*-isomer. The orthoester and glycoside were not easily separated by flash chromatography, but treatment of the mixture with 50% aqueous TFA prior to chromatography, to hydrolyse the orthoester to the hemiacetal, proved effective (Scheme 2). Standard deacetylation of per-*O*-acetylated octyl lactoside **3** with sodium methoxide in methanol gave the corresponding unprotected glycoside **6**[25] in near quantitative yield [$\delta_H$ 4.40, 4.41 (2×1H, 2d, $J_{1a,2a}=J_{1b,2b}=7.4$, 8.0 Hz, 1a-H, 1b-H)].

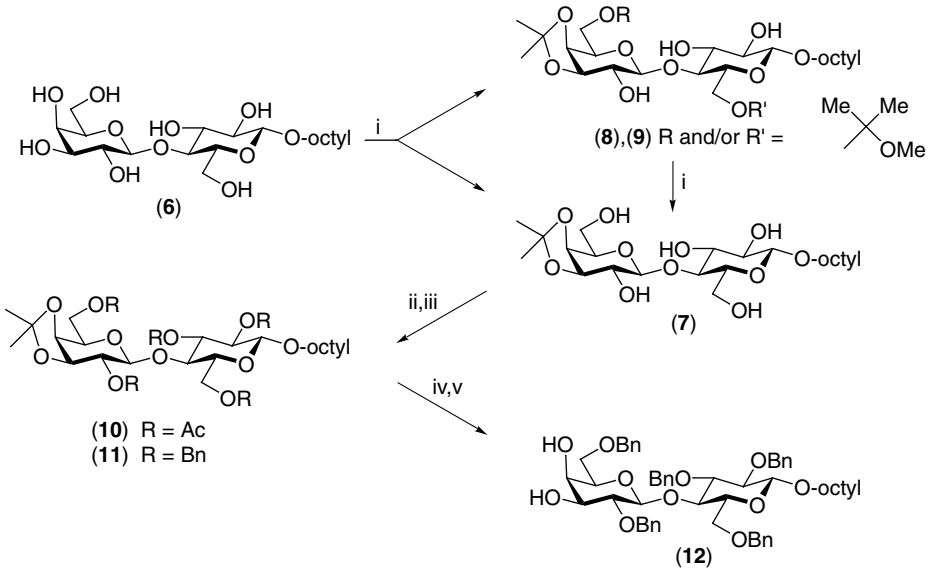
Attempts to improve on the above glycosylation chemistry produced unexpected results. In the presence of octanol, $AgClO_4$ suffered some degradation during overnight drying, giving a brown solution. Repeating the reaction without adding octanol to the other reagents until directly prior to the bromide addition gave no orthoester, but instead a different by-product was identified. This proved to be the α-configured, 2-de-*O*-acetyl octyl glycoside, **5** (Scheme 2), which was formed in 28% yield, along with 44% of the expected β-glycoside, **3**. α-Glycoside **5** presumably results from loss of octyl acetate from the orthoester and attack by octanol to give the thermodynamically favoured α-lactoside, there being no participating groups present on C-2 (Scheme 3).[§] Whilst the yield of α-glycoside **5** obtained in this study is not exceptional, it is hard to conceive of a more straightforward route to α-linked alkyl lactosides.

### 2.3. Chemical synthesis approach

**2.3.1. Acceptor synthesis for chemical coupling (Scheme 4).** Octyl lactoside **6** was protected with an isopropylidene ketal across the 3b-,4b-hydroxyl groups to give **7**

[‡] For di- and tri-saccharides, the monosaccharide residues are labelled a, b, c from the reducing terminus.

[§] Attempts to effect orthoester rearrangement in the glycosylation reaction by increasing the quantity of $AgClO_4$ to 1.5 mol equiv. (with respect to glycosyl bromide) had little impact on the ratio of products formed.




**Scheme 4.** Syntheses of lactoside acceptor **12**. *Reagents*: (i) (a) $Pr^{i}(OMe)_2$, *p*-toluenesulfonic acid, (b) TFA–$H_2O$, (66%); (ii) $Ac_2O$, pyridine (quant); (iii) NaH, BnBr, DMF (95%); (iv) AcOH(aq) (98%).

in 66% yield. This was achieved using an excess of dimethoxypropane and catalytic *p*-toluenesulfonic acid, with subsequent selective hydrolysis of any 6a- and/or 6b-based mixed methanol ketals, **8/9**, with aqueous TFA. The position of the protecting group was confirmed by acetylation of a small portion of the product, to give per-*O*-acetate **10**, which showed a clear downfield shift of the 6a-H and 6b-H signals. Ketal **7** was then benzylated under standard conditions, giving **11**, followed by hydrolysis of the acetonide to give the diol acceptor **12** in 90% yield (Scheme 4). Although two hydroxyl groups are still free in acceptor **12**, glycosylation has shown to be selective for the equatorial 3b position of galactosides over the less reactive, axial 4b-OH.[26]

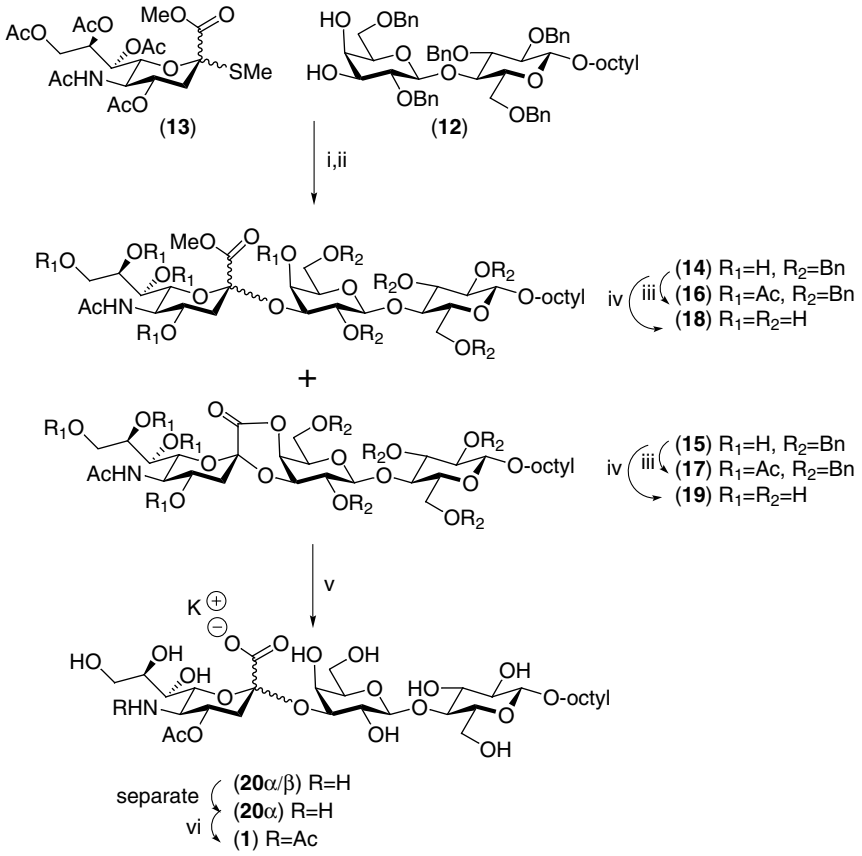
**2.3.2. Donor synthesis for chemical coupling.** The sialic acid donor **13** was prepared in three steps, by a literature procedure,[27] in 74% overall yield from sialic acid. Briefly, the per-*O*-acetylated sialic acid methyl ester, prepared from sialic acid by methyl esterification and per-*O*-acetylation, was treated with (methylthio)trimethylsilane and TMSOTf to give the methyl thioglycoside **13** as an anomeric mixture (α/β, 1:1).

**2.3.3. Chemical glycosylation (Scheme 5).** Glycosylation of diol acceptor **12** was performed with 2.5 equiv. of donor **13** and *N*-iodosuccinimide and catalytic triflic acid as promoter, according to a literature procedure.[26] It proved convenient to deacetylate the product mixture prior to column chromatography, which yielded a mixture of trisaccharides **14** and **15** (Scheme 5).

A small sample of the mixture was partially separated by careful chromatography and the fractions reacetylated to give an α/β mixture of the methyl ester **16** and the α-linked 3c→4b lactone **17**, which was identified by FAB-MS and by comparison of NMR spectral data for analogous literature compounds (Section 4).[28,29] It is unclear at what point the lactonisation occurred; acid-promoted lactonisation during the glycosylation reaction is one possibility, but the partial conversion of sialoside methyl esters to lactones under Zemplén deacetylation conditions has also been reported.[29] Hydrogenolysis of each of the deacetylated fractions using palladium hydroxide in MeOH gave a mixture of methyl ester(s) **18** and lactone **19**. The remaining product mixture was debenzylated in a similar fashion and then treated with hot 1 M potassium hydroxide solution to hydrolyse both the ester/lactone and also the sialic acid acetamide, as trial experiments had shown the α and β anomers to be best resolved on TLC as the amino compounds **20**α/β. The deprotected product was obtained in 49% yield from acceptor **12** as a 6:1, α:β mixture, whereas the published procedure noted total anomeric stereocontrol to give the α-sialoside in similar yield.[26] Although the anomers could be separated on a preparative TLC plate, or by repeated flash chromatography, this tedious final purification step gave the desired product in only 21% yield. Reacetylation of the amino group was achieved using 3 equiv. of acetic anhydride in MeOH solution giving trisaccharide **1** in high yield.

## 2.4. Enzymatic approach

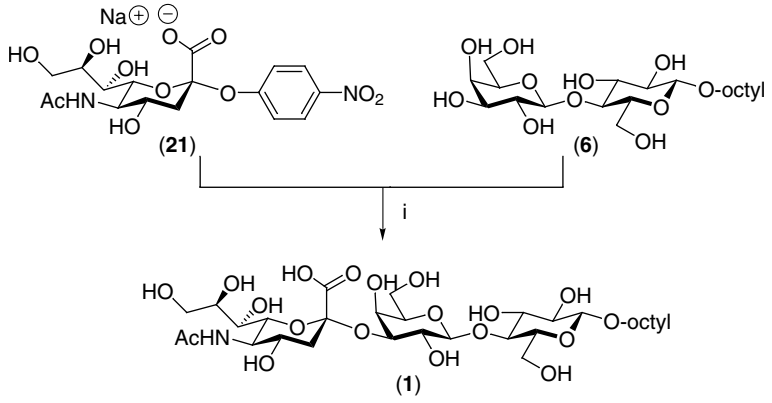
**2.4.1. Donor synthesis for enzymatic coupling.** Whilst we have been unable to determine reliable kinetic parameters for the hydrolysis or transfer of sialic acid from its *p*-nitrophenyl glycoside by *trans*-sialidase,[23] this substrate, which is straightforward to prepare on a gram scale, is very effective for milligram-scale biotransformations, which proceed with high efficiency when stoichiometric acceptor is present.[23] A more detailed account of this observation can be seen in the recent work of Crout and co-workers.[22c] The *p*-nitrophenyl sialoside glycosyl donor substrate **21**[30] was prepared from the corresponding protected glycosyl chloride under phase transfer conditions. Deacetylation and saponification of the resulting methyl ester gave the required Neu5AcαpNP donor **21** in 45% overall yield from sialic acid.




**Scheme 5.** Chemical synthesis of α-2,3-sialylated octyl β-lactoside, **1**, and its de-*N*-acetyl derivative, **18**. Reagents: (i) NIS, TfOH, MeCN; (ii) NaOMe, MeOH; (iii) $Ac_2O$, pyridine; (iv) $H_2$, $Pd(OH)_2$, MeOH; (v) KOH(aq) (49% from **14**. α/β, 6:1); (vi) $Ac_2O$, MeOH (88%).

**2.4.2. Enzymatic glycosylation (Scheme 6).** Biotransformations were conducted with a 70 kDa recombinant construct of the *Trypanosoma cruzi trans*-sialidase. This construct is truncated to remove C-terminal repeats, retains the catalytic N-terminal part of the enzyme, and is His-tagged to aid purification.[31] In our hands, *trans*-sialidase proved labile in both partially and fully purified form, and retained its activity far better, both in reactions and on frozen storage, as a crude *E. coli* lysate. For routine use, therefore, cleared cell lysates were used rather than purified enzyme for enzymatic syntheses.

The *trans*-glycosylation reaction catalysed by the *trans*-sialidase is fully reversible. One would therefore expect the equilibrium product mixture to reflect the relative concentrations of acceptors present. After incubating octyl lactoside, **6**, and 1.5 equiv. of the donor **21** with the *trans*-sialidase overnight at 30°C, TLC indicated that the reaction had gone about half way to completion. Although the reaction can be pushed further towards completion by increasing the concentration of the donor, excess unreacted donor can complicate product isolation. Reactions were therefore not generally pushed to complete consumption of the acceptor, which could be recovered during purification in a straightforward manner. It proved more convenient to stop the reaction after a relatively short reaction time and




**Scheme 6.** Enzymatic synthesis of α-2,3-sialylated octyl β-lactoside, **1**. Reagents: (i) *trans*-sialidase, phosphate buffer pH 7 (42%).

separate the product from the reactants. For these particular compounds (i.e. octyl glycosides) preparative reverse phase chromatography on C-18 silica proved to be highly efficient in this regard, and far less time consuming than gel filtration on Biogel resin, which has proved useful for reducing sugar products.[22c]

Having removed most of the protein by precipitation with ethanol, the supernatant was concentrated and redissolved in water before applying to a C-18 column. The excess Neu5AcαpNP and virtually all of the yellow *p*-nitrophenol were eluted from the column with approximately four column volumes of water. The product and acceptor were then eluted using a fairly crude stepwise methanol gradient; the sialylated product eluted at approximately 25–50% MeOH, the unreacted acceptor eluting with 75% MeOH. Unreacted lactoside acceptor was recovered in suitably pure form for direct re-use. Using the method outlined 10 mg batches of the tri-saccharide **1** could be prepared and purified within 24 h from the easily accessible donor and acceptor **21** and **6**, respectively. Treatment of acetamido-trisaccharide **1** with hot 1 M potassium hydroxide solution gave the amino-trisaccharide **20**α in near quantitative yield, so potentially providing access to a range of *N*-acyl trisaccharide derivatives.

## 3. Conclusions

The chemo-enzymatic synthesis of α-2,3-sialylated octyl lactoside **1** was completed in 10 steps from lactose and sialic acid, in an overall yield of 10% The longest linear sequence was seven steps from sialic acid. This should be compared with the purely chemical approach, which was four steps longer and gave an overall yield of only 5%. These two yields would have been almost identical had it not been for the poor α-selectivity of the chemical sialylation, which necessitated a difficult and low yielding chromatographic separation of anomers. It is also worth noting that the chemo-enzymatic synthesis required only three purifications by silica gel or reverse phase chromatography, whereas the chemical synthesis required at least nine chromatographic purification steps. A crude extrapolation from the preparations described herein suggests that a 10L culture of *E. coli* expressing *trans*-sialidase would provide sufficient enzyme for synthesis on a tens of grams scale. We conclude that *trans*-sialidase is a useful tool in oligosaccharide synthesis, allowing rapid and technically undemanding stereo- and regio-selective synthesis of α-2,3-linked sialosides in overall yields in excess of those routinely attainable by chemical synthesis, and on scales ranging from radiochemical to at least tens of grams.

## 4. Experimental

### 4.1. General

All reagents and solvents were dried prior to use according to standard methods.[32] Commercial reagents were used without further purification unless stated. Analytical TLC was performed on silica gel 60-$F_{254}$ (Merck or Whatman) with detection by fluorescence and/or by charring following immersion in a dilute ethanolic solution of sulfuric acid. An orcinol dip, prepared by the careful addition of concentrated sulfuric acid (20 $cm^3$) to an ice cold solution of 3,5-dihydroxytoluene (360 mg) in EtOH (150 $cm^3$) and water (10 $cm^3$), was used to detect deprotected compounds by charring. Flash chromatography was performed with silica gel 60 (Fluka). Reverse phase chromatography was typically performed on 15 g C-18 silica gel 100 (Fluka) eluting with $H_2O$ (80 $cm^3$) and then MeOH–$H_2O$, 1:3 (20 $cm^3$), 1:1 (20 $cm^3$), 3:1 (20 $cm^3$) and finally with MeOH (20 $cm^3$). For smaller scale work C-18 Sep-pak cartridges (500 mg; Waters) were used and eluted with 2 $cm^3$ of each of the above eluents.

During work-up, organic solutions were washed two or three times with equal volumes of each of the aqueous solutions listed. Standard work-up **A** involved washing organic solutions successively with water, saturated $NaHCO_3$ solution and water; standard work-up **B** involved washing organic solutions successively with 1 M HCl solution, saturated $NaHCO_3$ solution and water. All such organic solutions were then dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuo.

Optical rotations were measured at the sodium D-line and at ambient temperature, with an Optical Activity AA-1000 polarimeter. $[\alpha]_D$ values are given in units of $10^{-1}$ deg $cm^2$ $g^{-1}$. Melting points were measured using a Gallenkamp melting point apparatus and are uncorrected. IR spectra were recorded as thin films on NaCl plates using a Perkin–Elmer 1710 IRFT spectrometer. Fast atom bombardment (FAB) mass spectra were recorded on a Fisons VG Autospec spectrometer using a 3-nitrobenzyl alcohol matrix. Electrospray mass spectra (ES-MS) were recorded on a Fisons VG Biotech electrospray mass spectrometer. Unless stated otherwise, $^1$H NMR and $^{13}$C NMR spectra were recorded on a Varian Gemini 2000 spectrometer at 300 and 75 MHz, respectively. $^1$H NMR spectra were referenced to the following internal standards: $CHCl_3$, $\delta_H$ 7.26 in $CDCl_3$; $CD_2HOD$, $\delta_H$ 3.31 in $CD_3OD$; $CH_3OH$, $\delta_H$ 3.43 in $D_2O$. $^{13}$C NMR spectra were referenced to the following internal standards: $CDCl_3$, $\delta_C$ 76.9 in $CDCl_3$; $CD_3OD$, $\delta_C$ 49.15 in $CD_3OD$; $CH_3OH$ $\delta_C$ 49.9 in $D_2O$. *J*-values are given in Hz. Only partial NMR data are given for some compounds; other spectral features were in accord with the proposed structures but are not sufficiently resolved at 300 MHz to be useful. Signals for $CF_3$ were not recorded in $^{13}$C NMR experiments of compounds containing the trifluoromethanesulfonyl group.

### 4.2. Synthetic procedures

The following compounds were prepared essentially as described in the literature: 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide **2**;[24] methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α/β-D-*galacto*-2-nonulopyranosid)onate, **13**;[27] 4-nitrophenyl *O*-(sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate), **21**.[30,33]

**4.2.1. Octyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside, 3.** *Method*

*one*. A suspension of silver perchlorate (4.96 g, 22 mmol), 4 Å molecular sieves (10 g), octanol (4.56 $cm^3$, 50 mmol) and dry $CH_2Cl_2$ (110 $cm^3$) was stirred overnight at room temperature in a tin foil covered flask in order to exclude light. Silver carbonate (8.27 g, 30 mmol) was added, followed by glycosyl bromide **2** (13.99 g, 20 mmol) and the mixture was stirred at room temperature for 24 h. The mixture was then filtered through Celite and aqueous TFA (1 $cm^3$; 50% v/v solution) was added to the filtrate. After stirring for 2 h, the solution was subjected to standard work-up A. Concentration gave a syrup. Flash chromatography (silica gel; hexane–EtOAc, 3:1→1:1) gave the desired *acetylated octyl lactoside* **3** as an amorphous mass (8.45 g, 56%), $[\alpha]_D=-7.7$ (*c* 1.0, $CHCl_3$) (Found: C, 54.77; H, 7.26. $C_{34}H_{52}O_{18}$ requires C, 54.54; H, 7.00%); $\delta_H(CDCl_3)$ 0.86 (3H, t, *J*=6.9 Hz, $C_7H_{14}CH_3$), 1.25 (10H, m, $(CH_2)_5$ $CH_3$), 1.55 (2H, m, $OCH_2CH_2$), 1.94–2.14 (7×3H, 7s, 7×AcO), 3.45 (1H, m, $OCH_2$), 4.40, 4.50 (2×1H, 2d, $J_{1a,2a}=J_{1b,2b}=7.7$, 8.0 Hz, 1a-H, 1b-H), 4.86 (1H, dd, $J_{1a,2a}=J_{2a,3a}=9.3$ Hz, 2a-H), 4.94 (1H, dd, $J_{2b,3b}=10.2$ Hz, $J_{3b,4b}=3.2$ Hz, 3b-H), 5.09 (1H, dd, $J_{1b,2b}=J_{2b,3b}$, 2b-H), 5.17 (1H, t, $J_{2a,3a}=J_{3a,4a}=9.3$ Hz, 3a-H), 5.33 (1H, d, $J_{3b,4b}=3.3$ Hz, 4b-H); $\delta_C(CDCl_3)$ 14.1, 20.5, 20.7 (4), 20.9, 22.7, 25.85, 29.3 (2), 29.45 (2), 31.8, 60.9, 62.2, 66.8, 69.3, 70.3, 70.8, 71.1, 71.9, 72.7, 73.0, 76.5, 100.8, 101.2, 169.35, 169.85, 170.1, 170.3, 170.4, 170.6, 170.7.

*Method two*. A suspension of silver perchlorate (4.96 g, 22 mmol), 4 Å molecular sieves (10 g), and dry $CH_2Cl_2$ (125 $cm^3$) was stirred overnight at room temperature in a tin foil covered flask in order to exclude light. Octanol was added (4.56 $cm^3$, 50 mmol) followed by silver carbonate (8.27 g, 30 mmol), glycosyl bromide **2** (13.99 g, 20 mmol) and the mixture was stirred at room temperature for 24 h. The mixture was then filtered through Celite and gave a syrup on concentration. Flash chromatography (silica gel; hexane–EtOAc, 3:1→1:1) gave the desired *acetylated octyl lactoside* **3** as an amorphous mass (6.51 g, 44%) which gave identical analytical data to the above. Further elution (hexane–EtOAc, 1:2) gave *octyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-acetyl-α-D-glucopyranoside* **5** as a glassy solid (3.90 g, 28%), (Found: C, 54.16; H, 7.09. $C_{32}H_{50}O_{17}$ requires C, 54.18; H, 7.13%); $[\alpha]_D=+64.4$ (*c* 1 in $CHCl_3$); $\delta_H(CDCl_3)$ 0.86 (3H, m, $C_7H_{14}CH_3$), 1.26 (10H, m, $(CH_2)_5$ $CH_3$), 1.55 (2H, m, $OCH_2CH_2$), 1.94–2.14 (6×3H, 6s, 6×AcO), 4.49 (1H, d, $J_{1b,2b}=8.0$ Hz, 1b-H), 4.81 (1H, dd, $J_{1a,2a}=3.8$ Hz, 1a-H), 4.93 (1H, dd, $J_{2b,3b}=10.4$ Hz, $J_{3b,4b}=3.5$ Hz, 3b-H), 5.09 (1H, dd, $J_{1b,2b}=J_{2b,3b}$, 2b-H), 5.20 (1H, t, $J_{2a,3a}=J_{3a,4a}=9.5$ Hz, 3a-H), 5.33 (1H, dd, $J_{3b,4b}=J_{4b,5b}=1.0$ Hz, 4b-H). A sample was acetylated using pyridine–acetic anhydride and purified by flash chromatography (silica gel; hexane–EtOAc, 1:1) to give *octyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-acetyl-α-D-glucopyranoside*. The following $^1H$ NMR data are consistent with the *peracetylated α lactoside*: $\delta_H(CDCl_3)$ 0.85 (3H, m, $C_7H_{14}CH_3$), 1.28 (10H, m, $(CH_2)_5$ CH3), 1.55 (2H, m, $OCH_2CH_2$), 1.94–2.20 (7×3H, 7s, 7×AcO), 4.46 (1H, d, $J_{1b,2b}=8.0$ Hz, 1b-H), 4.74 (1H, dd, $J_{1a,2a}=3.6$ Hz, $J_{2a,3a}=10.1$ Hz, 2a-H), 4.86 (1H, dd, $J_{2b,3b}=10.4$ Hz, $J_{3b,4b}=3.6$ Hz, 3b-H), 4.94 (1H, d, $J_{1a,2a,}$ 1a-H), 5.09 (1H, dd, $J_{1b,2b}=J_{2b,3b}$, 2b-H), 5.32 (1H, br d, $J_{3b,4b}$, 4b-H), 5.44 (1H, t, $J_{2a,3a}=J_{3a,4a}=10.1$ Hz, 3a-H).

**4.2.2. Octyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside, 6.**[25] A solution of both compound **3** (8.35 g, 11.2 mmol) and sodium metal (100 mg, 4.3 mmol) in dry MeOH (80 $cm^3$) was stirred for 1 h at room temperature. More MeOH (120 $cm^3$) was added to dissolve the resulting precipitate and the solution was neutralised with Amberlite IRC-50 ($H^+$) resin (1 g). Filtration and concentration gave the fully deprotected compound **6** as an amorphous white solid (5.02 g, 99%); $[\alpha]_D=-9.2$ (*c* 1 in MeOH); $\delta_H[CD_3OD–D_2O$ (1:1)] 0.86 (3H, t, *J*=6.9 Hz, $C_7H_{14}CH_3$), 1.22–1.40 (10H, m, $(CH_2)_5$ $CH_3$), 1.62 (2H, m, $OCH_2CH_2$), 4.40, 4.41 (2×1H, 2d, $J_{1a,2a}=J_{1b,2b}=7.4$, 8.0 Hz, 1a-H, 1b-H); $\delta_C[CD_3OD–D_2O$ (1:1)] 13.7, 23.0, 26.4, 29.7, 29.9, 30.1, 32.0, 61.3, 61.8, 69.7, 70.3, 71.9, 74.15, 74.2, 75.9, 75.9, 76.5, 80.1, 103.6, 104.5; ES-MS (−ve): *m/z* 453 $(M-H)^-$, 489.5 $(M+Cl)^-$ ($C_{20}H_{38}O_{11}$ requires *m/z* 454). NMR data consistent with the literature.[25]

**4.2.3. Octyl 3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside, 7.** A solution of *octyl lactoside* **6** (2.00 g, 4.40 mmol) and *p*-toluenesulfonic acid (80 mg, 0.4 mmol) in 2,2-dimethoxypropane (40 $cm^3$) was stirred at room temperature for 60 h. The reaction was quenched with triethylamine (0.5 $cm^3$) and concentrated to a solid foam which was suspended in EtOAc (30 $cm^3$). Aqueous TFA (200 $mm^3$; 50% v/v) was added and after stirring for 1 h, the reaction was again quenched with triethylamine and evaporated onto silica (10 g). Flash chromatography (silica gel, 100 g; EtOAc then EtOAc–MeOH, 19:1) gave the title compound **7** as an amorphous white solid (1.44 g, 66%), (Found: C, 55.75; H, 8.4. $C_{23}H_{42}O_{11}$ requires C, 55.86; H, 8.56%); $[\alpha]_D=+9.9$ (*c* 0.87 in MeOH); $\delta_H(CD_3OD)$ 0.90 (3H, t, *J*=6.9 Hz, $C_7H_{14}Me$), 1.26–1.40 (13H, m, $(CH_2)_5CH_3$, $CMe_2$), 1.48 (3H, s, $CMe_2$), 1.62 (2H, m, $OCH_2CH_2$), 4.28, 4.37 (2×1H, 2d, $J_{1a,2a}=J_{1b,2b}=7.4$, 7.7 Hz, 1a-H, 1b-H); $\delta_C(CD_3OD)$ 14.5, 23.8, 26.6, 27.2, 28.5, 30.5, 30.7, 30.9, 33.1, 62.1, 62.6, 71.1, 74.6, 75.0, 75.2, 75.5, 76.5, 76.6, 81.0, 81.2, 104.4 (2), 111.3.

A small sample (80 mg) of the above was acetylated using pyridine–acetic anhydride and purified by flash chromatography (silica gel; toluene–EtOAc, 2:1) to give *octyl 2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside* **10** as a colourless syrup (105 mg, 95%), (Found: C, 56.48; H, 7.72. $C_{33}H_{52}O_{16}$ requires C, 56.25; H, 7.44%); $[\alpha]_D=+6.4$ (*c* 1 in $CHCl_3$); $\nu$max/$cm^{-1}$ 2930 ($CH_2$, $CH_3$), 1750 (C=O), 1370 ($Pr^i$, C–H), 1042 (C–O); $\delta_H(CDCl_3)$ 0.84 (3H, t, *J*=6.9 Hz, $C_7H_{14}CH_3$), 1.20–1.30 (13H, m, $(CH_2)_5CH_3$, $CMe_2$), 1.50 (5H, m, $OCH_2CH_2$, $CMe_2$), 2.00, 2.01, 2.05, 2.08, 3.00 (5×3H, 5s, 5×AcO), 3.42 (1H, m, $OCH_2$), 3.58 (1H, ddd, $J_{4a,5a}=9.9$ Hz, $J_{5a,6a}=4.9$ Hz, $J_{5a,6a'}=1.9$ Hz, 5a-H), 3.72 (1H, m, 4a-H), 3.80 (1H, m, $OCH_2$), 3.90 (1H, m, 5b-H), 4.09–4.17 (3H, m, 6a-H, 3b-H, 4b-H), 4.25 (1H, dd, $J_{5b,6b}=7.1$ Hz, $J_{6b,6b'}=11.5$ Hz, 6b-H), 4.30 (1H, dd, $J_{5b,6b'}=5.2$ Hz, $J_{6b,6b'}$, 6b′-H), 4.31 (1H, d, $J_{1b,2b}=7.7$ Hz, 1b-H), 4.42 (2H, m, 1a-H, 6a′-H), 4.82 (1H, m, 2b-H), 4.87 (1H, dd, $J_{1a,2a}=8.0$ Hz, $J_{2a,3a}=9.6$ Hz, 2a-H), 5.16 (1H, m, 3a-H).

**4.2.4. Octyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside, 11.** Sodium hydride (60% dispersion in oil;

350 mg, 8.6 mmol) was added in portions to a cooled (0°C), stirred solution of ketal **7** (660 mg, 1.33 mmol) in DMF (13 $cm^3$) and the mixture was stirred at 0°C for 30 min. Benzyl bromide (0.87 $cm^3$, 7.32 mmol) was added dropwise and the mixture was allowed to warm to room temperature and stirred for a further hour. After careful addition of MeOH (1 $cm^3$) and concentration, the residue was partitioned between diethyl ether and water, the aqueous phase being extracted twice with diethyl ether before washing the combined organic extracts with saturated NaCl solution, drying and concentration to a colourless oil. Flash chromatography (silica gel; hexane–EtOAc, 6:1) gave compound **11** as a colourless syrup (1.20 g, 95%), (Found: C, 73.89; H, 7.87. $C_{58}H_{72}O_{11}$ requires C, 73.70; H, 7.68%); $[\alpha]_D$=+15.8 (*c* 1.1 in $CHCl_3$); $\nu$max/$cm^{-1}$ 3030 (Ar-H), 2930, 2860 ($CH_2$, $CH_3$), 1370 (Pr*i* C–H), 735, 700 (Ar-H); $\delta_H$($CDCl_3$) 0.90 (3H, t, *J*=6.9 Hz, $C_7H_{14}CH_3$), 1.27–1.46 (16H, m, $(CH_2)_5CH_3$, $CMe_2$), 1.68 (2H, m, $OCH_2CH_2$), 4.32–4.99 (10×1H, 10 AB d, 5×$OCH_2$Ph), 4.41, 4.46 (2×1H, 2d, $J_{1a,2a}$=$J_{1b,2b}$=8.0, 7.7 Hz, 1a-H, 1b-H), 7.20–7.45 (25H, m, Ar-H); $\delta_C$($CDCl_3$) 13.9, 22.4, 25.9, 26.2, 27.7, 29.0, 29.2, 29.5, 31.6, 68.2, 68.7, 69.9, 71.8, 73.0 (2), 73.2, 73.5, 74.8, 74.9, 75.2, 76.3, 79.2, 80.5, 81.7, 82.8, 101.7, 103.6, 109.6, 127.2–128.2 (Ar C), 138.3, 138.4, 138.5, 138.7, 139.0.

**4.2.5. Octyl 2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside, 12.** Compound **11** (1.02 g, 1.08 mmol) was dissolved in aqueous acetic acid (50 $cm^3$; 80% v/v) and stirred at 80°C for 2 h. After cooling, the solution was concentrated and co-evaporated several times with toluene. Flash chromatography (silica gel; hexane–EtOAc, 3:2) gave compound **12** as a colourless syrup (0.96 g, 98%), (Found: C, 73.25; H, 7.61. $C_{55}H_{68}O_{11}$ requires C, 72.98; H, 7.57%); $[\alpha]_D$=+18.8 (*c* 1 in $CHCl_3$); $\nu$max/$cm^{-1}$ 3450 (OH br), 3030 (Ar-H), 2925, 2860 (CH2, CH3), 735, 700 (Ar-H); $\delta_H$($CDCl_3$) 0.90 (3H, t, *J*=6.9 Hz, $C_7H_{14}CH_3$), 1.25–1.46 (10H, m, $(CH_2)_5CH_3$), 1.67 (2H, m, $OCH_2CH_2$), 2.40 (2H, br s, OH), 4.38–5.02 (10×1H, 10 AB d, 5×$OCH_2$Ph), 4.40, 4.60 (2×1H, 2d, $J_{1a,2a}$=$J_{1b,2b}$=8.0, 8.0 Hz, 1a-H, 1b-H), 7.20–7.40 (25H, m, Ar-H); δ($CDCl_3$) 13.9, 22.4, 25.95, 29.0, 29.2, 29.5, 31.6, 68.2, 68.5, 68.6, 69.9, 72.7, 73.0, 73.3, 73.4, 74.7 (2), 75.0, 75.05, 76.7, 79.9, 81.7, 82.7, 102.5, 103.6, 127.2–128.4 (Ar C), 137.95, 138.25, 138.3, 138.6, 139.2.

A small sample of the *diol* (100 mg) was acetylated using pyridine–acetic anhydride and purified by flash chromatography (silica gel; hexane–EtOAc, 4:1) to give *octyl 3,4-di-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside* as a colourless syrup (100 mg, 92%), $\delta_H$($CDCl_3$) 0.89 (3H, t, *J*=6.9 Hz, $C_7H_{14}CH_3$), 1.26–1.44 (10H, m, $(CH_2)_5CH_3$), 1.67 (2H, m, $OCH_2CH_2$), 1.95, 1.99 (2×3H, 2s, 2×AcO), 3.71 (1H, dd, $J_{5a,6a}$=1.6 Hz, $J_{6a,6a'}$=11.0 Hz, 6a-H), 3.79 (1H, dd, $J_{5a,6a'}$=4.1 Hz, $J_{6a,6a'}$, 6a′-H), 3.91–4.03 (2H, m, $OCH_2$, 4a-H), 4.19–4.98 (10×1H, 10 AB d, 5×OC*H*2Ph), 4.38 (1H, d, $J_{1a,2a}$=7.7 Hz, 1a-H), 4.53 (1H, d, $J_{1b,2b}$=7.7 Hz, 1b-H), 4.87 (1H, dd, $J_{2b,3b}$=10.2 Hz, $J_{3b,4b}$=3.3 Hz, 3b-H), 5.39 (1H, d, $J_{3b,4b}$, 4b-H), 7.15–7.40 (25H, m, Ar-H).

**4.2.6. Octyl (potassium 5-amino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside, 20.** A suspension of acceptor **12** (200 mg, 220 μmol), donor **13** (288 mg, 550 μmol) and 3 Å molecular sieves (600 mg) in dry acetonitrile (2 $cm^3$) was stirred for 2 h at room temperature under nitrogen. The mixture was cooled to −35°C and *N*-iodosuccinimide (247 mg, 1.10 mmol) was added, followed by trifluoromethanesulfonic acid (10 $mm^3$, 110 μmol) and the mixture was stirred at this temperature for 2 h. The mixture was diluted with $CH_2Cl_2$ (50 $cm^3$), filtered through Celite and the filtrate was washed successively with 1 M solutions of sodium carbonate, sodium thiosulfate and sodium chloride, before drying and concentration to a syrup. In order to facilitate separation of products from starting materials, the mixture was deacetylated prior to chromatography: a solution of the crude product mixture and sodium methoxide (6 mg, 110 μmol) in MeOH (10 $cm^3$) was stirred for 2 h at room temperature. The mixture was neutralised with acetic acid and concentrated on to silica (2 g). Flash chromatography (silica gel; $CH_2Cl_2$–MeOH, 99:1→97:3) gave a mixture of tri-saccharides **14/15** (150 mg).

A small portion (20 mg) of this mixture was partially separated by careful column chromatography (silica gel; $CH_2Cl_2$–MeOH, 99:1→97:3) to give first, the lactone, *octyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyloyl-1c→4b-lactone)-(2→3)-2,6-di-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside* **15**; $\delta_H$($CD_3OD$; cf. compound **14** in Ref. 29) 1.69 (1H, dd, $J_{3cax,3ceq}$=13.8 Hz, $J_{3cax,4c}$=11.3 Hz, $3c_{ax}$-H), 2.01 (3H, s, AcN), 2.26 (1H, dd, $J_{3cax,3ceq}$=$J_{3ceq,4c}$=5.5 Hz, $3c_{eq}$-H), 3.25 (1H, dd, $J_{1a,2a}$=8.0 Hz, $J_{2a,3a}$=9.3 Hz, 2a-H), 4.10 (1H, dd, $J_{2b,3b}$=9.3 Hz, $J_{3b,4b}$=3.9 Hz, 3b-H), 4.42 (1H, d, $J_{1b,2b}$=7.7 Hz, 1b-H), 4.45 (1H, d, $J_{1a,2a}$, 1a-H), and then *octyl (methyl 5-acetamido-3,5-dideoxy-D-glycero-α/β-D-galacto-2-nonulopyranosylonate)-(2→3)-2,6-di-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside* **14** as an anomeric mixture (α/β, 7:3); $\delta_H$($CD_3OD$) 1.73 (m, $J_{3cax,3ceq}$=12.9 Hz, $J_{3cax,4c}$=11.8 Hz, β$3c_{ax}$-H), 2.01 (m, α/βAcN, α$3c_{ax}$-H), 2.68 (m, α/β$3c_{eq}$-H), 3.58 (s, β$CO_2Me$), 3.80 (s, α$CO_2Me$). Both samples were acetylated with pyridine and acetic anhydride to verify their structures.

*Lactone* **17**; $\delta_H$($CDCl_3$) 1.88 (3H, s, AcN), 1.92, 2.00, 2.03, 2.15, (4×3H, 4s, 4×AcO), 4.60 (1H, dd, $J_{2b,3b}$=9.3 Hz, $J_{3b,4b}$=3.9 Hz, 3b-H), 4.18 (1H, m, 5c-H), 4.37 (1H, d, $J_{1a,2a}$=7.7 Hz, 1a-H), 5.05 (1H, m, 8c-H), 5.25 (1H, dd, $J_{6c,7c}$=1.9 Hz, $J_{7c,8c}$=6.3 Hz, 7c-H), 5.30 (1H, d, $J_{5c,NH}$=10.2 Hz, NH), 5.49 (1H, m, 4c-H); FAB-MS: *m/z* 1369 $(M+Na)^+$ ($C_{74}H_{91}NO_{22}$ requires *m/z* 1346).

*α/β methyl esters* **16**; $\delta_H$($CDCl_3$, cf. compounds **25** and **26** in Ref. 28) 1.75, 1.96, 1.99, 2.00, 2.08 (5×3H, 5s, 5×βAcO), 1.85 (3H, s, α/βAcN), 2.60 (m, α/β$3c_{eq}$-H), 3.43 (s, β$CO_2Me$), 3.83 (s, α$CO_2Me$), 5.05 (d, $J_{3b,4b}$=3.3 Hz, α4b-H), 5.32 (dd, $J_{6c,7c}$=2.2 Hz, $J_{7c,8c}$=8.5 Hz, α7c-H), 5.39 (t, $J_{6c,7c}$=$J_{7c,8c}$=2.0 Hz, α7c-H), 5.59 (1H, m, α8c-H).

A suspension of the remaining tri-saccharide mixture (130 mg) and palladium hydroxide on charcoal (130 mg;

10% w/w) in MeOH (30 $cm^3$) was stirred for 24 h under an atmosphere of hydrogen. The mixture was filtered through Celite and concentrated. A solution of the residue in potassium hydroxide solution (10 $cm^3$;1 M) was heated for 20 h at 90°C. After cooling to room temperature, the solution was neutralised with acetic acid and desalted on a reverse phase column (C-18 silica gel; $H_2O \rightarrow$MeOH) to give *octyl (potassium 5-amino-3,5-dideoxy-D-glycero-α/β-D-galacto-2-nonulopyranosylonate)-(2→3)-β-D-galacto-pyranosyl-(1→4)-β-D-glucopyranoside* **20**α/β as a glassy solid (80 mg, 49%; α/β, 6:1) $\delta_H(CD_3OD)$ β anomer 2.34 (dd, $J_{3cax,3ceq}$=12.6 Hz, $J_{3ceq,4c}$=4.4 Hz, $3c_{eq}$-H), 2.90 (t, $J_{4c,5c}=J_{5c,6c}$=9.6 Hz, 5c-H), α anomer 2.73 (t, $J_{4c,5c}=J_{5c,6c}$=9.9 Hz, 5c-H), 2.79 (dd, $J_{3cax,3ceq}$=12.4 Hz, $J_{3ceq,4c}$=4.7 Hz, $3c_{eq}$-H).

Repeated flash chromatography (silica gel; $CH_2Cl_2$–MeOH–$H_2O$, 6:5:1) gave **20**α as a glassy solid (35 mg, 21%), $[\alpha]_D$=−24.8 (*c* 0.5 in MeOH); $\delta_H(CD_3OD)$ 0.90 (3H, t, *J*=6.9 Hz, $C_7H_{14}CH_3$), 1.26–1.42 (10H, m, $(CH_2)_5CH_3$), 1.47–1.70 (3H, m, $OCH_2CH_2$, $3c_{ax}$-H), 2.73 (1H, t, $J_{4c,5c}=J_{5c,6c}$=9.9 Hz, 5c-H), 2.79 (1H, dd, $J_{3cax,3ceq}$=12.4 Hz, $J_{3ceq,4c}$=4.7 Hz, $3c_{eq}$-H), 4.28 (1H, d, $J_{1a,2a}$=8.0 Hz, 1a-H), 4.43 (1H, d, $J_{1b,2b}$=8.0 Hz, 1b-H); $\delta_C(CD_3OD)$ 14.5, 23.8, 27.2, 30.5, 30.7, 30.9, 33.1, 42.1, 54.6, 62.3, 62.9, 64.9, 68.9, 70.1, 71.1 (2), 72.25, 73.7, 74.9, 76.6 (2), 77.2, 77.5, 81.3, 101.3, 104.5, 105.3, 175.75; ES-MS (+ve): *m/z* 704 $(M-K+2H)^+$ ($C_{29}H_{52}NO_{18}K$ requires *m/z* 741).

**4.2.7. Octyl (potassium 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside, 1-Re-*N*-acetylation of 20α.** Acetic anhydride (64 $mm^3$, 64 μmol; 1 M solution in MeOH) was added to a stirred solution of **20**α (15 mg, 21.3 μmol) in MeOH. After 18 h the solution was concentrated and the residue was redissolved in water (2 $cm^3$). Reverse phase chromatography (C-18 silica gel; $H_2O \rightarrow$MeOH) gave *octyl (potassium 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulo-pyranosylonate)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside*, **1** as a glassy solid (14 mg, 88%), $[\alpha]_D$=−7.2 (*c* 1 in MeOH); $\delta_H(CD_3OD)$ 0.90 (3H, t, *J*=6.9 , $C_7H_{14}CH_3$), 1.26–1.42 (10H, m, $(CH_2)_5CH_3$), 1.62 (2H, m, $OCH_2CH_2$), 1.73 (1H, m, $3c_{ax}$-H), 2.00 (3H, s, AcN), 2.86 (1H, dd, $J_{3cax,3ceq}$=12.4 Hz, $J_{3ceq,4c}$=4.4 Hz, $3c_{eq}$-H), 4.28 (1H, d, $J_{1a,2a}$=7.7 Hz, 1a-H), 4.43 (1H, d, $J_{1b,2b}$=7.7 Hz, 1b-H); $\delta_C(CD_3OD)$ 14.5, 22.7, 23.8, 27.2, 30.5, 30.7, 30.9, 33.1, 42.25, 54.1, 62.2, 62.9, 64.8, 69.2, 69.5, 70.3, 71.1, 73.2, 75.0, 75.1, 75.15, 76.6, 77.2, 77.9, 81.2, 101.3, 104.5, 105.3, 175.3, 175.8; ES-MS (−ve): *m/z* 744 $(M-K)^-$ ($C_{31}H_{54}NO_{19}K$ requires *m/z* 783).

**4.2.8. Octyl (potassium 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside, 1-enzymatic sialylation of 6.** Typical biotransformation: *pNP sialoside* **21**[30,33] (3 mg, 6.7 μmol) was added to a solution of *octyl lactoside* **1** (2 mg, 4.4 μmol) and *trans*-sialidase (~1 mg crude protein) in 50 mM phosphate buffer (pH 7, 1 $cm^3$). The reaction mixture was incubated for 18 h at 30°C. TLC ($CHCl_3$–MeOH–$H_2O$, 6:4:1) indicated ~50% turnover of the *octyl lactoside*. The reaction was quenched by addition of EtOH (0.5 $cm^3$) to precipitate the protein and the resulting mixture was centrifuged at 13,000*g* for 3 min. The supernatants from five such incubations were combined and concentrated. The residue was redissolved in water (2 $cm^3$) and subjected to reverse phase chromatography (C-18 silica gel; gradient $H_2O \rightarrow$MeOH). Elution at approximately 50% aqueous methanol gave the title compound **1** as a white powder following freeze-drying from water (1.4 mg, 42%). Analytical data were identical to those obtained for the chemically synthesised material.

**4.2.9. Octyl (potassium 5-amino-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-β-D-galac-topyranosyl-(1→4)-β-D-glucopyranoside, 20-de-*N*-acetylation of 1.** A solution of enzymatically synthesised **1** (5 mg) and potassium hydroxide (1 $cm^3$; 1 M) was heated for 20 h at 90°C. After cooling to room temperature, the solution was neutralised with acetic acid and desalted on a reverse phase column (C-18 silica gel; $H_2O \rightarrow$MeOH) to give the *de-N-acetyl* compound **20** as a glassy solid (4.5 mg, 95%) Analytical data were identical to those for the chemically synthesised material.

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