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# Synthetic access to spacer-linked 3,6-diamino-2,3,6-trideoxy-α-D-glucopyranosides—potential aminoglycoside mimics for the inhibition of the HIV-1 TAR-RNA/Tat-peptide complex

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**Abstract**—The synthesis of spacer-linked neoaminoglycoside **5** is described. Key steps of the synthesis are the introduction of nitrogen functionalities at C-3 and C-6 and the olefin cross metathesis of allyl glycoside **16**. Although it is known that Grubbs catalysts tolerate nitrogen functionalities, difficulties were encountered in the cross metathesis reaction. Factors that govern this dimerization are the steric and electronic demands of the catalyst and the substrate. Preliminary biological evaluation of homodimer **5**, by studying the inhibition of HIV-1 TAR-RNA/Tat-peptide complex using a method based on fluorescence titration, revealed an inhibitory effect of **5**.


*Keywords:* Olefin metathesis; Aminoglycosides; Antibiotics; Azidosugars

## 1. Introduction

Small molecule natural products and natural product-like analogues have aided in understanding the role and function of many biomacromolecules critical to the progression and maintenance of the cell cycle. The identification of the direct target of such small molecules gives investigators a tool to control a specific aspect of the cell cycle.[1] Aminoglycoside antibiotics like neomycin B (**1**), streptomycin (**2**), and analogues derived from them are highly potent antibiotics. By interacting with the 16S subunit of the bacterial ribosome they interfere with the fidelity of translation and translocation, which ultimately disrupts protein biosynthesis of bacteria and causes miscoding.[2] The structural basis of the recognition process has been determined.[3] Recently, many efforts of different research groups were dedicated to the synthesis of aminoglycoside antibiotics and derivatives as well as mimetics that are able to selectively bind to RNA. In this context, a wide range of modified aminoglycosides, in which substitution[4] of one or more carbohydrates[5] was systematically varied, have been prepared. Furthermore, orthogonally protected sugar diamino acids have been exploited as building blocks in the synthesis of linear and branched aminoglycoside derivatives.[6] Some of these efforts also demonstrated that synthetic derivatives of natural aminoglycosides can exert improved antibacterial performance while targeting resistance-causing enzymes at the same time (Fig. 1).[7]

Recently, we described the first preparation of novel macrocyclic 1,4-butanediol-linked aminodeoxyglycosides **3** and **4**.[8] The coupling of the aminodeoxyhexose units was achieved by olefin metathesis of appropriately allylated aminosugar precursors and finally, in the case of macrocycle **4**, by ring closing metathesis (RCM).

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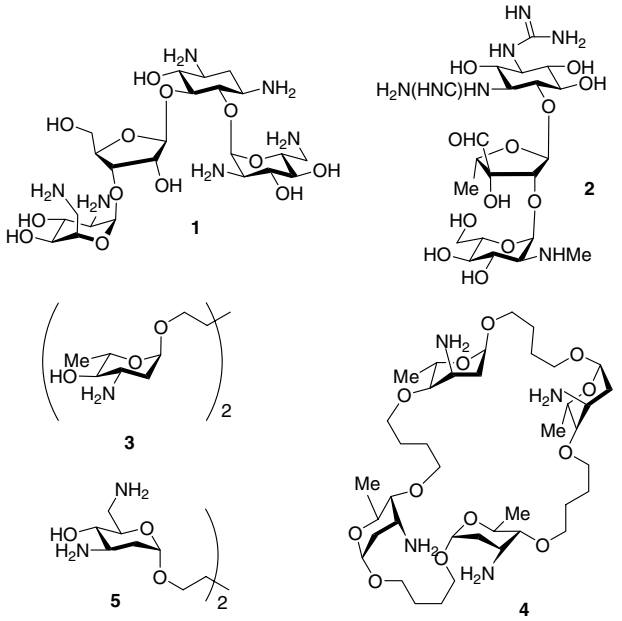


**Figure 1.** Structures of neomycin (**1**), streptomycin (**2**), linear and macrocyclic neoaminoglycosides **3** and **4** and target neoaminoglycoside **5**.

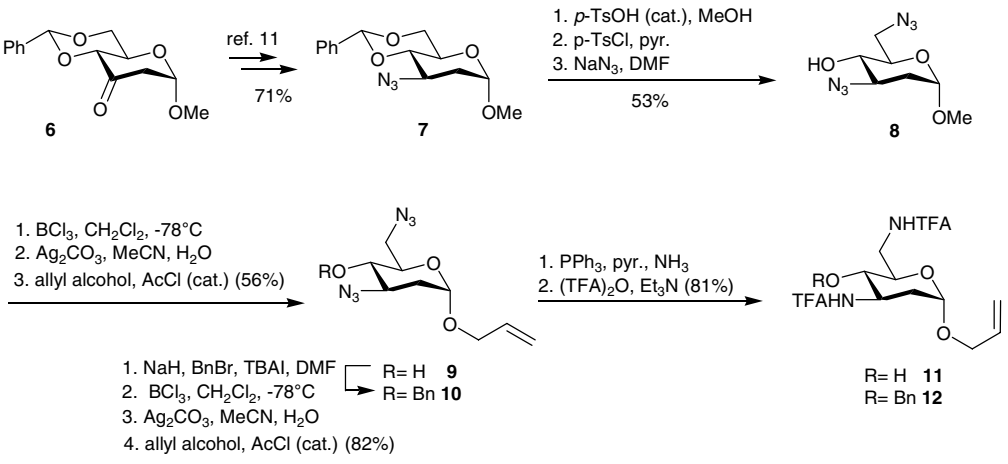
Dot blot binding experiments of **4** with TAR-RNA in the presence of the Tat protein and multi-dimensional heteronuclear NMR spectroscopic studies on the complex of HIV-2 TAR-RNA revealed that the novel macrocyclic aminoglycoside analogue simultaneously contacts the crucial residues of the bulge required for Tat binding and the A35 of the dynamic hexanucleotide loop.[9] This novel mode of binding, together with the easy accessibility of new aminoglycoside antibiotic mimetics, revealed that glycomimetics of type **3** and **4** are biologically and pharmaceutically interesting synthetic targets. From these structural studies we also concluded that additional amino groups in **4** should lead to an increased number of contact points with the RNA, hence leading to even tighter interaction.

Indeed, aminoglycoside antibiotics like **1** and **2** often contain more than one nitrogen functionality per hexose or cyclitol unit. All mimetics that we have prepared so far, including **3** and **4**, bear only one amino group per hexose unit. En route to new amino-rich macrocycles like **4**, we therefore first investigated synthetic routes to access spacer-linked glycomimetics like **5** that contain a larger density of functionalities with nitrogen. The synthetic challenge was to deal with more than one N-functionality while employing an olefin cross metathesis as the key step to combine the subunits.

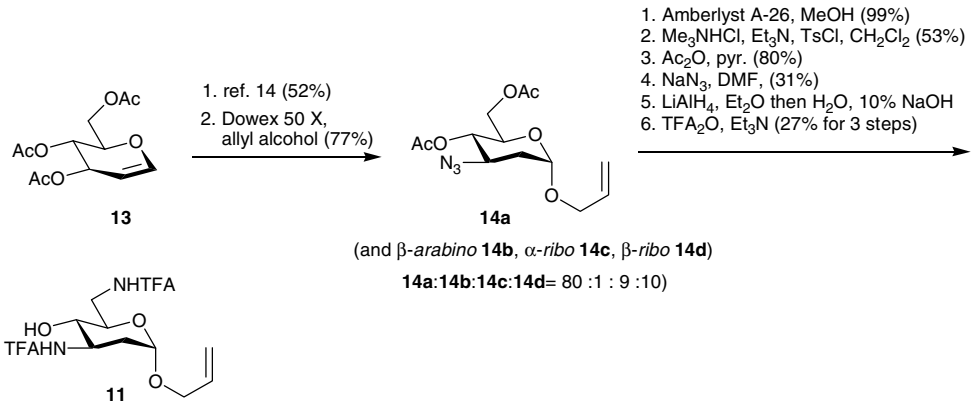
## 2. Results and discussion

The syntheses of neoaminoglycoside **5** commenced with the three step transformation of D-ulose **6** (available from D-mannose[10]) to the 3-azido-3-deoxy-glycoside **7** (Scheme 1).[11] Another three steps (removal of benzylidene protection, 6-O-tosylation and introduction of azido group) led to 3,6-bisazido glycoside **8**. At this point, we introduced the allyl moiety by first transforming the methyl glycoside into the glycosyl chloride, followed by hydrolysis and glycosidation in the presence of allyl alcohol, which furnished allyl glycoside **9**.[12] Transformation of the methyl glycoside into the free sugar by employing different Brønsted and Lewis acids, as well as direct conversion of the glycosyl chloride into the desired product via glycosidation, resulted in poor yields. Staudinger reaction employing the conditions first described by Letsinger and co-workers[13] yielded the corresponding diamine, which was directly transformed into bis(trifluoroacetamide) **11** in good yield for both steps. Alternatively, we also prepared the 4-*O*-benzyl protected derivative **12** via bisazido glycoside **10**.

An alternate synthetic approach, which is depicted in Scheme 2, started from tri-*O*-acetyl glucal **13**. It is well established that glycals can be converted into 3-azido-3-deoxy hexoses in aqueous acetic acid using sodium



**Scheme 1.** Preparation of allyl glycosides **9**–**12**.




**Scheme 2.** Alternate preparation of allyl glycoside **11** from glycal **13**.

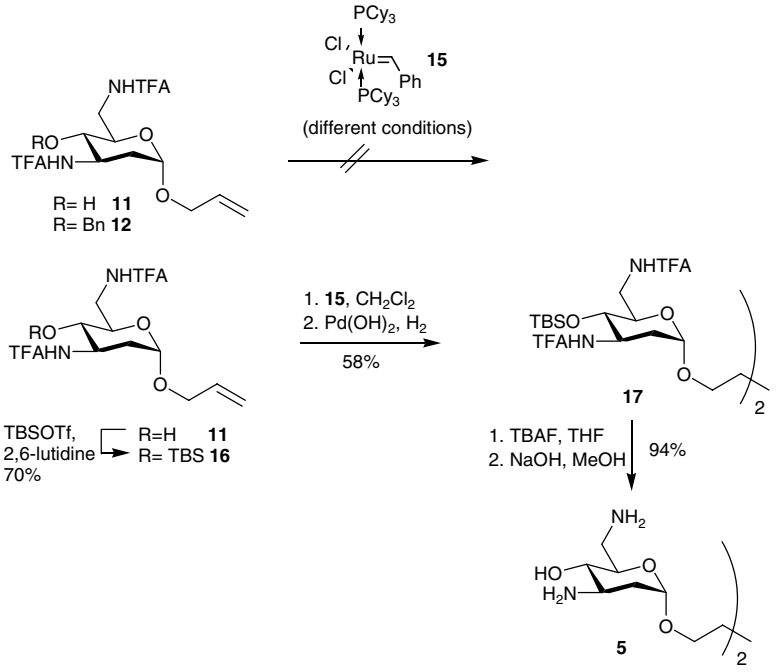
azide as the nitrogen source.[14] The mixture of stereoisomers was directly transformed into the corresponding allyl glycosides **14**, which could be separated by column chromatography. From here, the synthesis followed a similar route as described in Scheme 1. Complete de-O-acylation allowed us to introduce the tosyl group at C-6. Exchange by azide gave the bisazido compound, which is a 4-O-acylated analogue of glycoside **9**. Transformation into bisamide **11** was carried out under reductive conditions, which turned out to be less efficient compared to the Staudinger reaction. We ascribe this observation to severe difficulties when removing inorganic materials derived from lithium aluminium hydride after the reduction step.

With allyl glycosides **11** and **12** in hand, we approached the dimerization step. Earlier work has demonstrated that a conventional approach, which involves glycosylation of a spacer diol with suitable glycosyl donors, very inefficiently (low yield, mixture of anomers) leads to spacer-linked disaccharides.[8e] Although many ruthenium-based complexes for promoting olefin metathesis are available, only the first generation Grubbs catalyst **15** is suited to efficiently promote this transformation. The more reactive precatalysts (Grubbs II or Hoveyda-type complexes) tend to catalyze double bond migration yielding the corresponding enol ethers instead.[8,15]

To our surprise, allyl glycoside **11**, as well as the 4-*O*-benzyl derivative, did not furnish metathesis products under varying conditions (**12**, $CH_2Cl_2$/MeCN, 45 °C, starting material; **12**, benzene, 50 °C, starting material; **12**, $CH_2Cl_2$, 50 °C, decomp.; **11**, benzene, 80 °C, decomp.) (Scheme 3). Only after O-silylation of the hydroxyl group at C4 did the resulting allyl glycoside **16** undergo cross metathesis under conventional reaction conditions. The product was subjected to hydrogenation conditions to afford 1,4-butanediol linked homodimer **17**. The target aminoglycoside mimetic **5** was collected in excellent yield after removal of all protecting groups. At this point, it should be mentioned that direct dimerization of the intermediate chloro 4-*O*-benzyl-2,3,6-trideoxy-3,6-trifluoroacetamido-α/β-*arabino*-hexopyranoside with 1,4-butanediol in the presence of silver triflate only yielded about 30% of the desired homodimer, which was part of a complex reaction mixture. A similar result was obtained for the corresponding acetimidate.

Preliminary biological evaluation of homodimer **5** was conducted by studying the inhibition of HIV-1 TAR-RNA/Tat-peptide complex in the presence of homodimer **5**. We established a method based on fluorescence titration[16,17] by using the arginine-rich motif of the Tat-peptide composed of the nine amino acids, 49–58, which was labelled with rhodamine green. A solution of this peptide in the presence of **5** (60 μmol) was titrated with unlabelled TAR-RNA. The results collected from the fluorescence-titration measurements are listed in Figure 2. By increasing the amount of homodimer **5** with respect to the Tat-peptide, a strong signal reduction caused by homodimer **5** was encountered, which clearly reveals a pronounced inhibitory effect of **5** on the Tat/TAR-RNA complex. This preliminary result, along with our previous work,[9] is an accordance with Tor's rationale, as to why compounds should contain flexible linkers and rigid domains when interacting with RNAs in general.[18] For example, when two moderate or good RNA binders are covalently linked, the ribozyme inhibitory activity of the derivate surpasses that of any natural aminoglycoside antibiotic.[19] In principle, if two binding sites are in close proximity, a dimeric derivative can bind simultaneously to the two sites, resulting in stronger binding affinity.

In conclusion, we describe the first preparation of aminoglycoside mimetic **5**, which contains two 2,3,6-trideoxy-3,6-diamino glycoside units. Unlike analogues with only one amino group per unit such as homodimer **18**,[17,20] homodimer **5** showed an inhibitory effect on the Tat/TAR-RNA complex. Detailed studies are underway in our laboratories to structurally study this inhibitory effect[9] and to create macrocyclic derivatives of **5** for further in vitro testing.




**Scheme 3.** Olefin metathesis reactions.

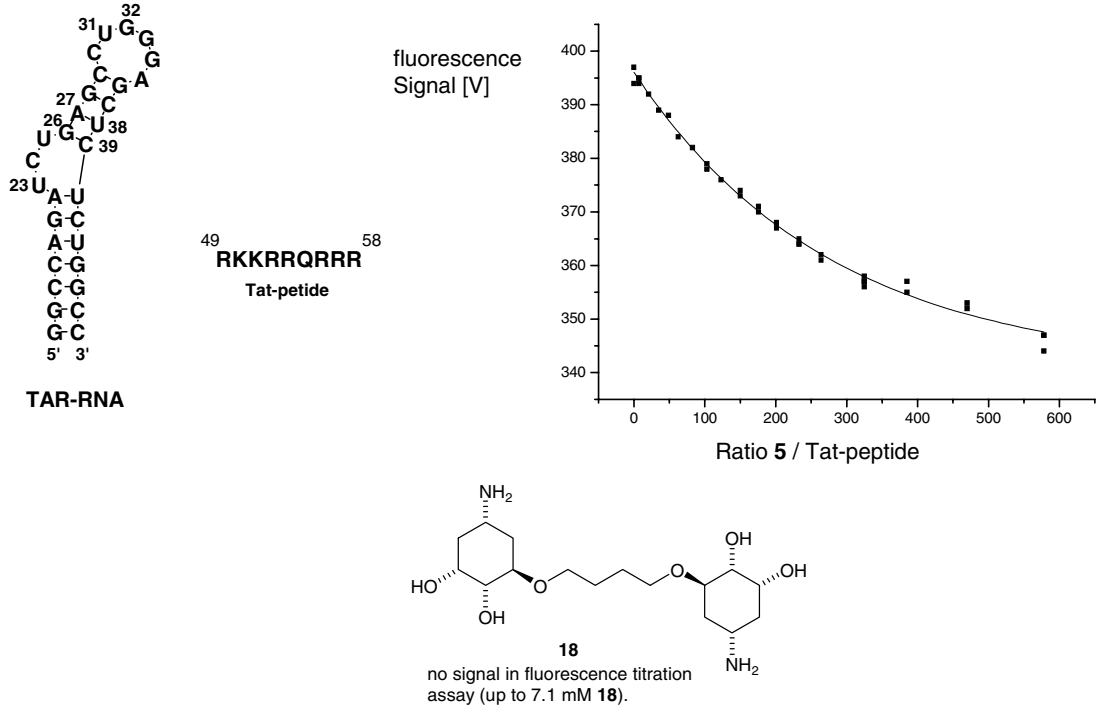



**Figure 2.** Structures of TAR-RNA and Tat-peptide; effect of dimer **5** on Tat/TAR-complex, determined by fluorescence titration (200 nM TAR-RNA, 100 nM Tat-peptide; maximum concentration of **5** = 60 μM); structure of homodimer **18**.

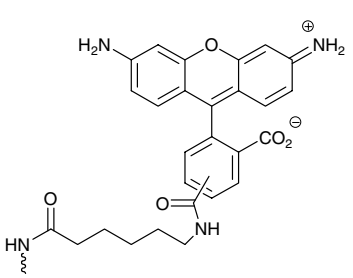
## 3. Experimental

### 3.1. General methods

$^{1}$H NMR, $^{13}$C NMR and $^{1}$H,$^{13}$C–COSY as well as NOESY spectra were measured on Avance 200/DPX (Bruker) with 200 MHz (50 MHz), Avance 400/DPX (Bruker) with 400 MHz (100 MHz) and Avance 500/DRX (Bruker), respectively, using tetramethylsilane as the internal standard. Unless otherwise noted, $CDCl_3$ is the solvent for all NMR experiments. Multiplicities are described using the following abbreviations: s = singlet,

d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Chemical shift values of $^{13}C$ NMR spectra are reported as values in parts per million relative to residual $CHCl_3$ (77 ppm) or $CD_3OD$ (49 ppm) as internal standards. The multiplicities refer to the resonances in the off-resonance spectra and were elucidated using the distortionless enhancement by polarization transfer (DEPT) spectral editing technique, with secondary pulses at 90° and 135°. Multiplicities are reported using the following abbreviations: s = singlet (due to quaternary carbon), d = doublet (methine), q = quartet (methyl), t = triplet (methylene). Mass spectra were recorded on a type LCT-spectrometer (Micromass) and on a type VG autospec (Micromass). Ion mass (*m*/*z*) signals are reported as values in atomic mass units followed, in parentheses, by the peak intensities relative to the base peak (100%). Optical rotations [α] were collected on a Polarimeter 341 (Perkin Elmer) at a wavelength of 589 nm and are given in $10^{-1}$ deg cm$^2$ g$^{-1}$. Combustion analyses were performed at the Institut für Organische Chemie, Leibniz Universität Hannover. The fluorescence titration was measured with a FP-6500 spectral photometer of JASCO. All solvents used were of reagent grade and were further dried. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 $F^{254}$ (E. Merck, Darmstadt) and spots were detected either by UV-absorption or by charring with $H_2SO_4$/4-methoxybenzaldehyde in MeOH. Preparative column chromatography was performed on silica gel 60 (E. Merck, Darmstadt). D-Glucal **13** is commercially available. D-Ulose **6** was prepared according to Ref. 10. The synthesis of homodimer **18** is described in Ref. 20. The *trans-activation-response* (TAR) fragment, used for the fluorescence-titration assay, was obtained as a non labelled oligonucleotide (31 nucleotides: 5′-GGC CAG AUC UGA GCC UGG GAG CUC GGC C-3′) from Purimex (Grebenstein, Germany). The extinction coefficient was 192,300 L/(mol cm) at 260 nm. The fluorescence-labelled Tat-peptide (sequence: RhoG-DOA-RKKR RQRRR-COOH; rhodamine green (RhoG) as fluorophore, attached via a linker with the peptide) was obtained as hydrochloride from NMI-Peptides (Reutlingen, Germany) (95% HPLC purity; MW = 1840 g/mol; extinction coefficient 74,000 L/(mol cm) at 530 nm).

The fluorophore RhoG showed an excitation wavelength at 504 nm and an emission wavelength at 530 nm.




### 3.2. 1′,4′-*O*-Di-(3,6-diamino-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside)-1,4-butane (5)

To a solution of 1′,4′-*O*-Di-(4-*tert*-butyldimethylsilyl-2,3,6-trideoxy-3,6-trifluoroacetamido-α-D-*arabino*-hexopyranoside)-1,4-butane (**17**) (40 mg, 40 μmol) in THF (1 mL) was added TBAF (37 mg, 118 μmol). The reaction mixture was stirred at room temperature for 2 h, preadsorbed on silica gel and purified by column chromatography over silica gel using petroleum ether–EtOAc (v/v = 4/1). The product was dissolved in MeOH (1 mL) and aq NaOH (2 mL, 1 M) was added. The reaction mixture was stirred at room temperature for 3 h. The solvent was removed in vacuo and the crude product was purified by column chromatography on reversed phase silica gel using $H_2O$–MeOH (v/v = 1/0 to 0/1) as eluent. Title compound **5** was obtained as a colourless powder (28 mg, 94%): $[\alpha]_D^{22}$ +18.5 (*c* 1.0, $H_2O$); $^1H$ NMR (400 MHz, MeOD, MeOD = 3.31 ppm): δ 1.45–1.55 (m, 1H, 2-H), 1.62–1.71 (m, 2H, $OCH_2CH_2$–H), 1.96–2.03 (m, 1H, 2-H), 2.60 (dd, *J* = 13.8, 7.5 Hz, 1H, 4-H), 2.93–3.15 (m, 3H, 3-H, 6-H), 3.36–3.49 (m, 2H, 5-H, $OCH_2CH_2$–H), 3.68–3.74 (m, 1H, $OCH_2CH_2$–H), 4.95 (d, *J* = 2.6 Hz, 1H, 1-H); $^{13}C$ NMR (100 MHz, MeOD, MeOD = 49.0 ppm): δ 24.2 (t, C–$OCH_2CH_2$), 27.7 (t, C-2), 38.4 (t, C-6), 44.0 (d, C-3), 68.1 (t, C–$OCH_2CH_2$), 74.1 (d, C-5), 75.5 (d, C-4), 98.2 (d, C-1); HRMS *m*/*z* calcd for $C_{16}H_{34}N_4O_6$ $[M+Na]^+$: 401.2376. Found: 401.2376.

### 3.3. Methyl 3-azido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-*arabino*-hexopyranoside (7)

To a solution of methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*erythro*-hexopyranoside-3-ulose (**6**) (5.7 g, 22 mmol) in dry MeOH (200 mL) was added sodium borohydride (1.65 mg, 43.7 mmol) at 0 °C. The suspension was stirred at room temperature for 2 h. $H_2O$ (50 mL) and $CH_2Cl_2$ were added, the phases were separated and the organic phase was washed with satd aq $NaHCO_3$, dried with $MgSO_4$ and concentrated in vacuo. The crude product was dissolved in $CH_2Cl_2$ (30 mL) and added dropwise to a solution of trifluoromethanesulfonic anhydride (5.0 mL, 29.5 mmol) in dry pyridine (14 mL). The reaction mixture was stirred at room temperature for 45 min and then poured into ice cold satd aq $NaHCO_3$. The aqueous phase was extracted with $CH_2Cl_2$, the organic phase was dried with $MgSO_4$ and concentrated in vacuo. The red residue was dissolved in dry DMF (100 mL). Sodium azide (5.5 g, 84.7 mmol) was added and the reaction mixture was stirred for 1.5 h at room temperature. The reaction was quenched with $H_2O$. The aqueous layer was extracted with $Et_2O$, the combined organic phases were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography over silica gel using petroleum ether–

EtOAc (v/v = 4/1) as eluent. Title compound **7** was obtained as a colourless syrup (4.5 g, 71%): $^1$H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm): $\delta$ 1.72 (ddd, $J$ = 13.5, 12.0, 3.5 Hz, 1H, 2-H$_{ax}$), 2.17 (ddd, $J$ = 13.5, 5.0, 1.0 Hz, 1H, 2-H$_{eq}$), 3.35 (s, 3H, OC$H_3$–H), 3.56 (t, $J$ = 9.4 Hz, 1H, 4-H), 3.76 (t, $J$ = 10.2 Hz, 1H, 6-H$_{ax}$), 3.88 (ddd, $J$ = 10.2, 9.4, 4.5 Hz, 1H, 5-H), 4.06 (ddd, $J$ = 12.0, 9.4, 5.0 Hz, 1H, 3-H), 4.30 (dd, $J$ = 10.2, 4.5 Hz, 1H, 6-H$_{eq}$), 4.78 (d, $J$ = 3.5 Hz, 1H, 1-H), 5.63 (s, 1H, PhC$H$–H), 7.31–7.54 (m, 5H, Ph–H); $^{13}$C NMR (100 MHz, $CDCl_3$, $CDCl_3$ = 77 ppm): $\delta$ 35.51 (t, 2-C), 54.80 (q, O$C$H$_3$–C), 56.37 (d, 3-C), 63.15 (d, 5-C), 69.06 (t, 6-C), 82.08 (d, 4-C), 98.14 (d, 1-C), 101.54 (d, Ph$C$H–C), 125.97 (d, Ph–C), 128.24 (d, Ph–C), 128.96 (s, Ph–C), 137.08 (s, Ph–C). $^1$H and $^{13}$C NMR spectral data matched those reported before.[8d]

### 3.4. Methyl 3,6-azido-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (8)

**3.4.1. Methyl 3-azido-2,3-dideoxy-α-D-*arabino*-hexopyranoside.** To a solution of methyl 3-azido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-*arabino*-hexopyranoside (**7**) (300 mg, 1.0 mmol) in MeOH (20 mL) was added *p*-toluenesulfonic acid (20 mg, 0.1 mmol). The reaction was stirred at room temperature for 5 h. $Et_3N$ (1 mL) was added, the reaction mixture was concentrated in vacuo and the residue was purified by column chromatography over silica gel using petroleum ether–EtOAc (v/v = 4/1) as eluent. Title compound was obtained as a yellow oil (163 mg, 80%): $^1$H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm): $\delta$ 1.64 (td, $J$ = 12.7, 3.4 Hz, 1H, 2-H), 2.11 (dd, $J$ = 12.7, 4.4 Hz, 1H, 2-H), 3.32 (s, 3H, MeO–H), 3.50 (t, $J$ = 9.0 Hz, 1H, 4-H), 3.57 (dt, $J$ = 9.6, 3.4 Hz, 1H, 5-H), 3.74–3.86 (m, 3H, 3-H, 6-H), 4.77 (d, $J$ = 3.1 Hz, 1H, 1-H); $^{13}$C NMR (100 MHz, $CDCl_3$, $CDCl_3$ = 77.2 ppm): $\delta$ 34.8 (t, C-2), 54.7 (q, C–OMe), 60.1 (d, C-3), 61.9 (t, C-6), 70.4 (d, C-4), 71.5 (d, C-5), 97.7 (d, C-1). $^1$H and $^{13}$C NMR spectral data matched those reported before.[21]

**3.4.2. Methyl 3,6-azido-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (8).** To a solution of methyl 3-azido-2,3-dideoxy-α-D-*arabino*-hexopyranoside described above (138 mg, 0.68 mmol) in dry pyridine (2 mL) was added *p*-toluenesulfonyl chloride (142 mg, 0.75 mmol) and the reaction mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo, EtOAc was added and washed successively with 1 N HCl and satd aq $NaHCO_3$. The organic phase was dried ($Na_2SO_4$) and concentrated in vacuo. The crude product was dissolved in DMF (2 mL) and sodium azide (221 mg, 3.4 mmol) was added. The reaction mixture was stirred at 50 °C for 20 h. $H_2O$ was added and the aqueous phase was extracted (3×) with EtOAc. The organic phase was dried with $MgSO_4$, concentrated in vacuo and purified by column chromatography over silica gel using petroleum ether–EtOAc (v/v = 6/1) as eluent. Title compound **8** was obtained as a colourless syrup (104 mg, 67%): $[\alpha]_D^{22}$ +139.4 (*c* 1.0, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm): $\delta$ 1.73 (td, $J$ = 12.7, 3.5 Hz, 1H, 2-H), 2.18 (ddd, $J$ = 13.2, 4.9, 1.1 Hz, 1H, 2-H), 3.03 (s, 1H, OH–H), 3.38 (s, 3H, OMe–H), 3.39 (t, $J$ = 9.7 Hz, 1H, 4-H), 3.48 (dd, $J$ = 13.1, 5.5 Hz, 1H, 6-H), 3.56 (dd, $J$ = 13.1, 2.6 Hz, 1H, 5-H), 3.73–3.81 (m, 2H, 3-H, 6-H), 4.82 (d, $J$ = 3.0 Hz, 1H, 1-H); $^{13}$C NMR (100 MHz, $CDCl_3$, $CDCl_3$ = 77.2 ppm): $\delta$ 34.5 (t, C-2), 51.3 (t, C-6), 54.8 (q, C–OMe), 60.3 (d, C-3), 70.8 (d, C-5), 70.9 (d, C-4), 97.5 (d, C-1); HRMS *m*/*z* calcd for $C_7H_{12}N_6O_3$ $[M]^-$: 227.0893. Found: 227.0897.

### 3.5. Allyl 3,6-azido-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (9)

A solution of methyl 3,6-azido-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (**8**) (200 mg, 0.88 mmol) in $CH_2Cl_2$ (2 mL) was cooled to −78 °C and treated with $BCl_3$ (1.1 mL, 1.1 mmol, 1 M in hexanes). The reaction mixture was stirred for 1 h while slowly warming to room temperature. It was cooled to −78 °C again and treated with $H_2O$. The aqueous phase was extracted with $CH_2Cl_2$ (3×) and the combined organic phases were concentrated in vacuo. The crude product was dissolved in acetonitrile (4.5 mL), treated with $H_2O$ (1 mL) and $Ag_2CO_3$ (728 mg, 2.6 mmol) and stirred at room temperature for 24 h. The reaction mixture was diluted with $Et_2O$ and $Na_2SO_4$ was added. After filtration, the solution was concentrated in vacuo and allyl alcohol (4.8 mL) was added, followed by the addition of acetyl chloride (150 μL). The reaction mixture was stirred at 50 °C for 16 h, treated with $Et_3N$ and purified by column chromatography on silica gel using petroleum ether–EtOAc (v/v = 6/1) as eluent. Title compound **9** was obtained as a colourless oil (40 mg, 56%): $[\alpha]_D^{22}$ +95.2 (*c* 1.0, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$, $CDCl_3$ = 7.26 ppm): $\delta$ 1.76 (ddd, $J$ = 13.0, 12.5, 3.5 Hz, 1H, 2-H), 2.21 (ddd, $J$ = 13.0, 5.0, 1.0 Hz, 1H, 2-H), 3.42 (t, $J$ = 9.5 Hz, 1H, 4-H), 3.48 (dd, $J$ = 13.1, 5.6 Hz, 1H, 6-H), 3.55 (dd, $J$ = 13.1, 2.6 Hz, 1H, 6-H), 3.78–3.86 (m, 2H, 5-H, 3-H), 3.99 (ddt, $J$ = 12.9, 6.1, 1.3 Hz, 1H, $CH_2$=CH–C$H_2$–H), 4.18 (ddd, $J$ = 12.9, 5.2, 1.5 Hz, 1H, $CH_2$=CH–C$H_2$–H), 4.98 (d, $J$ = 3.0 Hz, 1H, 1-H), 5.23 (dq, $J$ = 10.2, 1.2 Hz, 1H, C$H_2$=CH–CH$_2$–H), 5.32 (dd, $J$ = 17.2, 1.5 Hz, 1H, C$H_2$=CH–CH$_2$–H), 5.86–5.96 (m, 1H, $CH_2$=C$H$–CH$_2$–H); $^{13}$C NMR (100 MHz, $CDCl_3$, $CDCl_3$ = 77.2 ppm): $\delta$ 29.7 (t, C-2), 34.6 (t, C-6), 51.5 (d, C-3), 60.4 (d, C-5), 68.1 (t, C–$CH_2$=CH–$CH_2$), 71.1 (d, C-4), 95.6 (d, C-1), 117.7 (t, C–$CH_2$=CH–CH$_2$), 133.5

(d, C–$CH_2$=*C*H–$CH_2$); HRMS *m*/*z* calcd for $C_9H_{14}N_6O_3$ $[M]^-$: 253.1049. Found: 253.1057.

### 3.6. Allyl 2,3,6-trideoxy-3,6-trifluoroacetamido-α-D-*arabino*-hexopyranoside (11)

A solution of allyl 3,6-azido-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (**9**) (46 mg, 0.18 mmol) in dry pyridine (1 mL) was treated with $PPh_3$ (190 mg, 0.72 mmol) and stirred at room temperature for 1 h. $NH_3$ (105 μL, 1.45 mmol; 28% in $H_2O$) was added and the reaction mixture was allowed to stand at room temperature for 16 h. The solvents were removed in vacuo, the crude product was dried by azeotropic distillation with toluene and the dry crude product was dissolved in $CH_2Cl_2$ (1 mL). $Et_3N$ (2.9 mmol, 0.4 mL) and trifluoroacetic anhydride (1.45 mmol, 0.2 mL) were added and stirred at room temperature for 3 h. MeOH was added, the reaction mixture was preadsorbed on silica and purified by column chromatography on silica gel using petroleum ether–EtOAc (v/v = 2/1) as eluent. Title compound **11** was obtained as colourless oil (20 mg, 81%): $^1$H NMR (400 MHz, MeOD, MeOD = 3.31 ppm): δ 1.77 (td, *J* = 12.8, 3.5 Hz, 1H, 2-H), 1.98 (d, *J* = 4.8 Hz, 1H, 2-H), 3.23–3.28 (m, 1H, 4-H), 3.39 (dd, *J* = 13.9, 8.5 Hz, 1H, 6-H), 3.68–3.75 (m, 2H, 6-H, 5-H), 3.91 (ddt, *J* = 12.9, 5.9, 1.3 Hz, 1H, $CH_2$=CH–C*H*$_2$–H), 4.08 (ddd, *J* = 12.9, 5.3, 1.4 Hz, 1H, $CH_2$=CH–C*H*$_2$–H), 4.21 (ddd, *J* = 12.3, 10.0, 4.5 Hz, 1H, 4-H), 4.88 (d, *J* = 2.9 Hz, 1H, 1-H), 5.13 (dd, *J* = 10.4, 1.6 Hz, 1H, C*H*$_2$=CH–$CH_2$–H), 5.24 (dd, *J* = 17.3, 1.7 Hz, 1H, C*H*$_2$=CH–$CH_2$–H), 5.85 (ddt, *J* = 17.0, 10.9, 5.5 Hz, 1H, $CH_2$=C*H*–$CH_2$–H); $^{13}$C NMR (100 MHz, MeOD, MeOD = 49.0 ppm): δ 36.0 (t, C-2), 41.1 (t, C-6), 50.4 (d, C-3), 68.7 (t, C–$CH_2$=CH–*C*$H_2$), 71.6 (d, C-4), 72.3 (d, C-5), 96.8 (d, C-1), 117.4 (t, C–*C*$H_2$=CH–$CH_2$), 117.5 (q, *J* = 287.0 Hz, C–$CF_3$), 117.6 (q, *J* = 286.6 Hz, C–$CF_3$), 135.4 (d, C–$CH_2$=*C*H–$CH_2$), 159.0 (q, *J* = 36.8 Hz, C–$CF_3$*C*O), 159.2 (q, *J* = 36.9 Hz, C–$CF_3$*C*O); HRMS *m*/*z* calcd for $C_{13}H_{16}F_6N_2O_5$ $[M+Na+MeCN]^+$: 458.1135. Found: 459.1127.

### 3.7. Allyl 2,3,6-trideoxy-3,6-trifluoroacetamido-α-D-*arabino*-hexopyranoside (11)

**3.7.1. Allyl 3-azido-4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*arabino*-hexopyranoside (14a).** To a suspension of glycal (**13**) (100 g, 0.37 mol) in $H_2O$ (430 mL) were added glacial acetic acid (95 mL) and sodium azide (47.7 g, 0.74 mol). The mixture was stirred at 80 °C for 5 h and then cooled to room temperature. Satd aq $NaHCO_3$ was added and the aqueous phase was extracted with EtOAc (3×). The combined organic phases were dried ($MgSO_4$) and concentrated in vacuo. The mixture was filtered through silica gel and directly used for the next step. A suspension of the crude material (68 g) was refluxed in allyl alcohol (300 mL) for 30 min, filtered off and washed with abs. allyl alcohol several times. The crude product was dissolved in allyl alcohol (680 mL) and treated with the Dowex® 50X8 at 90 °C for 2 h. The reaction mixture was filtered and the ion exchange resin was washed several times with $CH_2Cl_2$. The combined organic phases were concentrated in vacuo and the mixture of title compounds was collected (46.1 g, 40%).

**3.7.2. Allyl 3-azido-2,3-dideoxy-6-*O*-tosyl-α-D-*arabino*-hexopyranoside.** 3-Azido-4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*arabino*-hexopyranoside described above (1 g, 3.19 mmol) was dissolved in MeOH (15 mL) and treated with Amberlyst IRA 900® (5 g) and was shaken overnight at rt. After filtration and washing of the resin with MeOH (25 mL), the combined organic phases were concentrated in vacuo to yield the crude product (0.73 g, 3.16 mmol), which was pure enough to be used for the next step. The crude product (65 mg, 0.28 mmol) was dissolved in $CH_2Cl_2$ (3 mL) and treated with trimethylammonium chloride (38.5 mg, 0.28 mmol) and $Et_3N$ (97 μL, 0.70 mmol). The solution was cooled to −10 °C and *p*-toluenesulfonyl chloride (65 mg, 0.34 mmol) in $CH_2Cl_2$ (3 mL) was slowly added. After 30 min, the reaction was terminated by the addition of satd aq NaCl and extracted with $CH_2Cl_2$ (4 × 5 mL). The combined organic phases were dried ($MgSO_4$) and purified by column chromatography over silica gel using petroleum ether–EtOAc (v/v = 3/1) as eluent. The title compound was obtained as a colourless oil (57 mg, 53%): $[\alpha]_D^{22}$ +64.9 (*c* 0.81, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$, $CDCl_3$ = 7.26 ppm): δ 1.67 (ddd, *J* = 12.8, 12.5, 3.4 Hz, 1H, 2b-H), 2.14 (dd, *J* = 12.8, 5.0 Hz, 1H, 2a-H), 2.45 (s, 3H, C*H*$_3$Ph–H), 2.64 (s, 1H, OH–H), 3.50 (dd, *J* = 9.7, 9.6 Hz, 1H, 4-H), 3.75 (ddd, *J* = 9.7, 3.9, 2.0 Hz, 1H, 5-H), 3.83 (ddd, *J* = 12.5, 9.6, 5.0 Hz, 1H, 3-H), 3.90 (ddt, *J* = 12.9, 5.4, 1.4 Hz, 1H, $CH_2$=CH–$CH_a$*H*$_b$O–H), 4.07 (ddt, *J* = 12.9, 5.4, 1.4 Hz, 1H, $CH_2$=CH–C*H*$_a$H$_b$O–H), 4.18 (dd, *J* = 11.1, 2.0 Hz, 1H, 6b-H), 4.41 (dd, *J* = 11.1, 3.9 Hz, 1H, 6a-H), 4.89 (d, *J* = 3.4 Hz, 1H, 1-H), 5.19 (dq, *J* = 10.4, 1.6 Hz, 1H, H$_{trans}$*H*$_{cis}$C=$CH_2$–$CH_2$O–H), 5.26 (dq, *J* = 16.8, 1.6 Hz, 1H, *H*$_{trans}$H$_{cis}$C=$CH_2$–$CH_2$O–H), 5.84 (ddt, *J* = 16.8, 10.4, 5.8 Hz, 1H, $CH_2$=C*H*–$CH_2$O–H), 7.38 (d, *J* = 8.3 Hz, 2H, Ts–H), 7.81 (d, *J* = 8.3 Hz, 2H, Ts–H); $^{13}$C NMR (100 MHz, $CDCl_3$, $CDCl_3$ = 77.2 ppm): δ 21.7 (q, C–*C*H$_3$Ph), 34.6 (t, C-2), 68.0, 68.7 (t, C-6, C–$CH_2$=CH–*C*$H_2$O), 59.8, 69.9, 70.0, (d, C-3, C-4, C-5), 95.8 (d, C-1), 117.6 (t, C–*C*$H_2$=CH–$CH_2$O), 128.0, 129.9 (d, C–Ts), 133.5 (d, C–$CH_2$=*C*H–$CH_2$O), 132.7, 145.1 (s, C–Ts). Elemental Anal. Calcd for $C_{16}H_{21}N_3O_6S$: C, 50.12; H, 5.52; N, 10.96. Found: C, 50.22; H, 5.39; N, 11.01.

**3.7.3. Allyl 4-*O*-acetyl-3-azido-2,3-dideoxy-6-*O*-tosyl-α-D-*arabino*-hexopyranoside.** Allyl 3-azido-2,3-dideoxy-6-*O*-tosyl-α-D-*arabino*-hexopyranoside described above (17 mg, 44.3 μmol) was dissolved in a mixture of pyridine–$Ac_2O$ (v/v = 2/1, 3 mL) and stirred for 16 h. The reaction was terminated by the addition of satd aq $NaHCO_3$ and extracted with $CH_2Cl_2$ (3 × 5 mL). The combined organic phases were dried ($MgSO_4$) and purified by column chromatography over silica gel using petroleum ether–EtOAc (v/v = 4/1) as eluent. The title compound was obtained as a colourless oil (15 mg, 80%): $[\alpha]_D^{22}$ +68.3 (*c* 0.73, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$, $CDCl_3$ = 7.26 ppm): δ 1.69 (ddd, *J* = 13.2, 12.6, 3.3 Hz, 1H, 2b-H), 2.15 (ddd, *J* = 13.2, 4.8, 0.8 Hz, 1H, 2a-H), 2.45 (s, 3H, C*H*$_3$Ph–H), 3.89 (ddd, *J* = 12.6, 9.7, 4.8 Hz, 1H, 3-H), 3.91 (ddt, *J* = 12.8, 5.9, 1.4 Hz, 1H, $CH_2$=CH–$CH_a$*H*$_b$O–H), 3.95 (ddd, *J* = 10.0, 5.6, 3.2 Hz, 1H, 5-H), 4.03 (dd, *J* = 10.8, 5.6 Hz, 1H, 6b-H), 4.08 (dd, *J* = 10.8, 3.2 Hz, 1H, 6a-H), 4.10 (ddt, *J* = 12.8, 5.5, 1.4 Hz, 1H, $CH_2$=CH–C*H*$_a$H$_b$O–H), 4.75 (dd, *J* = 10.0, 9.7 Hz, 1H, 4-H), 4.89 (d, *J* = 3.3 Hz, 1H, 1-H), 5.21 (dq, *J* = 10.4, 1.4 Hz, 1H, H$_{trans}$*H*$_{cis}$C=$CH_2$–$CH_2$O–H), 5.28 (dq, *J* = 17.1, 1.4 Hz, 1H, *H*$_{trans}$H$_{cis}$C=$CH_2$–$CH_2$O–H), 5.87 (ddt, *J* = 17.1, 10.4, 5.5 Hz, 1H, $CH_2$=C*H*–$CH_2$O–H), 7.34 (d, *J* = 8.2 Hz, 2H, Ts–H), 7.78 (d, *J* = 8.2 Hz, 2H, Ts–H); $^{13}$C NMR (100 MHz, $CDCl_3$, $CDCl_3$ = 77.2 ppm): δ 20.7 (q, C–*C*$H_3$CO), 21.7 (q, C–*C*$H_3$Ph–), 34.7 (t, C-2), 68.1, 68.3 (t, C-6, C–$CH_2$=CH–*C*$H_2$O), 57.5, 68.0, 70.4 (d, C-3, C-4, C-5), 128.1, 129.8 (d, C–Tos), 133.3 (d, C–$CH_2$=*C*H–$CH_2$O), 132.6, 144.9 (s, C–Ts), 169.8 (s, C–*C*OCH$_3$); LRMS *m*/*z* calcd for $C_{18}H_{23}N_3O_7S$ $[M+Na]^+$: 448.1. Found: 448.1.

**3.7.4. Allyl 4-*O*-acetyl-3,6-diazido-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside.** Allyl 4-*O*-acetyl-3-azido-2,3-dideoxy-6-*O*-tosyl-α-D-*arabino*-hexopyranoside described above (14 mg, 32.9 μmol) was dissolved in DMF (3 mL), treated with $NaN_3$ (10 mg, 150 μmol) and stirred at 80 °C for 4 h. The reaction was terminated by addition of satd aq NaCl and extracted with $CH_2Cl_2$ (2 × 5 mL). The combined organic phases were dried ($MgSO_4$) and purified by column chromatography over silica gel using petroleum ether–EtOAc (v/v = 10/1) as eluent. The title compound was obtained as a colourless oil (3 mg, 31%): $[\alpha]_D^{22}$ +101.1 (*c* 1.0, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$, $CDCl_3$ = 7.26 ppm): δ 1.77 (ddd, *J* = 13.1, 12.5, 3.3 Hz, 1H, 2b-H), 2.13 (s, 3H, C*H*$_3$CO–H), 2.21 (ddd, *J* = 13.1, 4.5, 1.3 Hz, 1H, 2a-H), 3.22 (dd, *J* = 13.3, 2.6 Hz, 1H, 6b-H), 3.33 (dd, *J* = 13.3, 7.1 Hz, 1H, 6a-H), 3.89 (ddd, *J* = 9.8, 7.1, 2.6 Hz, 1H, 5-H), 3.93 (ddd, *J* = 12.5, 9.7, 4.5 Hz, 1H, 3-H), 4.01 (ddt, *J* = 12.9, 5.6, 1.4 Hz, 1H, $CH_2$=CH–$CH_a$*H*$_b$O–H), 4.20 (ddt, *J* = 12.9, 5.6, 1.4 Hz, 1H, $CH_2$=CH–C*H*$_a$H$_b$O–H), 4.81 (dd, *J* = 9.8, 9.7 Hz, 1H, 4-H), 5.00 (d, *J* = 3.3 Hz, 1H, 1-H), 5.24 (dq, *J* = 10.3, 1.4 Hz, 1H, H$_{trans}$*H*$_{cis}$C=CH–$CH_2$O–H), 5.33 (dq, *J* = 17.1, 1.4 Hz, 1H, *H*$_{trans}$H$_{cis}$C=CH–$CH_2$O–H), 5.92 (ddt, *J* = 17.1, 10.3, 5.6 Hz, 1H, $CH_2$=C*H*–$CH_2$O–H); $^{13}$C NMR (100 MHz, $CDCl_3$, $CDCl_3$ = 77.2 ppm): δ 20.8 (q, C–CO*C*H$_3$), 34.9 (t, C-2), 51.4 (t, C-6), 68.2 (t, C–$CH_2$=CH–*C*$H_2$O), 57.2, 69.7, 71.6 (d, C-3, C-4, C-5), 95.3 (d, C-1), 117.9 (t, C–*C*$H_2$=CH–$CH_2$O), 133.4 (d, C–$CH_2$=*C*H–$CH_2$O), 169.9 (s, C–*C*OCH$_3$); LRMS *m*/*z* calcd for $C_{11}H_{16}N_6O_4$ $[M+Na]^+$: 319.1. Found: 319.1.

**3.7.5. Allyl 2,3,6-trideoxy-3,6-trifluoroacetamido-α-D-*arabino*-hexopyranoside.** Allyl 4-*O*-acetyl-3,6-diazido-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside described above (50 mg, 169 μmol) was dissolved in $Et_2O$ (7 mL) and treated with $LiAlH_4$ (51.2 mg, 1.35 mmol) at 0 °C. The reaction was stirred at 0 °C for 2 h and was then terminated by the addition of $H_2O$ (0.17 mL), NaOH (10%, 3 mL) and $H_2O$ (3 mL). The reaction was filtered through Celite™ and the organic phase was concentrated and dried in vacuo. The resulting crude product was directly used for the next step by dissolving it in $Et_3N$ (2 mL) and cooling to 0 °C. Trifluoroacetic acid (0.5 mL) was slowly added and the reaction was stirred at 0 °C for 1 h. The reaction was stopped by addition of satd aq $NaHCO_3$ and extracted with $CH_2Cl_2$ (3 × 5 mL). The combined organic phases were washed with satd aq NaCl, dried ($MgSO_4$) and purified by column chromatography over silica gel using petroleum ether–EtOAc (2/1) as eluent. Title compound **11** is obtained as a colourless oil (18 mg, 27%): $^1$H and $^{13}$C NMR spectral data matched those reported above.

### 3.8. Allyl 4-*O*-benzyl-2,3,6-trideoxy-3,6-trifluoroacetamido-α-D-*arabino*-hexopyranoside (12)

**3.8.1. Methyl 3,6-azido-4-*O*-benzyl-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside.** To a solution of methyl 3,6-azido-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (**8**) (100 mg, 0.44 mmol) in DMF (3 mL) were added successively NaH (24.5 mg, 0.61 mmol), BnBr (105 μL, 0.88 mmol) and tetra-*n*-butylammonium iodide (1 mg). The reaction mixture was stirred at room temperature for 24 h and was then treated with excess of $H_2O$. The aqueous phase was extracted with $CH_2Cl_2$ (3 × 10 mL), preadsorbed on silica gel and purified by column chromatography over silica gel using petroleum ether–EtOAc (v/v = 6/1) as eluent. The title compound was obtained as a colourless oil (134 mg, 95%): $[\alpha]_D^{22}$ +187.3 (*c* 1.0, MeOH); $^1$H NMR (400 MHz, $CDCl_3$, $CDCl_3$ = 7.26 ppm): δ 1.71 (td, *J* = 12.8, 3.4 Hz, 1H, 2-H), 2.16 (ddd, *J* = 13.6, 4.9, 0.3 Hz, 1H, 2-H), 3.31 (d, *J* = 9.6 Hz, 1H, 4-H), 3.34 (s, 3H, OMe–H), 3.38 (dd, *J* = 13.0, 5.1 Hz, 1H, 6-H), 3.49 (dd, *J* = 13.0, 2.4 Hz, 1H, 5-H), 3.76 (ddd, *J* = 9.7, 5.3, 2.4 Hz, 1H,

6-H), 3.90 (ddd, $J = 12.4$, 9.3, 2.1 Hz, 1H, 3-H), 4.59 (d, $J = 10.9$ Hz, 1H, Bn–H), 4.79 (d, $J = 3.1$ Hz, 1H, 1-H), 4.91 (d, $J = 10.9$ Hz, 1H, Bn–H); $^{13}$C NMR (100 MHz, $CDCl_3$, $CDCl_3 = 77.2$ ppm): $\delta$ 35.3 (t, C-2), 51.3 (t, C-6), 54.8 (q, C–OMe), 60.1 (d, C-3), 70.5 (d, C-5), 75.0 (t, C–Bn), 78.4 (d, C-4), 97.4 (d, C-1), 128.1 (d, C–Ph), 128.2 (d, C–Ph), 128.6 (d, C–Ph), 137.4 (s, C–Ph); HRMS $m/z$ calcd for $C_{14}H_{18}N_6O_3$ $[M+Na]^+$: 341.1338. Found: 341.1342.

**3.8.2. 3,6-Azido-4-*O*-benzyl-2,3,6-trideoxy-D-*arabino*-hexopyranose.** To a solution of methyl 3,6-azido-4-*O*-benzyl-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside described above (568 mg, 1.78 mmol) in $CH_2Cl_2$ (15 mL) was added $BCl_3$ (2 mL, 2.0 mmol) at −78 °C. The solution was allowed to warm to room temperature while being stirred for 1 h, cooled to −78 °C, treated with a phosphate buffer solution and stirred for additional 30 min at room temperature. The aqueous phase was extracted with $Et_2O$ ($3 \times 20$ mL) and concentrated in vacuo. The crude product was dissolved in MeCN (22 mL), and $H_2O$ (5 mL) and $Ag_2CO_3$ were added. The mixture was stirred at room temperature for 16 h. $Na_2SO_4$ and $Et_2O$ were added and the mixture was filtered through a pad of Celite™. The crude product was preadsorbed on silica gel and purified by column chromatography over silica gel using petroleum ether–EtOAc (v/v = 6/1) as eluent. The title compound was obtained as a colourless oil (485 mg, 90%): $^1$H NMR (400 MHz, $CDCl_3$, $CDCl_3 = 7.26$ ppm): $\delta$ 1.70 (ddd, $J = 13.0$, 12.6, 3.4 Hz, 1H, 2-H), 2.19 (ddd, $J = 13.3$, 4.9, 1.0 Hz, 1H, 2-H), 3.34 (d, $J = 9.5$ Hz, 1H, 4-H), 3.40 (d, 1H, 6-H), 3.52 (dd, $J = 13.1$, 2.5 Hz, 1H, 6-H), 3.97 (dd, $J = 9.4$, 4.8 Hz, 1H, 3-H), 4.03 (ddd, $J = 9.6$, 4.9, 2.6 Hz, 1H, 5-H), 4.60 (d, $J = 10.8$ Hz, 1H, Bn–H), 4.91 (d, $J = 10$-8 Hz, 1H, Bn–H), 5.36 (d, $J = 2.9$ Hz, 1H, 1-H). $^{13}$C NMR (100 MHz, $CDCl_3$, $CDCl_3 = 77.2$ ppm): $\delta$ 35.4 (t, C-2), 51.3 (t, C-6), 59.5 (d, C-3), 70.5 (d, C-5), 75.0 (t, C–Bn), 78.5 (d, C-4), 91.1 (d, C-1), 128.2 (d, C–Ph), 128.3 (d, C–Ph), 128.6 (d, C–Ph), 137.3 (s, C–Ph); LRMS $m/z$ calcd for $C_{13}H_{16}N_6O_3$ $[M+Na]^+$: 327.3 Found: 327.3.

**3.8.3. Allyl 3,6-azido-4-*O*-benzyl-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (10).** 3,6-Azido-4-*O*-benzyl-2,3,6-trideoxy-D-*arabino*-hexopyranose described above (200 mg, 0.66 mmol) was added to a solution of acetyl chloride (150 μL) in allyl alcohol (5 mL). The reaction was stirred for 18 h at room temperature and was stopped with $Et_3N$ (200 μL). The crude product was preadsorbed on silica gel and purified by column chromatography over silica gel using petroleum ether–EtOAc (v/v = 6/1) as eluent. Title compound **10** was obtained as a mixture of α- and β-anomers (4:1) (218 mg, 96%): $^1$H NMR (200 MHz, $CDCl_3$, $CDCl_3 = 7.26$ ppm): $\delta$ 1.72 (td, $J = 13.0$, 3.3 Hz, 1H, 2-H), 2.19 (dd, $J = 13.0$, 5.0 Hz, 1H, 2-H), 3.25–3.55 (m, 3H, 6-H, 6-H, 4-H), 3.73–4.06 (m, 3H, 3-H, 5-H, $CH_2$=CH–C$H_2$–H), 4.15 (ddt, $J = 13.0$, 5.0, 1.4 Hz, 1H, $CH_2$=CH–C$H_2$–H), 4.59 (d, $J = 10.8$ Hz, 1H, Bn–H), 4.91 (d, $J = 10.8$ Hz, 1H, Bn–H), 4.98 (d, $J = 3.0$ Hz, 1H, 1-H), 5.25 (dd, $J = 3.3$, 1.6 Hz, 1H, C$H_2$=CH–$CH_2$–H), 5.34 (dd, $J = 3.3$, 1.6 Hz, 1H, C$H_2$=CH–$CH_2$–H), 5.78–6.04 (m, 1H, $CH_2$=C$H$–$CH_2$–H), 7.29–7.50 (m, 5H, Ph–H); HRMS $m/z$ calcd for $C_{16}H_{20}N_6O_3$ $[M+Na]^+$: 367.1495. Found: 367.1488.

**3.8.4. Allyl 4-*O*-benzyl-2,3,6-trideoxy-3,6-trifluoroacetamido-α-D-*arabino*-hexopyranoside (12).** To a solution of allyl 3,6-azido-4-*O*-benzyl-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside **10** (218 mg, 0.36 mmol) in dry pyridine (4 mL) was added $PPh_3$ (665 mg, 2.5 mmol). The reaction mixture was stirred at room temperature for 1 h, was treated with $NH_3$ (410 μL, 5.1 mmol) and was allowed to stand for 16 h. The solvent was removed in vacuo, $CH_2Cl_2$ (20 mL), $Et_3N$ (1.4 mL, 10.1 mmol) and trifluoroacetic anhydride (705 μL, 5.1 mmol) were added and the reaction mixture was stirred at room temperature for 3 h. MeOH was added, the reaction mixture was preadsorbed on silica gel and purified by column chromatography over silica gel using petroleum ether–EtOAc (v/v = 4/1) as eluent. Title compound **12** was obtained as colourless crystals (194 mg, 63%): mp 237 °C; $[\alpha]_D^{22}$ +52.8 (*c* 1.0, MeOH); $^1$H NMR (400 MHz, acetone-$d_6$, acetone = 2.05 ppm): $\delta$ 1.95–2.10 (m, 1H, 2-H), 3.51 (t, $J = 9.8$ Hz, 1H, 4-H), 3.55–3.70 (m, 2H, 6-H), 3.87 (ddd, $J = 9.8$, 7.3, 2.8 Hz, 1H, 5-H), 4.91 (ddt, $J = 12.9$, 5.6, 1.4 Hz, 1H, $CH_2$=CH–C$H_2$–H), 4.10 (ddt, $J = 12.9$, 5.6, 1.4 Hz, 1H, $CH_2$=CH–C$H_2$–H), 4.45 (m, 1H, 3-H), 4.66 (d, $J = 4.8$ Hz, 2H, Bn–H), 4.91 (d, $J = 2.4$ Hz, 1H, 1-H), 5.11 (ddd, $J = 10.4$, 3.3, 1.6 Hz, 1H, C$H_2$=CH–$CH_2$–H), 5.23 (ddd, $J = 17.0$, 3.3, 1.6 Hz, 1H, C$H_2$=CH–$CH_2$–H), 5.85 (ddt, $J = 17.0$, 10.4, 5.6 Hz, 1H, $CH_2$=C$H$–$CH_2$–H), 8.45, 8.55 (m, 2H, NHTfa–H); $^{13}$C NMR (100 MHz, acetone-$d_6$, acetone = 29.8 ppm): $\delta$ 36.1 (t, C-2), 41.5 (t, C-6), 49.5 (d, C-3), 68.3 (t, C–$CH_2$=CH–$CH_2$), 70.7 (d, C-5), 74.8 (t, C–Bn), 78.7 (d, C-4), 96.2 (d, C-1), 117.2 (t, C–$CH_2$=CH–$CH_2$), 117.5 (q, $J = 287.0$ Hz, C–$CF_3$), 128.6, 128.9, 129.1, 132.8 (C–Ph), 139.0 (d, C–$CH_2$=$CH$–$CH_2$), 159.0 (q, $J = 36.8$ Hz, C–$CF_3CO$); HRMS $m/z$ calcd for $C_{20}H_{22}F_6N_2O_5$ $[M+Na+MeCN]^+$: 483.1355. Found: 483.1368.

### 3.9. 1′,4′-*O*-Di-(4-*tert*-butyldimethylsilyl-2,3,6-trideoxy-3,6-trifluoroacetamido-α-D-*arabino*-hexopyranoside)-1,4-butane (17)

**3.9.1. Allyl 4-*tert*-butyldimethylsilyl-2,3,6-trideoxy-3,6-trifluoroacetamido-α-D-*arabino*-hexopyranoside (16).** To a solution of allyl 2,3,6-trideoxy-3,6-trifluoroacetamido-α-D-*arabino*-hexopyranoside (**11**) (55 mg, 0.14 mmol) in $CH_2Cl_2$ (3 mL) were added 2,6-lutidine

(49 μL, 0.42 mmol) and TBSOTf (64 μL, 0.28 mmol) and the reaction mixture was stirred at room temperature for 16 h. Additional 2,6-lutidine (49 μL, 0.42 mmol) and TBSOTf (64 μL, 0.28 mmol) were added and the reaction mixture was stirred for another 2 h. $H_2O$ was added and the reaction was extracted with $CH_2Cl_2$ ($3 \times 10$ mL). The combined organic phases were preadsorbed on silica gel and purified by column chromatography over silica gel using petroleum ether–EtOAc (v/v = 6/1) as eluent. Title compound **16** was obtained as a colourless oil (50 mg, 70%): $^1$H NMR (400 MHz, MeOD, MeOD = 3.31 ppm): $\delta$ 0.06 (s, 3H, $CH_3Si$–H), 0.14 (s, 3H, $CH_3Si$–H), 0.90 (s, 9H, $(CH_3)_3CSi$–H), 1.85 (td, $J = 13.0$, 3.5 Hz, 1H, 2-H), 1.92 (ddd, $J = 13.0$, 5.2, 1.0 Hz, 1H, 2-H), 3.22 (dd, $J = 13.2$, 10 Hz, 1H, 4-H), 3.51 (t, $J = 9.3$ Hz, 1H, 6-H), 3.73 (td, $J = 9.6$, 2.2 Hz, 1H, 5-H), 3.86 (dd, $J = 13.2$, 2.1 Hz, 1H, 6-H), 3.93 (ddt, $J = 12.8$, 6.0, 1.3 Hz, 1H, $CH_2$=CH–C$H_2$–H), 4.10 (ddd, $J = 12.8$, 5.3, 1.4 Hz, 1H, $CH_2$=CH–C$H_2$–H), 4.27 (ddd, $J = 12.0$, 9.4, 5.1 Hz, 1H, 3-H), 4.88 (d, $J = 2.9$ Hz, 1H, 1-H), 5.17 (dd, $J = 10.3$, 1.3 Hz, 1H, C$H_2$=CH–$CH_2$–H), 5.27 (dd, $J = 17.2$, 1.6 Hz, 1H, C$H_2$=CH–$CH_2$–H), 5.86–5.98 (m, 1H, $CH_2$=C$H$–$CH_2$–H); $^{13}$C NMR (100 MHz, MeOD, MeOD = 49.0 ppm): $\delta$ −4.1 (q, C–$CH_3Si$), −3.6 (q, C–$CH_3Si$), 18.8 (q, C–$(CH_3)_3CSi$), 26.3 (s, C–$(CH_3)_3CSi$), 36.3 (t, C-2), 42.6 (t, C-6), 50.8 (d, C-3), 68.7 (t, C–$CH_2$=CH–$CH_2$), 72.4 (d, C-4), 73.0 (d, C-5), 96.3 (d, C-1), 117.4 (q, $J = 287.2$ Hz, C–$CF_3$), 117.6 (q, $J = 286.6$ Hz, C–$CF_3$), 117.6 (t, C–$CH_2$=CH–$CH_2$), 135.3 (d, C–$CH_2$=CH–$CH_2$), 158.5 (q, $J = 36.6$ Hz, C–$CF_3CO$), 159.0 (q, $J = 36.9$ Hz, C–$CF_3CO$); HRMS $m/z$ calcd for $C_{19}H_{29}F_6N_2O_5Si$ $[M]^-$: 508.1828. Found: 508.1835.

**3.9.2. 1′,4′-*O*-Di-(4-*tert*-butyldimethylsilyl-2,3,6-trideoxy-3,6-trifluoroacetamido-α-D-*arabino*-hexopyranoside)-1,4-butane (17).** To a solution of allyl 4-*tert*-butyldimethylsilyl-2,3,6-trideoxy-3,6-trifluoroacetamido-α-D-*arabino*-hexopyranoside (**16**) (44 mg, 86 μmol) in $CH_2Cl_2$ (2 mL) was added Grubbs precatalyst **15** (3 mg, 3.7 μmol). The reaction mixture was stirred at room temperature for 16 h and a second portion of Ru-complex **15** (3 mg, 3.7 μmol) was added. The reaction mixture was stirred at 40 °C for 8 h. A third portion of **15** (3 mg, 3.7 μmol) was added and the reaction mixture was stirred at 40 °C for 48 h. The reaction mixture was filtered through a pad of Celite™ and the solvent was removed in vacuo. The crude product was dissolved in a solvent mixture consisting of EtOAc–$CH_2Cl_2$–MeOH (1 mL, v/v/v = 16/8/1) and $PtO_2$ (10 mg) was added. The reaction mixture was stirred under an atmosphere of $H_2$ at room temperature for 16 h, was preadsorbed on silica gel and purified by column chromatography over silica gel using petroleum ether–EtOAc (v/v = 6/1) as eluent. Title compound **17** was obtained as a colourless oil (25 mg, 58%): $[\alpha]_D^{22}$ +87.0 (*c* 1.0, MeOH); $^1$H NMR (400 MHz, MeOD, MeOD = 3.31 ppm): $\delta$ 0.06 (s, 3H, $CH_3Si$–H), 0.13 (s, 3H, $CH_3Si$–H), 0.90 (s, 9H, $(CH_3)_3CSi$–H), 1.59–1.71 (m, 2H, OC$H_2CH_2$–H), 1.84 (td, $J = 12.6$, 3.3 Hz, 1H, 2-H), 1.92 (dd, $J = 12.6$, 5.1 Hz, 1H, 2-H), 3.22 (dd, $J = 13.1$, 9.9 Hz, 1H, 4-H), 3.37–3.44 (m, 1H, $OCH_2CH_2$–H), 3.51 (t, $J = 9.3$ Hz, 1H, 6-H), 3.58–3.64 (m, 1H, $OCH_2CH_2$–H), 3.73 (td, $J = 9.5$, 2.3 Hz, 1H, 5-H), 3.86 (dd, $J = 13.2$, 2.1 Hz, 1H, 6-H), 4.25 (ddd, $J = 12.1$, 9.4, 4.9 Hz, 1H, 3-H), 4.88 (d, $J = 2.9$ Hz, 1H, 1-H); $^{13}$C NMR (100 MHz, MeOD, MeOD = 49.0 ppm): $\delta$ −4.1 (q, C–$CH_3Si$), −3.6 (q, C–$CH_3Si$), 18.8 (q, C–$(CH_3)_3CSi$), 26.3 (s, C–$(CH_3)_3CSi$), 27.4 (t, C–OC$H_2CH_2$), 36.4 (t, C-2), 42.6 (t, C-6), 51.0 (d, C-3), 68.0 (t, C–OC$H_2CH_2$), 72.2 (d, C-4), 73.0 (d, C-5), 96.1 (d, C-1), 117.4 (q, $J = 286.9$ Hz, C–$CF_3$), 117.6 (q, $J = 286.7$ Hz, C–$CF_3$), 158.7 (q, $J = 36.7$ Hz, C–$CF_3CO$), 159.1 (q, $J = 36.9$ Hz, C–$CF_3CO$); HRMS $m/z$ calcd for $C_{36}H_{58}F_{12}N_4O_{10}Si_2$ $[M]^-$: 989.3422. Found: 989.3439.

### 3.10. Fluorescence titration

**3.10.1. TAR (*trans-activation-response*) fragment.** For the fluorescence titration an unlabelled 31-nucleotides containing TAR fragment (sequence: 5′-GGC CAG AUC UGA GCC UGG GAG CUC GGC C-3′) was used. The TAR fragment showed an extinction coefficient of 192300 L/(mol cm) at 260 nm.

**3.10.2. F-Tat (transactivator of transcription)-peptide.** For the fluorescence titration the Tat-peptide marked with a chromophore, namely rhodamine green, which was bound via a DOA-linker to the Tat-peptide, was used (sequence: RhoG-DOA-RKKR RQRRR-COOH). The peptide was purified by HPLC to 95% purity. It has a molecular weight of 1840 g/mol and an extinction coefficient of 74,000 L/(mol cm) at 530 nm. Rhodamine Green (RhoG) is excited at 504 nm and emitted at 530 nm.

The titration experiments were carried out in cuvettes, which had been pretreated with dimethyldichloro silane prior to use. A solution consisting of TAR-RNA (200 nM) and Tat-peptide (100 nM) was added to the cuvette. Under these conditions about 90% of the Tat/TAR-complex had formed. Defined concentrations (up to 60 μM) of **5** were titrated to the solution and the fluorescence signal was measured.

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