Tetrahedron 64 (2008) 9093–9100

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis of 1-C-linked diphosphate analogues of UDP-*N*-Ac-glucosamine and UDP-*N*-Ac-muramic acid

Andrej Babič [a,*], Stanislav Gobec [a], Christine Gravier-Pelletier [b], Yves Le Merrer [b], Slavko Pečar [a,c]

[a] *Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia*
[b] *Université Paris Descartes, UMR 8601 CNRS, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, 45 rue des Saints-Pères, 75006 Paris, France*
[c] *Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia*

ARTICLE INFO

Article history:
Received 22 March 2008
Received in revised form 12 June 2008
Accepted 3 July 2008
Available online 8 July 2008

Keywords:
C-Linked
glycosides substrate analogues
Diphosphate isosteres
Microwave-assisted synthesis

ABSTRACT

UDP-*N*-acetyl-glucosamine and UDP-*N*-acetyl-muramic acid are two important cytoplasmic precursors of bacterial peptidoglycan. The convergent synthesis of their analogues is reported. The α-1-C-linked-*N*-acetyl-glucosamine was synthesized using microwave-assisted Keck radical allylation. Oxidation of alkene derivatives to the corresponding carboxylic acids allowed attachment to *O*- and *N*-sulfamoyluridine giving stable diphosphate mimetics.

1. Introduction

N-Acetyl-glucosamine (GlcNAc) and *N*-acetyl-muramic acid (MurNAc) are present in almost all eubacteria as constituent units of the bacterial cell wall peptidoglycan.[1] In the biosynthesis of polysaccharides, glycolipids, glycoproteins and the bacterial cell wall, GlcNAc and MurNAc are usually utilized, in the form of precursors containing nucleoside diphosphates, by a variety of enzymes, in particular glycosyltransferases.[2–5] These enzymes are important targets for possible modulation of certain biochemical processes. We have focused on the enzymes involved in the cytoplasmic steps of peptidoglycan biosynthesis.[1,6] Since MurA, MurC and MurG enzymes transfer or modify UDP-GlcNAc or UDP-MurNAc, it was reasoned that analogues of these compounds could mimic their substrates and serve as useful starting points for designing new enzyme inhibitors.

The design and synthesis of nucleotide diphosphate sugar analogues are imposing challenges. Replacement of the diphosphate moiety is not straightforward. Ionic, steric and H-bond forming characteristics, as well as the distance between nucleoside and sugar moieties, all play an important role and affect the binding to the target enzymes. We have introduced the *O*- and *N*- sulfamoyl amide group in lieu of the diphosphate part of UDP as depicted in Figure 1. This was based on similar approaches that showed inhibition of protein glycosylation.[7]

To render the target compounds chemically and enzymatically stable, C-linked glycosides were chosen as a synthetically feasible alternative to natural α-*O*-glycosides.[5,8–10] However, to achieve this, maintaining the α-configuration at the anomeric position of the *N*-Ac-glucosamine would be crucial. Fortunately, synthetic strategies for making C-linked derivatives of *N*-Ac-glucosamine have been thoroughly investigated.[11–18] Keck radical allylation[19] was chosen as the key reaction to introduce the α-carbon–carbon bond at the anomeric position.

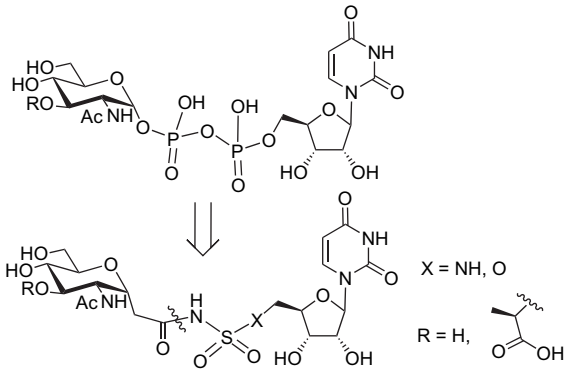


Figure 1. Design of *O*- and *N*-sulfamoyl amide UDP-GlcNAc and UDP-MurNAc analogues.

* Corresponding author. Tel.: +386 1 4769 601; fax: +386 1 4258 031.
E-mail address: andrej.babic@ffa.uni-lj.si (A. Babič).

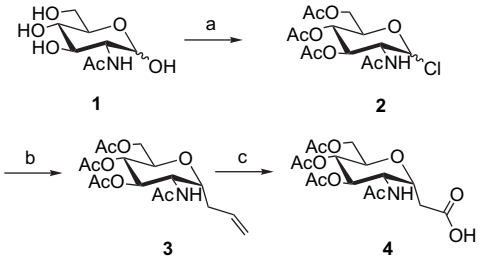
0040-4020/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2008.07.009

Here we describe the synthesis of the UDP-GlcNAc and UDP-MurNAc analogues where the uridine and C-1-linked sugar moieties are connected by an *O*- or *N*-sulfamoylamide bridge, which acts as a diphosphate isostere.

2. Results and discussion

The target compounds were synthesized using a convergent strategy. The *O*- and *N*-sulfamoyl uridine moieties were synthesized separately from 1-C-linked *N*-acetyl-α-D-glucosamine derivatives. Both moieties were then joined by a simple coupling reaction followed by deprotection to afford the end compounds.

Scheme 1 presents the synthesis of the desired α-1-C-linked-GlcNAc. According to known procedures, *N*-acetyl-glucosamine **1** was first converted to chloride derivative **2** in acetyl chloride saturated with hydrogen chloride gas.[14] The reaction proceeds as previously described albeit the instability of **2** prevents normal chromatographic purification. In the next reaction step we formed the desired carbon–carbon bond using Keck radical allylation. Despite the fact that this reaction proceeds via a free radical mechanism, it is not devoid of stereoselectivity and a number of authors have reported this reaction using chloride **2** with different resulting α/β anomer ratios.[13–15,17] To achieve as high an α/β anomer ratio as possible, only 3 equiv of allyltributyltin was used, and only 0.15 equiv of azobisisobutyronitrile (AIBN) initiator, as reported by Cui and Horton.[17] This lowered the yield slightly compared to that reported by Bouvet and Ben but the ^{1}H NMR spectrum indicated pure α-anomer of **3**. As previously observed very little side products were formed when THF was used as a solvent in the reaction.[14]

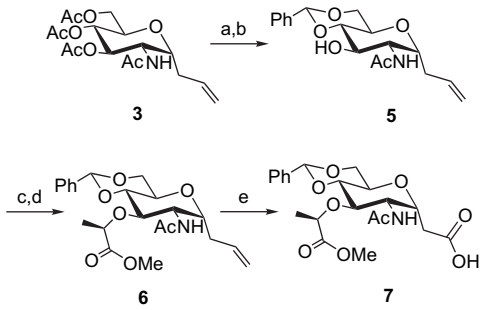


Scheme 1. Reaction conditions: (a) HCl(g), AcCl, 30 °C, 16 h; (b) $Bu_3Snallyl$, AIBN, THF, 10 min, MW; (c) $NaIO_4$, $RuCl_3$, H_2O/dioxane, rt, 4 h.

In addition to conventional oil-bath heating, the reaction was also attempted under microwave-assisted conditions. Using essentially the same amount of reagents and solvent, a significant reduction in reaction time was achieved, the reaction being complete in only 10 min at 100 °C compared to 7 h at 70 °C under the conventional procedure. A slight improvement in reaction yield was also achieved, but more importantly, and to our surprise, only the α-anomer was detected. Given the very short reaction time and relatively low temperature needed to achieve total conversion of the starting material, it appears that this novel use of microwave irradiation for Keck radical allylation is preferable to conventional heating.

In the next step, 1-*C*-allyl-*N*-acetyl-glucosamine **3** was oxidized to the corresponding carboxylic acid **4** using the sodium periodate and ruthenium trichloride oxidation system. Diluted dioxane/water could be used instead of the tricomponent tetrachlorocarbon, acetonitrile and water mixture to perform this reaction in very good yield.[21,22]

Alkene **3** was used as the starting material for the synthesis of 1-C-linked *N*-acetyl-muramic acid **7**. The standard two-step deprotection and reprotection protocol for peracetylated alkene derivative **3** was used (Scheme 2).

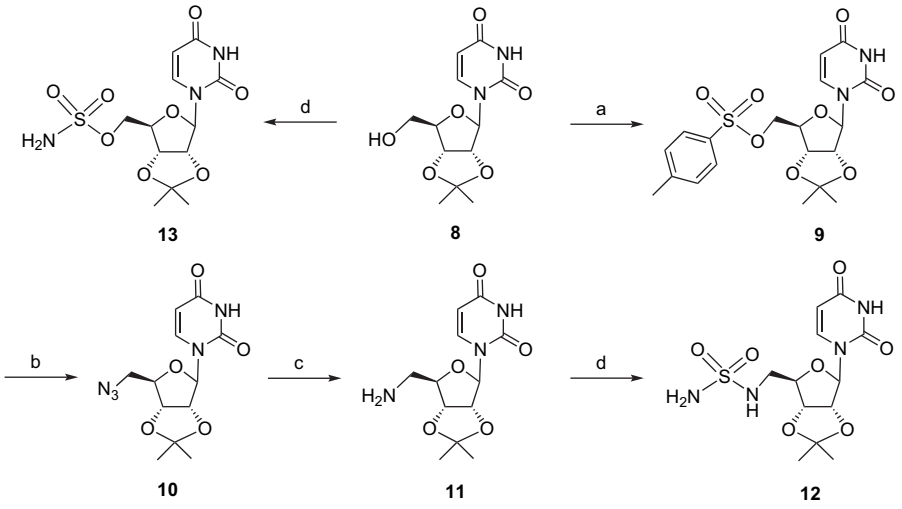


Scheme 2. Reaction conditions: (a) NaOMe, MeOH, rt, 4 h; (b) PhCHO, TFA, 0–25 °C, 3 h; (c) (*R*,*S*) 2-chloro propionic acid, NaH, dioxane, 50 °C, 16 h; (d) MeI, DMF, rt, 24 h; (e) $NaIO_4$, $RuCl_3$, H_2O/dioxane, rt, 4 h.

Acetyl groups were removed with sodium methoxide in absolute methanol at ambient temperature in quantitative yield. After the usual work up with Amberlyst® 15 ion exchange resin, the crude product was reprotected at the 4′- and 6′-hydroxy position with benzaldehyde and trifluoroacetic acid as catalyst.[14] This gave benzylidene derivative **5** with the 3′-hydroxy group conveniently unprotected and available for further synthesis.

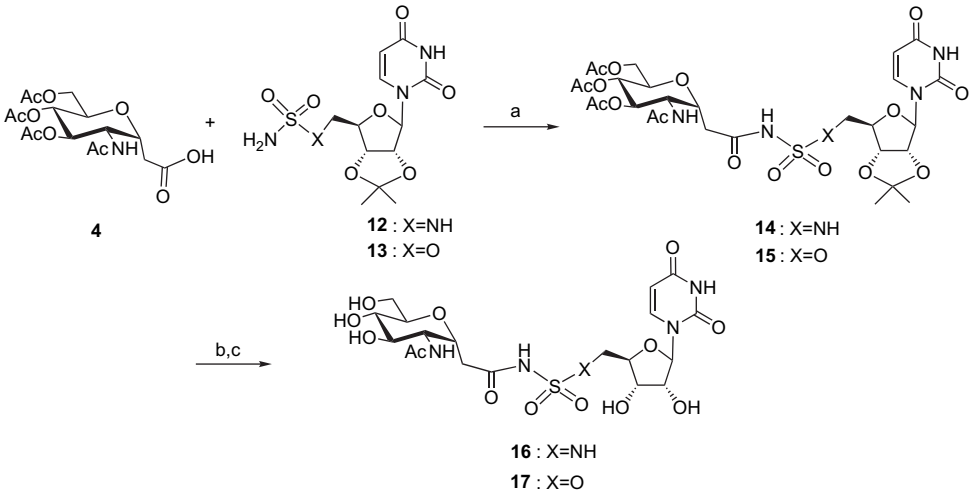
The muramic acid moiety was introduced to the free 3′-hydroxy group through the Williamson ether synthesis. Racemic (*R*,*S*)-2-chloropropionic acid was used as a reagent, since it was known that stereoselectivity could be achieved for this reaction.[23–25] As in the case of 2-acetamido-1-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucosamine,[25] the (*R*)-muramic acid derivative **6** was obtained from **5** in good yield with a diastereoisomeric ratio of 4.3:1. The ratio was determined from the benzylidene proton signal of the muramic and isomuramic derivatives mixture at 5.59 and 5.42 ppm, respectively. To ensure that the desired diastereoisomer with *R*-lactoyl configuration was the major reaction product, 6 equiv of sodium hydride was used as a base and 5 equiv of racemic (*R*,*S*)-2-chloropropionic acid, which was added quickly to the suspension. The obtained crude sodium salt was treated with methyl iodide in DMF at ambient temperature to give **6** as a diastereoisomeric mixture. The diastereoisomeric mixture was separated using flash chromatography under essentially the same chromatographic conditions as for the methyl ester of (*R*,*S*)-1-*O*-benzyl-4,6-*O*-benzylidene-*N*-acetyl-muramic acid described previously.[25] Carboxylic acid **7** was obtained in the same way as compound **4** in dioxane/water with the sodium periodate and ruthenium trichloride oxidation system, which left all the protecting groups intact.

The *N*- and *O*-sulfamoyluridine derivatives **12** and **13** were synthesized from commercially available 2′,3′-isopropylidene-uridine **8**. The 5′-*N*-sulfamoyl derivative **12** was obtained in four reaction steps (Scheme 3). The 5′-hydroxy group was first converted into a *p*-toluenesulfonyl ester **9** with *p*-toluenesulfonylchloride and pyridine as the catalyst/base. The following nucleophilic substitution with sodium azide gave azido derivative **10** in high yield. The ensuing catalytic hydrogenation proceeded to completion within 3 h, in complete contrast to the report that the azido derivative **10** was entirely resistant to reduction.[26] It was established that a dilute solution of the azido derivative **10** in methanol is required for fast catalytic hydrogenation. Compound **11** was found to be pure by ^{1}H NMR spectroscopy and was used without purification in the next reaction step due to its inherent instability. Treatment of **11** with sulfamoyl chloride[27] at 0 °C in dry dichloromethane, with triethylamine as the base, gave the desired 5′-*N*-sulfamoyl-2′,3′-*O*-isopropylidene-uridine **12** in relatively low yield. On the other hand, 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylidene-uridine **13** was synthesized in a straightforward direct sulfamoylation of 2′,3′-*O*-isopropylidene-uridine under reaction conditions similar to those used for **12**.

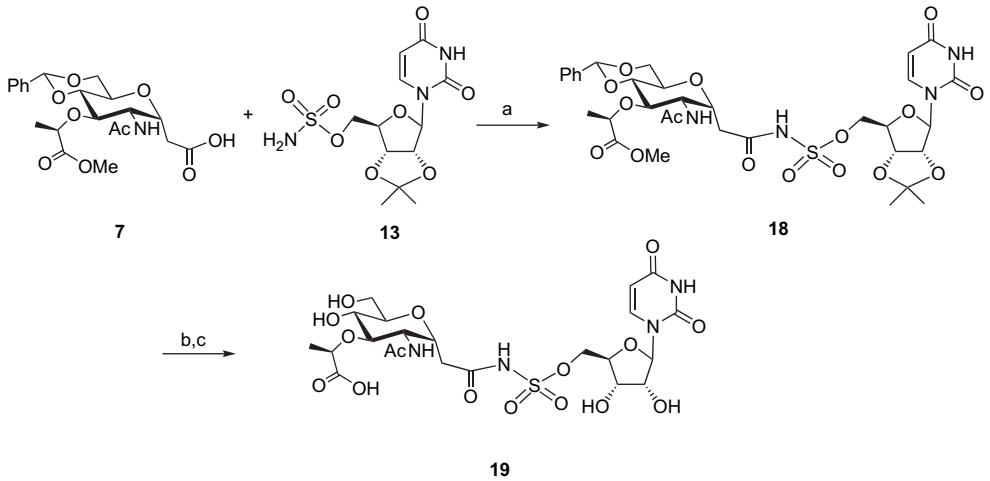


Scheme 3. Reaction conditions: (a) TsCl, pyridine, 40 °C, 3 h; (b) NaN_3, DMF, 50 °C, 16 h; (c) H_2, Pd/C, MeOH, rt, 3 h; (d) $ClSO_2NH_2$, TEA, CH_2Cl_2, 0–20 °C, 16 h.

The 2′,3′-*O*-isopropylidene uridine derivatives **12** and **13** were then coupled with the protected C-linked MurNAc acid **7** and GlcNAc **4** to give *N*-sulfamoyl compound **14** and *O*-sulfamoyl derivatives **15** and **18** (Schemes 4 and 5). The coupling reaction was tested with several coupling reagents, however, using equimolar amounts of carboxylic acid and *N*- or *O*-sulfamoyl-2′,3′-*O*-isopropylidene-uridine and small excesses of DCC and DMAP (1.2 equiv) gave the best yields in short reaction times at room



Scheme 4. Reaction conditions: (a) DCC, DMAP, CH_2Cl_2, RT, 24 h; (b) NaOMe, MeOH, rt, 4 h; (c) 70% TFA, rt, 1 h.



Scheme 5. Reaction conditions: (a) DCC, DMAP, CH_2Cl_2, rt, 24 h; (b) 70% TFA, rt, 1 h; (c) 1 M LiOH, rt, 2 h.

temperature. However, the use of DCC as the coupling agent made the isolation and purification difficult. Dicyclohexylurea had to be removed completely by precipitation and filtration prior to chromatographic purification. Only then could the products **14**, **15** and **18**, be isolated in a pure form without the co-elution of dicyclohexylurea.

The deprotection procedure for C-linked *N*-acetyl-glucosamine derivatives **14** and **15** (Scheme 4) proceeded smoothly. Acetyl groups were removed in the same manner as for compound **4** with sodium methoxide in dry methanol at room temperature. Then acidic hydrolysis of the isopropylidene protecting group was performed in 70% trifluoroacetic acid. The crude products were purified by gel filtration using Sephadex LH-20, which afforded pure compounds **16** and **17**.

In a similar manner the coupling reaction between the C-linked *N*-acetyl-muramic acid derivatives **7** and *O*-sulfamoyl-2′,3′-*O*-isopropylidene-uridine **13**, using DCC and DMAP, gave **18** (Scheme 5). The benzylidene and isopropylidene protecting groups were removed in 70% trifluoroacetic acid and then the ester group was hydrolyzed under basic conditions (1 M LiOH). The product **19** was isolated as the dilithium salt after pH adjustment with Amberlyst 15 and gel filtration on LH-20.

3. Conclusions

The synthesis of new 1-C-linked *O*- and *N*-sulfamoylamido analogues of UDP-GlcNAc and UDP-MurNAc is presented. The key compound **3** has been synthesized using the Keck radical allylation under microwave-accelerated conditions and converted into 1-C-linked-MurNAc and GlcNAc carboxylic acid derivatives. Coupling with protected *N*- and *O*-sulfamoyl uridine derivatives and the ensuing deprotection reactions gave new diphosphate analogues of UDP-GlcNAc and UDP-MurNAc that could provide starting points for further development of new inhibitors of the bacterial cell wall biosynthesis.

4. Experimental

4.1. General

Chemicals from Sigma–Aldrich and Fluka were used without further purification. Analytical TLC was performed on Merck silica gel (60 F_{254}) plates (0.25 mm) and components visualized with ultraviolet light and dyed with 20% sulfuric acid in ethanol, rhodamine G6, 2,4-dinitrophenylhydrazine and ninhydrin. Flash chromatography was performed using Merck silica gel (0.040–0.063 mm). Gel filtration was performed using LH-20 stationary phase and methanol as eluent. ^{1}H, ^{13}C, DEPT-135, gradient COSY and gradient HSQC NMR spectra were recorded on a Bruker AM250 and AVANCE DPX300 spectrometers in $CDCl_3$, DMSO-d_6, D_2O, MeOH-d_4 and acetone-d_6 solution with TMS as the internal standard. Microanalyses were performed on a Perkin–Elmer C, H, N analyzer 240C. Mass spectra were obtained using a VG-Analytical Autospec Q and Q-TOF Premier mass spectrometers.

4.2. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (2)

Compound **2** was synthesized according to Ref. 14 (α/β=4:1).

α-Anomer: ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 6.19 (d, J=3.7 Hz, 1H, H-1_g), 5.89 (d, J=8.7 Hz, 1H, NH), 5.33 (t, J=8.9 Hz, 1H, H-3_g), 5.20 (t, J=9.7 Hz, 1H, H-4_g), 4.58–4.50 (m, 1H, H-5_g), 4.30–4.26 (m, 2H, H-2_g, H-6_g), 4.14 (d, J=10.4 Hz, 1H, H-6′$_g$), 2.10 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 1.99 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ (ppm) 171.76, 170.96, 170.70, 169.49, 94.17, 71.18, 70.36, 68.03, 61.54, 53.64, 23.26, 21.02, 20.92, 20.80. LRMS (ESI), m/z 330.1 $(M-Cl)^+$.

4.3. 3-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl) propene (3)

4.3.1. Method A (classical heating)

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (1.2 g, 3.3 mmol) and AIBN (170 mg, 1.1 mmol) were suspended in allyltributyltin (3.86 mL, 12.6 mmol) and distilled THF (7.0 mL). The reaction mixture was flushed with argon for 10 min and then stirred at 70 °C for 7 h after which TLC analysis indicated the absence of starting material. The solvent was evaporated under reduced pressure and the oily residue dissolved in acetonitrile (50 mL) and extracted with pentane (5×30 mL). Flash chromatography (dichloromethane/acetone 10:1) afforded a colourless waxy solid, which crystallized overnight giving a colourless solid (0.60 g, 1.5 mmol, 49% yield).

4.3.2. Method B (microwave-accelerated)

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (365 mg, 1.0 mmol) and AIBN (25 mg, 0.15 mmol) were suspended in allyltributyltin (1.0 mL, 3.0 mmol) and distilled THF (2.0 mL). The reaction mixture was flushed with argon for 10 min and then stirred in a microwave reactor at 100 °C for 10 min. The power was set at 10 W. The reactor was cooled with compressed air during the reaction to reduce temperature fluctuations. The solvent was evaporated under reduced pressure and the oily residue dissolved in acetonitrile (20 mL) and extracted with pentane (5×15 mL). Flash chromatography (dichloromethane/acetone 10:1) afforded a colourless waxy solid, which crystallized overnight giving colourless solid (185 mg, 0.51 mmol, 51% yield). The ^{1}H NMR spectra are identical using method A or B. Mp 104–105 °C. IR (KBr, cm^{-1}): 3420, 2944, 1740, 1667, 1515, 1431, 1386, 1244, 1152, 1093, 1035, 988, 936, 924, 814, 668, 608. $[\alpha]_D^{20}$ +95.1 (c 0.20, DMF). ^{1}H NMR ($CDCl_3$, 300 MHz) α-anomer: δ (ppm) 6.23 (d, 1H, J=8.2 Hz, NH), 5.85–5.72 (m, 1H, CH), 5.18–5.10 (m, 2H, CH_2), 5.06 (t, 1H, J=7.8 Hz, H-3_g), 4.97 (t, 1H, J=6.8 Hz, H-4_g), 4.36–4.22 (m, 3H, H-2_g, H-6_g H-1_g), 4,13 (dd, 1H, J_{gem}=12.0 Hz, J=3.7 Hz, H-6′$_g$), 3.94–3.88 (dt, 1H, J=3.7, 6.5 Hz, H-5_g), 2.49–2.38 (m, 1H, CH_a), 2.34–2.25 (m, 1H, CH_b), 2.11 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 1.99 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ (ppm) 171.10, 170.94, 170.26, 169.47, 133.78, 117.90, 71.57, 70.59, 70.38, 68.57, 62.12, 50.77, 32.13, 23.34, 21.08, 20.99. LRMS (ESI), m/z 372.2 $(M+H)^+$, 394.2 $(M+Na)^+$. HRMS (ESI), m/z calcd for $C_{17}H_{26}NO_8$ 372.1658 $(M+H)^+$, found 372.1665.

4.4. 2-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)acetic acid (4)

3-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl) propene (210 mg, 0.54 mmol) was dissolved in water (20 mL) and dioxane (20 mL). While being stirred vigorously, sodium periodate (460 mg, 2.15 mmol) and ruthenium(III) chloride (6 mg, 0.03 mmol) were added. The same amount of sodium periodate was added again after 1 h. The reaction was complete in 4 h and the solvents were evaporated in vacuo. The dark green solid was resuspended in ethyl acetate and filtered. The crude product obtained after evaporation of ethyl acetate was purified with flash chromatography (dichloromethane/methanol 15:1 to 8:1). Colourless oil (157 mg, 0.40 mmol, 74% yield), which slowly solidified was obtained. Mp 148–150 °C. IR (KBr, cm^{-1}): 3547, 2939, 2363, 1748, 1558, 1374, 1239, 1139, 1088, 1043, 913, 602. $[\alpha]_D^{20}$ +50.2 (c 0.16, MeOH). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 6.53 (d, 1H, J=8.4 Hz, NH), 5.05 (t, 1H, J=6.8 Hz, H-3_g), 4.95 (t, 1H, J=6.0 Hz, H-4_g), 4.62 (m, 1H, H-1_g), 4.39–4.28 (m, 2H, H-2_g, H-6_g), 4.22 (dd, 1H, J=11.9, 4.2 Hz, H-6′$_g$), 4.00 (dd, 1H, J=10.4, 5.6 Hz, H-5_g), 2.62 (app t, 2H, J=6.4 Hz, CH_2), 2.10 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.00 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 173.95, 171.46, 171.31, 170.84, 169.47,

72.04, 69.67, 68.60, 67.88, 61.70, 49.83, 34.76, 23.21, 21.24, 21.21, 21.13. LRMS (ESI), m/z=390.1 $(M+H)^+$. HRMS (ESI), m/z calcd for $C_{16}H_{24}NO_{10}$ 390.1400 $(M+H)^+$, found 390.1409. Microanalysis calcd for $C_{16}H_{23}NO_{10}\times H_2O$ (%): C, 47.17; H, 6.19; N, 3.44. Found: C, 47.38; H, 6.36; N, 3.33.

4.5. 3-(2-Acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl) propene (5)

Compound **5** was synthesized according to Ref. 14.

3-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl) propene (200 mg, 0.54 mmol) was dissolved in dry methanol (2.0 mL). Under an argon atmosphere 30% sodium methoxide (100 μL) was added and the reaction mixture stirred at ambient temperature. After 2 h, the reaction was judged complete by TLC and the solution was neutralized using Amberlyst IR-15 ion exchange resin and concentrated to dryness under reduced pressure to obtain colourless oil. 3-(2-Acetamido-2-deoxy-α-D-glucopyranosyl) propene was judged pure by ^{1}H and ^{13}C NMR spectroscopy and was dissolved in benzaldehyde (1.8 mL) and cooled to 0 °C under argon. Anhydrous trifluoroacetic acid (100 μL) was then added dropwise and the temperature was raised to ambient. After 3 h, the reaction mixture was evaporated to dryness under reduced pressure, which gave a colourless solid (168 mg, 0.50 mmol, 92% yield). Mp >240 °C. IR (KBr, cm^{-1}): 3286, 1996, 1630, 1542, 1375, 1136, 1094, 1039, 993, 757, 697. $[\alpha]_D^{20}$ +64.1 (*c* 0.06, DMF). ^{1}H NMR (DMSO-d_6, 250 MHz): δ (ppm) 7.95 (d, 1H, *J*=7.27 Hz, NH), 7.47–7.36 (m, 5H, Ph–H), 5.81–5.65 (m, 1H, CH), 5.60 (s, 1H, CH–Ph), 5.13 (d, 1H, *J*=17 Hz, OH), 5.02 (d, 2H, *J*=10.2 Hz, CH_2), 4.06–3.84 (m, 3H, H-3_g, H-4_g, H-1_g), 3.75–3.61 (m, 2H, H-6_g, H-2_g), 3.55–3.40 (m, 2H, H-$6'_g$, H-5_g), 2.55–2.41 (m, 1H, CH_a), 2.24–2.14 (m, 1H, CH_b), 1.88 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 62.9 MHz): δ (ppm) 170.31, 138.70, 136.27, 129.75, 129.72, 129.45, 128.88, 127.29, 117.48, 101.70, 83.83, 75.31, 69.72, 68.00, 64.64, 55.50, 31.19, 24.01. LRMS (CI), m/z 333 $(M)^+$, (ESI) m/z 334.2 $(M+H)^+$. HRMS (ESI), m/z calcd for $C_{18}H_{24}NO_5$ 334.1654 $(M+H)^+$, found 334.1650.

4.6. 3-(2-Acetamido-4,6-*O*-benzylidene-2-deoxy-3-((*R*)-1-(methoxycarbonyl)ethoxy)-α-D-glucopyranosyl) propene (6)

3-(2-Acetamido-4,6-benzylidene-2-deoxy-α-D-glucopyranosyl) propene (420 mg, 1.26 mmol) was suspended in dioxane (40 mL) stored over molecular sieves. While being stirred under an argon atmosphere, sodium hydride (544 mg, 23 mmol) was added carefully to the suspension and the temperature raised to 50 °C. After 0.5 h, the reaction mixture was cooled to ambient temperature and 2-chloropropionic acid (580 μL, 6.3 mmol) was added dropwise to the suspension. The reaction mixture was heated at 50 °C for 4 h at which time TLC analysis indicated total absence of starting material. Water (1.0 mL) was added carefully at ambient temperature to quench the excess sodium hydride and the reaction mixture was evaporated to dryness under reduced pressure. The crude product was suspended in brine (30 mL), cooled on ice for 1 h and the precipitated solids filtered off. After extensive drying in vacuo for 24 h the sodium salt was dissolved in DMF (15 mL) and methyl iodide (400 μL, 6.4 mmol) was added in one portion at room temperature. The reaction mixture was stirred for 24 h. The solvents were evaporated and the crude product purified by flash chromatography (dichloromethane/hexane/ethyl acetate 1:1:1) yielding a colourless powder (253 mg, 0.60 mmol, 48% yield). Mp 209–210 °C. IR (KBr, cm^{-1}): 3311, 3083, 2948, 2873, 1758, 1736, 1651, 1553, 1450, 1372, 1341, 1316, 1211, 1174, 1132, 1094, 996, 922, 752, 697, 648. $[\alpha]_D^{20}$ +128.9 (*c* 0.10, DMF). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.86 (br s, 1H, NH), 7.45–7.37 (m, 5H, Ph–H), 5.83–5.69 (m, 1H, CH), 5.59 (s, 1H, CH–Ph), 5.09 (dd, 1H, *J*=17.0, 1.5 Hz, CH_a), 5.07 (d, 1H, *J*=9.9 Hz, CH_b), 4.78–4.71 (m, 1H, H-4_g), 4.59 (q, 1H, *J*=7.0 Hz, CH), 4.22 (dd, 1H, *J*=10.2, 4.7 Hz, H-3_g), 3.99–3.93 (m, 1H, H-1_g), 3.77 (s, 3H, CH_3), 3.74–3.63 (m, 3H, H-6_g, H-2_g, H-$6'_g$), 3.60–3.51 (m, 1H, H-5_g), 2.39–2.33 (m, 2H, CH_2), 2.04 (s, 3H, CH_3), 1.45 (d, 3H, *J*=7.0 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 176.06, 171.24, 137.25, 134.15, 128.95, 128.29, 125.74, 116.98, 101.19, 83.80, 75.07, 74.89, 73.75, 69.25, 63.64, 54.11, 52.35, 30.16, 23.10, 18.67. LRMS (ESI), m/z 420.2 $(M+H)^+$, 442.2 $(M+Na)^+$. HRMS (ESI), m/z calcd for $C_{22}H_{30}NO_7$ 420.2022 $(M+H)^+$, found 420.2012. Microanalysis calcd for $C_{22}H_{29}NO_7$ (%): C, 62.99; H, 6.97; N, 3.34. Found: C, 63.09; H, 7.20; N, 3.36.

4.7. 2-(2-Acetamido-4,6-*O*-benzylidene-2-deoxy-3-((*R*)-1-(methoxycarbonyl)ethoxy)-α-D-glucopyranosyl)acetic acid (7)

3-(2-Acetamido-4,6-benzylidene-2-deoxy-3-((*R*)-1-(methoxycarbonyl)ethoxy)-α-D-glucopyranosyl) propene (100 mg, 0.24 mmol) was dissolved in water (20 mL) and dioxane (20 mL). Sodium periodate (440 mg, 2.06 mmol) and ruthenium(III) chloride (5 mg, 0.02 mmol) were then added consecutively and the dark solution was stirred overnight. The solution was concentrated to dryness under reduced pressure and the dark green solid resuspended in ethyl acetate and filtered off. Ethyl acetate was evaporated and the crude product applied to a flash chromatography column. The product was eluted by gradient elution (dichloromethane/methanol 15:1 to 5:1) yielding colourless solid (98 mg, 0.22 mmol, 93% yield). Mp 158–160 °C. IR (KBr, cm^{-1}): 3339, 2938, 1734, 1655, 1546, 1450, 1372, 1314, 1278, 1212, 1130, 1093, 1010, 914, 877, 749, 697. $[\alpha]_D^{20}$ +47.5 (*c* 0.05, MeOH). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.01 (d, 1H, *J*=3.2 Hz, NH), 7.46–7.36 (m, 5H, Ph–H), 5.58 (s, 1H, CH–Ph), 5.21–5.14 (m, 1H, H-1_g), 4.57 (q, 1H, *J*=7.0 Hz, CH), 4.28–4.25 (m, 1H, H-4_g), 4.07–3.98 (m, 1H, H-2_g), 3.78 (s, 3H, CH_3), 3.74–3.55 (m, 4H, H-3_g, H-5_g, H-6_g, H-$6'_g$), 2.66 (d, 1H, *J*=2.8 Hz, CH_a), 2.63 (br s, 1H, CH_b), 2.05 (s, 3H, CH_3), 1.45 (d, 3H, *J*=7.0 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 176.53, 172.78, 137.62, 129.43, 128.72, 126.26, 101.73, 83.79, 75.58, 72.40, 70.46, 69.49, 66.48, 62.79, 54.07, 52.92, 32.81, 23.32, 19.05. LRMS (ESI), m/z 438.2 $(M+H)^+$, 460.2 $(M+Na)^+$. HRMS (ESI), m/z calcd for $C_{21}H_{28}NO_9$ 438.1764 $(M+H)^+$, found 438.1754. Microanalysis calcd for $C_{21}H_{27}NO_9$ (%): C, 57.66; H, 6.22; N, 3.20. Found: C, 57.46; H, 6.12; N, 3.16.

4.8. 2′,3′-*O*-Isopropylidene-5′-O-*para*-toluenesulfonyl-uridine (9)

Compound **9** was synthesized according to Ref. 26.

Colourless solid. Mp 93–94 °C. IR (KBr, cm^{-1}): 3220, 1695, 1490, 1379, 1273, 1214, 1190, 1177, 1096, 977, 813, 663, 554. $[\alpha]_D^{20}$ +39.3 (*c* 0.09, DMF). ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 9.22 (s, 1H, NH), 7.69 (d, 2H, *J*=8.1 Hz, Ph–H), 7.26 (d, 2H, *J*=8.1 Hz, Ph–H), 7.17 (d, 1H, *J*=8.0 Hz, CH), 5.64 (d, 1H, *J*=8.0 Hz, CH), 5.57 (d, 1H, *J*=1.3 Hz, H-1_r), 4.86 (dd, 1H, *J*=6.4, 1.6 Hz, H-2_r), 4.72 (dd, 1H, *J*=6.2, 3.6 Hz, H-3_r), 4.30–4.19 (m, 3H, H-4_r, H-5_r), 2.37 (s, 3H, CH_3–Ph), 1.47 (s, 3H, CH_3), 1.26 (s, 3H, CH_3). LRMS (ESI), m/z 439.1 $(M+H)^+$, 461.1 $(M+Na)^+$, 477.1 $(M+K)^+$.

4.9. 5′-Azido-5′-deoxy-2′,3′-*O*-isopropylidene-uridine (10)

Compound **10** was synthesized according to Ref. 26.

Colourless oil. IR (KBr, cm^{-1}): 3227, 2990, 2106, 1694, 1543, 1459, 1383, 1262, 1214, 1158, 1094, 936, 861, 807, 716, 569, 510. $[\alpha]_D^{20}$ +68.5 (c 0.02, DMF). ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 8.77 (s, 1H, NH), 7.29 (d, 1H, *J*=8.0 Hz, CH), 5.77 (d, 1H, *J*=8.0 Hz, CH), 5.66 (d, 1H, *J*=2.0 Hz, H-1_r), 5.00 (dd, 1H, *J*=6.5, 2.0 Hz, H-2_r), 4.81 (dd, 1H, *J*=6.5, 4.2 Hz, H-3_r), 4.27–4.21 (m, 1H, H-4_r), 3.62 (d, 2H, *J*=5.2 Hz, H-5_r), 1.57 (s, 3H, CH_3), 1.36 (s, 3H, CH_3). LRMS (ESI), m/z=332.1 $(M+Na)^+$.

4.10. 5′-Amino-5′-deoxy-2′,3′-*O*-isopropylidene-uridine (11)

5′-Azido-5′-deoxy-2′,3′-*O*-isopropylidene-uridine (650 mg, 2.1 mmol) was dissolved in methanol (150 mL) and argon was

bubbled through the solution for 10 min. Pd/C (50 mg, 10%) was added and the suspension stirred under hydrogen. After 3 h, TLC indicated the disappearance of the starting azide and the Pd/C was filtered off. The solvent was evaporated under reduced pressure at not more than 40 °C and the colourless solid (585 mg, 2.07 mmol, 98%) dried in vacuo. ^{1}H NMR spectroscopy indicated a high degree of purity so the product was used without purification for further synthesis. Mp 85–87 °C. $[\alpha]_D^{20}$ −22.16 (*c* 0.16, MeOH). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 7.83 (d, 1H, *J*=8.0 Hz, CH), 5.78 (d, 1H, *J*=2.8 Hz, H-1$_r$), 5.63 (d, 1H, *J*=8.0 Hz, CH), 4.94 (dd, 1H, *J*=6.5, 2.8 Hz, H-2$_r$), 4.74 (dd, 1H, *J*=6.5, 3.9 Hz, H-3$_r$), 3.95 (m, 1H, H-4$_r$), 3.17 (br s, 2H, NH_2), 2.77 (d, 2H, *J*=5.5 Hz, H-5$_r$), 1.48 (s, 3H, CH_3), 1.29 (s, 3H, CH_3). LRMS (ESI), *m*/*z* 284 $(M+H)^+$. HRMS (ESI), *m*/*z* calcd for $C_{12}H_{18}N_3O_5$ 284.1246 $(M+H)^+$, found 284.1249.

4.11. 2′,3′-*O*-Isopropylidene-5′-*N*-sulfamoyluridine (12)

5′-Amino-5′-deoxy-2′,3′-*O*-isopropylidene-uridine (100 mg, 0.35 mmol) was dissolved in dry dichloromethane (8 mL) and triethylamine (180 μL). Under an argon atmosphere, the solution was cooled to 0 °C in an ice bath. Sulfamoyl chloride (65 mg, 0.56 mmol) was added to the solution. The temperature was then raised to ambient temperature. After being stirred for 5 h, MeOH was used to quench the reaction mixture, which was concentrated to dryness under reduced pressure. Flash chromatography afforded a colourless oil (36 mg, 0.10 mmol, 29%). Mp 110–112 °C. IR (KBr, cm^{-1}): 1690, 1460, 1386, 1334, 1273, 1215, 1159, 1089, 860, 816, 550. $[\alpha]_D^{20}$ −3.3 (*c* 0.08, DMF). ^{1}H NMR (CD_3CN, 250 MHz): δ (ppm) 9.35 (br s, 1H, NH), 7.49 (d, 1H, *J*=8.0 Hz, CH), 5.69 (d, 1H, *J*=3.0 Hz, H-1$_r$), 5.67 (d, 1H, *J*=8.0 Hz, CH), 5.55 (t, 1H, *J*=6.2 Hz, NH), 5.35 (br s, 2H, NH_2), 5.04 (dd, 1H, *J*=6.6, 2.4 Hz, H-2$_r$), 4.84 (dd, 1H, *J*=6.6, 4.2 Hz, H-3$_r$), 4.25–4.19 (m, 1H, H-4$_r$), 3.35–3.29 (m, 2H, H-5$_r$), 1.55 (s, 3H, CH_3), 1.35 (s, 3H, CH_3). LRMS (ESI), *m*/*z* 385.1 $(M+Na)^+$, 413.3 $(M+K)^+$. HRMS (ESI), *m*/*z* calcd for $C_{12}H_{18}N_4O_7SNa$ 385.0794 $(M+Na)^+$, found 385.0814.

4.12. 2′,3′-*O*-Isopropylidene-5′-*O*-sulfamoyluridine (13)

2′,3′-*O*-Isopropylidene-uridine (300 mg, 1.06 mmol) was suspended in dichloromethane (20 mL). After the addition of triethylamine (1.0 mL), the solution was cooled to 0 °C. Sulfamoyl chloride (150 mg, 1.3 mmol) was added to the stirred reaction mixture. After 1 h another portion of sulfamoyl chloride (600 mg, 1.3 mmol) was added and the reaction mixture allowed to warm to ambient temperature. After stirring overnight, methanol (0.5 mL) was added and the reaction mixture filtered through a pad of Celite 545 and evaporated to dryness. The crude product was purified by flash chromatography (ethyl acetate/methanol 20:1), which afforded a colourless solid (220 mg, 0.61 mmol, 57% yield). Mp 90–92 °C. IR (KBr, cm^{-1}): 1686, 1560, 1465, 1380, 1274, 1216, 1183, 1070, 992, 932, 814, 668, 553. $[\alpha]_D^{20}$ −4.0 (*c* 0.14, DMF). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 11.43, (s, 1H, NH), 7.70 (d, 1H, *J*=8.0 Hz, CH), 7.59 (br s, 2H, NH_2), 5.82 (d, 1H, *J*=1.9 Hz, H-1$_r$), 5.64 (d, 1H, *J*=8.0 Hz, CH), 5.07 (dd, 1H, *J*=6.4, 1.9 Hz, H-2$_r$), 4.80 (dd, 1H, *J*=6.4, 3.7 Hz, H-3$_r$), 4.26–4.21 (m, 2H, H-4$_r$ H-5), 4.15 (dd, 1H, *J*=11.4, 7.8 Hz, H-5′), 1.50 (s, 3H, CH_3), 1.31 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm) 163.18, 150.26, 142.95, 113.38, 101.86, 92.81, 84.19, 83.50, 80.78, 68.37, 26.90, 25.11. LRMS (ESI), *m*/*z* 364 $(M+H)^+$. HRMS (ESI), *m*/*z* calcd for $C_{12}H_{18}N_3O_8S$ 364.0815 $(M+H)^+$, found 364.0828.

4.13. 2′,3′-*O*-Isopropylidene-5′-*N*-(2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)acetamidosulfamoyl)uridine (14)

2′,3′-*O*-Isopropylidene-5′-*N*-sulfamoyluridine (47 mg, 0.13 mmol), 2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-acetic acid (50 mg, 0.13 mmol), dicyclohexylcarbodiimide (32 mg, 0.15 mmol) and 4-dimethylaminopyridine (20 mg, 0.15 mmol) were suspended in dry dichloromethane (15 mL). The suspension dissolved in 15 min and precipitate started forming after 1 h. The reaction mixture was left stirring overnight and then concentrated to 3 mL and the precipitate filtered off and washed with the minimal volume of dichloromethane. The solution was concentrated to dryness. Flash chromatography (dichloromethane/methanol 10:1–7:1) afforded a colourless solid (49 mg, 0.07 mmol, 51% yield). Mp 148–150 °C. IR (KBr, cm^{-1}): 1746, 1692, 1647, 1561, 1458, 1378, 1236, 1150, 1043, 824, 728, 562. $[\alpha]_D^{20}$ +69.8 (*c* 0.04, DMF). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 10.04 (br s, 1H, NH), 7.32 (d, 1H, *J*=7.9 Hz, CH), 6.76 (br s, 1H, NH), 6.36 (br s, 1H, NH), 5.77 (d, 1H, *J*=7.9 Hz, CH), 5.47 (app s, 1H, H-1$_r$), 5.15–4.96 (m, 4H, H-3$_g$, H-4$_g$, H-2$_r$, H-3$_r$), 4.69–4.61 (m, 1H, H-1$_g$), 4.38–4.21 (m, 6H, H-2$_g$, H-6$_g$, H-6′$_g$, H-4$_r$, H-5$_r$, H-5′$_r$), 4.15–4.08 (m, 1H, H-5$_g$), 2.79–2.58 (m, 2H, CH_2), 2.11 (br s, 9H, 3×CH_3), 2.04 (s, 3H, CH_3), 1.56 (s, 3H, CH_3), 1.36 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 171.79, 171.07, 170.55, 169.57, 169.28, 163.96, 150.61, 144.02, 114.45, 102.67, 85.64, 83.79, 81.55, 77.20, 71.38, 71.34, 69.39, 68.66, 67.90, 61.60, 50.64, 35.48, 27.00, 25.15, 22.94, 20.82, 20.72. LRMS (ESI), *m*/*z* 734.2 $(M+H)^+$. HRMS (ESI), *m*/*z* calcd for $C_{28}H_{40}N_5O_{16}S$ 734.2191 $(M+H)^+$, found 734.2211.

4.14. 5′-*N*-(2-(2-Acetamido-2-deoxy-α-D-glucopyranosyl)acetamidosulfamoyl)uridine (16)

2′,3′-*O*-Isopropylidene-5′-*O*-(2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)acetamidosulfamoyl) uridine (37 mg, 0.050 mmol) was dissolved in dry methanol (2.0 mL) and flushed with argon. Sodium methoxide solution in methanol (150 μL, 30%) was added dropwise at ambient temperature. After being stirred for 4 h, the reaction mixture was diluted with methanol (5 mL) and Amberlyst 15 ion exchange resin was added to adjust the pH of the solution to 7. The resin was filtered off and methanol evaporated under reduced pressure. The obtained white solid was dissolved in bidistilled water (800 μL) and trifluoroacetic acid (1850 μL) and stirred at ambient temperature. After 1 h, the solvents were evaporated under reduced pressure and at temperature not exceeding 30 °C. Traces of water were removed by toluene co-evaporation under reduced pressure, which gave a brownish solid. The crude product was purified on a Sephadex LH-20 gel filtration column with methanol as mobile phase. The fractions containing the product were pooled and evaporated under reduced pressure to afford a colourless solid (17 mg, 0.030 mmol, 60% yield). Mp 158–159 °C. IR (KBr, cm^{-1}): 2930, 2345, 1686, 1467, 1441, 1273, 1160. $[\alpha]_D^{20}$ +80.0 (*c* 0.04, MeOH). ^{1}H NMR (MeOH-d_4, 300 MHz): δ (ppm) 7.72 (d, 1H, *J*=8.1 Hz, CH), 5.81 (d, 1H, *J*=4.8 Hz, H-1$_r$), 5.75 (d, 1H, *J*=8.1 Hz, CH), 4.64–4.57 (m, 1H, H-1$_g$), 4.25 (t, 1H, *J*=5.2 Hz, H-2$_r$), 4.11 (t, 1H, *J*=5.4 Hz, H-3$_r$), 4.06–3.98 (m, 2H, H-4$_r$, H-2$_g$), 3.85–3.73 (m, 2H, H-6$_g$, H-6′$_g$), 3.63–3.58 (m, 2H, H-3$_g$, H-5$_g$), 3.45–3.29 (m, 3H, H-5$_r$, H-5′$_r$, H-4$_g$), 2.68 (dd, 1H, *J*=15.3, 9.9 Hz, CH_a), 2.49 (dd, 1H, *J*=15.3, 4.1 Hz, CH_b), 2.01 (s, 3H, CH_3). ^{13}C NMR (MeOH-d_4, 75 MHz): δ (ppm) 173.50, 171.39, 165.95, 152.22, 143.12, 102.83, 91.85, 83.58, 76.51, 74.40, 71.92, 71.72, 71.68, 70.65, 62.19, 53.97, 45.93, 42.38, 35.59, 22.61. LRMS (ESI), *m*/*z* 590.1 $(M+Na)^+$. HRMS (ESI), *m*/*z* calcd for $C_{19}H_{29}N_5O_{13}SNa$ 590.1380 $(M+Na)^+$, found 590.1390. HPLC: Column C_{18} Phenomex Luna 10 μ; mobile phase: 20% acetonitrile, 80% trifluoroacetic acid (0.1%), flow rate 1.0 mL/min; injection volume: 10 μL; retention time: 2.46 min (96.47% at 220 nm, 97.38% at 254 nm).

4.15. 4-Dimethylaminopyridine salt of 2′,3′-*O*-isopropylidene-5′-*O*-(2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)acetamidosulfamoyl)uridine (15)

2′,3′-*O*-Isopropylidene-5′-*O*-sulfamoyluridine (100 mg, 0.27 mmol), 2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-

acetic acid (90 mg, 0.23 mmol), dicyclohexylcarbodiimide (74 mg, 0.36 mmol) and 4-dimethylaminopyridine (44 mg, 0.36 mmol) were suspended in dry dichloromethane (20 mL). The suspension dissolved in 15 min and a precipitate started forming after 0.5 h. The reaction mixture was left stirring overnight. The solvent was evaporated to concentrate the mixture to 3 mL. Then the precipitate was filtered off and washed with a minimal volume of dichloromethane. The solution was concentrated to dryness. Gradient flash chromatography (dichloromethane/methanol 10:1 to 7:1) afforded colourless solid (98 mg, 0.13 mmol, 57% yield). Mp 124–126 °C. IR (KBr, cm^{-1}): 1747, 1692, 1648, 1561, 1458, 1378, 1236, 1151, 1043, 824, 728, 562. $[\alpha]_D^{20}$ +38.6 (*c* 0.03, MeOH). ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.20 (d, 2H, *J*=7.5 Hz, CH–Ar), 7.45 (d, 1H, *J*=8.0 Hz, CH), 6.69 (d, 2H, *J*=7.5 Hz, CH–Ar), 6.64 (d, 1H, *J*=8.5 Hz, NH), 5.68 (d, 1H, *J*=8.0 Hz, CH), 5.64 (app s, 1H, H-1_r), 5.10 (t, 1H, *J*=7.5 Hz, H-3_g), 5.06–4.95 (m, 3H, H-2_r, H-3_r, H-4_g), 4.67–4.61 (m, 1H, H-1_g), 4.35–4.15 (m, 6H, H-4_r, H-5_r, H-$5'_r$, H-2_g, H-6_g, H-$6'_g$), 4.01 (m, 1H, H-5_g), 3.20 (s, 6H, CH_3), 2.71–2.67 (m, 2H, CH_2), 2.06 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 1.96 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 1.28 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 178.88, 170.80, 170.71, 169.30, 163.50, 156.93, 150.56, 142.63, 141.11, 114.06, 106.57, 102.55, 94.59, 84.99, 84.39, 80.52, 70.78, 70.18, 68.03, 67.78, 62.04, 50.18, 39.94, 39.19, 27.12, 25.13, 23.03, 20.83, 20.75, 20.72. LRMS (ESI), *m*/*z* 735.2 $(M+H)^+$. HRMS (ESI), *m*/*z* calcd for $C_{28}H_{39}N_4O_{17}S$ 735.2031 $(M+H)^+$, found 735.2061. HPLC: Column C_{18} Phenomex Luna 10 μ; mobile phase: 50% acetonitrile, 50% trifluroacetic acid (0.1%), flow rate 1.0 mL/min; injection volume: 10 μL; retention time: 3.36 min (95.98% at 220 nm, 98.86% at 254 nm).

4.16. 5′-*O*-(2-(2-Acetamido-2-deoxy-α-D-glucopyranosyl)acetamidosulfamoyl)uridine (17)

2′,3′-*O*-Isopropylidene-5′-*O*-(2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)acetamidosulfamoyl) uridine (65 mg, 0.088 mmol) was dissolved in dry methanol (4.0 mL) and flushed with argon. Sodium methoxide solution in methanol (300 μL, 30%) was added dropwise at ambient temperature. After being stirred for 4 h, the reaction mixture was diluted with methanol (10 mL) and Amberlyst 15 ion exchange resin was added to adjust the pH of the solution to 7. The resin was filtered off and methanol evaporated under reduced pressure. The obtained white solid was dissolved in bidistilled water (800 μL) and trifluoroacetic acid (1.85 mL) and stirred at ambient temperature. After 1 h, the solvents were evaporated under reduced pressure and at temperature not exceeding 30 °C. Traces of water were removed by toluene co-evaporation under reduced pressure, which gave a gummy white solid. The crude product was purified using Sephadex LH-20 gel filtration column and methanol as mobile phase. The fractions containing the product were pooled and evaporated under reduced pressure to afford a colourless solid (35 mg, 0.062 mmol, 70% yield). Mp 193–196 °C. IR (KBr, cm^{-1}): 2929, 2345, 1686, 1547, 1467, 1390, 1273, 1220, 814, 766. $[\alpha]_D^{20}$ +44.5 (*c* 0.08, MeOH). ^1H NMR (D_2O, 300 MHz): δ (ppm) 7.53 (d, 1H, *J*=8.1 Hz, CH), 5.77 (d, 1H, *J*=8.1 Hz, CH), 5.73 (d, 1H, *J*=4.2 Hz, H-1_r), 4.51–4.40 (m, 3H, H-1_g, H-5_r, H-$5'_r$), 4.20–4.11 (m, 3H, H-2_r, H-3_r, H-4_r), 3.84 (dd, 1H, *J*=5.9, 10.6 Hz, H-2_g), 3.62–3.51 (m, 3H, H-6_g, H-3_g, H-$6'_g$), 3.45–3.39 (m, 1H, H-5_g), 3.31 (m, 1H, H-4_g), 2.68 (dd, 1H, *J*=9.3, 15.6 Hz, CH_a), 2.56 (dd, 1H, *J*=5.1, 15.6 Hz, CH_b), 1.86 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 175.02, 171.17, 166.38, 151.86, 142.04, 102.79, 89.95, 80.99, 74.26, 73.40, 71.73, 70.73, 70.58, 70.50, 69.37, 61.02, 53.15, 34.32, 22.16. LRMS (ESI), *m*/*z* 569.1 $(M+H)^+$. HRMS (ESI), *m*/*z* calcd for $C_{19}H_{29}N_4O_{14}S$ 569.1401 $(M+H)^+$, found 569.1407. HPLC: Column C_{18} Phenomex Luna 10 μ; mobile phase: 15% acetonitrile, 85% trifluoroacetic acid (0.1%), flow rate 1.0 mL/min; injection volume: 10 μL; retention time: 2.98 min (98.84% at 254 nm).

4.17. 2′,3′-*O*-Isopropylidene-5′-*O*-(2-(2-acetamido-4,6-benzylidene-2-deoxy-3-((*R*)-1-(methoxycarbonyl)ethoxy)-α-D-glucopyranosyl)acetamidosulfamoyl)uridine (18)

2-(2-Acetamido-4,6-benzylidene-2-deoxy-3-((*R*)-1-(methoxycarbonyl)ethoxy)-α-D-glucopyranosyl)acetic acid (50 mg, 0.11 mmol), 2′,3′-*O*-isopropylidene-5′-*O*-sulfamoyluridine (42 mg, 0.11 mmol), dicyclohexylcarbodiimide (26 mg, 0.12 mmol) and 4-dimethylaminopyridine (15 mg, 0.12 mmol) were suspended in dry dichloromethane (10 mL). The suspension dissolved in 15 min and a precipitate started forming after 1 h. The reaction mixture was left stirring overnight and concentrated to 2 mL and the precipitate filtered off and washed with a minimal volume of dichloromethane. The solution was concentrated to dryness. Gradient flash chromatography (dichloromethane/methanol 10:1 to 7:1) afforded colourless solid (60 mg, 0.077 mmol, 70% yield). Mp 193–194 °C. IR (KBr, cm^{-1}): 1691, 1571, 1460, 1380, 1277, 1145, 1092, 1001, 832, 760, 698. $[\alpha]_D^{20}$ +49.3 (*c* 0.16, MeOH). ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 10.13 (br s, 1H, NH), 8.31 (s, 1H, NH), 7.48 (d, 2H, *J*=7.6 Hz, CH), 7.42–7.33 (m, 5H, Ph–H), 5.83 (d, 1H, *J*=7.6 Hz, CH), 5.74 (app s, 1H, H-1_r), 5.49 (s, 1H, CH–Ph), 5.20–5.19 (m, 1H, H-1_g), 5.00–4.91 (m, 2H, H-2_r, H-3_r), 4.51 (q, 1H, *J*=7.0 Hz, CH), 4.38–4.21 (m, 4H, H-4_g, H-4_r, H-5_r, H-$5'_r$), 3.91–3.88 (m, 1H, H-2_g), 3.76 (s, 3H, CH_3), 3.70–3.51 (m, 4H, H-3_g, H-5_g, H-6_g, H-$6'_g$), 2.65–2.43 (m, 2H, CH_2), 2.08 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 1.40 (d, 3H, *J*=7.0 Hz, CH_3), 1.32 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 176.03, 173.02, 172.95, 164.35, 157.19, 150.40, 140.18, 137.39, 128.87, 128.20, 125.93, 114.13, 106.52, 102.58, 101.24, 84.33, 83.34, 80.61, 77.20, 75.21, 72.72, 68.95, 68.22, 64.59, 53.96, 52.50, 39.99, 27.14, 25.27, 23.08, 18.62. LRMS (ESI), *m*/*z* 783.2 $(M+H)^+$, 805.2 $(M+Na)^+$. HRMS (ESI), *m*/*z* calcd for $C_{33}H_{43}N_4O_{16}S$ 783.2395 $(M+H)^+$, found 734.2418.

4.18. (*R*)-2-Methyl-2-(3-(2-acetamido-1-(5′-*O*-acetamidosulfamoyluridine))-2-deoxy-α-D-glucopyranosyl) acetic acid (19)

2′,3′-*O*-Isopropylidene-5′-*O*-(2-(2-acetamido-4,6-benzylidene-2-deoxy-3-((*R*)-1-(methoxycarbonyl)ethoxy)-α-D-glucopyranosyl)-acetamidosulfamoyl)uridine (20 mg, 0.026 mmol) was suspended in water (0.5 mL) and trifluoroacetic acid (1.5 mL) at 0 °C. The suspension dissolved immediately upon the addition of the trifluoroacetic acid and stirred for 1 h. The solvents were evaporated under reduced pressure using a rotary evaporator at temperature not exceeding 30 °C. Water and benzaldehyde traces were removed with toluene co-evaporation (2×5 mL), affording a colourless oil, which was dried for several hours in vacuo. Lithium hydroxide (1 M, 1.0 mL) and methanol (1.0 mL) were added at room temperature and the solution was stirred for 2 h. The solution was diluted with methanol (2 mL) and the pH adjusted to 7 with Amberlyst® 15 ion exchange resin. After filtration, the solvent was evaporated under reduced pressure. The crude product was purified using Sephadex LH-20 gel filtration column (flow rate 5 mL/h) with methanol as mobile phase. The fractions containing the product were pooled and evaporated under reduced pressure to afford a colourless solid (16 mg, 0.024 mmol, 94% yield). Mp 211–213 °C.IR (KBr, cm^{-1}): 3422, 2390, 1687, 1459, 1209, 1145, 849. $[\alpha]_D^{20}$ +38.5 (*c* 0.06, MeOH). ^1H NMR (MeOH-d_4, 300 MHz): δ (ppm) 7.93 (d, 1H, *J*=8.1 Hz, CH), 5.95 (d, 1H, *J*=4.9 Hz, H-1_r), 5.78 (d, 1H, *J*=8.1 Hz, CH), 4.92–4.84 (m, 1H, H-1_g), 4.48 (q, 1H, *J*=6.9 Hz, CH) 4.27–4.15 (m, 5H, H-2_r, H-3_r, H-2_g, H-3_g, H-4_g), 3.75–3.40 (m, 6H, H-4_r, H-5_r, H-$5'_r$, H-5_g, H-6_g, H-$6'_g$), 2.53 (dd, 1H, *J*=14.5, 10.9 Hz, CH_a), 2.31 (dd, 1H, *J*=14.5, 3.6 Hz, CH_b), 1.99 (s, 3H, CH_3), 1.36 (d, 3H, *J*=6.9 Hz, CH_3). ^{13}C NMR (MeOH-d_4, 75 MHz): δ (ppm) 179.65, 173.90, 166.00, 152.36, 142.49, 102.97, 89.49, 83.89, 78.15, 75.17, 75.16, 73.14, 72.67, 72.67,

71.50, 68.92, 62.47, 54.60, 36.58, 22.60, 19.79. LRMS (ESI), *m*/*z* 641.2 $(M+H)^+$, 647.2 $(M+Li)^+$, 653.2 $(M+2Li)^+$. HRMS (ESI), *m*/*z* calcd for $C_{22}H_{33}N_4O_{16}S_1$ 641.1612 $(M+H)^+$, found 641.1617, *m*/*z* calcd for $C_{22}H_{32}N_4O_{16}SLi$ 647.1694 $(M+Li)^+$, found 647.1697. HPLC: Column C_{18} Phenomex Luna 10 μ; mobile phase: 10% acetonitrile, 90% trifluoroacetic acid (0.1%), flow rate 1.0 mL/min; injection volume: 10 μL; retention time: 7.61 min (96.26% at 254 nm, 98.97 at 210 nm).

Acknowledgements

This work was supported by the European Union FP6 Integrated Project EUR-INTAFAR (Project no. LSHM-CT-2004-512138) under the thematic priority Life Sciences, Genomics and Biotechnology for Health, Institute Charles Nodier and the Ministry of Higher Education, Science and Technology of the Republic of Slovenia. The authors thank Dr. Roger H. Pain for critical reading of the manuscript.

References and notes

1. Barreteau, H.; Kovač, A.; Boniface, A.; Sova, M.; Gobec, S.; Blanot, D. *FEMS Microbiol. Rev.* **2008**, *32*, 168–207.
2. Wang, R.; Steensma, D. H.; Takaoka, Y.; Yun, J. W.; Kajimoto, T.; Wong, C. H. *Bioorg. Med. Chem.* **1997**, *5*, 661–672 and references therein.
3. Hajduch, J.; Nam, G.; Kim, E. J.; Frölich, R.; Hanover, J. A.; Kirk, K. L. *Carbohydr. Res.* **2008**, *343*, 189–195.
4. Bugg, T. D. H.; Walsh, C. T. *Nat. Prod. Rep.* **1992**, *9*, 199–215.
5. Lis, H.; Sharon, N. *Eur. J. Biochem.* **1993**, *218*, 1–27.
6. Zoeiby, A.; Sanschagrin, F.; Levesque, R. C. *Mol. Microbiol.* **2003**, *47*, 1–12.
7. Camarasa, M. J.; Fernández-Resa, P.; Garcia-López, M. T.; Heras, F. G.; Méndez-Castrillón, P. P.; Alarcón, B.; Carrasco, L. *J. Med. Chem.* **1985**, *28*, 40–46.
8. Horton, D.; Machinami, T.; Takagi, Y. *Carbohydr. Res.* **1983**, *121*, 135–161.
9. Ireland, R. E.; Wuts, P. G. M.; Ernst, B. *J. Am. Chem. Soc.* **1981**, *103*, 3205–3207.
10. Tatsuta, K.; Takahashi, H.; Amemiya, Y.; Kinoshita, M. *J. Am. Chem. Soc.* **1983**, *105*, 4096–4097.
11. Roe, B. A.; Boojamra, C. G.; Griggs, J. L.; Bertozzi, C. *J. Org. Chem.* **1996**, *61*, 6442–6445.
12. Postrema, M. H. D.; Piper, J. L.; Liu, L.; Shen, J.; Faust, M.; Andreana, P. *J. Org. Chem.* **2003**, *68*, 4748–4754.
13. McGarvey, G. J.; Schmidtmann, F. W.; Benedum, T. E.; Kizer, D. E. *Tetrahedron Lett.* **2003**, *44*, 3775–3779.
14. Bouvet, V. R.; Ben, R. N. *J. Org. Chem.* **2006**, *71*, 3619–3622.
15. Röhrig, C. H.; Takhi, M.; Schmidt, R. R. *Synlett* **2001**, 1170–1172.
16. Liu, L.; Gallagher, T. *Org. Lett.* **2004**, *6*, 2445–2448.
17. Cui, J.; Horton, D. *Carbohydr. Res.* **1998**, *309*, 319–330.
18. Ponten, F.; Magnusson, G. *J. Org. Chem.* **1996**, *61*, 7463–7466.
19. Keck, G. E.; Yates, J. B. *J. Am. Chem. Soc.* **1982**, *104*, 5829–5831.
21. Buskas, T.; Söderberg, E.; Konradsson, P.; Fraser-Reid, B. *J. Org. Chem.* **2000**, *65*, 958–963.
22. Virta, P.; Karskela, M.; Lönnberg, H. *J. Org. Chem.* **2006**, *71*, 1989–1999.
23. Gross, P.; Rimpler, M. *Liebigs Ann. Chem.* **1986**, *1*, 37–45.
24. Osawa, T.; Sinay, P.; Halford, M.; Jeanloz, R. W. *Biochemistry* **1969**, *8*, 3369–3375.
25. Babič, A.; Pečar, S. *Tetrahedron Lett.* **2007**, *48*, 4403–4405.
26. Winans, K. A.; Bertozzi, C. R. *Chem. Biol.* **2002**, *9*, 113–129.
27. Appel, R.; Berger, G. *Chem. Ber.* **1958**, *91*, 1339–1341.