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Modelling, synthesis and biological evaluation of novel glucuronide-based probes of *Vibrio cholerae* sialidase

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Abstract—The development of sialidase inhibitors is an area of continuing interest due to their potential use as therapeutic agents to combat viral and bacterial infections. Herein, we report our studies involving the sialidase from the pathogen *Vibrio cholerae*, through the modelling, synthesis and biological evaluation of mimetics of 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (Neu5Ac2en, **1**), a naturally occurring sialidase inhibitor. These mimetics are *O*- and *S*-glycosides of *N*-acetyl-D-glucosaminuronic acid in which the aglycone portion effectively replaces the C-6 glycerol side chain of Neu5Ac2en (**1**). The choice of aglycones was aided by use of the X-ray crystal structure of *V. cholerae* sialidase complexed with Neu5Ac2en (**1**). All Neu5Ac2en mimetics tested were found to inhibit *V. cholerae* sialidase as determined using a standard fluorometric assay.

1. Introduction

Sialic acids are a family of nine-carbon sugars, which are often found as the terminal components of cell-surface glycoconjugates. As such, they are well positioned for involvement in many molecular and cellular recognition events, generally by either acting as receptors or by effectively masking recognition sites.[1,2] The biological importance of sialic acids has been confirmed by the discovery of a wide range of proteins that recognise them. Sialidases form a widely studied group of sialic acid-recognising proteins, which catalyse the release of sialic acids from glycoconjugates by hydrolysis of the sialosyl glycosidic bond.[3] Sialidases from mammalian sources are important for catabolism of sialic acid-containing molecules and modification of receptors to alter cellular function. A number of pathogenic microorganisms produce sialidases, despite the fact that they do not biosynthesise sialic acids themselves.[3,4] This has led to the discovery of their role in the pathogenesis of various microbial diseases. In recent years, sialidases from viral sources have been the subject of intense research, leading to the structure-based discovery of therapeutic agents that act by sialidase inhibition.[5] In comparison, there has been little research effort directed towards the development of bacterial sialidase inhibitors.[6]

Vibrio cholerae sialidase is considered a potential drug target for therapeutic agents against cholera. The sialidase, secreted only by epidemic strains of the bacterium *V. cholerae*,[7] is believed to play an important role in infection. As part of a multi-enzyme mucinase complex,[8] the sialidase may facilitate the cholera toxin's entry into epithelial cells[9] by reducing the viscosity of the gastrointestine's protective mucosal coat.[10] *V. cholerae* sialidase also cleaves sialic acids from higher order gangliosides to form GM_1,[11] the receptor for cholera toxin on epithelial cells,[12] thereby increasing the number of available receptor sites to which the toxin may bind. *V. cholerae* sialidase is readily expressed[13] and purified,[14] and the X-ray crystal structure with 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (Neu5Ac2en, **1**), a natural inhibitor, bound in the catalytic site, has been solved to 1.9 Å resolution.[15] Thus, *V. cholerae* sialidase is an ideal representative bacterial sialidase for the development of inhibitors.

Research within our group has been recently directed towards the preparation of carbohydrate-based mimetics of Neu5Ac2en for sialidase inhibition. We have described in preliminary reports[16,17] the synthesis of

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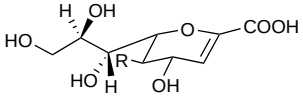
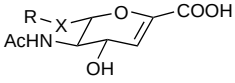
C-6 ether mimetics of Neu5Ac2en of the general structure **2** from *N*-acetylglucosamine, that contain different O-linked alkyl groups that effectively replace the glycerol side chain of Neu5Ac2en (**1**). During the preparation of Neu5Ac2en mimetics represented by **2**, we have developed an efficient synthetic strategy that allows for rapid access to a wide range of mimetics from a common, late stage intermediate.[16] In a preliminary screen ethyl and 3-pentyl derivatives **2a** and **2b**, respectively, prepared by this method were shown to inhibit *V. cholerae* sialidase.[16] We have now completed a more extensive investigation of a wider range of both C-6 ether and thioether Neu5Ac2en mimetics **2** and **3**, respectively, and their biological evaluation against *V. cholerae* sialidase using a fluorometric enzyme assay.



1 Neu5Ac2en; R = NHAc



2 X = O; R = alkyl
2a X = O; R = CH_2Me
2b X = O; R = $CH(CH_2Me)_2$
3 X = S; R = alkyl

2. Results and discussion

2.1. *Vibrio cholerae* sialidase active site

The X-ray crystal structure[15] of the complex between Neu5Ac2en (**1**) and *V. cholerae* sialidase was subjected to an energy-minimisation protocol as previously described.[18] Neu5Ac2en (**1**) and selected active site residues in the energy-minimised complex are depicted in Figure 1. The interactions observed in the energy-minimised complex suggested that there was little change in the relative positions of the catalytic site amino acids and Neu5Ac2en (**1**) compared with those reported[15] for the X-ray crystal structure. The C-5 *N*-acetyl group of Neu5Ac2en (**1**) is located in a hydrophobic pocket formed by Trp311, Asn318, Asn545, Gln317 and Pro251 residues. The hydrophobic residues Phe638 and Leu580 encase the C-8/C-9 region, whilst Asp637 has the potential to form a hydrogen bond to O-8 of the glycerol side chain of **1**. The triarginyl cluster (residues Arg224, Arg635 and Arg712) is well placed for strong interactions with the carboxyl group of Neu5Ac2en (**1**). Hydrogen bonding is possible between the ring hydroxyl group and both Arg245 and Asp250.

In the region of the glycerol side chain, active site residues including Asp637, Ser618, Asn318, Glu619 and Gln317 have the potential to form hydrogen bonds with hydroxyl groups of Neu5Ac2en mimetics containing hydrophilic glycerol side-chain mimics. Alternatively, hydrophobic residues Phe638 and Leu580 are located

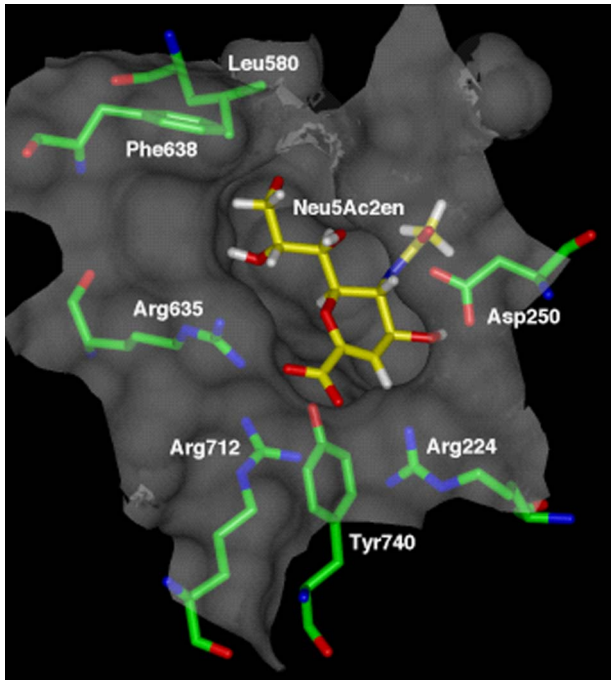
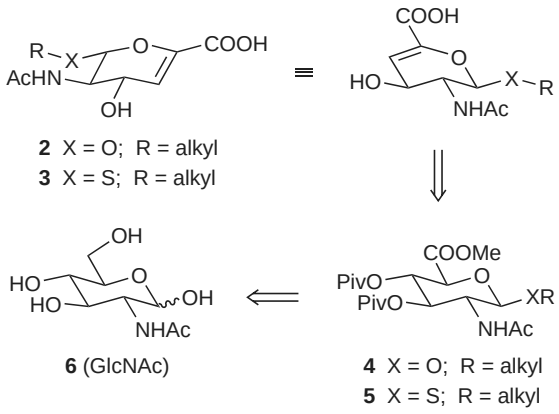


Figure 1. Neu5Ac2en (**1**) in the active site of *V. cholerae* sialidase generated by energy-minimisation[18] of the X-ray crystal structure.[15]

beyond the end of the glycerol side-chain pocket and could potentially form favourable interactions, particularly with hydrophobic groups that are of similar or slightly larger size compared with the glycerol side chain of Neu5Ac2en (**1**). This preliminary molecular modelling study suggested that Neu5Ac2en mimetics that contain either hydrophobic or hydrophilic aglycones could be accommodated in the active site of *V. cholerae* sialidase. With this information in hand, we aimed to prepare a range of glucuronide-based derivatives **2** and **3** that contained different functional groups (R), to probe the glycerol side-chain pocket of *V. cholerae* sialidase.

2.2. Chemistry

The general approach used for the synthesis of both C-6 ether and thioether Neu5Ac2en mimetics **2** and **3**, respectively, is outlined in retrosynthetic terms in

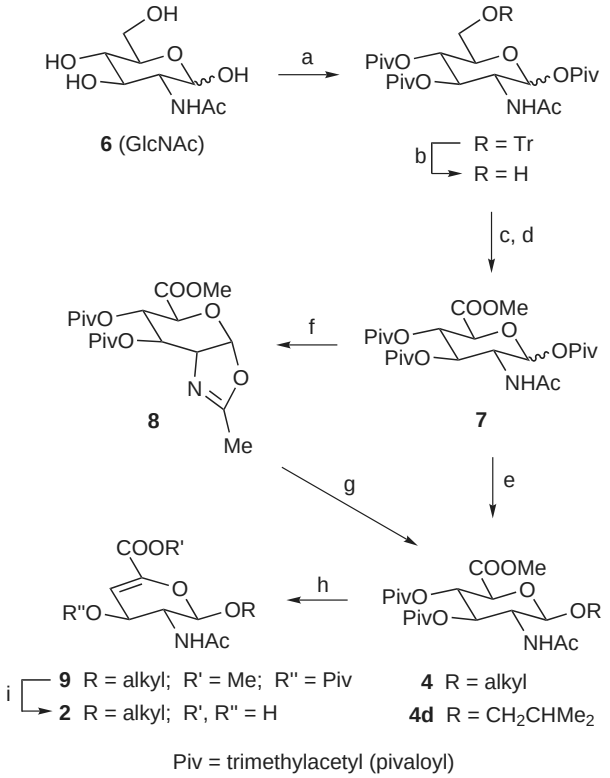


Scheme 1.

Scheme 1. *O*- and *S*-Glycosides **4** and **5**, respectively, of protected *N*-acetylglucosaminuronic acid were prepared from readily available *N*-acetylglucosamine (GlcNAc, **6**).

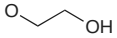
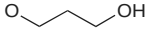
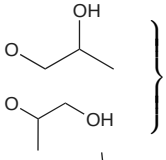
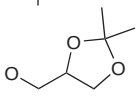
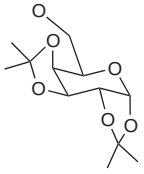
2.3. O-linked Neu5Ac2en mimetics

The method used for the preparation of C-6 ether Neu5Ac2en mimetics **2** is shown in Scheme 2. The pivaloylated glucosaminuronate **7** was made in 5 steps and 58% yield from GlcNAc (**6**) (see Section 3 for full details).[16] A series of 11 *O*-glycosides **4a**–**4k** (Table 1) was prepared by TMSOTf-promoted glycosidation of the pivaloylated glucosaminuronate **7** in 1,2-dichloroethane (DCE). Conversion, determined from recovered starting material **7** (α-anomer) and oxazoline **8**, ranged from 65% to 100%. While isolated yields were in the range of 42–78%, the corrected yields, based on recovered starting material **7** (α-anomer) and oxazoline **8**, ranged from 60% to 94%. In reactions in which the acceptor alcohol contained an isopropylidene-protecting group (i.e., formation of **4i** and **4j**), TLC analysis indicated the presence of polar by-products, suggesting that there may have been some partial loss of the isopropylidene-protecting groups in the presence of TfOH.



Scheme 2. *Reagents and conditions*: (a) i—TrCl, pyridine, ~100 °C, 20 min; ii—PivCl, DMAP, 0 °C → rt, 6 d (83%); (b) 80% aq AcOH, 65 °C, 1 h (92%); (c) TEMPO, KBr, Bu_4NBr, 10–15% aq NaOCl, aq $NaHCO_3$, aq NaCl, CH_2Cl_2, rt, 75 min; (d) $SOCl_2$, $CH(OMe)_3$, MeOH, rt, 30 min (76% over 2 steps); (e) i—TMSOTf, DCE, 50 °C, 3 d; ii—3 Å molecular sieves, rt, 30 min; iii—ROH, 24 h, rt (60–94%, based on recovered α-**7** or **8**); (f) TMSOTf, DCE, 50 °C, 3 d (90%, based on recovered α-**7**); (g) *i*-BuOH, TMSOTf, DCE, 60 °C, 24 h (72%); (h) DBU, CH_2Cl_2, rt, 18 h (80–99%); (i) 50% aq MeOH, aq NaOH, pH 13, 18 h (70–87%).

Table 1. β-Glycosidation of **7** with various alcohols

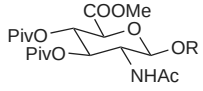
Product	Aglycone	Isolated yield of **4** (%)	Yield (%) based on recovered α-**7** and **8**
4a[c]		64	80
4b[c]		78[a]	94
4c[c]		53	82
4d[c]		77	88
4e		44	61
4f		42	66
4g		63[b]	70
4h			
4i[c]		64	81
4j		47	60
4k		47	68

[a] Some co-elution with unreacted α-**7** during chromatographic purification.
[b] Combined yield of a 3:2 mixture of **4g** and **4h**.
[c] Ref. 16.

Conversion of **7** to the isobutyl β-glycoside **4d** was also carried out over two steps with isolation of the intermediate oxazoline **8** (Scheme 2). Oxazoline **8** was isolated in 90% yield (based on recovered starting material) by reaction of **7** with TMSOTf[19] over 3 d, followed by quenching of the reaction with triethylamine. O-Glycosidation of oxazoline **8** with isobutyl alcohol was carried out in the presence of catalytic TMSOTf[20] at 60 °C to give the β-isobutyl glycoside **4d** in 72% yield. The 65% yield of this glycoside over two steps from **7** was significantly lower than the 88% yield (based on recovered α-**7** and **8**) from the one-pot reaction.

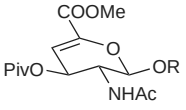
O-Glycosidation of **7** with propan-1,2-diol gave a 3:2 mixture of the primary alcohol and the secondary alcohol glycosidation products **4g** and **4h**, respectively, in 70% yield based on recovered α-**7**. These compounds proved to be inseparable by flash chromatography, and so selective derivatisation of **4h** in the mixture was

carried out. Selective acetylation of the primary hydroxyl group of **4h** was accomplished using AcCl and *i*-Pr_2EtN in CH_2Cl_2[21] to give **4h-Ac** in 95% yield. The acetylated derivative **4h-Ac** was easily separated from unreacted **4g** by chromatography. Other glycosides containing a free hydroxyl group in their aglycone portion (**4e**, **4f** and **4g**) were also acetylated, due to complications experienced with treatment of an unprotected derivative with DBU in the subsequent β-elimination step. Acetylation was achieved using Ac_2O in pyridine to give the corresponding acetates **4e-Ac**, **4f-Ac** and **4g-Ac**.

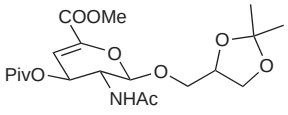


4e-Ac R = CH_2CH_2OAc
4f -Ac R = $CH_2CH_2CH_2OAc$
4g-Ac R = $CH_2CH(OAc)Me$
4h-Ac R = $CH(Me)CH_2OAc$

The final stages in the preparation of C-6 ether Neu5Ac2en mimetics **2** involved β-elimination of the *O*-glycosides **4** to give the 4,5-unsaturated derivatives **9**, followed by deprotection (Scheme 2). Treatment of compounds **4a-4d**, **4e-Ac-4h-Ac** and **4i** with DBU in CH_2Cl_2 afforded the 4,5-unsaturated derivatives **9a–9i** in 80–99% yield after chromatography. ^1H NMR spectroscopy of **9a–9i** showed olefinic H-4 resonances at $\delta \sim 6.2$ and the absence of resonances for H-5.

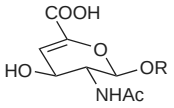


9a R = CH_2Me
9b R = $CH(CH_2Me)_2$
9c R = $CHMe_2$
9d R = CH_2CHMe_2
9e R = CH_2CH_2OAc
9f R = $CH_2CH_2CH_2OAc$
9g R = $CH_2CH(OAc)Me$
9h R = $CH(Me)CH_2OAc$
9i-OH R = $CH_2CH(OH)CH_2OH$



9i

Deprotection of the 4,5-unsaturated derivatives **9** was then carried out to give a series of C-6 ether Neu5Ac2en mimetics **2**. Acid-catalysed hydrolysis of the isopropylidene group in **9i** (50% aq TFA, 0 °C) afforded **9i-OH** in 78% yield after chromatography. Base-catalysed deacylation and de-esterification of each of the unsaturated derivatives **9a–9h** and **9i-OH** were achieved at pH 13 using aqueous NaOH, with TLC analysis indicating a clean conversion to the final products within 18 h (Scheme 2). Purification by HPLC afforded the final derivatives **2a–2i** in 70–87% yield. Thus, a series of C-6 ether Neu5Ac2en mimetics **2a–2i** were prepared in 14–41% yield over 8–9 steps from GlcNAc (**6**).

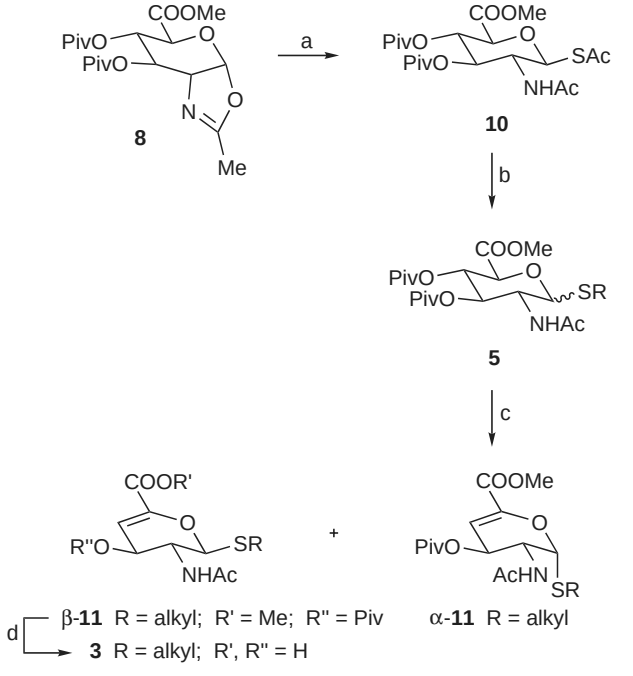


2a R = CH_2Me
2b R = $CH(CH_2Me)_2$
2c R = $CHMe_2$
2d R = CH_2CHMe_2
2e R = CH_2CH_2OH
2f R = $CH_2CH_2CH_2OH$
2g R = $CH_2CH(OH)Me$
2h R = $CH(Me)CH_2OH$
2i R = $CH_2CH(OH)CH_2OH$

2.4. S-linked Neu5Ac2en mimetics

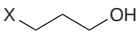
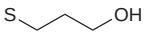
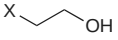
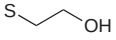
We have had a long-standing interest in the synthesis of thioglycosides of various carbohydrates.[22,23] We thought it of value to utilise this expertise to investigate the possible influence of replacement of oxygen with sulfur on the biological activity of these mimetics. Synthesis of thioether mimetics of the general structure **3** (Scheme 3) was achieved via the intermediate β-*S*-glucosaminuronides **5**, which in turn were prepared from the β-thiolacetate derivative **10**. Treatment of oxazoline **8** with thiolacetic acid in DMF at 80 °C[24] provided the thiolacetate **10** in 64% yield after chromatography. A characteristic anomeric *S*-acetyl resonance was observed at δ2.34 in the ^1H NMR spectrum of **10**, and the $J_{1,2}$ coupling (10.3 Hz) was consistent with a β-1-thiolacetate of a 1,2-*trans*-configured sugar.[22]

With the β-1-thiolacetate **10** in hand, the synthesis of thioglycosides was attempted using $HNEt_2$ to generate



Scheme 3. *Reagents and conditions*: (a) HSAc, DMF, 80 °C, 18 h (64%); (b) alkyl halide, $HNEt_2$, DMF, −20 °C-rt, 4–48 h (57–82%); (c) DBU, CH_2Cl_2, rt, 18 h (90–100%, based on recovered β-**5**); (d) 50% aq MeOH, aq NaOH, pH 13, rt, 18 h (70–85%).

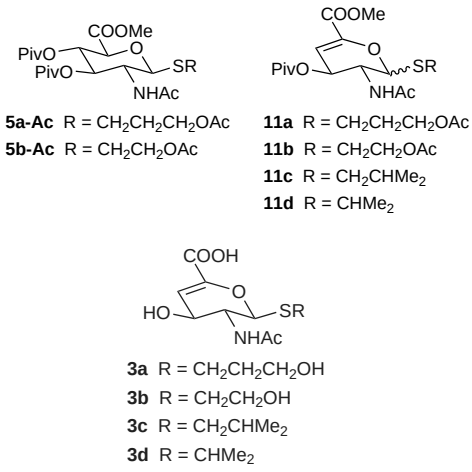
Table 2. Diethylamine-mediated coupling between β-1-thiolacetate **10** and different alkyl halides (R-X) to give thioglycosides **5**

Entry	Alkyl halide coupling partner			Reaction conditions		Thioglycoside product			
	R-X	X	Equivalent	Temperature (°C)	Time (h)	Product	S–R	Yield (%)	α/β ratio
1		Br	1.5	rt	4	**5a**		78	1:1
2		Br	1.5	−20	48			57	1:3
3		Br	5	rt	4			69	1:8
4		Br	5	4	24			74	Only β
5		Br	1.5	rt	4	**5b**		82	1:1
6		Br	5	4	24			76	1:8
7		Br	5	4	24	**5c**		77	1:1
8		I	5	4	24			73	1:9
9		Br	1.5	rt	4	**5d**		65	Only α
10		I	1.5	rt	4			72	Only α
11		I	5	4	24			73	1:2

the intermediate 1-thiolate anion (Scheme 3).[23] Originally developed for the synthesis of thioglycosides of *N*-acetylneuraminic acid,[23] this method has since been used to prepare thioglycosides of mannopyranose,[25] lactose [26] and various glycosylsulfenamides.[27] The method has been reported to be consistently stereoselective, except in the synthesis of thiomannopyranosides where anomeric mixtures of the products were observed.[25] According to the published procedure,[23] a solution of β-1-thiolacetate **10** in DMF containing 1.5 equivalents of 3-bromopropan-1-ol was treated with $HNEt_2$ at room temperature and monitored by TLC. Work-up of the reaction mixture after 4 h gave the thioglycoside product **5a** in 78% yield after chromatography (Table 2, Entry 1). Unexpectedly, analysis by NMR spectroscopy revealed that the product was a mixture of two components in approximately 1:1 ratio. The two components were identified as the α- and β-anomers of the 3-hydroxypropyl thioglycoside **5a**. In the ^{1}H NMR spectrum of the mixture, doublets at δ4.58 (J 10.3 Hz) and at δ5.48 (J 5.1 Hz) were assigned to H-1 of the β- and α-anomers of **5a**, respectively (confirmed by a COSY experiment). The signal for H-5 of the α-anomer at δ4.70 (d, J 9.0 Hz) was downfield compared to the H-5 signal for the β-anomer at δ4.01 (d, 9.5 Hz), which is consistent with an anomeric mixture of 2-deoxy-2-*N*-thioglucopyranosides.[28] Analysis by LRMS showed a single ion (*m*/*z* 514) corresponding to $[M+Na]^+$ of **5a**. In subsequent investigations (Table 2), it was found that lowering the reaction temperature (Entry 2), or increasing the amount of coupling partner (Entry 3), increased the proportion of β-thioglycoside β-**5a**. The combination of reduced temperature (4 °C) and increased amount of coupling partner (5 equivalents) produced the desired result (Entry 4), giving pure β-thioglycoside product **5a**. Similar results were obtained during the synthesis of **5b** from coupling between **10** and 2-bromoethan-1-ol (Entries 5 and 6). Interestingly, reaction of 5 equivalents of isobutyl bromide with the β-1-thiolacetate **10** at 4 °C (Entry 7) gave a 1:1 mixture of anomers. Use of the more reactive isobutyl iodide was required to produce a higher ratio of the desired isobutyl β-thioglycoside β-**5c** (Entry 8). In the case of a secondary alkyl halide, the use of 5 equivalents of 2-iodopropane at lower temperature was required to produce any of the β-thioglycoside **5d** (Entry 11).

The formation of anomeric mixtures of thioglycosides during the one-pot de-S-acetylation and coupling of a β-1-thiolacetate has only been reported for reactions of mannose.[25] Consistent with our observations, Bundle and co-workers[25] obtained anomeric mixtures of thiomannopyranosides from $HNEt_2$-mediated coupling between a β-1-thiolacetate of mannose and various acceptors when the reactions were done at room temperature. Lowering the reaction temperature and using a more reactive coupling partner increased the formation of β-thiomannopyranosides. In the present work, it is presumed that the presence of the electronegative C-6 ester moiety in the glucuronic acid precursor **10** enhances the thermodynamic preference for the formation (by mutarotation) of the α-anomer of the intermediate thiolate compared with the equivalent glucose derivative. However, lowering the reaction temperature, increasing the concentration of coupling partner, or increasing the reactivity of the coupling partner drives the formation of the kinetically favoured β-thioglycoside product (β-**5**).

Thioglycosides **5** were treated with DBU in CH_2Cl_2 (Scheme 3) to give the corresponding unsaturated derivatives **11** in 75–90% isolated yield or higher yield (90–100%) based on recovered thioglycoside. The β-thioglycosides, β-**5**, appeared to be less reactive than the α-thioglycosides, α-**5**, as seen by the recovery of a small amount of β-thioglycoside β-**5** after chromatography. The hydroxyl groups of compounds **5a** and **5b** were acetylated (Ac_2O/pyridine), to give **5a-Ac** and **5b-Ac**, respectively, prior to β-elimination, due to complications that were encountered after treating an unprotected derivative with DBU. The α- and β-anomers of the 4,5-unsaturated thioglycoside products **11a**–**11d** were easily separated from each other by flash chromatography due to a difference in R_f of ~0.2. In each case, the least mobile component was identified using ^{1}H NMR spectroscopy to be the desired unsaturated β-thioglycoside. Base-catalysed deprotection of each of the β-ano-



mers of the unsaturated thioglycosides **11a**–**11d** was carried out in a manner similar to the deprotection of the O-glycosides to give the final derivatives **3a**–**3d** in 70–85% yield after HPLC purification.

2.5. Biological evaluation

Sialidase activity was assessed using a fluorometric assay, which was based upon a method developed by Potier et al.[29] and measures the hydrolysis of 4-methylumbelliferyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosidonic acid (MUN).[29,30] To investigate the nature of the inhibition of the Neu5Ac2en mimetics, Neu5Ac2en (**1**) and the *S*-isopropyl derivative **3d** were, separately, pre-incubated with *V. cholerae* sialidase at 37 °C for 0, 30 or 80 min prior to the addition of MUN (at a final concentration of 50 μM), followed by a further 20 min incubation period. It was found that the maximum inhibition was achieved when the enzyme and inhibitor were incubated for 30 min prior to MUN addition. This suggested that both Neu5Ac2en (**1**) and the *S*-isopropyl derivative **3d** may have slow-binding characteristics.[31] Neu5Ac2en mimetics **2a**–**2e**, **2g**–**2i** and **3a**–**3d** were assessed for inhibition against *V. cholerae* sialidase (see Section 3 for details), and the results are shown in Table 3. The K_i estimates were calculated based on inhibition observed at an inhibitor concentration of 1 mM (i.e., [I] = 1 mM), using the following equation:[32]

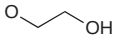
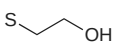
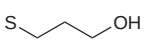
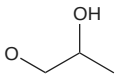
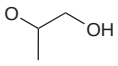
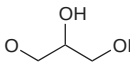
$$\%\text{Inhibition} = [\text{I}]/\{[\text{I}] + K_i(1 + [\text{S}]/K_m)\},^{32}$$

where [I] is the inhibitor concentration, K_i is the inhibition constant, [S] is the substrate concentration and K_m is the Michaelis–Menten constant.

In previous studies, inhibition for Neu5Ac2en mimetics and derivatives was determined to be of a linear competitive type.[17,33] The K_m for *V. cholerae* sialidase using MUN as the substrate was determined to be 1.5 mM.

The fluorometric assay represented a first screen of our series of C-6 modified Neu5Ac2en mimetics and provided an estimation of inhibitory activity against *V. cholerae* sialidase compared with Neu5Ac2en (**1**). The estimated K_i value (3×10^{-5} M) for Neu5Ac2en (**1**) in the present study is comparable to that reported in the literature.[17,33–37] All Neu5Ac2en mimetics tested showed similar inhibitory activity, being approximately one order of magnitude less potent compared with Neu5Ac2en (**1**). Interestingly, derivatives that contain a hydrophobic side chain were generally found to be more potent compared to derivatives with more hydrophilic side chains. The S-linked derivatives **3c** (*i*-Bu), **3d** (*i*-Pr) and **3b** (2-OH-Et) were of similar potency to the O-linked derivatives containing the same aglycone moiety **2d** (*i*-Bu), **2c** (*i*-Pr) and **2e** (2-OH-Et), respectively. This observed similarity in potency is not surprising considering that the thioether and ether derivatives adopt a similar ring conformation in solution. This ring conformation is different to the conformation adopted by Neu5Ac2en (**1**)[38,39] in which all substituents are in an equatorial position.[38,39] A Neu5Ac2en-like ring conformation is required for binding to both influenza virus sialidase[40] and *V. cholerae* sialidase.[15] The magnitude of the coupling constants in the ^{1}H NMR spectra of the

Table 3. Inhibitory activity of Neu5Ac2en mimetics determined against *V. cholerae* sialidase

Mimetic X-R		% Inhibition[a,b] (1 mM)	K_i estimate (M)
Neu5Ac2en	**1**	97	3×10^{-5}
	**2a**	71	4×10^{-4}
	**2b**	90	1×10^{-4}
	**2c**	75	3×10^{-4}
	**3d**	85	2×10^{-4}
	**2d**	82	2×10^{-4}
	**3c**	75	3×10^{-4}
	**2e**	64	6×10^{-4}
	**3b**	70	4×10^{-4}
	**3a**	70	4×10^{-4}
	**2g**	55	8×10^{-4}
	**2h**	79	3×10^{-4}
	**2i**	57	8×10^{-4}

[a] All assays were performed in duplicate or triplicate.
[b] Neu5Ac2en (**1**) was included in every assay for comparison.

presently described series of C-6 modified Neu5Ac2en mimetics, and in particular their small $J_{2,3}$ values, is characteristic[41] of a $^1H_2$ conformation in which the substituents are *quasi*-axial. It is possible that there is an energy penalty incurred on binding of the C-6 ether and C-6 thioether Neu5Ac2en mimetics to *V. cholerae* sialidase in the appropriate Neu5Ac2en-like half-chair conformation, which results in weaker inhibitory activity compared with Neu5Ac2en (**1**). A similar rationale was proposed by Smith et al.[42] to explain the marked differences in inhibition potency between structurally similar 4-amino-4-deoxy-Neu5Ac2en mimetics.[42]

In summary, we have successfully synthesised an extended array of Neu5Ac2en mimetics that are useful probes of *V. cholerae* sialidase. Our preliminary molecular modelling study suggested that alternative, more hydrophobic, functionalities could be accommodated in the glycerol side-chain pocket of the active site and the presented inhibition data support this premise. The chemistry developed in this study provides a useful starting point for the synthesis of designed mimetics, which may significantly inhibit this important enzyme.

3. Experimental section

3.1. General

Reactions were monitored by TLC using Merck silica gel plates 60 F_{254}. Detection was typically effected under UV light where applicable, followed by treatment with H_2SO_4 in EtOH (5% v/v) and charring at ~180 °C. Purification by flash chromatography was achieved with Merck silica gel 60 (0.040–0.063 mm). HPLC was performed using an Agilent HP1100 instrument, and ChemStation for LC 3D software (revision A.09.01 [1206]). Analytical HPLC was carried out using a Phenomenex Aqua 5 μ C18 124 Å column (250 × 4.60 mm). Semi-preparative chromatography was performed using a Phenomenex Aqua 5 μ C18 124 Å column (250 × 10.00 mm). ^{1}H and ^{13}C NMR spectra were recorded using a Brüker Avance 300 spectrometer. All ^{1}H NMR spectra were recorded at 300 MHz, and all ^{13}C NMR spectra were recorded at 75.5 MHz. Chemical shifts are expressed as parts per million (ppm, δ) and are relative to the solvent as an internal reference ($CDCl_3$: δ7.27 for ^{1}H; δ77.0 for ^{13}C; CD_3OD: δ4.78 for ^{1}H; δ49.0 for ^{13}C; D_2O: δ4.67 for ^{1}H). In the NMR assignments, (′) and (″) refer to the atoms of the aglycone unit. Where (#) is used, the assignment is tentative, while (*) designates the minor component or diastereomer in a mixture. Two-dimensional COSY and HMQC experiments were recorded in order to assist with spectral assignment. LRMS were recorded in electrospray ionisation mode on a Brüker Esquire 3000 spectrometer using Brüker Esquire Control software (version 5.0). All spectra were recorded in positive ion mode at a concentration of 0.1 mg/mL using 0.1% AcOH. HRMS and elemental analyses were recorded at the Department of Chemistry at the University of Queensland, Australia. All HRMS were run in positive ion electrospray ionisation mode. All solvents were distilled prior to use or were of analytical grade.

3.2. Methyl 2-acetamido-2-deoxy-1,3,4-tri-*O*-pivaloyl-α,β-D-glucopyranuronate (7) from GlcNAc (6)

A stirred suspension containing anhyd GlcNAc (**6**) (4.0 g, 18 mol), TrCl (6.0 g, 22 mmol) and DMAP (50 mg, 0.5 mmol) in anhyd pyridine (50 mL) under an atmosphere of N_2 was warmed to 100 °C and monitored by TLC analysis (EtOAc/MeOH/H_2O 7:2:1). After 30 min, the reaction mixture was allowed to cool to rt, followed by addition of PivCl (8.0 mL, 65 mmol). The reaction was monitored by TLC analysis (EtOAc/hexane 3:5). After 6 d, the reaction mixture was quenched with MeOH and concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 (200 mL), washed with water (2× 200 mL), dried (Na_2SO_4), filtered and concentrated. Purification of the crude residue by flash chromatography (EtOAc/hexane 1:4 → 1:1) gave an anomeric mixture (α/β 2:3) of 2-acetamido-2-deoxy-6-*O*-triphenylmethyl-1,3,4-tri-*O*-pivaloyl-α,β-D-glucopyranose as a white foam (10.8 g, 83%). R_f = 0.29 (EtOAc/hexane 3:5); ^{1}H NMR ($CDCl_3$) α-anomer: δ 0.88, 1.14, 1.36 (3× 9H, 3× s, 3× OPiv), 1.92 (3H, s, NAc), 3.05 (1H, dd, $J_{6a,6b}$ 10.2, $J_{6a,5}$ 1.8 Hz, H-6a), 3.13 (1H, dd, $J_{6b,6a}$ 10.2, $J_{6b,5}$ 6.0 Hz, H-6b), 3.98–4.06 (1H, m, H-5), 4.57 (1H, ddd, $J_{2,3}$ 10.8, $J_{2,NH}$ 9.0, $J_{2,1}$ 3.6 Hz, H-2), 5.18–5.29 (2H, m, H-3, H-4), 5.43 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH), 6.33 (1H, d, $J_{1,2}$ 3.6 Hz, H-1), 7.17–7.48 (15H, m, OTr); β-anomer: δ 0.86, 1.14, 1.27 (3× 9H, 3× s, 3× OPiv), 1.90 (3H, s, NAc), 3.07 (1H, dd, $J_{6a,6b}$ 10.2, $J_{6a,5}$ 5.1 Hz, H-6a), 3.16 (1H, dd, $J_{6b,6a}$ 10.2, $J_{6b,5}$ 1.8 Hz, H-6b), 3.77 (1H, ddd, $J_{5,4}$ 9.6, $J_{5,6a}$ 5.1, $J_{5,6b}$ 1.8 Hz, H-5), 4.53 (1H, ddd, $J_{2,3}$ 10.6, $J_{2,NH}$ 10.2, $J_{2,1}$ 9.0 Hz, H-2), 5.13 (1H, dd, $J_{3,2}$ 10.6, $J_{3,4}$ 9.6 Hz, H-3), 5.31 (1H, dd, $J_{4,3} = J_{4,5}$ 9.6 Hz, H-4), 5.69 (1H, d, $J_{1,2}$ 9.0 Hz, H-1), 5.72 (1H, br d, $J_{NH,2}$ 10.2 Hz, NH), 7.16–7.47 (15H, m, OTr); ^{13}C NMR ($CDCl_3$) δ 23.6, 23.7 (NC(O)*Me* α/β), 27.4, 27.6, 27.7, 27.8 (3× OC(O)C*Me*$_3$ α/β), 39.1, 39.5, 39.6, 40.0 (3× OC(O)*C*Me$_3$ α/β), 52.5 (C-2 α), 53.5 (C-2 β), 62.5 (C-6 α/β), 67.8, 67.9 (C-4 α/β), 71.2 (C-3 α), 72.6 (C-5 α), 73.1 (C-3 β), 75.1 (C-5 β), 87.0 (O*C*Ph$_3$ α/β), 91.2 (C-1 α), 93.4 (C-1 β), 127.6, 128.4, 129.4, 144.2 (OC*Ph*$_3$ α/β), 170.1, 170.3, 176.4, 176.5, 176.6, 177.8, 179.6, 180.2 (N*C*(O)Me α/β, 3× O*C*(O)CMe$_3$ α/β). LRMS *m*/*z* 738 ([M+Na]$^+$, 30%), 243 ([Ph$_3$C]$^+$, 100). HRMS calcd for $C_{42}H_{53}NNaO_9$ [M+Na] 738.3618. Found 738.3601. A solution of 2-acetamido-2-deoxy-6-*O*-triphenylmethyl-1,3,4-tri-*O*-pivaloyl-α,β-D-glucopyranose (628 mg, 0.88 mmol) in dilute AcOH (80%, 30 mL) was stirred at 60 °C. After 1 h, the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (EtOAc/hexane 3:5 → 1:1) to give 2-acetamido-2-deoxy-1,3,4-tri-*O*-pivaloyl-α,β-D-glucopyranose as an amorphous mass (383 mg, 92%). Recrystallisation of the product from EtOAc/hexane gave pure α-anomer. R_f = 0.15 (EtOAc/hexane 3:5); ^{1}H NMR ($CDCl_3$) α-anomer: δ 1.15, 1.19, 1.31 (3× 9H, 3× s, 3× OPiv), 1.90 (3H, s, NAc), 2.34 (1H, dd, $J_{OH,6a}$ 8.7, $J_{OH,6b}$ 5.7 Hz, OH), 3.52 (1H, ddd, $J_{6b,6a}$ 12.6, $J_{6b,OH}$ 5.7, $J_{6b,5}$ 5.1 Hz, H-6b), 3.65 (1H, ddd, $J_{6a,6b}$ 12.6, $J_{6a,OH}$ 8.7, $J_{6a,5}$ 2.1 Hz, H-6a), 3.77 (1H, ddd, $J_{5,4}$ 9.9, $J_{5,6b}$ 5.1, $J_{5,6a}$ 2.1 Hz, H-5), 4.50 (1H, ddd, $J_{2,3}$ 10.8, $J_{2,NH}$ 9.0, $J_{2,1}$ 3.6 Hz, H-2), 5.20 (1H, dd, $J_{4,5} = J_{4,3}$ 9.9 Hz, H-4),

5.32 (1H, dd, $J_{3,2}$ 10.8, $J_{3,4}$ 9.9 Hz, H-3), 5.46 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH), 6.17 (1H, d, $J_{1,2}$ 3.6 Hz, H-1); β-anomer: δ 1.14, 1.17, 1.20 (3× 9H, 3× s, 3× OPiv), 1.87 (3H, s, NAc), 2.45 (1H, br s, OH), 3.54 (1H, dd, $J_{6a,6b}$ 12.3, $J_{6a,5}$ 4.8 Hz, H-6a), 3.64 (1H, ddd, $J_{5,4}$ 9.0, $J_{5,6a}$ 4.8, $J_{5,6b}$ 2.1 Hz, H-5), 3.71 (1H, dd, $J_{6b,6a}$ 12.3, $J_{6b,5}$ 2.1 Hz, H-6b), 4.41 (1H, ddd, $J_{2,3}$ 10.5, $J_{2,NH}$ 9.9, $J_{2,1}$ 9.0 Hz, H-2), 5.12 (1H, dd, $J_{4,3}$ 9.3, $J_{4,5}$ 9.0 Hz, H-4), 5.20 (1H, dd, $J_{3,2}$ 10.5, $J_{3,4}$ 9.3 Hz, H-3), 5.60 (1H, br d, $J_{NH,2}$ 9.9 Hz, NH), 5.63 (1H, d, $J_{1,2}$ 9.0 Hz, H-1); ^{13}C NMR ($CDCl_3$) δ 23.3 (NC(O)*Me* α/β), 27.1, 27.2, 27.3, 27.4 (3× OC(O)C*Me*$_3$ α/β), 39.1, 39.2, 39.3, 39.7 (3× OC(O)*C*Me$_3$ α/β), 52.0 (C-2 α), 53.1 (C-2 β), 61.3, 61.5 (C-6 α/β), 67.6 (C-4 α), 68.1 (C-4 β), 70.2 (C-3 α), 72.3 (C-3 β), 72.8 (C-5 α), 75.6 (C-5 β), 90.9 (C-1 α), 93.0 (C-1 β), 169.8, 170.0, 176.5, 177.5, 177.6, 177.7, 179.1, 179.9 (N*C*(O)Me α/β, 3× O*C*(O)CMe$_3$ α/β). LRMS *m/z* 496 ([M+Na]$^+$, 100%). HRMS calcd for $C_{23}H_{39}NNaO_9$ [M+Na] 496.2522. Found 496.2531. Anal. Calcd for $C_{23}H_{39}NO_9$: C, 58.33; H, 8.30; N, 2.96. Found: C, 58.25; H, 8.42; N, 2.75. To a solution of 2-acetamido-2-deoxy-1,3,4-tri-*O*-pivaloyl-α,β-D-glucopyranose (6.3 g, 13 mmol) and TEMPO (21 mg, 0.13 mmol) in CH_2Cl_2 (37 mL) was added a solution of saturated aq $NaHCO_3$ (25 mL) containing KBr (141 mg, 1.3 mmol) and Bu_4NBr (216 mg, 0.67 mmol). The biphasic mixture was stirred vigorously at rt, while a solution of aq NaOCl (10–15%, 32 mL), containing saturated aq $NaHCO_3$ (14 mL) and saturated aq NaCl (27 mL), was added over 15 min. After 45 min, a further portion of aq NaOCl (10–15%, 32 mL) was added. After a further 15 min, the reaction mixture was adjusted to pH 2 using dilute HCl (4 M) and diluted with $CHCl_3$ (50 mL). The layers were separated, and the organic layer was washed with water (2× 150 mL), dried (Na_2SO_4), filtered and concentrated. To the residue in anhyd MeOH (40 mL) was added $CH(OMe)_3$ (2.1 mL, 19 mmol), followed by cautious addition of $SOCl_2$ (0.7 mL, 9.6 mmoL) and the solution was stirred under N_2 at rt for 30 min and then concentrated. The crude product was purified by flash chromatography (EtOAc/hexane 2:3) to give the title compound **7** as a white foam (5.10 g, 76%). R_f = 0.25 (EtOAc/hexane 2:3); ^{1}H NMR ($CDCl_3$) α-anomer: δ 1.16, 1.17, 1.32 (3× 9H, 3× s, 3× OPiv), 1.90 (3H, s, NAc), 3.73 (3H, s, OMe), 4.25 (1H, d, $J_{5,4}$ 9.6 Hz, H-5), 4.57 (1H, ddd, $J_{2,3}$ 10.8, $J_{2,NH}$ 9.3, $J_{2,1}$ 3.6 Hz, H-2), 5.27–5.38 (2H, m, H-3, H-4), 5.40 (1H, br d, $J_{NH,2}$ 9.3 Hz, NH), 6.25 (1H, d, $J_{1,2}$ 3.6 Hz, H-1); β-anomer: δ 1.15, 1.20 (3× 9H, 3× s, 3× OPiv), 1.88 (3H, s, NAc), 3.73 (3H, s, OMe), 4.16 (1H, d, $J_{5,4}$ 9.3 Hz, H-5), 4.46 (1H, ddd, $J_{2,3}$ 10.2, $J_{2,NH}$ 9.9, $J_{2,1}$ 8.7 Hz, H-2), 5.20 (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 9.3 Hz, H-3), 5.29 (1H, dd, $J_{4,3} = J_{4,5}$ 9.3 Hz, H-4), 5.44 (1H, br d, $J_{NH,2}$ 9.9 Hz, NH), 5.67 (1H, d, $J_{1,2}$ 8.7 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 23.3, 23.4 (NC(O)*Me* α/β), 27.1, 27.3, 27.4 (3× OC(O)C*Me*$_3$ α/β), 39.1, 39.3, 39.4, 39.7 (3× OC(O)*C*Me$_3$ α/β), 51.6 (C-2 α), 52.8 (C-2 β), 53.2, 53.3 (CO_2*Me* α/β), 68.6 (C-4 α), 69.1 (C-4 β), 69.7 (C-3 α), 71.3 (C-5 α), 71.6 (C-3 β), 73.9 (C-5 β), 90.6 (C-1 α), 92.8 (C-1 β), 167.2, 167.6, 169.7, 169.9, 176.0, 176.8, 179.8, 177.4, 178.3, 178.9 (*C*O$_2$Me α/β, N*C*(O)Me α/β, 3× O*C*(O)CMe$_3$ α/β). LRMS *m/z* 524 ([M+Na]$^+$, 100%). Anal. Calcd for $C_{24}H_{39}NO_{10}$: C, 57.47; H, 7.84; N, 2.79. Found: C, 56.92; H, 8.01; N, 2.79.

3.3. General procedure for the synthesis of 4a–4k

TMSOTf (99 μL, 0.55 mmol) was added to a stirred solution of **7** (α/β ~2:3) (250 mg, 0.50 mmol) in anhyd DCE (2.5 mL) under Ar. The clear yellow solution was warmed to 50 °C. After 3 d, TLC analysis (EtOAc/hexane 1:3) indicated that the starting material was nearly all consumed. The resulting brown reaction mixture was cooled to rt, and 3 Å molecular sieves were added. After 30 min, anhyd alcohol (1.50 mmol) was added and the reaction mixture was stirred at rt under Ar for 24 h. NEt_3 was added to adjust to pH 9, the reaction mixture was filtered through Celite®, the residue was washed with $CHCl_3$/MeOH 10:1 (75 mL) and the filtrate was concentrated to give a brown gum. Purification of the crude product by flash chromatography afforded **4a**–**4k** (60–94%, based on recovered α-**7** and **8**).

3.4. Methyl (ethyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-β-D-glucopyranosid)uronate (4a)

Prepared from reaction between **7** and EtOH in 64% yield after chromatography (EtOAc/hexane 1:1 → 3:2) as a clear colourless gum. Starting material **7** (α-anomer) (7%) and oxazoline **8** (13%) were also isolated. R_f = 0.13 (EtOAc/hexane 1:1); ^{1}H NMR ($CDCl_3$): δ 1.12, 1.13 (2× 9H, 2× s, 2× OPiv), 1.17 (3H, t, $J_{2',1'}$ 7.1 Hz, H-2′), 1.91 (3H, s, NAc), 3.57 (1H, dd, $J_{1'a,1'b}$ 9.7, $J_{1'a,2'}$ 7.1 Hz, H-1′a), 3.90 (1H, dd, $J_{1'b,1'a}$ 9.7, $J_{1'b,2'}$ 7.1 Hz, H-1′b), 3.71 (3H, s, OMe), 3.93 (1H, ddd, $J_{2,3}$ 10.4, $J_{2,NH}$ 9.0, $J_{2,1}$ 8.1 Hz, H-2), 4.09 (1H, d, $J_{5,4}$ 9.7 Hz, H-5), 4.73 (1H, d, $J_{1,2}$ 8.1 Hz, H-1), 5.21 (1H, dd, $J_{4,5}$ 9.7, $J_{4,3}$ 9.5 Hz, H-4), 5.38 (1H, dd, $J_{3,2}$ 10.4, $J_{3,4}$ 9.5 Hz, H-3), 5.92 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 14.9 (C-2′), 23.1 (NC(O)*Me*), 27.0 (2× OC(O)C*Me*$_3$), 38.6, 38.8 (2× OC(O)*C*Me$_3$), 52.6 (CO_2*Me*), 54.2 (C-2), 65.3 (C-1′), 69.4 (C-4), 71.2 (C-3), 72.9 (C-5), 100.6 (C-1), 167.6, 169.9, 176.5, 178.2 (*C*O$_2$Me, N*C*(O)Me, 2× O*C*(O)CMe$_3$). LRMS *m/z* 468 ([M+Na]$^+$, 100%). HRMS calcd for $C_{21}H_{36}NO_9$ [M+H] 446.2390. Found 446.2402. Anal. Calcd for $C_{21}H_{35}NO_9 \cdot 0.5H_2O$: C, 55.49; H, 7.98; N, 3.08. Found C, 55.77; H, 8.04; N, 3.08.

3.5. Methyl (3-pentyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-β-D-glucopyranosid)uronate (4b)

Prepared from reaction between **7** and pentan-3-ol in 78% yield after chromatography (EtOAc/hexane 1:3 → 2:3) as a white amorphous mass. Starting material **7** (α-anomer) (12%) and oxazoline **8** (5%) were also isolated. R_f = 0.28 (EtOAc/hexane 2:3); ^{1}H NMR ($CDCl_3$): δ 0.76–0.85 (6H, m, H-3′, H-3″), 1.08, 1.10 (2× 9H, 2× s, 2× OPiv), 1.37–1.58 (4H, m, H-2′, H-2″), 1.85 (3H, s, NAc), 3.43 (1H, tt, $J_{1',2'} = J_{1',2''}$ 5.7 Hz, H-1′), 3.67 (3H, s, OMe), 3.91 (1H, ddd, $J_{2,3}$ 10.6, $J_{2,NH}$ 9.1, $J_{2,1}$ 8.3 Hz, H-2), 4.06 (1H, d, $J_{5,4}$ 9.9 Hz, H-5), 4.74 (1H, d, $J_{1,2}$ 8.3 Hz, H-1), 5.15 (1H, dd, $J_{4,3} = J_{4,5}$ 9.7 Hz, H-4), 5.40 (1H, dd, $J_{3,2}$ 10.6, $J_{3,4}$ 9.5 Hz, H-3), 6.40 (1H, br d, $J_{NH,2}$ 9.1 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 8.9, 9.4 (C-3′, C-3″), 23.0 (NC(O)*Me*), 25.6, 26.7 (C-2′, C-2″),

26.9, 27.0 (2× OC(O)C*Me*$_3$), 38.6, 38.8 (2× OC(O)*C*Me$_3$), 52.8 (CO_2*Me*), 54.7 (C-2), 69.4 (C-4), 71.2 (C-3), 72.6 (C-5), 82.9 (C-1′), 100.2 (C-1), 167.6, 169.9, 176.5, 178.2 (*C*O_2Me, N*C*(O)Me, 2× O*C*(O)CMe$_3$). LRMS *m*/*z* 510 ([M+Na]$^+$, 100%). HRMS calcd for $C_{24}H_{41}NNaO_9$ [M+Na] 510.2679. Found 510.2686.

3.6. Methyl (isopropyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-β-D-glucopyranosid)uronate (4c)

Prepared from reaction between **7** and *i*-PrOH in 53% yield after chromatography (EtOAc/hexane 2:3 → 1:1) as a white amorphous mass. Starting material **7** (α-anomer) (32%) and oxazoline **8** (3%) were also isolated. R_f = 0.20 (EtOAc/hexane 1:1); ^{1}H NMR ($CDCl_3$): δ 1.13 (3H, d, $J_{2',1'}$ 6.3 Hz, H-2′), 1.22 (3H, d, $J_{2'',1'}$ 6.3 Hz, H-2″), 1.13, 1.14 (2× 9H, 2× s, 2× OPiv), 1.92 (3H, s, NAc), 3.73 (3H, s, OMe), 3.79 (1H, ddd, $J_{2,3}$ 10.4, $J_{2,1} = J_{2,NH}$ 8.4 Hz, H-2), 3.96 (1H, sept, $J_{1',2'} = J_{1',2''}$ 6.3 Hz, H-1′), 4.09 (1H, d, $J_{5,4}$ 9.8 Hz, H-5), 4.87 (1H, d, $J_{1,2}$ 8.2 Hz, H-1), 5.21 (1H, dd, $J_{4,5} = J_{4,3}$ 9.6 Hz, H-4), 5.47 (1H, dd, $J_{3,2}$ 10.0, $J_{3,4}$ 9.5 Hz, H-3), 5.65 (1H, br d, $J_{NH,2}$ 8.5 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 21.7, 23.0 (C-2′, C-2″), 23.2 (NC(O)*Me*), 27.0, 27.1 (2× OC(O)C*Me*$_3$), 38.6, 38.8 (2× OC(O)*C*Me$_3$), 52.6 (CO_2*Me*), 54.9 (C-2), 69.5 (C-4), 71.7 (C-3), 72.4 (C-1′), 72.8 (C-5), 99.3 (C-1), 167.6, 170.1, 176.5, 178.1 (*C*O_2Me, N*C*(O)Me, 2× O*C*(O)CMe$_3$). LRMS *m*/*z* 482.5 ([M+Na]$^+$, 100%). Anal. Calcd for $C_{22}H_{37}NO_9$: C, 57.50; H, 8.12; N, 3.05. Found: C, 57.34; H, 8.30; N, 3.03.

3.7. Methyl (2-methylpropyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-β-D-glucopyranosid)uronate (4d)

Prepared from reaction between **7** and *i*-BuOH in 77% yield after chromatography (EtOAc/hexane 1:3 → 1:1) as a white foam. Starting material **7** (α-anomer) (6%) and oxazoline **8** (6%) were also isolated. R_f = 0.24 (EtOAc/hexane 2:3); ^{1}H NMR ($CDCl_3$): δ 0.87 (3H, d, $J_{3',2'}$ 6.7 Hz, H-3′), 0.88 (3H, d, $J_{3'',2'}$ 6.7 Hz, H-3″), 1.14, 1.15 (2× 9H, 2× s, 2× OPiv), 1.85 (1H, m, H-2′), 1.91 (3H, s, NAc), 3.20 (1H, dd, $J_{1'a,1'b}$ 9.3, $J_{1'a,2'}$ 7.1 Hz, H-1′a), 3.70 (1H, dd, $J_{1'b,1'a}$ 9.3, $J_{1'b,2'}$ 7.1 Hz, H-1′b), 3.72 (3H, s, OMe), 3.99 (1H, ddd, $J_{2,3}$ 10.3, $J_{2,NH}$ 9.0, $J_{2,1}$ 8.1 Hz, H-2), 4.07 (1H, d, $J_{5,4}$ 9.6 Hz, H-5), 4.68 (1H, d, $J_{1,2}$ 8.1 Hz, H-1), 5.23 (1H, dd, $J_{4,5} = J_{4,3}$ 9.4 Hz, H-4), 5.36 (1H, dd, $J_{3,2}$ 10.3, $J_{3,4}$ 9.4 Hz, H-3), 5.59 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 18.9, 19.1 (C-3′, C-3″), 23.1 (NC(O)*Me*), 27.0 (2× OC(O)C*Me*$_3$), 28.3 (C-2′), 38.7, 38.9 (2× OC(O)*C*Me$_3$), 52.7 (CO_2*Me*), 54.4 (C-2), 69.3 (C-4), 71.7 (C-3), 73.0 (C-5), 76.6 (C-1′), 101.2 (C-1), 167.6, 169.8, 176.5, 178.3 (*C*O_2Me, N*C*(O)Me 2× O*C*(O)CMe$_3$). LRMS *m*/*z* 496 ([M+Na]$^+$, 100%). HRMS calcd for $C_{23}H_{29}NNaO_9$ [M+Na] 496.2523. Found 496.2530.

3.8. Methyl (2-*O*-acetyl-2-hydroxyethyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-β-D-glucopyranosid)uronate (4e-Ac)

Prepared from reaction between **7** and ethylene glycol. To remove excess ethylene glycol, the crude product was diluted with EtOAc and washed twice with water, dried (Na_2SO_4), filtered and concentrated. Purification of the crude product by flash chromatography (EtOAc → EtOAc/MeOH 10:1) gave **4e** [R_f = 0.23 (EtOAc); 44%] as a clear yellow syrup. Starting material **7** (α-anomer) (28%) was also isolated. To **4e** (212 mg, 0.51 mmol) dissolved in anhyd pyridine (3 mL) was added Ac_2O (0.1 mL, 1 mmol) and the solution was stirred at rt overnight under N_2. The reaction was quenched using MeOH (0.5 mL) and then concentrated. The crude product was dissolved in EtOAc (15 mL), washed with dilute HCl (1 M, 2× 15 mL), water (15 mL), dried (Na_2SO_4), filtered and concentrated. Purification of the crude product by flash chromatography (EtOAc/hexane 3:2 → 3:1) gave the title compound **4e-Ac** (211 mg, 91%) as an amorphous mass. R_f = 0.21 (EtOAc/hexane 3:2); ^{1}H NMR ($CDCl_3$): δ 1.14, 1.15 (2× 9H, 2× s, 2× OPiv), 1.93 (3H, s, NAc), 2.07 (3H, s, OAc), 3.74 (3H, s, OMe), 3.80 (1H, ddd, $J_{1'a,1'b}$ 11.5, $J_{1'a,2'a}$ 6.5, $J_{1'a,2'b}$ 3.2 Hz, H-1′a), 3.92–4.06 (2H, m, H-1′b, H-2), 4.09 (1H, d, $J_{5,4}$ 9.6 Hz, H-5), 4.13 (1H, ddd, $J_{2'a,2'b}$ 12.1, $J_{2'a,1'a}$ 6.5, $J_{2'a,1'b}$ 3.0 Hz, H-2′a), 4.33 (1H, ddd, $J_{2'b,2'a}$ 12.1, $J_{2'b,1'b}$ 6.6, $J_{2'b,1'a}$ 3.2 Hz, H-2′b), 4.80 (1H, d, $J_{1,2}$ 8.1 Hz, H-1), 5.24 (1H, dd, $J_{4,5}$ 9.6, $J_{4,3}$ 9.3 Hz, H-4), 5.36 (1H, dd, $J_{3,2}$ 10.3,$J_{3,4}$ 9.3 Hz, H-3), 5.57 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 20.9 (NC(O)*Me*), 23.2 (OC(O)*Me*), 27.0 (2× OC(O)C*Me*$_3$), 38.7, 38.9 (2× OC(O)*C*Me$_3$), 52.7 (CO_2*Me*), 54.0 (C-2), 62.8 (C-2′), 67.0 (C-1′), 69.2 (C-4), 70.9 (C-3), 73.0 (C-5), 100.3 (C-1), 167.4, 169.9, 171.0, 176.5, 178.1 (*C*O_2Me, O*C*(O)Me, N*C*(O)Me, 2× O*C*(O)CMe$_3$). LRMS *m*/*z* 526 ([M+Na]$^+$, 100%). Anal. Calcd for $C_{23}H_{37}NO_{11}$: C, 54.86; H, 7.41; N, 2.78. Found: C, 54.62; H, 7.48; N, 2.68.

3.9. Methyl (3-*O*-acetyl-3-hydroxypropyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-β-D-glucopyranosid)uronate (4f-Ac)

Prepared from reaction between **7** and propan-1,3-diol. To remove excess propan-1,3-diol, the crude product was diluted with EtOAc and washed twice with water, dried (Na_2SO_4), filtered and concentrated. The crude product was purified by flash chromatography (EtOAc → EtOAc/MeOH 10:1) to give **4f** [R_f = 0.21 (EtOAc); 42%] as a clear colourless residue. Starting material **7** (α-anomer) (36%) was also isolated. Ac_2O (0.3 mL, 3 mmol) was added to a solution of **4f** (432 mg, 0.91 mmol) in anhyd pyridine (4 mL) under N_2. The solution was stirred at rt overnight. The reaction was quenched with MeOH (0.5 mL), and then the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc (15 mL), washed with dilute HCl (1 M, 2× 15 mL), water (15 mL), dried (Na_2SO_4), filtered and concentrated. The crude product was purified by flash chromatography (EtOAc/hexane 3:2) to give the title compound **4f-Ac** (447 mg, 95%) as an amorphous mass. R_f = 0.24 (EtOAc/hexane 3:2); ^{1}H NMR ($CDCl_3$): δ 1.04, 1.06 (2× 9H, 2× s, 2× OPiv), 1.72–1.87 (2H, m, H-2′, H-2″), 1.83 (3H, s, NAc), 1.95 (3H, s, OAc), 3.46 (1H, ddd, $J_{1'a,1'b}$ 9.9, $J_{1'a,2'a}$ 6.9, $J_{1'a,2'b}$ 6.0 Hz, H-1′a), 3.63 (3H, s, OMe), 3.89 (1H, ddd, $J_{1'b,1'a}$ 9.9, $J_{1'b,2'a}$ 5.7, $J_{1'b,2'b}$ 5.4 Hz, H-1′b), 3.91–4.04 (2H, m, H-2, H-3′a), 4.03 (1H, d, $J_{5,4}$ 9.9 Hz, H-5), 4.12 (1H, ddd, $J_{3'b,3'a}$ 10.8, $J_{3'b,2'a} = J_{3'b,2'a}$ 6.6 Hz, H-3′b), 4.64 (1H, d, $J_{1,2}$ 8.1 Hz, H-1), 5.11 (1H, dd,

$J_{4,5} = J_{4,3}$ 9.6 Hz, H-4), 5.32 (1H, dd, $J_{3,2}$ 10.5, $J_{3,4}$ 9.6 Hz, H-3), 6.49 (1H, br d, $J_{NH,2}$ 9.3 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 20.7 (OC(O)*Me*), 22.8 (NC(O)*Me*), 26.8 (2× OC(O)C*Me*$_3$), 28.5 (C-2′), 38.4, 38.6 (2× OC(O)*C*Me$_3$), 52.5 (CO_2*Me*), 53.6 (C-2), 60.8 (C-3′), 65.9 (C-1′), 69.3 (C-4), 71.0 (C-3), 72.5 (C-5), 100.7 (C-1), 167.5, 170.1, 171.0, 176.4, 177.8 (*C*O$_2$Me, N*C*(O)Me, O*C*(O)Me, 2× O*C*(O)CMe$_3$). LRMS *m*/*z* 540 ([M+Na]$^+$, 100%). Anal. Calcd for $C_{24}H_{39}NO_{11}$: C, 55.69; H, 7.60; N, 2.71. Found: C, 55.70; H, 7.72; N, 2.66.

3.10. Methyl (1-*O*-acetyl-1-hydroxyisopropyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-β-D-glucopyranosid)uronate (4h-Ac) and methyl (2-*O*-acetyl-2-hydroxypropyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-β-D-glucopyranosid)uronate (4g-Ac)

Prepared from reaction between **7** and propan-1,2-diol. To remove excess propan-1,2-diol, the crude product was diluted with EtOAc and washed twice with water, dried (Na_2SO_4), filtered and concentrated. The crude product was purified by flash chromatography (EtOAc/hexane 1:3 → EtOAc/MeOH 5:1) to give a 3:2 mixture of **4g** and **4h** [R_f = 0.20 (EtOAc)] as a clear colourless residue in 63% yield. Starting material **7** (α-anomer) (10%) was also isolated. *i*-Pr$_2$EtN (26 μL, 0.37 mmol) was added to a 3:2 mixture of **4g** and **4h** (85 mg, 0.18 mmol) in anhyd CH_2Cl_2 (0.88 mL) under Ar. The solution was cooled to −78 °C, and AcCl (5.0 mL, 0.11 mmol) was added dropwise. The reaction mixture was stirred for 2.5 h at −78 °C and then warmed to rt. After a further 1 h at rt, the reaction mixture was diluted with $CHCl_3$ (5 mL), washed with dilute HCl (1 M, 5 mL), water (5 mL), saturated aq NaCl (5 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure to give a pale yellow residue. Purification of the crude product by flash chromatography (EtOAc → EtOAc/MeOH 5:1) afforded a 5:1 diastereomeric mixture of **4h-Ac** (36 mg, 95% from **4h**) as a colourless residue. A 1:1 diastereomeric mixture of unreacted **4g** (35 mg, 66% from **4g**) was also isolated. Compound **4h-Ac**: R_f = 0.43, 0.50* (EtOAc); ^{1}H NMR ($CDCl_3$) including partial assignment of minor diastereomer: δ 1.13 (9H, s, OPiv), 1.14 (9H, s, OPiv*), 1.23 (3H, d, $J_{3',1'}$ 7.5 Hz, H-3′*), 1.25 (3H, d, $J_{3',1'}$ 6.3 Hz, H-3′), 1.91 (3H, s, NAc), 1.92 (3H, s, NAc*), 2.07 (3H, s, OAc*), 2.08 (3H, s, OAc), 3.71 (3H, s, OMe*) 3.73 (3H, s, OMe), 3.88 (1H, dd, $J_{2'a,2'b}$ 11.7, $J_{2'a,1'}$ 5.7 Hz, H-2′a), 3.90 (1H, ddd, $J_{2,3}$ 9.9, $J_{2,NH}$ 9.0, $J_{2,1}$ 8.4 Hz, H-2), 4.04 (1H, ddd, $J_{1',3'}$ 6.3, $J_{1',2'a}$ 5.7, $J_{1',2'b}$ 3.3 Hz, H-1′), 4.07 (1H, d, $J_{5,4}$ 9.9 Hz, H-5), 4.07 (1H, d, $J_{5,4}$ 9.9 Hz, H-5*), 4.26 (1H, dd, $J_{2'b,2'a}$ 11.7, $J_{2'b,1'}$ 3.3 Hz, H-2′b), 4.88 (1H, d, $J_{1,2}$ 8.4 Hz, H-1), 4.93 (1H, d, $J_{1,2}$ 8.4 Hz, H-1*), 5.20 (1H, dd, $J_{4,5}$ 9.9, $J_{4,3}$ 9.6 Hz, H-4), 5.39 (1H, dd, $J_{3,2}$ 9.9, $J_{3,4}$ 9.6 Hz, H-3), 5.50 (1H, dd, $J_{3,2}$ 9.9, $J_{3,4}$ 9.6 Hz, H-3*), 5.62 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH), 5.65 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH*); ^{13}C NMR ($CDCl_3$) including partial assignment of minor diastereomer: δ 16.7 (C-3′), 18.1 (C-3′), 20.8 (OC(O)*Me**), 20.9 (OC(O)*Me*), 23.1 (NC(O)*Me*, NC(O)*Me**), 26.9, 27.0, 27.1 (2× OC(O)C*Me*$_3$, 2× OC(O)C*Me*$_3$*), 38.7, 38.8 (2× OC(O)*C*Me$_3$), 52.6 (CO_2*Me**), 52.7 (CO_2*Me*), 54.5 (C-2), 55.2 (C-2*), 66.2 (C-2′), 67.0 (C-2′*), 69.3 (C-4), 70.6 (C-3*), 71.1 (C-3), 72.8, 73.8 (C-5*, C-1′*), 72.9, 73.6 (C-5, C-1′), 99.2 (C-1*), 99.8 (C-1), 167.4, 169.9, 171.0, 176.5, 178.0 (N*C*(O)Me, O*C*(O)Me, *C*O$_2$Me, 2× O*C*(O)CMe$_3$), *minor diastereomer. LRMS *m*/*z* 540 ([M+Na]$^+$, 100%). HRMS calcd for $C_{24}H_{39}NNaO_{11}$ [M+Na] 540.2421. Found 540.2428. Acetic anhydride (0.2 mL, 2 mmol) was added to a solution of a 1:1 diastereomeric mixture of **4g** (337 mg, 0.71 mmol) in anhyd pyridine (3 mL). The solution was stirred at rt overnight under N_2. MeOH (1 mL) was added and the solution was concentrated under reduced pressure. The crude product was dissolved in EtOAc (15 mL) and then washed with dilute HCl (1 M, 2× 15 mL), water (2× 15 mL), saturated aq NaCl (15 mL), dried (Na_2SO_4), filtered and concentrated. The crude product was purified by flash chromatography (EtOAc/hexane 3:2) to give a 1:1 diastereomeric mixture of **4g-Ac** as an amorphous mass (285 mg, 78%). Compound **4g-Ac**: R_f = 0.24 (EtOAc/hexane 3:2); ^{1}H NMR ($CDCl_3$): δ 1.13, 1.14 (2× 18H, 2× s, 4× OPiv), 1.19 (3H, d, $J_{3',2'}$ 6.6 Hz, H-3′), 1.20 (3H, d, $J_{3',2'}$ 6.6 Hz, H-3′), 1.91 (2× 3H, 2× s, 2× NAc), 2.04 (2× 3H, 2× s, 2× OAc), 3.57 (1H, dd, $J_{1'a,1'b}$ 10.8, $J_{1'a,2'}$ 6.3 Hz, H-1′a), 3.67 (1H, dd, $J_{1'a,1'b}$ 11.7, $J_{1'a,2'}$ 3.6 Hz, H-1′a), 3.73 (6H, s, 2× OMe), 3.76 (1H, dd, $J_{1'b,1'a}$ 11.7, $J_{1'b,2'}$ 4.5 Hz, H-1′b), 3.85 (1H, dd, $J_{1'b,1'a}$ 10.8, $J_{1'b,2'}$ 4.5 Hz, H-1′b), 3.94 (1H, ddd, $J_{2,3}$ 10.2, $J_{2,NH}$ 9.0, $J_{2,1}$ 8.4 Hz, H-2), 4.05 (1H, ddd, $J_{2,3}$ 10.2, $J_{2,NH}$ 9.0, $J_{2,1}$ 8.4 Hz, H-2), 4.06 (1H, d, $J_{5,4}$ 9.6 Hz, H-5), 4.07 (1H, d, $J_{5,4}$ 9.6 Hz, H-5), 4.73 (1H, d, $J_{1,2}$ 8.4 Hz, H-1), 4.76 (1H, d, $J_{1,2}$ 8.4 Hz, H-1), 4.96-5.07 (1H, m, H-2′), 5.08–5.18 (1H, m, H-2′), 5.21 (1H, dd, $J_{4,5}$ 9.6, $J_{4,3}$ 9.3 Hz, H-4), 5.21 (1H, dd, $J_{4,5}$ 9.6, $J_{4,3}$ 9.3 Hz, H-4), 5.31 (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 9.3 Hz, H-3), 5.38 (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 9.3 Hz, H-3), 5.64 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH), 5.67 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 16.3, 16.5 (2× C-3′), 21.2, 21.3 (2× OC(O)*Me*), 23.1 (2× NC(O)*Me*), 27.0 (4× OC(O)C*Me*$_3$), 38.7, 38.8 (4× OC(O)*C*Me$_3$), 52.7 (2× CO_2*Me*), 53.7, 54.2 (2× C-2), 67.8, 69.2, 69.3 (2× C-2′, 2× C-4), 70.7, 71.2 (2× C-3), 70.9, 71.4 (2× C-1′), 72.9, 73.0 (2× C-5), 99.8, 100.8 (2× C-1), 167.4, 169.8, 170.5, 170.9, 176.5, 178.1 (2× *C*O$_2$Me, 2× N*C*(O)Me, 2× O*C*(O)Me, 4× O*C*(O)CMe$_3$). LRMS *m*/*z* 540.5 ([M+Na]$^+$, 100%). HRMS calcd for $C_{24}H_{39}NNaO_{11}$ [M+Na] 540.2421. Found 540.2421.

3.11. Methyl (2,3-dihydroxy-2,3-*O*-isopropylidenepropyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-β-D-glucopyranosid) uronate (4i)

Prepared from reaction between **7** and 2,3-dihydroxy-2,3-*O*-isopropylidenepropanol. After addition of 2,3-dihydroxy-2,3-*O*-isopropylidenepropanol the reaction mixture was stirred for 48 h before work-up by the usual method, to give a 3:2 diastereomeric mixture of the title compound **4i** in 64% yield after chromatography (EtOAc/hexane 3:2) as a white amorphous mass. Starting material **7** (α-anomer) (14%) and oxazoline **8** (7%) were also isolated. R_f = 0.20 (EtOAc/hexane 3:2); ^{1}H NMR ($CDCl_3$): δ 1.13, 1.14 (2× 18H, 2× s, 2× OPiv, 2× OPiv*), 1.33, 1.34, 1.40, 1.41 (4× 3H, 4× s, O_2CMe_2, O_2CMe_2*), 1.91, 1.92 (2× 3H, 2× s, NAc, NAc*), 3.63 (1H, dd, $J_{1'a,1'b}$ 10.8, $J_{1'a,2'}$ 5.4 Hz,

H-1′a[#]), 3.64 (1H, dd, $J_{1'a,1'b}$ 10.5, $J_{1'a,2'}$ 6.6 Hz, H-1′a*[#]), 3.67 (1H, dd, $J_{3'a,3'b}$ 8.4, $J_{3'a,2'}$ 6.3 Hz, H-3′a[#]), 3.72 (6H, s, OMe, OMe*), 3.80 (1H, dd, $J_{3'a,3'b}$ 8.1, $J_{3'a,2'}$ 6.0 Hz, H-3′a*[#]), 3.84 (1H, dd, $J_{1'b,1'a}$ 10.8, $J_{1'b,2'}$ 5.4 Hz, H-1′b[#]), 3.89 (1H, dd, $J_{1'b,1'a}$ 10.5, $J_{1'b,2'}$ 4.5 Hz, H-1′b*[#]), 3.97-4.15 (4H, m, H-2, H-2*, H-3′b[#], H-3′b*[#]), 4.06 (1H, d, $J_{5,4}$ 9.3 Hz, H-5), 4.07 (1H, d, $J_{5,4}$ 9.6 Hz, H-5*), 4.20-4.30 (2H, m, H-2′, H-2′*), 4.77 (1H, d, $J_{1,2}$ 8.4 Hz, H-1), 4.77 (1H, d, $J_{1,2}$ 8.1 Hz, H-1*), 5.21 (1H, dd, $J_{4,5} = J_{4,3}$ 9.3 Hz, H-4), 5.21 (1H, dd, $J_{4,5}$ 9.6, $J_{4,3}$ 9.3 Hz, H-4*), 5.31 (1H, dd, $J_{3,2}$ 10.5, $J_{3,4}$ 9.3 Hz, H-3), 5.31 (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 9.3 Hz, H-3*), 5.70 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH), 5.71 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH*); ^{13}C NMR ($CDCl_3$): δ 23.1, 23.2 (2× NC(O)*Me*), 25.1, 25.4, 26.6, 26.8 (2× O_2CMe_2), 27.0 (4× OC(O)C*Me*$_3$), 38.7, 38.8 (4× OC(O)*C*Me$_3$), 52.7 (2× CO_2*Me*), 53.8 (2× C-2), 66.0, 66.5 (2× C-3′[#]), 69.1 (C-1′[#]), 69.1 (2× C-4), 70.6 (C-1′[#]), 71.1, 71.2 (2× C-3), 72.9, 73.0 (2× C-5), 74.3, 74.9 (2× C-2′), 101.0, 101.1 (2× C-1), 109.3, 109.6 (2× O_2*C*Me$_2$), 167.4, 169.9, 170.0, 176.5, 178.2 (2× *C*O_2Me, 2× N*C*(O)Me, 4× O*C*(O)CMe$_3$), *minor diastereomer, [#]assignments tentative. LRMS *m/z* 555 ([M+Na]$^+$, 100%). Anal. Calcd for $C_{25}H_{41}NO_{11}$: C, 56.48; H, 7.77; N, 2.63. Found: C, 56.10; H, 7.81; N, 2.38.

3.12. Methyl (1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranosyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-β-D-glucopyranosid)uronate (4j)

Prepared from reaction between **7** and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose[43,44] in 47% yield after chromatography (EtOAc/hexane 2:3 → EtOAc) as an amorphous mass. Starting material **7** (α-anomer) (16%) and oxazoline **8** (5%) were also isolated. R_f = 0.15 (EtOAc/hexane 1:1); ^{1}H NMR ($CDCl_3$) GlcNAcA unit: δ 1.14, 1.15 (2× 9H, 2× s, 2× OPiv), 1.94 (3H, s, NAc), 3.74 (3H, s, OMe), 4.06 (1H, d, $J_{5,4}$ 9.6 Hz, H-5[#]), 4.19 (1H, ddd, $J_{2,3}$ 9.6, $J_{2,NH}$ 9.3, $J_{2,1}$ 8.4 Hz, H-2), 4.74 (1H, d, $J_{1,2}$ 8.4 Hz, H-1), 5.17-5.26 (2H, m, H-3, H-4), 5.47 (1H, br d, $J_{NH,2}$ 9.3 Hz, NH); Gal unit: 1.31, 1.33, 1.45, 1.51 (4× 3H, 4× s, 4× Me), 3.77 (1H, dd, $J_{6a,6b}$ 12.9, $J_{6a,5}$ 9.3 Hz, H-6a), 3.92-4.01 (2H, m, H-5, H-6b), 4.14 (1H, dd, $J_{4,3}$ 7.8, $J_{4,5}$ 1.5 Hz, H-4), 4.31 (1H, dd, $J_{2,1}$ 5.1, $J_{2,3}$ 2.4 Hz, H-2), 4.58 (1H, dd, $J_{3,4}$ 7.8, $J_{3,2}$ 2.4 Hz, H-3), 5.53 (1H, d, $J_{1,2}$ 5.1 Hz, H-1); ^{13}C NMR ($CDCl_3$) GlcNAcA unit: δ 23.2 (NC(O)*Me*), 26.9 (2× OC(O)C*Me*$_3$), 38.6, 38.8 (2× OC(O)*C*Me$_3$), 52.6 (CO_2*Me*), 53.3 (C-2), 69.3 (C-4), 71.7 (C-3), 73.0 (C-5), 101.9 (C-1), 167.4, 169.9, 176.4, 178.2 (*C*O_2Me, N*C*(O)Me, 2× O*C*(O)CMe$_3$); Gal unit: δ 24.1, 24.9, 25.9, 26.0 (4× Me), 68.3 (C-5), 69.0 (C-6), 70.1 (C-2), 70.5 (C-3), 70.9 (C-4), 96.1 (C-1), 108.5, 109.2 (2× O_2*C*Me$_2$), [#]assignments tentative. LRMS *m/z* 682 ([M+Na]$^+$, 100%). Anal. Calcd for $C_{31}H_{49}NO_{14}$: C, 56.44; H, 7.49; N, 2.12. Found: C, 56.20; H, 7.64; N, 2.11.

3.13. Methyl (allyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-β-D-glucopyranosid)uronate (4k)

Prepared from reaction between **7** and allyl alcohol in 47% yield after chromatography (EtOAc/hexane 2:3 → 1:1) as a clear colourless gum. Compounds **7** (α-anomer) (26%) and **8** (5%) were also isolated. R_f = 0.12 (EtOAc/hexane 2:3); ^{1}H NMR ($CDCl_3$): δ 1.12, 1.13 (2× 9H, 2× s, 2× OPiv), 1.91 (3H, s, NAc), 3.72 (3H, s, OMe), 4.00–4.13 (2H, m, H-2, H-1′a), 4.07 (1H, d, $J_{5,4}$ 9.6 Hz, H-5), 4.35 (1H, ddd, $J_{1'b,1'a}$ 13.1, $J_{1'b,2'}$ 4.9, $J_{1'b,3'}$ 1.4 Hz, H-1′b), 4.71 (1H, d, $J_{1,2}$ 8.1 Hz, H-1), 5.15–5.38 (4H, m, H-3, H-4, H-3′), 5.76–5.90 (2H, m, NH, H-2′); ^{13}C NMR ($CDCl_3$): δ 23.2 (NC(O)*Me*), 27.0 (2× OC(O)C*Me*$_3$), 38.7, 38.9 (2× OC(O)*C*Me$_3$), 52.7 (CO_2*Me*), 54.0 (C-2), 69.3 (C-4), 69.9 (C-1′), 71.1 (C-3), 72.9 (C-5), 99.8 (C-1), 117.9 (C-3′), 133.4 (C-2′), 167.5, 169.9, 176.5, 178.3 (*C*O_2Me, N*C*(O)Me, 2× O*C*(O)CMe$_3$). LRMS *m/z* 480 ([M+Na]$^+$, 100%). HRMS calcd for $C_{22}H_{35}NNaO_9$ [M+Na] 480.2210. Found 480.2204.

3.14. 2-Methyl-4,5-dihydro-(methyl 1,2-dideoxy-3,4-di-*O*-pivaloyl-α-D-glucopyranuronato)[2,1-*d*]-1,3-oxazole (8)

TMSOTf (198 μL, 1.1 mmol) was added to a solution of **7** (α/β 2:3) (500 mg, 1.0 mmol) in anhyd DCE (5 mL). The clear yellow solution was stirred under Ar at 50 °C and monitored by TLC analysis (toluene/acetone 8:1). After 3 d, NEt_3 was added to the brown reaction mixture to adjust to pH 9, and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (EtOAc/hexane 1:3 → 1:1) to give the title compound **8** as a colourless syrup (273 mg, 69%). Starting material **7** (α-anomer) (117 mg, 23%) was also isolated. R_f = 0.38 (toluene/acetone 8:1); R_f = 0.33 (EtOAc/hexane 2:3); ^{1}H NMR ($CDCl_3$): δ 1.15, 1.16 (2× 9H, 2× s, 2× OPiv), 2.04 (3H, d, $J_{Me,2}$ 1.5 Hz, NAc), 3.71 (3H, s, OMe), 3.97 (1H, d, $J_{5,4}$ 7.8 Hz, H-5), 4.07–4.09 (1H, m, H-2), 5.05 (1H, ddd, $J_{4,5}$ 7.8, $J_{4,3}$ 2.9, $J_{4,2}$ 1.2 Hz, H-4), 5.21 (1H, dd, $J_{3,2} = J_{3,4}$ 2.9 Hz, H-3), 6.03 (1H, d, $J_{1,2}$ 7.1 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 13.6 (NC(O)*Me*), 26.7, 26.8 (2× OC(O)C*Me*$_3$), 38.4, 38.5 (2× OC(O)*C*Me$_3$), 52.6 (CO_2*Me*), 64.9 (C-2), 68.2 (C-4), 68.7 (C-3), 69.4 (C-5), 98.6 (C-1), 166.0, 168.5, 176.4, 176.5 (*C*O_2Me, N*C*(O)Me, 2× O*C*(O)CMe$_3$). LRMS *m/z* 422 ([M+Na]$^+$, 100%). HRMS calcd for $C_{19}H_{29}NNaO_8$ [M+Na] 422.1791. Found 422.1799.

3.15. General procedure for the synthesis of 9a–9i

DBU (150 μL, 0.76 mmol) was added to a solution of compound **4a**–**4d**, **4e**–**Ac**—**4h**–**Ac** or **4i** (0.38 mmol) in anhyd CH_2Cl_2 (2 mL) under N_2. The pale yellow solution was stirred at rt and monitored by TLC analysis. After 18 h, the reaction mixture was concentrated under reduced pressure to give a clear yellow syrup. Purification of the crude product by flash chromatography afforded **9a**–**9i** (80–99%).

3.16. Methyl (ethyl 2-acetamido-2,4-dideoxy-3-*O*-pivaloyl-α-L-threo-hex-4-enopyranosid)uronate (9a)

Prepared from **4a** in 99% yield after chromatography (EtOAc/hexane 3:2) as a colourless syrup. R_f = 0.23 (EtOAc/hexane 3:2); ^{1}H NMR ($CDCl_3$): δ 1.15 (3H, t, $J_{2',1'}$ 7.1 Hz, H-2′), 1.16 (9H, s, OPiv), 1.96 (3H, s, NAc), 3.55 (1H, dq, $J_{1'a,1'b}$ 9.4, $J_{1'a,2'}$ 7.1 Hz, H-1′a),

3.79 (1H, dq, $J_{1'b,1'a}$ 9.4, $J_{1'b,2'}$ 7.1 Hz, H-1′b), 3.81 (3H, s, OMe), 4.38 (1H, dddd, $J_{2,NH}$ 9.0, $J_{2,3}$ 3.5, $J_{2,1}$ 1.9, $J_{2,4}$ 1.3 Hz, H-2), 4.95 (1H, ddd, $J_{3,4}$ 4.9, $J_{3,2}$ 3.5, $J_{3,1}$ 0.6 Hz, H-3), 5.17 (1H, dd, $J_{1,2}$ 1.9, $J_{1,3}$ 0.6 Hz, H-1), 5.90 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH), 6.19 (1H, dd, $J_{4,3}$ 4.9, $J_{4,2}$ 1.3 Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 14.8 (C-2′), 23.0 (NC(O)*Me*), 26.9 (OC(O)C*Me*$_3$), 38.7 (OC(O)*C*Me$_3$), 48.4 (C-2), 52.6 (CO_2*Me*), 64.2 (C-3), 65.0 (C-1′), 98.1 (C-1), 107.5 (C-4), 142.2 (C-5), 162.6, 170.0, 177.5 (*C*O$_2$Me, N*C*(O)Me, O*C*(O)CMe$_3$). LRMS *m*/*z* 366 ([M+Na]$^+$, 100%). HRMS calcd for $C_{16}H_{25}NNaO_7$ [M+Na] 366.1529. Found 366.1532.

3.17. Methyl (3-pentyl 2-acetamido-2,4-dideoxy-3-*O*-pivaloyl-α-L-threo-hex-4-enopyranosid)uronate (9b)

Prepared from **4b** in 82% yield after chromatography (EtOAc/hexane 3:2) as a colourless syrup. R_f = 0.20 (EtOAc/hexane 1:1); ^{1}H NMR ($CDCl_3$): δ 0.80 (3H, t, $J_{3',2'}$ 7.4 Hz, H-3′), 0.89 (3H, t, $J_{3'',2''}$ 7.4 Hz, H-3″), 1.19 (9H, s, OPiv), 1.37–1.58 (4H, m, H-2′, H-2″), 1.98 (3H, s, NAc), 3.57–3.65 (3H, s, OMe), 4.41 (1H, dddd, $J_{2,NH}$ 9.0, $J_{2,1}$ 1.8, $J_{2,4}$ 1.5, $J_{2,3}$ 0.9 Hz, H-2), 4.97 (1H, ddd, $J_{3,4}$ 5.1, $J_{3,2}$ 0.9, $J_{3,1}$ < 1 Hz, H-3), 5.27 (1H, dd, $J_{1,2}$ 1.8, $J_{1,3}$ < 1 Hz, H-1), 5.59 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH), 6.26 (1H, dd, $J_{4,3}$ 5.1, $J_{4,2}$ 1.5 Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 9.2, 9.6 (C-3′, C-3″), 23.2 (NC(O)*Me*), 25.9, 26.6 (C-2′, C-2″), 27.0 (OC(O)C*Me*$_3$), 38.7 (OC(O)*C*Me$_3$), 48.6 (C-2), 52.6 (CO_2*Me*), 64.2 (C-3), 81.7 (C-1′), 96.9 (C-1), 107.5 (C-4), 142.5 (C-5), 162.7, 169.5, 177.5 (*C*O$_2$Me, N*C*(O)Me, O*C*(O)CMe$_3$). LRMS *m*/*z* 408 ([M+Na]$^+$, 100%). HRMS calcd for $C_{19}H_{31}NNaO_7$ [M+Na] 408.1998. Found 408.1995.

3.18. Methyl (isopropyl 2-acetamido-2,4-dideoxy-3-*O*-pivaloyl-α-L-threo-hex-4-enopyranosid)uronate (9c)

Prepared from **4c** in 80% yield after chromatography (EtOAc/hexane 3:2) as a colourless syrup. R_f = 0.22 (EtOAc/hexane 3:2); ^{1}H NMR ($CDCl_3$): δ 1.15 (3H, d, $J_{2',1'}$ 6.5 Hz, H-2′), 1.17 (3H, d, $J_{2'',2'}$ 6.5 Hz, H-2″), 1.19 (9H, s, OPiv), 1.97 (3H, s, NAc), 3.83 (3H, s, OMe), 3.98 (1H, sept, $J_{1',2'} = J_{1',2''}$ 6.2 Hz, H-1′), 4.33–4.40 (1H, m, H-2), 4.97 (1H, br dd, $J_{3,4}$ 5.1, $J_{3,2}$ 1.5 Hz, H-3), 5.28 (1H, br d, $J_{1,2}$ 2.4 Hz, H-1), 5.64 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH), 6.23 (1H, dd, $J_{4,3}$ 5.1, $J_{4,2}$ 1.2 Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 21.6, 23.1 (C-2′, C-2″), 23.2 (NC(O)*Me*), 26.9 (OC(O)C*Me*$_3$), 38.7 (OC(O)*C*Me$_3$), 48.8 (C-2), 52.6 (CO_2*Me*), 64.3 (C-3), 71.6 (C-1′), 96.6 (C-1), 107.5 (C-4), 142.3 (C-5), 162.6, 169.6, 177.5 (*C*O$_2$Me, N*C*(O)Me, O*C*(O)CMe$_3$). LRMS *m*/*z* 380 ([M+Na]$^+$, 100%), 278 (56). HRMS calcd for $C_{17}H_{27}NNaO_7$ [M+Na] 380.1685. Found 380.1692.

3.19. Methyl (2-methylpropyl 2-acetamido-2,4-dideoxy-3-*O*-pivaloyl-α-L-threo-hex-4-enopyranosid)uronate (9d)

Prepared from **4d** in 95% yield after chromatography (EtOAc/hexane 4:3) as a colourless syrup. R_f = 0.17 (EtOAc/hexane 1:1); ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, d, $J_{3',2'}$ 6.9 Hz, H-3′), 0.89 (3H, d, $J_{3'',2'}$ 6.9 Hz, H-3″), 1.21 (9H, s, OPiv), 1.84 (1H, septt, $J_{2',3'} = J_{2',3''}$ 6.9, $J_{2',1'a} = J_{2',1'b}$ 6.6 Hz, H-2′), 1.99 (3H, s, NAc), 3.33 (1H, dd, $J_{1'a,1'b}$ 9.3, $J_{1'a,2'}$ 6.6 Hz, H-1′a), 3.56 (1H, dd, $J_{1'b,1'a}$ 9.3, $J_{1'b,2'}$ 6.6 Hz, H-1′b), 3.86 (3H, s, OMe), 4.46 (1H, dddd, $J_{2,NH}$ 9.3, $J_{2,1}$ 3.9, $J_{2,3}$ 1.8, $J_{2,4}$ 1.2 Hz, H-2), 5.00 (1H, ddd, $J_{3,4}$ 4.8, $J_{3,2}$ 1.8, $J_{3,1}$ 0.9 Hz, H-3), 5.18 (1H, dd, $J_{1,2}$ 2.1, $J_{1,3}$ 0.6 Hz, H-1), 5.44 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH), 6.27 (1H, dd, $J_{4,3}$ 4.8, $J_{4,2}$ 1.2 Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 19.0, 19.1 (C-3′, C-3″), 23.1 (NC(O)*Me*), 27.0 (OC(O)C*Me*$_3$), 28.3 (C-2′), 38.7 (OC(O)*C*Me$_3$), 48.7 (C-2), 52.6 (CO_2*Me*), 64.4 (C-3), 76.1 (C-1′), 98.3 (C-1), 107.9 (C-4), 142.2 (C-5), 162.6, 170.0, 177.6 (*C*O$_2$Me, N*C*(O)Me, O*C*(O)CMe$_3$). LRMS *m*/*z* 394 ([M+Na]$^+$, 100%). HRMS calcd for $C_{18}H_{29}NNaO_7$ [M+Na] 394.1842. Found 394.1848. Anal. Calcd for $C_{18}H_{29}NO_7 \cdot 0.5H_2O$: C, 56.83; H, 7.95; N, 3.68. Found: C, 56.95; H, 8.08; N, 3.63.

3.20. Methyl (2-*O*-acetyl-2-hydroxyethyl 2-acetamido-2,4-dideoxy-3-*O*-pivaloyl-α-L-threo-hex-4-enopyranosid)uronate (9e)

Prepared from **4e-Ac** in 87% yield after chromatography (EtOAc/hexane 3:2 → 3:1) as a colourless syrup. R_f = 0.33 (EtOAc/hexane 3:1); ^{1}H NMR ($CDCl_3$): δ 1.16 (9H, s, OPiv), 1.97 (3H, s, NAc), 2.01 (3H, s, OAc), 3.77 (1H, ddd, $J_{1'a,1'b}$ 11.2, $J_{1'a,2'a}$ 6.6, $J_{1'a,2'b}$ 3.8 Hz, H-1′a), 3.83 (3H, s, OMe), 3.93 (1H, ddd, $J_{1'b,1'a}$ 11.2, $J_{1'b,2'a}$ 5.3, $J_{1'b,2'b}$ 3.4 Hz, H-1′b), 4.12–4.20 (2H, m, H-2′a, H-2′b), 4.43 (1H, dddd, $J_{2,NH}$ 8.9, $J_{2,1}$ 2.4, $J_{2,3}$ 2.0, $J_{2,4}$ 1.3, Hz, H-2), 5.00 (1H, ddd, $J_{3,4}$ 4.8, $J_{3,2}$ 2.0, $J_{3,1}$ 0.7 Hz, H-3), 5.22 (1H, dd, $J_{1,2}$ 2.4, $J_{1,3}$ 0.7 Hz, H-1), 5.75 (1H, br d, $J_{NH,2}$ 8.9 Hz, NH), 6.22 (1H, dd, $J_{4,3}$ 4.8, $J_{4,2}$ 1.3 Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 20.7 (OC(O)*Me*), 23.0 (NC(O)*Me*), 26.9 (OC(O)C*Me*$_3$), 38.7 (OC(O)*C*Me$_3$), 48.4 (C-2), 52.6 (CO_2*Me*), 63.0 (C-2′), 64.1 (C-3), 66.9 (C-1′), 98.0 (C-1), 107.7 (C-4), 142.1 (C-5), 162.3, 170.0, 170.1, 177.5 (*C*O$_2$Me, O*C*(O)Me, N*C*(O)Me, O*C*(O)CMe$_3$). LRMS *m*/*z* 424 ([M+Na]$^+$, 100%), 322 (30). Anal. Calcd for $C_{18}H_{27}NO_9 \cdot H_2O$: C, 51.55; H, 6.97; N, 3.34. Found: C, 51.54; H, 6.78; N, 3.21.

3.21. Methyl (3-*O*-acetyl-3-hydroxypropyl 2-acetamido-2,4-dideoxy-3-*O*-pivaloyl-α-L-threo-hex-4-enpopyranosid)uronate (9f)

Prepared from **4f-Ac** in 99% yield after chromatography (EtOAc/hexane 3:1) as a colourless syrup. R_f = 0.16 (EtOAc/hexane 3:2); ^{1}H NMR ($CDCl_3$): δ 1.15 (9H, s, OPiv), 1.78–1.92 (2H, m, H-2′), 1.96 (3H, s, NAc), 2.00 (3H, s, OAc), 3.60 (1H, dt, $J_{1'a,1'b}$ 9.3, $J_{1'a,2'}$ 6.0 Hz, H-1′a), 3.81 (3H, s, OMe), 3.84 (1H, dt, $J_{1'b,1'a}$ 9.3, $J_{1'b,2'}$ 6.0 Hz, H-1′b), 4.07 (2H, t, $J_{3',2'}$ 6.3 Hz, H-3′), 4.39 (1H, dddd, $J_{2,NH}$ 9.0, $J_{2,3}$ 3.6, $J_{2,1}$ 2.1, $J_{2,4}$ 1.2 Hz, H-2), 4.98 (1H, ddd, $J_{3,4}$ 4.8, $J_{3,2}$ 1.2, $J_{3,1}$ 0.8 Hz, H-3), 5.16 (1H, dd, $J_{1,2}$ 2.1, $J_{1,3}$ 0.8 Hz, H-1), 5.91 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH), 6.19 (1H, dd, $J_{4,3}$ 4.8, $J_{4,2}$ 1.2 Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 20.8 (OC(O)*Me*), 22.9 (NC(O)*Me*), 26.9 (OC(O)C*Me*$_3$), 28.6 (C-2′), 38.6 (OC(O)*C*Me$_3$), 48.5 (C-2), 52.6 (CO_2*Me*), 60.9 (C-3′), 64.3 (C-3), 65.4 (C-1′), 98.0 (C-1), 107.8 (C-4), 142.0 (C-5), 162.4, 169.6, 170.9, 177.5 (*C*O$_2$Me, N*C*(O)Me, O*C*(O)Me, O*C*(O)CMe$_3$). LRMS

m/z 438.5 ([M+Na]$^+$, 100%). HRMS calcd for $C_{19}H_{29}NNaO_9$ [M+Na] 438.1740. Found 438.1743.

3.22. Methyl (2-*O*-acetyl-2-hydroxypropyl 2-acetamido-2,4-dideoxy-3-*O*-pivaloyl-α-L-threo-hex-4-enopyranosid)uronate (9g)

Prepared as a 1:1 diastereomeric mixture from **4g-Ac** in 81% yield after chromatography (EtOAc/hexane 3:2 → 3:1) as a colourless syrup. R_f = 0.31 (EtOAc/hexane 3:1); ^{1}H NMR ($CDCl_3$): δ 1.11, 1.12 (2× 9H, 2× s, 2× OPiv), 1.13 (3H, d, $J_{3',2'}$ 6.6 Hz, H-3′), 1.14 (3H, d, $J_{3',2'}$ 6.6 Hz, H-3′), 1.92 (2× 3H, s, 2× NAc), 1.93, 1.94 (2× 3H, 2× s, 2× OAc), 3.55 (1H, dd, $J_{1'a,1'b}$ 10.5, $J_{1'a,2'}$ 6.6 Hz, H-1′a), 3.58 (1H, dd, $J_{1'a,1'b}$ 10.8, $J_{1'a,2'}$ 4.2 Hz, H-1′a), 3.68 (1H, dd, $J_{1'b,1'a}$ 10.5, $J_{1'b,2'}$ 3.9 Hz, H-1′b), 3.71 (1H, dd, $J_{1'b,1'a}$ 10.8, $J_{1'b,2'}$ 5.1 Hz, H-1′b), 3.77 (6H, s, 2× OMe), 4.31–4.38 (2H, m, 2× H-2), 4.86–4.99 (4H, m, 2× H-3, 2× H-2′), 5.13–5.16 (2H, m, 2× H-1), 6.08–6.17 (4H, m, 2× H-4, 2× NH); ^{13}C NMR ($CDCl_3$): δ 16.3 (2× C-3′), 20.9 (2× OC(O)*Me*), 22.8 (2× NC(O)*Me*), 26.8 (2× OC(O)C*Me*$_3$), 38.5, 38.6 (2× OC(O)*C*Me$_3$), 48.4, 48.5 (2× C-2), 52.5 (2× CO_2*Me*), 64.0, 64.2 (2× C-3), 68.7, 69.0 (2× C-2′), 70.6, 71.1 (2× C-1′), 97.6, 98.0 (2× C-1), 107.8, 107.9 (2× C-4), 141.8, 141.9 (2× C-5), 162.3, 169.7, 170.2, 177.4 (2× *C*O$_2$Me, 2× N*C*(O)Me, 2× O*C*(O)Me, 2× O*C*(O)CMe$_3$). LRMS *m/z* 438 ([M+Na]$^+$, 100%). HRMS calcd for $C_{19}H_{29}NNaO_9$ [M+Na] 438.1740. Found 438.1744.

3.23. Methyl (1-*O*-acetyl-1-hydroxyisopropyl 2-acetamido-2,4-dideoxy-3-*O*-pivaloyl-α-L-threo-hex-4-enopyranosid)uronate (9h)

Prepared as a 5:1 diastereomeric mixture from **4h-Ac** in 80% yield after chromatography (EtOAc/hexane 3:2 → 3:1) as a colourless syrup. R_f = 0.11 (EtOAc/hexane 1:1); ^{1}H NMR ($CDCl_3$) including partial assignment of the minor diastereomer: δ 1.12 (3H, d, $J_{3',1'}$ 6.3 Hz, H-3′), 1.15 (18H, s, OPiv, OPiv*), 1.95 (3H, s, NAc), 2.06 (3H, s, OAc), 3.81 (6H, s, OMe, OMe*), 3.94 (1H, dd, $J_{2'a,2'b}$ 12.1, $J_{2'a,1'}$ 7.8 Hz, H-2′a), 4.04 (1H, dd, $J_{2'b,2'a}$ 12.1, $J_{2'b,1'}$ 3.3 Hz, H-2′b), 4.04 (1H, ddd, $J_{1',2'a}$ 7.8, $J_{1',3'}$ 6.3, $J_{1',2'b}$ 3.3 Hz, H-1′), 4.37 (1H, dddd, $J_{2,NH}$ 9.0, $J_{2,1}$ 2.4, $J_{2,3}$ 1.5, $J_{2,4}$ 1.2 Hz, H-2), 4.95 (1H, ddd, $J_{3,4}$ 4.8, $J_{3,2}$ 1.5, $J_{3,1}$ < 1 Hz, H-3), 5.27 (1H, dd, $J_{1,2}$ 2.1, $J_{1,3}$ < 1 Hz, H-1*), 5.33 (1H, dd, $J_{1,2}$ 2.4, $J_{1,3}$ < 1 Hz, H-1), 5.80 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH), 5.85 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH*), 6.20 (1H, dd, $J_{4,3}$ 4.8, $J_{4,5}$ 1.2 Hz, H-4), 6.21 (1H, m, H-4*); ^{13}C NMR ($CDCl_3$): δ 16.1 (C-3′*), 17.9 (C-3′), 20.5 (OC(O)*Me**), 20.7 (OC(O)*Me*), 23.0 (NC(O)*Me*, NC(O)*Me**), 26.8 (OC(O)C*Me*$_3$), 27.0 (OC(O)C*Me*$_3$*), 38.6 (OC(O)*C*Me$_3$, OC(O)*C*Me$_3$*), 48.5 (C-2), 48.6 (C-2*), 52.5 (CO_2*Me*), 52.6 (CO_2*Me**), 64.0 (C-3), 64.1 (C-3*), 67.1 (C-2′*), 67.2 (C-2′), 71.8 (C-1′*), 73.6 (C-1′), 95.6 (C-1*), 97.8 (C-1), 107.6 (C-4, C-4*), 142.1 (C-5, C-5*), 162.4, 162.5, 169.5, 169.6, 170.6, 170.8 177.4 (N*C*(O)Me, N*C*(O)Me*, O*C*(O)Me, O*C*(O)Me*, *C*O$_2$Me, *C*O$_2$Me*, O*C*(O)CMe$_3$, O*C*(O)CMe$_3$*), *minor diastereomer. LRMS *m/z* 438 ([M+Na]$^+$, 100%). HRMS calcd for $C_{19}H_{29}NNaO_9$ [M+Na] 438.1740. Found 438.1747.

3.24. Methyl (2,3-dihydroxy-2,3-*O*-isopropylidenepropyl 2-acetamido-2,4-dideoxy-3-*O*-pivaloyl-α-L-threo-hex-4-enopyranosid)uronate (9i)

Prepared as a 3:2 diastereomeric mixture from **4i** in 99% yield after chromatography (EtOAc/hexane 3:1) as a colourless syrup. R_f = 0.27 (EtOAc/hexane 3:1); ^{1}H NMR ($CDCl_3$): δ 1.19 (18H, s, 2× OPiv), 1.33, 1.39 (2× 6H, 2× s, 4× O_2C*Me*$_2$), 1.98 (6H, s, 2× NAc), 3.59 (1H, dd, $J_{1'a,1'b}$ 10.2, $J_{1'a,2'}$ 6.3 Hz, H-1′a), 3.65 (1H, dd, $J_{3'a,3'b}$ 8.4, $J_{3'a,2'}$ 6.0 Hz, H-3′a), 3.67 (1H, dd, $J_{1'a,1'b}$ 10.2, $J_{1'a,2'}$ 6.0 Hz, H-1′a), 3.72 (1H, dd, $J_{1'b,1'a}$ 10.2, $J_{1'b,2'}$ 6.0 Hz, H-1′b), 3.80 (1H, dd, $J_{3'a,3'b}$ 8.4, $J_{3'a,2'}$ 6.0 Hz, H-3′a), 3.84 (1H, dd, $J_{1'b,1'a}$ 10.2, $J_{1'b,2'}$ 6.3 Hz, H-1′b), 3.84 (6H, s, 2× OMe), 3.98 (1H, dd, $J_{3'b,3'a}$ 8.4, $J_{3'b,2'}$ 6.3 Hz, H-3′b), 4.01 (1H, dd, $J_{3'b,3'a}$ 8.4, $J_{3'b,2'}$ 6.3 Hz, H-3′b), 4.16–4.26 (2H, m, 2× H-2′), 4.42–4.49 (2H, m, 2× H-2), 4.99–5.04 (2H, m, 2× H-3), 5.22–5.26 (2H, m, 2× H-1), 5.60 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH), 5.60 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH), 6.21–6.26 (2H, m, 2× H-4); ^{13}C NMR ($CDCl_3$): δ 22.9 (2× NC(O)*Me*), 25.2, 26.7 (2× O_2C*Me*$_2$), 26.7, 26.9 (2× OC(O)C*Me*$_3$), 38.7 (2× OC(O)*C*Me$_3$), 48.4 (2× C-2), 52.6, 52.7 (2× CO_2*Me*), 64.2, 64.3 (2× C-3), 66.5, 66.8 (2× C-3′), 69.4, 69.9 (2× C-1′), 73.9, 74.0 (2× C-2′), 98.0, 98.3 (2× C-1), 107.9 (2× C-4), 109.3, 109.5 (2× O_2CMe$_2$), 141.9, 142.0 (2× C-5), 162.3, 169.6, 177.5 (2× *C*O$_2$Me, 2× N*C*(O)Me, 2× O*C*(O)CMe$_3$). LRMS *m/z* 452.5 ([M+Na]$^+$, 100%). HRMS calcd for $C_{20}H_{31}NNaO_9$ [M+Na] 452.1897. Found 452.1900.

3.25. Methyl (2,3-dihydroxypropyl 2-acetamido-2,4-dideoxy-3-*O*-pivaloyl-α-L-threo-hex-4-enopyranosid)uronate (9i-OH)

Compound **9i** (147 mg, 0.342 mmol) was dissolved in TFA/H_2O 1:1 (2 mL) at 0 °C. The solution was stirred at 0 °C and monitored by TLC analysis (EtOAc/MeOH 20:1). After 2 h, the reaction mixture was diluted with toluene (0.5 mL) and then concentrated in vacuo. The crude product was purified by flash chromatography (EtOAc/MeOH 20:1) to give a 3:2 diastereomeric mixture of the title compound **9i-OH** (104 mg, 78%) as an amorphous mass. R_f = 0.22 (EtOAc/MeOH 20:1); ^{1}H NMR ($CDCl_3$): δ 1.18 (18H, s, 2× OPiv), 1.99 (6H, s, 2× NAc), 2.99 (4H, br s, 4× OH), 3.52–3.82 (10H, m, 4× H-1′, 2× H-2′, 4× H-3′), 3.87 (6H, s, 2× OMe), 4.39–4.48 (2H, m, 2× H-2), 5.12–5.21 (2H, m, 2× H-3), 5.22–5.28 (2H, m, 2× H-1), 6.21–6.20 (4H, 2× NH, 2× H-4); ^{13}C NMR ($CDCl_3$): δ 23.1 (2× NC(O)*Me*), 27.0 (2× OC(O)C*Me*$_3$), 38.8 (2× OC(O)*C*Me$_3$), 49.1, 49.2 (2× C-2), 52.8 (2× CO_2*Me*), 63.3 (2× C-3′), 65.1, 65.2 (2× C-3), 70.4, 70.6 (2× C-2′), 70.8 (2× C-1′), 98.8, 98.9 (2× C-1), 108.1, 108.2 (2× C-4), 142.2, 142.3 (2× C-5), 162.3, 162.4, 170.4, 177.8 (2× *C*O$_2$Me, 2× N*C*(O)Me, 2× O*C*(O)CMe$_3$). LRMS *m/z* 412 ([M+Na]$^+$, 100%). HRMS calcd for $C_{17}H_{27}NNaO_9$ [M+Na] 412.1584. Found 412.1584.

3.26. General procedure for the synthesis of 2a–2i

A solution of compound **9a**–**9h** or **9i**–**OH** (~0.4 mmol) in aq MeOH (50%, 5 mL) was adjusted to pH 13 using

aq NaOH (0.5 M). The solution was stirred at rt and monitored by TLC analysis (EtOAc/MeOH/H_2O 7:2:1). After 18 h, Amberlite® IR-120 (H^+) resin was added to adjust to pH 3, the reaction mixture was filtered, the resin was washed with MeOH/H_2O 1:1 (30 mL) and the filtrate was concentrated to dryness. PivOH was then removed by evaporation under reduced pressure (~1 mmHg) at 40 °C for 3 h. The residue was dissolved in water (5 mL), aq NaOH was added to adjust to pH 7.3 and the solution was lyophilised to afford an amorphous solid. The crude product was purified by HPLC and then lyophilised to give **2a**–**2i** (70–87%).

3.27. Sodium (ethyl 2-acetamido-2,4-dideoxy-α-L-threo-hex-4-enopyranosid)uronate (2a)

Prepared from **9a** in 86% yield after reverse-phase HPLC (1% CH_3CN in water) as a creamy-coloured amorphous mass. $R_f = 0.14$ (EtOAc/MeOH/H_2O 7:2:1); ^1H NMR (D_2O): δ 1.04 (3H, t, $J_{2',1'}$ 7.2 Hz, H-2′), 1.87 (3H, s, NAc), 3.54 (1H, dq, $J_{1'a,1'b}$ 10.2, $J_{1'a,2'}$ 7.2 Hz, H-1′a), 3.71 (1H, dq, $J_{1'b,1'a}$ 10.2, $J_{1'b,2'}$ 7.2 Hz, H-1′b), 3.94 (1H, ddd, $J_{2,1}$ 4.8, $J_{2,3}$ 4.2, $J_{2,4}$ 0.9 Hz, H-2), 4.01 (1H, ddd, $J_{3,2} = J_{3,4}$ 4.2, $J_{3,1}$ 0.6 Hz, H-3), 5.01 (1H, dd, $J_{1,2}$ 4.8, $J_{1,3}$ 0.6 Hz, H-1), 5.75 (1H, dd, $J_{4,3}$ 4.2, $J_{4,2}$ 0.9 Hz, H-4); ^{13}C NMR (D_2O): δ 15.1 (C-2′), 22.9 (NC(O)*Me*), 53.0 (C-2), 65.1 (C-3), 66.9 (C-1′), 99.7 (C-1), 113.2 (C-4), 141.6 (C-5), 166.4, 175.2 (CO_2Na, N*C*(O)Me). LRMS *m*/*z* 268 ($[M+H]^+$, 100%). HRMS calcd for $C_{10}H_{14}NNa_2O_6$ [M+Na] 290.0617. Found 290.0615.

3.28. Sodium (3-pentyl 2-acetamido-2,4-dideoxy-α-L-threo-hex-4-enopyranosid)uronate (2b)

Prepared from **9b** in 75% yield after reverse-phase HPLC (18% CH_3CN in water) as a creamy-coloured amorphous mass. $R_f = 0.19$ (EtOAc/MeOH/H_2O 7:2:1); ^1H NMR (D_2O): δ 0.70 (3H, t, $J_{3',2'}$ 7.5 Hz, H-3′), 0.75 (3H, t, $J_{3'',2''}$ 7.5 Hz, H-3″), 1.30–1.48 (4H, m, H-2′, H-2″), 1.88 (3H, s, NAc), 3.43–3.58 (1H, m, H-1′), 3.96–4.02 (2H, m, H-3, H-2), 5.10 (1H, br d, $J_{1,2}$ 4.5, H-1), 5.78 (1H, dd, $J_{4,3}$ 4.2, $J_{4,2}$ 0.9 Hz, H-4); ^{13}C NMR (CD_3OD): δ 9.6, 10.1 (C-3′, C-3″), 22.6 (NC(O)*Me*), 27.1, 27.8 (C-2′, C-2″), 53.7 (C-2), 65.5 (C-3), 83.9 (C-1′), 99.5 (C-1), 112.8 (C-4), 142.5 (C-5), 165.7, 173.4 (CO_2Na, N*C*(O)Me). LRMS *m*/*z* 310 ($[M+H]^+$, 100%). HRMS calcd for $C_{13}H_{20}NNa_2O_6$ [M+Na] 332.1086. Found 332.1084.

3.29. Sodium (isopropyl 2-acetamido-2,4-dideoxy-α-L-threo-hex-4-enopyranosid)uronate (2c)[17]

Prepared from **9c** in 80% yield after reverse-phase HPLC (1% CH_3CN in water) as a creamy-coloured amorphous mass. $R_f = 0.15$ (EtOAc/MeOH/H_2O 7:2:1); ^1H NMR (D_2O): δ 1.03 (3H, d, $J_{2',1'}$ 6.3 Hz, H-2′), 1.05 (3H, d, $J_{2'',1'}$ 6.3 Hz, H-2″), 1.87 (3H, s, NAc), 3.87 (1H, ddd, $J_{2,1}$ 5.4, $J_{2,3}$ 5.1, $J_{2,4}$ 0.6 Hz, H-2), 3.93 (1H, sept, $J_{1',2'} = J_{1',2''}$ 6.3 Hz, H-1′), 4.05 (1H, ddd, $J_{3,2}$ 5.1, $J_{3,4}$ 3.9, $J_{3,1}$ 0.6 Hz, H-3), 5.06 (1H, dd, $J_{1,2}$ 5.4, $J_{1,3}$ 0.6 Hz, H-1), 5.71 (1H, dd, $J_{4,3}$ 3.9, $J_{4,2}$ 0.6 Hz, H-4); ^{13}C NMR (D_2O): δ 21.0, 21.9 (C-2′, C-2″), 22.3 (NC(O)*Me*), 52.5 (C-2), 65.0 (C-3), 73.0 (C-1′), 97.5 (C-1), 107.2 (C-4), 145.1 (C-5), 169.3, 174.3 (CO_2Na, N*C*(O)Me). LRMS *m*/*z* 282 ($[M+H]^+$, 100%). HRMS calcd for $C_{11}H_{16}NNa_2O_6$ [M+Na] 304.0773. Found 304.0772.

3.30. Sodium (2-methylpropyl 2-acetamido-2,4-dideoxy-α-L-threo-hex-4-enopyranosid)uronate (2d)

Prepared from **9d** in 87% yield after HPLC (5% CH_3CN in water) as an amorphous solid. $R_f = 0.20$ (EtOAc/MeOH/H_2O 7:2:1); ^1H NMR (D_2O): δ 0.68 (3H, d, $J_{3',2'}$ 6.6 Hz, H-3′), 0.69 (3H, d, $J_{3'',2'}$ 6.6 Hz, H-3″), 1.68 (1H, septt, $J_{2',1'}$ 6.9, $J_{2',3'} = J_{2',3''}$ 6.6 Hz, H-2′), 1.84 (3H, s, NAc), 3.25 (1H, dd, $J_{1'a,1'b}$ 9.9, $J_{1'a,2'}$ 6.9 Hz, H-1a′), 3.48 (1H, dd, $J_{1'b,1'a}$ 9.9, $J_{1'b,2'}$ 6.9 Hz, H-1′b), 3.90 (1H, br dd, $J_{2,1} = J_{2,3}$ 5.4 Hz, H-2), 4.04 (1H, br dd, $J_{3,2}$ 5.4, $J_{3,4}$ 3.9 Hz, H-3), 4.91 (1H, br d, $J_{1,2}$ 5.4 Hz, H-1), 5.69 (1H, br d, $J_{4,3}$ 3.9 Hz, H-4); ^{13}C NMR (CD_3OD): δ 19.5 (C-3′, C-3″), 22.6 (NC(O)*Me*), 29.4 (C-2′), 49.9 (C-2), 66.0 (C-3), 77.3 (C-1′), 100.7 (C-1), 113.0 (C-4), 142.6 (C-5), 165.8, 173.4 (CO_2Na, N*C*(O)Me). LRMS *m*/*z* 296 ($[M+H]^+$, 100%). Anal. Calcd for $C_{12}H_{18}NNaO_6 \cdot H_2O$: C, 46.01; H, 6.43; N, 4.47. Found: C, 45.46; H, 6.42; N, 4.16.

3.31. Sodium (2-hydroxyethyl 2-acetamido-2,4-dideoxy-α-L-threo-hex-4-enopyranosid)uronate (2e)

Prepared from **9e** in 80% yield after reverse-phase HPLC (1% CH_3CN in water) as a creamy-coloured amorphous mass. $R_f = 0.09$ (EtOAc/MeOH/H_2O 7:2:1); ^1H NMR (D_2O): δ 1.80 (3H, s, NAc), 3.48–3.59 (3H, m, H-1′, H-2′a), 3.63–3.73 (1H, m, H-2′b), 3.92 (1H, ddd, $J_{3,4}$ 4.2, $J_{3,2}$ 3.6, $J_{3,1}$ 0.6 Hz, H-3), 3.96 (1H, ddd, $J_{2,1}$ 3.9, $J_{2,3}$ 3.6, $J_{2,4}$ 0.9 Hz, H-2), 5.00 (1H, dd, $J_{1,2}$ 3.9, $J_{1,3}$ 0.6 Hz, H-1), 5.80 (1H, dd, $J_{4,3}$ 4.2, $J_{4,2}$ 0.9 Hz, H-4); ^{13}C NMR (D_2O): δ 22.8 (NC(O)*Me*), 52.7 (C-2), 61.5 (C-2′), 65.0 (C-3), 71.6 (C-1′), 99.4 (C-1), 109.0 (C-4), 144.8 (C-5), 169.4, 175.1 (CO_2Na, N*C*(O)Me). LRMS *m*/*z* 306 ($[M+Na]^+$, 53%), 284 ($[M]^+$, 100), 261 (38). HRMS calcd for $C_{10}H_{14}NNa_2O_7$ [M+Na] 306.0566. Found 306.0564.

3.32. Sodium (3-hydroxypropyl 2-acetamido-2,4-dideoxy-1-α-L-threo-hex-4-enopyranosid)uronate (2f)

Prepared from **9f** in 85% yield after reverse-phase HPLC (5% CH_3CN in water) as a creamy-coloured amorphous mass. $R_f = 0.08$ (EtOAc/MeOH/H_2O 7:2:1); ^1H NMR (D_2O): δ 1.65 (2H, m, H-2′), 1.84 (3H, s, NAc), 3.41-3.54 (2H, m, H-3′), 3.56 (1H, dt, $J_{1'a,1'b}$ 10.2, $J_{1'a,2'}$ 6.0 Hz, H-1′a), 3.76 (1H, dt, $J_{1'b,1'a}$ 10.2, $J_{1'b,2'}$ 6.0 Hz, H-1′b), 3.92 (1H, ddd, $J_{3,2}$ 4.5, $J_{3,4}$ 3.9, $J_{3,1}$ 0.6 Hz, H-3), 3.99 (1H, ddd, $J_{2,3}$ 4.5, $J_{2,1}$ 4.2, $J_{2,4}$ 0.6 Hz, H-2), 4.96 (1H, dd, $J_{1,2}$ 4.2, $J_{1,3}$ 0.6 Hz, H-1), 5.73 (1H, dd, $J_{4,3}$ 3.9, $J_{4,2}$ 0.6 Hz, H-4); ^{13}C NMR (CD_3OD): δ 22.6 (NC(O)*Me*), 33.2 (C-2′), 53.6 (C-2), 60.1 (C-3′), 65.9 (C-3), 67.8 (C-1′), 100.2 (C-1), 111.4 (C-4), 143.7 (C-5), 167.1, 173.4 (CO_2Na, N*C*(O)Me). LRMS *m*/*z* 298 ($[M+H]^+$, 100%). HRMS calcd for $C_{11}H_{16}NNa_2O_7$ [M+Na] 320.0722. Found 320.0721.

3.33. Sodium (2-hydroxypropyl 2-acetamido-2,4-dideoxy-α-L-threo-hex-4-enopyranosid)uronate (2g)

Prepared as a 1:1 diastereomeric mixture from **9g** in 85% yield after reverse-phase HPLC (5% CH_3CN in water) as a creamy-coloured amorphous mass. $R_f = 0.08$ (EtOAc/MeOH/H_2O 7:2:1); ^1H NMR (CD_3OD): δ 0.97 (6H, d, $J_{3',2'}$ 6.6 Hz, 2× H-3′), 1.81 (6H, s, 2× NAc), 3.31 (1H, dd, $J_{1'a,1'b}$ 10.5, $J_{1'a,2'}$ 7.2 Hz, H-1′a), 3.37 (1H, dd, $J_{1'a,1'b}$ 9.9, $J_{1'a,2'}$ 3.9 Hz, H-1′a), 3.60 (1H, dd, $J_{1'b,1'a}$ 9.9, $J_{1'b,2'}$ 6.9 Hz, H-1′b), 3.59 (1H, dd, $J_{1'b,1'a}$ 10.5, $J_{1'b,2'}$ 3.3 Hz, H-1′b), 3.79–3.80 (2H, m, 2× H-2′), 3.84–3.89 (2H, m, 2× H-3), 4.01–4.06 (2H, m, 2× H-2), 4.98–5.03 (2H, m, 2× H-1), 6.00–6.04 (2H, m, 2× H-4); ^{13}C NMR (CD_3OD): δ 19.3, 19.4 (2× C-3′), 22.5 (2× NC(O)*Me*), 53.5 (2× C-2), 65.4 (2× C-3), 67.1, 67.4 (2× C-2′), 76.0, 76.1 (2× C-1′), 100.1, 100.5 (2× C-1), 112.6 (2× C-4), 142.5 (2× C-5), 165.9, 166.0, 173.4 (2× CO_2Na, 2× N*C*(O)Me). LRMS *m/z* 298 ($[M+H]^+$, 100%). HRMS calcd for $C_{11}H_{16}NNa_2O_7$ [M+Na] 320.0722. Found 320.0725.

3.34. Sodium (1-hydroxyisopropyl 2-acetamido-2,4-dideoxy-α-L-threo-hex-4-enopyranosid)uronate (2h)

Prepared as a 5:1 diastereomeric mixture from **9h** in 78% yield after reverse-phase HPLC (5% CH_3CN in water) as a creamy-coloured amorphous mass. $R_f = 0.12$ (EtOAc/MeOH/H_2O 7:2:1); ^1H NMR (D_2O) including partial assignment of the minor diastereomer: δ 1.05 (3H, d, $J_{3',1'}$ 6.3 Hz, H-3′), 1.07 (3H, d, $J_{3',1'}$ 6.3 Hz, H-3′*), 1.91 (3H, s, NAc), 1.93 (3H, s, NAc*), 3.42 (1H, m, H-2′a*), 3.43 (1H, dd, $J_{2'a,2'b}$ 12.0, $J_{2'a,1'}$ 6.6 Hz, H-2′a), 3.53 (1H, dd, $J_{2'b,2'a}$ 12.0, $J_{2'b,1'}$ 3.6 Hz, H-2′b), 3.89 (1H, ddd, $J_{1',2'a}$ 6.6, $J_{1',3'}$ 6.3, $J_{1',2'b}$ 3.6 Hz, H-1′), 3.98 (1H, m, H-3*), 4.01 (1H, br dd, $J_{3,4}$ 4.2, $J_{3,2}$ 3.6 Hz, H-3), 4.08 (1H, br dd, $J_{2,1} = J_{2,3}$ 3.6 Hz, H-2), 4.10 (1H, m, H-2*), 5.14 (1H, br d, $J_{1,2}$ 5.1 Hz, H-1*), 5.22 (1H, br d, $J_{1,2}$ 3.6 Hz, H-1), 5.80 (1H, br d, $J_{4,3}$ 3.9 Hz, H-4*), 5.85 (1H, br d, $J_{4,3}$ 4.5 Hz, H-4); ^{13}C NMR (D_2O): δ 15.0 (C-3′*), 16.6 (C-3′), 21.8 (NC(O)*Me*, NC(O)*Me**), 51.8 (C-2), 52.3 (C-2*), 63.7 (C-3), 64.2 (C-3*), 64.8 (C-2′, C-2′*), 76.5 (C-1′*), 77.8 (C-1′), 97.5 (C-1*), 98.7 (C-1), 111.7 (C-4, C-4*), 140.8 (C-5), 141.0 (C-5*), 165.6, 174.3 (CO_2Na*, N*C*(O)Me*), 165.7, 174.2 (CO_2Na, N*C*(O)Me), *minor diastereomer. LRMS *m/z* 298 ($[M+H]^+$, 100%). HRMS calcd for $C_{11}H_{16}NNa_2O_7$ [M+Na] 320.0722. Found 320.0724.

3.35. Sodium (2,3-dihydroxypropyl 2-acetamido-2,4-dideoxy-α-L-threo-hex-4-enopyranosid)uronate (2i)

Prepared as a 3:2 diastereomeric mixture from **9i-OH** in 70% yield after reverse-phase HPLC (5% CH_3CN in water) as an amorphous solid. $R_f = 0.04$ (EtOAc/MeOH/H_2O 7:2:1); ^1H NMR (D_2O): δ 1.84 (6H, s, 2× NAc), 3.33-3.77 (10H, m, 2× H-1′, 2× H-2′, 2× H-3′), 3.93–4.01 (4H, m, 2× H-2, 2× H-3), 4.98–5.02 (2H, m, 2× H-1), 5.74–5.78 (2H, m, 2× H-4); ^{13}C NMR (D_2O): δ 21.7 (NC(O)*Me*, NC(O)Me*), 51.6 (C-2, C-2*), 62.1 (C-3′), 62.2 (C-3′*), 63.6 (CO_2Me, CO_2Me*), 69.9 (C-2*), 70.2 (C-2′), 70.3 (C-1′*), 70.6 (C-1′), 98.6 (C-1*), 99.1 (C-1), 112.2 (C-4, C-4*), 140.2 (C-5, C-5*), 165.1, 174.1 (CO_2Na, CO_2Na*, N*C*(O)Me, N*C*(O)Me*), *minor diastereomer. LRMS *m/z* 314 ($[M+H]^+$, 100%). Anal. Calcd for $C_{11}H_{16}NNaO_8 \cdot H_2O$: C, 39.88; H, 5.48; N, 4.23. Found: C, 40.28; H, 5.69; N, 4.10.

3.36. Methyl 2-acetamido-1-*S*-acetyl-2-deoxy-3,4-di-*O*-pivaloyl-1-thio-β-D-glucopyranuronate (10)

Thiolacetic acid (133 μL, 1.86 mmol) was added to a solution of oxazoline **8** (248 mg, 0.622 mmol) in anhyd DMF (2.5 mL). The solution was stirred at 80 °C under N_2. After 18 h, the dark brown reaction mixture was concentrated in vacuo. Purification of the crude product by flash chromatography (EtOAc/hexane 1:1) afforded **10** as an amorphous mass (189 mg, 64%). For analytical purposes, recrystallisation from EtOAc/hexane gave **10** as fine white needles. $R_f = 0.16$ (EtOAc/hexane 1:1); ^1H NMR ($CDCl_3$): δ 1.13, 1.14 (2× 9H, 2× s, 2× OPiv), 1.88 (3H, s, NAc), 2.34 (3H, s, SAc), 3.70 (3H, s, OMe), 4.18 (1H, d, $J_{5,4}$ 9.6 Hz, H-5), 4.47 (1H, ddd, $J_{2,1} = J_{2,3}$ 10.3, $J_{2,NH}$ 9.9 Hz, H-2), 5.22–5.32 (3H, m, H-1, H-3, H-4), 5.96 (1H, br d, $J_{NH,2}$ 9.9 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 23.0 (NC(O)*Me*), 27.0 (2× OC(O)C*Me*$_3$), 30.7 (SC(O)*Me*), 38.7, 38.9 (2× OC(O)*C*Me$_3$), 51.5 (C-2), 52.8 (CO_2Me), 68.9 (C-4), 72.7 (C-3), 76.8 (C-5), 81.6 (C-1), 167.0, 170.0, 176.5, 178.6, 193.3 (*C*O_2Me, N*C*(O)Me, 2× O*C*(O)CMe$_3$, S*C*(O)Me). LRMS *m/z* 498 ($[M+Na]^+$, 100%). Anal. Calcd for $C_{21}H_{33}NO_9S$: C, 53.04; H, 6.99; N, 2.95. Found: C, 53.02; H, 7.10; N, 2.93.

3.37. General procedure for the synthesis of 5a–5d

Alkyl halide (2.1 mmol) was added to a stirred solution of **10** (200 mg, 0.42 mmol) in anhyd DMF (2 mL) containing 3 Å molecular sieves under Ar. The solution was cooled to 4 °C, and then $HNEt_2$ (0.9 mL, 8 mmol) was added. After 24 h, the reaction mixture was concentrated to remove $HNEt_2$. The residue was diluted with EtOAc (15 mL), and then filtered. The filtrate was washed with dilute HCl (1 M, 15 mL), water (15 mL), saturated aq NaCl (15 mL), dried (Na_2SO_4), filtered and concentrated. Purification of the crude product by flash chromatography afforded **5a**–**5d** (73–76%).

3.38. Methyl (3-hydroxypropyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-1-thio-β-D-glucopyranosid)uronate (β-5a)

Prepared from reaction between **10** and 3-bromopropan-1-ol in 74% yield after chromatography (EtOAc/hexane 3:1) as an amorphous mass. $R_f = 0.20$ (EtOAc/hexane 3:1); ^1H NMR ($CDCl_3$): δ 1.13 (18H, s, 2× OPiv), 1.72–2.02 (2H, m, H-2′), 1.94 (3H, s, NAc), 2.52–2.59 (1H, m, OH) 2.82–2.99 (2H, m, H-1′), 3.67–3.78 (2H, m, H-3′), 3.72 (3H, s, OMe), 4.01 (1H, d, $J_{5,4}$ 9.5 Hz, H-5), 4.29 (1H, ddd, $J_{2,1}$ 10.3, $J_{2,3}$ 10.0, $J_{2,NH}$ 9.7 Hz, H-2), 4.58 (1H, d, $J_{1,2}$ 10.3 Hz, H-1), 5.18–5.29 (2H, m, H-3, H-4), 6.07 (1H, br d, $J_{NH,2}$ 9.7 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 23.0 (NC(O)*Me*), 25.5 (C-1′), 26.9 (2× OC(O)C*Me*$_3$), 31.6 (C-2′), 38.6, 38.8 (2× OC(O)*C*Me$_3$), 52.2 (C-2), 52.7 (CO_2Me), 59.8 (C-3′), 69.1 (C-4), 72.3 (C-3), 76.2 (C-5), 84.3 (C-1), 167.5, 170.5, 176.5, 178.3 (*C*O_2Me, N*C*(O)Me, 2×

OC(O)CMe$_3$). LRMS *m/z* 513.5 ([M+Na]$^+$, 100%). Anal. Calcd for $C_{22}H_{37}NO_9S$: C, 53.75; H, 7.59; N, 2.85. Found: C, 53.57; H, 7.65; N, 2.65.

3.39. Methyl (2-hydroxyethyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-1-thio-α,β-D-glucopyranosid)uronate (5b)

Prepared as an anomeric mixture (α/β 1:8) from reaction between **10** and 2-bromoethan-1-ol in 76% yield after chromatography (EtOAc/MeOH 10:1) as an amorphous mass. $R_f = 0.20$ (EtOAc/MeOH 10:1); ^{1}H NMR ($CDCl_3$) β-anomer: δ 1.11 (18H, s, 2× OPiv), 1.92 (3H, s, NAc), 2.04 (1H, br s, OH), 2.77 (1H, ddd, $J_{1'a,1'b}$ 14.0, $J_{1'a,2'a}$ 6.3, $J_{1'a,2'b}$ 4.7 Hz, H-1′a), 3.04 (1H, dt, $J_{1'b,1'a}$ 14.0, $J_{1'b,2'}$ 5.4 Hz, H-1′b), 3.7 (3H, s, OMe), 3.75–3.83 (2H, m, H-2′), 4.15 (1H, d, $J_{5,4}$ 9.9 Hz, H-5), 4.26 (1H, ddd, $J_{2,1} = J_{2,3} = J_{2,NH}$ 9.9 Hz, H-2), 4.74 (1H, d, $J_{1,2}$ 10.2 Hz, H-1), 5.19 (1H, dd, $J_{4,3} = J_{4,5}$ 9.9 Hz, H-4), 5.30 (1H, dd, $J_{3,2} = J_{3,4}$ 9.9 Hz, H-3), 6.47 (1H, br d, $J_{NH,2}$ 9.6 Hz, NH); α-anomer: δ 1.13, 1.18 (2× 9H, 2× s, 2× OPiv), 1.95 (3H, s, NAc), 2.81 (1H, dt, $J_{1'a,1'b}$ 14.0, $J_{1'a,2'}$ 5.8 Hz, H-1′a), 2.94 (1H, dt, $J_{1'b,1'a}$ 14.0, $J_{1'b,2'}$ 6.0 Hz, H-1′b), 3.74 (3H, s, OMe), 3.76–3.87 (2H, m, H-2′), 4.59 (1H, ddd, $J_{2,3}$ 10.2, $J_{2,NH}$ 9.0, $J_{2,1}$ 5.0 Hz, H-2), 4.73 (1H, d, $J_{5,4}$ 9.1 Hz, H-5), 5.12 (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 8.4 Hz, H-3), 5.26 (1H, dd, $J_{4,5}$ 9.1, $J_{4,3}$ 8.4 Hz, H-4), 5.55 (1H, d, $J_{1,2}$ 5.0 Hz, H-1), 5.90 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 23.0 (NC(O)*Me* α/β), 26.9, 27.0 (2× OC(O)C*Me*$_3$ α/β), 33.6 (C-1′ β), 34.6 (C-1′ α), 38.6, 38.8 (2× OC(O)*C*Me$_3$ α/β), 51.4 (C-2 α), 52.5 (C-2 β), 52.7 (CO_2*Me* α/β), 61.4 (C-2′ α), 62.1 (C-2′ β), 68.6 (C-4 α), 69.0 (C-4 β), 69.6 (C-3 α), 72.3 (C-3 β), 69.9 (C-5 α), 76.0 (C-5 β), 84.5 (C-1 α/β), 167.3, 168.0, 169.9, 170.3, 176.4, 178.4, 176.5, 178.2 (*C*O$_2$Me α/β, N*C*(O)Me α/β, 2× O*C*(O)CMe$_3$ α/β). LRMS *m/z* 500 ([M+Na]$^+$, 100%). Anal. Calcd for $C_{21}H_{35}NO_9S{\cdot}1/3H_2O$: C, 52.16; H, 7.43; N, 2.90. Found: C, 52.28; H, 7.46; N, 2.70.

3.40. Methyl (isobutyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-1-thio-α,β-D-glucopyranosid)uronate (5c)

Prepared as an anomeric mixture (α/β 1:9) from reaction between **10** and isobutyl iodide in 73% yield after chromatography (EtOAc/hexane 2:3) as an amorphous mass. $R_f = 0.26$ (EtOAc/hexane 2:3); ^{1}H NMR ($CDCl_3$): δ 0.98 (3H, d, $J_{3',2'}$ 6.6 Hz, H-3′ β), 0.99 (3H, d, $J_{3'',2'}$ 6.6 Hz, H-3″ β), 1.00 (3H, d, $J_{3',2'}$ 6.6 Hz, H-3′ α), 1.01 (3H, d, $J_{3'',2'}$ 6.6 Hz, H-3″ α), 1.14, 1.15, 1.17 (36H, 3× s, 4× OPiv α/β), 1.74–1.91 (2H, m, H-2′ α/β), 1.94 (3H, s, NAc β), 1.95 (3H, s, NAc α), 2.50 (1H, dd, $J_{1'a,1'b}$ 12.9, $J_{1'a,2'}$ 7.2 Hz, H-1′a α), 2.61 (1H, dd, $J_{1'b,1'a}$ 12.9, $J_{1'b,2'}$ 7.2 Hz, H-1′b α), 2.59 (1H, dd, $J_{1'a,1'b}$ 9.3, $J_{1'a,2'}$ 6.6 Hz, H-1′a β), 2.65 (1H, dd, $J_{1'b,1'a}$ 9.3, $J_{1'b,2'}$ 6.6 Hz, H-1′b β), 3.73 (3H, s, OMe β), 3.74 (3H, s, OMe α), 4.04 (1H, d, $J_{5,4}$ 9.6 Hz, H-5 β), 4.23 (1H, ddd, $J_{2,1}$ 10.5, $J_{2,3}$ 9.9, $J_{2,NH}$ 9.3 Hz, H-2 β), 4.54 (1H, d, $J_{1,2}$ 10.5 Hz, H-1 β), 4.59 (1H, ddd, $J_{2,3}$ 10.5, $J_{2,NH}$ 9.3, $J_{2,1}$ 5.1 Hz, H-2 α), 4.71 (1H, d, $J_{5,4}$ 9.3 Hz, H-5 α), 5.13 (1H, dd, $J_{3,2}$ 10.5, $J_{3,4}$ 9.0 Hz, H-3 α), 5.18–5.28 (3H, m, H-4 α, H-3 β, H-4 β), 5.40 (1H, d, $J_{1,2}$ 5.1 Hz, H-1 α), 5.68 (1H, br d, $J_{NH,2}$ 9.3 Hz, NH α), 5.72 (1H, br d, $J_{NH,2}$ 9.3 Hz, NH β); ^{13}C NMR ($CDCl_3$): δ 21.6, 21.7, 22.1 (C-3′ α/β, C-3″ α/β), 23.0 (NC(O)*Me* α/β), 26.9, 27.0 (2× OC(O)C*Me*$_3$ α/β), 28.5 (C-2′ β), 28.6 (C-2′ α), 38.5 (C-1′ β), 38.6, 38.8 (2× OC(O)*C*Me$_3$ α/β), 40.4 (C-1′ α), 51.6 (C-2 α), 52.6 (C-2 β, CO_2*Me* β), 52.7 (CO_2*Me* α), 68.8 (C-4 α), 69.2, 72.6 (C-3 β, C-4 β), 69.7, 69.8 (C-3 α, C-5 α), 76.5 (C-5 β), 85.0 (C-1 α/β), 167.2, 168.0, 169.6, 169.7, 176.3, 178.5, 176.4, 178.6 (*C*O$_2$Me α/β, N*C*(O)Me α/*β*, 2× O*C*(O)CMe$_3$ α/β). LRMS *m/z* 512.5 ([M+Na]$^+$, 100%). Anal. Calcd for $C_{23}H_{39}NO_8S$: C, 56.42; H, 8.03; N, 2.86. Found: C, 56.40; H, 8.26; N, 2.73.

3.41. Methyl (isopropyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-1-thio-α,β-D-glucopyranosid)uronate (5d)

Prepared as an anomeric mixture (α/β 1:2) from reaction between **10** and 2-iodopropane in 73% yield after chromatography (EtOAc/hexane 2:3) as an amorphous mass. Crystallisation of the product from EtOAc/hexane afforded pure β-anomer of **5d**. Compound α-**5d**: $R_f = 0.23$ (EtOAc/hexane 2:3); ^{1}H NMR ($CDCl_3$): δ 1.14, 1.17 (2× 9H, 2× s, 2× OPiv), 1.32 (3H, d, $J_{2',1'}$ 6.8 Hz, H-2′), 1.34 (3H, d, $J_{2'',1'}$ 6.8 Hz, H-2″), 1.94 (3H, s, NAc), 3.12 (1H, sept, $J_{1',2'} = J_{1',2''}$ 6.8 Hz, H-1′), 3.74 (3H, s, OMe), 4.61 (1H, ddd, $J_{2,3}$ 10.7, $J_{2,NH}$ 9.4, $J_{2,1}$ 5.2 Hz, H-2), 4.72 (1H, d, $J_{5,4}$ 9.4 Hz, H-5), 5.11 (1H, dd, $J_{3,2}$ 10.7, $J_{3,4}$ 8.9 Hz, H-3), 5.25 (1H, dd, $J_{4,5}$ 9.4, $J_{4,3}$ 8.9 Hz, H-4), 5.51 (1H, d, $J_{1,2}$ 5.2 Hz, H-1), 5.66 (1H, br d, $J_{NH,2}$ 9.2 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 23.1 (NC(O)*Me*), 23.6, 23.8 (C-2′, C-2″), 27.0 (2× OC(O)C*Me*$_3$), 36.4 (C-1′), 38.7, 38.9 (2× OC(O)*C*Me$_3$), 51.6 (C-2), 52.7 (CO_2*Me*), 68.8 (C-4), 70.0 (C-3, C-5), 83.4 (C-1), 168.1, 169.5, 176.4, 178.6 (*C*O$_2$Me, N*C*(O)Me, 2× O*C*(O)CMe$_3$). LRMS *m/z* 498 ([M+Na]$^+$, 100%), 476 (66), 153 (82). Compound β-**5d**: $R_f = 0.23$ (EtOAc/hexane 2:3); ^{1}H NMR ($CDCl_3$): δ 1.14, 1.15 (2× 9H, 2× s, 2× OPiv), 1.27 (3H, d, $J_{2',1'}$ 6.8 Hz, H-2′), 1.32 (3H, d, $J_{2'',1'}$ 6.8 Hz, H-2″), 1.93 (3H, s, NAc), 3.23 (1H, sept, $J_{1',2'} = J_{1',2''}$ 6.8 Hz, H-1′), 3.73 (3H, s, OMe), 4.05 (1H, d, $J_{5,4}$ 9.6 Hz, H-5), 4.17 (1H, ddd, $J_{2,1}$ 10.2, $J_{2,NH}$ 9.6, $J_{2,3}$ 10.5 Hz, H-2), 4.71 (1H, d, $J_{1,2}$ 10.2 Hz, H-1), 5.23–5.31 (2H, m, H-3, H-4), 5.38 (1H, br d, $J_{NH,2}$ 9.6 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 23.2 (NC(O)*Me*), 23.5, 24.2 (C-2′, C-2″), 27.0 (2× OC(O)C*Me*$_3$), 34.8 (C-1′), 38.7, 38.9 (2× OC(O)*C*Me$_3$), 52.7 (CO_2*Me*), 53.0 (C-2), 69.1 (C-4), 72.6 (C-3), 76.5 (C-5), 84.4 (C-1), 167.2, 169.7, 176.4, 178.5 (*C*O$_2$Me, N*C*(O)Me, 2× O*C*(O)CMe$_3$). LRMS *m/z* 498 ([M+Na]$^+$, 100%), 476 (66), 153 (82). Anal. Calcd for $C_{22}H_{37}NO_8S$: C, 55.56; H, 7.84; N, 2.95. Found: C, 53.34; H, 7.95; N, 2.90.

3.42. General procedure for the synthesis of 11a–11d

DBU (150 μL, 0.76 mmol) was added to a solution of an anomeric mixture of compound **5a**–**Ac**, **5b**–**Ac**, **5c**, or **5d** (0.38 mmol) in anhyd CH_2Cl_2 (2 mL) under N_2. The pale yellow solution was stirred at rt and monitored by TLC analysis. After 18 h, the reaction mixture was concentrated under reduced pressure to give a clear yellow syrup. Purification of the crude product by flash chromatography afforded **11a**–**11d**

[90–100%, based on recovered starting material (β-anomer)].

3.43. Methyl (3-*O*-acetyl-3-hydroxypropyl 2-acetamido-2,4-dideoxy-3-*O*-pivaloyl-1-thio-α-L-threo-hex-4-enopyranosid)uronate (11a)

Acetic anhydride (0.1 mL, 1 mmol) was added to a solution of an anomeric mixture of **5a** (162 mg, 0.33 mmol) in anhyd pyridine (3 mL). The solution was stirred overnight at rt under N_2. The reaction was quenched by addition of MeOH (0.5 mL) and then concentrated. The residue was diluted with EtOAc (10 mL), washed with dilute HCl (0.1 M, 10 mL), water (2× 10 mL), dried (Na_2SO_4), filtered and concentrated. Purification of the crude product by flash chromatography (EtOAc/hexane 3:2) afforded **5a-Ac** [$R_f = 0.24$ (EtOAc/hexane 1:1); 155 mg, 88%] as an amorphous mass. Treatment of an anomeric mixture of **5a**–**Ac** (α/β 2:3) with DBU afforded pure β-anomer of **11a** (40% from **5a-Ac**) after chromatography (EtOAc/hexane 2:1) as a colourless syrup. A mixture containing recovered starting material **5a**–**Ac** (β-anomer) (20% from **5a-Ac**) and the α-anomer of **11a** (40% from **5a-Ac**) was also isolated. Compound α-**11a**: $R_f = 0.37$ (EtOAc/hexane 2:1); ^{1}H NMR ($CDCl_3$) distinguishable signals in a mixture containing β-**5a-Ac**: δ 1.21 (9H, s, OPiv), 1.95–2.19 (2H, m, H-2′), 1.99 (3H, s, NAc), 2.07 (3H, s, OAc), 2.79–2.92 (2H, m, H-1′), 3.84 (3H, s, OMe), 4.12–4.20 (2H, m, H-3′), 4.58 (1H, ddd, $J_{2,NH}$ 8.4, $J_{2,3}$ 7.5, $J_{2,1}$ 3.3 Hz, H-2), 5.36 (1H, dd, $J_{3,2}$ 7.5, $J_{3,4}$ 3.6 Hz, H-3), 5.70 (1H, br d, $J_{NH,2}$ 8.4 Hz, NH), 6.12 (1H, d, $J_{4,3}$ 3.6 Hz, H-4). Compound β-**11a**: $R_f = 0.20$ (EtOAc/hexane 2:1); ^{1}H NMR ($CDCl_3$): δ 1.24 (9H, s, OPiv), 1.95–2.08 (2H, m, H-2′), 1.99 (3H, s, NAc), 2.06 (3H, s, OAc), 2.76 (1H, dt, $J_{1'a,1'b}$ 13.5, $J_{1'a,2'}$ 7.2 Hz, H-1′a), 2.83 (1H, dt, $J_{1'b,1'a}$ 13.5, $J_{1'b,2'}$ 7.2 Hz, H-1′b), 3.85 (3H, s, OMe), 4.14 (2H, t, $J_{3',2'}$ 6.2 Hz, H-3′), 4.52 (1H, dddd, $J_{2,NH}$ 9.0, $J_{2,3} = J_{2,1} = 1.8$, $J_{2,4}$1.2 Hz, H-2), 4.98 (1H, ddd, $J_{3,4}$ 5.1, $J_{3,2}$ 1.5, $J_{3,1}$ 1.2 Hz, H-3), 5.47 (1H, dd, $J_{1,2}$ 2.1, $J_{1,3}$ 1.2 Hz, H-1), 5.59 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH), 6.32 (1H, dd, $J_{4,3}$ 5.1, $J_{4,2}$ 1.2 Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 20.9 (OC(O)*Me*), 23.0 (NC(O)*Me*), 27.0 (OC(O)C*Me*$_3$), 28.7 (C-2′), 29.4 (C-1′), 38.9 (OC(O)*C*Me$_3$), 49.5 (C-2), 52.6 (OMe), 62.8 (C-3′), 64.3 (C-3), 83.2 (C-1), 107.2 (C-4), 143.0 (C-5), 162.1, 169.3, 170.1, 177.3 (*C*O$_2$Me, N*C*(O)Me, O*C*(O)Me, O*C*(O)CMe$_3$). LRMS *m*/*z* 454.5 ([M+Na]$^+$, 100%). HRMS calcd for $C_{19}H_{29}NNaSO_8$ [M+Na] 454.4909. Found 454.1522.

3.44. Methyl (2-*O*-acetyl-2-hydroxyethyl 2-acetamido-2,4-dideoxy-3-*O*-pivaloyl-1-thio-α-L-threo-hex-4-enopyranosid)uronate (11b)

Acetic anhydride (0.1 mL, 1 mmol) was added to a solution of an anomeric mixture of **5b** (165 mg, 0.35 mmol) in anhyd pyridine (3 mL). The solution was stirred overnight at rt under N_2. The reaction was quenched by addition of MeOH (0.5 mL) and then concentrated. The residue was diluted with EtOAc (10 mL), washed with dilute HCl (0.1 M, 10 mL), water (2× 10 mL), dried (Na_2SO_4), filtered and concentrated. Purification of the crude product by flash chromatography (EtOAc/hexane 3:2) afforded **5b-Ac** [$R_f = 0.30$ (EtOAc/hexane 3:2); 174 mg, 97%] as an amorphous mass. Treatment of an anomeric mixture of **5b-Ac** (α/β 3:2) with DBU afforded pure β-anomer of **11b** (37% from **5b-Ac**) after chromatography (EtOAc/hexane 3:2 → 3:1) as a colourless syrup. The α-anomer of **11b** (53% from **5b-Ac**) was also isolated. Compound α-**11b**: $R_f = 0.22$ (EtOAc/hexane 3:2); ^{1}H NMR ($CDCl_3$): δ 1.20 (9H, s, OPiv), 1.99 (3H, s, NAc), 2.07 (3H, s, OAc), 2.94 (1H, dt, $J_{1'a,1'b}$ 14.1, $J_{1'a,2'a} = J_{1'a,2'b}$ 6.6 Hz, H-1′a), 3.08 (1H, dt, $J_{1'b,1'a}$ 14.1, $J_{1'b,2'a} = J_{1'b,2'b}$ 6.6 Hz, H-1′b), 3.83 (3H, s, OMe), 4.23 (1H, dt, $J_{2'a,2'b}$ 11.4, $J_{2'a,1'a} = J_{2'a,1'b}$ 6.6 Hz, H-2′a), 4.40 (1H, dt, $J_{2'b,2'a}$ 11.4, $J_{2'b,1'a} = J_{2'b,1'b}$ 6.6 Hz, H-2′b), 4.58 (1H, ddd, $J_{2,NH}$ 8.4, $J_{2,3}$ 6.9, $J_{2,1}$ 3.6 Hz, H-2), 5.33 (1H, dd, $J_{3,2}$ 6.9, $J_{3,4}$ 3.6 Hz, H-3), 5.51 (1H, d, $J_{1,2}$ 3.6 Hz, H-1), 5.71 (1H, br d, $J_{NH,2}$ 8.4 Hz, NH), 6.12 (1H, d, $J_{4,3}$ 3.6 Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 20.7 (OC(O)*Me*), 22.9 (NC(O)*Me*), 26.9 (OC(O)C*Me*$_3$), 30.1 (C-1′), 38.9 (OC(O)*C*Me$_3$), 49.5 (C-2), 52.5 (CO$_2$*Me*), 63.3 (C-2′), 65.6 (C-3), 83.9 (C-1), 108.5 (C-4), 143.7 (C-5), 161.7, 170.0, 170.7, 178.0 (*C*O$_2$Me, N*C*(O)Me, O*C*(O)CMe$_3$, O*C*(O)Me). LRMS *m*/*z* 440 ([M+Na]$^+$, 100%). Compound β-**11b**: $R_f = 0.11$ (EtOAc/hexane 3:2); ^{1}H NMR ($CDCl_3$): δ 1.23 (9H, s, OPiv), 1.99 (3H, s, NAc), 2.07 (3H, s, OAc), 2.86 (1H, ddd, $J_{1'a,1'b}$ 14.1, $J_{1'a,2'a}$ 7.2, $J_{1'a,2'b}$ 6.0 Hz, H-1′a), 3.02 (1H, ddd, $J_{1'b,1'a}$ 14.1, $J_{1'b,2'a} = J_{1'b,2'b}$ 7.2 Hz, H-1′b), 3.85 (3H, s, OMe), 4.21 (1H, ddd, $J_{2'a,2'b}$ 11.4, $J_{2'a,1'a} = J_{2'a,1'b}$ 7.2 Hz, H-2′a), 4.36 (1H, ddd, $J_{2'b,2'a}$ 11.4, $J_{2'b,1'a}$ 6.0, $J_{2'b,1'b}$ 7.2 Hz, H-2′b), 4.52 (1H, dddd, $J_{2,NH}$ 8.7, $J_{2,1} = J_{2,3}$ 1.8, $J_{2,4}$ 1.2 Hz, H-2), 4.98 (1H, ddd, $J_{3,4}$ 4.8, $J_{3,2}$ 1.8, $J_{3,1}$ 0.9 Hz, H-3), 5.56 (1H, dd, $J_{1,2}$ 1.8, $J_{1,3}$ 0.9 Hz, H-1), 5.69 (1H, br d, $J_{NH,2}$ 8.7 Hz, NH), 6.31 (1H, dd, $J_{4,3}$ 4.8, $J_{4,2}$ 1.2 Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 20.7 (OC(O)*Me*), 23.0 (NC(O)*Me*), 27.0 (OC(O)C*Me*$_3$), 31.1 (C-1′), 38.9 (OC(O)*C*Me$_3$), 49.3 (C-2), 52.7 (CO$_2$*Me*), 63.3 (C-2′), 64.3 (C-3), 83.2 (C-1), 107.2 (C-4), 142.9 (C-5), 162.1, 169.3, 170.7, 177.2 (*C*O$_2$Me, N*C*(O)Me, O*C*(O)Me, O*C*(O)CMe$_3$). LRMS *m*/*z* 440 ([M+Na]$^+$, 100%). HRMS calcd for $C_{18}H_{27}NNaSO_8$ [M+Na] 440.1355. Found 440.1358.

3.45. Methyl (2-methylpropyl 2-acetamido-2,4-dideoxy-3-*O*-pivaloyl-1-thio-α-L-threo-hex-4-enopyranosid)uronate (11c)

Treatment of anomeric mixture of **5c** (α/β 2:3) with DBU afforded pure β-anomer of **11c** (40%) after chromatography (EtOAc/hexane 1:1) as a colourless syrup. A mixture of recovered starting material **5c** (β-anomer) (18%) and the α-anomer of **11c** (35%) was also isolated. Compound α-**11c**: $R_f = 0.40$ (EtOAc/hexane 1:1); ^{1}H NMR ($CDCl_3$) distinguishable signals in a mixture containing β-**5c**: δ 0.95–1.01 (6H, m, H-3′, H-3″), 1.74–1.91 (1H, m, H-2′), 1.93 (3H, s, NAc), 2.55–2.72 (2H, m, H-1′), 3.72 (3H, s, OMe), 4.56 (1H, ddd, $J_{2,NH}$ 8.7, $J_{2,3}$ 7.2, $J_{2,1}$ 3.3 Hz, H-2), 5.37 (1H, dd, $J_{3,2}$ 7.2, $J_{3,4}$ 3.3 Hz, H-3), 5.41 (1H, d, $J_{1,2}$ 3.3 Hz, H-1), 5.75 (1H, br

d, $J_{NH,2}$ 8.7 Hz, NH), 6.10 (1H, d, $J_{4,3}$ 3.3 Hz, H-4). LRMS *m/z* 410 ([M+Na]$^+$, 80%). Compound β-**11c**: R_f = 0.22 (EtOAc/hexane 1:1); ^{1}H NMR ($CDCl_3$): δ 0.98 (3H, d, $J_{3',2'}$ 6.6 Hz, H-3′), 0.99 (3H, d, $J_{3'',2'}$ 6.6 Hz, H-3″), 1.24 (9H, s, OPiv), 1.88 (1H, septt, $J_{2',1'a} = J_{2',1'b}$ 6.9, $J_{2',3'} = J_{2',3''}$ 6.6 Hz, H-2′), 1.99 (3H, s, NAc), 2.57 (1H, dd, $J_{1'a,1'b}$ 12.6, $J_{1'a,2'}$ 6.9 Hz, H-1′a), 2.64 (1H, dd, $J_{1'b,1'a}$ 12.6, $J_{1'b,2'}$ 6.9 Hz, H-1′b), 3.85 (3H, s, OMe), 4.53 (1H, dddd, $J_{2,NH}$ 9.0, $J_{2,1} = J_{2,3}$ 1.8, $J_{2,4}$ 1.2 Hz, H-2), 4.97 (1H, ddd, $J_{3,4}$ 4.8, $J_{3,2}$ 1.8, $J_{3,1}$ 1.2 Hz, H-3), 5.47 (1H, dd, $J_{1,2}$ 1.8, $J_{1,3}$ 1.2 Hz, H-1), 5.65 (1H, br d, $J_{NH,2}$ 9.0 Hz, NH), 6.32 (1H, dd, $J_{4,3}$ 4.8, $J_{4,2}$ 1.2 Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 21.8, 21.9 (C-3′, C-3″), 23.2 (NC(O)*Me*), 27.0 (OC(O)C*Me*$_3$), 28.5 (C-2′), 38.9 (OC(O)*C*Me$_3$), 41.8 (C-1′), 49.5 (C-2), 52.7 (CO_2*Me*), 64.4 (C-3), 83.6 (C-1), 107.2 (C-4), 143.0 (C-5), 162.3, 169.1, 177.3 (*C*O$_2$Me, N*C*(O)Me, O*C*(O)CMe$_3$). LRMS *m/z* 410 ([M+Na]$^+$, 100%). HRMS calcd for $C_{18}H_{29}NNaSO_6$ [M+Na] 410.1613. Found 410.1609.

3.46. Methyl (isopropyl 2-acetamido-2,4-dideoxy-3-*O*-pivaloyl-1-thio-α-L-threo-hex-4-enopyranosid)uronate (11d)

Treatment of an anomeric mixture of **5d** (α/β 2:3) with DBU afforded pure β-anomer of **11d** in 47% yield after chromatography (EtOAc/hexane 1:1 → 3:2) as a colourless syrup. A mixture of recovered starting material **5d** (β-anomer) (12%) and the α-anomer of **11d** (41%) was also isolated. Compound α-**11d**: R_f = 0.31 (EtOAc/hexane 1:1); ^{1}H NMR ($CDCl_3$) distinguishable signals in a mixture containing β-**5d**: δ 1.19 (9H, s, OPiv), 1.34 (3H, d, $J_{2',1'}$ 6.8 Hz, H-2′), 1.35 (3H, d, $J_{2'',1'}$ 6.8 Hz, H-2″), 1.97 (3H, s, NAc), 3.25 (1H, sept, $J_{1',2'} = J_{1',2''}$ 6.8 Hz, H-1′), 3.82 (3H, s, OMe), 4.56 (1H, ddd, $J_{2,NH}$ 8.5, $J_{2,3}$ 7.2, $J_{2,1}$ 3.5 Hz, H-2), 5.33 (1H, dd, $J_{3,2}$ 7.2, $J_{3,4}$ 3.5 Hz, H-3), 5.49 (1H, d, $J_{1,2}$ 3.5 Hz, H-1), 5.76 (1H, br d, $J_{NH,2}$ 8.5 Hz, NH), 6.09 (1H, d, $J_{4,3}$ 3.5 Hz, H-4); ^{13}C NMR ($CDCl_3$) distinguishable signals in a mixture containing β-**5d**: δ 23.1 (NC(O)*Me*), 23.6, 23.9 (C-2′, C-2″), 27.0 (OC(O)C*Me*$_3$), 36.2 (C-1′), 38.8 (OC(O)*C*Me$_3$), 49.7 (C-2), 52.5 (CO_2*Me*), 66.0 (C-3), 82.8 (C-1), 108.5 (C-4), 144.0 (C-5), 161.9, 169.9, 178.1 (*C*O$_2$Me, N*C*(O)Me, O*C*(O)CMe$_3$). LRMS *m/z* 396 ([M+Na]$^+$, 100%). Compound β-**11d**: R_f = 0.19 (EtOAc/hexane 1:1); ^{1}H NMR ($CDCl_3$): δ 1.23 (9H, s, OPiv), 1.32 (3H, d, $J_{2',1'}$ 6.8 Hz, H-2′), 1.34 (3H, d, $J_{2'',1'}$ 6.8 Hz, H-2″), 1.99 (3H, s, NAc), 3.15 (1H, sept, $J_{1',2'} = J_{1',2''}$ 6.8 Hz, H-1′), 3.84 (3H, s, OMe), 4.50 (1H, dddd, $J_{2,NH}$ 8.7, $J_{2,4} = J_{2,1} = J_{2,3}$ 1.8 Hz, H-2), 4.96 (1H, ddd, $J_{3,4}$ 5.1, $J_{3,2} = J_{3,1}$ 1.8 Hz, H-3), 5.56–5.59 (1H, m, H-1), 5.72 (1H, br d, $J_{NH,2}$ 8.4 Hz, NH), 6.31 (1H, dd, $J_{4,3}$ 5.1, $J_{4,2}$1.5 Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 23.2 (NC(O)*Me*), 23.4, 23.8 (C-2′, C-2″), 27.0 (OC(O)C*Me*$_3$), 36.9 (C-1′), 38.9 (OC(O)*C*Me$_3$), 49.7 (C-2), 52.6 (CO_2*Me*), 64.4 (C-3), 82.1 (C-1), 107.1 (C-4), 143.1 (C-5), 162.3, 169.2, 177.3 (*C*O$_2$Me, N*C*(O)Me, O*C*(O)CMe$_3$). LRMS *m/z* 396 ([M+Na]$^+$, 100%), 294 (30). HRMS calcd for $C_{17}H_{27}NNaSO_6$ [M+Na] 396.1457. Found 396.1461.

3.47. General procedure for the synthesis of 3a–3d

A solution of **11a**–**11d** (~0.4 mmol) in aq MeOH (50%, 5 mL) was adjusted to pH 13 using aq NaOH (0.5 M). The solution was stirred at rt and monitored by TLC analysis (EtOAc/MeOH/H_2O 7:2:1). After 18 h, Amberlite® IR-120 (H^+) resin was added to adjust to pH 3, the reaction mixture was filtered, the resin was washed with MeOH/H_2O 1:1 (30 mL), and the filtrate was concentrated to dryness. PivOH was then removed by evaporation under high vacuum (~1 mmHg) at 40 °C for 3 h. The residue was dissolved in water (5 mL), aq NaOH was added to adjust to pH 7.3 and the solution was lyophilised to afford an amorphous solid. The crude product was purified by HPLC and then lyophilised to give **3a**–**3d** (70–85%).

3.48. Sodium (3-hydroxypropyl 2-acetamido-2,4-dideoxy-1-thio-α-L-threo-hex-4-enopyranosid)uronate (3a)

Prepared from β-**11a** in 85% yield after reverse-phase HPLC (5% CH_3CN in water) as a creamy-coloured amorphous solid. R_f = 0.09 (EtOAc/MeOH/H_2O 7:2:1); ^{1}H NMR (D_2O): δ 1.67–1.82 (2H, m, H-2′), 1.95 (3H, s, NAc), 2.64 (1H, dt, $J_{1'a,1'b}$ 13.2, $J_{1'a,2'}$ 7.2 Hz, H-1′a), 2.74 (1H, dt, $J_{1'b,1'a}$ 13.2, $J_{1'b,2'}$ 7.2 Hz, H-1′b), 3.55 (2H, t, $J_{3',2'}$ 6.3 Hz, H-3′), 4.00 (1H, ddd, $J_{2,1}$ 5.4, $J_{2,3}$ 4.5, $J_{2,4}$ 0.3 Hz, H-2), 4.10 (1H, ddd, $J_{3,2}$ 4.5, $J_{3,4}$ 3.9, $J_{3,1}$ 0.6 Hz, H-3), 5.28 (1H, dd, $J_{1,2}$ 5.4, $J_{1,3}$ 0.6 Hz, H-1), 5.84 (1H, dd, $J_{4,3}$ 3.9, $J_{4,2}$ 0.3 Hz, H-4); ^{13}C NMR (D_2O): δ 21.9 (NC(O)*Me*), 27.8 (C-1′), 31.3 (C-2′), 52.4 (C-2), 60.0 (C-3′), 64.7 (C-3), 82.4 (C-1), 107.6 (C-4), 145.7 (C-5), 168.4, 173.9 (CO_2Na, N*C*(O)Me). LRMS *m/z* 336 ([M+Na]$^+$, 100%). Anal. Calcd for $C_{11}H_{16}NNaO_6S{\cdot}H_2O$: C, 39.88; H, 5.48; N, 4.23. Found: C, 39.70; H, 5.47; N, 4.05.

3.49. Sodium (2-hydroxyethyl 2-acetamido-2,4-dideoxy-1-thio-α-L-threo-hex-4-enopyranosid)uronate (3b)

Prepared from β-**11b** in 79% yield after reverse-phase HPLC (1% CH_3CN in water) as a creamy-coloured amorphous solid. R_f = 0.12 (EtOAc/MeOH/H_2O 7:2:1); ^{1}H NMR (D_2O): δ 1.89 (3H, s, NAc), 2.72 (1H, dt, $J_{1'a,1'b}$ 14.1, $J_{1',2'}$ 6.3 Hz, H-1′a), 2.84 (1H, dt, $J_{1'b,1'a}$ 14.1, $J_{1'b,2'}$ 6.3 Hz, H-1′b), 3.64 (1H, dt, $J_{2'a,2'b}$ 11.4, $J_{2'a,1'}$ 6.3 Hz, H-2′a), 3.70 (1H, dt, $J_{2'b,2'a}$ 11.4, $J_{2'b,1'}$ 6.3 Hz, H-2′b), 4.01 (1H, ddd, $J_{2,1}$ 5.1, $J_{2,3}$ 4.5, $J_{2,4}$ 0.6 Hz, H-2), 4.08 (1H, ddd, $J_{3,2}$ 4.5,$J_{3,4}$ 3.9, $J_{3,1}$ 0.6 Hz, H-3), 5.32 (1H, dd, $J_{1,2}$ 5.1, $J_{1,3}$ 0.6 Hz, H-1), 5.84 (1H, dd, $J_{4,3}$ 3.9, $J_{4,2}$ 0.6 Hz, H-4); ^{13}C NMR (D_2O) δ 21.8 (NC(O)*Me*), 33.7 (C-1′), 52.4 (C-2), 60.7 (C-2′), 64.6 (C-3), 82.2 (C-1), 107.5 (C-4), 145.7 (C-5), 168.6, 173.9 (N*C*(O)Me, CO_2Na). LRMS *m/z* 300 ([M+H]$^+$, 100%). Anal. Calcd for $C_{10}H_{14}NNaO_6S{\cdot}H_2O$: C, 37.86; H, 5.08; N, 4.41. Found: C, 37.88; H, 5.26; N, 4.29.

3.50. Sodium (isobutyl 2-acetamido-2,4-dideoxy-1-thio-α-L-threo-hex-4-enopyranosid)uronate (3c)

Prepared from β-**11c** in 80% yield after reverse-phase HPLC (15% CH_3CN in water) as a creamy-coloured

amorphous mass. $R_f = 0.31$ (EtOAc/MeOH/H_2O 7:2:1); ^1H NMR (D_2O): δ 0.82 (3H, d, $J_{3',2'}$ 6.6 Hz, H-3′), 0.82 (3H, d, $J_{3'',2'}$ 6.6 Hz, H-3″), 1.62–1.80 (1H, m, H-2′), 2.48 (1H, dd, $J_{1'a,1'b}$ 12.6 Hz, $J_{1'a,2'}$ 7.2, H-1′a), 2.57 (1H, dd, $J_{1'b,1'a}$ 12.6, $J_{1'b,2'}$ 6.9 Hz, H-1′b), 4.00 (1H, br dd, $J_{2,1}$ 5.7, $J_{2,3}$ 4.5 Hz, H-2), 4.11 (1H, br dd, $J_{3,2}$ 4.5, $J_{3,4}$ 3.9 Hz, H-3), 5.26 (1H, br d, $J_{1,2}$ 5.7 Hz, H-1), 5.98 (1H, br d, $J_{4,3}$ 3.9 Hz, H-4); ^{13}C NMR (D_2O): δ 21.6, 21.7 (C-3′, C-3″), 22.5 (NC(O)*Me*), 28.6 (C-2′), 40.8 (C-1′), 53.1 (C-2), 65.3 (C-3), 83.7 (C-1), 110.6 (C-4), 144.4 (C-5), 167.2, 174.5 (CO_2Na, N*C*(O)Me). LRMS *m/z* 312 ([M+H]$^+$, 100%). HRMS calcd for $C_{12}H_{18}NNa_2SO_5$ [M+Na] 334.0701. Found 334.0701.

3.51. Sodium (isopropyl 2-acetamido-2,4-dideoxy-1-thio-α-L-threo-hex-4-enopyranosid)uronate (3d)

Prepared from β-**11d** in 70% yield after reverse-phase HPLC (5% CH_3CN in water) as an amorphous creamy-coloured solid. $R_f = 0.24$ (EtOAc/MeOH/H_2O 7:2:1); ^1H NMR (D_2O): δ 1.10 (3H, d, $J_{2',1'}$ 6.6 Hz, H-2′), 1.14 (3H, d, $J_{2'',1'}$ 6.6 Hz, H-2″), 1.84 (3H, s, NAc), 3.06 (3H, sept, $J_{1',2'} = J_{1',2''}$ 6.6 Hz, H-1′), 3.92 (1H, br dd, $J_{2,1}$ 5.1, $J_{2,3}$ 4.5 Hz, H-2), 4.02 (1H, br dd, $J_{3,2}$ 4.5, $J_{3,4}$ 3.9 Hz, H-3), 5.31 (1H, br d, $J_{1,2}$ 5.1 Hz, H-1), 5.73 (1H, br d, $J_{4,3}$ 3.9 Hz, H-4); ^{13}C NMR (D_2O) δ 21.8 (NC(O)*Me*), 22.8 (C-2′, C-2″), 36.2 (C-1′), 52.5 (C-2), 64.3 (C-3), 81.9 (C-1), 111.7 (C-4), 142.3 (C-5), 165.3, 174.0 (CO_2Na, N*C*(O)Me). LRMS *m/z* 320 ([M+Na]$^+$, 100%), 298 ([M+H]$^+$, 39), 265 (33), 229 (56). Anal. Calcd for $C_{11}H_{16}NO_5SNa$ 1.5H_2O: C, 40.74; H, 5.90; N, 4.32. Found: C, 40.96; H, 5.58; N, 4.03.

3.52. Sialidase assay

Vibrio cholerae sialidase was expressed and purified using previously published methods.[13,45] Sialidase activity was assayed using a modified fluorometric assay[33] developed by Potier et al..[29] The substrate used for the enzyme assay, MUN, was prepared using published methods.[30] Solutions were prepared in Eppendorf tubes containing sialidase (6.2×10^5 U,[46] 10 μL) in the presence or absence of inhibitor (1 mM final concentration, 10 μL). Samples were made up to 95 μL using 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (50 mM, pH 5.6, containing 6 mM $CaCl_2$). The samples were incubated at 37 °C for 30 min with shaking, prior to the addition of MUN (50 μM final concentration, 5 μL). The reactions were stopped after a further 20 min incubation period at 37 °C with shaking using glycine solution (0.25 M, pH 10, 2.4 mL). Fluorescence was measured using a TD-700 Fluorometer (Turner Design, CA, USA) at emission and excitation wavelengths of 400 and 365 nm, respectively. The assay was performed at least in duplicate. Inhibition was measured as a percentage of the control (incubations performed in the absence of inhibitor). Sample measurements were corrected for background fluorescence that was not produced by the enzyme-catalysed hydrolysis of the substrate, by subtracting a blank sample that contained MUN in MES buffer. Neu5Ac2en (**1**) was included in every assay as a comparison.

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