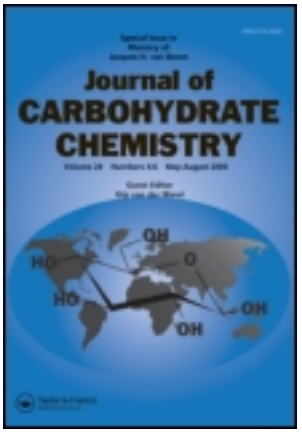

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Synthesis and Chemistry of Noeuromycin and Isofagomine Analogues

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CONTENTS

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ABSTRACT

Several N-substituted analogues of noeuromycin ((2*RS*,3*S*,4*R*,5*R*)-2,3,4-trihydroxy-5-hydroxymethylpiperidine) and isofagomine ((3*R*,4*R*,5*R*)-3,4-dihydroxy-5-hydroxymethylpiperidine) were synthesised. The isofagomine analogues (3*RS*,4*RS*,5*RS*)-*N*-(2-phosphonoethyl)-3,4-dihydroxy-5-hydroxymethyl-piperidine, (3*SR*,4*SR*,5*RS*)-*N*-(2-phosphonoethyl)-3,4-dihydroxy-5-hydroxy-methylpiperidine, and (3*R*,4*R*,5*R*)-*N*-(10-chloro-9-anthracenemethyl)-3,4-dihydroxy-5-hydroxy-methylpiperidine were synthesised by direct alkylation of the corresponding azasugar. N-Substituted noeuromycin derivatives could not be made in this straightforward manner, but were made by modification of a synthesis intermediate. By this method (2*RS*,3*S*,4*R*,5*R*)-*N*-(4-methoxyphenyl)-2,3,4-trihydroxy-5-hydroxymethylpiperidine and (2*RS*,3*S*,4*R*,5*R*)-*N*-nonyl-2,3,4-trihydroxy-5-hydroxymethylpiperidine were synthesised. The stability of noeuromycin was studied and was found to depend on stereochemistry and pH. The L-fuco isomer ((2*RS*,3*R*,4*R*,5*R*)-2,3,4-trihydroxy-5-methylpiperidine) was observed to undergo a particularly facile Amadori rearrangement at neutral pH to the 3-ketopiperidine. A noeuromycin analogue, that could not undergo the Amadori rearrangement, was synthesised.

Key Words: Noeuromycin; Isofagomine; Glycoside metabolism; Gaucher's disease; Glycosidase inhibitor; 1-N-Iminosugar; 1-Azasugar; N-Alkyl.

INTRODUCTION

Selective inhibition of glycoside metabolism has very large potential applications. Not only are several glycosidase inhibitors currently used in treatment of diabetes, but also a number of others are under clinical investigation to treat a variety of diseases and disorders such as HIV, Gaucher's disease, hepatitis, and cancer.[1] Success in this area have been difficult however because most of the potent glycosidase inhibitors are azasugars (imino- or aminosugars) of various kinds[2] that are very polar and difficult to absorb making them unsuitable drugs. This problem is frequently overcome by attaching lipophilic groups, such as a butyl group to the nitrogen of 1-deoxynojirimycin,[3,4] though these modifications may compromise enzyme binding.

Noeuromycin (**1**)[5] and isofagomine (**2**)[6] are members of a new type of azasugars with high biological activity (Fig. 1). However in order to use these potent enzyme ligands as drugs it may be necessary to improve lipophilicity, stability, or specificity.

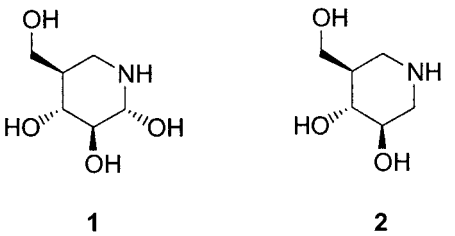


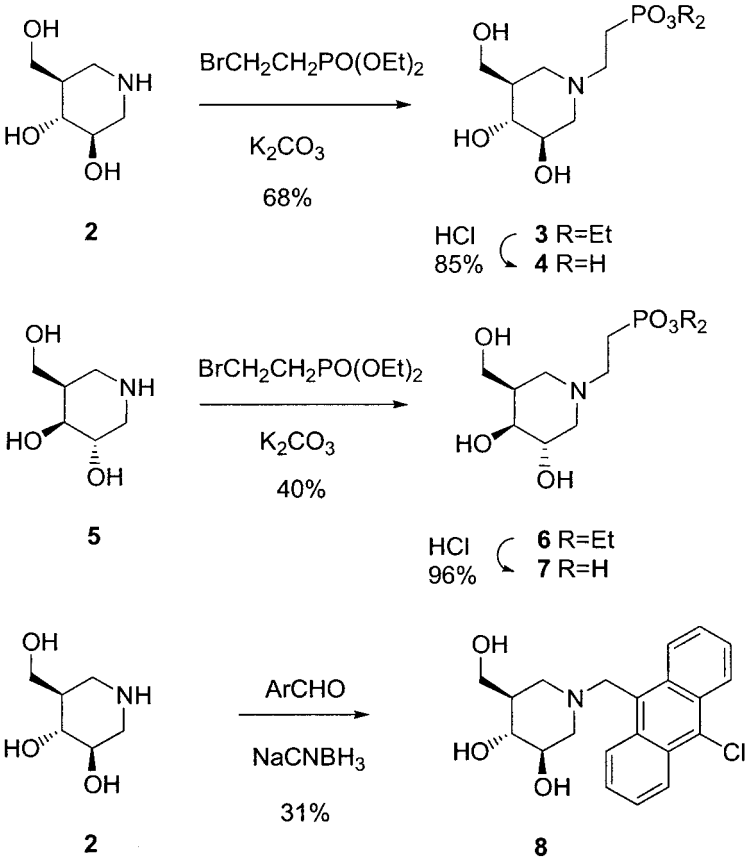
Figure 1. Structure of noeuromycin and isofagomine.

In this paper, we have investigated various structural modifications that improve some of these properties in **1** or **2**.

RESULTS AND DISCUSSION

N-Substitution of 2

Isofagomine (**2**) and noeuromycin (**1**) are potent glycogen phosphorylase inhibitors.[1] Binding of the inhibitors to the enzyme is presumably associated with simultaneous binding of phosphate since the crystal structure of **1** shows the presence of a phosphate molecule coordinated to N1.[1] Joining the phosphate and azasugar together, possibly through a ethylphosphonate, might improve binding and specificity towards this enzyme. For this reason, we investigated the incorporation of this group at N1 in **2**. Reaction of (±)-**2** with diethyl 2-bromoethylphosphonate in EtOH in the presence of anhydrous K_2CO_3 gave the alkylation product **3** in 68% yield (Sch. 1). By the same

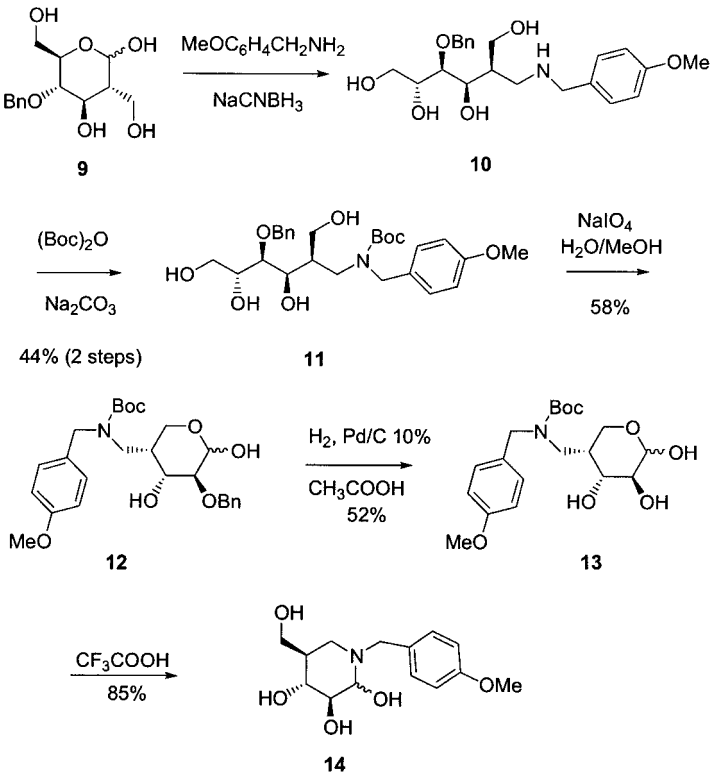


Scheme 1.

procedure the stereoisomer (±)-**5** was converted to **6** in 40% yield. The two esters **3** and **6** were hydrolysed by hydrochloric acid to form the phosphonic acids **4** and **7** in 85% and 96% yield, respectively. A similar modification of **2** was made in the fluorescence labelled inhibitor **8** (Sch. 1). Reductive amination of **2** with 10-chloro-9-anthralaldehyde and sodium cyanoborohydride gave the product **8** in modest yield.

N-Substitution of 1

Derivatives of **1** with lipophilic groups at nitrogen could convey better absorption characteristics to this very polar compound. We have synthesised two such derivatives. Modification of the nitrogen of **1** could not be carried out on **1** for obvious reasons. Reductive amination reaction similar to the synthesis of **8** results in destruction of the hemiaminal function, while an alkylation reaction such as that used in the synthesis of **3** and **6** is problematic due to the poor nucleophilicity of the nitrogen atom. Substitution of the nitrogen therefore had to be carried out at an earlier stage in the synthesis, and we have used two different protocols. The synthesis of the *N*-*p*-methoxybenzyl modified compound **14** was carried out from **9** (Sch. 2), which is obtained from levoglucosan.[7] Treatment of **9** with *p*-methoxybenzylamine and $NaCNBH_3$ gave the crude secondary amine **10**, which



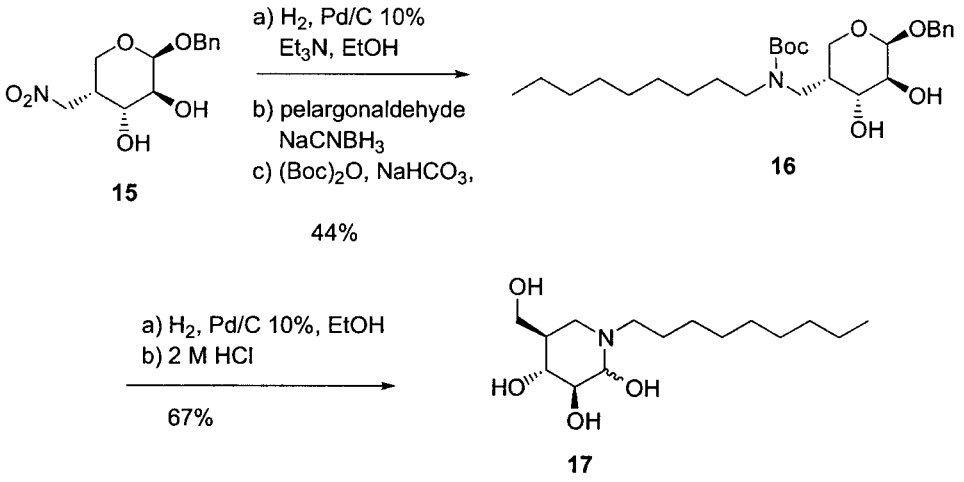
Scheme 2.

was protected with a Boc group to provide **11** in 44% yield. Cleavage of the diol in **11** with sodium periodate in $MeOH/H_2O$ gave the pyranose **12** in 58% yield. Hydrogenolysis of the benzyl group gave **13** in 52% yield, which after deprotection of the Boc group with TFA gave **14** in 85% yield.

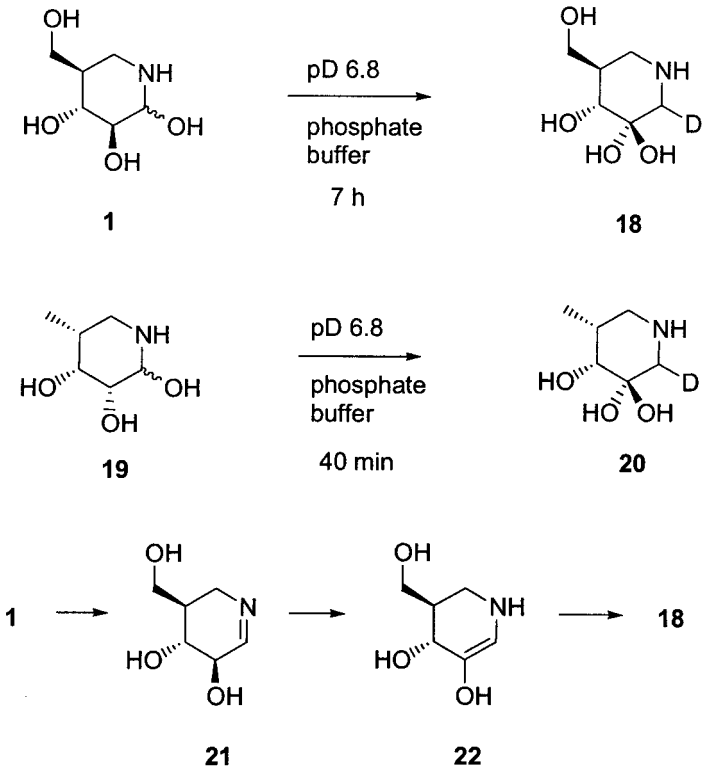
The *N*-nonyl analogue **17** was made in a different manner (Sch. 3). The nitro derivative **15**, which is obtained from D-arabinose,[8] was reduced to the amine by catalytic hydrogenation. Reductive amination with pelargonaldehyde and $NaCNBH_3$ followed by Boc protection afforded the Boc derivative **16** in 44% yield. Hydrogenolysis of the benzyl group followed by treatment with hydrochloric acid gave **17** as a hydrochloride in 67% yield.

Stability Study with 1

In his classical work Paulsen found that nojirimycin and similar compounds[9] underwent pH dependant Amadori rearrangement. Similar reactions could be expected to occur in compound **1** and analogues, but pH dependence might be different due to the different base strength of the amine in **1** and nojirimycin. Noeuromycin (**1**) was found to be stable for a month when kept as the hydrochloride. However at neutral pH the compound began to rearrange: in D_2O at pD 6.8, as maintained by a buffer, the compound was converted to the hydrated ketone **18** over 7 hr (Sch. 4). The incorporation of deuterium is stereospecific, but its configuration not determined. The rearrangement must occur by elimination of water from **1** to imine **21**, rearrangement to **22**, and addition of D_2O to **22** to reach **18**. Even faster, the fuco isomer **19** rearranged in D_2O to ketone hydrate **20** again with stereospecific incorporation of deuterium. The same rearrangement has recently been observed by Nishimura's group starting from various 2-amino derivatives that presumably hydrolyse to **19**.[10]



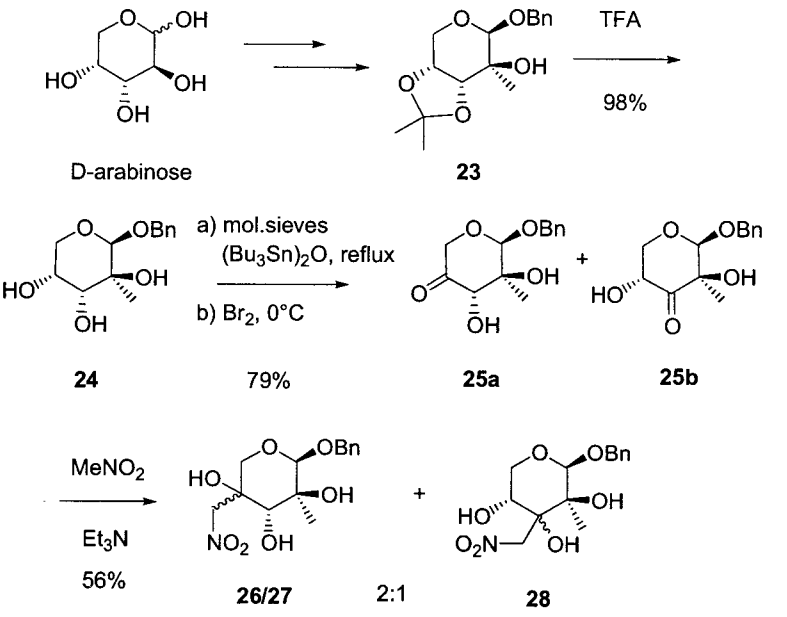
Scheme 3.



Scheme 4.

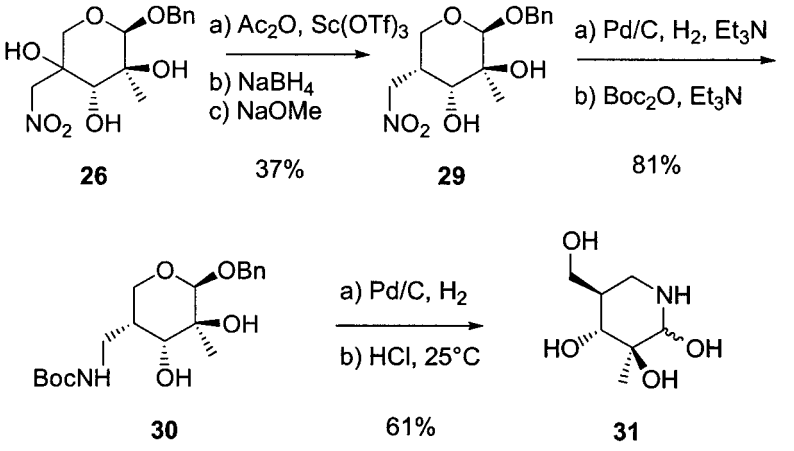
Synthesis of 31; a 3-C-Methyl Analogue of 1

The instability of **1** is a possible problem that limits its suitability as a drug. We therefore considered ways to prevent the rearrangement of **1**. A C-3 methyl group would prevent the rearrangement of **21** to **22** and might not affect inhibition significantly. We therefore synthesised a C-3 methyl analogue starting from the known D-arabinose derivative **23**. Compound **23** as well as its C-2 epimer is readily obtained from D-arabinose by stereoselective addition of organometallic reagents to the corresponding ketone.[11] Removal of the acetonide group in **23** was accomplished with aqueous TFA to give the triol **24** in 98% yield (Sch. 5). Oxidation of **24** with dibutyltin oxide and bromine gave a crude product containing mainly **25**. The reaction is not as regioselective as the corresponding oxidation of benzyl β-D-arabinoside[8] and oxidation at C-3 is also occurring. The product is however difficult to analyse and separate due to the presence of intermolecular hemiacetal formation. Treatment of this product with nitromethane and triethylamine gave a mixture of three different isomers **26**, **27**, and **28** in a combined yield of 56%. By chromatography the individual compounds were isolated in 11%, 18%, and 10% yield, respectively. The absolute stereochemistries at the newly formed tertiary centers were not determined. Compound **26** was acetylated by treatment with Ac_2O and scandium triflate, and then treated with $NaBH_4$ which gave β-elimination and conjugate reduction of the



Scheme 5.

resulting nitroalkene (Sch. 6). Complete deacetylation with NaOMe in methanol gave diol **29** in 37% yield. Catalytic hydrogenation of the nitro group followed by Boc protection gave the Boc protected amine **30** in 81% yield. Finally hydrogenolysis of the benzyl group and removal of the Boc group with hydrochloric acid gave the 3-C methyl analogue of noeuromycin **31** in 61% yield.



Scheme 6.

Glycosidase Inhibition

The new analogues were investigated for inhibition of α- and β-glucosidases (Table 1). (Solutions of **18** were prepared by incubating **1** in phosphate buffer overnight.) The compounds **14**, **18**, and **31** were found to be 100–1000 times weaker than **1**. In the case of **14**, it is clear that the bulky p-methoxybenzyl group disrupts the efficient binding of the azasugar moiety. The weaker binding of **18** and **31** suggests that there is no space for an axial substituent in the 3-position of the azasugar. The isofagomine analogue **8** inhibited almond β-glucosidase with a K_i of 24 μM. The phosphonates **4** and **7** were found to be very poor inhibitors of glycogen phophorylase.

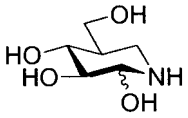
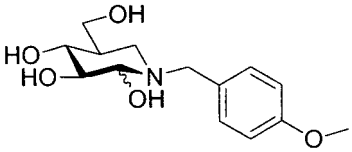
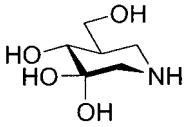
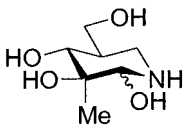
In summary we have established a number of synthetic methods that can be used to prepare analogues of **1** and **2**, which will be important for obtaining pharmaceutically acceptable derivatives of these compounds. The stability of **1** has also been investigated and it has been found to undergo Amadori rearrangement at neutral pH.

EXPERIMENTAL

General

^{1}H (200 or 400 MHz) and ^{13}C NMR (50 MHz) spectra were recorded on a Varian Gemini 200 or on a Varian 400 spectrometer using the signal of the solvent as reference.

Table 1. K_i values, in μM, of some noeuromycin analogues towards various glycosidases.

Compound	Structure	Yeast α-glu'ase	Almond β-glu'ase	Yeast isomaltase
1		0.022	0.069	0.025
14		3.0	45.0	—
18		8.5	7.5	5.3
31		110.0	3.0	—

Chemical shifts were expressed in δ values (ppm). Where a compound is present as a mixture of anomers NMR signals may be assigned to integrate to a fraction of a proton reflecting the fraction present of that anomer. Optical rotations were measured with a Perkin–Elmer Model 241 polarimeter. The mass spectra and the high-resolution mass spectra were taken by a Micromass LC-TOF. For K_i determination a Milton Roy Genesys 5 spectrophotometer was used. Unless otherwise stated, all reactions were performed under a normal atmosphere and pressure. THF was distilled prior to use from sodium and benzophenone, and CH_2Cl_2 was distilled with CaH_2. The acetone used was HPLC grade, and DMF was dried using 4 Å molecular sieves. Other solvents and reagents from stock or purchased were used as received. When dry glassware was necessary it was heated in an oven at a temperature above 125°C for atleast 6 hr, then quickly assembled and cooled under a stream of dry inert gas. Flash column chromatography was performed on silica gel (Merck silica gel 60, 230–400 mesh). TLC plates were viewed under an ultraviolet lamp or treated with one of the reagents listed here: (i) 3 g of $KMnO_4$, 20 g of K_2CO_3, 5 mL of NaOH (5%, aq.) and 300 mL of H_2O; (ii) 10 g of Ce(IV)sulphate, 15 g of $(NH_4)_2MoO_4$ in 1 L 10% H_2SO_4. Ion-exchange chromatography was performed with Amberlite IR-120, H^+ and eluted with 2.5% NH_3 (aq.). Rotary evaporation was performed under reduced pressure (minimum 25 mbar) and maximum temperature 40°C.

(±)-2-[3,4-*trans*-4,5-*trans*)-5-Hydroxymethyl-3,4-dihydroxypiperidine-1-yl]ethylphosphono acid diethylester (3). A mixture of (±)-isofagomine (**2**, 30 mg, 0.2 mmol), diethyl 2-bromoethylphosphonate (0.12 mL, 0.6 mmol), and K_2CO_3 (138 mg, 1.0 mmol) in 3 mL of EtOH was heated reflux for 20 hr. After the reaction, the solid was filtered off and the obtained solution was concentrated. The residue was purified by chromatography ($CHCl_3$/EtOH, 3 : 1) to provide product **3** (43 mg, 68%) as an oil. ^{1}H NMR (200 MHz, CD_3OD): δ 3.97 (q, 4H, J 7.5 Hz, CH_2CH$_3$), 3.70 (dd, 1H, $J_{5'a,5'b}$11.3, $J_{5,5'a}$ 3.80, H-5′a), 3.57–3.26 (m, 3H, H-3, H-4, H-5′b), 3.04–2.80 (m, 2H, H-2eq, H-6eq), 2.64–2.40 (m, 2H, H-2ax, H-6ax), 2.00–1.70 (m, 4H, H-1′a, H-1′b, H-1″a, H-1b″), 1.60 (m, 1H, H-5), 1.20 (t, 6H, CH_3). ^{13}C NMR (50 MHz, CD_3OD): δ 74.1 (C-4), 71.3 (C-3), 61.4 (d, 2C, CH$_2$CH$_3$), 60.8 (C-5′), 57.4 (C-2), 54.0 (C-6), 50.3 (NCH$_2$), 43.3 (C-5), 22.0 (d, PCH$_2$), 14.9 (d, 2C, CH$_3$). HRMS (ES): calcd for $C_{12}H_{26}NO_6P + Na^+$ 334.1395, found 334.1389.

(±)-2-[(3,4-*trans*-4,5-*cis*)-5-Hydroxymethyl-3,4-dihydroxypiperidine-1-yl]-ethylphosphono acid diethylester (6). Compound **5** (25 mg, 0.17 mmol) was processed as described above for **2**. Chromatography ($CHCl_3$/EtOH, 3 : 1) provided **6** (20 mg, 40%) as an oil. ^{1}H NMR (200 MHz, CD_3OD): δ 4.22–3.90 (m, 4H, CH_2CH$_3$), 3.70–3.45 (m, 4H, H-3, H-4, H-5′a, H-5′b), 2.6–2.27 (m, 6H, H-2ax, H-2eq, H-6ax, H-6eq, H-1″a, H-1″b), 2.10–1.85 (m, 3H, H-1′a, H-1′b, H-5), 1,23 (t, 6H, CH$_2$CH_3, J = 7.5 Hz). ^{13}C NMR (50 MHz, CD_3OD): δ 69.6 (C-4), 68.4 (C-3), 61.4 (d, 2C, CH$_2$ of Et), 60.7 (C-5′), 60.0 (C-2), 54.1 (C-6), 50.4 (NCH$_2$CH$_2$), 39.1 (C-5), 22.0 (d, PCH$_2$), 14.8 (d, 2C, CH$_3$). HRMS (ES): calcd for $C_{12}H_{26}NO_6P + H^+$ 312.1576, found 312.1579.

(±)-2-[(3,4-*trans*-4,5-*trans*)-5-Hydroxymethyl-3,4-dihydroxypiperidine-1-yl]-ethylphosphono acid (4). A solution of **3** (36 mg, 0.12 mmol) in 2 mL of 6 N HCl was heated reflux for 8 hr. Concentration of the reaction solution provided **4** (25 mg, 85%) as a colourless oil. ^{1}H NMR (400 MHz, D_2O): δ 3.80–3.50 (m, 5H, H-2eq, H-3, H-5′a, H-5′b, H-6eq), 3.50–3.23 (m, 3H, H-4, H-2ax, H-6ax), 3.04–2.70 (m, 2H, H-1′a, H-1′b), 2.26-1.97 (m, 2H, H-1″a, H-1″b), 1.88 (m, 1H, H-5). ^{13}C NMR(50 MHz, D_2O): δ 70.3 (C-4), 68.1

(C-3), 58.3 (C-5′), 53.9 (C-2), 52.7 (N*C*H$_2$CH$_2$), 51.8 (C-6), 40.5 (C-5), 22.4 (d, PCH$_2$). HRMS (ES): calcd for $C_8H_{18}NO_6P + H^+$ 256.0949, found 256.0946.

(±)-2-[(3,4-*trans*-4,5-*cis*)-5-Hydroxymethyl-3,4-dihydroxypiperidine-1-yl]-ethylphosphono acid diethylester (7). Compound **6** (20 mg, 0.064 mmol) was processed as described above for **3** to provide **7** as a colourless oil (18 mg, 96%). ^{1}H NMR (200 MHz, D_2O): δ 4.08 (bs, 1H, H-3), 3.95 (bs, 1H, H-4), 3.75–3.20 (m, 7H, H-1′a, H-1′b, H-2ax, H-2eq, H-5′a, H-5′b, H-6eq), 2.97 (t, 1H, H-6ax), 2.42 (m, 1H, H-5), 2.28–1.96 (m, 2H, H-1″a, H-1″b). ^{13}C NMR (50 MHz, D_2O): δ 65.7 (C-4), 64.1 (C-3), 59.6 (C-5′), 52.2 (C-2), 51.9 (N*C*H$_2$), 48.6 (C-6), 37.4 (C-5), 22.3 (d, PC*H*$_2$). HRMS (ES): calcd for $C_8H_{18}NO_6P + H^+$ 256.0949, found 256.0948.

***N*-(10-Chloro-9-anthracenemethyl] isofagomine (8).** A mixture of isofagomine (**2**, 22 mg, 0.15 mmol) and 10-chloro-9-anthralaldehyde (36 mg, 0.15 mmol) in methanol (15 mL) and $CHCl_3$ (1 mL) was heated to reflux until a homogeneous solution was formed. Then $NaCNBH_3$ (18 mg, 0.29 mmol) was added. After 10 min HOAc (0.1 mL) was added, and the mixture heated to about 60°C for 4 hr and furthermore stirred at room temperature for 24 hr. The solution was evaporated and the residue chromatographed with $CHCl_3$ until the lipophilic compounds were eluted (7 mg 20% 10-chloro-9-anthralaldehyde and 16 mg 44% 10-chloro-9-anthracenemethanol) and then changed to $CHCl_3$/MeOH (8 : 1) giving 20 mg (31%) of *N*-(10-chloro-9-anthracenemethyl)isofagomine (**8**) as yellow acetic acid salt (R_F 0.35, $CHCl_3$/MeOH 8 : 1). Evaporation of a solution of the acetic acid salt in HCl (1 mL, 1.0 M) provided the corresponding hydrochloride. ^{1}H NMR (200 MHz, D_2O): δ 8.08 (d, 2H, *J* 8.8 Hz), 7.90 (d, 2H, *J* 8.8 Hz), 7.46 (m, 4H), 4.95 (s, 2H, ArC*H*$_2$), 3.33–3.71 (m, 6H, H-2eq, H-6eq, H-3, H-4, H-5′a, H-5′b), 3.07 (m, 2H, H-2ax, H-6ax), 1.74 (m, 1H, H-5). UV (nm): 399 (3.93), 378 (3.96), 360 (3.76), 344* (3.46), 327* (3.14), 251 (>4.32), 220 (4.13). HRMS (ES): calcd for $M + H^+$ 372.1366, found 372.1364.

2-[(*N*-*tert*-Butylcarbonyl)-(*p*-methoxyphenyl)amino]methyl-4-*O*-benzyl-2-deoxy-D-mannitol (11). **9** (0.23 g, 0.81 mmol) and *p*-methoxybenzylamine (0.2 mL, 1.5 mmol) in H_2O (0.3 mL) was heated to 60°C. After the mixture became homogeneous, the heating was continued for 3 hr at the same temperature. Then methanol (1 mL) was added, and the obtained solution cooled to room temperature. Sodium borohydride (0.06 g, 1.6 mmol) was added slowly in portion so that the temperature did not exceed 30°C. After stirring for 18 hr at room temperature, the reaction solution was concentrated in vacuo, redissolved in methanol (2 mL) and acidified to pH 2 with 3 M HCl followed by concentration twice with methanol (2 × 2 mL). The residue was taken up to 2 mL of methanol, and the obtained mixture was filtered. Concentration of the filtrate provided a crude product of **10**. (^{1}H NMR (200 MHz, CD_3OD): δ 7.33–7.25 (m, 5H, Ph), 7.16 (d, 2H, Ar, *J* 8.5 Hz), 6.82 (d, 2H, Ar), 4.67 (d, 1H, PhC*H*$_2$, *J* 11.0 Hz), 4.46 (d, 1H, PhC*H*$_2$), 3.93–3.53 (m, 9H, H-2′a, H-2′b, H-3, H-4, H-5, H-6a, H-6b, NCH$_2$Ar), 3.65 (s, 3H, OCH_3), 3.16 (dd, 1H, $J_{1a,2}$ 7.0 Hz, $J_{1a,1b}$ 12.5 Hz, H-1a), 3.01 (dd, 1H, $J_{1b,2}$ 7.0 Hz, H-1b), 2.28 (m, 1H, H-2). ^{13}C NMR (50 MHz, CD_3OD): δ 161.5, 138.7, 131.4, 132.6, 131.6, 129.5, 129.0 (Ph, Ar), 126.1, 124.0, 115.3 (Ar), 80.2 (C-4), 73.7, 73.2, 71.5 (C-3, C-5, PhCH$_2$), 64.0, 62.0 (C-6, C-2′), 55.8 (OCH_3), 51.7 (PhCH$_2$N), 43.8 (C-1), 42.3 (C-2). MS (ES): *m*/*z* 406.1 (M + H)). To the crude **10** (0.45 g, 0.81 mmol) in a solution of 1,4-dioxane/H_2O (1 : 1, 30 mL) was added Na_2CO_3 (0.26 g, 2.5 mmol). Subsequently a solution of $(Boc)_2O$ (0.47 g, 2.2 mmol) was added dropwise to the mixture during 2 min at room temperature. After stirring for 18 hr at the same temperature,

the reaction mixture was concentrated in vacuo. The residue was taken up into ethyl acetate and washed twice with H_2O (30 mL). The organic phase was dried over $MgSO_4$, concentrated in vacuo and purified by chromatography firstly with ethyl acetate, and then with ethyl acetate/MeOH 99 : 1 to give **11** (0.18 g, 44% over two steps). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.34–7.25 (m, 5H, Ph), 7.00 (d, 2H, *J* 7.5 Hz, Ar), 6.74 (d, 2H, Ar), 4.64 (d, 1H, *J* 11.5 Hz, $PhCH_2$), 4.51 (d, 1H, $PhCH_2$), 4.36 (d, 1H, *J* 15.5 Hz, $ArCH_2$), 4.07 (d, 1H, $ArCH_2$), 3.86–3.75 (m, 3H, H-3, H-6a, H-6b), 3.71 (s, 3H, CH_3O), 3.65–3.41 (m, 5H, H-1a, H-2′a, H-2′b, H-4, H-5), 3.01 (dd, 1H, $J_{1b,2}$ 5.0 Hz, $J_{1a,1b}$ 14.5 Hz, H-1b), 1.80 (m, 1H, H-2), 1.40 (s, 9H, $(CH_3)_3C$). ^{13}C NMR (50 MHz, $CDCl_3$): δ 158.9 (Ar), 157.2 (CO), 138.0, 129.8, 128.4–127.8 (Ph, Ar), 114.0 (Ar), 80.9 ($(CH_3)_3CO$), 79.1 (C-4), 73.2 ($PhCH_2$), 71.7, 70.4 (C-3, C-5), 63.6, 59.5 (C-2′, C-6), 55.3 (OCH_3), 50.5 ($PhCH_2N$), 44.7 (C-5), 41.2 (C-2), 28.4 ($(CH_3)_3C$). HRMS (ES): calcd for $C_{27}H_{39}NO_8 + Na$ 528.2573, found 528.2587.

3-*O*-Benzyl-4-[*N*-(*tert*-butyloxycarbonyl)-*N*-(*p*-methoxybenzyl)amino]methyl-4-deoxy-α/β-D-arabino-pyranose (12). To a solution of **11** (101 mg, 0.20 mmol) in H_2O (0.6 mL) was added dropwise a solution of $NaIO_4$ (214 mg, 1.0 mmol) in H_2O (2 mL) over 10 min and subsequently MeOH (1 mL) was added. The obtained mixture was stirred at 45°C for 3 hr and then filtered. The solution was concentrated in vacuo. The residue was taken up into ethyl acetate/EtOH (1 : 1, 3 mL), and undissolved solid filtered off. The obtained solution was concentrated in vacuo and purified by chromatography (ethyl acetate/$CHCl_3$, 1 : 5) to give **12** as a yellow oil (54 mg, 58%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.42–7.16 (m, 5H, Ph), 7.08 (d, 2H, *J* 8.5 Hz, Ar), 6.80 (d, 2H, Ar), 5.37 (bs, 1H, H-1), 5.12–4.79 (m, 1H, OH), 4.70 (m, 4H, CH_2Ph, CH_2Ar), 4.00–3.20 (m, 6H, H-2, H-3, H-5a, H-5b, H-4′a, H-4′b), 3.70 (s, 3H, CH_3), 2.53 (m, 1H, H-4), 1.41 (s, 9H, $(CH_3)_3C$). HRMS (ES): calcd for $C_{26}H_{35}NO_7 + Na^+$ 496.2311, found 496.2307.

4-[*N*-(*tert*-Butyloxycarbonyl)-*N*-(*p*-methoxybenzyl)amino]methyl-4-deoxy-α/β-D-arabino-pyranose (13). The mixture of **12** (50 mg, 0.11 mmol) and Pd/C (10%, 20 mg) in CH_3COOH (2 mL) was hydrogenolysed for 18 hr at room temperature. After the reaction, the mixture was filtered and the solution was concentrated in vacuo. The residue was purified by chromatography ($CHCl_3/CH_3OH$, 20 : 1) to give **13** (21 mg, 52%) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.08 (d, 2H, *J* 8.5 Hz, Ar), 6.80 (d, 2H, Ar), 5.48 (d, 0.5 H, $J_{1,2}$ 8.0 Hz, H-1α), 5.42 (d, 0.5H, $J_{1,2}$ 1.5 Hz, H-1β), 4.93 (s, 0.5H, OH), 4.85 (d, 0.5H, OH), 4.70 (s, 0.5H, OH), 4.56 (d, 1H, *J* 15.0 Hz, CH_2Ar), 3.98 (d, 1H, *J* 15.0 Hz, CH_2Ar), 3.80–3.64 (m, 2H, H-3, H-4), 3.71 (s, 3H, OCH_3), 3.60–3.40 (m, 4.5H, H-3, H-5a, H-5b, H-4′a, H-4′bα), 3.22 (dd, 0.5H, $J_{4'a,4'b}$12.5, $J_{4,4'b}$ 3.5 Hz, H-4′bβ), 2.54 (m, 1H, H-4), 1.41 (s, 9H, $(CH_3)_3C$). ^{13}C NMR (50 MHz, $CDCl_3$): δ 128.9–114.3 (Ar), 95.2 (C-1α), 92.7 (C-1β), 82.0, 81.5 ($(CH_3)_3C$), 70.0, 67.5, 67.0 (C-3, C-4), 63.4 (C-5α), 56.8 (C-5β), 55.5 (OCH_3), 50.8 (CH_2Ar), 43.5, 42.7 (C-4′), 34.6, 33.7 (C-4), 28.5 ($(CH_3)_3C$). HRMS (ES): calcd for $C_{19}H_{29}NO_7 + Na^+$ 406.1842, found 406.1845.

(2*R*/*S*,3*S*,4*R*,5*R*)-1-(*p*-Methoxybenzyl)-5-(hydroxymethyl)-2,3,4-trihydroxy-piperidine tri-fluoroacetate (14). Compound **13** (10 mg, 0.026 mmol) was dissolved in CF_3COOH (0.5 mL). The obtained solution was stirred for 5 min and concentrated in vacuo to furnish **14** (8.8 mg, 85%) as a yellow oil. ^{1}H NMR (200 MHz, D_2O): δ 7.40–7.04 (d, 4H, *J* 8.6 Hz, Ar), 5.08 (d, 0.33H, $J_{2,3}$ 2.9 Hz, H-2β), 4.88 (d, 0.67H, $J_{2,3}$ 7.7 Hz, H-2α), 4.20 (bs, 2H, CH_2Ar), 4.10–3.48 (m, 3H, H-3, H-4, H-6a), 3.83 (s, 3H, CH_3O), 3.40–3.00 (m, 3H, H-5′a, H-5′b, H-6b), 2.37 (m, 1H, H-5). HRMS (ES): calcd for $(C_{14}H_{21}NO_5 - H_2O) + H^+$ 266.1392, found 266.1395.

Benzyl 4-(*N*-*tert*-butyloxycarbonyl-*N*-nonyl)methyl-4-deoxy-β-D-arabino-pyranoside (16). A mixture of **15** (110 mg, 0.39 mmol), Et_3N and Pd/C (10%, 20 mg) in EtOH (10 mL) was hydrogenated under 1 atm at room temperature for 2 hr. The catalyst was filtered off and the solution was concentrated in vacuo. The residue was dissolved in CH_3OH (2.5 mL). The pH value of the solution was adjusted to about 6 by using CH_3CO_2H. Then, to the solution were added pelargonaldehyde (78 mg, 0.55 mmol) in CH_3OH (1.5 mL) and $NaCNBH_3$ (27 mg, 0.43 mmol) at room temperature. The obtained mixture was stirred at room temperature for 18 hr and then concentrated in vacuo. The residue was taken up to acetone/H_2O (1 : 1, 5 mL). To this mixture was added $NaHCO_3$ (0.2 g, 2.38 mmol) and $(Boc)_2O$ (174 mg, 0.80 mmol). The reaction mixture was stirred at room temperature for 2hr, and the acetone was removed in vacuo. To this aqueous residue was added H_2O (5 mL). The aqueous mixture was extracted three times with ethyl acetate. The combined organic phases were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by chromatography (pentane/ethyl acetate 3 : 1) to furnish **16** (82 mg, 44%) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.40–7.13 (m, 5H, Ph), 4.80 (d, 1H, *J* 11.6HZ, CH_2Ph), 4.79 (bs, 1H, H-1), 4.55 (d, 1H, *J* 11.6, CH_2Ph), 4.55 (m, 1H, H-3), 3.73 (bs, 1H, H-2), 3.60–3.40 (m, 3H, H-4′a, H-5a, H-5b), 3.23–2.86 (m, 2H, CH_2CH_2N), 2.64 (dd, 1H, H-4′b), 2.35 (bs, 1H, OH), 2.20 (m, 1H, H-4), 1.60–1.10 (m, 14H, $7CH_2$), 1.40 (s, 9H, $(CH_3)_3C$), 0.90 (t, 3H, CH_2CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 137.5, 128.6, 128.2, 121.1 (Ph), 97.8 (C-1), 80.6 ($(CH_3)_3C$), 70.6 (CH_2Ph), 69.8, 67.4 (C-2, C-3), 62.9 (C-5), 47.9 (C-4′), 43.5 (CH_2CH_2N), 36.0 (C-4), 32.1, 29.7, 29.4, 27.0, 22.9 ($7CH_2$), 28.6 ($(CH_3)_3C$), 14.3 (CH_2CH_3).

(2*R*/*S*,3*S*,4*R*,5*R*)-5-(Hydroxymethyl)-1-nonyl-2,3,4-trihydroxy-piperidine hydrochloride (17). The mixture of **16** (57 mg, 0.12 mmol) and Pd/C (10%, 20 mg) in EtOH (22 mL) was hydrogenolysed under 1 atm of H_2 pressure at room temperature for 20 hr. After reaction, the catalyst was filtered off, and the solution was concentrated in vacuo. The residue was purified by chromatography ($CHCl_3$/MeOH 10 : 1) to give an oil. The oil was treated with aqueous HCl (2 M, 1.5 mL) at room temperature for 5 min and concentrated in vacuo to furnish **17** (30 mg, 67%) as a brownish oil. ^{1}H NMR (200 MHz, D_2O): δ 5.10 (s, 0.33 H, H-2β), 4.50 (d, 0.67H, $J_{2,3}$ 8.5 Hz, H-2α), 4.1–2.9 (m, 8H, H-3, H-4, H-6a, H-6b, H-5′a, H-5′b, CH_2CH_2N), 2.43–2.26 (m, 1H, H-5), 1.90–1.00 (m, 14H, $7CH_2$), 0.9 (bs, 3H, CH_3). ^{13}C NMR (50 MHz, D_2O): δ 96.5 (C-2β), 91.5 (C-2α), 71.6, 71.1, 68.5, 68.0 (C-3α, C-3β, C-4α, C-4β), 62.4 (C-5′β), 60.0 (C-5′α), 47.9 (C-6), 45.1 (CH_2CH_2N), 37.2 (C-5), 30.7, 28.0, 27.8, 25.3, 24.9, 21.7 ($7CH_2$), 13.0 (CH_3). HRMS (ES): calcd for $(C_{15}H_{31}NO_4 - H_2O) + H^+$: 272.2226, found 272.2220.

Hydrolysis of 1. A 10 mg of **1** was dissolved in pD 6.8 phosphate buffer (0.2 M, ~0.3 mL). After standing for 7 hr at room temperature, **1** had been completely converted to the rearrangement product **18** according to ^{1}H- and ^{13}C NMR. The stereochemistry at C-2 was not determined. ^{1}H NMR (200 MHz, D_2O): δ 3.83–3.66 (m, 2H, H-5′a, H-5′b), 3.62 (d, 1H, $J_{4,5}$ 10.5 Hz, H-4), 3.44 (dd, 1H, $J_{5,6eq}$ 4.1, $J_{6,6}$ 12.5 Hz, H-6eq), 3.34 (s, 1H, H-2), 2.96 (t, 1H, $J_{5,6ax}$ 12.5 Hz, H-6ax), 2.10 (m, 1H, H-5).^{13}C NMR (50 MHz, D_2O): δ 90.6 (C-3), 70.7 (C-4), 58.8 (C-5′), 49.9 (t, C-3), 44.4 (C-6), 39.3 (C-5).

Hydrolysis of 19. A 10 mg of **19** was dissolved in pD 6.8 phosphate buffer (0.2 M, ~0.3 mL). After standing for 40 min at room temperature, **19** had been completely converted to the rearranged product **20** according to ^{1}H- and ^{13}C NMR spectra. The stereochemistry of C-2 was not determined. ^{1}H NMR (200 MHz, D_2O): δ 3.52 (d, 1H, $J_{4,5}$

2.0 Hz, H-4), 3.00 (s, 1H, H-2), 2.98 (dd, 1H, $J_{5,6eq}$ 4.2, $J_{6,6}$ 12.1 Hz, H-6eq), 2.70 (t, 1H, $J_{5,6ax}$ 12.1 Hz, H-6ax), 2.20 (m, 1H, H-5), 0.89 (d, 3H, $J_{5,5'}$ 6.0 Hz, CH_3).[13]C NMR (50 MHz, D_2O): δ 91.6 (C-3), 71.8 (C-4), 45.8 (t, C-3), 43.3 (C-6), 30.1 (C-5), 13.3 (CH_3). These NMR data are identical to those given in Ref.[10].

Benzyl 2-*C*-methyl-β-D-arabinopyranoside (24). To a solution of CF_3CO_2H/H_2O (2/1, 3 mL) was added benzyl 3,4-*O*-isopropylidene-2-*C*-methyl-β-D-arabinopyranoside (1.0 g, 3.54 mmol, **23**) at room temperature. The obtained solution was stirred at the same temperature for 10 min and concentrated in vacuo to provide **24** (0.88 g, 98%) as a yellowish oil. ^{1}H NMR (200 MHz, $CDCl_3/D_2O$): δ 7.40–7.20 (bs, 5H, Ph), 4.73 (d, 1H, *J* 11.4 Hz, CH_2Ph), 4.46 (d, 1H, CH_2Ph), 4.60 (s, 1H, H-1), 3.98 (ddd, 1H, $J_{3,4}$ 3.6, $J_{4,5eq}$ 2.8, $J_{4,5ax}$ 4.2 Hz, H-4), 3.80 (dd, 1H, $J_{5ax,5eq}$ 12.4 Hz, H-5eq), 3.78 (d, 1H, H-3), 3.63 (dd, 1H, H-5ax), 1.29 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 136.9, 128.8, 128.3 (Ph), 101.7 (C-1), 73.1 (CH_2Ph), 72.6 (C-2), 70.4, 67.9 (C-3, C-4), 63.0 (C-5), 20.3 (CH_3). HRMS (ES): calcd for $C_{13}H_{18}O_5 + Na^+$ 277.1052, found 277.1053.

Benzyl 4-*C*-nitromethyl-2-*C*-methylpentopyranoside (26 and 27, and benzyl 3-*C*-nitromethyl-2-*C*-methylpentopyranoside (28). To a mixture of **24** (2.0 g, 7.86 mmol) and molecular sieves (3 Å, 3.5 g) in CH_2Cl_2 (40 mL) was added $(Bu_3Sn)_2O$ (9.4 g, 15.7 mmol) at room temperature. The mixture was refluxed for 3.5 hr and then cooled to 0°C. To this mixture was added bromine (0.9 mL, 17.9 mmol) dropwise until the solution was faintly coloured. The mixture was poured on a column with silica and washed thoroughly with $CHCl_3$ in order to remove tin compounds. The product **25** was washed down with ethyl acetate and was obtained after concentration as a mixture (1.5 g, 79%) of several different forms that could not be separated. These were not characterised but used directly in the next step.

To a suspension of **25** (1.0 g, 4.3 mmol) in dry CH_3NO_2 (30 mL) was added Et_3N (0.45 g, 4.45 mmol). The solution was stirred at room temperature for 18 hr and then concentrated in vacuo. The residue was chromatographed on silica gel by using pentane/ethylacetate 3 : 2 to furnish **27** (220 mg, 18%), **28** (120 mg, 10%), **26** (140 mg, 11%), and a mixture of **27** and **28** (200 mg). **26**: ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.35–7.12 (m, 5H, Ph), 4.70–4.39 (m, 5H, H-1, H-4′a, H-4′b, CH_2Ph), 3.90 (d, 1H, $J_{5ax,5eq}$ 12.3 Hz, H-5eq), 3.60 (d, 1H, H-5ax), 3.48 (s, 1H, H-3), 1.20 (s, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 136.9, 127.6, 127.4, 127.0 (Ph), 101.5 (C-1), 79.5 (C-4′), 71.7, 71.6, 71.2 (C-2, C-3, C-4), 69.1 (CH_2Ph), 63.5 (C-5), 19.1 (CH_3). HRMS (ES): calcd for $C_{14}H_{19}NO_7 + Na^+$ 336.1059, found 336.1057. **27**: ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.40–7.24 (m, 5H, Ph), 4.84 (dd, 1H, $J_{4'a,5eq}$ 1.7, $J_{4'a,4'b}$ 10.9 Hz, H-4′a), 4.77 (d, 1H, *J* 11.3 Hz, CH_2Ph), 4.71 (d, 1H, H-4′b), 4.68 (s, 1H, H-1), 4.52 (d, 1H, CH_2Ph), 4.17 (s, 1H, H-3), 4.14 (dd, 1H, $J_{5ax,5eq}$ 13.5 Hz, H-5eq), 4.0 (bs, 1H, OH), 3.13 (bs, 1H, OH), 2.80 (bs, 1H, OH), 3.72 (d, 1H, H-5ax), 1.32 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 135.9, 129.0, 128.8, 128.4 (Ph), 103.6 (C-1), 76.1 (C-4′), 75.6 (C-4), 71.1 (C-3), 70.1 (C-2), 68.9 (CH_2Ph), 60.7 (C-5), 21.3 (CH_3). HRMS (ES): calcd for $C_{14}H_{19}NO_7 + Na^+$ 336.1059, found 336.1057. **28**: ^{1}H NMR (200 MHz, $CDCl_3/D_2O$): δ 7.40–7.20 (m, 5H, Ph), 4.86 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.76 (s, 2H, H-3′a, H-3′b), 4.60 (s, 1H, H-1), 4.57 (d, 1H, CH_2Ph), 4.20 (dd, 1H, $J_{4,5aq}$ 10.1, $J_{4,5eq}$ 5.6 Hz, H-4), 3.88 (dd, 1H, $J_{5ax,5eq}$ 11.4 Hz, H-5eq), 3.52 (dd, 1H, H-5ax), 1.24 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 137.0, 128.7, 128.3 (Ph), 99.5 (C-1), 75.4 (C-3′), 74.5 (C-2), 71.0 (C-4), 66.3 (CH_2Ph), 63.5 (C-5), 18.3 (CH_3).

Benzyl 4-nitromethyl-4-deoxy-2-methyl-β-D-arabinopyranoside (29). To a solution of **26** (110 mg, 0.35 mmol) and Ac_2O (0.16 mL) in CH_3CN (4 mL) was added $Sc(OTf)_3$ (8 mg, 0.016 mmol) at room temperature. The obtained mixture was stirred at the same temperature for 2 hr and then quenched with saturated aqueous solution of $NaHCO_3$. The mixture was extracted three times with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and concentrated in vacuo to afford a crude triacetate (150 mg). This product was dissolved in EtOH (6 mL), and to this solution was added $NaBH_4$ (20 mg, 0.53 mmol) at room temperature. The reaction mixture was stirred at the same temperature for 1 hr and then concentrated in vacuo. The residue was dissolved in MeOH (6 mL) and NaOMe (20 mL) was added. The mixture was stirred at room temperature for 0.5 hr, and Amberlite IR 120 H^+ was added until pH reached 6. The resin was removed by filtration. The filtrate was concentrated in vacuo. The residue was purified by chromatography (pentane/ethyl acetate 2 : 1) to furnish **29** (39 mg, 37%) as a colourless solid. ^{1}H NMR (200 MHz, $CDCl_3/D_2O$): δ 7.35–7.17 (m, 5H, Ph), 4.78–4.50 (d, 2H, J 12.5 Hz, CH_2Ph), 4.56 (bs, 1H, H-1), 4.47 (dd, 1H, $J_{4,4'a}$ 6.5, $J_{4'a,4'b}$ 12.5 Hz, H-4′a), 4.28 (dd, 1H, $J_{4,4'b}$ 8.7 Hz, H-4′b), 3.80 (dd, 1H, $J_{4,5eq}$ 4.2, $J_{5ax,5eq}$ 12.9 Hz, H-5eq), 3.76 (d, 1H, $J_{3,4}$ 4.0 Hz, H-3), 3.60 (dd, 1H, $J_{4,5ax}$ 8.4 Hz, H-5ax), 2.88 (m, 1H, H-4), 1.15 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 137.0, 128.7, 128.3 (Ph), 100.4 (C-1), 73.7 (C-4′), 72.0 (C-3), 71.6 (C-2), 70.7 (CH_2Ph), 61.4 (C-5), 36.8 (C-4), 20.8 (CH_3). HRMS (ES): calcd for $C_{14}H_{19}NO_6 + Na^+$ 320.1110, found 320.1110.

Benzyl 4-(*N-tert*-butyloxycarbonyl)methyl-4-deoxy-2-methyl-β-D-arabinopyranoside (30). A mixture of **29** (20 mg, 0.07 mmol), Pd/C (10%, 15 mg) and Et_3N (0.03 mL) in MeOH (1.5 mL) was hydrogenated under 1 atm at room temperature for 2 hr. The catalyst was filtered off and the solution was concentrated in vacuo. The residue was dissolved in CH_2Cl_2. To this solution was added Et_3N (0.10 mL) and $(Boc)_2O$ (30 mg, 0.14 mmol). The obtained solution was stirred at room temperature for 2 hr. Afterwards, the reaction solution was concentrated in vacuo. The residue was purified by chromatography (pentane/ethyl acetate 2 : 1) giving **30** (20 mg, 81%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36–7.20 (m, 5H, Ph), 4.82–4.52 (d, 2H, J 11.6 Hz, CH_2Ph), 4.76 (dd, 1H, $J_{NH,4'a}$ 8.8, $J_{NH,4'b}$ 5.2 Hz, NH), 4.52 (bs, 1H, H-1), 3.60–3.40 (m, 3H, H-3, H-5a, H-5b), 3.25 (ddd, 1H, $J_{4,4'a}$ 10.8 Hz, $J_{4'a,4'b}$ 14.8 Hz, H-4′a), 2.69 (ddd, 1H, $J_{4,4'b}$ 5.2 Hz, H-4′b), 2.18 (m, 1H, H-4), 1.38 (s, 9H, $C(CH_3)_3$), 1.25 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 137.9, 128.6, 128.2, 128.0 (Ph), 100.4 (C-1), 80.6 ($C(CH_3)_3$), 71.5 (C-2), 71.1 (C-3), 70.9 (CH_2Ph), 63.0 (C-5), 38.5 (C-4′), 37.6 (C-4), 28.5 ($C(CH_3)_3$), 22.3 (CH_3). HRMS (ES): calcd for $C_{19}H_{29}NO_6 + Na^+$ 390.1893, found 390.1892.

(2*R*/*S*,3*S*,4*R*,5*R*)-5-(Hydroxymethyl)-3-methyl-2,3,4-trihydroxy-piperidine trifluoroacetate (31). A mixture of **30** (20 mg, 0.054 mmol), Pd/C (10%, 10 mg) in ethanol (10 mL) was hydrogenated under 1 atm at room temperature for 18 hr. The catalyst was filtered off, and the solution was concentrated in vacuo. The residue was treated with aqueous HCl (0.2 M, 1 mL) for 5 min, and the reaction solution was concentrated in vacuo to give **31** (7 mg, 61%) as a brownish oil. ^{1}H NMR (200 MHz, D_2O): δ 4.90 (s, 0.5H, H-2β), 4.30 (s, 0.5H, H-2α), 3.8–3.2 (m, 4H, H-4, H-5′a, H-5′b, H-6eq), 2.95 (t, 1H, $J_{5,6ax} = J_{6ax,6eq}$ 12.2 Hz, H-6ax), 1.95 (m, 1H, H-5), 1.20 (s, 3H, CH_3). ^{13}C NMR (50 MHz, D_2O): δ 82.5 (C-2β), 81.4 (C-2α), 74.3 (C-4β), 71.9 (C-3α), 71.8 (C-4α), 69.3 (C-3β), 59.3 (C-5′β), 58.9 (C-5′α), 40.9 (C-6β), 39.5 (C-6α), 38.7 (C-5), 17.1 (CH_3).

K_i Determination

Each glycosidase assay was performed by preparing fourteen 2 mL samples in cuvettes, containing 1 mL sodium phosphate buffer (0.1 M) of pH 6.8 or acetate buffer of pH 4.0, along with 0.05–0.70 mL of different substrate. The concentration of the substrate was in the range of $0.25K_m$–$5K_m$. The substrates used were 4-nitrophenyl β-D-glucopyranoside or 4-nitrophenyl α-D-glucopyranoside. Also added was 0.025–0.1 mL of a solution of either the inhibitor or water, and finally each cuvette was filled up to a total volume of 1.9 mL with distilled water. Seven of the samples contained the inhibitor at a fixed concentration but with varying concentrations of nitrophenyl glycoside. The other seven samples contained no inhibitor, but also varying concentrations of nitrophenyl glycoside. Finally the reaction was started by adding 0.1 mL of a diluted solution of enzyme solution. The formation of 4- or 2-nitrophenol was monitored for 2 min at 25°C by measurement of the absorbance at 400 nm. Initial velocities were calculated from the slopes from each reaction and used to construct two Hanes plots ([S]/v vs. [S]), one with and one without inhibitor. From the two Michaelis–Menten constants, K_m and K_m', thus obtained, the inhibition constant, K_i, was calculated.

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