ARTICLE

OBC
www.rsc.org/obc

Preparation of macrocyclic ^{15}N-labelled oligoaminodeoxysaccharides as probes for RNA-binding

Janis Jaunzems, Benjamin Oelze and Andreas Kirschning*

Institut für Organische Chemie der Universität Hannover, Schneiderberg 1B, D-30167, Hannover, Germany. E-mail: andreas.kirschning@oci.uni-hannover.de

Received 13th August 2004, Accepted 7th October 2004
First published as an Advance Article on the web 10th November 2004

Two macrocyclic aminoglycosides were prepared from a 1,4-butanediol linked 2-deoxy-L-rhamnal which was *O*-allylated at the 4- and 4′-positions *via* the precursor allyl 3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*arabino*-hexoside employing olefin metathesis and ring closing metathesis in a sequential manner. The macrocycles were ^{15}N-labelled at all four amino groups in order to study interactions with regulatory RNA structures in solution by NMR spectroscopy. A key step for the introduction of the ^{15}N-label was a reductive amination step using commercially available $^{15}NH_4OAc$. The reductive amination proceeds with excellent stereocontrol. As a by-product the unusual acyclic amino nitrile was isolated which originated from intramolecular imine formation followed by cyanide addition to the intermediate C=N double bond.

Introduction

Small-molecule natural products and natural product-like analogues have aided understanding of the role and function of many biomacromolecules critical to the progression and maintenance of the cell cycle. Successfully identifying the direct target of a particular molecule provides a tool with which to control specific aspects of the cell cycle.[1] In this context, RNA has been regarded as a particularly important macromolecule, since it exhibits a great functional and structural variety, comparable to that of proteins. The vast conformational range of RNA should allow for specific binding by small molecules. Recently, the effort of many research groups was dedicated to the synthesis of molecules that are able to selectively bind to RNA.[1] Among the various ligands that have been considered for this task,[2] aminoglycosides such as neomycin B (**1**) are the most promising substructures (Fig. 1).[3a–c] In this context, the interactions between aminoglycosides and various RNA targets, including the HIV-1 TAR-RNA used in competitive binding studies in the presence of the Tat protein, have been studied by various groups.[3–8]

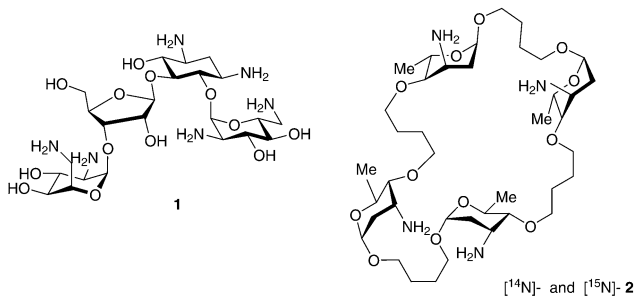


Fig. 1 Structures of the aminoglycoside neomycin B (**1**) and macrocycle **2**.

The results of those studies suggest that particular RNA regions containing either asymmetric internal loops or hairpin loop–stem junctions are preferential binding sites for aminoglycosides. Indeed, their basic scaffold and the potential for synthetic assembly utilizing various monomeric building blocks, make aminoglycosides ideal for the synthesis of new selective and, potentially, less toxic drugs that can be used for studying RNA binding. These efforts also demonstrated that synthetic derivatives of aminoglycosides like neomycin **1** can exert improved antibacterial performance whilst targeting resistance-causing enzymes at the same time.[9]

Recently, we described the first preparation of a novel macrocyclic 1,4-butanediol-linked aminodeoxyglycoside **2** (Fig. 1).[10] The coupling of the aminodeoxyhexose units was achieved by olefin metathesis of appropriately allylated aminosugar precursors and then by ring-closing metathesis.[11] In addition, we conducted competitive dot blot binding experiments with TAR-RNA and the Tat protein in the presence of macrocycle **2**. Furthermore, two aptamers selected for binding to the REV protein were included in these affinity studies. These investigations revealed that unlike other butanediol linked neoaminoglycosides obtained from allylated aminohexoses, macrocycle **2** is able to inhibit the TAR-RNA–Tat-protein interaction.[12] In order to obtain a deeper understanding of the nature and mode of binding, we planned to study the complex in solution, formed upon mixing the TAR-RNA with macrocycle **2** by NMR spectroscopy.

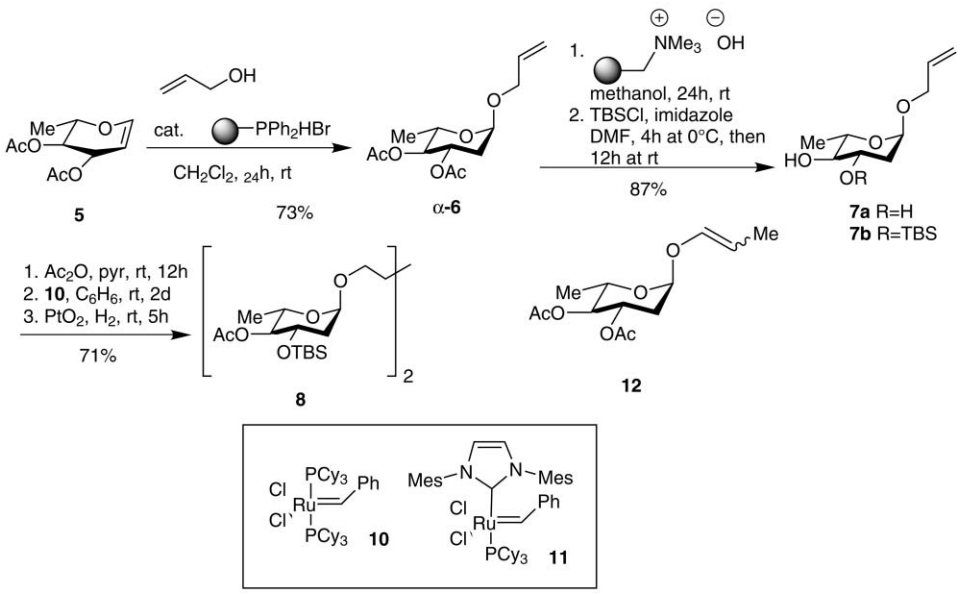
Our original synthetic strategy had one major drawback in view, that the NMR experiments required the ^{15}N-labelled aminoglycoside analogue for localizing proximities between functional groups and structural elements of the RNA and the aminoglycoside **2**. The ^{15}N-labelled aminoglycosides will only be conveniently accessible if the nitrogen label is introduced at a late stage of the synthesis and a readily available ^{15}N source can be utilized. We reckoned that the successful introduction of a ^{15}N-labelled amino group into complex glycosides should be of general utility in the context of aminoglycosides and binding studies to nucleic acids in solution. Here, we report on the efficient synthesis of labelled macrocycle [^{15}N]-**2** by an alternate route to that previously reported.

Results and discussion

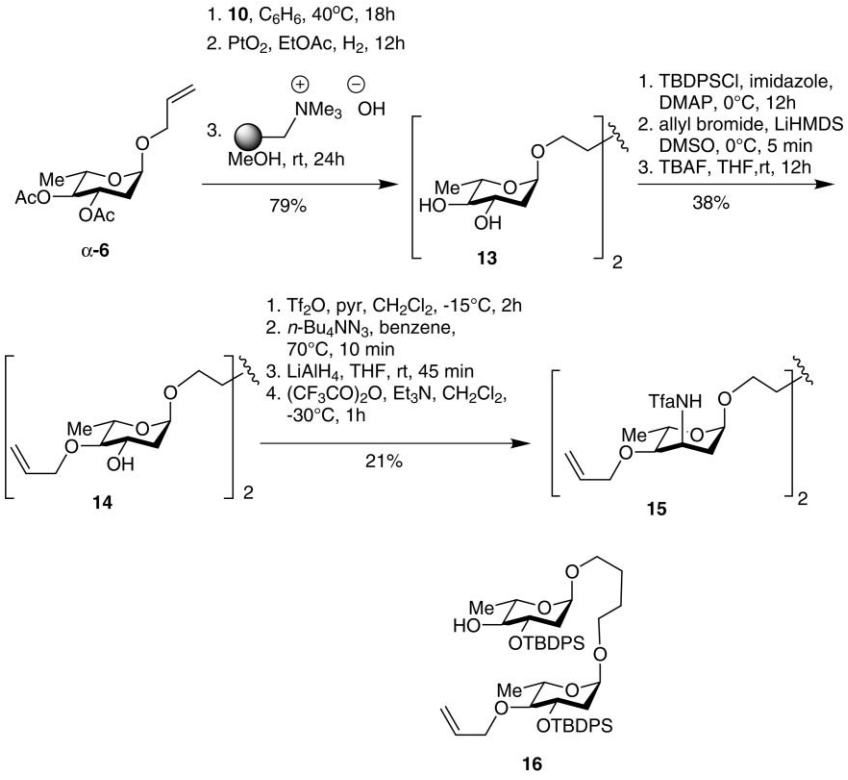
At first, we had to design a new synthetic route which allowed introduction of the ^{15}N label at a late stage. Thus, L-rhamnal **5** was chosen as a starting point for the preparation of the target macrocycle [^{15}N]-**2** (Scheme 1). Polymer-bound triphenyl phosphonium bromide served as a mild proton source in the allylation process to yield allyl glycosides α/β-**6** (α : β = 1.3 : 1) in 73% which could easily be separated by column chromatography.[13] Formation of the Ferrier rearranged products was substantially suppressed by the polymer-bound proton source (19%).[14] Functional group manipulations became necessary prior to olefin metathesis. After deacylation of glycoside α-**6**, selective silylation at O-3 yielded allyl glycoside **7b** which, however, did not yield homodimers upon treatment with the

DOI: 10.1039/b412436g

 This journal is © The Royal Society of Chemistry 2004



Scheme 1



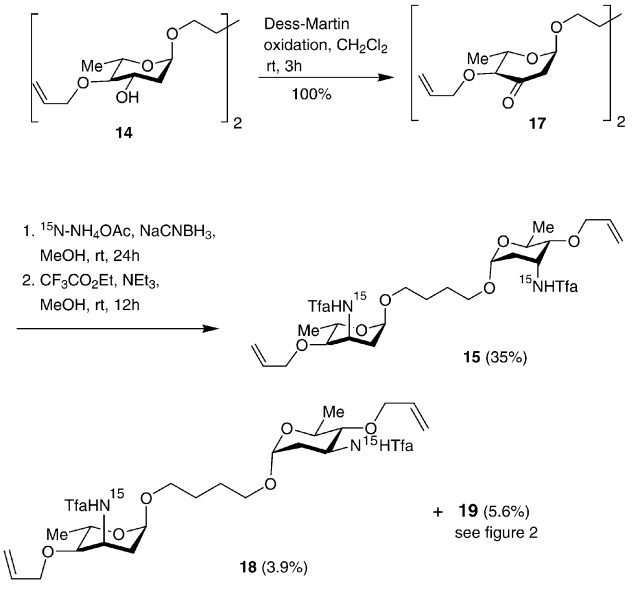
Scheme 2

Grubbs' catalyst **10**. The two donor hydroxyl groups at both C-4 positions in **7b** could explain this result. The more reactive ruthenium catalyst **11** was also ill suited for the olefin metathesis reaction with the allyl glycosides, as substantial double bond migration and formation of enol ether **12** (73%; $E : Z = 1.6 : 1$; along with recovery of 20% of starting material) occurred.[15] Acylation of glycoside **7b** yielded a fully protected allyl glycoside which gave homodimer **8** after olefin metathesis with catalyst **10** and reductive hydrogenation. However, introduction of the allyl group at both O-4 positions failed because the deacylation of dimer **8** yielded complex mixtures due to silyl migration and silyl removal.

Therefore, we had to alter the synthetic strategy and locate the dimerization at an earlier stage of the synthesis, namely using allyl glycoside α-**6** as substrate (Scheme 2). The olefinic double bond in the resulting dimer was hydrogenated and methanolysis of the acetyl groups afforded 1,4-butane diol linked glycoside **13**. Selective 3-*O*-silylation on both pyranose rings, this time using the bulkier *tert*-butyldiphenylsilyl group proceeded smoothly (94%), was followed by 4-*O*-allylation. The allylation turned out to be troublesome because under classical conditions (NaH, allyl bromide or $CH_2{=}CH{-}CH_2OC(NH)CCl_3$, H^+) either silyl migration or desilylation and cleavage of the glycosidic bond occurred, producing a complex mixture of products. Wong's procedure[16] using allyl bromide in DMSO and LiHMDS as base gave satisfactory results without resulting in these side reactions. The target bisallylated product could be isolated in 48% yield. Monoallylated product **16** was formed in 50% yield and could be resubjected to the conditions to achieve complete allylation. The homodimeric bisallylated product was liberated from the

silyl protection which furnished diol **14**. Below we investigated a stereospecific introduction of the nitrogen source on both pyran rings. The carbohydrate-derived bistriflate was reacted with the nucleophilic azide anion which yielded the bisazido substitution product. This intermediate was reduced to the corresponding diamine and hence further transformed to the diamide **15**. Indeed, the introduction of the nitrogen functionality proceeded with excellent stereoselectivity, but the yield for this process was low (36%) which made the transformation from **14** to **15** and hence the whole route uneconomical in view of the price of ^{15}N-labelled azide. As an alternative, we tested the Barton conditions of the Ritter reaction using chlorodiphenylmethyl hexachloroantimonate as a free carbocation source to induce activation of the hydroxyl groups in the presence of acetonitrile.[17] Although the procedure works well with various alcohols, such as menthol, it failed here and caused complete decomposition of diol **14**.

Therefore, we directed our efforts towards 1,4-butanediol-linked diketone **17** (readily available from diol **14**) (Scheme 3). The advantage of this route is that a low cost ^{15}N source (ammonium-based) or derivatives can be utilized. Below reductive amination using ^{15}N-labelled NH_4OAc (>98% purity) afforded an intermediate diamine which, after trifluoroacetylation, furnished the homodimeric L-ristosamine derivative [^{15}N]-**15** in 35% yield. The spectroscopic data are identical with those collected for the reaction product obtained from the alternate route *via* diol **14** and the corresponding ditriflate (Scheme 2). The reductive amination step proceeded with high stereoselectivity (*ribo* : *arabino* = 12 : 1). The *ribo–arabino*-mixed heterodimer [^{15}N]-**18** was formed as a minor isomer (4%) and could be separated by column chromatography.



Scheme 3

Interestingly, the cyclic by-product [^{15}N]-**19** was also isolated in about 6% yield (single isomer). The structure was elucidated by IR (ν= 2279 cm^{-1}, CN) and NMR spectroscopy as well as mass spectrometry (ESI-MS: $M^+ = 438.26$; >98% purity in ^{15}N). The ^{1}H and ^{13}C NMR spectra revealed that an unsymmetrical molecule was formed which contained only one ^{15}N nitrogen atom (1H, 4.7 ppm, J_{NH} = 81.5 Hz). 2D, TOCSY and NOE experiments helped to fully elucidate the structure. Molecular modelling calculations using the MMFF ForceField within MacroModel 7.2 were conducted yielding a 3D structure which supports the recorded NOE-data (Fig. 2).[18–20,22,23]

A possible mechanism for the formation of the unusual macrocycle **19** is proposed in Scheme 4. Initially, only one keto group underwent reductive amination. Then, intramolecular

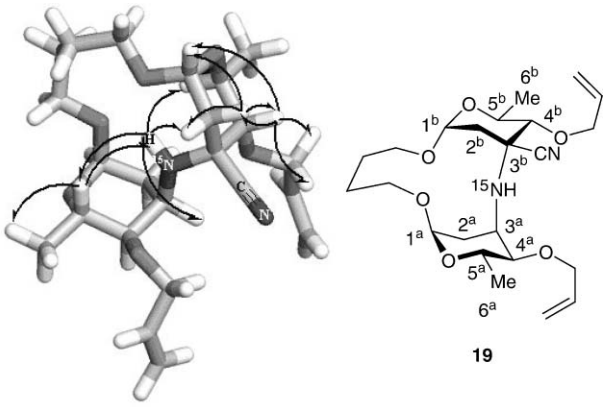
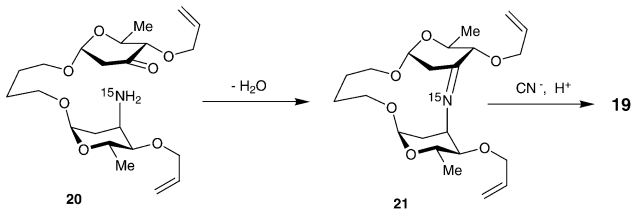


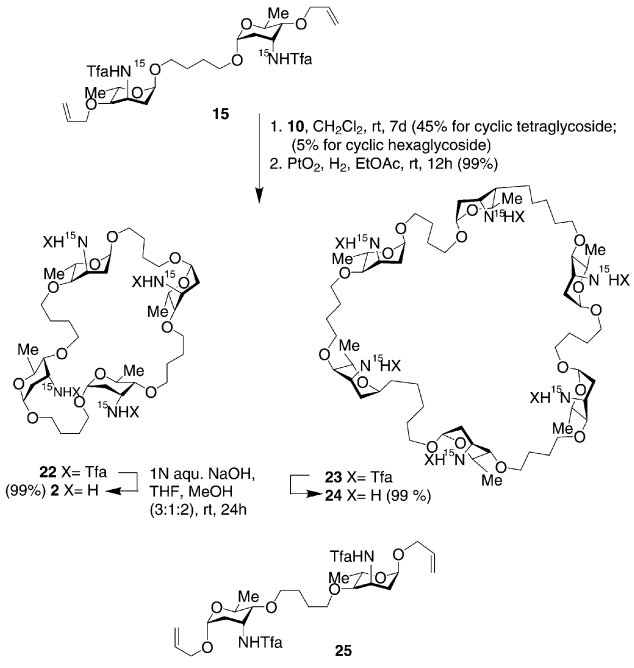
Fig. 2 Calculated structure and main NOE connectivities of aminocyanide **19** (these connectivities are also depicted in the graphic created by a molecular modelling study): 5-H^a (6-H^a, ^{15}NH), ^{15}NH (5-H^a, 3-H^a, 2-H$^b_{eq}$, 5-H^b), 2-H$^b_{ax}$ (2-H$^b_{eq}$, 1-H^b, 4-H^b), 4-H^b (1-H^b, 2-H^b, –O–CH_2–CH=CH_2^{b}).



Scheme 4

imine formation between the amino group of [^{15}N]-**20** and the remaining keto group took place which yielded macrocycle [^{15}N]-**21**. Subsequent nucleophilic attack of cyanide onto the imino carbon generated amino nitrile [^{15}N]-**19**. Surprisingly, no macrocycle was detected with hydrogen instead of the cyano group, which would have originated from hydride attack onto the cyclic imine **21**. However, under these conditions formation of a symmetrical product would have occurred. Surprisingly, the source of the cyanide ion in the present case is the reducing agent.

When the L-ristosamine-derived head-to-head dimer [^{15}N]-**15** was subjected to the metathesis protocol Scheme 5 the



Scheme 5

corresponding cyclic neo-tetrasaccharide [^{15}N]-**22** was formed as the major product, while hexasaccharide [^{15}N]-**23** was the only by-product that could be isolated. These structures were unequivocally confirmed by NMR spectroscopy and importantly by mass spectrometry {[^{15}N]$_4$-**22**: LC-MS (ESI) (+*c*): *m*/*z* (%): 1215.45 (100) [M + Na]$^+$; [^{15}N]$_6$-**23**: LC-MS (ESI) (+*c*): *m*/*z* (%): 1811.18 (100) [M + Na]$^+$}. Finally, reduction and deblocking yielded the target macrocyclic aminoglycosides [^{15}N]-**2** and [^{15}N]-**24**. Formation of these macrocycles can be rationalized by assuming that the axial amido substituent at C-3 prevents direct intramolecular olefin metathesis reaction of dimer **15**. Instead, dimerization and trimerization, respectively, affording the linear tetra- and hexasaccharide intermediates, occur prior to intramolecular ring closure.

Interestingly, the structurally reversed tail-to-tail dimer **25** yields the same set of macrocyclic tetramer **22** and hexamer **23** with similar ratio upon treatment with the catalyst **10**.[10] Thus, the outcome of RCM is irrespective of the mode of connectivity (butanediol bridge between C-4–C-4′ or between C-1–C-1′) and location of the allyl groups (C-1 or C-4) present in the dimeric precursor.

Conclusion

In summary, we have developed and evaluated new synthetic routes towards macrocyclic 1,4-butanediol linked ^{15}N-labelled aminoglycosides. The synthetic strategy allows the preparation of complex derivatives with good efficiency by introducing the nitrogen functionality at a late stage of the synthesis using ^{15}N-labelled ammonium acetate, which is readily available at comparably low cost. Structural studies on the binding properties of these labelled aminoglycosides with biomacromolecules (TAR-RNA) in solution are currently underway using NMR spectroscopy.[21] In essence, we believe that this synthetic work is of general importance for the field of synthetic aminoglycosides and their use as tools for studying RNA–ligand interactions.

Experimental

General remarks and starting materials

^{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) with 500 MHz (125 MHz), using tetramethylsilane as the internal standard. If not 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 ppm 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 distortionless enhancement by polarisation transfer (DEPT) spectral editing techniques, with secondary pulses at 90 and 135°. Multiplicities are reported using the following abbreviations: s = singlet (due to quaternary carbon), d = doublet (methine), t = triplet (methylene), q = quartet (methyl). 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, Universität Hannover. 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 methanol. Preparative column chromatography was performed on silica gel 60 (E. Merck, Darmstadt). ^{15}N-Ammonium acetate was purchased from Aldrich (>98% purity).

Allyl 3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*arabino*-pyranoside (6)

To a solution of 3,4-di-*O*-acetyl-L-rhamnal **5** (5 g, 23 mmol) and allyl alcohol (2 g, 34.5 mmol, 1.5 eq) in dry CH_2Cl_2 (50 ml) was added polymer-bound PPh_3·HBr (20 mg, 0.02 mmol).[12] The reaction mixture was stirred at rt for 24 h, filtered through a pad of Celite and concentrated under reduced pressure. The crude product mixture was finally separated by column chromatography (silica gel; ethyl acetate–petroleum ether 1 : 6) to yield α-**6** (2.6 g, 9.56 mmol; 41%), β-**6** (2.0 g, 7.35 mmol; 31.6%) and the Ferrier rearrangement product (1.0 g, 4.72 mmol; 18.7%). $[\alpha]_D^{26} = -156.3°$ (*c* 1, $CHCl_3$); data for α-anomer **6** δ_H (400 MHz; $CDCl_3$) 1.17 (3 H, d, *J* 6.2, 6-H), 1.79 (1 H, ddd, *J* 12.8, 11.7 and 3.0, 2-H$_{eq}$), 1.99 and 2.04 (6 H, 2 × s, 2 × AcO), 2.24 (1 H, ddd, *J* 12.8, 5.4 and 2.7, 2-H$_{ax}$), 3.86 (1 H, dq, *J* 9.7 and 6.2, 5-H), 3.94 (1 H, ddt, *J* 13.0, 5.9 and 1.5, –O–C*H*H′–CH=), 4.13 (1 H, ddt, *J* 13.0, 5.2 and 1.5, –O–CH*H*′–CH=), 4.73 (1 H, t, *J* 9.7, 4-H), 4.90 (1 H, d, *J* 3.0, 1-H), 5.18 (1 H, dq, *J* 10.5 and 1.5, –CH=C*H*H′), 5.29 (1 H, dq, *J* 17.1 and 1.5, –CH=CH*H*′), 5.30 (1 H, ddd, *J* 9.7, 5.4 and 2.7, 3-H), 5.89 (1 H, dddd, *J* 17.1, 10.5, 5.9 and 5.2, –C*H*=CH$_2$); δ_C (100 MHz, $CDCl_3$) 17.8 (q, C-6), 21.1 and 21.3 (2 × q, 2 × CO*C*H$_3$), 35.6 (t, C-2), 68.2 (t, –*C*H$_2$–CH=), 66.0, 69.4 and 75.2 (3 × d, C-4, C-3, C-5), 117.5 (t, –CH=*C*H$_2$), 96.2 (d, C-1), 134.3 (d, –*C*H=CH$_2$), 170.5 (2 × s, 2 × *C*OCH$_3$); LC-MS (ESI) (+*c*): *m*/*z* (%): 336.15 (100) [M + CH_3CN + Na]$^+$.

1,4-Di-(2′,6′-dideoxy-α-L-*arabino*-pyranosyl)-1,4-butanediol (13)

Bisallyl glycoside α-**6** (4 g, 15 mmol) was dissolved in dry benzene (30 ml) and catalyst **10** (5 mg, 1 mol%) was added under argon in one portion. The reaction mixture was stirred at 40 °C for 6 h and a second portion of catalyst **10** (5 mg, 1 mol%) was added. Stirring was continued for additional 12 h at 40 °C after which the solvent was removed under reduced pressure. The crude product was subjected to column chromatography (silica gel; ethyl acetate–petroleum ether 1 : 7) to yield the unsaturated homodimer (3.1 g, 6.0 mmol; 80%). This material was directly hydrogenated with H_2 (1 bar) in ethyl acetate (25 ml) using PtO_2 (7 mol%). After 12 h at rt the reaction mixture was filtered and concentrated *in vacuo* to yield the corresponding saturated homodimeric saccharide (3.1 g, 5.97 mmol; >99%). This material was directly employed for the next deacetylation step.

Selected spectroscopic data of the crude material: δ_H (200 MHz, $CDCl_3$) 1.16 (6 H, d, *J* 6.4, 2 × 6-H), 1.64 (4 H, m, 2 × –O–CH$_2$–C*H*$_2$–), 1.77 (2 H, ddd, *J* 12.7, 11.7 and 3.8, 2 × 2-H$_{eq}$), 2.04 and 1.99 (12 H, 2 × s, 4 × AcO), 2.20 (2 H, ddd, *J* 12.8, 5.4 and 0.9, 2 × 2-H$_{ax}$), 3.35 (2 H, m, 2 × –O–C*H*H′–CH$_2$–), 3.63 (2 H, m, 2 × –O–CH*H*′–CH$_2$–), 3.83 (2 H, dq, *J* 9.7 and 9.5, 2 × 5-H), 4.72 (2 H, dd, *J* 9.7 and 9.5, 2 × 4-H), 4.84 (2 H, d, *J* 2.9, 2 × 1-H), 5.25 (2 H, ddd, *J* 11.6, 9.6 and 5.5, 2 × 3-H).

Deacetylation of the hydrogenated homodimer was achieved by treatment of starting peracetate (3.1 g, 5.97 mmol) in methanol (25 ml) with amberlite A-26 (hydroxide form, 0.6 g). Stirring was continued at rt for 24 h. After filtration and removal of the solvent *in vacuo* the crude product (2.08 g, 5.95 mmol; 99%) was dried and used for the next step without additional purification.

δ_H (200 MHz, CD_3OD = 3.35 ppm) 1.24 (6 H, d, *J* 6.3, 2 × 6-H), 1.59 (2 H, ddd, *J* 12.9, 11.6 and 3.5, 2 × 2-H$_{eq}$), 1.70 (4 H, m, 2 × –O–C*H*$_2$–CH$_2$–), 2.05 (2 H, ddd, *J* 12.9, 5.1 and 0.9, 2 × 2-H$_{ax}$), 2.93 (2 H, t, *J* 9.3, 2 × 4-H), 3.44 (2 H, m, 2 × –O–C*H*H′–CH$_2$–), 3.58 (2 H, dq, *J* 9.3 and 6.3, 2 × 5-H), 3.66 (2 H, m, 2 × –O–CH*H*′–CH$_2$–), 3.77 (2 H, ddd, *J* 11.6, 9.3 and 5.1, 2 × 3-H), 4.80 (2 H, d, *J* 3.5, 1-H).

Allyl 3-*O*-(*tert*-butyldimethylsilyl)-2,6-dideoxy-α-L-*arabino*-pyranoside (7b)

To a stirred solution of allylated sugar **6** (3.36 g 10.0 mmol) in methanol (50 ml) was added Amberlite A-26 (OH^- form) (1 g). After 24 h the reaction mixture was filtered and the solvent was removed under reduced pressure. The product was dried under reduced pressure for 4 h and directly used for the next step. Yield: 1.90 g (10.0 mmol, >98%).

To a stirred solution of 2-deoxy-α-allyl-L-rhamnoside (1.90 g, 0.01 mol), imidazole (1.0 g, 0.015 mol, 1.5 eq) and DMAP (cat.) in DMF (15 ml) was slowly added TBSCl (1.55g, 0.01 mol, 1 eq) at 0 °C. The solution was stirred at 0 °C for 4 h and then at rt for additional 12 h. The pure product **7b** was isolated after purification by flash column chromatography as colorless oil. Yield: 2.68 g (8.86 mmol, 89%).

δ_H (400 MHz, $CDCl_3$) 0.09 and 0.11 (6 H, 2 × s, $Si(CH_3)_2$), 0.89 (9 H, s, *t*-Bu–Si), 1.28 (3 H, d, *J* 6.2, 6-H), 1.68 (1 H, ddd, *J* 12.9, 11.3 and 3.4, 2-H_{eq}), 2.02 (1 H, ddd, *J* 12.9, 5.1 and 1.1, 2-H_{ax}), 2.23 (1 H, d, *J* 2.0, OH), 3.10 (1 H, dt, *J* 9.1 and 2.0, 4-H), 3.68 (1 H, dq, *J* 9.1 and 6.2, 5-H), 3.93 (2 H, m, –O–C*H*H′–CH=, 3-H), 4.12 (1 H, ddt, *J* 13.2, 4.9 and 1.5, –O–CH*H*′–CH=), 4.85 (1 H, d, *J* 3.4, 1-H), 5.16 (1 H, dq, *J* 10.5 and 1.5, –CH=C*H*H′), 5.27 (1 H, dq, *J* 17.1 and 1.6, –CH=CH*H*′), 5.90 (1 H, dddd, *J* 17.1, 10.5, 5.8 and 4.9, –C*H*=CH_2); δ_C (100 MHz, $CDCl_3$) −4.64 and −4.20 (2 × q, $Si(CH_3)_2$), 17.84 (q, C-6), 17.98 (s, Si-$C(CH_3)_3$), 25.76 (q, Si–C(CH_3)$_3$), 38.63 (t, C-2), 67.55 (t, –CH_2–CH=), 67.46, 70.56 and 78.02 (3 × d, C-3, C-4, C-5), 96.61 (d, C-1), 116.58 (t, –CH=CH_2), 134.40 (d, –CH=CH_2).

1,4-Di-(4′-*O*-acetyl-3′-*O*-(*tert*-butyldimethylsilyl)-2′,6′-dideoxy-α-L-*arabino*-pyranosyl)-1,4-butanediol (8)

Alcohol **7b** (2.58 g, 8.5 mmol) was dissolved in dry pyridine (10 ml) and Ac_2O (1.02 ml, 11.05 mmol, 1.3 eq) was added. The reaction mixture was stirred overnight at rt. The solvent was evaporated under reduced pressure and the residue was extracted with ethyl acetate, washed with water, dried ($MgSO_4$) and again concentrated under reduced pressure to yield allyl 4-*O*-acetyl-3-*O*-(*tert*-butyldimethylsilyl)-2,6-dideoxy-α-L-*arabino*-pyranoside (2.9 g, 8.4 mmol; 99%).

δ_H (400 MHz, $CDCl_3$) 0.03 and 0.04 (6 H, 2 × s, Me_2Si), 0.84 (9.H, s, *t*-BuSi), 1.13 (3 H, d, *J* 6.2, 6-H), 1.75 (1 H, ddd, *J* 13.1, 11.3 and 3.3, 2-H_{eq}), 2.06 (1 H, ddd, *J* 13.1, 5.3 and 1.1, 2-H_{ax}), 2.06 (3 H, s, CH_3CO), 3.73 (1 H, dq, *J* 9.5 and 6.2, 5-H), 3.92 (1 H, ddt, *J* 13.1, 5.8 and 1.3, –*O*–C*H*H′–CH=), 4.05 (1 H, ddd, *J* 11.3, 9.5 and 5.3, 3-H), 4.11(1 H, ddt, *J* 13.1, 5.0 and 1.5, –*O*–CH*H*′–CH=), 4.63 (1 H, t, *J* 9.5, 4-H), 4.86 (1 H, d, *J* 3.3, 1-H), 5.18 (1 H, dq, *J* 10.5 and 1.4, –CH=C*H*H′), 5.27 (1 H, dq, *J* 17.2 and 1.6, –CH=CH*H*′), 5.90 (1 H, dddd, *J* 17.2, 10.5, 5.8 and 5.0, –C*H*=CH_2); δ_C (100 MHz, $CDCl_3$) −4.6 and −4.2 (2 × q, $Si(CH_3)_2$), 17.9 (q, C-6), 18.1 (s, Si–$C(CH_3)_3$), 21.5 (q, COCH_3), 25.9 (q, Si–C(CH_3)$_3$), 39.4 (t, C-2), 66.3, 67.8 and 78.1 (3 × d, C-3, C-4, C-5), 68.0 (t, –CH_2–CH=), 96.7 (d, C-1), 117.1 (t, –CH=CH_2), 134.6 (d, –CH=CH_2), 170.3 (s, *C*OCH$_3$).

This allyl glycoside (100 mg, 0.29 mmol) was dissolved in dry benzene (3 ml) and Grubbs' catalyst **10** (5 mg, 1 mol%) was added under argon. The reaction mixture was stirred for 6 h, then a second portion of the catalyst (5 mg, 1 mol%) was added and the mixture was stirred for additional 12 h. Then, the reaction temperature was elevated to 40 °C and an additional portion of the catalyst was added (10 mg, 2 mol% in two portions within 24 h). The solvent was evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (ethyl acetate–petroleum ether 1 : 10) to yield the target cross coupling olefin (69 mg, 0.104 mmol; 72%) and unreacted starting material (20 mg, 0.058 mmol, 20%).

This dimeric alkene (69 mg, 0.104 mmol) was dissolved in a mixture of solvents (ethyl acetate–CH_2Cl_2–MeOH 16 : 8 : 1; 5 ml) and PtO_2 (1.6 mg, 7 mol%) was added. This suspension was stirred for 5 h under H_2 atmosphere. The reaction mixture was filtered and concentrated under reduced pressure to give the product **8** (70 mg, 0.103 mmol; 99%) as a colorless oil.

δ_H (400 MHz, $CDCl_3$) 0.02 and 0.03 (12 H, 4 × s, 2 × $SiMe_2$), 0.83 (18 H, s, 2 × *t*-Bu), 1.12 (6 H, d, *J* 6.3, 2 × 6-H), 1.62 (4 H, m, 2 × –O–CH_2–CH_2–), 1.72 (2 H, ddd, *J* 13.3, 10.8 and 3.2, 2 × 2-H_{eq}), 2.01 (2 H, dd, *J* 13.3 and 5.2, 2 × 2-H_{ax}), 2.05 (6 H, s, 2 × CH_3CO), 3.36 (2 H, m, 2 × –O–C*H*H′–CH_2–), 3.60 (2 H, m, 2 × –O–CH*H*′–CH_2–), 3.69 (2 H, dq, *J* 9.5 and 6.3, 2 × 5-H), 4.00 (2 H, ddd, *J* 10.8, 9.1 and 5.2, 2 × 3-H), 4.61 (2 H, dd, *J* 9.1 and 9.5, 2 × 4-H), 4.79 (2 H, d, *J* 3.2, 2 × 1-H); δ_C (100 MHz, $CDCl_3$) −4.9 and −4.5 (2 × q, $Si(CH_3)_2$), 17.6 (q, 2 × C-6), 17.8 (s, 2 × Si*C*$(CH_3)_3$), 21.1 (q, 2 × CO*C*H_3), 25.5 (q, 2 × SiC(CH_3)$_3$), 26.4 (t, 2 × –O–CH_2–*C*H_2–), 39.2 (t, 2 × C-2), 67.0 (t, 2 × –O–*C*H_2–CH_2–), 67.5, 65.9 (2 × d, 2 × C-3, 2 × C-5), 77.8 (d, 2 × 4-H), 97.1 (d, 2 × 1-H), 169.9 (s, 2 × *C*OCH$_3$); elemental analysis calcd. (%) for $C_{15}H_{30}O_4Si$ (302.48): C 59.56, H 10.00; found C 59.44 H 10.10.

1-*O*-Prop-1-(*E*/*Z*)enyl-3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*arabino*-pyranoside (12)

When allyl glycoside (α)-**6** was subjected to identical reaction conditions as described above (except that Grubbs' catalyst **11** was employed) pyranoside **12** was isolated after column chromatography (73%, *E* : *Z*= 1.6 : 1) and 20% of recovered starting allyl glycoside **6**.

δ_H (400 MHz; $CDCl_3$) 1.18 and 1.19 (6 H, 2 × d, *J* 6.3, 6-H, 6-H′), 1.48 and 1.50 (6H, 2 × dd, J 7.0 and 1.6, J 6.3 and 1.6, =C*H*–CH_3, =C*H*′–CH_3), 1.74 and 1.79 (2 H, 2 × ddd, *J* 12.8, 11.7 and 3.0, 2-H_{eq}, 2-H′$_{eq}$), 1.97, 1.99, 2.03 and 2.04 (12 H, 4 × s, 4 × AcO), 2.24 (2 H, ddd, *J* 12.8, 5.4 and 2.7, 2-H_{ax}, 2-H′$_{ax}$), 3.79 and 3.86 (2 H, 2 × dq, *J* 9.7 and 6.3, 5-H, 5-H′), 4.42 (1 H, dq, J 6.3 and 6.3, =C*H*′–CH_3), 4.73 and 4.78 (2 H, 2 × t, *J* 9.7, 4-H, 4-H′), 4.90 and 5.12 (2 H, 2 × d, *J* 3.0, 1-H, 1-H′), 4.92 (1 H, dq, J 12.1 and 7.0, =C*H*–CH_3), 5.30 (2 H, m, 3-H, 3-H′), 5.92 (1 H, dq, *J* 6.3 and 1.6, O–C*H*′=CH–), 6.10 (1 H, dq, *J* 12.1 and 1.6, O–C*H*=CH–); δ_C (100 MHz, $CDCl_3$) 8.9 and 11.8 (2 × q, =CH–CH_3, =CH–CH_3′), 17.8 and 17.9 (2 × q, C-6, C-6′), 21.1, 21.2 and 21.3 (4 × q, 4 × CO*C*H_3), 35.5 and 35.6 (2 × t, C-2, C-2′), 65.9, 66.0, 69.2, 69.4, 75.2 and 75.6 (6 × d, C-4, C-4′, C-3, C-3′, C-5, C-5′), 95.3 and 96.2 (2 × d, C-1, C-1′), 102.8 and 103.0 (2 × d, =C*H*–CH_3, =C*H*′–CH_3), 145.3 and 146.1 (2 × d, –O–C*H*=, –O–C*H*′=), 170.5 (4 × s, 4 × *C*OCH$_3$); LC-MS (ESI) (+*c*): *m*/*z* (%): 273.1283 (100) $[M+H]^+$.

1,4-Di-(4′-*O*-allyl-2′,6′-dideoxy-α-L-*arabino*-pyranosyl)-1,4-butanediol (14)

To a solution of tetrol **13** (393 mg, 1.13 mmol), imidazole (223 mg, 3.3 mmol), DMAP (10 mg, cat.) in dry DMF (10 ml) was slowly added *tert*-butyldiphenylsilyl chloride (565 mg, 2.26 mmol) at 0 °C. The solution was stirred at 0 °C for 4 h and then allowed to warm up to rt. After additional 12 h the reaction was terminated by addition of *n*-hexane (10 ml). The DMF phase was extracted with *n*-hexane (2 × 5 ml) and the combined hexane phases were concentrated under reduced pressure. The crude material was subjected to silica gel filtration (petroleum ether–ethyl acetate 10 : 1) after which 1,4-di-(3′-*O*-*tert*-butyldiphenylsilyl-2′,6′-dideoxy-α-L-*arabino*-pyranosyl)-1,4-butanediol was isolated (857 mg, 1.034 mmol; 94%).

δ_H (200 MHz, $CDCl_3$) 1.08 (9 H, s, *t*-Bu), 1.25 (6 H, d, *J* 6.3, 2 × 6-H), 1.33 (4 H, m, 2 × –O–CH_2–CH_2–), 1.67 (2 H, ddd, *J* 12.6, 11.2 and 2.8, 2 × 2-H_{eq}), 1.84 (2 H, ddd, *J* 12.6, 5.1 and 0.9, 2 × 2-H_{ax}), 2.14 (2 H, d, *J* 2.6, 2 × OH), 3.15 (2 H, m, 2 × –O–C*H*H′–CH_2–), 3.23 (2 H, ddd, *J* 9.1, 9.0 and 2.5, 2 × 4-H), 3.44 (2 H, m, 2 × –O–CH*H*′–CH_2–), 3.50 (2 H, dq, *J* 9.1 and 6.3, 2 × 5-H), 4.01 (2 H, ddd, *J* 11.2, 9.0 and 5.1, 2 × 3-H), 4.63 (2 × H, d, *J* 2.8, 2 × 1-H), 7.68 (8 H, m, Ar), 7.38 (12 H, m, Ar).

To a vigorously stirred solution of this homodimeric diol (4.6 g, 5.5 mmol) and allyl bromide (9.4 ml, 110.0 mmol) was

added $LiN(SiMe_3)_2$ (6.45 g, 38.5 mmol) at 0 °C. After 5 min the reaction was diluted with ethyl acetate and water. The organic layer was washed with water until it reached neutral pH. The organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude oil was purified by column chromatography on silica gel (ethyl acetate–petroleum ether 1 : 10) to yield bisallylated homodimer, along with monoallylated product **16** (2.39 g, 2.75 mmol; 50%). The latter can be used for another allylation step according to the procedure described above.

1st fraction. Bisallylated homodimer [1,4-di-(4′-*O*-allyl-3′-*O*-*tert*-butyldiphenylsilyl-2′,6′-dideoxy-α-L-*arabino*-pyranosyl)-1,4-butanediol] (2.4 g, 2.64 mmol, 48%); colorless oil; δ_H (400 MHz, $CDCl_3$) 1.10 (18 H, s, 2 × *t*-Bu), 1.27 (4 H, m, 2 × –O–CH_2–CH_2–), 1.28 (6 H, d, *J* 6.3, 2 × 6-H), 1.54 (4 H, m, 2 × 2-H), 3.02 (2 H, dd, *J* 9.3 and 9.0, 2 × 4-H), 3.05 (2 H, m, 2 × –O–C*H*H′–CH_2–), 3.39 (2 H, m, 2 × –O–CH*H*′–CH_2–), 3.60 (2 H, dq, *J* 9.3 and 6.3, 2 × 5-H), 4.18 (2 H, dd, *J* 12.0 and 6.0, 2 × –O–C*H*H′–CH=), 4.25 (2 H, ddd, *J* 10.1, 9.0 and 5.9, 2 × 3-H), 4.50 (2 H, dd, *J* 12.0 and 5.4, 2 × –O–CH*H*′–CH=), 4.52 (2 H, br s, 2 × 1-H), 5.20 (2 H, dd, *J* 10.3 and 1.7, 2 × –CH=C*H*H′), 5.28 (2 H, dd, *J* 17.2 and 1.7, 2 × –CH=CH*H*′), 5.97 (2 H, dddd, *J* 17.2, 10.3, 6.0 and 5.4, 2 × –C*H*=CH_2), 7.71 (4 H, dd, *J* = 7.9 and 1.5, Ar), 7.36 (12 H, m, Ar), 7.76 (4 H, dd, *J* = 7.9 and 1.5, Ar); δ_C (100 MHz, $CDCl_3$) 18.2 (q, 2 × C-6), 19.2 (s, 2 × Si*C*$(CH_3)_3$), 26.0 (t, 2 × –O–CH_2–*C*H_2–), 27.0 (q, 2 × SiC(CH_3)$_3$), 38.9 (t, 2 × C-2), 66.5 (t, 2 × –O–*C*H_2–CH_2–), 67.2 (d, 2 × C-5), 71.1 (d, 2 × C-3), 74.3 (t, 2 × –*C*H_2–CH=), 85.7 (d, 2 × C-4), 96.9 (d, 2 × C-1), 116.8 (t, 2 × –CH=*C*H_2), 129.6, 129.5, 127.4 (3 × d, 6 × Ar), 133.8 (s, 2 × Ar), 134.9 (s, 2 × Ar), 135.1 (d, 2 × –*C*H=CH_2), 135.8 (d, 2 × Ar), 135.9 (d, 2 × Ar).

2nd fraction. [1-(4′-*O*-allyl-3′-*O*-*tert*-butyldiphenylsilyl-2′,6′-dideoxy-α-L-*arabino*-pyranosyl)-4-(3′-*O*-*tert*-butyldiphenylsilyl-2′,6′-dideoxy-α-L-*arabino*-pyranosyl)-1,4-butanediol] (**16**); δ_H (500 MHz, $CDCl_3$) 1.09 (9 H, s, *t*-Bu), 1.25 (3 H, d, *J* 6.1, 6-H′), 1.26 (3 H, d, *J* 6.2, 6-H), 1.62 (6 H, m, 2 × –O–CH_2–CH_2–, 2-H_{eq}, 2-H′$_{eq}$), 2.01 (2 H, ddd, *J* 13.0, 4.0 and 3.7, 2-H_{ax}, 2-H$_{ax}$′), 2.26 (1 H, d, *J* 2.0, OH), 2.85 (1 H, dd, *J* 9.2 and 9.1, 4-H′), 3.11 (1 H, ddd, *J* 9.2, 8.7 and 2.0, 4-H), 3.63 (4 H, m, 5-H, 5-H′, –O–CH_2–CH_2–), 3.34 (2 H, m, –O–CH_2–CH_2–), 3.90 (1 H, ddd, *J* 11.1, 8.7 and 4.9, 3-H), 3.96 (1 H, ddd, *J* 10.9, 9.2 and 5.1, 3-H′), 4.03 (1 H, dd, *J* 12.2 and 6.0, –*O*–C*H*H′–CH=), 4.33 (1 H, dd, *J* 12.2 and 5.4, –*O*–CH*H*′–CH=), 4.77, 4.75 (2 H, 2 × d, 2 × *J* 3.0, 1-H, 1-H′), 5.17 (1 H, d, *J* 10.6, –CH=C*H*H′), 5.26 (1 H, dd, *J* 17.1 and 1.5, –CH=CH*H*′), 5.95 (1 H, dddd, *J* 17.1, 10.6, 5.8 and 5.8, –C*H*=CH_2), 7.34 (6 H, m, Ar), 7.67 (2 H, dd, *J* 7.9 and 1.5, Ar), 7.73 (2 H, dd, *J* 7.9 and 1.5, Ar); δ_C (125 MHz, $CDCl_3$) 17.8 and 17.9 (2 × q, C-6, C-6′), 19.2 (s, Si*C*$(CH_3)_3$), 26.4 and 26.4 (2 × t, 2 × –O–CH_2–*C*H_2–), 27.0 (q, SiC(*C*H_3)$_3$), 38.7 and 39.5 (2 × t, C-2, C-2′), 66.8 and 66.9 (2 × t, 2 × –O–*C*H_2–CH_2–), 67.2 and 67.3 (2 × d, C-5, C-5′), 70.2 (d, C-3′), 70.6 (d, C-3), 74.3 (t, –*C*H_2–CH=), 78.0 (d, C-4), 85.3 (d, C-4′), 97.1 (d, C-1′), 97.3 (d, C-1), 116.8 (t, –CH=*C*H_2), 129.3 and 129.4 (2 × d, 3 × Ar), 133.8 (s, Ar), 134.9 (s, Ar), 135.2 (d, –*C*H=CH_2), 135.8 (d, Ar), 135.9 (d, Ar).

A solution (1 M) of tetra-*n*-butyl ammonium fluoride (1.65 mmol, 1.65 ml, 3 eq) in THF was added to a solution of 1,4-di-(4′-*O*-allyl-3′-*O*-*tert*-butyldiphenylsilyl-2′,6′-dideoxy-α-L-*arabino*-pyranosyl)-1,4-butanediol (0.5 g, 0.55 mmol) in THF (10 ml). The solution was stirred overnight, the solvent was removed under reduced pressure and the crude material was purified by column chromatography (silica gel; petroleum ether–ethyl acetate 1 : 3) to give the corresponding diol **14** (200 mg, 0.465 mmol; 85%) as a colorless, amorphous powder.

δ_H (500 MHz, $CDCl_3$) 1.30 (6 H, d, *J* 6.3, 2 × 6-H), 1,63 (4 H, ddd *J* 6.4, 6.4 and 6.4, 2 × –O–CH_2–CH_2–), 1.69 (2 H, ddd, *J* 12.9, 11.7 and 3.5, 2 × 2-H_{eq}), 2.13 (2 H, ddd, *J* 12.9, 5.2 and 1.1, 2 × 2-H_{ax}), 2.34 (2 H, d, *J* 3.1, 2 × OH), 2.87 (2 H, br dd, *J* 9.3 and 9.1, 2 × 4-H), 3.36 (2 H, dt, *J* 9.5 and 6.0, 2 × –O–C*H*H′–CH_2–), 3.63 (2 H, dt, *J* 9.4 and 6.4, 2 × –O–CH*H*′–CH_2–), 3.66 (2 H, dq, *J* 9.3 and 6.3, 2 × 5-H), 4.00 (2 H, dddd, *J* 11.7, 9.1, 5.2 and 3.2, 2 × 3-H), 4.25 (4 H, dq, *J* 5.7 and 1.4, 2 × –O–CH_2–CH=), 4.83 (2 H, d, *J* 3.5, 2 × 1-H), 5.22 (2 H, dq, *J* 10.4 and 1.4, 2 × –CH=C*H*H′), 5.32 (2 H, dq, *J* 17.2 and 1.4, 2 × –CH=CH*H*′), 5.97 (2 H, dddd, *J* 17.2, 10.4, 5.7 and 5.7, 2 × –C*H*=CH_2); δ_C (125 MHz, $CDCl_3$) 18.2 (q, 2 × C-6), 26.4 (t, 2 × –O–CH_2–*C*H_2–), 37.7 (t, 2 × C-2), 66.9 (t, 2 × –O–*C*H_2–CH_2–), 67.0 (d, 2 × C-5), 68.7 (d, 2 × C-3), 74.0 (t, 2 × –*C*H_2–CH=), 86.4 (d, 2 × C-4), 97.1 (d, 2 × C-1), 117.2 (t, 2 × –CH=*C*H_2), 134.9 (d, 2 × –*C*H=CH_2); elemental analysis calcd. (%) for $C_{22}H_{38}O_8$ (430.53): C 61.37, H 8.90; found C 61.29, H 8.78.

1,4-Di-(4′-*O*-allyl-2′,3′,6′-trideoxy-3′-trifluoroacetamido-α-L-*ribo*-hexopyranosyl)-1,4-butanediol (15)

To a solution of diol **14** (100 mg, 0.23 mmol) in dry CH_2Cl_2 (20 ml) with pyridine as a base (100 μl) at −15 °C Tf_2O (138 mg, 0.49 mmol) in dry CH_2Cl_2 (5 ml) was added. The reaction mixture was stirred for 2 h, diluted with water and extracted with petroleum ether (2 × 40 ml). The organic extracts were combined, washed with water, dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The crude product was dissolved in a small amount of CH_2Cl_2 and filtered through a small column of silica gel to yield the highly labile bistriflate (125 mg, 0.22 mmol; 96%) as a colorless oil.

δ_H (200 MHz, $CDCl_3$) 1.29 (6 H, d, *J* 6.2, 2 × 6-H), 1.38 (4 H, m, 2 × –O–CH_2–CH_2–), 1.63 (2 H, dt, *J* 12.3 and 3.1, 2 × 2-H_{eq}), 2.29 (2 H, ddd, *J* 12.3, 5.3 and 0.9, 2 × 2-H_{ax}), 2.90 (2 H, t, *J* 9.2, 2 × 4-H), 3.05 (2 H, m, 2 × –O–C*H*H′–CH_2–), 3.38 (2 H, m, 2 × –O–CH*H*′–CH_2–), 3.77 (2 H, dq, *J* 9.2 and 6.2, 2 × 5-H), 3.91 (2 H, ddt, *J* 12.1, 5.5 and 1.0, 2 × =CH–C*H*H′–O–), 4.18 (2 H, ddt, *J* 12.1, 5.5 and 1.2, 2 × =CH–CH*H*′–O–), 4.51 (2 H, d, *J* 3.1, 2 × 1-H), 5.11 (2 H, dq, *J* 10.3 and 1.5, 2 × –CH=C*H*H′), 5.27 (2 H, dq, *J* 17.2 and 1.5, 2 × –CH=CH*H*′), 5.41 (2 H, ddd, *J* 12.3, 9.2 and 5.3, 2 × 3-H), 5.93 (2 H, ddt, *J* 17.2, 10.3 and 5.5, 2H, 2 × –C*H*=).

A solution of this bistriflate (200 mg, 0.465 mmol) in dry benzene (1 ml) was treated with tetra-*n*-butylammonium azide (300 mg, 1.06 mmol, 2.3 eq). The reaction mixture was heated at 70 °C for 15 min, diluted with water (10 ml) and extracted with ethyl acetate (3 × 30 ml). The organic extracts were combined, dried ($MgSO_4$) and solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel; ethyl acetate–petroleum ether 1 : 12) to yield the 1,4-di-(4′-*O*-allyl-3′-azido-2′,3′,6′-trideoxy-α-L-*ribo*-hexopyranosyl)-1,4-butanediol (80 mg, 0.167 mmol; 36%).

δ_H (500 MHz, C_6D_6, C_6D_6 = 7.16 ppm) 1.31 (6 H, d, *J* 6.2, 2 × 6-H), 1.33 (2 H, dt, *J* 14.5 and 4.1, 2 × 2-H_{eq}), 1.76 (4 H, m, 2 × –O–CH_2–CH_2–),1.85 (2 H, ddd, *J* 14.5, 3.4 and 0.9, 2 × 2-H_{ax}), 2.82 (2 H, dd, *J* 9.1 and 3.8, 2 × 4-H), 3.26 (2 H, m, 2 × –O–C*H*H′–CH_2–), 3.41 (2 H, dt, *J* 3.8 and 3.4, 2 × 3-H), 3.60 (2 H, dd, *J* 12.7 and 5.4, 2 × –O–C*H*H′–CH=), 3.70 (2 H, m, 2 × –O–CH*H*′–CH_2–), 3.85 (2 H, dd, *J* 12.7 and 5.4, 2 × –O–CH*H*′–CH=), 4.25 (2 H, dq, *J* 9.1 and 6.2, 2 × 5-H), 4.53 (2 H, d, *J* 4.1, 2 × 1-H), 5.01 (2 H, br dd, *J* 10.4 and 1.4, 2 × –CH=C*H*H′), 5.18 (2 H, dq, *J* 17.2 and 1.4, 2 × –CH=CH*H*′), 5.78 (2 H, ddt, *J* 17.2, 10.4 and 5.4, 2 × –C*H*=CH_2); δ_C (125 MHz, C_6D_6, C_6D_6 = 128.06 ppm) 18.2 (q, 2 × C-6), 26.9 (t, 2 × –O–CH_2–*C*H_2–), 33.0 (t, 2 × C-2), 54.8 (d, 2 × C-3), 63.4 (d, 2 × C-5), 67.4 (t, 2 × –O–*C*H_2–CH_2–), 69.9 (t, 2 × –*C*H_2–CH=), 80.1 (d, 2 × C-4), 95.6 (d, 2 × C-1), 116.7 (t, 2 × –CH=*C*H_2), 135.1 (d, 2 × –*C*H=CH_2); LC-MS (ESI) (+*c*): *m*/*z* (%): 453.26 (100) $[M–N_2]^+$, 487.26 (92) $[M + Li]^+$, 503.24 (88) $[M + Na]^+$; HR-MS $C_{22}H_{36}N_6O_6$ + ^{23}Na: calcd. 503.2594, found 503.2593.

A solution of 1,4-di-(4′-*O*-allyl-3′-azido-2′,3′,6′-trideoxy-α-L-*ribo*-hexopyranosyl)-1,4-butanediol (80 mg, 0.167 mmol) in dry THF (5 ml) is added to a solution of $LiAlH_4$ (25 mg, 0.66 mmol)

in dry THF (10 ml). The reaction mixture was stirred for 45 min. at rt, then NaF (500 mg) was added followed by a careful addition of a water–THF mixture (1 ml water, 4 ml THF). The resulting suspension was stirred for an additional hour, filtered and the solvent was evaporated under reduced pressure. The crude amine was dissolved in CH_2Cl_2 (25 ml) and dry triethylamine (50 mg, 0.5 mmol, 3 eq) was added at 0 °C followed by addition of trifluoroacetic anhydride (105 mg, 0.5 mmol). The mixture was stirred at rt for 30 min, whereupon TLC (ethyl acetate–petroleum ether 1 : 5) revealed that acylation was complete. The mixture was concentrated under reduced pressure to afford a crude product which was purified by column chromatography (silica gel; ethyl acetate–petroleum ether 1 : 7) to yield pure diamide **15** (62 mg, 0.1 mmol; 60% for two steps).

δ_H (500 MHz, C_6D_6, C_6D_6 = 7.16 ppm) 1.41 (6 H, d, *J* 6.2, 2 × 6-H), 1.3–1.6 (8 H, m, 2 × 2-H_{ax}, 2 × 2-H_{eq}, 2 × –O–CH_2–CH_2–), 2.89 (2 H, dd, *J* 9.5 and 3.9, 2 × 4-H), 3.06 (2 H, m, 2 × –O–C*H*H′–CH_2–), 3.56 (2 H, m, 2 × –O–CH*H*′–CH_2–), 3.84 (2 H, dq, *J* 9.5 and 6.2, 2 × 5-H), 3.85 (2 H, dd, *J* 12.5 and 6.0, 2 × –O–C*H*H′–CH=), 4.38 (2 H, dd, *J* 12.5 and 5.3, 2 × –O–CH*H*′–CH=), 4.48 (2 H, d, *J* 3.0, 2 × 1-H), 4.50 (2 H, m, 2 × 3-H), 5.15 (2 H, br dd, *J* 10.4 and 1.3, 2 × –CH=C*H*H′), 5.34 (2 H, dq, *J* 17.2 and 1.3, 2 × –CH=CH*H*′), 5.97 (2 H, dddd, *J* 17.2, 10.4, 6.0 and 5.3, 2 × –C*H*=CH_2), 8.00 (2 H, d, *J* 8.5, 2 × NH); δ_C (125 MHz, C_6D_6, C_6D_6 = 128.06 ppm), 18.3 (q, 2 × C-6), 26.6 (t, 2 × –O–CH_2–*C*H_2–), 32.7 (t, 2 × C-2), 43.7 (d, 2 × C-3), 63.5 (d, 2 × C-5), 67.4 (t, 2 × –O–*C*H_2–CH_2–), 70.5 (t, 2 × –*C*H_2–CH=), 78.1 (d, 2 × C-4), 96.8 (d, 2 × C-1), 117.1 (t, 2 × –CH=*C*H_2), 135.1 (d, 2 × –*C*H=CH_2); HR-MS $C_{26}H_{38}F_6N_2O_8{}^{23}$Na: calcd. 643.2430, found 643.2435.

1,4-Di-(4′-*O*-allyl-2′,6′-dideoxy-3′-oxo-α-L-*arabino*-pyranosyl)-1,4-butanediol (17)

To a solution of diol **14** (51 mg, 0.118 mmol) in dry CH_2Cl_2 was added Dess–Martin periodinane (111 mg, 0.261 mmol). The mixture was stirred at rt for 3 h, after which TLC (ethyl acetate–petroleum ether 1 : 3) revealed that oxidation was completed. Remaining oxidant was destroyed by washing with aqueous $Na_2S_2O_3$ solution. The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (ethyl acetate–petroleum ether 1 : 5) yielding bisulose **17** (50 mg, 0.117 mmol; >99%) as fine colorless crystals which was used directly in the next step.

δ_H (500 MHz, C_6D_6, C_6D_6 = 7.16 ppm) 1.43 (4 H, br q, *J* 5.8, 2 × –O–CH_2–CH_2–), 1.52 (6 H, d, *J* 6.2, 2 × 6-H), 2.27 (2 H, dd, *J* 14.0 and 4.3, 2 × 2-H_{eq}), 2.50 (2 H, br d, *J* 14.0, 2 × 2-H_{ax}), 3.10 (2 H, dt, *J* 9.4 and 5.8, 2 × –O–C*H*H′–CH_2–), 3.43 (2 H, br d, *J* 9.5, 2 × 4-H), 3.46 (2 H, dt, *J* 10.0 and 6.3, 2 × –O–CH*H*′–CH_2–), 3.99 (2 H, br dd, *J* 12.7 and 6.2, 2 × –O–C*H*H′–CH=), 4.17 (2 H, dq, *J* 9.5 and 6.2, 2 × 5-H), 4.56 (2 H, br ddt, *J* 12.7, 5.0 and, 1.4, 2 × –O–CH*H*′–CH=), 4.79 (2 H, d, *J* 4.3, 2 × 1-H), 5.13 (2 H, dq, *J* 10.6 and 1.4, 2 × –CH=C*H*H′), 5.31 (2 H, dq, *J* 17.0 and 1.6, 2 × –CH=CH*H*′), 5.98 (2 H, dddd, *J* 17.0, 10.6, 6.0 and 5.0, 2 × –C*H*=CH_2); δ_C (125 MHz, C_6D_6, C_6D_6 = 128. ppm) 19.1 (q, 2 × C-6), 26.4 (t, 2 × –O–CH_2–*C*H_2–), 46.9 (t, 2 × C-2), 67.2 (t, 2 × –O–*C*H_2–CH_2–), 69.4 (d, 2 × C-5), 72.4 (t, 2 × –*C*H_2–CH=), 85.0 (d, 2 × C-4), 98.6 (d, 2 × C-1), 116.8 (t, 2 × –CH=*C*H_2), 135.2 (d, 2 × –*C*H=CH_2), 202.2 (s, 2 × C-3).

[^{15}N]$_2$-1,4-Di-(4′-*O*-allyl-2′,3′,6′-trideoxy-3′-trifluoroacetamido-α-L-*ribo*-hexopyran-osyl)-1,4-butanediol (15) [^{15}N]$_2$-1-(4′-*O*-allyl-2′,3′,6′-trideoxy-3′-trifluoroacetamido-α-L-*ribo*-hexopyranosyl)-4-(4′-*O*-allyl-2′,3′,6′-trideoxy-3′-trifluoroacetamido-α-L-*arabino*-hexopyranosyl)-1,4-butanediol 18 and macrocycle (19)

The bisulose **17** (350 mg, 0.82 mmol), [^{15}N]-ammonium acetate (700 mg, 9.0 mmol) and $NaBH_3CN$ (105 mg, 1.64 mmol) were stirred in methanol (100 ml) for 24 h, followed by the addition of the second portion of $NaBH_3CN$ (105 mg, 1.64 mmol). The reaction mixture was allowed to stir for another 24 h after which triethylamine (1.5 ml, 10 mmol) and CF_3COOEt (3.6 ml, 30 mmol) were added. The reaction mixture was stirred overnight at rt. The solvent was evaporated under reduced pressure. Prior to column chromatography the crude product was separated from trifluoroacetamide by sublimation in high vacuum (0.01 mbar, 60 °C, 30 min). The remaining residue was purified by column chromatography (silica gel; ethyl acetate–petroleum ether 1 : 5) to yield three fractions:

1st fraction. [^{15}N]$_2$-**15** (177 mg, 0.284 mmol; 35%); δ_H (500 MHz, C_6D_6, C_6D_6 = 7.16 ppm) 1.30 (6 H, d, *J* 6.2, 2 × 6-H), 1.2–1.5 (8 H, m, 2 × 2-H_{ax}, 2 × 2-H_{eq}, 2 × –O–CH_2–CH_2–), 2.77 (2 H, ddd, *J* 9.6, 3.8 and 3.8, 2 × 4-H), 2.95 (2 H, m, 2 × –O–C*H*H′–CH_2–), 3.44 (2 H, m, 2 × –O–CH*H*′–CH_2–), 3.85 (4 H, m, 2 × –O–C*H*H′–CH=, 2 × 5-H), 4.27 (2 H, dd, *J* 12.4 and 5.3, 2 × –O–CH*H*′–CH=), 4.37 (2 H, d, *J* 3.2, 2 × 1-H), 4.38 (2 H, m, 2 × 3-H), 5.03 (2 H, br dd, *J* 10.3 and 1.34, 2 × –CH=C*H*H′), 5.22 (2 H, dq, *J* 17.2 and 1.6, 2 × –CH=CH*H*′), 5.86 (2 H, dddd, *J* 17.2, 10.3, 6.4 and 5.3, 2 × –C*H*=CH_2), 7.89 (2 H, dd, *J* 92.1 and 9.1, 2 × NH); δ_C (125 MHz, C_6D_6, C_6D_6 = 128 ppm) 18.3 (q, 2 × C-6), 26.6 (t, 2 × –O–CH_2–*C*H_2–), 32.7 (t, 2 × C-2), 43.8, 43.7 (d, 2 × C-3), 63.5 (d, 2 × C-5), 67.4 (t, 2 × –O–*C*H_2–CH_2–), 70.5 (t, 2 × –*C*H_2–CH=), 78.1 (d, 2 × C-4), 96.8 (d, 2 × C-1), 117.1 (t, 2 × –CH=*C*H_2), 135.1 (d, 2 × –*C*H=CH_2), 157.1, 156.9, 156.8, 156.7, 156.5, 156.4, 156.2 (s, 2 × *C*OCF_3);

2nd fraction. **18** (20 mg, 32 μmol; 3.9%); the *ribo*-configured pyran is labelled with the letter [a] while the *arabino*-configured pyran is labelled as [b]; δ_H (500 MHz, C_6D_6, C_6D_6 = 7.16 ppm) 1.38 (1 H, m, 2-$H_{eq}{}^a$), 1.38 (3 H, d, *J* 6.1, 6-H^b), 1.39 (3 H, d, *J* 6.1, 6-H^a), 1.55 (5 H, m, 2-$H_{ax}{}^a$, 2 × –O–CH_2–CH_2–), 1.68 (1 H, dt, *J* 13.2 and 3.6, 2-$H_{eq}{}^b$), 1.97 (1 H, dd, *J* 12.9 and 4.6, 2-$H_{ax}{}^b$), 2.88 (1 H, ddd, *J* 9.6, 3.7 and 3.7, 4-H^a), 2.95 (1 H, t, *J* 9.5, 4-H^b), 3.08, 3.22 and 3.60 (4 H, 3 × m, 2 × –O–CH_2–CH_2–), 3.8–3.9 (3 H, m, –O–C*H*H′–CH=[a], –O–C*H*H′–CH=[b], 5-H^a), 3.91 (1 H, dq, *J* 9.5 and 6.1, 5-H^b), 4.01 (1 H, dd, *J* 12.7 and 5.3, –O–CH*H*′–CH=[b]), 4.32 (1 H, m, 3-H^b), 4.38 (1 H, dd, *J* 12.3 and 5.4, –O–CH*H*′–CH=[a]), 4.50 (1 H, m, 3-H^a), 4.51 (1 H, d, *J* 3.0, 1-H^a), 4.65 (1 H, d, *J* 2.8, 1-H^b), 5.11 (1 H, dq, *J* 10.7 and 1.4, –CH=C*H*H′[a]), 5.15 (1 H, dq, *J* 10.5 and 1.6, –CH=C*H*H′[b]), 5.26 (1 H, dq, *J* 17.2 and 1.6, –CH=CH*H*′[a]), 5.33 (1 H, dq, *J* 17.1 and 1.6, –CH=CH*H*′[b]), 5.83 (1 H, dddd, *J* 17.1, 10.7, 5.4 and 5.4, –C*H*=$CH_2{}^a$), 5.97 (1 H, dddd, *J* 17.1, 10.5, 6.4 and 5.3, –C*H*=$CH_2{}^b$), 6.00 (1 H, dd, *J* 90.9 and 8.1, ^{15}NH^b), 8.08 (1 H, dd, *J* 92.2 and 9.2, ^{15}NH^a); δ_C (125 MHz, C_6D_6, C_6D_6 = 128.06 ppm) 18.5 and 18.6 (2 × q, C-6[a,b]), 26.6 and 27.1 (2 × t, 2 × –O–CH_2–*C*H_2–), 32.9 (t, C-2[a]), 35.4 (t, C-2[b]), 43.8 and 43.9 (2 × d, C-3[a]), 49.7 and 49.8 (2 × d, C-3[b]), 63.6 (d, C-5[a]), 67.1 and 67.8 (2 × t, 2 × –O–*C*H_2–CH_2–), 68.2 and 68.2 (2 × d, C-5[b]), 70.61 (t, –*C*H_2–CH=[a]), 73.4 (t, –*C*H_2–CH=[b]), 78.3 (d, C-4[a]), 82.1 and 82.2 (2 × d, C-4[b]), 96.5 and 96.5 (2 × d, C-1[b]), 96.9 and 97.0 (2 × d, C-1[a]), 117.2 and 117.3 (2 × t, –CH=*C*$H_2{}^a$, –CH=*C*$H_2{}^b$), 135.0 and 135.3 (2 × d, –*C*H=$CH_2{}^a$, –*C*H=$CH_2{}^b$), 156.5 and 156.7 (2 × s, 2 × *C*OCF_3); LC-MS (ESI) (+*c*): *m*/*z* (%): 645.15 (100) [M + Na]$^+$.

3rd fraction. **19** (20 mg, 46 μmol, 5.6%); the cyanide free pyran is labelled with the letter [a] while the cyanide containing pyran is labelled as [b]; δ_H (500 MHz, C_6D_6, C_6D_6 = 7.16 ppm) 1.34 (3 H, d, *J* 6.3, 6-H^b), 1.45 (1 H, m, –O–CH_2–C*H*H′–[b]), 1 52 (2 H, m, –O–CH_2–CH_2–[a]), 1.54 (3 H, d, *J* 6.1, 6-H^a), 1.65 (1 H, m, –O–CH_2–CH*H*′–[b]), 1.78 (1 H, ddt, *J* 14.1, 3.7 and 3.7, 2-$H_{eq}{}^a$), 1.94 (1 H, ddd, *J* 13.7, 5.7 and 3.9, 2-$H_{eq}{}^b$), 2.46 (1 H, dd, *J* 14.1 and 2.9, 2-$H_{ax}{}^a$), 2.48 (1 H, br d, *J* 13.7, 2-$H_{ax}{}^b$), 3.01 (1 H, ddd, *J* 9.6, 4.0 and 4.0, 4-H^a), 3.09 (1 H, ddd, *J* 9.4, 7.0 and 2.1, –O–C*H*H′–CH_2–[b]), 3.17 (1 H, m, –O–CH*H*′–CH_2–[a]), 3.22 (1 H, dd, *J* 9.6 and 1.3, 4-H^b), 3.53 (1 H, ddd, *J* 9.2, 7.6 and 3.3, –O–C*H*H′–CH_2–[a]), 3.72 (1 H, ddd, *J* 9.7, 8.4 and

1.7, –O–CH*H′*–CH_2–[b]), 3.91 (1 H, m, 3-H[a]), 4.05 (2 H, m, –O–C*H*H′–CH=[a], –O–C*H*H′–CH=[b]), 4.30 (1 H, dq, *J* 9.6 and 6.1, 5-H[a]), 4.33 (1 H, dq, *J* 9.6 and 6.3, 5-H[b]), 4.42 (1 H, d, *J* 3.9, 1-H[b]), 4.49 (1 H, ddt, *J* 12.7, 5.2 and 1.5, –O–CH*H′*–CH=[a]), 4.70 (1 H, ddt, *J* 12.3, 5.3 and 1.5, –O–CH*H′*–CH=[b]), 4.70 (1 H, dd, *J* 81.6 and 9.3, ^{15}NH), 4.76 (1 H, d, *J* 3.7, 1-H[a]), 5.16 (1 H, dq, *J* 10.5 and 1.6, –CH=C*H*H′[a]), 5.22 (1 H, dq, *J* 10.4 and 2.0, –CH=C*H*H′[b]), 5.35 (1 H, dq, *J* 17.2 and 1.7, –CH=CH*H′*[a]), 5.47 (1 H, dq, *J* 17.3 and 1.5, –CH=CH*H′*[b]), 5.94 (1 H, ddt, *J* 17.2, 10.5 and 5.2, –C*H*=CH_2[a]), 6.29 (1 H, dddd, *J* 17.3, 10.4, 6.2 and 5.3, –C*H*=CH_2[b]); δ_C (125 MHz, C_6D_6, C_6D_6 = 128 ppm) 17.9 (q, C-6[b]), 18.7 (q, C-6[a]), 28.7 (t, –O–CH_2–*C*H_2–[a]), 29.1 (t, –O–CH_2–*C*H_2–[b]), 36.4 (t, C-2[a]), 41.4 and 41.5 (2 × t, C-2[b]), 49.3 and 49.4 (2 × d, C-3[a]), 57.5 and 57.6 (2 × s, C-3[b]), 63.9 and 64.0 (2 × d, C-5[a], C-5[b]), 68.5 (t, –O–*C*H_2–CH_2–[a]), 68.7 (t, –O–*C*H_2–CH_2–[b]), 71.5 (t, –*C*H_2–CH=[b]), 74.7 (t, –*C*H_2–CH=[a]), 81.9 and 82.0 (2 × d, C-4[a]), 87.1 (d, C-4[b]), 96.2 (d, C-1[b]), 98.4 (d, C-1[a]), 116.5 (t, –CH=*C*H_2[a]), 116.6 (t, –CH=*C*H_2[b]), 122.3 (s, *C*≡N), 135.1 (d, –*C*H=CH_2[a]), 136.6 (d, –*C*H=CH_2[b]); LC-MS (ESI) (+*c*): *m*/*z* (%): 438.26 (100) $[M + H]^+$.

Macrocyclization of ^{15}N-labelled bisallylated homodimer 15 and catalytic hydrogenation: Synthesis of macrocycles 22 and 23

The bisallylated aminodeoxysaccharide **15** (150 mg, 0.24 mmol) was dissolved in dry CH_2Cl_2 (150 ml) and Grubbs' catalyst **10** (100 mg, 20 mol%) was added in one portion. After the reaction mixture was stirred under N_2 at rt for seven days the solvent was removed under reduced pressure. The mixture of crude products was directly hydrogenated with H_2 (1 bar) in ethyl acetate (5 ml) using PtO_2 (7 mol%). After 12 h at rt the reaction mixture was filtered and concentrated *in vacuo* to yield two macrocycles after column chromatography (silica gel; petroleum ether–ethyl acetate 5 : 1).

1st fraction. **22** (65 mg, 54.3 μmol; 45%); $[\alpha]_D^{25} = -115.6°$ (*c* 1 in $CHCl_3$); the term ^{head} refers to linker attached at C-1 while the label ^{tail} refers to butanediol linker attached at C-4; δ_H (500 MHz, C_6D_6, C_6D_6 = 7.16 ppm) 1.28 (12 H, d, *J* 6.0, 4 × 6-H), 1.2 – 1.6 (16 H, m, 4 × 2-H, 4 × –O–CH_2–CH_2–^{head}), 1.66 (8 H, m, 4 × –O–CH_2–CH_2–^{tail}), 2.66 (4 H, ddd, *J* 9.4, 3.8 and 3.3, 4 × 4-H), 2.94 (4 H, m, 4 × –O–C*H*H′–CH_2–^{head}), 3.08 (4 H, m, 4 × –O–C*H*H′–CH_2–^{tail}), 3.51 (4 H, m, 4 × –O–CH*H′*–CH_2–^{head}), 3.63 (4 H, dq, *J* 9.4 and 6.0, 4 × 5-H), 3.82 (4 H, m, 4 × –O–CH*H′*–CH_2–^{tail}), 4.35 (4 H, br s, 4 × 1-H), 4.40 (4 H, br dd, *J* 8.0 and 3.3, 4 × 3-H), 7.84 (4 H, dd, *J* 91.8 and 9.3, 4 × NH); δ_N (50 MHz, C_6D_6, CH_3NO_2 = 0 ppm) −266.56 (d, *J* = 91.8 Hz, 4 × NH); δ_C (125 MHz, C_6D_6, C_6D_6 = 128 ppm) 18.4 (q, 4 × C-6), 27.2 (t, 4 × –O–CH_2–*C*H_2–^{head}), 27.7 (t, 4 × –O–CH_2–*C*H_2–^{tail}), 32.9 (t, 4 × C-2), 44.1 and 44.2 (2 × d, 4 × C-3), 63.8 (d, 4 × C-5), 67.5 (t, 4 × –O–*C*H_2–CH_2–^{head}), 70.2 (t, 4 × –O–*C*H_2–CH_2–^{tail}), 79.3 (d, 4 × C-4), 96.9 (d, 4 × C-1), 117.2 (q, *J* 288.7, 4 × CO*C*F_3), 156.67 (q, *J* 35.9, 4 × *C*O*C*F_3); LC-MS (ESI) (+*c*): *m*/*z* (%): 1215.45 (100) $[M + Na]^+$; HR-MS $C_{48}H_{72}{}^{15}N_4O_{16}$ +Na: calcd. 1215.4531, found 1215.4532.

2nd fraction. **23** (7 mg, 4 μmol, 5%); in the following text the term ^{head} refers to linker attached at C-1 while the label ^{tail} refers to butanediol linker attached at C-4; δ_H (500 MHz, C_6D_6, C_6D_6 = 7.16 ppm) 1.34 (18 H, d, *J* 6.2, 6 × 6-H), 1.2 – 1.5 (24 H, m, 6 × 2-H, 6 × –O–CH_2–CH_2–^{head}), 1.74, 1.65 (12 H, 2 × m, 6 × –O–CH_2–CH_2–^{tail}), 2.75 (6 H, ddd, *J* 9.6, 3.6 and 3.6, 6 × 4-H), 2.95 (6 H, m, 6 × –O–C*H*H′–CH_2–^{head}), 3.17 (6 H, m, 6 × –O–C*H*H′–CH_2–^{tail}), 3.48 (6 H, m, 6 × –O–CH*H′*–CH_2–^{head}), 3.71 (6 H, dq, *J* 9.6 and 6.2, 6 × 5-H), 3.90 (6 H, m, 6 × –O–CH*H′*–CH_2–^{tail}), 4.38 (6 H, d, *J* 2.5, 6 × 1-H), 4.57 (6 H, br dd, *J* 8.4 and 3.6, 6 × 3-H), 7.90 (6 H, dd, *J* 92.2 and 9.2, 6 × NH); δ_N (50 MHz, C_6D_6, CH_3NO_2 = 0 ppm) −266.36 (d, *J* 92.2, 6 × NH); δ_C (125 MHz, C_6D_6, C_6D_6 = 128 ppm) 18.3 (q, 6 × C-6), 26.8 (t, 6 × –O–CH_2–*C*H_2–^{head}), 26.9 (t, 6 × –O–CH_2–*C*H_2–^{tail}), 32.7 (t, 6 × C-2), 43.8 and 43.9 (2 × d, 6 × C-3), 63.6 (d, 6 × C-5), 67.3 (t, 6 × –O–*C*H_2–CH_2–^{head}), 69.7 (t, 6 × –O–*C*H_2–CH_2–^{tail}), 79.2 (d, 6 × C-4), 96.8 (d, 6 × C-1), 117.2 (q, *J* 288.8, 6 × CO*C*F_3), 156.5 (q, *J* 35.9, 6 × *C*O*C*F_3); LC-MS (ESI) (+*c*): *m*/*z* (%): 1811.18 (100) $[M + Na]^+$.

The macrocylces were first prepared from [^{14}N]$_2$-**15**. Isolated yields were almost identical to those described for the ^{15}N-labelled macrocycles ([^{14}N]-**22** = 44%; [^{14}N]-**23** = 4.5%).

Preparation of spacer-linked cyclotetraristosamine 2 by *N*-deacylation

The hydrogenated product **22** (130 mg, 0.109 mmol) was dissolved in THF–1.0 M aqueous NaOH (1 : 3, 72 ml) and methanol (20 ml) was added in order to obtain a clear solution. This mixture was allowed to stir at rt for 20 h, at which time TLC (CH_3CN–25% aqueous NH_3 10 : 1, R_f 0.5 [TLC was exposed over an aq. ammonia bath prior to use]) indicated that the deprotection step was complete. The mixture was extracted with CH_2Cl_2 (3 × 50 ml), the organic phase dried over Na_2SO_4 and evaporated under reduced pressure. The amine was lyophilized from benzene to yield macrocycle **2** (88 mg, 0.109 mmol, >99%) as a hygroscopic gray crystalline powder.

$[\alpha]_D^{25} = -139.9°$ (*c* 1 in $CHCl_3$); in the following text the term ^{head} refers to linker attached at C-1 while the label ^{tail} refers to butanediol linker attached at C-4; δ_H (500 MHz, C_6D_6, C_6D_6 = 7.16 ppm) 1.41 (12 H, d, *J* 6.0, 4 × 6-H), 1.54 (16 H, m, 4 × –O–CH_2–CH_2–^{tail}, 4 × –O–CH_2–CH_2–^{head}), 1.64 (4 H, ddd, *J* 14.1, 4.7 and 3.7, 4 × 2-H_{eq}), 2.10 (4 H, br d, *J* 14.1, 4 × 2-H_{ax}), 2.77 (4 H, dd, *J* 9.3 and 3.0, 4 × 4-H), 3.08 (8 H, m, 4 × –O–C*H*H′–CH_2–^{head}, 4 × –O–C*H*H′–CH_2–^{tail}), 3.25 (4 H, br s, 4 × 3-H), 3.39 (4 H, m, 4 × –O–CH*H′*–CH_2–^{head}), 3.62 (4 H, m, 4 × –O–CH*H′*–CH_2–^{tail}), 3.98 (4 H, dq, *J* 9.3 and 6.0, 4 × 5-H), 4.63 (4 H, d, *J* 3.7, 4 × 1-H); δ_N (50 MHz, C_6D_6, CH_3NO_2 = 0 ppm) −360.81 (s, 4 × NH); δ_C (125 MHz, C_6D_6, C_6D_6 = 128.06 ppm) 18.7 (q, 4 × C-6), 27.5 (t, 4 × –O–CH_2–*C*H_2–^{tail}), 27.8 (t, 4 × –O–CH_2–*C*H_2–^{head}), 34.9 (t, 4 × C-2), 45.9 (d, 4 × C-3), 62.1 (d, 4 × C-5), 67.7 (t, 4 × –O–*C*H_2–CH_2–^{tail}), 68.9 (t, 4 × –O–*C*H_2–CH_2–^{head}), 82.7 (d, 4 × C-4), 97.8 (d, 4 × C-1); LC-MS (ESI) (+*c*): *m*/*z* (%): 809.54 (90) $[M + H]^+$, 831.49 (100) $[M + Na]^+$; HR-MS $C_{40}H_{77}{}^{15}N_4O_{12}$: calcd. 809.5419, found 809.5403.

Preparation of spacer-linked cyclohexaristosamine 24 by *N*-deacylation

The hydrogenated product **23** (11 mg, 6.2 μmol) was dissolved in a mixture of THF–1.0 M aqueous NaOH (1 : 3, 10 ml) and methanol (5 ml) was added in order to obtain a clear solution. This mixture was allowed to stir at rt for 20 h, at which time TLC (CH_3CN–25% aqueous NH_3 10 : 1, R_f 0.5 [TLC was exposed over an aq. ammonia bath prior to use]) indicated that the deprotection was complete. The mixture was extracted with CH_2Cl_2 (3 × 10 ml), the organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The amine was lyophilized from benzene to yield macrocycle **24** (7.4 mg, 6.1 μmol, 99%) a hygroscopic gray crystalline powder.

$[\alpha]_D^{25} = -112.3°$ (*c* 0.3 in $CHCl_3$); in the following text the term ^{head} refers to linker attached at C-1 while the label ^{tail} refers to butanediol linker attached at C-4; δ_H (500 MHz, C_6D_6, C_6D_6 = 7.16 ppm) 1.43 (18 H, d, *J* 6.2, 6 × 6-H), 1.2–1.8 (30 H, m, 6 × –O–CH_2–CH_2–^{tail}, 6 × –O–CH_2–CH_2–^{head}, 6 × 2-H_{eq}), 2.12 (6 H, br d, *J* 14.0, 6 × 2-H_{ax}), 2.79 (6 H, dd, *J* 9.5 and 3.1, 6 × 4-H), 3.12 (12 H, m, 6 × –O–C*H*H′–CH_2–^{head}, 6 × –O–C*H*H′–CH_2–^{tail}), 3.27 (6 H, br s, 6 × 3-H), 3.40 (6 H, m, 6 × –O–CH*H′*–CH_2–^{head}), 3.61 (6 H, m, 6 × –O–CH*H′*–CH_2–^{tail}), 4.01 (6 H, dq, *J* 9.5 and 6.2, 6 × 5-H), 4.65 (6 H, d, *J* 3.7, 6 × 1-H); δ_N (50 MHz, C_6D_6, CH_3NO_2 = 0 ppm) −360.77 (s, 6 × NH); δ_C (125 MHz, C_6D_6, C_6D_6 = 128.06 ppm) 18.7 (q, 4 × C-6), 27.3 (t, 4 × –O–CH_2–*C*H_2–^{tail}), 27.6 (t, 4 × –O–CH_2–*C*H_2–^{head}), 35.0 (t, 4 × C-2), 45.9 and 45.9 (2 × d, 4 × C-3), 62.1 (d, 4 × C-5), 67.7 (t, 4 × –O–*C*H_2–CH_2–^{tail}), 68.8 (t, 6 × –O–*C*H_2–CH_2–^{head}),

82.7 (d, 6 × C-4), 97.9 (d, 6 × C-1); HR-MS $C_{60}H_{114}{}^{15}N_6O_{18}$: calcd. 1213.8090, found 1213.8068.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 416, project B2) and by the Fonds der Chemischen Industrie. We thank Solvay Pharmaceutical Research Laboratories (Hannover) for donation of chemicals.

References

1 Reviews: (*a*) W. D. Wilson and K. Li, *Curr. Med. Chem.*, 2000, **7**, 73–98; (*b*) G. Werstuck and M. R. Green, *Science*, 1998, **282**, 296–298; (*c*) D. T. Hung, T. F. Jamison and S. L. Schreiber, *Chem. Biol.*, 1996, **3**, 623–639.

2 (*a*) K. Michael and Y. Tor, *Chem. Eur. J.*, 1998, **4**, 2091–2098; (*b*) Y. Tor, *ChemBioChem*, 2003, **4**, 998–1007; (*c*) Q. Vicens and E. Westhof, *ChemBioChem*, 2003, **4**, 1018–1023; also non aminoglycoside-based oligoamines have served as potent inhibitors of HIV-1 TAR RNA-Tat peptide binding and other RNAs; (*d*) L. Yu, T. K. Oost, J. M. Schkeryantz, J. Yang, D. Janowick and S. W. Fesik, *J. Am. Chem. Soc.*, 2003, **125**, 4444–4450; (*e*) D. Moazed and H. F. Noller, *Nature*, 1987, **327**, 389–394.

3 Neomycin was shown to be the most effective triplex groove binder that stabilizes DNA and RNA triple helices with little effect on the double helices. In further studies hybrid structures were designed, based on BBQ and neomycin, with dual recognition ligands that had the potential to be more selective and potent ligands for nucleic acids: (*a*) D. P. Arya, L. Xue and P. Tennant, *J. Am. Chem. Soc.*, 2003, **125**, 8070–8071; (*b*) D. P. Arya, R. L. Coffee Jr., B. Willis and A. I. Abramovitch, *J. Am. Chem. Soc.*, 2001, **123**, 5385–5395; (*c*) U. von Ahsen, J. Davies and R. Schroeder, *J. Mol. Biol.*, 1992, **226**, 935–941; (*d*) J. Davies, U. von Ahsen, R. Schroeder, in *The RNA World*, Cold Spring Harbor Laboratory Press, New York, 1993, p. 185–204; (*e*) U. von Ahsen, J. Davies and R. Schroeder, *Nature*, 1991, **353**, 368–370; (*f*) U. von Ahsen and H. F. Noller, *Science*, 1993, **260**, 1500–1503.

4 (*a*) M. L. Zapp, S. Stern and M. R. Green, *Cell (Cambridge, Mass.)*, 1993, **74**, 969–978; (*b*) M. L. Zapp, D. W. Young, A. Kumar, R. Singh, D. W. Boykin, W. D. Wilson and M. R. Green, *Bioorg. Med. Chem.*, 1997, **5**, 1149–1155; (*c*) M. Hendrix, E. S. Priestly, G. F. Joyce and C. Wong, *J. Am. Chem. Soc.*, 1997, **119**, 3641–3648.

5 (*a*) H.-Y. Mei, A. A. Galan, N. S. Halim, D. P. Mack, D. W. Moreland, K. B. Sanders, H. N. Truong and A. W. Czarnik, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 2755–2760; (*b*) S. Wang, P. W. Huber, M. Cui, A. W. Czarnik and H.-Y. Mei, *Biochemistry*, 1998, **37**, 5549–5557; (*c*) F. Hamy, V. Brondani, A. Flörsheimer, W. Stark, M. J. J. Blommers and T. Klimkait, *Biochemistry*, 1998, **37**, 5086–5095; (*d*) H.-Y. Mei, M. Cui, A. Heldsinger, S. M. Lemrow, J. A. Loo, K. A. Sannes-Lowry, L. Sharmeen and A. W. Czarnik, *Biochemistry*, 1998, **37**, 14204–14212.

6 (*a*) D. Fourmy, M. I. Rech, S. C. Blanchard and J. D. Puglisi, *Science*, 1996, **274**, 1269–1432; (*b*) J. Haddad, P. K. Lakshmi, B. Llano-Sotelo, C. Kim Jr., E. F. Azucenca, M. Liu, S. B. Vakulenko, C. S. Chow and S. Mobashery, *J. Am. Chem. Soc.*, 2002, **124**, 3229–3237; (*c*) H. Wang and Y. Tor, *Angew. Chem. Int. Ed.*, 1998, **37**, 117–120; H. Wang and Y. Tor, *Angew. Chem. Int. Ed.*, 1998, **37**, 109–111; (*d*) D. J. Earnshaw and M. J. Gait, *Nucleic Acids Res.*, 1998, **26**, 5551–5561.

7 (*a*) T. K. Stage, K. J. Hertel and O. C. Uhlenbeck, *RNA*, 1995, **1**, 95–101; (*b*) H. Wang and Y. Tor, *J. Am. Chem. Soc.*, 1997, **119**, 8734–8735; (*c*) B. Clouet-d'Orval, T. K. Stage and O. C. Uhlenbeck, *Biochemistry*, 1995, **34**, 11186–11190; (*d*) T. Hermann and E. Westhof, *J. Mol. Biol.*, 1998, **276**, 903–912; (*e*) D. J. Earnshaw and M. J. Gait, *Nucleic Acids Res.*, 1998, **26**, 5551–5561; (*f*) A. Krebs, V. Ludwig, O. Boden and M. W. Goebel, *ChemBioChem*, 2003, **4**, 972–978.

8 E. Kikuta, S. Aoki and E. Kimura, *J. Am. Chem. Soc.*, 2001, **123**, 7911–7912.

9 (*a*) N. W. Luedtke, P. Carmichael and Y. Tor, *J. Am. Chem. Soc.*, 2003, **125**, 12374–12375; (*b*) M. Fridman, V. Belakhov, S. Yaron and T. Baasov, *Org. Lett.*, 2003, **5**, 3575–3578.

10 G.-W. Chen and A. Kirschning, *Chem. Eur. J.*, 2002, **8**, 2717–2729.

11 M. A. Leeuwenburgh, G. A. Van der Marel and H. S. Overkleeft, *Curr. Opin. Chem. Biol.*, 2003, **7**, 757–765.

12 G.-W. Chen, J. Jaunzems, M. Jesberger, M. Kalesse, M. Lindner and A. Kirschning, *Tetrahedron*, 2004, **60, 15**, 3505–3521.

13 J. Jaunzems, D. Kashin, A. Schönberger and A. Kirschning, *Eur. J. Org. Chem.*, 2004, 3435–3446.

14 D. M. Ciment and R. J. Ferrier, *J. Chem. Soc. C*, 1966, 441–445.

15 (*a*) B. Schmidt, *Eur. J. Org. Chem.*, 2004, 1865–1880; (*b*) M. K. Gurjar and P. Yakambram, *Tetrahedron Lett.*, 2001, **42**, 3633–3636; (*c*) D. C. Braddock and A. J. Wildsmith, *Tetrahedron Lett.*, 2001, **42**, 3239–3242; (*d*) T. R. Hoye and H. Zhao, *Org. Lett.*, 1999, **1**, 1123–1125.

16 W. K. C. Park, M. Auer, H. Jaksche and C.-H. Wong, *J. Am. Chem. Soc.*, 1996, **118**, 10150–10155.

17 (*a*) D. H. R. Barton, P. D. Magnus, J. A. Garbarino and R. N. Young, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2101–2107; (*b*) G. A. Olah and J. J. Svoboda, *Synthesis*, 1972, 307–308.

18 The starting structure of molecule **19** was first minimized employing Macro Model 7.2[20] using the MMFF ForceField[19] and $CHCl_3$ as solvent model[22]. The conformation obtained was used as an input for a Monte Carlo search to find the global minimum[23]. Within 10 000 steps structure **19** was found seven times to occupy the lowest energy conformation (354.3199 kJ mol^{-1}).

19 T. A. Halgren, *J. Comput. Chem.*, 1996, **17**, 490–519.

20 F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson and W. C. Still, *J. Comput. Chem.*, 1990, **11**, 440–467.

21 T. Carlomagno, A. Kirschning, unpublished investigations..

22 W. C. Still, A. Tempczyk, R. C. Hawley and T. Hendrickson, *J. Am. Chem. Soc.*, 1990, **112**, 6127.

23 (*a*) J. M. Goodman and W. C. Still, *J. Comput. Chem.*, 1991, **12**, 1110–1117; (*b*) H. Senderowitz, F. Guarnieri and W. C. Still, *J. Am. Chem. Soc.*, 1995, **117**, 8211–8219.