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Note

A short and efficient transformation of rhamnose into activated daunosamine, acosamine, ristosamine and *epi*-daunosamine derivatives, and synthesis of an anthracycline antibiotic acosaminyl-ε-*iso*-rhodomycinone

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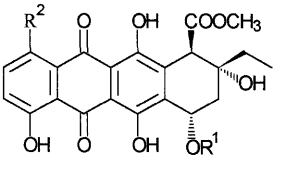
Abstract

3-Amino-2,3,6-trideoxyhexopyranoses are essential constituents of most anthracycline antitumour antibiotics. For an investigation of structure–activity relationships, the four diastereomeric amino sugars daunosamine, acosamine, ristosamine, and *epi*-daunosamine were synthesised in short and efficient routes starting from commercially available rhamnose. Several glycosyl donors were provided and their use was exemplified in the synthesis of acosaminyl-ε-*iso*-rhodomycinone.

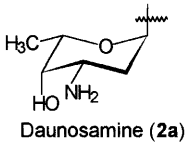
Keywords: Acosamine; Daunosamine; Ristosamine; Anthracycline; Acosaminyl-ε-*iso*-rhodomycinone

Many anthracycline antibiotics show strong antitumour activity [1,2]: the highest intracellular concentration of the drug is found in the nucleus, where it intercalates into the DNA double helix forming a ternary complex with DNA topoisomerase II [3], with consequent inhibition of replication and transcription. Because of the small difference between normal DNA and DNA from cancer cells, anthracyclines are generally very toxic also to hosts, and administration of the drug may cause serious side-effects such as nausea, vomiting, gastrointestinal toxicity, and most of all, cumulative cardiotoxicity (Scheme 1).

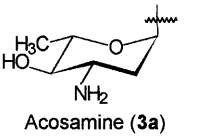
Anthracycline antibiotics like daunomycin or the ε-*iso*-rhodomycinone derivative D-788-6 (**1a**) consist of a tetracyclic chromophore and one or more sugar residues; the first moiety is usually an amino sugar, which may be the only sugar residue, or be part of an oligosaccharide chain. The configuration of the amino



1a: R^1 = Daunosamine, R^2 = H
1b: R^1 = H, R^2 = OH
1c: R^1 = Acosamine, R^2 = OH



Daunosamine (**2a**)



Acosamine (**3a**)

Scheme 1.

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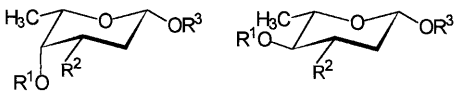
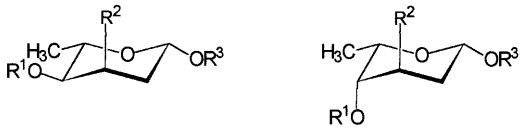


Daunosamine (**2**) Acosamine (**3**)

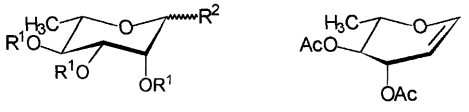


Ristosamine (**4**) 3-*epi*-Daunosamine (**5**)

2-5	a	b	c	d	e	f	g	h	I
R^1	H	Ac	Ac	H	H	$BzNO_2$	Ac	Tf	Bz
R^2	NH_2	N_3	N_3	N_3	NHTfAc	NHTfAc	NHTfAc	N_3	N_3
R^3	H	H	TBD	TBD	TBD	TBD	TBD	TBD	TBD

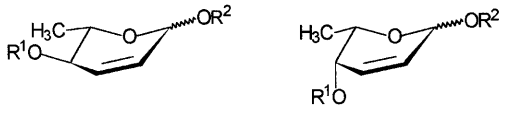
Ac = Acetyl, $BzNO_2$ = *p*-Nitrobenzoyl, TBD = *tert.*-Butyldimethylsilyl, TfAc = Trifluoroacetyl, Tf = Trifluoromethansulfonyl

Scheme 2.



6a: R^1 = H, R^2 = OH **7**

6b: R^1 = Ac, R^2 = Br



8a: R^1 = Ac, R^2 = H **9a**: R^1 = Bz, R^2 = Me

8b: R^1 = Ac, R^2 = Me **9b**: R^1 = Ac, R^2 = Me

8c: R^1 = H, R^2 = Me **9c**: R^1 = Ac, R^2 = H

Scheme 3.

sugar influences strongly the bioactivity of the drug: replacement of L-daunosamine (**2a**) with L-acosamine (**3a**) or L-ristosamine (**4a**) in anthracyclines produces analogues having similar antitumour activity but reduced cardiotoxicity [4]. In this paper we describe an optimised short synthesis for the L-amino sugars **2a**–**5a** in an activated form useful for the glycosylation of anthracyclinones, as is exemplified in the coupling reaction of the acosamine derivative **3f** with **1b** affording acosaminyl-ε-*iso*-rhodomycinone (**1c**).

The amino sugars **2a**–**4a** occur naturally as constituents of anthracycline antibiotics and have been synthesised from commercially available cheap D-monosaccharides, however, with rather long reaction sequences or low yields [5]. The L-amino hexose **5a** bearing *xylo* configuration is the C-3 epimer of L-daunosamine and has often been referred as 3-*epi*-daunosamine. It is not a naturally occurring isomer, and has been obtained most usually as a minor by-product during the synthesis of other amino sugars [6] (Scheme 2).

The first total syntheses of **5a** were reported by Cheung [7] and Boivin [8], starting from α-D-glucopyranoside. In our synthesis of amino sugar derivatives **2a**–**5a**, L-di-*O*-acetylrhamnal (**7**) was used as the starting material. The latter was easily obtained in a 100 g scale, when a literature procedure for the synthesis of acetobromo sugars [9] and the reductive dehalogenation were optimised and performed in a one-pot-reaction. L-(+)-Rhamnose (**6a**) was first peracetylated and transformed into acetobromorhamnose (**6b**) using PBr_3–H_2O as the brominating agent and then reduced by a zinc–copper alloy giving the expensive L-(+)-di-*O*-acetyl-rhamnal (**7**) in more than 85% yield (Scheme 3).

Heating the aqueous suspension of **7** at 80 °C [10] delivered the hydrolysis product **8a** which was used for the synthesis of acosamine (**3a**) and ristosamine (**4a**) derivatives. In contrast to the literature [11], addition of azide on **8a** occurred with only moderate stereoselectivity, and both **3b** and **4b** were obtained in a ratio of merely 2.1:1. Silylation of the crude product with *tert*-butyl(dimethyl)silyl chloride–imidazole[1] using DMF as the solvent afforded a mixture of α and β anomers [12]; in dichloromethane or 1,2-dichloroethane, however, both **3c** and **4c** (84%) were obtained as pure β anomers [13]. After removal of the acetyl groups using K_2CO_3–MeOH, the ob-

[1] The condensation of ε-rhodomycinone (**1b**) with an α-1-*O*-trimethylsilylated daunosamine donor, catalyzed by TMS–triflate, gave a complex mixture of products, from which α,α- and α,β-disaccharide as well as an ε-rhodomycin with β-glycosidic linkage have been isolated. As these side reactions could be avoided in the case of daunosamine donor **2f** by using the more stable β-1-*O*-*tert*-butyldimethylsilyl glycoside, we selected TBDMS as the protective group in the syntheses of other glycosyl donors as well.

tained diastereomeric mixture of azides could be separated easily by column chromatography to yield pure **3d** and **4d**.

The result that azide addition on **8a** gave both **3b** and **4b**, allowed a divergent synthesis of the diastereomeric L-amino sugars **2b** and **5b** in the same way. Thus, compound **7** was first converted in a Ferrier reaction to **8b** (α and β anomer in a ratio of ~4:1), using zinc chloride in methanol as a catalyst [14], followed by deacetylation to afford compound **8c**. Epimerisation of the 4-OH group by the Mitsunobu reaction furnished compound **9b** with an axial 4-OH group. In the presence of hydrochloric acid in acetic acid and THF, the acetal group in **9b** was cleaved, the resulting **9c** was then subjected to azide addition. The resulting mixture was again protected with TBDMS without further purification and characterisation. After column chromatography, a colourless viscous oil was obtained. The ^{1}H NMR spectrum showed that **2c** and **5c** were obtained in a ratio of about 1:2.4, very similar to the synthesis of **3c** and **4c**. Removal of the acetate groups delivered **2d** and **5d**.

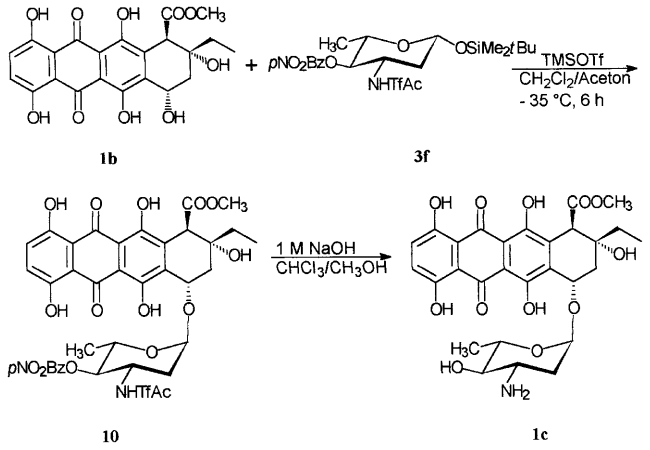
Another facile access to the daunosamine series was possible starting from the acosamine derivative **3d**: first, this was converted to the triflate **3h**, followed by epimerisation at C-4 using AcOCs and sodium acetate. Although the reaction failed to give a high yield of **2c** (43% overall yield from **3d**), it is still more efficient than results reported previously [15].

The low stereoselectivity during azide addition on **8a** or **9c** is due to the small steric shielding of their 4-*O*-acetyl group, and reaction of benzoate **9a** with azide should give much better yields of the 3-*epi*-daunosamine configuration, therefore. The hexenopyranoside **9a** was obtained, however, by mesylation of **8c** and subsequent nucleophilic substitution of the mesylate with caesium benzoate in only 39% yield; better results (80–90%) were obtained using the Mitsunobu reaction [16]: when the acetal group in **9a** was cleaved with 1 N HCl in HOAc–THF and subsequently azide was added, only a single product was detectable by TLC. The 1-hydroxy group of this intermediate was finally protected as *tert*-butyl(dimethyl)silyl (TBDMS) derivative. TLC analysis showed that again only one product was formed, which was further confirmed as the β anomer **5i**.

From the ^{1}H NMR spectrum of the final product it was not clear whether the compound had the *lyxo* or the *xylo* configuration. Removal of the benzoyl group with K_2CO_3–CH_3OH, however, gave a compound, the ^{1}H NMR spectrum of which was *not* identical with that of the authentic *lyxo*-compound **2d**, identifying the acetal as being the *xylo* derivative **5d** with an axial azido group. This stereochemistry was further confirmed by NMR experiments with the derivative **5f**: significant nuclear Overhauser effects (NOE) of NH with 1-H and 5-H allowed the assignment of the $NHCOCF_3$ group as being axial, and a strong NOE between one 2-H_2 signal and a weak NOE with the other allowed the assignment of chemical shifts of 2-H_e and 2-H_a. The 1-proton was assigned as being axial according to the coupling constant and also the strong NOE with the NH-group.

Reduction of all azides was done in the same way: when catalytic hydrogenation of **3d**–**4d** was carried out in methanol in the presence of two equivalents of ethyl trifluoroacetate and triethyl amine, trifluoro-acetylation of the amino group occurred simultaneously to give **3e** and **4e**, and the yield was 12% higher than using an improved literature procedure [17]. Protection of the 4-hydroxyl group as acetate or *p*-nitrobenzoate gave the acosamine derivatives **3f**–**3g** and the ristosamine derivative **4g** in good yields (> 90%). With similar results, compound **2d** was finally converted into the glycosyl donor **2f**, and from **5d**, **5f** was obtained. All four protected L-amino sugars are versatile intermediates for the synthesis of anthracycline type anticancer antibiotics.

The glycosylation of ε-*iso*-rhodomycinone (**1b**) with the acosamine donor **3f** proceeded smoothly in 1:1 dichloromethane–acetone in the presence of 1.6 equivalents of Me_3Si–triflate and powdered molecular sieve (4 Å), when a temperature of about −35 °C was maintained. At lower temperature (−45 to −50 °C), the sparingly soluble **1b** precipitated, and prolonged reaction time lead to side reactions, such as enhanced bisglycosylation, and decomposition of the glycosyl donor **3f**.



Scheme 4.

By column chromatography on silica gel, **10** was obtained in 70% yield along with about 15% of the bisglycosylated product as an inseparable mixture. Removal of the trifluoroacetyl and *p*-nitrobenzoyl groups with 1 N sodium hydroxide [18] gave the pure anthracycline analogue **1c** in 68% yield (Scheme 4).

As expected, the in vitro cytotoxicity of **1c** is lower than that of doxorubicin and of ε-*iso*-rhodomycin (**1b**) (see Tables 1 and 2). In an in vitro-cytotoxicity study with six human tumour cell lines, acosaminyl-ε-*iso*-rhocomycinon (**1c**) showed a very similar IC_{70}-pattern like doxorubicin (see Table 2). The colon cancer model (CCL HT29) and the lung cancer (LXF 529) were the most sensitive in contrast to the gastric cancer (GXF 251), lung cancer (LXF 629) and the melanoma (MEXF 462).

1. Experimental

General.—IR spectra (KBr pellet or neat) were recorded on Perkin–Elmer 297 spectrometer, ^{1}H NMR spectra were recorded on Varian VXR 200 (200 MHz), Bruker AM 300 (300 MHz); ^{13}C NMR spectra were recorded on Varian VXR 200 (50.3 MHz), Bruker AM 300 (75.5 MHz), coupling constants J in Hertz, chemical shifts in δ related to tetramethylsilane as internal standard. Mass spectra were obtained on Varian MAT 73 and Varian 311A at 70 eV by electron impact. TLC plates (Silica Gel PF_{254}, E. Merck) and silica gel for column chromatography (0.05–0.2 mm, 70–270 mesh) was purchased from Macherey–Nagel & Co (Düren, Germany). L-(+)-rhamnose monohydrate (**6a**, $[\alpha]_{20}^{D} = +8.20°$, $c = 10$, water, 1 h) was purchased from Acros (Geel, Belgium) and used without further purification.

Table 1
In-vitro-cytotoxicity (IC_{50}, μg/mL) of doxorubicin, ε-*iso*-rhodomycin and acosaminyl-ε-*iso*-rhodomycinone (**1c**) against L1210 leukemia cells in short time and long-termed exposure tests

Time of exposure	Doxorubicin	ε-*iso*-Rhodomycin	Acosaminyl-ε-*iso*-rhodomycinone (**1c**)
1 h	>1	>1	>1
7 days	0.01	0.6	0.8

Table 2
In-vitro-cytotoxicity (IC_{70}, μg/mL) of doxorubicin, ε-*iso*-rhodomycinone and acosaminyl-ε-*iso*-rhodomycinone (**1c**) against six human tumour cell lines in a modified propidium iodide assay [19]

Compound	Colon cancer	Gastric cancer	Melanoma xenograft	Non-small cell lung cancer		
	HT29	GXF 251	MEXF 462NL	LXF 529L	LXF 629L	LXF 66NL
Doxorubicin	<0.30	3.81	1.45	<0.30	1.47	0.75
1c	0.71	2.21	2.19	0.33	>3.00	1.58
ε-*iso*-Rhodomycinone (**1b**)	>3.00	>3.00	>3.00	>3.00	>3.00	>3.00
β-Rhodomycinone	>3.00	>3.00	>3.00	>3.00	3.14	1.88

tert-*Butyldimethylsilyl 4-O-acetyl-3-azido-2,3,6-trideoxy-β-L-lyxo-hexopyranoside* (**2c**) *and* tert-*butyldimethylsilyl 3-azido-4-O-acetyl-2,3,6-trideoxy-β-L-xylo-hexopyranoside* (**5c**)

Method A. A solution of **9b** (3.70 g, ca. 20 mmol) in 1 N HCl (20 mL), AcOH (8 mL) and THF (30 mL) was stirred for 15 h at 20 °C. TLC analysis showed complete cleavage of the acetal group. Sodium azide (2.30 g, ca. 35 mmol) was added and the reaction mixture was stirred for 10 h. The organic layer was separated and the water phase was extracted with CH_2Cl_2. The combined organic layer was washed with satd $NaHCO_3$ and dried with Na_2SO_4. The solvent was removed and the crude product was redissolved in dry CH_2Cl_2 (100 mL), together with *tert*-butyl(dimethyl)silyl chloride (6.00 g, ca. 40 mmol) and imidazole (2.72 g, ca. 40 mmol). After stirring for 2 h, the mixture was washed with water and dried with Na_2SO_4. The crude product was purified by column chromatography (silica gel: 80 g, 20:1 cyclohexane–EtOAc) to give a colourless viscous oil (3.30 g, 50%) of both **2c** and **5c** in a ratio of about 1:2.4. Compound **2c**: ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.04$ (dd, $J_{4,3} = 3.2$, $J_{4,5} = 1$, 1 H, 4-H), 4.77 (dd, $J_{1,2ax} = 8.5$, $J_{1,2eq} = 3.0$, 1 H, 1-H), 3.58 (dq, $J_{5,6} = 6.5$, 1 H, 5-H), 3.40 (ddd, $J_{3,2ax} = 12.0$, $J_{3,2eq} = 5.0$, 1 H, 3-H), 2.17 (s, 3 H, MeCO), 2.00 (ddd, 1 H, 2_{eq}-H), 1.93 (ddd, $J_{2ax,2eq} = 12.0$, 1 H, 2_{ax}-H), 1.16 (d, 3 H, 6-H_3), 0.90 (br s, 9 H, $SiCMe_3$), 0.12, 0.10 (2s, 6 H, $SiMe_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta =$ 170.64 (MeCO), 94.93 (C-1), 70.33 (C-5), 69.19 (C-4), 57.50 (C-3), 33.66 (C-2), 25.77 (Me_3C), 20.83 (MeCO), 18.17 (Me_3C), 16.78 (C-6), −4.13, −5.07 (Me_2Si). Compound **5c**: ^{1}H NMR (300 MHz, $CHCl_3$): $\delta = 4.95$ (dd, $J_{1,2eq} = 3.9$, $J_{1,2ax} = 7.3$, 1 H, 1-H), 4.54 (dd, $J_{4,3} = 3.2$, $J_{4,5} = 1.5$, 1 H, 4-H), 3.95 (m, 2 H, 3-, 5-H), 2.10 (s, 3 H, MeCO), 1.82 (m, 2 H, 2-H_2), 1.15 (d, $J_{6,5} = 6.5$, 3 H, 6-H_3), 0.89 (s, 9 H, $SiC(Me)_3$), 0.17, 0.16 (2 s, 6 H, $SiMe_2$). ^{13}C NMR (75 MHz, $CHCl_3$: $\delta = 170.39$ (MeCO), 92.49 (C-1), 68.88 (C-4), 68.00 (C-5), 58.10 (C-3), 33.55 (C-2), 25.81 (Me_3C), 20.92 (MeCO), 18.19 (Me_3C-), 16.63 (C-6), −4.20, −5.12 (Me_2Si).

Method B. Anhydrous AcONa (1.64 g, 20 mmol) and anhyd AcOCs (1.92 g, 10 mmol, vacuum dried at 150 °C/0.05 Torr) was added to a solution of crude triflate **3h** (8.1 g, ca. 19.3 mmol) dissolved in dry DMF (50 mL). After stirring for 18 h at 20 °C, the reaction mixture was diluted with CH_2Cl_2 (200 mL) and washed three times with water (50 mL each time). The organic layer was dried with Na_2SO_4, concentrated in vacuo, and the crude product was purified by column chromatography (1:30 EtOAc–cyclohexane) to give **2c** (3.55 g) as a colourless oil, along with 20% unidentified impurity which could not be separated.

tert-*Butyldimethylsilyl 3-azido-2,3,6-trideoxy-β-L-lyxo-hexopyranoside* (**2d**).—Deacetylation of **2c** (3.40 g, ca. 8 mmol, containing ca. 20% impurity) carried out as described in the synthesis of **3d** gave, after column chromatography (1:15 EtOAc–cyclohexane), **2d** (2.02 g, 88%) as a colourless solid with mp 69–70 °C (Lit.: 72 °C [17]). $[\alpha]_D^{20} = -3.6°$ ($c = 0.80$, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 4.74$ (dd, $J_{1,2ax} = 8.9$, $J_{1,2eq} = 2.8$, 1 H, 1-H), 3.60 (br s, 1 H, 4-H), 3.46 (dq, $J_{5,4} = 1.0$, $J_{5,6} = 6.5$, 1 H, 5-H) 3.30 (ddd, $J_{3,4} = 2.7$, $J_{3,2ax} = 12.5$, $J_{3,2eq} = 5.1$, 1 H, 3-H), 1.99 (ddd, $J_{2ax,2eq} = 12.7$, 1 H, 2_{eq}-H), 1.97 (br s, 1 H, 4-OH), 1.84 (ddd, 1 H, 2_{ax}-H), 1.29 (d, 3 H, 6-H_3), 0.91 (br s, 9 H, $SiCMe_3$), 0.13 and 0.11 (2 s, 2 H, $SiMe_2$).

1-O-tert-*Butyldimethylsilyl 2,3,6-trideoxy-3-trifluoroacetamido-β-L-lyxo-hexopyranoside* (**2e**).—Catalytic hydrogenation of **2d** (1.87 g, 6.5 mmol) carried out as described in the synthesis of **3e** gave, after column chromatography (1:5 EtOAc–cyclohexane), **2e** (2.11 g, 91%) as a colourless solid with mp 54 °C (Lit.: 55 °C [18]). $[\alpha]_D^{25} = +16.2°$ ($c = 0.92$, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 6.82$ (br d, $J_{NH,3} = 8$, 1 H, NH), 4.77 (dd, $J_{1,2ax} = 9.5$, $J_{1,2eq} = 2.5$, 1 H, 1-H), 4.10 (dddd, $J_{3,2ax} = 13$, $J_{3,2eq} = 5.0$, $J_{3,4} = 2.3$, 1 H, 3-H), 3.62 (qd, $J_{5,4} = 1.0$, $J_{5,6} = 6.5$, 1 H, 5-H), 3.48 (dd, $J_{4,OH} = 10$, 1 H, 4-H), 2.12 (d, 1 H, 4-OH), 2.04 (ddd, $J_{2eq,2ax} = 13$, 1 H, 2_{eq}-H), 1.54 (ddd, 1 H, 2_{ax}-H), 1.28 (d, 3 H, 6-H_3), 0.88 (s, 9 H, $SiCMe_3$), 0.11 and 0.10 (2 s, 6 H, $SiMe_2$).

1-O-tert-*Butyldimethylsilyl 4-O-p-nitrobenzoyl-2,3,6-trideoxy-3-trifluoroacetamido-β-L-lyxo-hexopyranoside* (**2f**).—*p*-Nitrobenzoylation of **2e** (1.97 g, 5.5 mmol) carried out as

described in the synthesis of **3f** gave, after column chromatography (1:6 EtOAc–cyclohexane) of the crude product, **2f** (2.59 g, 93%) as a colourless solid, mp 73 °C (Lit.: 75 °C [18]). $[\alpha]_D^{25} = -90.1°$ ($c = 0.935$, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.32$, 8.28 (A_2B_2, $J = 9$, 4 H, Ar–H), 6.35 (d br, $J_{NH,3} = 7.5$, 1 H, NH), 5.34 (d br, $J_{4,3} = 3$, 1 H, 4-H), 4.94 (dd, $J_{1,2ax} = 8.7$, $J_{1,2eq} = 2.0$, 1 H, 1-H), 4.37 (m, 1 H, 3-H), 3.85 (qd, $J_{5,4} = 1.0$, $J_{5,6} = 6.5$, 1 H, 5-H), 2.08 (ddd, $J_{2eq,3} = 4.5$, $J_{2eq,2ax} = 12.3$, 1 H, 2_{eq}-H), 1.83 (ddd, $J_{2ax,3} = 12.5$, 1 H, 2_{ax}-H), 1.24 (d, 3 H, 6-H_3), 0.94 (s, 9 H, $SiCMe_3$), 0.18 and 0.16 (2 s, 6 H, $SiMe_2$).

4-O-Acetyl-3-azido-2,3,6-trideoxy-L-arabino-hexopyranose (**3b**) *and 4-O-acetyl-3-azido-2,3,6-trideoxy-L-ribo-hexopyranose* (**4b**).—A suspension of **7** (17.14 g, 80 mmol) in water (100 mL) was stirred for 2 h at 80 °C. At 20 °C, AcOH (16 mL) and NaN_3 (8.1 g, 0.12 mol) was added. After stirring for 24 h at 20 °C the reaction mixture was poured into a satd aq $NaHCO_3$ solution and extracted three times with EtOAc (100 mL each time). The organic layer was dried with Na_2SO_4 and evaporated to dryness. The crude mixture of **3b** and **4b** (16.5 g) was used without further purification and characterisation.

tert-*Butyldimethylsilyl 4-O-acetyl-3-azido-2,3,6-trideoxy-β-L-arabino-hexopyranosid* (**3c**) *and* tert-*butyldimethylsilyl 4-O-acetyl-3-azido-2,3,6-trideoxy-β-L-ribo-hexopranoside* (**4c**).—*tert*-Butyldimethylsilyl chloride (13.9 g, 92 mmol) in dry 1,2-dichloroethane (50 mL) was added dropwise to a solution of a crude **3b**–**4b** mixture (16.5 g) and imidazole (10.5 g, 154 mmol) in dry CH_2Cl (200 mL) at 0 °C. After stirring for 24 h, the reaction mixture was washed with water, satd $NaHCO_3$, and again water. After drying with Na_2SO_4, the crude product was purified by column chromatography (silica gel: 200 g, 30:1 cyclohexane–EtOAc) to give **3c**–**4c** (21.7 g 2.1:1 mixture, 83% from **7**) as a colourless oil. ^1H NMR (200 MHz, $CDCl_3$), **3c**: $\delta = 4.81$ (dd, $J_{1,2ax} = 9.2$, $J_{1,2eq} = 2.0$, 1 H, 1-H), 4.66 (dd, $J_{4,3} = J_{4,5} = 9.5$, 1 H, 4-H), 3.50 (ddd, $J_{3,2ax} = 12.8$, $J_{3,2eq} = 5.0$, 1 H, 3-H), 3.44 (qd, $J_{5,6} = 6.2$, 1 H, 5-H), 2.20 (ddd, $J_{2eq,2ax} = 13.0$, 1 H, 2_{eq}-H), 2.12 (s, 3 H, MeCO), 1.68 (ddd, 1 H, 2_{ax}-H), 1.20 (d, 3 H, 6-H_3), 0.90 (s, 9 H, $SiCMe_3$), 0.12 and 0.11 (2 s, 6 H, $SiMe_2$). Compound **4c**: $\delta = 4.99$ (dd, $J_{1,2ax} = 8.8$, $J_{1,2eq} = 2.2$, 1 H, 1-H), 4.63 (dd, $J_{4,3} = 3.5$, $J_{4,5} = 9.4$, 1 H, 4-H), 4.16 (ddd, $J_{3,2ax} = 3.5$, $J_{3,2eq} = 3.5$, 1 H, 3-H), 3.94 (qd, $J_{5,6} = 6.2$, 1 H, 5-H), 2.12 (s, 3 H, MeCO), 2.00 (ddd, $J_{2eq,2ax} = 13.0$, 1 H, 2_{eq}-H), 1.82 (ddd, 1 H, 2_{ax}-H), 1.20 (d, 3 H, 6-H_3), 0.90 (s, 9 H, $SiCMe_3$), 0.12 and 0.11 (2 s, 6 H, $SiMe_2$).

tert-*Butyldimethylsilyl 3-azido-2,3,6-trideoxy-β-L-arabino-hexopyranoside* (**3d**) *and* tert-*butyldimethylsilyl 3-azido-2,3,6-trideoxy-β-L-ribo-hexopyranoside* (**4d**).—A solution of the previously obtained **3c**–**4c** mixture (19.8 g, 60 mmol) and K_2CO_3 (0.5 g) in dry MeOH (200 mL) was stirred for 15 h. The solvent was removed in vacuo to give a viscous oil (17.4 g) which was purified by column chromatography (silica gel: 400 g, 15:1 cyclohexane–EtOAc). Two fractions were obtained.

From the faster moving fraction, compound **3d** (10.2 g, 59%) was obtained as a colourless viscous oil. $[\alpha]_D^{25} = +27.9°$ ($c = 1.87$, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): $\delta = 4.80$ (dd, $J_{1,2ax} = 9.4$, $J_{1,2eq} = 2.4$, 1 H, 1-H), 3.38 (ddd, $J_{2ax,3} = 12.5$, $J_{3,2eq} = 5.0$, $J_{3,4} = 9.5$, 1 H, 3-H), 3.32 (dq, $J_{5,4} = 9.2$, $J_{5,6} = 6.5$, 1 H, 5-H), 3.15 (ddd, $J_{4,OH} = 3.0$, 1 H, 4-H), 2.17 (ddd, $J_{2eq,2ax} = 12.5$, 1 H, 2_{eq}-H), 2.14 (d, 1 H, 4-OH), 1.65 (ddd, 1 H, 2_{ax}-H), 1.32 (d, 3 H, 6-H_3), 0.90 (s, 9 H, $SiCMe_3$), 0.13 and 0.12 (2 s, 6 H, $SiMe_2$). Anal. Calcd for $C_{12}H_{25}N_3O_3Si$: C, 50.14; H, 8.77; N, 14.62. Found C, 49.97; H, 9.00; N, 14.55.

From the slower moving fraction, compound **4d** (4.9 g, 28%) was obtained as a colourless viscous oil. $[\alpha]_D^{25} = -36.6°$ ($c = 0.90$, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): $\delta = 5.00$ (dd, $J_{1,2ax} = 9.0$, $J_{1,2eq} = 2.3$, 1 H, 1-H), 4.07 (ddd, $J_{3,2eq} = J_{3,2ax} = J_{3,4} = 3.5$, 1 H, 3-H), 3.63 (dq, $J_{5,4} = 9.2$, $J_{5,6} = 6.2$, 1 H, 5-H), 3.40 (m, 1 H, 4-H; after D_2O-exchange: m → dd), 2.12 (ddd, $J_{2eq,2ax} = 14$, 1 H, 2_{eq}-H), 1.83 (s br, 1 H, 4-OH), 1.82 (ddd, 1 H, 2_{ax}-H), 1.29 (d, 3 H, 6-H_3), 0.91 (s, 9 H, $SiCMe_3$), 0.13 and 0.12 (2 s, 6 H, $SiMe_2$). Anal. Calcd for $C_{12}H_{25}N_3O_3Si$: C, 50.14; H, 8.77; N, 14.62. Found C, 50.34; H, 8.66; N, 14.68.

1-O-tert-Butyldimethylsilyl 2,3,6-trideoxy-3-trifluoroacetamido-β-L-arabino-hexopyranoside (**3e**).—Triethylamine (1 mL) and ethyl tri-

fluoroacetate (5.11 g, 36 mmol) was added to a solution of **3d** (5.17 g, 18 mmol) in MeOH (50 mL), and the reaction mixture was shaken for 4 h in a hydrogen atmosphere in the presence of Pd–C (10% Pd, 400 mg). After filtering off the catalyst, the solvent was removed in vacuo and the green–brown oil was purified on silica gel (1:5 EtOAc–cyclohexane) to give **3e** (5.28 g, 82%) as a colourless solid, mp 140–141 °C (Lit.: 142 °C [18]). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 6.46$ (d br, $J_{NH,3} = 7.5$ Hz, 1 H, NH), 4.87 (dd, $J_{1,2ax} = 8.7$, $J_{1,2eq} = 2.1$ Hz, 1 H, 1-H), 3.96 (m, 1 H, 3-H), 3.37 (qd, $J_{5,4} = 9.1$, $J_{5,6} = 6.2$ Hz, 1 H, 5-H), 3.17 (t br, $J_{4,3} = 10.0$, 1 H, 4-H), 2.47 (s br, 1 H, 4-OH), 2.23 (ddd, $J_{2eq,3} = 4.8$, $J_{2eq,2ax} = 12.7$, 1 H, 2_{eq}-H), 1.62 (ddd, $J_{2ax,3} = 12.7$, 1 H, 2_{ax}-H), 1.33 (d, 3 H, 6-H_3), 0.89 (s, 9 H, $SiCMe_3$), 0.12 and 0.11 (2 s, 6 H, $SiMe_2$).

1-O-tert-*Butyldimethylsilyl 4*-O-p-*nitrobenzoyl-2,3,6-trideoxy-3-trifluoro-acetamido-β*-L-arabino-*hexopyranoside* (**3f**).—*p*-Nitrobenzoyl chloride (2.26 g, 12.2 mmol) was added to a solution of **3e** (3.57 g, 10 mmol) and 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 and pyridine (6:1, 70 mL) at 0 °C. After stirring for 24 h at 20 °C, the reaction mixture was washed with water, satd $NaHCO_3$, and again water. After drying with Na_2SO_4, the solvent was removed in vacuo and the residue was co-evaporated three times with toluene (30 mL each time) to remove pyridine. Column chromatography (1:6 EtOAc–cyclohexane) of the crude product gave **3f** (4.60 g, 91%) as a colourless solid, mp 88–90 °C. $[\alpha]_D^{25} = -5.5°$ ($c = 1.96$, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.29$, 8.14 (A_2B_2, $J =$ 9, 4 H, Ar–H), 6.74 (d br, $J_{NH,3} = 8.3$, 1 H, NH), 4.97 (dd, $J_{1,2ax} = 9.0$, $J_{1,2eq} = 2.2$, 1 H, 1-H), 4.84 (dd, $J_{4,5} = J_{4,3} = 9.7$, 1 H, 4-H), 4.38 (m, 1 H, 3-H), 3.79 (qd, $J_{5,6} = 6.2$, 1 H, 5-H), 2.37 (ddd, $J_{2eq,3} = 4.7$, $J_{2eq,2ax} = 12.5$, 1 H, 2_{eq}-H), 1.76 (ddd, $J_{2ax,3} = 12.5$, 1 H, 2_{ax}-H), 1.31 (d, 3 H, 6-H_3), 0.92 (s, 9 H, $SiCMe_3$), 0.16 and 0.14 (2 s, 6 H, $SiMe_2$). Anal. Calcd for $C_{21}H_{29}F_3N_2O_7Si$: C, 49.79; H, 5.77; N, 5.53. Found C, 50.03; H, 5.76; N, 5.47.

tert-*Butyldimethylsilyl 4*-O-*acetyl-2,3,6-trideoxy-3-trifluoroacetamido-β*-L-arabino-*hexopyranoside* (**3g**).—Acetic anhydride (4.8 mL, 48 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a solution of **3e** (4.29 g, 12 mmol) and pyridine (5 mL) in CH_2Cl_2 (40 mL) at 0 °C. After stirring for 12 h at 20 °C, CH_2Cl_2 (50 mL) and water (50 mL) was added and the mixture was stirred for 30 min. The organic layer was separated, washed with water and dried with Na_2SO_4. After concentrating in vacuo, the residue was co-evaporated three times with toluene (30 mL each time), and the crude product was purified on silica gel (1:10 EtOAc–cyclohexane) to give **3g** (4.45 g, 92%) as a colourless solid, mp 76 °C (Lit. 78 °C [18]). $[\alpha]_D^{25} = -23.9°$ ($c = 1.99$, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 6.74$ (d br, $J_{NH,3} = 7.5$, 1 H, NH), 4.87 (dd, $J_{1,2ax} = 9.2$, $J_{1,2eq} = 2.1$, 1 H, 1-H), 4.54 (dd, $J_{4,3} = J_{4,5} =$ 9.5, 4-H), 4.12 (m, 1 H, 3-H), 3.58 (qd, 1 H, $J_{5,6} = 6.4$, 5-H), 2.33 (ddd, 1 H, $J_{2eq,3} = 4.8$, $J_{2ax,2eq} = 13.0$, 2_{eq}-H), 2.08 (s, 3 H, MeCO), 1.62 (ddd, $J_{2ax,3} = 13$, 1 H, 2_{ax}-H), 1.24 (d, 3 H, 6-H_3), 0.90 (s, 9 H, $SiCMe_3$), 0.12 and 0.11 (2 s, 6 H, $SiMe_2$).

tert-*Butyldimethylsilyl 3-azido-2,3,6-trideoxy-4*-O-*trifluoromethanesulfonyl-β*-L-arabino-*hexopyranoside* (**3h**).—Dry pyridine (3.96 g, 50 mmol) and DMAP (100 mg) was added to **3d** (5.75 g, 20 mmol) in dry CH_2Cl_2 (150 mL). At −40 °C, a solution of trifluoromethanesulfonic acid anhydride (3.4 mL, 6.77 g, 24 mmol) in dry CH_2Cl_2 (50 mL, containing 1% pyridine) was added dropwise to the reaction mixture. After stirring for 4 h at −20 °C (reaction was monitored by thin layer chromatography (TLC), 1:5 EtOAc–cyclohexane), the reaction mixture was washed twice with 10% aq AcONa, 5% $NaHCO_3$, and finally with water. The solvent was removed in vacuo, and residue was co-evaporated three times with toluene (30 mL each time) to remove pyridine. The crude product **3h** was used directly for the next step (inversion) without further purification. For NMR measurement, a small amount was purified by column chromatography (1:5 EtOAc–cyclohexane) to give **3h** as a colourless unstable oil which decomposed visibly within some hours by going dark. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 4.86$ (dd, $J_{1,2ax} = 9.2$, $J_{1,2eq} = 2.2$, 1 H, 1-H), 4.34 (dd, $J_{3,4} = J_{4,5} = 9.5$, 1 H, 4-H), 3.64 (ddd, $J_{3,2ax} = 13$, $J_{3,2eq} = 5.0$, 1 H, 3-H), 3.60 (qd, $J_{5,6} = 6.2$, 1 H, 5-H), 2.38 (ddd, $J_{2eq,2ax} = 13.0$, 1 H, 2_{eq}-H), 1.83 (ddd, 1 H, 2_{ax}-H), 1.38 (d, 3

H, 6-H_3), 0.90 (s, 9 H, $SiCMe_3$), 0.13 and 0.12 (2 s, 6 H, $SiMe_2$).

tert-*Butyldimethylsilyl 2,3,6-trideoxy-3-trifluoroacetamido-β-*L*-ribo-hexopyranoside* (**4e**).—Catalytic hydrogenation of **4d** (4.00 g, 13.9 mmol) carried out as described in the synthesis of **3e** gave, after column chromatography (1:5 EtOAc–cyclohexane), **4e** (4.32 g, 87%) as colourless viscous oil. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 6.46$ (d br, 1 H, NH), 5.07 (dd, $J_{1,2ax} = 6.0$, $J_{1,2eq} = 3$, 1 H, 1-H), 4.45 (m, 1 H, 3-H), 3.79 (qd, $J_{5,4} = J_{5,6} = 6.5$, 1 H, 5-H), 3.65 (s br, 1 H, 4-H), 2.20 (ddd, $J_{2eq,3} = 7.5$, $J_{2eq,2ax} = 13.5$, 1 H, 2_{eq}-H), 2.10 (s br, 1 H, 4-OH), 1.81 (ddd, $J_{2ax,3} = 4$, 1 H, 2_{ax}-H), 1.39 (d, 3 H, 6-H_3), 0.89 (s, 9 H, $SiCMe_3$), 0.12 and 0.12 (2 s, 6 H, SiMe). Anal. Calcd for $C_{14}H_{26}F_3NO_4Si$: C, 47.04; H, 7.33; N, 3.92. Found: C, 47.46; H, 7.46; N, 3.93.

tert-*Butyldimethylsilyl 4-*O*-p-nitrobenzoyl-2,3,6-trideoxy-3-trifluoroacetamido-β-*L*-ribo-hexopyranoside* (**4f**).—*p*-Nitrobenzoylation of **4e** (5.36 g, 15 mmol) carried out as described in the synthesis of **3f** gave, after column chromatography (1:6 EtOAc–cyclohexane), **4f** (6.26 g, 82%) as a colourless solid with mp 106 °C. $[\alpha]_D^{25} = +46.2°$ ($c = 2.09$, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.30$, 8.14 (m, 4H, A_2B_2, $J = 9$, 4 H, Ar–H), 6.47 (d br, $J_{NH,3} = 7.5$, 1 H, NH), 5.20 (dd, $J_{1,2ax} = 6.5$, $J_{1,2eq} = 2.7$, 1 H, 1-H), 5.09 (dd, $J_{4,3} = 3.8$, $J_{4,5} = 6.7$, 1 H, 4-H), 4.85 (m, 1 H, 3-H), 4.06 (qd, $J_{5,6} = 6.7$, 1 H, 5-H), 2.19 (ddd, $J_{2eq,3} = 6.8$, $J_{2eq,2ax} = 13.5$, 1 H, 2_{eq}-H), 2.04 (ddd, $J_{2ax,3} = 4.5$, 1 H, 2_{ax}-H), 1.42 (d, 3 H, 6-H_3), 0.92 (s, 9 H, $SiCMe_3$), 0.16 and 0.15 (2 s, 6 H, $SiMe_2$). Anal. Calcd for $C_{21}H_{29}F_3N_2O_7Si$: C, 49.79; H, 5.77; N, 5.53. Found C, 50.07; H, 5.85; N, 5.59.

tert-*Butyldimethylsilyl 4-*O*-acetyl-2,3,6-trideoxy-3-trifluoroacetamido-β-*L*-ribo-hexopyranoside* (**4g**).—Acetylation of **4e** (1.79 g, 5 mmol) carried out as described in the synthesis of **3g** gave, after column chromatography, **4g** (1.77 g, 89%) as a colourless solid with mp 122 °C. $[\alpha]_D^{25} = +21.4°$ ($c = 0.94$, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 6.38$ (d br, $J_{NH,3} = 7.5$, 1 H, NH), 5.09 (dd, $J_{1,2ax} = 6.5$, $J_{1,2eq} = 2.9$, 1 H, 1-H), 4.75 (dd, $J_{4,3} = 4.0$, $J_{4,5} = 6.7$, 4-H), 4.66 (m, 1 H, 3-H), 3.88 (qd, 1 H, $J_{5,6} = 6.7$, 5-H), 2.13 (ddd, 1 H, $J_{2eq,3} = 2.8$, $J_{2ax,2eq} = 13.0$, 2_{eq}-H), 2.08 (s, 3 H, MeCO), 1.90 (ddd, $J_{2ax,3} = 4.5$, 1 H, 2_{ax}-H), 1.33 (d, 3 H, 6-H_3), 0.90 (s, 9 H, $SiCMe_3$), 0.13 and 0.12 (2 s, 6 H, $SiMe_2$). Anal. Calcd for $C_{16}H_{28}F_3NO_5Si$: C, 48.11; H, 7.06; N, 3.51. Found: C, 48.33; H, 7.19; N, 3.64.

tert-*Butyldimethylsilyl 3-azido-2,3,6-trideoxy-β-*L*-xylo-hexopyranoside* (**5d**)

Method A. A solution of **5i** (6.00 g, 15.3 mmol) and K_2CO_3 (0.5 g) in dry MeOH (50 mL) was stirred overnight and solvent was removed in vacuo. Column chromatography (80 g of silica gel, 5:1 cyclohexane–EtOAc) of the crude product yielded **5d** (4.00 g, 91%) as a white solid, mp 85–86 °C. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 4.93$ (dd, $J_{1,2eq} = 4.3$ $J_{1,2ax} = 7.3$, 1 H, 1-H), 3.98 (dd, $J_{3,4} = 3.2$, $J_{3,2ax} = 6.6$, 1 H, 3-H), 3.91 (dq, $J_{5,6} = 6.6$, $J_{5,4} = 1$, 1 H, 5-H), 3.32 (ddd, $J_{4\text{-OH}} = 10$, $J_{3-4} = 3.2$, $J_{4-5} = 1$, 1 H, 4-H), 2.24 (d, 1 H, OH), 1.85 (m, 2 H, 2_{eq}-, 2_{ax}-H), 1.25 (d, 3 H, 6-H_3) 0.90 (s, 9 H, $SiC(CH_3)_3$), 0.13, 0.12 (2 s, 2 × 3 H, $SiMe_2$). ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = -4.24$, -5.21 (–$SiCH_3$), 16.59 (C-6), 18.13 [$(CH_3)_3C$–], 25.75 [–$(CH_3)_3$], 32.91 (C-2), 60.47 (C-3), 69.22/68.31(C-4, C-5), 93.12 (C-1).

Method B. Deacetylation of a mixture of **2c**–**5c** was carried out as depicted in the deacetylation of **3c**–**4c**. Thus, when 2.50 g (7.59 mmol) of the mixture was deacetylated in anhyd MeOH (50 mL), using K_2CO_3 (0.5 g) as the base, a white solid (1.90 g) was obtained. Further separation of the mixture by column chromatography (silica gel: 200 g, 15:1 cyclohexane–EtOAc) afforded two fractions: from the faster moving fraction, compound **2d** (0.50 g, 24%) was obtained as a colourless solid with mp 68–70 °C. From the slower moving fraction, compound **5d** (1.20 g, 56%) was obtained as a colourless solid, mp 85–86 °C. Anal. Calcd for $C_{12}H_{25}N_3O_3Si$: C, 50.14; H, 8.77. Found: C, 50.11; H, 8.50.

tert-*Butyldimethylsilyl 3-trifluoroacetamido-2,3,6-trideoxy-β-*L*-xylo-hexopyranoside* (**5e**).—Hydrogenation of **5d** (2.40 g, 8.35 mmol) carried out as described in the synthesis of **3e** gave, after column chromatography (silica gel: 60 g, 5:1 cyclohexane–EtOAc), **5e** (2.65 g, 92%) as a colourless solid, mp 125–126 °C. $[\alpha]_D^{25} = +16.4°$ ($c = 1.97$, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 6.26$ (br, 1 H, NH), 4.92 (dd, $J_{1,2e} = 2.4$, $J_{1-2a} = 8.5$, 1 H, 1-H),

4.29 (m, 1 H, 3-H), 3.80 (dq, $J_{5-4} = 1.7$, $J_{5-6} = 6.6$, 1 H, 5-H), 3.50 (m, $J_{4-3} = 3.6$, 1 H, 4-H), 2.30 (d, $J = 9.1$, 1 H, OH), 2.07 (ddd, $J_{2a,2e} = 13.6$, $J_{2a-1} = 8.5$, $J_{2a,3} = 4.7$, 1 H, 2a-H), 1.79 (ddd, $J_{2a,2e} = 13.6$, $J_{2e,3} = 2.7$, $J_{2e,1} = 2.4$, 1 H, 2e-H), 1.31 (d, $J = 6.6$, 3 H, 6-H), 0.90 (s, 9 H, *tert*-Bu), 0.14 and 0.12 (2 s, 2 × 3 H, –Si–Me). ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = -4.05$, -5.17 (–SiMe), 16.73 (C-6), 18.01 [$(Me)_3C$–], 25.70 [–C(Me)$_3$], 33.25 (C-2), 49.92 (C-3), 69.65/68.10 (C-4, C-5), 92.97 (C-1), 115.60 ($J_{CF} = 287.9$ Hz, CF_3), 156.88 ($J_{CF} = 37.4$ Hz, –COCF_3). MS (70 eV): m/z (%) = 356.2 ([$M^+ - 1$], 0.5), 119 (100), 101 (20), 75 (36), 73 (26). Anal. Calcd for $C_{14}H_{26}F_3NO4Si$: C, 47.04; H, 7.34. Found: C, 46.95; H, 7.20.

tert-*Butyldimethylsilyl 3-trifluoracetamido-4-*O*-(*p*-nitrobenzoyl)-2,3,6-trideoxy-*L*-xylo-hexopyranoside* (**5f**).—*p*-Nitrobenzoylation of **5e** (1.55 g, 4.34 mmol) carried out as described in the synthesis of **3f** gave, after column chromatography (silica gel: 60 g, 20:1 cyclohexane–EtOAc), **5f** (2.0 g, 91%) as a pale yellow foam. $[\alpha]_D^{25} = -12.1°$ ($c = 2.05$, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 8.33$, 8.24 (A_2B_2, $J = 9$, 4 H, Ar–H), 6.45 (d, $J = 7.3$, NH), 5.13 (m, 2 H, 1-, 4-H), 4.61 (m, 1 H, 3-H), 4.18 (dq, $J_{5-6} = 6.6$, $J_{4-5} = 3.4$, 1 H, 5-H), 2.15 (ddd, $J_{2a-2e} = 13.7$, $J_{1,2a} = 6.4$, $J_{3,2a} = 4.7$, 1 H, 2a-H), 1.93 (ddd, 1 H, $J_{2a-2e} = 13.7$, $J_{3-2e} = 6.4$, $J_{1-2e} = 2.7$, 2e-H), 1.34 (d, 3H, 6-H_3), 0.94 (s, 9 H, *tert*-Bu), 0.18 and 0.16 (2 s, 2 × 3 H, –Si–Me). ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = -4.08$, -5.08 (SiMe), 17.34 (C-6), 18.08 [$(Me)_3C$–], 25.75 [–C(Me)$_3$], 35.64 (C-2), 46.36 (C-3), 69.17–71.16 (C-4, C-5), 92.47 (C-1), 115.52 (q, $J^1_{CF} = 288$ Hz, CF_3), 156.86 (q, $J^2_{CF} = 38$ Hz, –COCF_3), 123.74 (C-3′, C-5′), 131.04 (C-2′, C-6′), 134.46 (C-1′), 150.89 (C-4′), 164.19 (–COO). MS (70 eV): m/z (%) = 505.1 ([$M^+ - 1$], 491 (32), 449 (36), 336 (48), 238 (50), 224 (36), 208 (20), 150 (100), 101 (16). Anal. Calcd for $C_{21}H_{29}N_2O_7F_3Si$: C, 49.79; H, 5.77; Found: C, 49.79; H, 5.71.

tert-*Butyldimethylsilyl 3-azido-4-*O*-benzoyl-2,3,6-trideoxy-β-*L*-xylo-pyranoside* (**5i**).—Compound **9a** (5.00 g, ca. 20 mmol) was dissolved in a mixture of 1 N HCl (20 mL), HOAc (8 mL) and THF (30 mL), and stirred for 15 h at 20 °C until TLC analysis showed complete cleavage of the acetal group. Sodium azide (2.30 g, ca. 35 mmol) was added and the reaction mixture was stirred for 10 h. The organic layer was separated and the water phase was extracted with CH_2Cl_2. The combined organic layers were washed with satd $NaHCO_3$ and dried with Na_2SO_4. The solvent was removed and the crude product was redissolved in dry CH_2Cl_2 (100 mL), together with of *tert*-butyl(dimethyl)silyl chloride (3.00 g, ca. 20 mmol) and imidazole (2.72 g, ca. 40 mmol). After stirring for 2 h, the mixture was washed with water and dried with Na_2SO_4. The crude product was purified by column chromatography (80 g of silica gel, 20:1 cyclohexane–EtOAc) to give **5i** (7.0 g, 88%) as a colourless viscous oil. $[\alpha]_D^{25} = -60.4°$ ($c = 0.37$, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 8.09$ (m, 2 H) and 7.65–7.42 (m, 3 H, phenyl–H), 5.06 (dd, $J_{1,2eq} = 3.9$, $J_{1,2ax} = 7.3$, 1 H, 1-H), 4.81 (dd, $J_{4,3} = 3.2$, $J_{4,5} = 1.5$, 1 H, 4-H), 4.10 (m, 2 H, 3-, 5-H), 1.93 (m, 2 H, 2-H_2), 1.25 (d, $J_{6,5} = 6.5$, 3 H, 6-H_3), 0.94 (s, 9 H, $SiC(CH_3)_3$), 0.17, 0.16 (2 s, 2 × 3 H, $SiMe_2$).

*3,4-Di-*O*-acetyl-1,5-anhydro-2,6-dideoxy-*L*-arabino-hex-1-enol (di-*O*-acetyl-*L*-(+)-rhamnal)* (**7**).—Rhamnose (**6a**) (100 g, 0.55 mol) was added to a mixture of Ac_2O (340 mL) and 70% perchloric acid (2 mL) in a 1-L three-necked flask in such a way that the reaction temperature was maintained between 30 and 40 °C. After stirring for 2 h, phosphorus tribromide (70 mL) was added dropwise below 10 °C. Finally water (33 mL) was added dropwise below 15 °C, and the reaction mixture was then stirred for another 2 h at 20 °C.

Sodium acetate (226 g), water (700 mL) and AcOH (295 mL) were added to a 4-L three-necked round bottom flask equipped with mechanic stirrer, thermometer and dropping funnel. The mixture was cooled to −10 °C using MeOH–dry ice bath. Zinc powder (200 g) and finally aq $CuSO_4$ solution (16.5 g $CuSO_4 \times 5\ H_2O$ in 65 mL water) were added.

After disappearance of the blue colour and development of hydrogen, the crude bromoaceto–rhamnose solution prepared above was added dropwise to this mixture and the reaction temperature was maintained between

−5 and −10 °C. After stirring for 6 h at −10 °C, 1 L of ice–water was added and the reaction mixture was stirred for another 5 min. Unreacted zinc powder and Cu were filtered off, and the filtrate was extracted five times with CH_2Cl_2 (300 mL each time). The combined organic layer was washed with ice–water, satd $NaHCO_3$, again ice–water, and dried with Na_2SO_4. The solvent was removed in vacuo and the crude product was vacuum-distilled at 78–79 °C/0.1 Torr to give **7** (100 g, 85%) as a colourless liquid (Lit.: 68–69 °C/0.06 Torr [9]). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 6.43$ (dd, 1 H, $J_{1,2} = 6.1$, 1-H), 5.34 (m, 1 H, 2-H), 5.03 (dd, 1 H, $J_{4,5} = 8.1$, $J_{3,4} = 6.1$, 4-H), 4.78 (dd, 1 H, $J_{3,4} = 6.1$, $J_{2,3} = 3.0$, 3-H), 4.11 (dq, 1 H, $J_{4,5} = 6.1$, $J_{5,6} = 6.6$, 5-H), 2.09, 2.05 (2 s, 6 H, 2 AcO), 1.32 (d, 3 H, $J_{5,6} = 6.6$, 6-H).

Methyl 4-O-acetyl-2,3,6-trideoxy-α,β-L-erythro-hex-2-enopyranoside (**8b**).—Anhydrous ZnCl (14.0 g, c.a. 0.10 mol) was added to a solution of **7** (43 g, 0.20 mol) in dry CH_2Cl_2 (330 mL) and dry MeOH (20 mL). After stirring overnight, satd $NaHCO_3$ (100 mL) was added to the reaction mixture and the organic layer was washed with water. After drying with Na_2SO_4, the crude product was vacuum-distilled at 55–56 °C/0.1 Torr to give colourless oily **8b** (30.0 g, 81%) as mixture of α and β anomer (α:β = 4:1). ^{1}H NMR (200 MHz, $CDCl_3$), α-**8b**: $\delta = 5.83$ (m, 2 H, 2-, 3-H), 5.05 (m, 1 H, 4-H), 4.83 (dd, $J_{1,2} = 1.5$, $J_{1,3} = 0.9$, 1 H, 1-H), 3.94 (dq, $J_{5,6} = 6.5$, $J_{5,4} = 9.5$, 1 H, 5-H), 3.44 (s, 3 H, OMe), 2.09 (s, 3 H, MeCO), 1.24 (d, 3 H, 6-H_3). β-**8b**: $\delta = 5.90$ (m, 2 H, 2-, 3-H), 5.05 (m, 1 H, 4-H), 4.78 (d, $J_{1,2} = 4$, 1 H, 1-H), 3.85 (m, 1 H, 5-H), 3.47 (s, 1 H, OMe), 2.10 (s, 3 H, MeCO), 1.32 (d, $J_{6,5} = 6.5$, 3 H, 6-H_3).

Methyl 4-O-benzoyl-2,3,6-trideoxy-α,β-L-threo-hex-2-enopyranoside (**9a**)

Method A. Compound **8b** (8.00 g, ca. 10 mmol) was dissolved in dry MeOH (30 mL) and anhyd K_2CO_3 (0.1 g) was added. After stirring overnight, the solvent was removed in vacuo. The residue was dissolved in dry pyridine (10 mL) and mesyl chloride (2.06 g, ca. 18 mmol) was added dropwise at 0 °C. After stirring for 15 h at 20 °C, water (30 mL) was added and the mixture was extracted with CH_2Cl_2. The crude product was dried with Na_2SO_4, and redissolved in DMF (50 mL). Caesium benzoate (3.81 g) was added and the suspension was stirred for 5 h at 80 °C. At 20 °C, water (50 mL) was then added and the reaction mixture was extracted four times with diethyl ether (50 mL of each time). The organic phase was washed with water, dried with Na_2SO_4, and the crude product was purified by column chromatography to give **9a** (0.95 g, 39%) as a colourless oil. $[\alpha]_D^{25} = +214.0°$ ($c = 0.86$, $CHCl_3$).

Method B (Mitsunobu reaction). To a solution of **8c** (9.6 g, 66.6 mmol), benzoic acid (17.0 g, 0.14 mol) and triphenylphosphine (36.5 g, 0.14 mol) in anhyd THF (100 mL), DEAD (22.0 g, ca. 0.13 mol) dissolved in THF (30 mL) was added dropwise at 0 °C. The reaction mixture was stirred for 15 h and the solvent was removed in vacuo, 100 mL of ether was added to the residue and the insoluble part was filtered off. After removal of the solvent, the crude product was purified by column chromatography (200 g of silica gel, 10:1 cyclohexane–EtOAc) to give **9a** (15.00 g, 90%) as a colourless viscous liquid. ^{1}H NMR (200 MHz, $CDCl_3$): α-**9a**: $\delta = 8.09$ (m, 2 H, Ar–H), 7.61–7.39 (m, 3 H, Ar–H), 6.21 (dd, $J_{3,4} = 5$, $J_{3,2} = 10$, 1 H, 3-H), 6.06 (dd, $J_{2,1} = 3$, 1 H, 2-H), 5.15 (dd, $J_{4,5} = 5.2$, 1 H, 4-H), 4.98 (d, 1 H, 1-H), 4.33 (dq, $J_{5,6} = 6.5$, 1 H, 5-H), 3.47 (s 3 H, OMe), 1.32 (d, 3 H, 6-H_3). β-**9a**: $\delta = 8.09$ (m, 2 H, Ar–H), 7.61–7.39 (m, 3 H, Ar–H), 6.18 (ddd, $J_{3,1} = 1.5$, $J_{3,4} = 4.8$, $J_{3,2} = 10$, 1 H, 3-H), 6.06 (ddd, $J_{2,1} = J_{2,4} = 1$, 1 H, 2-H), 5.26 (m, 1 H, 4-H), 5.09 (br q, 1 H, 1-H), 4.01 (dq, $J_{5,4} = 2.8$, $J_{5,6} = 6.5$, 1 H, 5-H), 3.53 (s 3 H, OMe), 1.34 (d, 3 H, 6-H_3).

Methyl 4-O-acetyl-2,3,6-trideoxy-α,β-L-threo-hex-2-enopyranoside (**9b**)

Deacetylation reaction. A mixture of anhyd K_2CO_3 (0.1 g) and **8b** (6.50 g, ca. 35 mmol) in dry MeOH (30 mL) was stirred for 15 h at 20 °C and solvent was removed in vacuo. The resulting yellowish oil was purified on silica gel (column, 10:1 cyclohexane–EtOAc) to give **8c** (4.70 g) as a colourless oily mixture of both anomers.

Mitsunobu reaction.—Diethyl azodicarboxylate (11.40 g, ca. 65 mmol) in dry THF (15 mL) was added dropwise to the solution

of **8c** (4.7 g, 65 mmol) obtained above, AcOH (4.0 g, ca. 65 mmol) and triphenylphosphine (17.10 g, 65 mmol) in dry THF (100 mL) at 0 °C. The reaction mixture was stirred 15 h and solvent was removed in vacuo, diethyl ether (50 mL) was added to the residue and the insoluble part was filtered off. After removal of solvent, the crude product was purified by column chromatography (200 g silica, 10:1 cyclohexane–EtOAc) to give **9b** (4.85 g, 85%) as a colourless viscous liquid.

7-O-(2,3,6-Trideoxy-4-O-p-nitrobenzoyl-3-trifluoroacetamido-α-L-arabino-hexopyranosyl)-ε-iso-rhodomycinone (7-O-acosaminyl-ε-iso-rhodomycinone) (**10**).—Me_3Si–triflate (1.50 g, 6.72 mmol) was added dropwise at −35 °C to a suspension of **1b** (1.80 g, 4.05 mmol), **2f** (2.60 g, 5.13 mmol) and powdered molecular sieves (4 Å, 4.5 g) in 10:1 CH_2Cl_2–acetone (210 mL). After stirring for 6 h at this temperature, the reaction was quenched by addition of triethylamine (2.8 mL). The molecular sieves were filtered off, and the filtrate was washed twice with water (100 mL each time). After drying with Na_2SO_4, the crude product was purified by column chromatography (170 g of silica gel, 200:10:1 chloroform–EtOAc–formic acid) to give **10** (2.32 g, 70%) as a red powder, mp 268–271 °C (Lit. 279–281 °C [11]) which contained ca. 12% bis-glycoside. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 13.02$, 12.84, 12.30, 12.29 (4 s, 4 H, 1-, 4-, 6-, 11-OH), 8.32, 8.18 (A_2B_2, 4 H, nitrobenzoyl–H), 7.30 (s, 2 H, 2-, 3-H), 6.60 (d, $J_{3',NH} = 8$, 1 H, NH), 5.52 (d, $J_{1',2ax} = 4$, 1 H, 1′-H), 5.26 (dd, $J_{7,8a} = 10$, $J_{7,8b} = 2$, 1 H, 7-H), 4.91 (dd, $J_{3',4'} = J_{4',5'} = 10$, 1 H, 4′-H), 4.48–4.38 (m, 2 H, 5′-, 3′-H), 4.34 (s, 1 H, 10-H), 4.09 (s, 1 H, 9-OH), 3.74 (s, 3 H, COOMe), 2.44 (m, 2 H, $2'_{eq}$-, 8_a-H), 2.30 (dd, 1 H, 8_b-H), 1.92 (m, 2 H, $2'_{ax}$-, 13_b-H), 1.34 (d, $J_{5',6'} = 6$, 6′-H_3), 1.18 (t, $J_{14,13} = 7.3$, 3 H, 14-H_3).

7-O-(3-Amino-2,3,6-trideoxy-α-L-arabino-hexopyranosyl)-ε-iso-rhodomycinone (7-acosaminyl-ε-iso-rhodomycinone) (**1c**).—To a solution of **10** (2.21 g, 2.7 mmol) in CH_2Cl_2–MeOH (2: 1, 25 mL), 1 N NaOH (9.5 mL) of was added and the reaction mixture was homogenised by adding MeOH. After stirring 20 °C for 30 min, the reaction mixture was neutralised by adding 1 N HCl (9.5 mL), concentrated in vacuo, redissolved in 2:1 CH_2Cl_2–butanol (200 mL), washed with satd $NaHCO_3$ and with water (50 mL). The organic phase was concentrated in vacuo and the residue was purified by column chromatography (60 g of silica gel, 100:5:0.5 CH_2Cl_2–MeOH–concentrated NH_3) giving **1c** (1.04 g, 67%) as a red sparingly soluble powder, mp 169–171 °C (Lit. 173–175 °C).

Biological testing.—The samples were solved in DMSO and stored at −20 °C. For the in vitro study the compounds were further diluted with culture medium under consideration that the final DMSO concentration never exceeds 0.3%. The supplied drugs were tested in a final concentration of 0.3 and 3 μg/mL.

Human tumor cell lines.—For the in vitro cytotoxicity study the following six human tumor cell lines were used:

Colon cancer:	HT 29
Gastric cancer:	GXF 251
Melanoma:	MEXF 462NL
Non-small cell lung cancer:	LXFL 529L, LXF 629, LXF 66

Cell culture.—Human tumor cells were grown at 37 °C in a humidified atmosphere (95% air, 5% CO_2) in monolayer cultures in RPMI 1640 medium with phenol red (Life Technologies, Karlsruhe, Germany) supplemented with 10% of fetal calf serum. Cells were trypsinised and maintained weekly.

Cytotoxicity assay.—A modified propidium iodide assay was used to examine the antiproliferative activity of the studied compounds [19]. Briefly, cells were harvested from exponential phase cultures growing in RPMI 1640 medium supplemented with 10% of fetal calf serum by trypsination, counted and plated in 96 well flat-bottomed microtitre plates (100 μl cell suspension, 1×10^5 and 5×10^4 cells/mL). After a 24 h recovery, to allow cells to resume exponential growth, 50 μl culture medium (six control wells per plate) or culture medium containing the test drug were added to the wells. Each drug concentration was plated in triplicate. After 3–7 days of incubation, depending on cell doubling time, culture medium

was replaced by fresh medium containing propidium iodide (6 μg/mL). Microplates were then kept at −18 C for 24 h, resulting in a total cell kill. After thawing of the plates, fluorescence was measured using a Millipore Cytofluor 2350-microplate reader (excitation 530 nm, emission 620 nm) in order to quantify the total cell number. The assay included untreated and positive controls (Doxorubicin).

Growth inhibition was expressed as treated/control × 100 (T/C%). IC_{50} and IC_{70} values were determined by plotting compound concentration versus cell number. Mean IC_{50} and IC_{70} values were calculated according to the formula:

$$\text{mean } IC_{50,70} = 10^{\frac{\sum_{x=1}^{n} \log(IC_{50,70})_x}{n}}$$

With x = specific tumor cell line and n = total number of cell lines studied. If IC_{50} or IC_{70} could not be determined within the examined dose range, the lowest or highest concentration studied was used for the calculation.

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