Thio-oligosaccharides of sialic acid – synthesis of an α(2→3) sialyl galactoside *via* a gulofuranose/galactopyranose approach

W. Bruce Turnbull and Robert A. Field *

School of Chemistry, University of St. Andrews, Biomolecular Sciences Building, St. Andrews, UK KY16 9ST

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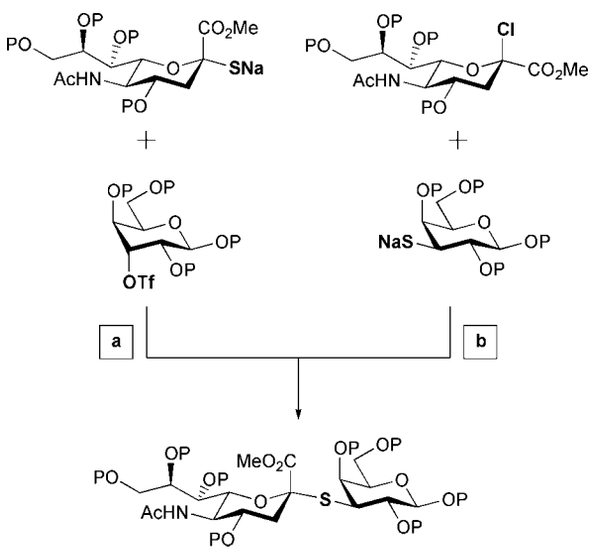
A new approach to the synthesis of thio-oligosaccharides containing the *N*-acetylneuraminic acid-α(2→3)-galactopyranose linkage is described. 3-*O*-(Trifluoromethylsulfonyl)gulofuranose derivative **5** can be converted into the α-2,3-sialyl-3-thiogalactofuranose derivative **8** in good yield. Partial deprotection of the thiodisaccharide provides an α/β mixture of both galactofuranose and galactopyranose isomers, but this mixture can be transformed efficiently into the desired pyranose-ring form to allow further elaboration into other glycosides *via* trichloroacetimidate donor **21**. This strategy avoids introducing sulfur into 3-*O*-(trifluoromethylsulfonyl)gulopyranose derivatives, which can be prone to elimination side reactions.

Introduction

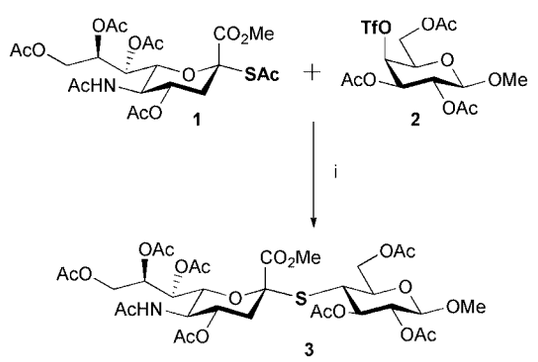
Our understanding of the importance of cell-surface glycoconjugates[1] to many biological processes has advanced, hand in hand, with developments in the efficient synthesis[2] of naturally occurring oligosaccharides and their analogues. Although attention has been drawn to the potential of carbohydrate-based therapeutics,[3] their application may be limited, in some cases, as a consequence of their susceptibility to enzymic hydrolysis by glycosidases. One strategy towards circumventing this problem is to replace the interglycosidic oxygen atom with a sulfur atom to give sulfur-linked oligosaccharides.[4] However, as many glycosidases are exo-hydrolases – *i.e.*, they remove monosaccharide residues in a stepwise manner from the non-reducing terminus – often it may be necessary only to 'protect' the terminal glycosidic linkage from enzymic hydrolysis.[4]

Sialic acids[5] (derivatives of the nine-carbon sugar neuraminic acid) are typically found located at the non-reducing terminus of both glycolipids and glycoproteins and, as such, they are rarely substituted by other saccharides other than another sialic acid. Sialic acid-containing glycosphingolipids, gangliosides,[6] have been implicated in a wide range of biological processes that are central to the development and spread of cancer.[7] In some cases, structural modifications of glycoconjugate-bound sialic acids are associated with changes in their biological activities.[5,8] Thus, in the course of studies relating to enzymes that modify α(2→3)galactose-linked sialosides, we considered that glycosidase-resistant oligosaccharides, *e.g.* thio-oligosaccharides, could serve as useful substrate probes and/or enzyme inhibitors.

Synthesis of the *N*-acetylneuraminic acid-α(2→3)-galactopyranose thioglycosidic [Neu5Acα(2→3)*S*Gal] linkage has been reported by both Hasegawa[9] and Schmidt,[10] employing anomeric *S*-alkylation and thioglycosylation strategies (Scheme 1a and b), respectively. Unfortunately, reaction schemes analogous to those already reported initially failed to provide us with the desired oligosaccharide analogues – rather, they resulted in elimination products, no reaction, or occasionally in disulfide formation. von Itzstein and co-workers have reported[11] a convenient procedure for thiosialoside synthesis *via in situ* *S*-deacetylation of the known 2-thiosialic acid methyl ester per-acetate **1**, and subsequent reaction with a sugar triflate, *e.g.* galactose derivative **2** (Scheme 2), to give the corresponding thioglycoside **3** in good yield. In contrast, however, attempts to prepare Neu5Acα(2→3)*S*Gal by this approach gave only the elimination product,[10b,12] and von Itzstein recently reported[13] the synthesis of a Neu5Acα(2→3)*S*Gal disaccharide employing the approach first described by Schmidt rather than by 'in



Scheme 1 Synthesis of Neu5Acα(2→3)*S*Gal *via* (**a**) anomeric *S*-alkylation and (**b**) thioglycosylation. P = Protecting group.



Scheme 2 *Reagents and conditions*: i, Et_2NH, DMF.

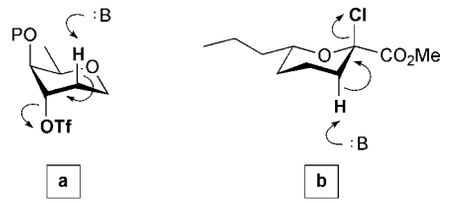


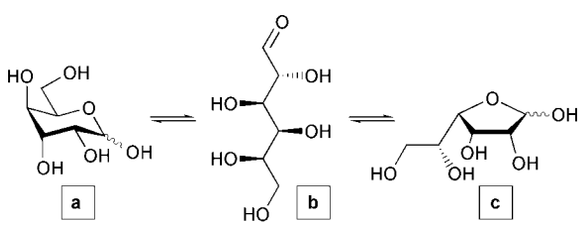
Fig. 1 Base-promoted elimination reactions of (**a**) 3-*O*-(trifluoromethylsulfonyl)gulofuranose and (**b**) 2-chlorosialic acid derivatives. P = Protecting group.

house' methods. Frustrated by our initial lack of success, we were drawn to investigate alternative approaches to the synthesis of Neu5Acα(2→3)*S*Gal.

Results and discussion

All synthetic approaches to the Neu5Acα(2→3)*S*Gal linkage reported thus far have relied on the introduction of sulfur into a suitably activated gulopyranose derivative. As ^{1}H NMR coupling constants indicate[9] ($J_{1,2}$ 8.8 Hz, $J_{2,3}$ 2.9 Hz, $J_{3,4}$ 2.9 Hz), the gulopyranosyl unit adopts, principally, the $^4C_1$ conformation and as such, it is set up perfectly for elimination of triflate with a *trans*-diaxial arrangement of H-2 and the leaving group (Fig. 1a). Overlap of the (C-4)–O bonding orbital with the (C-3)–O antibonding orbital may further facilitate the loss of triflate by weakening the (C-3)–OTf bond – such an effect may explain the difference in reactivities observed for 3-*O*-(trifluoromethylsulfonyl)gulose derivatives and for 4-*O*-(trifluoromethylsulfonyl)galactose derivative **2**.[11*b*] Similarly, 2-chlorosialic acid derivatives are prone to elimination (Fig. 1b) which may compete with nucleophilic displacement of chloride,[14] and, in any case, the sterically crowded ketose anomeric centre of sialic acid, whether as the chloride or thiolate, will be less reactive than analogous aldose derivatives. Thus, we would attribute some of the difficulties that we encountered in forming the Neu5Acα(2→3)*S*Gal linkage to the relative reactivities of the carbohydrate derivatives to substitution and competing reactions, *i.e.* elimination and/or oxidation of thiolate to disulfide. Synthetic difficulties have been reported by others,[15] in systems involving similarly hindered sugar triflates.

Gulose, as with all aldohexoses, can exist in a six-membered (pyranose) ring, an open-chain form and a five-membered (furanose) ring (Scheme 3). In its furanose form, gulose exhibits



Scheme 3 Interconversion of (**a**) gulopyranose and (**c**) gulofuranose isomers *via* (**b**) its acyclic form.

a number of structural properties that might suggest that a 3-*O*-(trifluoromethylsulfonyl)-D-gulofuranose derivative could be more suitable for nucleophilic displacement reactions than a corresponding gulopyranose derivative. First, for an α-gulofuranose derivative, all ring substituents point to the lower, α-face of the molecule, which should allow a relatively unhindered line of attack for an incoming sulfur nucleophile from the β-face. Secondly, a 3-*O*-(trifluoromethylsulfonyl)-gulofuranose derivative should be less prone to elimination than the analogous gulopyranose derivatives. In order to attain an anti-periplanar arrangement of H-2 and the triflate group that is required for elimination to occur, the furanose ring would have to adopt a less favoured conformation in which other ring substituents would be eclipsed. Furthermore, S_N2-type nucleophilic displacement reactions usually occur more readily in five-membered-ring systems than in six-membered-ring systems[16] as the former experience a reduction in substituent eclipsing on proceeding to the transition state, whereas for the latter the converse is true.

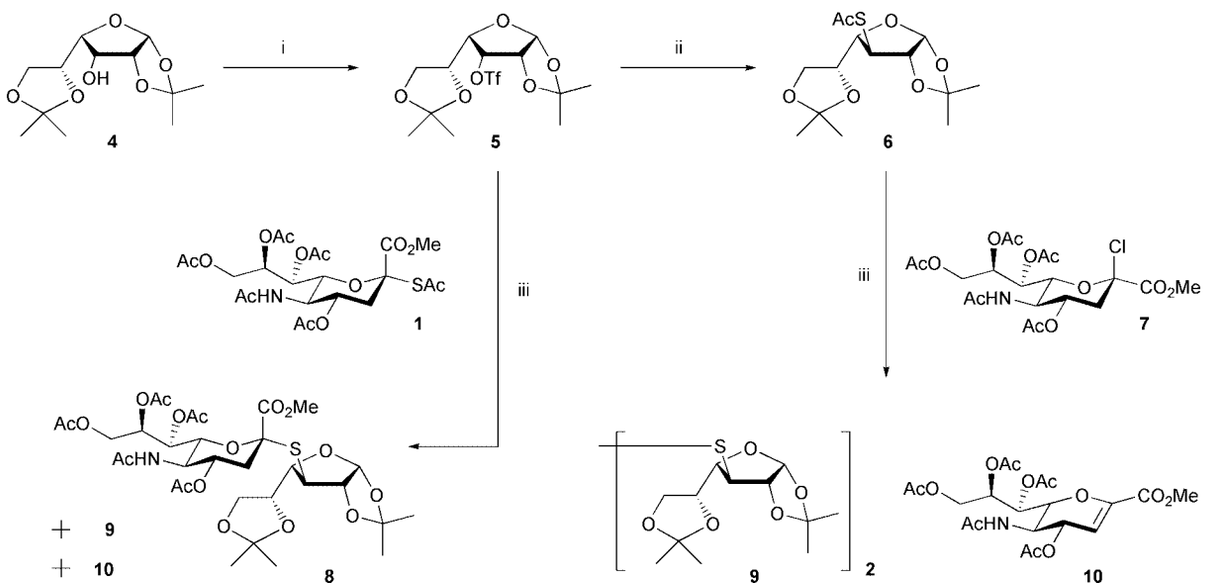
Here, we report a new strategy for the synthesis of Neu5Acα(2→3)*S*Gal containing oligosaccharides based on the reaction of a sialic acid 2-thiolate with an activated gulofuranose to give the Neu5Acα(2→3)Gal*f* thioglycoside, which was converted to its galactopyranose form for use as a donor in subsequent glycosylation reactions.

1,2:5,6-Di-*O*-isopropylidene-α-D-gulofuranose **4**, was chosen as a suitable gulose starting material. The fused bicyclic ring system was expected to further decrease the risk of elimination as a consequence of both the reduced flexibility of the furanose ring and the increased ring strain that would result on formation of an sp^2 centre at C-2. Compound **4** was obtained readily from commercially available diacetone glucose by a modified four-step literature procedure,[17] and activated as the triflate **5**[18] under standard conditions. Treatment of sialic acid thioacetate **1**[19] and 1.3 equivalents of the triflate **5** with diethylamine in DMF gave the desired thiodisaccharide **8** in 68% yield, along with 14% unchanged triflate, and surprisingly, the galactofuranose disulfide **9** in 4% yield (Scheme 4). In contrast, on subjecting a mixture of 3-(thioacetyl)galactose derivative **6** – prepared by reaction of triflate **5** with potassium thioacetate under phase-transfer conditions – and 2-chlorosialic acid derivative **7**[20] to the same reaction conditions as before, only the disulfide **9** and the sialic acid 2,3-elimination product **10** were recovered. As the thiodisaccharide product **8** was found to be stable to the reaction conditions (Et_2NH–DMF), we presume that disulfide **9** that was formed during the successful anomeric S-alkylation reaction results from one of the mechanisms outlined in Scheme 5. Although disaccharide **8** was contaminated with a small amount of the sialic acid elimination product **10** which proved impossible to remove by flash chromatography, an analytical sample was obtained, by gel-permeation chromatography on Sephadex LH20 in methanol. As the impurity proved easy to remove by flash chromatography following partial deprotection of the disaccharide, the product was used in subsequent steps without further purification.

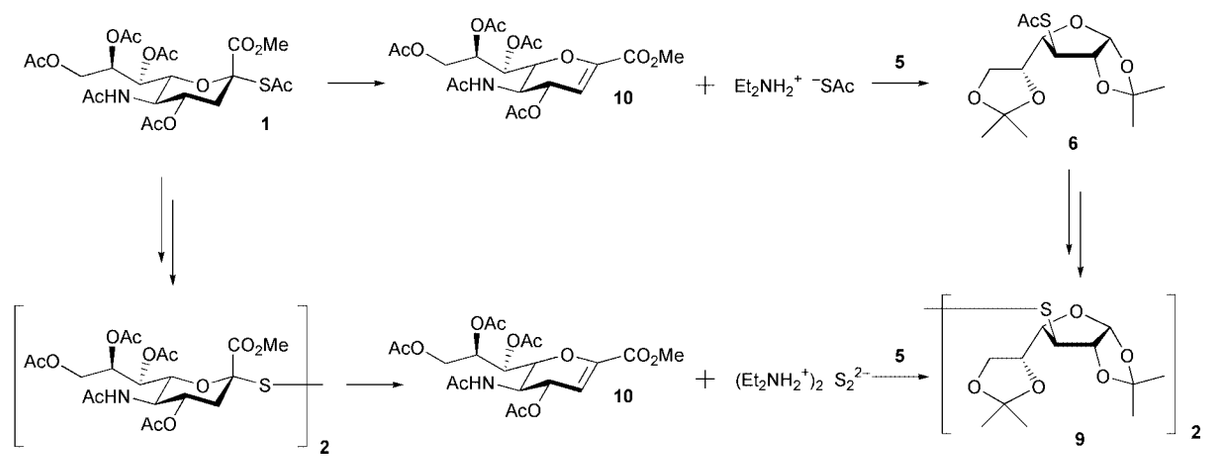
Although the 5,6-isopropylidene group of **8** is very acid labile, the 1,2-acetal was not removed completely when using hot, aq. 80% acetic acid. However, on treating the protected disaccharide with 90% TFA at room temperature for 15–30 minutes both acetals were hydrolysed readily (Scheme 6) in an acceptable 70% yield. The resulting reducing sugar **11** gave complex ^{1}H NMR spectra in a variety of deuterated solvents and acetylation of this crude hydrolysis product mixture resulted in a 4:1 pyranose:furanose mixture of α- and β-per-acetates **12(a, b)**α/β (Fig. 2a).

In order to confirm which signals related to furanose and which to pyranose isomers, a mixture of α- and β-furanose per-acetates **12b**α/β was synthesised by an independent route (Scheme 6). Thus, the 5,6-*O*-isopropylidene group was hydrolysed selectively with aq. acetic acid and the resulting 5- and 6-hydroxy compound **13** was acetylated to give **14** prior to removal of the 1,2-*O*-isopropylidene group with 90% TFA. Acetylation of the 1- and 2-hydroxy compound **15**α/β gave a 1:2 mixture of the α- and β-anomers **12b**α/β in 45% yield from **8** (Fig. 2b).

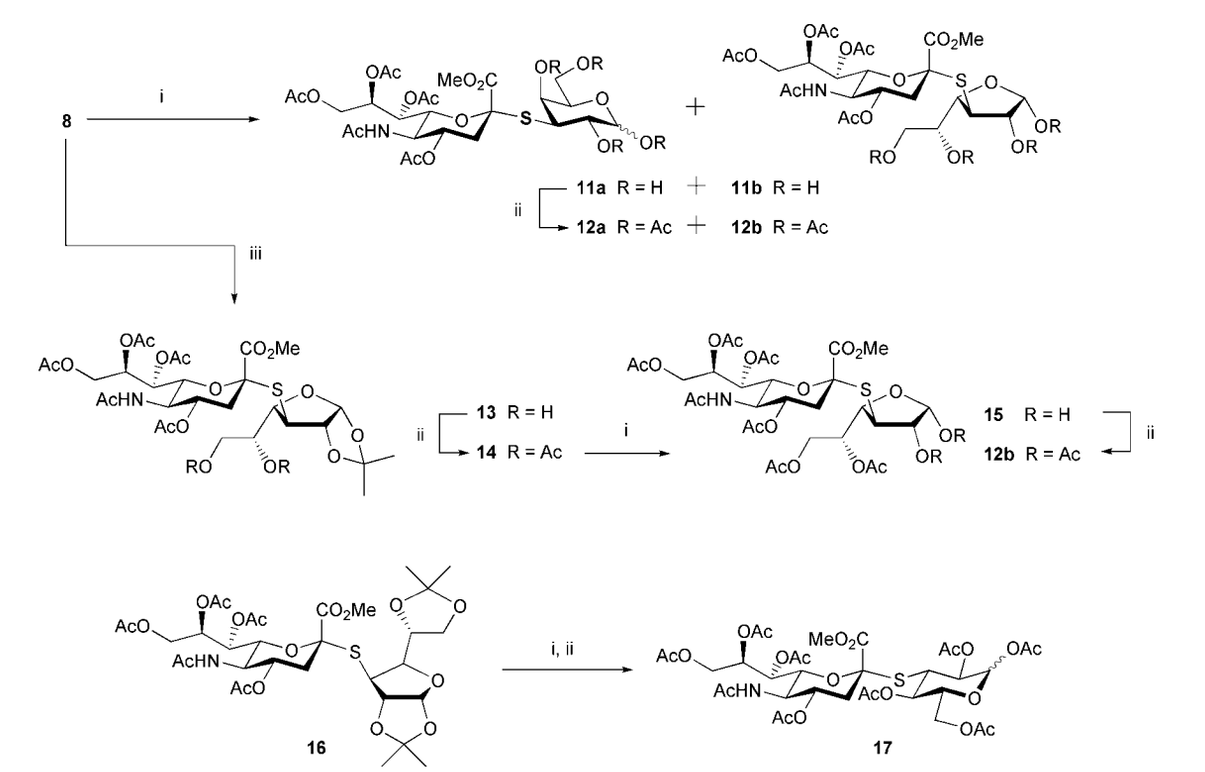
Acid-catalysed removal of the acetonide protecting groups (Scheme 6) from *S*-[methyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate]-(2→3)-1,2:5,6-di-*O*-isopropylidene-3-thio-α-D-glucofuranose **16**,[11*b*] the *gluco*-isomer of disaccharide **8**, followed by acetylation under the same conditions used previously, gave a mixture of only two per-acetate isomers. Both ^{1}H (Fig. 2d) and ^{13}C NMR spectra ($CDCl_3$) were consistent with the α- and



Scheme 4 *Reagents and conditions*: i, Tf_2O, pyridine, CH_2Cl_2; ii, KSAc, K_2CO_3, $TBAHSO_4$, EtOAc, H_2O; iii, Et_2NH, DMF.



Scheme 5 Possible routes by which disaccharide **9** could be formed under the conditions of the coupling reaction.



Scheme 6 *Reagents and conditions*: i, 90% $TFA_{(aq)}$; ii, Ac_2O, pyridine; iii, 50% $AcOH_{(aq)}$, 75 °C.

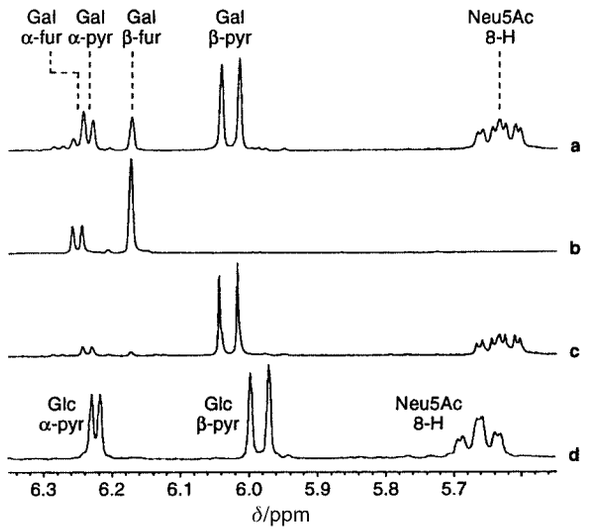


Fig. 2 Partial ^{1}H NMR spectra (300 MHz; $CDCl_3$) of isomeric mixtures of thiodisaccharide per-acetates. (**a**) **12a/12b**; (**b**) **12b**; (**c**) **12a**; (**d**) **17**.

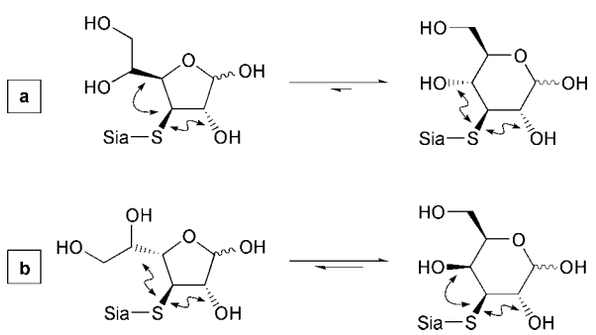
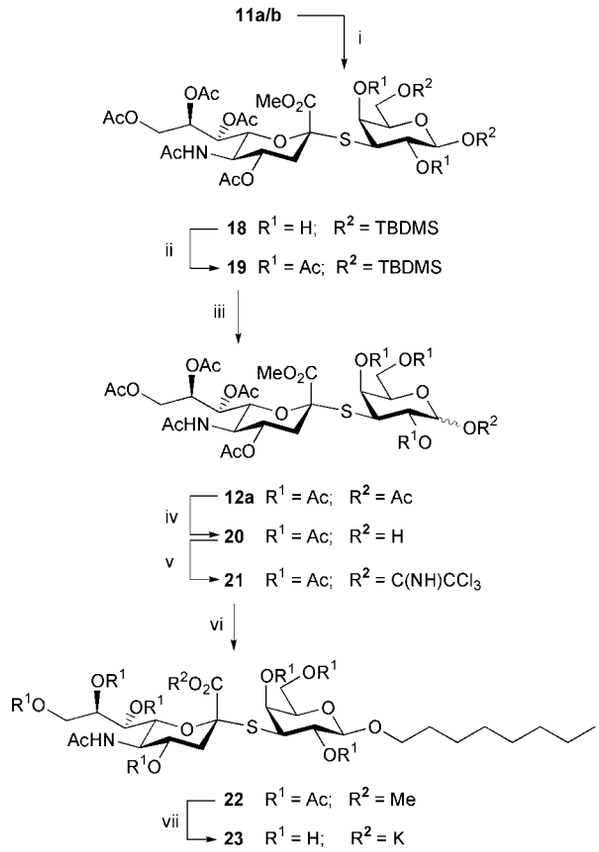


Fig. 3 Comparison of the *cis*- and *trans*-arrangements of substituents around the thioglycosidic linkage for (**a**) glucofuranose–pyranose and (**b**) galactofuranose–pyranose.

β-glucopyranose structures **17**α/β; δ_H 5.98 (d, $J_{1,2}$ 8.2, 1a-H β), 6.22 (d, $J_{1,2}$ 3.8, 1a-H α); δ_C 88.7 (1a-C α), 92.5 (1a-C β).

The difference in configuration at C-4 between disaccharides **8** and **16** seems to be sufficient to govern to what extent the compound should adopt the pyranose or the furanose ring forms. In the *gluco*-configuration (Fig. 3a), the disaccharide can avoid a *cis* interaction of the interglycosidic sulfur atom and C-5 – present in the furanose form – by adopting, exclusively, a pyranose structure with its fully equatorial arrangement of substituents. In other words, there is a significant difference in the relative energies of the pyranose and furanose forms, and the equilibrium lies, therefore, very much in favour of the pyranose isomer. However, for the *galacto*-configuration (Fig. 3b), it is the furanose ring that has a *trans* arrangement of substituents around the thioglycosidic linkage, and the pyranose ring that has a *cis* arrangement of sulfur and O-4. Presumably, the net effect is to bring the relative energies of the two ring forms closer together, resulting in the equilibrium mixture of isomers thus observed. *The properties of the furanose ring that were advantageous to the coupling reaction constitute the basis of the problem encountered in trying to convert completely the reducing sugar to its pyranose-ring form.*

Varying the acetylation conditions gave no favourable change in product mixture. However, on treating the isomeric mixture of reducing sugars **11(a,b)**α/β with an excess of *tert*-butyldimethylsilyl chloride in pyridine (Scheme 7), a first silyl ether is formed at the free primary position, as expected, but a second silylation then takes place at the anomeric position in such a way as to give, almost exclusively, the β-pyranose derivative **18** (^{1}H NMR, $CDCl_3$; $J_{1a,2a}$ 7.4 Hz, $J_{2a,3a}$ 11.8 Hz, $J_{3a,4a}$ 3.3 Hz). The second silylation occurs considerably more slowly than the



Scheme 7 *Reagents and conditions*: i, TBDMSCl, pyridine; ii, Ac_2O, pyridine; iii, Ac_2O, AcOH, H_2SO_4; iv, N_2H_4·AcOH, DMF; v, Cl_3CCN, DBU, CH_2Cl_2; vi, octan-1-ol, BF_3·OEt_2, CH_2Cl_2, 4 Å molecular sieves; vii, NaOMe, MeOH then $KOH_{(aq)}$.

first, and perhaps, could be thought of as a kinetic resolution of the disaccharide isomers, occurring alongside a re-equilibration of the other components of the mixture.

Having tied up the galactose into the pyranose ring successfully, the remaining hydroxy groups (positions 2 and 4) were acetylated to give **19** in 90% yield from **11**. This second protection step prevents re-equilibration of the hemiacetal to give both furanose and pyranose isomers on hydrolysis of the TBDMS ethers, as O-4 – which would otherwise become the furanose ring oxygen – is now blocked as an ester. A minor isomer visible in the ^{1}H NMR spectrum (≈5%) could not be identified clearly, but following conversion to the per-acetate, a comparison with the product mixture resulting from the direct acetylation of hemiacetals **11(a,b)** confirmed its identity as β-furanose **12b**.

Although the primary silyl ether protecting group was removed readily under standard conditions – 80% aq. acetic acid at 80 °C or tetrabutylammonium fluoride in THF – clean removal of the anomeric TBDMS group proved more difficult. The more harsh conditions of an acetolysis reaction gave the per-acetate **12** in 76% as a 83:14:3 mixture of β-pyranose:α-pyranose:β-furanose (Fig. 2c). Spectral data for the major component of this mixture were in good agreement with those published previously by Hasegawa for the β-pyranose per-acetate.[9]

The per-acetate was deprotected selectively using hydrazinium acetate[21] to give the hemiacetal **20** in 70% yield which was then converted into an anomeric mixture (β-*p*:α-*p*, 92:6) of trichloroacetimidates **21** in 90% yield using DBU as base. The anomeric ratio of the imidate is perhaps unexpected, as DBU, when used as the base for this reaction, usually gives principally the thermodynamic product *i.e.* the α-anomer.[22] ^{1}H NMR spectroscopy indicated that the galactopyranose imidate was contaminated with less than 2% of a furanose derivative.

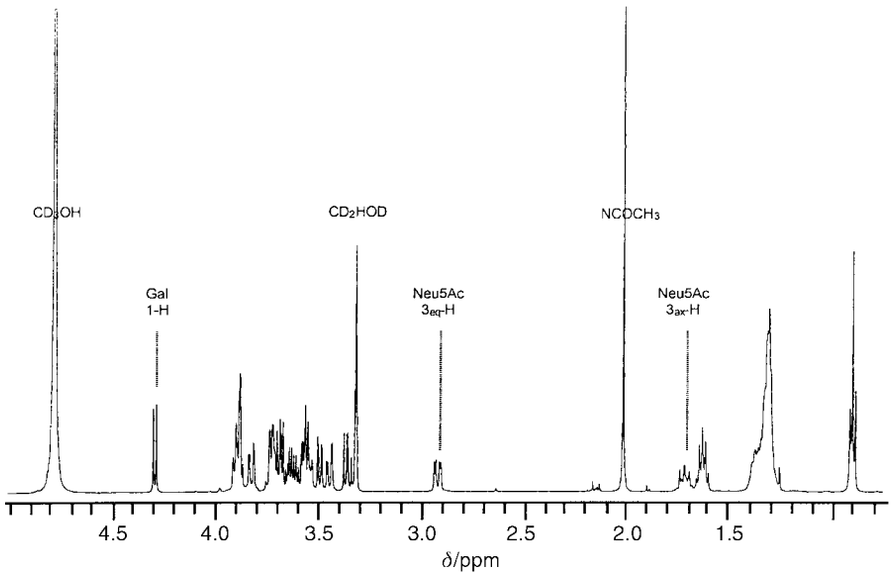


Fig. 4 ^{1}H NMR spectrum (500 MHz; CD_3OD) of octyl glycoside **23**.

We chose to convert glycosyl donor **21** into octyl glycoside **23** as this compound would be sufficiently hydrophobic to allow its capture on C-18 reversed-phase silica as a work-up step in various bioassays.[23] Thus, imidate donor **21** was treated with octan-1-ol in the presence of boron trifluoride–diethylether to give the protected octyl glycoside **22**. However, purification of this per-acetyl compound proved difficult and so the product mixture was de-acetylated and the methyl ester was saponified prior to purification by reversed-phase chromatography. ^{1}H NMR spectroscopy showed (Fig. 4) that the product **23**, obtained in 50% yield from imidate **21**, was exclusively the β-pyranoside.

Conclusions

The known Neu5Acα(2→3)*S*Gal donor **21** was synthesised successfully in a total of 15 steps from diacetone glucose and *N*-acetylneuraminic acid. This synthesis compares favourably with published procedures[9,10b,21] in terms of both length and overall yield. Glycosyl donor **21** was converted into octyl glycoside **23** to give the desired single isomer. Evaluation of compound **23** and similar thio-oligosaccharides as sialidase and *trans*-sialidase inhibitors, and the use of hydrophobic glycoside **23** in solid-phase extraction assays, will be reported in due course.

Experimental

All reagents and solvents were dried prior to use according to standard methods. Otherwise, commercial reagents were used without further purification. 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 5% ethanolic solution of sulfuric acid. Flash chromatography was performed with silica gel 60 (Fluka). Reversed-phase chromatography was performed on 15 g of C-18 silica gel 100 (Fluka). 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 aq. $NaHCO_3$ and water; standard **work-up B** involved washing organic solutions successively with 1 M HCl solution, saturated aq. $NaHCO_3$ and water. All such organic solutions were then dried over anhydrous Na_2SO_4 and concentrated. All concentrations were performed *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}. 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 MHz and 75 MHz, respectively. ^{1}H NMR and ^{13}C NMR spectra were referenced using their residual solvent signals as internal standards.[24] *J*-Values are given in Hz. For disaccharides, the monosaccharide residues are labelled *a*, *b* from the reducing terminus. Only partial NMR data are given for some compounds; other spectral features are in accord with the proposed structures.

3-*S*-Acetyl-1,2:5,6-di-*O*-isopropylidene-3-thio-α-D-galactofuranose 6

A mixture of triflate **5**[18] (760 mg, 1.94 mmol), potassium thioacetate (434 mg, 3.80 mmol), potassium carbonate (1.31 g, 9.5 mmol) and tetrabutylammonium hydrogensulfate ($TBAHSO_4$) (645 mg, 1.90 mmol) in a mixture of EtOAc (8 ml) and H_2O (8 ml) was vigorously stirred for 2 h at room temperature. The mixture was diluted with EtOAc (50 ml) and washed with water before drying and concentration to give a syrup. Flash chromatography (silica gel; hexane–EtOAc, 8:2) gave the thioacetate **6** as a glassy solid which was freeze-dried from 1,4-dioxane to give a white powder (524 mg, 85%), $[\alpha]_D$ −3.4 (*c* 1 in $CHCl_3$); ν_{max}/cm^{-1} 2985, 2935 (CH_2, CH_3), 1700 (C=O), 1375 (Pr^i, C–H), 1065 (C–O); $\delta_H(CDCl_3)$ 1.33, 1.38, 1.43, 1.60 (4 × 3 H, 4 s, 2 × CMe_2), 2.36 (3 H, s, AcS), 3.69 (1 H, dd, $J_{2,3}$ 1.6, $J_{3,4}$ 4.7, 3-H), 3.84 (1 H, dd, $J_{5,6'}$ 6.6, $J_{6,6'}$ 8.5, 6′-H), 3.98 (1 H, dd, $J_{3,4}$, $J_{4,5}$ 7.1, 4-H), 4.07 (1 H, dd, $J_{5,6}$ 6.6, $J_{6,6'}$, 6-H), 4.41 (1 H, m, 5-H), 4.61 (1 H, dd, $J_{1,2}$ 3.8, $J_{2,3}$, 2-H), 5.91 (1 H, d, $J_{1,2}$, 1-H); CI-MS *m*/*z* 319 $(M + H)^+$ (Found: $[M + H]^+$, 319.1222. $C_{14}H_{23}O_6S$ requires *m*/*z*, 319.1215).

Attempted reaction of 3-*S*-acetyl-1,2:5,6-di-*O*-isopropylidene-3-thio-α-D-galactofuranose 6 and methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosyl)onate chloride 7

Diethylamine (0.3 ml) was added dropwise to a stirred solution of glycosyl chloride **7**[20] (86 mg, 0.17 mmol) and thioacetate **6** (70 mg, 0.22 mmol) in DMF (0.6 ml) at 0 °C. The reaction mixture was allowed to warm to room temperature overnight. The mixture was concentrated to a syrup, re-dissolved in EtOAc (10 ml), and subjected to standard **work-up B**. No formation of

the desired *thiodisaccharide* was observed. However, flash chromatography (silica gel; hexane–EtOAc, 7:3) gave *bis(3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-galactofuranos-3-yl) disulfide* **9** as a glassy solid (50 mg, 83%) (Found: C, 52.86; H, 7.14. $C_{24}H_{38}O_{10}S_2$ requires C, 52.35; H, 6.96%); $[a]_D$ −96.4 (*c* 1.025 in $CHCl_3$); $\delta_H(CDCl_3)$ 1.34, 1.36, 1.44, 1.56 (4 × 6 H, 4 s, 4 × CMe_2), 3.34 (2 H, dd, $J_{2,3}$ 2.2, $J_{3,4}$ 5.8, 3-H), 3.87 (4 H, m, 4-, 6′-H), 4.06 (2 H, dd, $J_{5,6}$ 6.9, $J_{6,6'}$ 8.5, 6-H), 4.40 (2 H, m, 5-H), 4.77 (2 H, dd, $J_{1,2}$ 3.8, $J_{2,3}$, 2-H), 5.84 (2 H, d, $J_{1,2}$, 1-H); $\delta_C(CDCl_3)$ 25.1, 26.25, 26.6, 27.25, 54.1, 65.6, 75.4, 83.9, 86.7, 105.3, 110.1, 114.0; FAB-MS *m*/*z* 573 $(M + Na)^+$. Further elution (EtOAc) gave methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-eno-pyranosyl)onate **10**; $\delta_H(CDCl_3)$ 1.93 (3 H, s, AcN), 2.05, 2.06, 2.07, 2.12 (4 × 3 H, 4 s, 4 × AcO), 3.80 (3 H, s, CO_2Me) 4.19 (1 H, dd, $J_{8,9}$ 7.1, $J_{9,9'}$ 12.3, 9-H), 4.38 (2 H, m, 5-, 6-H), 4.58 (1 H, dd, $J_{8,9'}$ 3.2, $J_{9,9'}$, 9′-H), 5.21 (1 H, dd, $J_{7,8}$ 4.4, $J_{8,9}$, $J_{8,9'}$, 8-H), 5.50 (2 H, m, 4-, 7-H), 5.81 (1 H, d, $J_{5,NH}$ 8.3, NH), 6.01 (1 H, d, $J_{3,4}$ 3.3, 3-H). NMR data in agreement with literature data.[25]

S-[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate]-(2→3)-1,2:5,6-di-*O*-isopropylidene-3-thio-α-D-galactofuranose 8

Diethylamine (12.5 ml) was added dropwise to a stirred solution of triflate **5**[18] (2.27 g, 5.80 mmol) and thioacetate **1**[19] (2.44 g, 4.44 mmol) in DMF (25 ml) at 0 °C. The reaction mixture was allowed to warm to room temperature overnight. The mixture was concentrated to a syrup, re-dissolved in EtOAc (250 ml) and subjected to standard **work-up B** before evaporation, leaving the residue on silica (5 g). Flash chromatography (silica gel, 50 g; hexane–EtOAc, 7:3) gave, first, bis[3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranos-3-yl] disulfide **9** (63 mg) and then the unchanged triflate **5** (313 mg); $\delta_H(CDCl_3)$ 1.37, 1.43, 1.45, 1.61 (4 × 3 H, 4 s, 2 × CMe_2), 3.65 (1 H, dd, $J_{5,6'}$ 6.3, $J_{6,6'}$ 8.8, 6′-H), 4.05 (1 H, dd, $J_{3,4}$ 5.8, $J_{4,5}$ 8.8, 4-H) 4.15 (1 H, dd, $J_{5,6}$ 6.6, $J_{6,6'}$, 6-H), 4.56 (1 H, m, 5-H), 4.80 (1 H, dd, $J_{1,2}$ 4.1, $J_{2,3}$ 5.8, 2-H), 5.09 (1 H, t, $J_{2,3}$, $J_{3,4}$, 3-H), 5.83 (1 H, d, $J_{1,2}$, 1-H). Further elution (EtOAc) gave the desired *thiodisaccharide* **8** as an amorphous solid (2.26 g, 68%) contaminated with the 2,3-*dehydrosialic acid* **10** (≈5% by ^1H NMR). The product was used in subsequent reactions without further purification, but an analytical sample of title compound **8** was obtained by gel-permeation chromatography (Sephadex LH-20; MeOH) (Found: C, 50.92; H, 6.25, N, 1.74. $C_{32}H_{47}NO_{17}S$ requires C, 51.26; H, 6.32, N, 1.87%); $[a]_D$ −5.0 (*c* 1 in $CHCl_3$); ν_{max}/cm^{-1} 2990 (CH_2, CH_3), 1740 (C=O, ester), 1660, 1540 (C=O, amide), 1065 (C–O); $\delta_H(CDCl_3)$ 1.30, 1.34, 1.40, 1.56 (4 × 3 H, 4 s, 2 × CMe_2), 1.87 (4 H, m, AcN, $3b_{ax}$-H), 1.99, 2.00, 2.05, 2.10 (4 × 3 H, 4 s, 4 × AcO), 2.76 (1 H, dd, $J_{3bax,3beq}$ 12.5, $J_{3beq,4b}$ 4.7, $3b_{eq}$-H), 3.56 (1 H, m, 3a-H), 3.59 (1 H, dd, $J_{5a,6a'}$ 6.6, $J_{6a,6a'}$ 8.5, 6a′-H), 3.74–3.83 (5 H, m, 4a-, 6b-H, CO_2Me), 3.94 (1 H, q, $J_{4b,5b} = J_{5b,6b} = J_{5b,NH}$ = 10.2, 5b-H), 4.06–4.14 (2 H, m, 6a-, 9b-H), 4.23 (1 H, dd, $J_{8b,9b'}$ 2.5, $J_{9b,9b'}$ 12.6, 9b′-H), 4.43 (1 H, m, 5a-H), 4.76 (1 H, dd, $J_{1a,2a}$ 3.6, $J_{2a,3a}$ 0.8, 2a-H), 4.91 (1 H, m, 4b-H), 5.29 (1 H, dd, $J_{6b,7b}$ 1.6, $J_{7b,8b}$ 9.3, 7b-H), 5.34 (1 H, d, $J_{5b,NH}$, NH), 5.58 (1 H, m, 8b-H), 5.82 (1 H, d, $J_{1a,2a}$, 1a-H); FAB-MS *m*/*z* 772 $(M + Na)^+$ ($C_{32}H_{47}NO_{17}S$ requires *M*, 749).

S-[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate]-(2→3)-3-thio-D-galactose 11

Compound **8** (400 mg, 533 μmol) was dissolved in aq. trifluoroacetic acid (4 ml; 90% v/v) and the solution was stirred at room temperature for 15 min, concentrated and the residue co-evaporated several times with toluene. Flash chromatography (silica gel; EtOAc then MeOH) gave compound **11**, which was freeze dried from water as an α/β mixture of both furanose and pyranose isomers (251 mg, 70%); $[a]_D$ +51.0 (*c* 1 in MeOH); ν_{max}/cm^{-1} 3375 (OH, NH br), 2925, 2853 (CH_2, CH_3), 1735 (C=O, ester), 1660, 1560 (C=O, amide), 1065 (C–O); δ_H(pyridine-d_5) 5.64 (d, $J_{1,2}$ 4.4, 1a-H α-pyr/fur), 5.69 (d, $J_{1,2}$ 7.4, 1a-H β-pyr), 5.88 (d, $J_{1,2}$ 4.4, 1a-H α-fur/pyr), 5.90 (s, 1a-H β-fur); FAB-MS *m*/*z* 692 $(M + Na)^+$ (Found: $[M + Na]^+$ 692.1841. $C_{26}H_{39}NNaO_{17}S$ requires *m*/*z*, 692.1836).

S-[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate]-(2→3)-1,2,4,6-tetra-*O*-acetyl-3-thio-α/β-D-galactopyranose and -(2→3)-1,2,5,6-tetra-*O*-acetyl-3-thio-α/β-D-galactofuranose 12a, 12b

11 (100 mg, 149 μmol) was dissolved in a mixture of pyridine (1.0 ml) and acetic anhydride (1.0 ml) and the solution was stirred at room temperature overnight. The mixture was concentrated to an oil and flash chromatography (silica gel; CH_2Cl_2–acetone, 9:1→3:1) gave the *per-acetate* **12a/12b** (107 mg, 86%) as an α/β mixture in both pyranose and furanose ring forms (α-*p*:β-*p*:α-*f*:β-*f*, 20:60:10:10) (Found: C, 48.65; H, 5.74; N, 1.62. $C_{34}H_{47}NO_{21}S$ requires C, 48.74; H, 5.65; N, 1.67%); $[a]_D$ +35.4 (*c* 1 in $CHCl_3$); ν_{max}/cm^{-1} 2965 (CH_2, CH_3), 1745 (C=O, ester), 1670, 1540 (C=O, amide), 1060 (C–O); $\delta_H(CDCl_3)$ 2.60–2.76 (1 H, m, $3b_{eq}$-H), 3.83 (3 H, s, CO_2Me), 5.64 (ddd, $J_{7b,8b}$ 9.6, $J_{8b,9b}$ 2.5, $J_{8b,9b'}$ 6.9, 8b-H β-pyr), 6.03 (d, $J_{1,2}$ 8.2, 1a-H β-pyr), 6.17 (s, 1a-H β-fur), 6.23 (d, $J_{1,2}$ 4.1, 1a-H α-pyr), 6.25 (d, $J_{1,2}$ 4.1, 1a-H α-fur); $\delta_C(CDCl_3)$ 36.8 (3b-C β-pyr), 37.3 (3b-C α-pyr), 37.4 (3b-C β-fur), 38.0 (3b-C α-fur), 80.45 (2b-C α-pyr), 80.8 (2b-C β-pyr), 82.6 (2b-C α-fur), 83.25 (2b-C β-fur), 89.1 (1a-C α-pyr), 92.7 (1a-C β-pyr), 92.8 (1a-C α-fur), 99.4 (1a-C β-fur) – see Fig. 2.

S-[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate]-(2→3)-5,6-di-*O*-acetyl-1,2-*O*-isopropylidene-3-thio-α-D-galactofuranose 14

Compound **8** (200 mg, 267 μmol) was dissolved in aq. acetic acid (4 ml; 50% v/v) and the solution was stirred for 15 min at 75 °C. After cooling, the solution was concentrated and co-evaporated several times with toluene. Flash chromatography (silica gel; CH_2Cl_2 then CH_2Cl_2–MeOH, 98:2→95:5) gave *S*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate]-(2→3)-1,2-*O*-isopropylidene-3-thio-α-D-galactofuranose **13** as a solid foam (114 mg); $\delta_H(CDCl_3)$ 1.30, 1.55 (2 × 3 H, 2 s, CMe_2), 1.86 (3 H, s, AcN), 1.99, 2.00, 2.08, 2.12 (4 × 3 H, 4 s, 4 × AcO), 2.75 (1 H, dd, $J_{3bax,3beq}$ 12.6, $J_{3beq,4b}$ 4.4, $3b_{eq}$-H), 2.90 (2 H, br s, OH), 3.59 (1 H, dd, $J_{5a,6a'}$ 5.2, $J_{6a,6a'}$ 11.8, 6a′-H), 3.68 (1 H, dd, $J_{5a,6a}$ 3.0, $J_{6a,6a'}$, 6a-H), 3.79 (3 H, s, CO_2Me), 4.26 (1 H, dd, $J_{8b,9b'}$ 2.6, $J_{9b,9b'}$ 12.5, 9b′-H), 4.80 (1 H, d, $J_{1a,2a}$, 3.85, 2a-H), 4.88 (1 H, m, 4b-H), 5.28 (1 H, dd, $J_{6b,7b}$ 1.6, $J_{7b,8b}$ 9.0, 7b-H), 5.55 (2 H, m, 8b-H, NH), 5.81 (1 H, d, $J_{1a,2a}$, 1a-H).

The diol was dissolved in a mixture of pyridine (2 ml) and acetic anhydride (1.5 ml) and stirred overnight at room temperature. The mixture was concentrated, re-dissolved in EtOAc and subjected to standard **work-up B**. Flash chromatography (silica gel; CH_2Cl_2–acetone, 9:1→2:1) gave the *title compound* **14** as a glassy solid (124 mg, 58% from **8**) (Found: C, 50.28; H, 6.13; N, 1.70. $C_{33}H_{47}NO_{19}S$ requires C, 49.93; H, 5.97; N, 1.76%); $[a]_D$ +13.8 (*c* 1 in $CHCl_3$); $\delta_H(CDCl_3)$ 1.33, 1.63 (2 × 3 H, 2 s, CMe_2), 1.88 (4 H, m, AcN, $3b_{ax}$-H), 1.99–2.16 (6 × 3 H, 6 s, 6 × AcO), 2.76 (1 H, dd, $J_{3bax,3beq}$ 12.6, $J_{3beq,4b}$ 4.7, $3b_{eq}$-H), 3.60 (1 H, m, 3a-H), 3.74–3.80 (5 H, m, 4a-, 6b-H, CO_2Me), 3.89 (1 H, m, 5b-H), 4.12–4.19 (2 H, m, 6a′-, 9b-H), 4.25 (1 H, dd, $J_{8b,9b'}$ 2.5, $J_{9b,9b'}$ 12.6, 9b′-H), 4.32 (1 H, dd, $J_{5a,6a}$ 3.8, $J_{6a,6a'}$ 12.1, 6a-H), 4.76 (1 H, dd, $J_{1a,2a}$ 3.6, $J_{2a,3a}$ 1.1, 2a-H), 4.95 (1 H, m, 4b-H), 5.21–5.30 (2 H, m, 5a-, 7b-H), 5.32 (1 H, d, $J_{5b,NH}$ 9.6, NH), 5.64 (1 H, m, 8b-H), 5.73 (1 H, d, $J_{1a,2a}$, 1a-H); $\delta_C(CDCl_3)$ 20.44, 20.47, 20.51, 20.56, 20.8, 23.0, 26.75, 27.0, 38.0, 46.0, 49.7, 61.2, 62.95, 53.2, 66.5, 68.3, 69.1, 69.3, 73.9, 80.6, 82.6, 89.8, 105.3, 114.4, 168.4, 169.3, 169.7, 170.2, 170.4, 170.5, 170.7.

S-[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate]-(2→3)-1,2,5,6-tetra-*O*-acetyl-3-thio-α/β-D-galactofuranose 12b

Compound **14** (60 mg, 75 μmol) was dissolved in aq. trifluoroacetic acid (1 ml; 90% v/v) and the solution was stirred for 15 min at room temperature, concentrated, and the residue co-evaporated several times with toluene. Flash chromatography (silica gel; CH_2Cl_2 then CH_2Cl_2–acetone, 2:1) gave *S*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate]-(2→3)-5,6-di-*O*-acetyl-3-thio-α/β-D-galactofuranose **15** as a glassy solid (45 mg).

The diol was dissolved in a mixture of pyridine (2 ml) and acetic anhydride (1.5 ml) and the solution was stirred overnight at room temperature. The mixture was concentrated, re-dissolved in EtOAc and subjected to standard **work-up B**. Flash chromatography (silica gel; CH_2Cl_2–acetone, 9:1→3:1) gave the title compound **12b** as a glassy solid (50 mg, 78% from **14**; α:β, 1:2) (Found: C, 48.69; H, 5.49; N, 1.54. $C_{34}H_{47}NO_{21}S$ requires C, 48.74; H, 5.65; N, 1.67%); $[\alpha]_D$ −5.6 (*c* 1 in $CHCl_3$); $\delta_H(CDCl_3)$ 1.87 (3 H, s, AcN), 1.99–2.18 (24 H, overlapping signals, AcO), 2.68–2.76 (1 H, m, $3b_{eq}$-H), 3.82 (s, CO_2Me α), 3.83 (s, CO_2Me β), 6.17 (s, 1a-H β), 6.25 (d, $J_{1,2}$ 4.1, 1a-H α) – see Fig. 2; $\delta_C(CDCl_3)$ 20.25, 20.5, 20.6, 20.65, 20.75, 20.8, 23.0, 30.7, 37.4, 38.0, 41.7, 44.1, 49.4, 53.3, 53.4, 61.4, 62.0, 62.6, 66.3, 66.75, 67.2, 67.4, 68.6, 69.1, 69.3, 73.7, 74.0, 79.8, 82.2, 82.6, 83.25, 85.6, 92.8, 99.4, 168.6, 168.8, 169.2, 169.75, 170.1, 170.2, 170.3, 170.4, 170.6, 170.75.

S-[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate]-(2→3)-1,2,4,6-tetra-*O*-acetyl-3-thio-α/β-D-glucopyranose 17

Compound **16**[11b] (116 mg, 155 μmol) was dissolved in aq. trifluoroacetic acid (1.0 ml; 90% v/v) and the solution was stirred at room temperature for 15 min. The solution was concentrated and co-evaporated several times with toluene. Flash chromatography (silica gel; EtOAc then EtOAc–MeOH, 2:3) gave a glassy solid (84 mg), which was dissolved in a mixture of pyridine (1.0 ml) and acetic anhydride (1.0 ml) and this solution was stirred at room temperature overnight. The mixture was concentrated to an oil, which was re-dissolved in EtOAc (10 ml) and subjected to standard **work-up B**. Flash chromatography (silica gel; CH_2Cl_2–acetone, 9:1→3:1) gave the per-acetylated α/β pyranose **17** (104 mg, 80%; α-*p*:β-*p*, 42:58) as an amorphous solid (Found: C, 48.73; H, 5.96; N, 1.61. $C_{34}H_{47}NO_{21}S$ requires C, 48.74; H, 5.65; N, 1.67%); $[\alpha]_D$ +23.2 (*c* 1 in $CHCl_3$); $\delta_H(CDCl_3)$ 1.83–2.25 (28 H, m, AcN, AcO, $3b_{ax}$-H), 2.69, 2.75 (1 H, 2 dd, $3b_{eq}$-H α, β), 3.82 (3 H, s, CO_2Me), 5.98 (d, $J_{1,2}$ 8.2, 1a-H β), 6.22 (d, $J_{1,2}$ 3.8, 1a-H α) – see Fig. 2; $\delta_C(CDCl_3)$ 20.4, 20.5, 20.55, 20.6, 21.0, 21.2, 22.8, 22.9, 37.6, 38.3, 43.9, 45.9, 48.8, 49.3, 52.8, 61.3, 61.7, 61.8, 62.4, 66.9, 67.4, 67.5, 68.0, 68.7, 69.0, 69.2, 69.3, 71.0, 74.15, 74.3, 74.7, 83.1, 83.3, 88.7, 92.5, 168.5, 169.0, 169.2, 169.4, 169.5, 169.6, 169.8, 169.85, 170.4, 170.5, 170.8.

tert-Butyldimethylsilyl *S*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)-onate]-(2→3)-2,4-di-*O*-acetyl-6-*O*-(*tert*-butyldimethylsilyl)-3-thio-β-D-galactopyranoside 19

tert-Butyldimethylsilyl chloride (281 mg, 1.87 mmol) was added to a solution of compound **11** (250 mg, 0.373 mmol) in pyridine (2.5 ml) at 0 °C. After stirring of the mixture for 2 h at 0 °C more *tert*-butyldimethylsilyl chloride (281 mg, 1.87 mmol) was added and the mixture was allowed to warm slowly to room temperature overnight before being quenched with MeOH (1 ml) and concentrated to give the crude diol **18**. This residue was re-dissolved in pyridine (2 ml) and acetic anhydride (1.5 ml) and the solution was stirred at room temperature overnight. The mixture was concentrated again, and the residue was taken up in CH_2Cl_2 (20 ml), subjected to standard **work-up B** and concentrated to give a syrup. Flash chromatography (silica gel; CH_2Cl_2 then CH_2Cl_2–MeOH, 100:3) gave the *bis-silylated compound* **19** (331 mg, 90%) predominantly in the β-pyranose form, but contaminated with a small quantity (≈5%) of a furanose derivative (Found: C, 51.11; H, 7.46; N, 1.36. $C_{42}H_{71}NO_{19}SSi_2$ requires C, 51.36; H, 7.28; N, 1.43%); $[\alpha]_D$ +9.9 (*c* 0.75 in $CHCl_3$); ν_{max}/cm^{-1} 2960, 2860 (CH_2, CH_3), 1750 (C=O, ester), 1660, 1540 (C=O, amide), 1370 (Bu^t C–H), 1225, 840 (Si–C), 1055 (C–O); $\delta_H(CDCl_3)$ −0.01, 0.03 (2 × 3 H, 2 s, $SiMe_2$), 0.11 (6 H, s, $SiMe_2$), 0.85, 0.87 (2 × 9 H, 2 s, 2 × $SiCMe_3$), 1.85 (4 H, m, AcN, $3b_{ax}$-H), 2.00, 2.03, 2.04, 2.05, 2.14, 2.18 (18 H, 6 s, 6 × AcO), 2.64 (1 H, dd, $J_{3bax,3beq}$ 12.6, $J_{3beq,4b}$ 4.4, $3b_{eq}$-H), 3.46 (1 H, dd, $J_{5a,6a'}$ 7.7, $J_{6a,6a'}$, 6a′-H), 3.56 (1 H, dd, $J_{5a,6a}$ 5.8, $J_{6a,6a'}$ 9.9, 6a-H), 3.58 (1 H, dd, $J_{2a,3a}$ 11.8, $J_{3a,4a}$ 3.3, 3a-H), 3.69 (1 H, dd, $J_{5b,6b}$ 11.0, $J_{6b,7b}$ 2.2, 6b-H), 3.78–3.86 (4 H, m, 5a-H, CO_2Me), 4.05 (1 H, m, 5b-H), 4.08 (1 H, dd, $J_{8b,9b}$ 4.1, $J_{9b,9b'}$ 12.6, 9b-H), 4.18 (1 H, dd, $J_{8b,9b'}$ 3.0, $J_{9b,9b'}$, 9b′-H), 4.77 (1 H, dd, $J_{1a,2a}$ 7.4, $J_{2a,3a}$, 2a-H), 4.82 (1 H, m, 4b-H), 5.01 (1 H, dd, $J_{3a,4a}$, $J_{4a,5a}$ 0.8, 4a-H), 5.08 (1 H, d, $J_{1a,2a}$, 1a-H), 5.16 (1 H, d, $J_{5b,NH}$ 10.2, NH), 5.35 (1 H, dd, $J_{6b,7b}$, $J_{7b,8b}$ 10.2, 7b-H), 5.56 (1 H, ddd, $J_{7b,8b}$, $J_{8b,9b}$, $J_{8b,9b'}$, 8b-H).

S-[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate]-(2→3)-1,2,4,6-tetra-*O*-acetyl-3-thio-α/β-D-galactopyranose 12a

A stirred solution of **19** (100 mg, 101 μmol) in a mixture of acetic anhydride (0.5 ml) and acetic acid (0.25 ml) was cooled to 0 °C. Dil. sulfuric acid (0.25 ml; 10% v/v in acetic acid) was added and the mixture was stirred for 10 min at 0 °C and for 1 h at room temperature. The mixture was diluted with CH_2Cl_2 (20 ml) and subjected to standard **work-up A**. Flash chromatography (silica gel; CH_2Cl_2 then CH_2Cl_2–MeOH, 99:1→95:5) gave the *per-acetate* **12a** as a glassy solid (65 mg, 76%; β:α, 83:14), but contaminated by a small quantity (≈3%) of the β furanose derivative **12b**. ^{1}H NMR for β pyranose; $\delta_H(CDCl_3)$ 1.85 (3 H, s, AcN), 1.86 (1 H, m, $3b_{ax}$-H), 1.99–2.20 (8 × 3 H, 8 s, 8 × AcO), 2.63 (1 H, dd, $J_{3bax,3beq}$ 12.6, $J_{3beq,4b}$ 4.4, $3b_{eq}$-H), 3.69 (1 H, dd, $J_{5b,6b}$ 10.7, $J_{6b,7b}$ 2.2, 6b-H), 3.79 (1 H, dd, $J_{2a,3a}$ 11.5, $J_{3a,4a}$ 3.6, 3a-H), 3.83 (3 H, s, CO_2Me), 3.89 (1 H, dd, $J_{8b,9b}$ 6.6, $J_{9b,9b'}$ 12.0, 9b-H), 3.95 (1 H, dd, $J_{5a,6a}$ 6.9, $J_{6a,6a'}$, 11.5, 6a-H), 4.05 (1 H, dd, $J_{5a,6a'}$ 3.3, $J_{6a,6a'}$, 6a′-H), 4.07 (1 H, m, 5b-H), 4.30 (1 H, m, 5a-H), 4.33 (1 H, dd, $J_{8b,9b'}$ 2.2, $J_{9b,9b'}$, 9b′-H), 4.80 (1 H, m, 4b-H), 4.91 (1 H, m, 4a-H), 4.95 (1 H, dd, $J_{1a,2a}$ 8.0, $J_{2a,3a}$, 2a-H), 5.18 (1 H, d, $J_{5b,NH}$ 10.4, NH), 5.24 (1 H, dd, $J_{6b,7b}$ 2.2, $J_{7b,8b}$ 10.2, 7b-H), 5.63 (1 H, m, 8b-H), 6.02 (1 H, d, $J_{1a,2a}$, 1a-H) – see Fig. 2 – NMR data in agreement with literature data.[9]

S-[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate]-(2→3)-2,4,6-tri-*O*-acetyl-3-thio-β-D-galactopyranose 20

A solution of both **12a** (210 mg, 0.25 mmol) and hydrazinium acetate (25 mg, 0.275 mmol) in DMF (3.5 ml) was heated at 50 °C for 7 h. The mixture was cooled to room temperature and concentrated to a syrup, which was re-dissolved in CH_2Cl_2 and subjected to standard **work-up B**. Flash chromatography (silica gel; toluene–MeOH, 9:1) gave the *title compound* **20** as a colourless syrup (140 mg, 70%) contaminated with a small amount of a furanose isomer which proved difficult to remove despite repeated chromatography; $[\alpha]_D$ +30.0 (*c* 0.7 in $CHCl_3$) (lit.,[21] +33.0); $\delta_H(CDCl_3)$ 1.86 (3 H, s, AcN), 1.88 (1 H, dd, $J_{3ax,3eq}$ 12.6, $J_{3ax,4}$ 11.8, $3b_{ax}$-H), 2.01, 2.03, 2.04, 2.06, 2.07, 2.19, 2.22 (7 × 3 H, 7 s, 7 × AcO), 2.64 (1 H, dd, $J_{3bax,3beq}$ 12.6, $J_{3beq,4b}$ 4.7, $3b_{eq}$-H), 3.66–3.74 (2 H, m, 3a-, 6b-H), 3.83 (3 H, s, CO_2Me), 3.93–4.19 (5 H, m, 5a-H, 6-H_2, 5b-, 9b-H), 4.29 (1 H, dd, $J_{8b,9b'}$ 2.7, $J_{9b,9b'}$ 12.4, 9b′-H), 4.70 (1 H, dd, $J_{1a,2a}$ 7.7, $J_{2a,3a}$ 11.8, 2a-H), 4.83 (1 H, m, 4b-H), 4.91 (1 H, dd, $J_{3a,4a}$ 3.6, $J_{4a,5a}$ 1.1, 4a-H), 5.04 (1 H, d, $J_{1a,2a}$, 1a-H), 5.22 (1 H, d, $J_{5b,NH}$ 10.2, NH), 5.31 (1 H, dd, $J_{6b,7b}$ 2.2, $J_{7b,8b}$ 10.2, 7b-H), 5.59 (1 H, m, 8b-H).

S-[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate]-(2→3)-2,4,6-tri-*O*-acetyl-3-thio-α/β-D-galactopyranosyl trichloroacetimidate 21

1,8-Diazabicyclo[5.4.0]undec-7-ene (20 μl) was added to a stirred solution of **20** (63 mg, 67 μmol) in CH_2Cl_2 (2.5 ml) and trichloroacetonitrile (0.5 ml). After 2 h at room temperature the dark brown solution was concentrated to a syrup and passed down a short column (silica gel; CH_2Cl_2 then acetone) to remove most of the colour. Careful column chromatography (silica gel; CH_2Cl_2–MeOH, 100:3→95:5) gave the desired imidate **21** as a syrup (67 mg, 90%; β:α, 92:6) contaminated with ≈2% of a furanose isomer (as judged by ^{1}H NMR); $[\alpha]_D$ +26.4 (*c* 1.1 in $CHCl_3$) (lit.,[21] [β anomer] +27.5); $\delta_H(CDCl_3)$ 1.85 (3 H, s, AcN), 1.88 (1 H, m, $3b_{ax}$-H), 1.99, 2.00, 2.02, 2.05, 2.09, 2.14, 2.21 (7 × 3 H, 7 s, 7 × AcO), 2.64 (1 H, dd, $J_{3bax,3beq}$ 12.6, $J_{3beq,4b}$ 4.4, $3b_{eq}$-H), 3.70 (1 H, dd, $J_{5b,6b}$ 10.7, $J_{6b,7b}$ 2.2, 6b-H), 3.81 (1 H, dd, $J_{2a,3a}$ 11.5, $J_{3a,4a}$ 3.6, 3a-H), 3.84 (3 H, s, CO_2Me), 3.93 (1 H, dd, $J_{8b,9b}$ 6.0, $J_{9b,9b'}$ 12.4, 9b-H), 4.01 (1 H, dd, $J_{5a,6a}$ 6.9, $J_{6a,6a'}$ 11.3, 6a-H), 4.02–4.12 (2 H, m, 6a′-, 5b-H), 4.30 (1 H, dd, $J_{8b,9b'}$ 2.2, $J_{9b,9b'}$, 9b′-H), 4.33 (1 H, m, 5a-H), 4.82 (1 H, m, 4b-H), 4.95 (1 H, d, $J_{3a,4a}$, 4a-H), 5.10 (1 H, dd, $J_{1a,2a}$ 8.0, $J_{2a,3a}$, 2a-H), 5.16 (1 H, d, $J_{5b,NH}$ 10.2, NH), 5.28 (1 H, dd, $J_{6b,7b}$, $J_{7b,8b}$ 10.2, 7b-H), 5.62 (1 H, ddd, $J_{7b,8b}$, $J_{8b,9b}$ 2.2, $J_{8b,9b'}$, 8b-H), 6.15 (1 H, d, $J_{1a,2a}$, 1a-H), 8.43 (1 H, s, C=NH).

Octyl *S*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate]-(2→3)-2,4,6-tri-*O*-acetyl-3-thio-β-D-galactopyranoside 22

A suspension of **21** (50 mg 53 μmol) and 4 Å molecular sieves (200 mg) in dry CH_2Cl_2 (2 ml) was stirred for 3 h at room temperature. The mixture was cooled to 0 °C and octan-1-ol (21 μl, 130 μmol) was added, followed by boron trifluoride–diethyl ether (7.5 μl, 58.5 μmol) and the mixture was stirred at this temperature for 3 h. The mixture was then diluted with CH_2Cl_2 (8 ml), filtered through Celite and subjected to standard **work-up B**. Repeated column chromatography (silica gel; CH_2Cl_2–MeOH, 97:3) failed to purify the disaccharide fully and so the crude product was used directly in the preparation of compound **23**. Crude octyl glycoside **22** (37 mg) showed $\delta_H(CDCl_3)$ 0.86 (3 H, t, *J* 6.9, $C_7H_{14}CH_3$), 1.26–1.44 (10 H, m, $[CH_2]_5CH_3$), 1.56 (2 H, m, OCH_2CH_2), 1.86 (4 H, m, AcN, $3b_{ax}$-H), 2.00–2.20 (21 H, 6 s, 7 × AcO), 2.63 (1 H, dd, $J_{3bax,3beq}$ 12.6, $J_{3beq,4b}$ 4.7, $3b_{eq}$-H), 3.51 (1 H, m, OCH_2), 3.63 (1 H, dd, $J_{2a,3a}$ 10.7, $J_{3a,4a}$ 3.3, 3a-H), 3.69 (1 H, dd, $J_{5b,6b}$ 10.7, $J_{6b,7b}$ 2.2, 6b-H), 3.81–3.89 (4 H, m, CO_2Me, OCH_2), 3.96–4.12 (5 H, m, 5a-H, 6a-H_2, 5b-, 9b-H), 4.29 (1 H, dd, $J_{8b,9b'}$ 2.7, $J_{9b,9b'}$ 12.4, 9b′-H), 4.75–4.86 (3 H, m, 1a-, 2a-, 4b-H), 4.88 (1 H, d, $J_{3a,4a}$ 3.0, 4a-H), 5.16 (1 H, d, $J_{5b,NH}$ 10.2, NH), 5.31 (1 H, dd, $J_{6b,7b}$, $J_{7b,8b}$ 10.2, 7b-H), 5.59 (1 H, ddd, $J_{7b,8b}$, $J_{8b,9b'}$ 2.7, $J_{8b,9b}$ 5.2, 8b-H); $\delta_C(CDCl_3)$ 13.8, 20.4–20.5 (6), 21.2, 22.4, 22.9, 25.6, 29.0, 29.1, 29.3, 31.6, 36.8, 45.4, 49.0, 53.0, 62.2, 62.25, 66.9, 67.2, 68.4, 69.1, 69.35, 70.0, 72.0, 73.5, 80.8, 101.6, 169.0, 169.7, 170.0, 170.2, 170.3, 170.4 (3), 170.9.

Octyl *S*-[potassium (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate]-(2→3)-3-thio-β-D-galacto-pyranoside 23

A solution of both the crude octyl glycoside **22** (37 mg) and sodium methoxide (2 mg) in dry MeOH (3 ml) was stirred for 2 h at room temperature. The solution was neutralised with acetic acid and concentrated to a solid. The mixture was re-dissolved in aq. potassium hydroxide (3 ml; 1 M). After storage for 3 h at room temperature, the solution was desalted on a reversed-phase column (C-18 silica gel; H_2O→MeOH) to give the fully deprotected compound **23** as a glassy solid (17 mg, 50% from **21**), $[\alpha]_D$ +42.9 (*c* 0.9 in MeOH); $\delta_H(CD_3OD$; 500 MHz) 0.90 (3 H, t, *J* 6.8, $C_7H_{14}CH_3$), 1.26–1.40 (10 H, m, $[CH_2]_5CH_3$), 1.62 (2 H, m, OCH_2CH_2), 1.71 (1 H, m, $3b_{ax}$-H), 2.00 (3 H, s, AcN), 2.91 (1 H, dd, $J_{3bax,3beq}$ 12.6, $J_{3beq,4b}$ 4.2, $3b_{eq}$-H), 3.35 (1 H, dd, $J_{1a,2a}$ 7.4, $J_{2a,3a}$ 11.5, 2a-H), 3.44 (1 H, dd, $J_{2a,3a}$, $J_{3a,4a}$ 2.6, 3a-H), 3.49 (1 H, dd, $J_{6b,7b}$ 1.6, $J_{7b,8b}$ 9.5, 7b-H), 3.52–3.59 (3 H, m, OCH_2CH_2, 5a-, 6b-H), 3.62 (1 H, dd, $J_{8b,9b}$ 5.3, $J_{9b,9b'}$ 11.6, 9b-H), 3.66 (1 H, dd, $J_{5a,6a}$ 5.3, $J_{6a,6a'}$ 11.6, 6a-H), 3.68–3.75 (3 H, m, 6a′-, 4b-, 5b-H), 3.82 (1 H, dd, $J_{8b,9b'}$ 2.6, $J_{9b,9b'}$, 9b′-H), 3.86–3.91 (3 H, m, OCH_2CH_2, 4a-, 8b-H), 4.28 (1 H, d, $J_{1a,2a}$, 1a-H) – see Fig. 4; $\delta_C(CD_3OD)$ 14.5 ($C_7H_{14}CH_3$), 22.7 ($NCOCH_3$), 23.8, 27.3, 30.5, 30.7, 30.9, 33.1 ($[CH_2]_6CH_3$), 43.15 (3b-C), 52.9 (3a-C), 54.0 (5b-C), 63.2 (6a-C), 64.7 (9b-C), 69.4 (4b-C), 70.2 (7b-C), 70.3 (2a-C), 70.4 (4a-C), 70.7 (OCH_2CH_2), 73.2 (8b-C), 77.1 (6b-C), 79.2 (5a-C), 85.9 (2b-C), 106.4 (1a-C), 175.5, 175.75 (1b-C, $NCOCH_3$); ES-MS (−ve) *m*/*z* 598 $(M - K)^-$ ($C_{25}H_{44}KNO_{13}S$ requires *M*, *m*/*z* 637).

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