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Parallel synthesis of a small library of novel aminoglycoside analogs based on 2-amino-2-deoxy-D-glucose and D-ribose scaffolds

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Abstract—The synthesis of a small library of aminoglycoside analogues based on 2-amino-2-deoxy-D-glucose and D-ribose was achieved using reductive amination reactions as key transformations.

1. Introduction

The vast majority of the drugs known today owe their pharmaceutical activity to interactions with proteins and only very few rely on interactions with RNA. In the last years the interest in RNA as a drug receptor has grown significantly due to the discovery that RNA is involved in many catalytic processes[1] and to the approval of the first antisense drug.[2] The increased awareness of the central role of RNA has led to research into small molecules that interact with RNA. The potency of aminoglycoside antibiotics and the importance of circumventing the emerging problems of resistance has led to studies on the mechanism of action of these antibiotics focusing on understanding the molecular interactions leading to activity.[3] It was shown early that aminoglycosides interact with the bacterial ribosome leading to miscoding and/or termination of growing peptide chains.[4] By using a foot-printing technique, Moazed and Noller were the first to describe the secondary structure of the 16S rRNA[5] subunit of the ribosome and later the site of aminoglycoside binding to the A-site.[6] The A-site together with the P-site ensure the fidelity of tRNA selection and the binding of aminoglycosides interferes with the selection and proof-reading mechanism thereby lowering the accuracy of protein synthesis. Chemically, the natural aminoglycosides are a group of structurally related polyamine oligosaccharides (Fig. 1).[7] Their common structural element is a *meso*-1,3-diaminocyclitol 2-deoxystreptamine unit. The 2-deoxystreptamine is glycosylated with a variety of saccharides both with and without amino groups at the 4-position as well as the 5-position as in the neomycin class, or at the 6-position as in the kanamycin–gentamicin class.

Most of the information about the binding of aminoglycosides to the 16S rRNA subunit has been obtained from two NMR structures, one of the free As-wt oligonucleotide model for the 16S rRNA subunit[8] and one of a complex between paromomycin and the As-wt.[9] One of the striking features of these structures is that it is the paromamine part of paromomycin that is responsible for virtually all of the specific hydrogen bonding contacts to the RNA. The two other rings are apparently responsible for orienting the paromamine/neamine part in an optimal way. Aminoglycosides bind to a pocket in the bacterial 16S rRNA subunit created by a mismatched base pair and to a host of other RNA targets. Tok and Rando have furthermore shown that simple 1,3-hydroxylamine containing aminols are also able to bind the As-wt model with low micro molar affinities which is comparable to the binding affinities of some of the natural aminoglycosides.[10] Further investigations on the role of the different parts of the natural aminoglycosides on binding have been performed by Alper et al.[11] who have synthesized a series of neomycin B analogues in which the 2,6-dideoxy-2,6-diamino-L-idose unit was replaced by different groups. Further work by Wong and coworkers has shown that 2-amino-2-deoxy-D-glucose[12] and neamine[13] are effective scaffolds for the synthesis of libraries of aminoglycoside analogues with good RNA binding properties. A differently structured library based on neamine in which the 6′-amino group was functionalized by reductive aminations or Ugi type couplings has been prepared in order to further study RNA binding.[14]

Keywords: carbohydrate libraries; aminoglycoside analogues; 2-amino-2-deoxy-D-glucose; reductive amination reactions.

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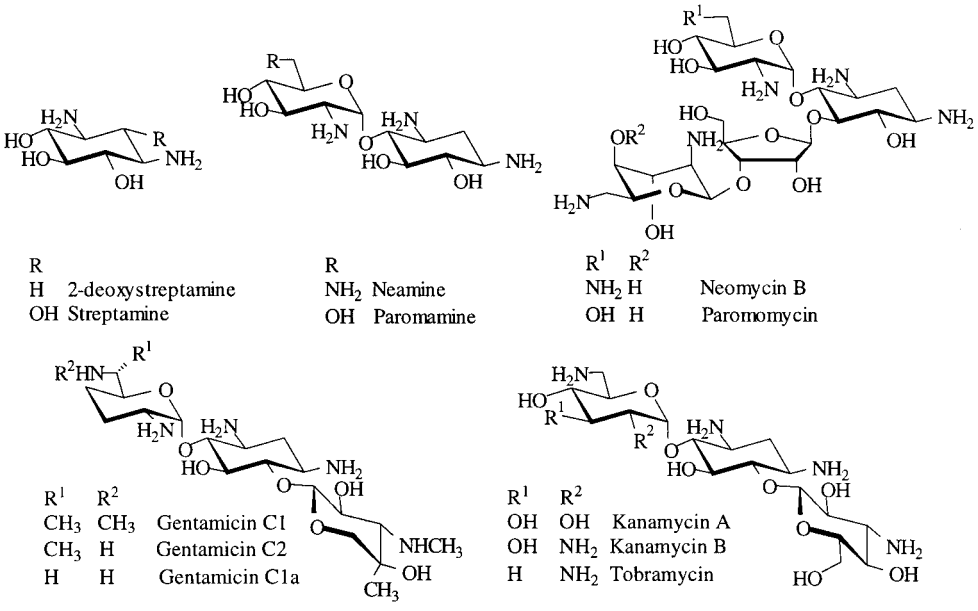


Figure 1. Examples of the natural aminoglycosides.

2. Results and discussion

Based on the above outlined knowledge regarding the binding motifs and the functional groups which are important for the interaction between aminoglycosides and the bacterial ribosome A-site, we have designed and synthesized a series of novel structurally simple aminoglycoside analogues and studied their antibacterial activity (Fig. 2). The 2-amino group was left free to mimic the natural aminoglycosides and the 6-position was modified with different amines by various methods. The diversity was further increased by allylation of the anomeric position followed by functionalization by ozonolysis and reductive amination. In addition, derivatives containing a ribose core were also included in the library.[15] In contrast to the known RNA-binding aminoglycoside analogues all having α-glycosidic bonds to a moiety containing an amino functionality, all the novel compounds synthesised and investigated herein are β-configured. However, we believed that the obtained results should add novel knowledge to the structure–activity relationship available for aminoglycoside analogues. For an exploratory library we decided to use parallel solution phase synthesis of individual compounds in order to facilitate analysis of both reactions and preliminary biological activities.

The synthesis of the core molecules **4** and **7** started with the hydrochloride of 2-amino-2-deoxy-D-glucose (**1**) which was first treated with 1.05 equiv. of triethylamine followed by ethyl trifluoroacetate.[16] Under these conditions the amino group is chemoselectively trifluoroacetylated yielding derivative **2**. Without purification, the trifluoroacetamide derivative **2** was subjected to glycosidation in anhydrous allyl alcohol at reflux for 8 h with boron trifluoride etherate as Lewis acid catalyst. After complete conversion according to analytical TLC, the reaction mixture was concentrated and the residue coevaporated with anhydrous pyridine before treatment with excess acetic anhydride in anhydrous pyridine affording the fully protected allyl glucoside **4** after purification by column chromatography (β-glucoside, 47% yield from **1**). The β-configuration of compounds **4**–**19** and **25**–**30** was assigned based on the $J_{1,2}$ coupling constants

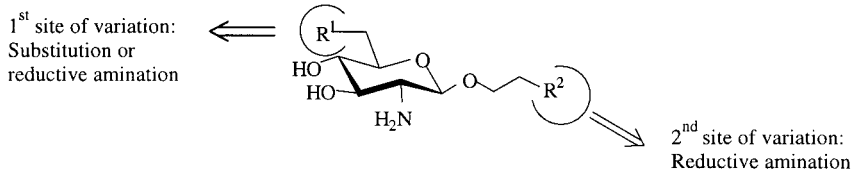
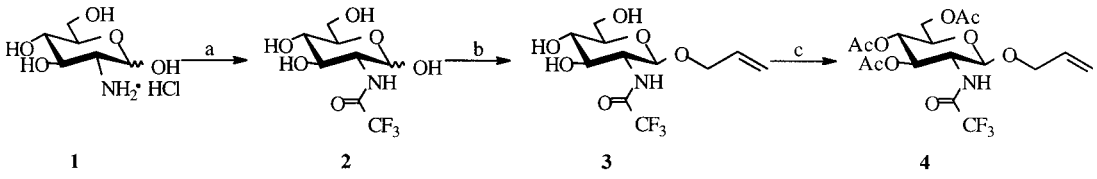
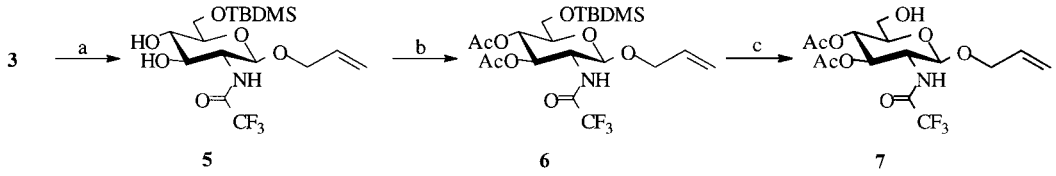


Figure 2. Design of the 1,6-disubstituted 2-amino-2-deoxy-D-glucose library.



Scheme 1. (a) Ethyl trifluoroacetate, Et_3N, MeOH; (b) Allyl alcohol, $BF_3{\cdot}OEt_2$ and (c) Ac_2O, pyridine (47% from **1**).



Scheme 2. (a) TBDMSCl (1.1 equiv.), pyridine; (b) Ac_2O, pyridine (50% from **1**) and (c) I_2, MeOH (86%).

observed in the ^1H NMR spectra ($J_{1,2}$=≈9 Hz) and the chemical shift value observed in the ^{13}C NMR spectra for C1 (at ≈99 ppm) being in the typical range observed for β-2-amino-2-deoxy-D-glycopyranosides[17] (Scheme 1).

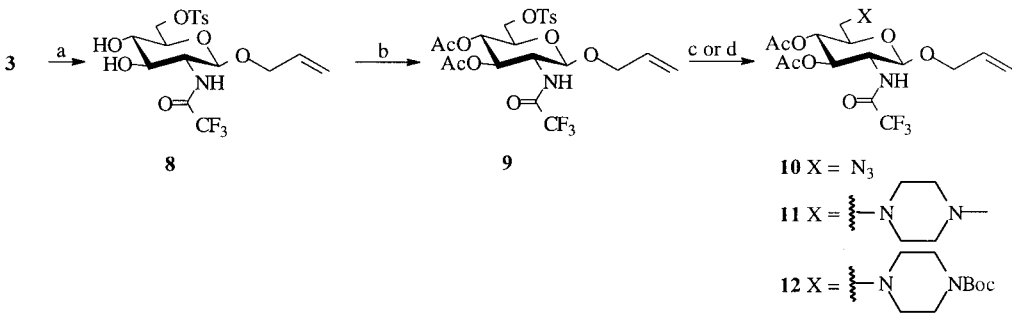
Compound **4** was now ready for the synthesis of paromamine-like compounds by ozonolysis of the allyl group followed by reductive amination. In order to functionalize the 6-position, the introduction of an orthogonal protection group at this site was needed. Silyl ethers are well known and much used in carbohydrate chemistry. When treating compound **3** with 1.1 equiv. of TBDMSCl in pyridine, monosilylation of the primary hydroxyl group was achieved. When TLC showed complete conversion to **5**, 4 equiv. of acetic anhydride were added to give derivative **6** which was obtained in 50% yield from **1** after purification by column chromatography (Scheme 2).

There is a wealth of methods available for the deprotection of TBDMS ethers, among these are KF and 18-crown-6, HF in pyridine, trifluoroacetic acid, HCl in ethanol, and $BF_3{\cdot}Et_2O$.[18] Strongly acidic conditions, e.g. the use of $BF_3{\cdot}Et_2O$, were ruled out for the deprotection of **6** because of the risk of deallylation and/or anomerisation. The most common method for the deprotection of a silyl ether is the treatment with excess fluoride ions usually provided in the form of tetrabutylammonium fluoride (TBAF) because of the solubility of this salt in most organic solvents. However, even with a very large excess of fluoride (10 equiv.) and long reaction times (>24 h) it was not possible to drive the desilylation of compound **6** to completion. As an alternative, compound **6** was treated with a 1% (w/v) solution of iodine in methanol[19] at reflux for 3 h leading to clean conversion of **6** giving compound **7** in 86% yield after chromatographic purification.

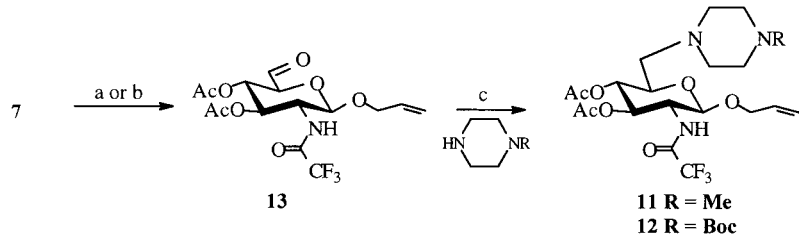
Two different methods for the introduction of amines at the 6-position were investigated. The classical method for the synthesis of primary amines is the Gabriel synthesis in which an alkyl halide is treated with the sodium salt of phthalimide. To obtain the free amine, the phthalimide is then either hydrolyzed or cleaved with hydrazine. A similar reaction was developed by Harland et al. and used by Overman et al. in the total synthesis of strychnine. In this reaction, an alkyl halide or similar is treated with the sodium salt of trifluoroacetamide generated in situ by reacting trifluoroacetamide with sodium hydride.[20] The latter reaction was attempted on **7** because it introduces the amine already protected as the trifluoroacetamide group which, as a similar group is already present in compound **7**, would eliminate an additional deprotection step. Three different leaving groups were tested in the substitution reaction, namely the mesyl, the triflate and the iodo group, but in all cases the substitution failed to give the desired 6-amino-6-deoxy-6-*N*-trifluoroacetyl derivative. A small scale reaction, in which **7** was tosylated and then treated with sodium azide in dimethylformamide yielded the azide **10** (Scheme 3). Therefore, inspired by the success of the selective silylation of compound **3**, a selective tosylation of this compound was performed. Thus, by treatment of compound **3** with tosyl chloride in pyridine (to give intermediate **8**) followed by the addition of an excess of acetic anhydride, compound **9** was obtained in 40% from **1** (Scheme 3) after column chromatographic purification.

Heating tosylate **9** in dimethylformamide with an excess of sodium azide afforded azide **10** in 76% yield. The tosylate **9** was also used for the reaction with the two secondary amines *N*-methylpiperazine and *N*-Boc-piperazine. In the first attempt, dimethylformamide was used as solvent but with both amines none of the desired product was observed, and heating only lead to decomposition and the formation of dimethylamine.[21] However, changing the solvent to pyridine allowed the introduction of the two secondary amines to proceed in moderate yields of 45% for **11** and 70% for **12** (Scheme 3).

As an alternative method for the introduction of amines at



Scheme 3. (a) TsCl, pyridine; (b) Ac_2O (40% from **1**); (c) NaN_3, DMF, 90°C (76% for **10**) and (d) *N*-methylpiperazine (45% for **11**) or Boc-piperazine (70% for **12**), pyridine (90°C).



Scheme 4. (a) Oxalyl chloride, DMSO, TEA, CH_2Cl_2 (32%); (b) CrO_3, Ac_2O, pyridine, CH_2Cl_2 (92%); (c) $NaCNBH_3$, AcOH, MeOH, amine (**11**: 25% and **12**: 18%).

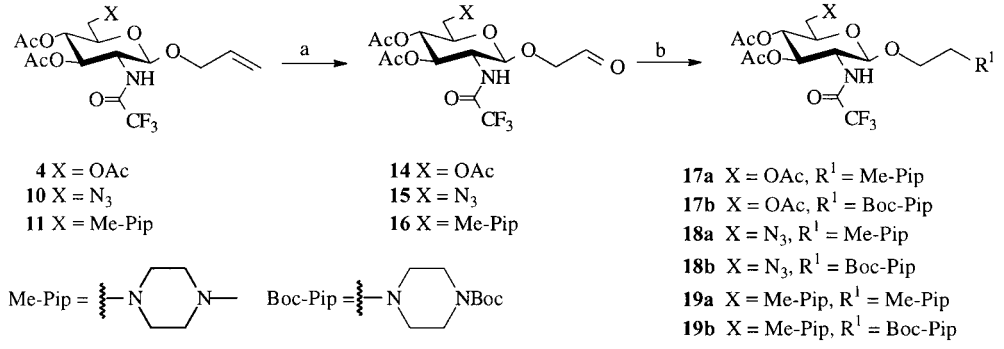
the 6-position of 2-amino-2-deoxy-D-glucose derivatives, reductive amination reactions were studied (Scheme 4). Oxidation of **7** with PDC[22] only proceeded slowly (about 50% conversion after 7 days according to analytical TLC) and a Swern oxidation[23] did not afford more than 32% yield of the desired aldehyde. However, oxidation with chrom-(VI)oxide, pyridine, and acetic anhydride[24] was successful yielding the aldehyde **13** in 92% after filtration of the reaction mixture through a plug of silica. The reductive amination of aldehyde **13** with *N*-methylpiperazine and *N*-Boc-piperazine proceeded in only poor yields (25% for **11** and 18% for **12**) and were therefore abandoned.

Compounds **4**, **10**, and **11** were subjected to ozonolysis of the allyl side chain in order to unmask an aldehyde for reductive amination reactions (Scheme 5). The reductive aminations to give the compound **17** and **18** shown in Scheme 5 proceeded in 34–85% yield which is within the normal range for this type of reaction.[25] Derivatives **19** were only obtained in very poor yields. Mattson et al.[26] have observed improvement in yields of reductive amination reactions by using titanium(IV)isopropoxide as Lewis acid but in this case the yields of **19** were not increased.

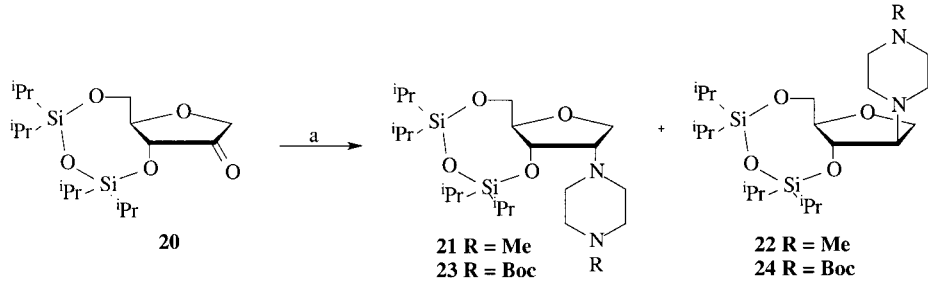
In order to expand the library, a D-ribose moiety as found in streptomycin, ribostamycin, and neomycin B was included. The very efficient synthesis of the ketone **20** was known from our previous work[15] and this ketone was subjected to reductive amination with both *N*-methylpiperazine and *N*-Boc-piperazine (Scheme 6) yielding pairs of diastereo-isomers. The reaction with *N*-methylpiperazine proceeded in 49% yield (**21/22**=1:1.2) whereas the reaction with *N*-Boc-piperazine proceeded in 63% yield (**23/24**=1:1.4).

The configuration of the individual diastereoisomers was assigned based on ^1H–^1H COSY, ^1H–^{13}C COSY and NOE experiments. Especially important for the configurational assignments were NOE contacts, or lack of the same, between α-protons of the piperazine moieties and H-3 of the ribose ring. Thus, the NOE experiment for **22** showed mutual and strong contacts between the α-protons of the piperazine ring and H-3. No such NOE contacts were observed for compound **21**. A similar pattern was seen for the diastereoisomeric pair **23** and **24**.

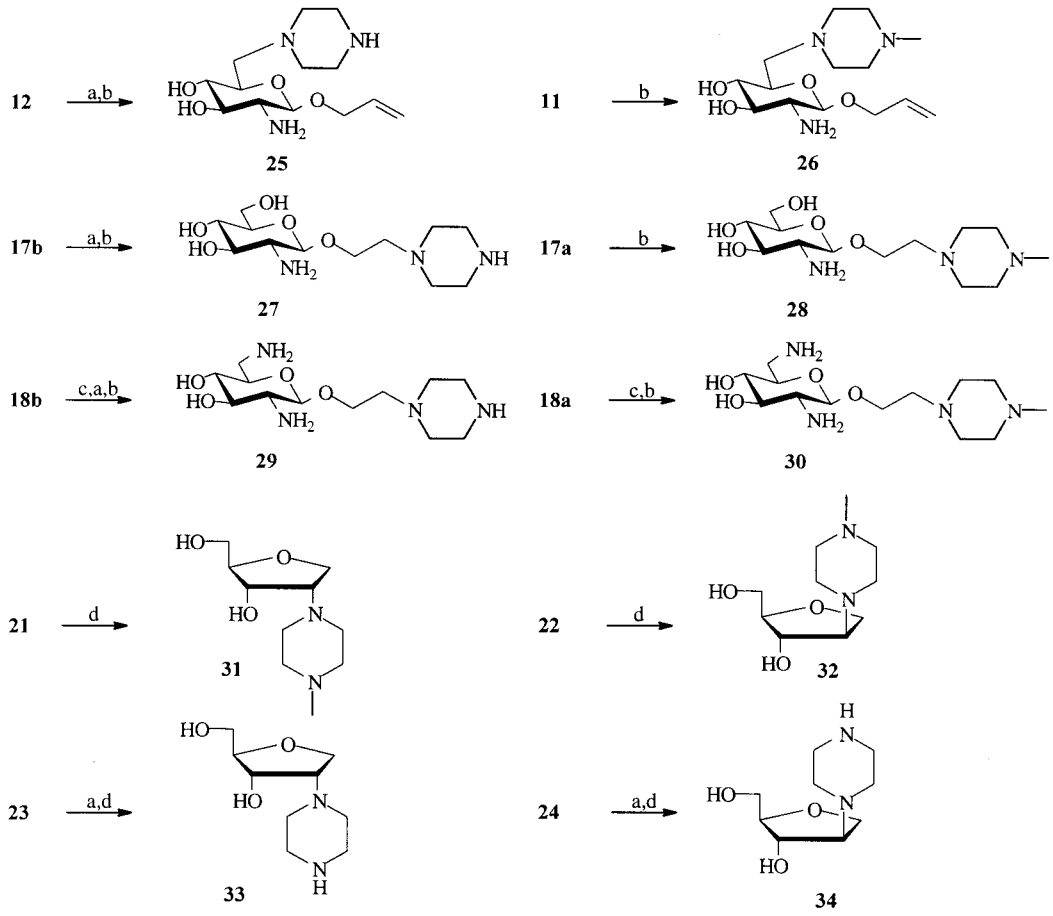
All the library compounds were finally deprotected, and depending on the protecting groups present different conditions had to be employed (Scheme 7). Complete deprotection was



Scheme 5. (a) O_3, MeOH, −78°C, CH_3SCH_3; (b) *N*-methylpiperazine or *N*-Boc-piperazine, $NaCNBH_3$, AcOH, MeOH.



Scheme 6. (a) *N*-methylpiperazine or *N*-Boc-piperazine, $NaCNBH_3$, AcOH, MeOH.



Scheme 7. (a) CF_3COOH; (b) NH_3, MeOH; (c) H_2, 20% $Pd(OH)_2/C$, 1:1 EtOH/dioxane and (d) TBAF, THF.

carried out with no purification between the individual steps, and the completely deprotected compounds **25**–**34** were purified by cation-exchange chromatography using ammonium hydroxide/water as eluent. The pure compounds were converted into their hydrochloride salts by adding an excess of 1 M hydrochloric acid followed by lyophilization to give off-white powders.

Compounds **25**–**34** were obtained in good yields and in excellent purities as verified by mass spectrometry and NMR spectroscopy. In the present study, compounds **25**–**34** were tested for their potential antibacterial, antifungal and antiviral properties by in vitro standard methods.[27] All compounds showed minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC) higher than 100 μg/ml. Some of the compounds showed weak antiviral properties against herpes simplex virus type 1 (HSV-1) (compounds **31**, **32** and **33**), Semliki forest virus L10 (compounds **33** and **34**) and vesicular stomatitis virus (VSV) (compound **33**) in concentrations of 100–50 μg/ml.

3. Conclusion

The synthesis of a small library of aminoglycoside analogues based on 2-amino-2-deoxy-D-glucose and D-ribose was achieved by applying nucleophilic substitution and reductive amination reactions. Only weak antiviral activities were obtained, and only for a few of the compounds evaluated.

4. Experimental

4.1. General

All reagents were obtained from commercial suppliers and were used without further purification. Silica gel (0.040–0.063 mm) used for column chromatography was purchased from Merck. FAB mass spectra were recorded on a Kratos MS 50 RF spectrometer, EI mass spectra on a Varian Mat 311A spectrometer, and NMR spectra on a Varian Gemini 2000 spectrometer (δ-values are reported relative to Me_4Si as internal reference for ^1H NMR (300 MHz) and ^{13}C NMR (62.9 MHz) whereas 1,4-dioxane was used as internal reference for ^{13}C NMR recorded in D_2O). The atoms numbered 1–6 for compounds **4**–**19** and **25**–**30** denote the pyranose ring (using standard carbohydrate nomenclature) with subsequent numbering of the atoms of the O1 substituent and, when applicable, the C6 substituent. The atoms numbered 1–5 for compounds **21**–**24** and **31**–**34** denote the furanose ring (using standard carbohydrate nomenclature) with subsequent numbering of the atoms of the C2 substituent. In the ^{13}C NMR spectra of the trifluoroacetamido derivatives the signals of the trifluoroacetyl

group were detectable in some cases and in others not as reflected in the data reported for the individual compounds. Microanalyses were performed at the Microanalytical Laboratory, Department of Chemistry, University of Copenhagen.

4.1.1. Allyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranoside (4). 2-Amino-2-deoxy-D-glucose as its hydrochloride salt **1** (5.39 g, 25 mmol) was suspended in methanol (25 ml) and triethylamine (3.57 ml, 26.2 mmol) was added followed by ethyl trifluoroacetate (3.27 ml, 27.5 mmol) and the solution was stirred for 18 h at rt. The solution was concentrated in vacuo and the resulting oil coevaporated three times with anhydrous allyl alcohol. The resulting gum was dissolved in anhydrous allyl alcohol (40 ml) and $BF_3{\cdot}Et_2O$ (0.5 ml) was added and the solution was heated under reflux for 8 h. The solvent was removed and the residue was coevaporated three times with anhydrous pyridine. The residue was dissolved in anhydrous pyridine (40 ml) and acetic anhydride (14.03 ml, 150 mmol) was added and the mixture was stirred for 30 min at rt. The solvent was removed under reduced pressure, the resulting oil was redissolved in DCM (100 ml) and washed with a saturated aqueous solution of $NaHCO_3$ (3×100 ml). The organic phase was separated, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography using 0–4% MeOH in DCM (v/v) as eluent to afford compound **4** (5.20 g, 47%) as a white solid material. ^{1}H NMR ($CDCl_3$) δ: 6.88 (br. s, 1H, NH), 5.88–5.73 (m, 1H, H8), 5.34 (d, J=10.0 Hz, 1H, H3), 5.29–5.15 (m, 2H, H9), 5.08 (t, J=10 Hz, 1H, H4), 4.67 (d, J=8.2 Hz, 1H, H1), 4.37–4.27 (m, 1H, H7′), 4.24 (d, J=4.9 Hz, 1H, H6′), 4.17 (d, J=2.5 Hz, 1H, H6″), 4.14–4.08 (m, 1H, H7″), 4.05–3.99 (m, 1H, H2), 3.76–3.71 (m, 1H, H5), 2.08 (s, 3H, CH_3CO), 2.02 (s, 6H, 2×CH_3CO); ^{13}C NMR ($CDCl_3$) δ: 171.4, 170.9, 169.5 (3×CH_3CO); 157.5 (q, J=37.5 Hz, CF_3CO), 133.1 (C8), 118.2 (C), 116.1 (q, J=262.5 Hz, CF_3CO), 99.1 (C1), 71.8 (C7, C5), 70.1 (C3), 68.5 (C4), 62.0 (C6), 54.7 (C2), 20.5, 20.4, 20.2 (3×CH_3CO); ^{19}F NMR ($CDCl_3$) δ: 116.0. MS (FAB^+/NBA) m/z: 384 ([M−(Oallyl)]$^+$). Found C: 46.23 H: 5.01 N: 3.11; $C_{17}H_{22}F_3NO_9$ requires C: 46.30 H: 5.02 N: 3.17.

4.1.2. Allyl 3,4-di-*O*-acetyl-6-*O*-(*t*-butyldimethylsilyl)-2-deoxy-2-trifluoroacetamido-β-D-glycopyranoside (6). 2-Amino-2-deoxy-D-glucose as its hydrochloride salt **1** (5.39 g, 25 mmol) was suspended in methanol (25 ml) and triethylamine (3.57 ml, 26.2 mmol) was added followed by ethyl trifluoroacetate (3.27 ml, 27.5 mmol) and the solution was stirred for 18 h at rt. The solution was concentrated in vacuo and the resulting oil coevaporated three times with anhydrous allyl alcohol. The resulting gum was dissolved in anhydrous allyl alcohol (40 ml) and $BF_3{\cdot}Et_2O$ (0.5 ml) was added and the solution was heated under reflux for 8 h. The solvent was removed and the residue was coevaporated three times with anhydrous pyridine. The resulting oil was dissolved in anhydrous pyridine (40 ml) and *t*-butyldimethylsilyl chloride (4.14 g, 27.5 mmol) was added and when TLC showed complete conversion after 16 h at rt acetic anhydride (9.35 ml, 100 mmol) was added and the mixture was stirred for 30 min at rt. The solvent was removed under reduced pressure, the resulting oil was redissolved in DCM (100 ml) and washed with a saturated aqueous solution of $NaHCO_3$ (3×100 ml). The organic phase was separated, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography using 0–4% MeOH in DCM (v/v) as eluent to afford compound **6** (6.42 g, 50%) as a white solid material. ^{1}H NMR ($CDCl_3$) δ: 6.42 (br. s, 1H, NH), 5.88–5.76 (m, 1H, H8), 5.31–5.17 (m, 3H, H3, H9), 5.02 (t, J=9.8 Hz, 1H, H4), 4.59 (d, J=8.2 Hz, 1H, H1), 4.32 (ddt, J=13.2, 4.8, 1.5 Hz, 1H, H2), 4.11–3.99 (m, 2H, H7), 3.70 (d, J=4.5 Hz, 2H, H6), 3.59–3.53 (m, 1H, H5), 2.02 (s, 6H, 2×CH_3CO), 0.95–0.82 (m, 9H, $SiC(CH_3)_3$), 0.11–0.01 (m, 6H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ: 171.6, 169.4 (2×CH_3CO); 157.2 (q, J=37.5 Hz, CF_3CO), 133.3 (C8), 118.0 (C9), 98.9 (C1), 74.9 (C5), 72.3 (C7), 69.4 (C3), 68.9 (C4), 62.5 (C6), 54.7 (C2), 25.5 ($C(CH_3)_3$), 20.6, 20.3 (2×CH_3CO), 18.2 ($C(CH_3)_3$), −5.5 (SiMe); ^{19}F NMR ($CDCl_3$) δ: 116.0. MS (FAB^+/NBA) m/z: 512 ([M]$^+$). Found C: 48.89 H: 6.57 N: 2.73; $C_{21}H_{34}F_3NO_8Si$ requires C: 49.11 H: 6.67 N: 2.73.

4.1.3. Allyl 3,4-di-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranoside (7). Glucoside **6** (1.66 g, 3.24 mmol) was dissolved in methanol (20 ml) and a 1% (w/v) solution of iodine in methanol (150 ml) was added. The solution was heated under reflux for 3 h whereupon the reaction was quenched by addition of 1 M $Na_2S_2O_3$ (aqueous) until decolorization was observed. The solvents were removed in vacuo and the resulting oil was taken up in DCM, washed with brine, separated, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography using 5% MeOH in DCM (v/v) as eluent to afford compound **7** (1.19 g, 92%) as a white solid material. ^{1}H NMR ($CDCl_3$) δ: 5.83–5.69 (m, 1H, H8), 5.27–5.12 (m, 3H, H3, H9), 5.01–4.94 (m, 1H, H4), 4.65 (d, J=8.2 Hz, 1H, H1), 4.32 (dd, J=13.2, 5.2 Hz, 1H, H2), 4.08–3.93 (m, 2H, H7), 3.67–3.49 (m, 3H, H6, H5), 2.02 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO); ^{13}C NMR ($CDCl_3$) δ: 171.2, 170.3 (2×CH_3CO), 133.3 (C8), 117.7 (C9), 99.1 (C1), 74.0 (C5), 71.9 (C7), 70.1 (C3), 68.8 (C4), 60.9 (C6), 54.3 (C2), 20.4, 20.2 (2×CH_3CO); ^{19}F NMR ($CDCl_3$) δ: 116.2. MS (FAB^+/NBA) m/z: 342 ([M−Oallyl]$^+$). Found C: 44.89 H: 4.78 N: 3.29; $C_{15}H_{20}F_3NO_8$ requires C: 45.12 H: 5.05 N: 3.51.

4.1.4. Allyl 3,4-di-*O*-acetyl-2-deoxy-2-trifluoroacetamido-6-*O*-tosyl-β-D-glucopyranoside (9). 2-Amino-2-deoxy-D-glucose as its hydrochloride salt **1** (10.78 g, 50 mmol) was suspended in methanol (50 ml) and triethylamine (7.15 ml, 52.5 mmol) was added followed by ethyl trifluoroacetate (6.54 ml, 55 mmol) and the solution was stirred for 18 h at rt. The solution was concentrated in vacuo and the resulting oil coevaporated three times with anhydrous allyl alcohol. The resulting gum was dissolved in anhydrous allyl alcohol (75 ml) and $BF_3{\cdot}Et_2O$ (1.0 ml) was added and the solution was heated under reflux for 8 h. The solvent was removed and the residue was coevaporated three times with anhydrous pyridine. The residue was dissolved in anhydrous pyridine (75 ml) and tosyl chloride (11.4 g, 60.0 mmol) was added and when TLC showed complete conversion after 3 h at rt acetic anhydride (10.29 ml, 110 mmol) was added and the mixture was stirred for further 30 min at rt. The solvent was removed

under reduced pressure, the resulting oil was redissolved in DCM (100 ml) and washed with a saturated aqueous solution of $NaHCO_3$ (3×100 ml). The organic phase was separated, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography first using 0–2% MeOH in DCM (v/v) and then 20–50% EtOAc in petroleum ether (v/v) as eluents to yield compound **9** (11.07 g, 40%) as an off-white solid material. ^{1}H NMR ($CDCl_3$) δ: 7.77 (d, J=8.3 Hz, 2H, H2′(Ts)), 7.34 (d, J=8.0 Hz, 2H, H3′(Ts)), 7.00 (d, J=9.2 Hz, 1H, NH), 5.85–5.71 (m, 1H, H8), 5.35–5.20 (m, 3H, H3, H9), 4.93 (app. t, J=9.8 Hz, 1H, H4), 4.63 (d, J=9.8 Hz, 1H, H1), 4.28–3.95 (m, 5H, H7, H6, H2), 3.85–3.79 (m, 1H, H5), 2.44 (s, 3H, CH_3 (Ts)) 2.01 (br. s, 6H, 2×CH_3CO); ^{13}C NMR ($CDCl_3$) δ: 171.4, 169.7 (2×CH_3CO); 157.5 (q, J=36 Hz, CF_3CO), 145.4 (C1′(Ts)), 133.0 (C8), 134.4 (C4′(Ts)), 130.0 (C2′(Ts)), 128.1 (C3′(Ts)), 118.1 (C9), 115.9 (q, J=255 Hz, CF_3CO), 98.9 (C1), 71.7 (C5, C6), 69.9 (C3), 68.8 (C7), 67.9 (C4), 54.4 (C2), 21.5 (CH_3(Ts)), 20.6, 20.3 (2×CH_3CO), ^{19}F NMR ($CDCl_3$) δ: 115.9. MS (FAB$^+$/NBA) m/z: 496 ([M−Oallyl]$^+$). Found C: 47.99 H: 4.50 N: 2.54; $C_{22}H_{26}F_3NSO_{10}$ requires C: 47.73 H: 4.73 N: 2.53.

4.1.5. Allyl 3,4-di-*O*-acetyl-6-azido-2,6-dideoxy-2-trifluoroacetamido-β-D-glucopyranoside (10). Derivative **9** (1.00 g, 1.81 mmol) was dissolved in anhydrous DMF (50 ml) and NaN_3 (352 mg, 5.42 mmol) was added. The solution was stirred at 90°C for 3 h. The solvent was removed under reduced pressure, and the resulting oil was redissolved in EtOAc (50 ml) and washed with brine (3×50 ml). The organic phase was separated, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by column chromatography using 20% EtOAc in petroleum ether (v/v) as eluent to afford compound **10** (589 mg, 76%) as a clear oil. ^{1}H NMR ($CDCl_3$) δ: 6.93 (d, J=8.9 Hz, 1H, NH), 5.89–5.76 (m, 1H, H8), 5.37–5.22 (m, 3H, H3, H9), 4.98 (app. t, J=9.5 Hz, 1H, H4), 4.71 (d, J=8.2 Hz 1H, H1), 4.35 (dd, J=12.9, 3.8 Hz, 1H, H5), 4.13–4.03 (m, 2H, H7), 3.43 (dd, J=13.4, 7.5 Hz, 1H, H6)), 3.20 (dd, J=13.5, 1.75 Hz, 1H, H6), 2.03 (br. s, 6H, 2×CH_3CO); ^{13}C NMR ($CDCl_3$) δ: 171.4, 169.7 (2×CH_3CO); 157.5 (q, J=33 Hz, CF_3CO), 133.0 (C8), 118.3 (C9), 115.6 (q, J=287 Hz, CF_3CO), 98.8 (C1), 73.7 (C5), 71.6 (C3), 69.9, 69.7 (C7, C4), 54.7 (C6) 51.1 (C2), 20.5, 20.4 (2×CH_3CO), ^{19}F NMR ($CDCl_3$) δ: 116.2. MS (FAB$^+$/NBA) m/z: 367 ([M−Oallyl]$^+$). IR (KBr): 3405, 3305, 3115, 2930, 2873, 2102, 1752, 1708, 1566, 1433, 1376, 1325, 1258, 1218, 1183, 1073, 1045, 994, 931, 895, 734 cm^{-1}. Found C: 42.58 H: 4.60 N: 13.33; $C_{15}H_{19}F_3N_4O_7$ requires C: 42.46 H: 4.51 N: 13.20.

4.1.6. Allyl 3,4-di-*O*-acetyl-2,6-dideoxy-6-(*N*-methylpiperazino)-2-trifluoroacetamido-β-D-glucopyranoside (11). Glucoside **9** (553 mg, 1.00 mmol) was dissolved in anhydrous pyridine (25 ml) and *N*-methylpiperazine (0.33 ml, 3.0 mmol) was added. The solution was stirred at 90°C for 7 h. The solvent was removed under reduced pressure, and the resulting oil was redissolved in EtOAc (25 ml) and washed with brine (3×25 ml). The organic phase was separated, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by column chromatography using 2–5% MeOH in DCM (v/v) as eluent to yield compound **11** (217 mg, 45%) as a clear oil. ^{1}H NMR ($CDCl_3$) δ: 7.19 (d, J=8.8 Hz, 1H, NH), 5.86–5.73 (m, 1H, H8), 5.31–5.13 (m, 3H, H3, H9), 4.98 (app. t, J=9.4 Hz, 1H, H4), 4.60 (d, J=8.3 Hz, 1H, H1), 4.26 (dd, J=13.1, 4.9 Hz, 1H, H5), 4.05–3.92 (m, 3H, H7, H6), 3.69–3.60 (m, 1H, H2), 2.45–2.31 (m, 8H, CH_2-pip), 2.24 (s, 3H, Me-pip), 2.00 (br. s, 6H, 2×CH_3CO); ^{13}C NMR ($CDCl_3$) δ: 171.4, 169.6 (2×CH_3CO), 157.5 (q, J=33 Hz, CF_3CO), 133.2 (C8), 117.9 (C9), 115.6 (q, J=287 Hz, CF_3CO), 98.9 (C1), 73.6, 72.9 (C5, C3), 70.5, 69.9 (C7, C4), 58.5 (C6), 55.0 (C10), 54.7 (C2), 53.6 (C11), 45.8 (C12), 20.7, 20.3 (2×CH_3CO); ^{19}F NMR ($CDCl_3$) δ: 116.2. MS (FAB$^+$/NBA) m/z: 482 ([M]$^+$). Found C: 49.67 H: 6.14 N: 8.68; $C_{20}H_{30}F_3N_3O_7$ requires C: 49.90 H: 6.28 N: 8.73.

4.1.7. Allyl 3,4-di-*O*-acetyl-6-(*N*-*t*-butoxycarbonyl)piperazino-2,6-dideoxy-2-trifluoroacetamido-β-D-glucopyranoside (12). Glucoside **9** (553 mg, 1.00 mmol) was dissolved in anhydrous pyridine (25 ml) and *N*-(*t*-butoxycarbonyl)-piperazine (279.0 mg, 3.0 mmol) was added. The solution was stirred at 90°C for 10 h. The solvent was removed under reduced pressure, and the resulting oil was redissolved in EtOAc (25 ml) and washed with brine (3×25 ml). The organic phase was separated, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by column chromatography using 2–5% MeOH in DCM (v/v) as eluent to afford compound **12** (198 mg, 70%) as a clear oil. ^{1}H NMR ($CDCl_3$) δ: 5.86–5.75 (m, 1H, H8), 5.29–5.15 (m, 3H, H3, H9), 5.00 (app. t, J=9.6 Hz, 1H, H4), 4.66 (d, J=8.1 Hz, 1H, H1), 4.32–4.25 (m, 1H, H5), 4.06–3.92 (m, 2H, H7), 3.67–3.61 (m, 1H, H2), 3.57–3.42 (m, 2H, H6), 2.43 (d, J=2.7 Hz, 4H, CH_2-pip), 2.15–2.04 (m, 4H, CH_2-pip), 2.00 (br. s, 6H, 2×CH_3CO), 1.44 (s, 9H, ($C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ: 170.9, 169.6 (2×CH_3CO), 154.6(NCOO), 133.2 (C8), 117.9 (C9), 99.0 (C1), 72.7, 71.9 (C5, C3), 70.4, 70.0 (C7, C4), 58.6 (C6), 54.8 (C2), 53.7 (C10), 43.6 (br. peak, C11), 29.2 ($C(CH_3)_3$), 20.7, 20.3 (2×CH_3CO); ^{19}F NMR ($CDCl_3$) δ: 116.3. MS (FAB$^+$/NBA) m/z: 568 ([M]$^+$). Found C: 50.50 H: 6.13 N: 7.29; $C_{20}H_{30}F_3N_3O_7$ requires C: 50.79 H: 6.39 N: 7.40.

4.2. General procedure for the ozonolysis reactions

The starting allyl glucoside was dissolved in anhydrous MeOH (30 ml/mmol starting allyl glucoside). After cooling to −78°C, purging with O_2 followed by O_3 was performed until a blue color appeared which was followed by purging once more with O_2. Dimethylsulfide (0.5 ml/mmol starting allyl glucoside) was added and the temperature was allowed to rise to rt. The resulting mixture was concentrated and dried in high vacuum overnight. The crude material obtained by this procedure was pure enough to be used directly in the reductive amination reactions.

4.3. General procedure for the reductive amination reactions

The carbonyl compound was dissolved in anhydrous MeOH (8 ml/1.0 mmol carbonyl compound) followed by addition of a 1.0 M solution of the amine (3 equiv.) in MeOH. Subsequently, a 1.0 M solution of AcOH (4 equiv.) in anhydrous MeOH and a 0.3 M solution of $NaCNBH_3$ (0.44 equiv.) in MeOH were added. The resulting mixture was stirred under Ar for 18 h at rt whereupon H_2O (0.2 ml) was added. The

resulting mixture was stirred for 20 min at rt followed by evaporation under reduced pressure and addition of brine. This mixture was extracted with DCM two or three times, the organic phases combined, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by column chromatography using 0–2% MeOH in DCM (v/v) as eluent to afford the products as white solid materials.

4.3.1. 2-(*N*-Methylpiperazino)ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranoside (17a). Yield: 52 mg (55%) which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ: 6.82 (d, J=11.0 Hz, 1H, NH), 5.44 (app. t, J=8.3 Hz, 1H, H3), 5.18 (app. t, J=9.0 Hz, 1H, H4), 4.90 (d, J=8.3 Hz, 1H, H1), 4.40 (dd, J=11.9, 4.1 Hz, 1H, H6), 4.21 (dd, J=12.1, 2.2 Hz, 1H, H6b), 4.15–4.00 (m, 2H, H7), 3.82–3.74 (m, 2H, H5, H2), 3.20 (br. s, 4H, H10), 3.00 (br. s, 2H, H8), 2.65–2.50 (m, 4H, H9), 2.22 (s, 3H, H11), 2.06 (br. s, 9H, 3×H_3CO); ^{13}C NMR ($CDCl_3$) δ: 170.8, 170.7, 169.5 (3×CH_3CO), 99.5 (C1), 72.3, 71.9 (C3, C5), 68.4 (C4), 65.7 (C7), 61.9 (C6), 56.0 (C8), 54.3 (C2), 52.8 (C9), 45.9 (C11), 43.7 (C10), 20.5, 20.3, 20.2 (3×CH_3CO); ^{19}F NMR ($CDCl_3$) δ: 115.9. MS (FAB$^+$/NBA) m/z: 528 ([MH]$^+$).

4.3.2. 2-(*N*-(*t*-Butoxycarbonyl)piperazino)ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranoside (17b). Yield: 96 mg (47%) which was used in the next step without further purification. δ: 6.90 (d, J=12.0 Hz, 1H, NH), 5.24 (app. t, J=9.3 Hz, 1H, H3), 5.06 (app. t, J=9.7 Hz, 1H, H4), 4.70 (d, J=8.4 Hz, 1H, H1), 4.25 (dd, J=12.2, 4.6 Hz, 1H, H6), 4.11 (dd, J=12.5, 2.4 Hz, 1H, H6), 4.06–4.00 (m, 2H, H7), 3.82–3.75 (m, 1H, H5), 3.70 (app. dq, J=9.9, 2.3 Hz, 1H, H2), 3.43 (br. s, 4H, H10), 2.72 (t, J=4.6 Hz, 2H, H8), 2.66–2.50 (m, 4H, H9), 2.01 (br. s, 9H, 3×CH_3CO), 1.43 (s, 9H, (C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$) δ: 170.8, 170.7, 169.5 (3×CH_3CO); 157.5 (m, CF_3CO), 154.6 (NCOO), 116.2 (q, J=264 Hz, CF_3CO), 99.9 (C1), 80.1 (C(CH_3)$_3$), 72.1, 72.1 (C3, C5), 68.3 (C4), 66.6 (C7), 61.3 (C6), 57.5 (C8), 54.4 (C2), 53.0 (C9), 43.0 (C10), 28.2 (C(CH_3)$_3$), 20.3, 20.3, 20.2 (3×iH_3CO); ^{19}F NMR ($CDCl_3$) δ: 116.2. MS (FAB$^+$/NBA) m/z: 614 ([MH]$^+$).

4.3.3. 2-(*N*-Methylpiperazino)ethyl 3,4-di-*O*-acetyl-6-azido-2,6-dideoxy-2-trifluoroacetamido-β-D-glucopyranoside (18a). Yield: 152 mg (85%) which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ: 5.28 (app. t, J=9.4 Hz, 1H, H3), 4.96 (app. t, J=9.8 Hz, 1H, H4), 4.82 (d, J=8.4 Hz, 1H, H1), 4.05–3.96 (m, 2H, H2, H5), 3.88–3.81 (m, 1H, H7), 3.76–3.70 (m, 1H, H7), 3.49–3.37 (m, 1H, H6), 3.19–3.14 (m, 1H, H6), 2.97–2.73 (m, 10H, H8, H9, H10), 2.43 (s, 3H, H11), 2.02 (br. s, 6H, 2×CH_3CO); ^{13}C NMR ($CDCl_3$) δ: 170.6, 169.7 (2×CH_3CO), 99.6 (C1), 74.0, 72.0 (C3, C5), 69.7 (C4), 66.1 (C7), 56.1 (C8), 54.4 (C6), 53.3 (C2), 51.1 (C9), 50.7 (C10), 44.4 (C11), 20.5, 20.3 (2×CH_3CO); ^{19}F NMR ($CDCl_3$) δ: 116.0. MS (FAB$^+$/NBA) m/z: 511 ([MH]$^+$).

4.3.4. 2-(*N*-(*t*-Butoxycarbonyl)piperazino)ethyl 3,4-di-*O*-acetyl-6-azido-2,6-dideoxy-2-trifluoroacetamido-β-D-glucopyranoside (18b). Yield: 115 mg (55%) which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ: 7.59 (d, J=8.9 Hz, 1H, NH), 5.30 (app. t, J=9.8 Hz, 1H, H3), 4.95 (app. t, J=9.8 Hz, 1H, H4), 4.77 (d, J=8.5 Hz, 1H, H1), 4.02–3.93 (m, 2H, H2, H5), 3.74–3.63 (m, 2H, H7), 3.37 (t, J=3.6 Hz, 4H, H10), 3.21–3.16 (m, 2H, H6), 2.62–2.58 (m, 2H, H8), 2.41 (t, J=4.6 Hz, 4H, H9), 2.01 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO); ^{13}C NMR ($CDCl_3$) δ: 170.9, 169.6 (2×CH_3CO), 154.8 (NCOO), 99.7 (C1), 79.7 (C12), 73.7, 71.6 (C3, C5), 69.7 (C4), 67.0 (C7), 57.3 (C8), 54.6 (C6), 53.3 (C2), 51.0 (C9), 42.8 (C10), 28.1 (C14), 20.4, 20.2 (2×CH_3CO); ^{19}F NMR ($CDCl_3$) δ: 116.1. MS (FAB$^+$/NBA) m/z: 597 ([MH]$^+$).

4.3.5. 1,2-Dideoxy-2-(*N*-methylpiperazino)-3-*O*,5-*O*-(tetraisopropyldisiloxan-1,3-diyl)-D-ribofuranose (21). Yield: 50 mg (21%) which was used in the next step without further purification. ^{1}H NMR (DMSO-d_6) δ: 4.23 (app. t, J=5.7 Hz, 1H, H4), 3.89–3.72 (m, 4H, H1, H2, H5′), 3.59–3.53 (m, 1H, H5), 3.03 (app. q, J=5.9 Hz, 1H, H3), 2.49 (br. s, 4H, H7), 2.47–2.44 (m, 2H, H6), 2.40–2.26 (br. s, 2H, H6), 2.11 (s, 3H, H8), 1.03–0.85 (m, 28H, ^{i}Pr); ^{13}C NMR (DMSO-d_6) δ: 83.7 (C2), 75.1 (C4), 71.7 (C3), 66.3 (C1), 62.4 (C5), 54.8 (C7), 49.8 (C6), 45.6 (C8), 17.1, 17.0, 16.8, 13.1, 12.9, 12.8, 12.6 (^{i}Pr). MS (FAB$^+$/NBA) m/z: 459 ([M]$^+$).

4.3.6. 1,2-Dideoxy-2-(*N*-methylpiperazino)-3-*O*,5-*O*-(tetraisopropyldisiloxan-1,3-diyl)-D-arabinofuranose (22). Yield: 67 mg (28%) which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ: ^{1}H NMR (DMSO-d_6) δ: 4.35 (app. t, J=4.9 Hz, 1H, H4), 3.92–3.82 (m, 2H, H1), 3.81–3.76 (m, 1H, H2), 3.73–3.61 (m, 2H, H5), 2.92 (app. q, J=6.5 Hz, 1H, H3), 2.76 (br. s, 2H, H7), 2.59 (br. s, 2H, H7), 2.28 (br s, 4H, H6), 2.13 (s, 3H, H8), 1.04–0.87 (m, 28H, ^{i}Pr); ^{13}C NMR (DMSO-d_6) δ: 85.1 (C2), 74.9 (C4), 68.1 (C3), 65.6 (C1), 63.8 (C5), 55.1 (C7), 50.5 (C6), 45.8 (C8), 17.3, 17.1, 16.9, 16.8, 12.9, 12.8, 12.3, 11.9 (^{i}Pr). MS (FAB$^+$/NBA) m/z: 459 ([M]$^+$).

4.3.7. 1,2-Dideoxy-2-(*N*-(*t*-butoxycarbonyl)piperazino)-3-*O*,5-*O*-(tetraisopropylidisiloxan-1,3-diyl)-D-ribofuranose (23). Yield: 240 mg (26%). ^{1}H NMR ($CDCl_3$) δ: 4.34 (app. t, J=5.9 Hz, 1H, H4), 3.97 (dd, J=11.9, 3.7 Hz, 1H, H1), 3.89–3.78 (m, 3H, H1, H2, H5), 3.74–3.69 (m, 1H, H5), 3.39 (t, J=4.7 Hz, 4H, H7), 3.39 (app. q, J=6.0 Hz, 1H, H3), 2.61–2.56 (m, 2H, H6), 2.43–2.37 (m, 2H, H6), 1.44 (s, 9H, H9), 1.07–0.91 (m 28H, ^{i}Pr); ^{13}C NMR ($CDCl_3$) δ: 154.9 (NCOO), 84.8 (C2), 79.7 (C(CH_3)$_3$), 75.5 (C4), 72.8 (C3), 67.6 (C1), 62.9 (C5), 50.5 (C6), 44.2 (br, C7), 28.8 ((C(CH_3)$_3$), 17.3, 17.1, 16.9, 16.8, 13.7, 12.9, 12.8, 12.3 (^{i}Pr). MS (FAB$^+$/NBA) m/z: 545 ([M]$^+$). Found C: 56.95 H: 9.54 N: 4.94; $C_{26}H_{52}N_2O_6Si_2$ requires C: 57.27 H: 9.62 N: 5.14.

4.3.8. 1,2-Dideoxy-2-(*N*-(*t*-butoxycarbonyl)piperazino)-3-*O*,5-*O*-(tetraisopropyldisiloxan-1,3-diyl)-D-arabinofuranose (24). Yield: 344 mg (37%). ^{1}H NMR ($CDCl_3$) δ: 4.43 (app. t, J=5.8 Hz, 1H, H4), 4.03–3.99 (m, 1H, H2), 3.98–3.92 (m, 2H, H5), 3.89–3.84 (m, 1H, H1), 3.70 (dd, J=10.9, 7.5 Hz, 1H, H1), 3.42 (br s, 4H, H7), 2.97 (app. q, J=6.6 Hz, 1H, H3), 2.85–2.82 (m, 2H, H6), 2.68–2.61 (m, 2H, H6), 1.47 (s, 9H, H9), 1.17–1.00 (m, 28H, ^{i}Pr); ^{13}C NMR ($CDCl_3$) δ: 154.8 (NCOO), 85.2 (C2), 79.5 (C(CH_3)$_3$), 75.1 (C4), 68.9 (C1), 66.4 (C3), 64.0 (C5), 51.0 (C6), 44.8 (br, C7), 28.7 ((C(CH_3)$_3$), 17.3, 17.1, 17.0, 16.9, 13.5, 12.9, 12.8, 12.5 (^{i}Pr). MS (FAB$^+$/NBA) m/z: 545

([M]$^+$). Found C: 56.98 H: 9.62 N: 4.98; $C_{26}H_{52}N_2O_6Si_2$ requires C: 57.27 H: 9.62 N: 5.14.

4.4. General purification procedure for compounds 25–34

Compounds **25**–**34** were purified by cation exchange chromatography on Amberlite CG-50 (NH_4^+-form) using 0–2% conc. aqueous NH_3 in H_2O (v/v) as eluent, and subsequently converted into their hydrochloride salts by addition of excess of 1.0 M aqueous HCl followed by lyophilization.

4.4.1. Allyl 2-amino-2,6-dideoxy-6-piperazino-β-D-glucopyranoside hydrochloride salt (25). Compound **12** (78 mg, 0.13 mmol) was dissolved in TFA (1 ml) and the mixture was stirred for 2 h at rt followed by evaporation under reduced pressure and coevaporation with toluene to afford a reddish gum which was dissolved in methanol saturated with NH_3 (5 ml) and stirred for 18 h at rt. The solvent was removed under reduced pressure and purification afforded compound **25** (28 mg, 51%) as a white solid material. ^{1}H NMR (D_2O) δ: 6.03–5.91 (m, 1H, H8), 5.35 (br. d, J=17.2 Hz, 1H, H9), 5.28 (br. d, J=10.3 Hz, 1H, H9), 4.41 (d, J=8.2 Hz, 1H, H1), 4.35 (dd, J=13.6, 6.8 Hz, 1H, H7), 4.20 (dd, J=12.5, 6.3 Hz, 1H, H7), 3.62 (app. t, J=9.3 Hz, 1H, H3), 3.35 (app. t, J=9.0 Hz, 1H, H4), 3.20 (app. t, J=9.1 Hz, 1H, H5), 2.95–2.84 (m, 5H, H2, H10) 2.65–2.51 (m, 6H, H6, H11); ^{13}C NMR (D_2O) δ: 134.0 (C8), 119.4 (C9), 102.5 (C1), 76.2 (C5), 74.0 (C3), 72.5 (C7), 71.4 (C4), 59.4 (C6), 57.0 (C2), 53.5 (C10), 44.4 (C11). HRMS (ESI) m/z: 288.1941 ([MH]$^+$, calculated 288.1923).

4.4.2. Allyl 2-amino-2,6-dideoxy-6-(*N*-methyl)piperazino-β-D-glucopyranoside hydrochloride salt (26). Compound **11** (50 mg, 0.10 mmol) was dissolved in methanol saturated with NH_3 (5 ml) and the mixture was stirred at rt for 18 h. The solvent was removed under reduced pressure and purification afforded compound **26** (35 mg, 84%) as a white solid material. ^{1}H NMR (D_2O) δ: 6.05–5.91 (m, 1H, H8), 5.35 (dd, J=17.2, 1.5 Hz, 1H, H9), 5.28 (br. d, J=10.3, 1.15 Hz, 1H, H9), 4.41 (d, J=8.3 Hz, 1H, H1), 4.35 (dd, J=12.6, 6.0 Hz, 1H, H7), 4.20 (dd, J=12.5, 6.3 Hz, 1H, H7), 3.61 (app. t, J=8.4 Hz, 1H, H3), 3.35 (dd, J=9.4, 7.6 Hz, 1H, H4), 3.20 (app. t, J=9.6 Hz, 1H, H5), 2.94 (d, J=13.8 Hz, 2H, H6) 2.77–2.51 (m, 9H, H2, H10, H11), 2.25 (s, 3H, H12); ^{13}C NMR (D_2O) δ: 134.0 (C8), 119.5 (C9), 102.5 (C1), 76.2 (C5), 74.1 (C3), 72.5 (C7), 71.5 (C4), 58.7 (C6), 57.0 (C2), 53.9 (C10), 52.8 (C11), 44.9 (C12). MS (ESI) m/z: 302 ([MH]$^+$, calculated 302). To verify the identity and purity of this product, a copy of the ^{13}C NMR spectrum was enclosed when submitting the revised manuscript.

4.4.3. 2-Piperazinoethyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride salt (27). Compound **17b** (37 mg, 0.06 mmol) was dissolved in TFA (1 ml) and the mixture was stirred for 2 h followed by evaporation under reduced pressure and coevaporation with toluene to afford a reddish gum which was dissolved in methanol saturated with NH_3 (5 ml) and stirred for 18 h at rt. The solvent was removed under reduced pressure and purification afforded compound **27** (17 mg, 71%) as a white solid material. ^{1}H NMR (CD_3OD) δ: 4.50 (d, J=8.4 Hz, 1H, H1), 3.73 (app. t, J=9.3 Hz, 1H, H3), 3.54–3.37 (m, 3H, H7, H4), 3.21 (app. t, J=9.0 Hz, 1H, H5), 2.97–2.87 (m, 5H, H2, H10), 2.72–2.68 (m 2H, H8), 2.65–2.51 (m, 6H, H6, H9); ^{13}C NMR (CD_3OD) δ: 102.0 (C1), 78.2 (C5), 75.1 (C3), 72.4 (C4), 67.8 (C7), 62.5 (C6), 58.1 (C2), 57.6 (C8), 51.1 (C9), 44.3 (C10). HRMS (ESI) m/z: 292.1893 ([MH]$^+$, calculated 292.1872).

4.4.4. 2-(*N*-Methylpiperazino)ethyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride salt (28). Compound **17a** (80 mg, 0.15 mmol) was dissolved in methanol saturated with NH_3 (5 ml) and the mixture was stirred for 18 h at rt. The solvent was removed under reduced pressure and the purification afforded compound **28** (42 mg, 66%) as a white solid material. ^{1}H NMR (CD_3OD) δ: 4.47 (d, J=8.2 Hz, 1H, H1), 3.63 (app. t, J=9.1 Hz, 1H, H3), 3.56–3.39 (m, 3H, H7, H4), 3.24 (app. t, J=9.0 Hz, 1H, H5), 3.00–2.87 (m, 5H, H2, H10), 2.75–2.69 (m, 2H, H8), 2.62–2.49 (m, 6H, H6, H9), 2.22 (s, 3H, H11); ^{13}C NMR (CD_3OD) δ: 101.8 (C1), 78.0 (C5), 75.4 (C3), 72.9 (C4), 67.6 (C7), 62.0 (C6), 58.0 (C2), 57.6 (C8), 53.3 (C10), 51.1 (C9), 45.8 (C11). HRMS (ESI) m/z: 306.2018 ([MH]$^+$, calculated 306.2029).

4.4.5. 2-Piperazinoethyl 2,6-diamino-2,6-dideoxy-β-D-glucopyranoside hydrochloride salt (29). Compound **18b** (100 mg, 0.17 mmol) was dissolved in a 1:1 mixture of abs. EtOH and dioxane (5 ml) and 20% $Pd(OH)_2$/C (20 mg) was added and the mixture was stirred under H_2 for 18 h at rt. The catalyst was filtered off through celite and the solution was concentrated. The residue was dissolved in TFA (1 ml) and the mixture was stirred for 2 h at rt followed by evaporation under reduced pressure and coevaporation with toluene to afford a reddish gum. This gum was dissolved in methanol saturated with NH_3 (5 ml) and the mixture was stirred for 18 h at rt. The solvent was removed under reduced pressure and purification afforded compound **29** (54 mg, 73%) as a white solid material. ^{1}H NMR (D_2O) δ: 4.62 (d, J=8.2 Hz, 1H, H1), 4.21–4.05 (m, 1H, H7), 3.83–3.70 (m, 1H, H7), 3.66 (app. t, J=9.3 Hz, 1H, H3), 3.60–3.37 (m, 6H, H4, H5, H6, H8), 3.30 (app. t, J=8.2 Hz, 1H, H2), 2.96–2.70 (m, 8H, H9, H10); ^{13}C NMR (D_2O) δ: 100.8 (C1), 74.9 (C5), 76.3 (C3), 71.9 (C4), 67.1 (C7), 66.2 (C6), 56.9 (C2), 56.5 (C8), 49.6 (C9), 43.3 (C10). HRMS (ESI) m/z: 291.2001 ([MH]$^+$, calculated 291.2032).

4.4.6. 2-(*N*-Methylpiperazino)ethyl 2,6-diamino-2,6-dideoxy-β-D-glucopyranoside hydrochloride salt (30). Compound **18a** (119 mg, 0.23 mmol) was dissolved in a 1:1 mixture of abs. EtOH and dioxane (5 ml), 20% $Pd(OH)_2$/C (20 mg) was added and the mixture was stirred under H_2 for 18 h at rt. The catalyst was filtered off through celite and the solution was concentrated. The residue was dissolved in methanol saturated with NH_3 (5 ml) and the resulting mixture was stirred for 18 h at rt. The solvent was removed under reduced pressure and purification afforded compound **30** (49 mg, 47%) as a white solid material. ^{1}H NMR (D_2O) δ: 4.72 (d, J=8.0 Hz, 1H, H1), 4.21–3.83 (m, 2H, H7), 3.64 (app. t, J=8.3 Hz, 1H, H3), 3.69–3.32 (m, 6H, H4, H5, H6, H8), 3.25 (app. t, J=7.9 Hz, 1H, H2), 2.91–2.71 (m, 8H, H9, H10), 2.29 (s, 3H, H11); ^{13}C NMR (D_2O) δ: 100.5 (C1), 75.2 (C5), 74.8 (C3), 71.5

(C4), 67.2 (C7), 66.2 (C6), 56.4 (C2), 56.1 (C10), 56.0 (C8), 50.5 (C9), 43.4 (C11). HRMS (ESI) *m/z*: 305.2173 ($[MH]^+$, calculated 305.2189).

4.4.7. 1,2-Dideoxy-2-(*N*-methylpiperazino)-D-ribofuranose hydrochloride salt (31). Compound **21** (34 mg, 0.07 mmol) was dissolved in THF (5 ml) and 1 M TBAF in THF (0.15 ml) was added and the solution was stirred for 2 h at rt. The solvent was removed under reduced pressure and purification afforded compound **31** (19 mg, 90%) as a white solid material. ^{1}H NMR (D_2O) δ: 4.40 (br. d, J= 7.0 Hz, 1H, H4), 4.30–4.22 (m, 2H, H1), 4.02–3.98 (m, 1H, H2), 3.90–3.62 (m, 3H, H3, H5), 3.59–3.40 (m, 8H, H6, H7), 2.29 (s, 3H, H8); ^{13}C NMR (D_2O) δ: 84.2 (C2), 72.1 (C4), 71.5 (C3), 66.6 (C1), 60.9 (C5), 53.5 (C7), 48.2 (C6), 45.2 (C8). HRMS (ESI) *m/z*: 217.1578 ($[MH]^+$, calculated 217.1552).

4.4.8. 1,2-Dideoxy-2-(*N*-methylpiperazino)-D-arabinofuranose hydrochloride salt (32). Compound **22** (21 mg, 0.04 mmol) was dissolved in THF (5 ml) and 1 M TBAF in THF (0.15 ml) was added and the solution was stirred for 2 h at rt. The solvent was removed under reduced pressure and purification afforded compound **32** (12 mg, 88%) as a white solid material. ^{1}H NMR (D_2O) δ: 4.70–4.59 (m, 1H, H4), 4.50–4.42 (m, 1H, H1), 4.32–4.22 (m, 1H, H1), 4.05–3.98 (m, 2H, H2, H3), 3.86–3.72 (m, 2H, H5), 3.57–3.37 (m, 8H, H6, H7), 2.25 (s, 3H, H8); ^{13}C NMR (D_2O) δ: 88.0 (C2), 70.0 (C4), 67.0 (C3), 66.1 (C1), 61.9 (C5), 53.1 (C7), 47.9 (C6), 45.1 (C8). HRMS (ESI) *m/z*: 217.1567 ($[MH]^+$, calculated 217.1552).

4.4.9. 1,2-Dideoxy-2-piperazino-D-ribofuranose hydrochloride salt (33). Compound **23** (200 mg, 0.37 mmol) was dissolved in TFA (5 ml) and the mixture was stirred for 2 h at rt followed by evaporation under reduced pressure and coevaporation with toluene which afforded a reddish gum. This gum was dissolved in THF (10 ml) and 1 M TBAF in THF (0.78 ml) was added and the solution was stirred for 2 h at rt. The solvent was removed under reduced pressure and purification afforded compound **33** (81 mg, 76%) as a white solid material. ^{1}H NMR (D_2O) δ: 4.50 (dd, J=7.2, 5.2 Hz, 1H, H4), 4.35–4.23 (m, 2H, H1), 4.09–4.03 (m, 1H, H2), 3.93–3.82 (m, 3H, H3, H5), 3.79–3.70 (m, 8H, H6, H7); ^{13}C NMR (D_2O) δ: 84.9 (C2), 72.6 (C4), 71.9 (C3), 66.3 (C1), 60.3 (C5), 48.2 (C6), 41.2 (C7). HRMS (ESI) *m/z*: 203.1370 ($[MH]^+$, calculated 203.1396).

4.4.10. 1,2-Dideoxy-2-piperazino-D-arabinofuranose hydrochloride salt (34). Compound **24** (242 mg, 0.44 mmol) was dissolved in TFA (5 ml) and the mixture was stirred for 2 h at rt followed by evaporation under reduced pressure and coevaporation with toluene which afforded a reddish gum. This gum was dissolved in THF (10 ml) and 1 M TBAF in THF (0.88 ml) was added and the solution was stirred for 2 h at rt. The solvent was removed under reduced pressure and purification afforded compound **34** (106 mg, 82%) as a white solid material. ^{1}H NMR (D_2O) δ: 4.60–4.50 (m, 1H, H4), 4.45–4.42 (m, 1H, H1), 4.21–4.18 (m, 1H, H1), 4.11–4.02 (m, 2H, H2, H3), 3.83–3.74 (m, 2H, H5), 3.69–3.60 (m, 8H, H6, H7); ^{13}C NMR (D_2O) δ: 88.2 (C2), 69.0 (C4), 67.1 (C3), 66.3 (C1), 61.7 (C5), 48.8 (C6), 40.8 (C7). HRMS (ESI) *m/z*: 203.1367 ($[MH]^+$, calculated 203.1396).

4.5. Chemotherapeutical testing

The antibacterial and antifungal screening was carried out according to the agar dilution test as described earlier.[27a] The activity was determined against a gram-positive coccus including *Staphylococcus aureus* ATCC 6538, a gram-positive bacillus including *Bacillus cereus* ATCCC 14579, gram-negative enteric bacilli including *Escherichia coli* ATCCC 8739, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 15442 and *Salmonella typhimurium* ATCC 13311, the yeast *Candida albicans* ATCC 10231 and the fungus *Aspergillus niger* ATCC 16404. The antiviral activity was determined according to the end point titration technique (EPTT) as described earlier.[27b] The following viruses were used: Herpes simplex virus (type 1 (HSV-1), Coxsackie B_2, virus (Cox B_2), Measles Edmondston A, poliomyelitis virus type 1, Semliki forest virus L10, and vesicular stomatitis virus (VSV). The antiviral activity against immunodeficiency virus type 1 (HIV-1) was evaluated in a microtiter assay as reported before.[27c]

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