Conference paper

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2-Deoxyglycosylation towards more effective and bioavailable neuroprotective molecules inspired by nature

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Abstract: The neuroprotective role of natural polyphenols is well established but phenolics poor water solubility affects their bioavailability and bioactivity. Aiming to overcome this issue, we were encouraged to investigate the 2-deoxyglycosylation of natural or nature inspired neuroprotective molecules, using glycals as easily accessed glycosyl donors. This robust methodology allowed the generation of a set of new resveratrol and caffeic acid ester glycosides, envisioning more effective and bioavailable compounds. Resveratrol 2-deoxyglycosides were more effective at protecting the neuronal cells from peroxide-induced cytotoxicity than resveratrol itself, while the caffeic acid ester glycoside also showed extraordinary neuroprotection activity. Coefficient partition measurements demonstrated the moderate lipophilicity of resveratrol glycosides, which Log D values are typical of a central nervous system (CNS) drug and ideal for blood-brain barrier (BBB) penetration. Passive permeation assessed by the parallel artificial membrane permeability assay (PAMPA) revealed that 2,6-dideoxy-L-*arabino*-hexopyranosides were more effective than 2-deoxy-D-*arabino*-hexopyranosides. The lack of toxicity of the neuroprotective glycosides and their promising physicochemical properties revealed the usefulness of sugar coupling towards the modulation of natural product properties and bioactivity.

Keywords: bioactive molecules; biomolecular chemistry; caffeic acid ester; cytotoxicity; deoxyglycoside; ICS-29; lipophilicity; medicinal chemistry; neuroprotection; organic synthesis; resveratrol.

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Introduction

Naturally occurring phenolic compounds are known for their beneficial health-promoting effects in chronic and degenerative diseases. Stilbenes, particularly resveratrol, are amongst the best documented and emblematic neuroprotective natural products. In addition to resveratrol antioxidant and anti-inflammatory activities, typical of polyphenols, this compound is able to inhibit Aβ oligomeric cytotoxicity and to reduce neuronal cell death [1, 2]. Despite the promising activities of resveratrol, its bioavailability in humans is less than 1 %, as a consequence of a quick and extensive metabolism, mainly through glucuronidation and sulfation. In addition, the water-insolubility of stilbenes limits their further pharmacological exploitation [3, 4].

Recently, concerns have been raised on the promiscuity and on the lack of therapeutic selectivity of some natural compounds, the so-called pan-assay interference compounds (PAINs) [5]. In the case of resveratrol, its planarity is allegedly guilty to induce membrane intercalation effects and interfere with bioassays.

Coupling sugars to bioactive polyphenols can be a resourceful way to improve the bioavailability and pharmacological activity of such molecular entities [6] while minimizing concerns associated with PAINS, by compromising their planarity. In addition, carbohydrates can prevent oxidation by masking phenolic groups [7], favor drugs' access to the brain via hexose transporters (GLUTs) at the blood–brain barrier [8] and retard amyloid fibril formation by stabilizing the native state through preferential hydration [9].

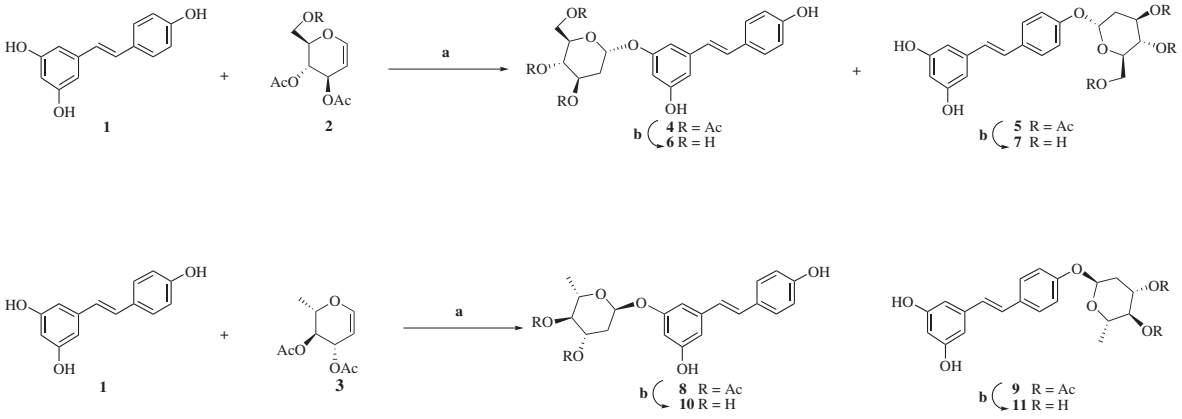
Literature shows a number of efforts to improve resveratrol water solubility and bioavailability via enzymatic glucosylation. Indeed, water solubility of the α-glucosylated derivatives (at 3 or 4-position) was at least 65- and five-fold higher than that of resveratrol and the natural β-glucosylated derivative (piceid), respectively [7]. On the other hand, rosmarinic acid, previously identified by our research group as the active principle of the neuroprotective plant *Salvia sclareoides*, prevents amyloid aggregation, and reduces a number of other events underlying AD pathology [10–12]. Interestingly, methyl caffeate itself, a sub structural unit of rosmarinic acid, reduces significantly Aβ oligomeric cytotoxicity and promotes disaggregation of Aβ oligomers, while having low bioavailability [12], making it also an interesting candidate for glycosylation. These findings encouraged us to investigate glycosylation of natural or nature inspired neuroprotective molecules, using glycals as easily accessed glycosyl donors to afford a set of new resveratrol and caffeic acid ester 2-deoxyglycosides, envisioning more effective and bioavailable compounds. Two glycosyl moieties were selected, namely 2,6-dideoxy-L-*arabino*-hexopyranosyl and 2-deoxy-D-*arabino*-hexopyranosyl, and corresponding glycoside synthesis is here fully disclosed. Glycosides' ability to protect neuronal cells from oxidative stress was assessed as well as their permeability properties, which will also be discussed.

Results and discussion

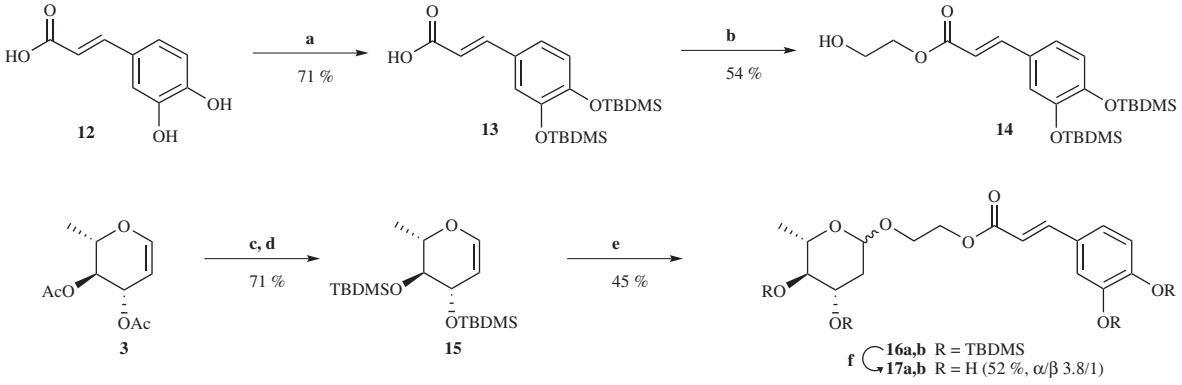
Chemistry

Resveratrol 3-*O*- and 4′-*O*-glycosides **4**, **5**, **8** and **9** were prepared by reaction of resveratrol with the appropriate acetyl protected glycal (**2** and **3**, respectively, Scheme 1), in the presence of triphenylphosphane hydrobromide (TPHB), a well-known catalyst for 2-deoxygenation [13–16], while catalysis by other Brønsted and Lewis acids gives the 2,3-unsaturated glycosides resulting from the Ferrier rearrangement. The respective β-anomers were detected only as traces at the TLC plate and were not isolated. Separation of 3-*O*- and 4′-*O*-glycosides was only possible after consecutive column chromatography, resulting in isolated yields of 21 % and 6 % for **4** and **5**, respectively, and 22 % and 8 % for **8** and **9**. After purification, Zemplén deprotection afforded glycosides **6**, **7**, **10** and **11** in quantitative yield.

For the synthesis of the caffeic acid ester, aromatic hydroxy groups of caffeic acid were protected with a *tert*-butyldimethylsilyl (TBDMS) group, and the aglycone was prepared by Steglich esterification, prior to sugar coupling (Scheme 2). Compound **13** was converted in 2-hydroxyethyl ester by DCC, in an overall yield of 54 %. Ester **14** was transformed into its 2,6-dideoxy-*arabino*-hexopyranoside, following the same procedure



Scheme 1: Synthesis of resveratrol 2-deoxyglycosides. (a) TPHB, THF, 40 °C, 48 h; (b) NaOMe, MeOH.



Scheme 2: Synthesis of the caffeic acid esters 17a,b. (a) TBDMSCl, DCM; (b) ethyleneglycol, DCC, DMAP, DCM; (c) NaOMe, MeOH, (d) TBDMSCl, imidazole, DMF; (e) TPHB, THF; (f) TBAF, THF.

described above, to give an anomeric mixture α/β in ratio 3:1, used for testing due to the difficulty of separating both anomers. The acetyl protecting groups were changed to TBDMS in the glycosyl donor **15**, for a mild final deprotection with TBAF. Reaction of the alcohol **14** with **15** in the presence of TPHB afforded glycosides **16a,b**, which were then deprotected to give the target compounds **17a,b** isolated in a 3.8:1 α/β ratio.

Neuroprotective effects

The link between oxidative stress and the pathogenesis of acute or chronic neurodegenerative processes has been extensively studied. The excessive production of free radicals damages cellular essentials such as lipids, proteins, and DNA [17–19], leading ultimately to the induction of apoptosis in neuronal cells [20]. In fact, hydrogen peroxide overproduction has been associated with events such as amyloid aggregation [21, 22], dopamine oxidation [23], and brain ischemia/reperfusion [24].

In this study, hydrogen peroxide was used to cause oxidative stress in neuroblastoma cells (SH-SY5Y) to assess the neuroprotective effects of the synthesized glycosides.

Incubating cells with 100 μM of H_2O_2 led to a decrease in cell viability of ca. 60 %, as expected. Incubating the cells with both hydrogen peroxide (100 μM) and resveratrol glycosides **6** and **11** resulted in a statistically significant increase in the percentage of cells remaining viable (Fig. 1). Surprisingly, resveratrol glycosides **7** and **10** had no statistically significant effect on cell viability.

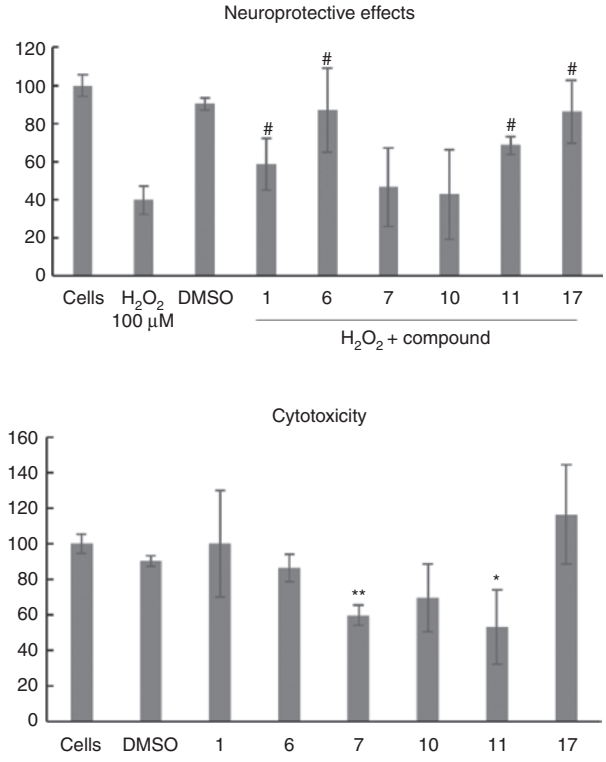


Fig. 1: Neuroprotective and cytotoxic effects of synthesized compounds in neuroblastoma (SH-SY5Y) cells. *, Significantly different when compared to cells control (p-value < 0.05); #, significantly different when compared to hydrogen peroxide controls (p-value < 0.05). Results are expressed as the mean ± SEM of at least three independent experiments.

Our results show that resveratrol glycosides **6** and **11** were more effective at protecting neuronal cells from peroxide-induced cytotoxicity than resveratrol itself, although it does not seem to exist any relationship between sugar moiety/glycosylation position and the neuroprotective activity. Caffeic acid ester **17** showed the best neuroprotection activity, being able to maintain cellular viability similar to that of the control. Although the mechanism of neuroprotection of compounds **6** and **11** remains unknown, resveratrol had been previously shown to protect embryonic neural stem cells, since it decreases oxidative stress by inducing higher activity of antioxidant enzymes, decreasing high nitric oxide production and nitric oxide synthase activity, and alleviating both nuclear and mitochondrial DNA damage in embryonic neural stem cells [25]. Resveratrol glycosides may also act through this mechanism, further enhanced by the sugar moiety.

The cytotoxic effects of compounds **1**, **6**, **7**, **11** and **17** were first assessed in SH-SY5Y cells (Fig. 1). Only compounds **7** and **11**, led to a statistically different loss of viability at 50 µM, although it remained above 50 % in all cases. The toxicity of the two most promising compounds was also assessed in Caco-2 and in HepG2 cell lines, used as models of intestinal and liver toxicity, respectively (Table 1). There was no decrease in the viability of either HepG2 or Caco-2 cells following 24 h and 48 h incubation with varying concentrations of compounds **6** and **17**, with concentrations ranging from 0.1 to 100 µM.

Physicochemical properties and intestinal permeability

To evaluate the drugability of the newly synthesized compounds, lipophilicity and permeability parameters were evaluated, by determining the partition coefficient at physiological pH (Log $D_{7.4}$) and the parallel artificial membrane permeability assay (PAMPA) permeability values, respectively (Table 2).

Table 1: Caco-2 and HepG2 cell viability (%) of compounds **6** and **17**.

Compound Nr.	Cytotoxicity IC_{50} (μM)	
	Caco-2	HepG2
6	>100	>100
17	>100	>100

Table 2: Physicochemical properties and intestinal wall permeability of lead candidates.

Compound	HBA	HBD	Log $D_{7.4}$	PAMPA (–Log P *e*)/cm s^{-1})
1	0	3	n.d.[a]	3.97 ± 0.02
6	2	5	1.748 ± 0.074	10.00 ± 0.00
7	2	5	1.747 ± 0.097	6.73 ± 0.18
10	2	4	2.013 ± 0.061	5.56 ± 0.13
11	2	4	2.813 ± 0.112	4.82 ± 0.02
17	3	4	n.d.[a]	10.00 ± 0.00

[a]Not detected due to poor ionization; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; Log $D_{7.4}$, distribution coefficient at pH 7.4.

The measured Log $D_{7.4}$ values for resveratrol glycosides indicate a moderate lipophilicity, essential to their bioavailability and blood-brain barrier (BBB) penetration. Log D values between 0 and 3 have are associated with a good balance between solubility and permeability, optimal oral absorption and low metabolic instability. Indeed, Log D values close to 2 have been established as ideal for BBB penetration [26, 27]. In fact, the median cLog D for marketed central nervous system (CNS) drugs is 1.7 [27]. In addition, MWs and numbers of HBA and HBD of all assessed molecules were in the acceptable ranges for drug-likeness, respecting Lipinski rule of 5 [28].

From the synthesized compounds, 2,6-dideoxy-*arabino*-hexopyranosides **10** and **11** (L-series) presented the best permeability results, predicting for permeability potential. In addition, glycosylation at position 4 seems to benefit permeation, when compared to 3′-*O*-glycosyation. Glycosylation of resveratrol did not improve passive permeation, but it is important to highlight that, although PAMPA assay highly correlates with permeation across a variety of barriers, it only accounts for passive permeation [29]. The conjugation of sugars may improve active permeation via hexose transporters, which would require further studies.

Conclusions

The synthesis of 2-deoxyglycosides embodying natural neuroprotective polyphenols as aglycones was carried out successfully, expecting to improve the bioavailability and neuroprotective activity of such molecular entities. Resveratrol glycosides **6** and **11** were more effective at protecting the neuronal cells from peroxide-induced cytotoxicity than resveratrol itself, and caffeic acid ester **17** also showed a remarkable neuroprotection activity. Compounds **6** and **17** were the most promising ones in terms of neuroprotection, and were not toxic to neuroblastoma, intestinal or liver cells at all concentrations tested. Coefficient partition measurements demonstrated the moderate lipophilicity of resveratrol glycosides, which Log D values are typical of CNS drug and ideal for BBB penetration, while passive permeation assessed by the PAMPA revealed that 2,6-dideoxy-L-*arabino*-hexopyranosides were more effective than 2-deoxy-D-*arabino*-hexopyranosides to permeate the intestinal barrier. This work highlights the beneficial role of coupling deoxyglycosides to improve bioactivity and physicochemical properties of the studied polyphenols, resulting in the generation of new lead molecules for further development.

Experimental section

Chemical synthesis

Starting materials and reagents were purchased from Sigma–Aldrich, Fluka and Acros. The solvents were dried prior to use with 4 Å or 3 Å (methanol) molecular sieves. Reactions were followed by UPLC-MS and/or TLC. TLC was carried out on aluminum sheets (20 cm × 20 cm) coated with 0.2 mm silica gel 60 F-254 (Merck) and detection was accomplished by spraying the plates with a solution of H_2SO_4 in ethanol (10 %) followed by heating at 120 °C.

Glycosylation of resveratrol was followed by UPLC-MS, using method A or B (%CP stands for chromatographic purity). Methods are as follows:

Method A (high pH): The column used was an XBridge C18 column (2.1 × 50 mm) with a 3.5 µm-particle size. The mobile phase consisted of eluent A: 10 mM ammonium bicarbonate; and eluent B: MeCN. The flow rate was maintained at 1.2 mL/min at 50 °C. The gradient was ramped up from 10 % B to 95 % B over 1.5 min, with additional 0.5 min hold time.

Method B (low pH): The column used was a Gemini NX C18 column (2.1 × 50 mm) with a 3 µm-particle size. The mobile phase consisted of eluent A: 0.1 % formic acid in water; and eluent B: 0.1 % formic acid in MeCN. The flow rate was maintained at 1.2 mL/min at 50 °C. The gradient was ramped up from 5 % B to 95 % B over 1.5 min, with additional 0.5 min hold time.

Compounds were purified by flash chromatography using silica gel 60G (0.040–0.063 mm, Merck), or by preparative HPLC.

Melting points were obtained with a SMP3 Melting Point Apparatus, Stuart Scientific, Bibby. Optical rotations were measured with a Perkin–Elmer 343 polarimeter. NMR spectra were recorded with a Bruker Avance 400 spectrometer at 298 K operating at 100.62 MHz for ^{13}C NMR and at 400.13 MHz for ^{1}H NMR. The solvents used were $CDCl_3$ with 0.03 % TMS and CD_3OD (Sigma–Aldrich). The chemical shifts are reported as δ (ppm) and the coupling constants (J) are given in Hz. ^{1}H and ^{13}C NMR spectra of final compounds are given in Supplementary Material.

High resolution mass spectra of new compounds were acquired on a Bruker Daltonics HR QqTOF Impact II mass spectrometer (Billerica, MA, USA). The nebulizer gas (N_2) pressure was set to 1.4 bar, and the drying gas (N_2) flow rate was set to 4.0 L/min at a temperature of 200 °C. The capillary voltage was set to 4500 V and the charging voltage was set to 2000 V.

Compound names are given according to the IUPAC recommendations but atom numbering used in NMR spectral data is shown in Fig. 2.

Glycosylation procedure

The appropriate glycal (**2** or **3**, 2.0 mmol) was dissolved in THF (5 mL), and resveratrol (1.5 equiv.) was added. The reaction mixture was stirred overnight at 40 °C and reaction course followed by LC-MS and TLC. The

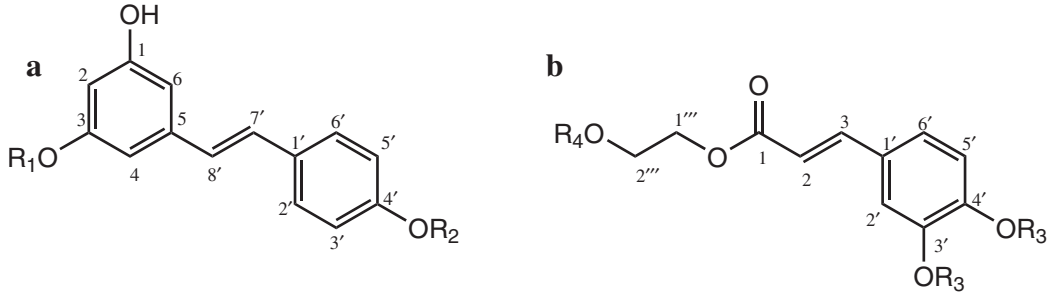


Fig. 2: Atom numbering used for the NMR signal assignment of compounds type (a) and (b): R_1, R_2 = H, glycosyl; R_3 = TBDMS, H; R_4 = H, glycosyl. Sugar atoms are numbered from 1″ to 6″, starting with the anomeric position.

reaction mixture was diluted with DCM (20 mL), washed with saturated sodium hydrogenocarbonate (30 mL), and then extracted with DCM (2×20 mL). The organic layers were combined, dried with magnesium sulphate, which was filtered off along with precipitated resveratrol. Solvent was evaporated and, to remove excess resveratrol, it was again suspended in EtOH (2 mL) and $CHCl_3$ (20 mL), filtered again, and the filtrate was evaporated to give a residue which was purified by consecutive flash chromatography with $CHCl_3 \rightarrow$ 95:5 $CHCl_3$/EtOH, to afford the corresponding 3-*O*- and 4′-*O*-resveratrol glycosides.

3-(3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranosyloxy)-5-[(*E*)-(4-hydroxyphenyl)ethenyl]phenol (4)
Reaction of glycal **2** with resveratrol afforded compound **4**, isolated in 21 % yield (0.210 g), as yellowish oil. LC-MS data (low pH): RT: 0.96, [M+H] = 501.00 *m/z*, [M+Na] = 523.00 *m/z*, [M − H] = 499.00 *m/z*; Rf ($CHCl_3$/EtOH 95:5) = 0.35; ^{1}H NMR ($CDCl_3$) δ 7.33 (d, 2H, $J_{2',3'} = J_{5',6'} = 8.3$ Hz, H-2′, H-6′), 6.94 (d, 1H, $J_{7',8'} = 16.4$ Hz, H-7′), 6.82 (d, 2H, H-3′, H-5′), 6.77 (d, 1H, H-8′), 6.74 (br s, 1H, H-4), 6.64 (br s, 1H, H-6), 6.49 (br t, 1H, $J_{2,6} = J_{2,4} = 2.0$ Hz, H-2), 5.64 (d, 1H, $J_{1'',2''ax} = 1.9$ Hz, H-1″), 5.52 (td, 1H, $J_{2''eq,3''} = 5.1$ Hz, $J_{3'',4''} = J_{3'',2''ax} = 9.6$ Hz, H-3″), 5.09 (t, 1H, $J_{3'',4''} = J_{4'',5''} = 9.6$ Hz, H-4″), 4.31 (dd, 1H, $J_{5'',6''a} = 4.9$ Hz, $J_{6''a,b} = 12.0$ Hz, H-6″a), 3.99–4.10 (m, 2H, H-5″, H-6″b), 2.45 (dd, 1H, $J_{2''ax,2''eq} = 12.6$ Hz, $J_{2''eq,3''} = 4.9$ Hz, H-2″eq), 2.06 (s, 3H, CH_3 −OAc), 2.08–2.05 (m, 7H, H-2″ax, 2 CH_3 −OAc), 2.02 (br s, 3H, CH_3 −OAc); ^{13}C NMR ($CDCl_3$) δ 171.4, 170.9, 170.3 (C=O, OAc), 157.4 (C-3), 157.2 (C-1), 155.9 (C-4′), 140.1 (C-5), 129.6 (C-1′), 129.1 (C-7′), 128.1 (C-2′, C-6′), 125.8 (C-8′), 115.7 (C-3′, C-5′), 107.6 (C-6), 106.5 (C-4), 102.8 (C-2), 95.1 (C-1″), 69.2, 69.1 (C-4″, C-3″), 68.6 (C-5″), 62.2 (C-6″) 35.0 (C-2″), 21.0, 20.7, 20.7 (CH_3, OAc).

5-{[(*E*)-4-(3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranosyloxy)phenyl]ethenyl}benzene-1,3-diol (5)
Reaction of glycal **2** with resveratrol afforded compound **5**, isolated in 3 % yield (0.031 mg), as yellowish oil. LC-MS data (low pH): RT: 1.07, [M+H] = 501.00 *m/z*, [M+Na] = 523.00 *m/z*, [M − H] = 499.00 *m/z*; R_f ($CHCl_3$/EtOH 95:5) = 0.27. ^{1}H NMR (MeOD) δ 7.50 (d, 2H, $J_{2',3'} = J_{5',6'} = 8.8$ Hz, H-2′, H-6′), 7.11 (d, 2H, H-3′, H-5′), 7.02 (d, 1H, $J_{7',8'} = 16.4$ Hz, H-7′), 6.90 (d, 1H, H-8′), 6.49 (d, 2H, $J_{4,2} = J_{2,6} = 2.0$ Hz, H-4, H-6), 6.20 (br t, 1H, $J_{2,6} = J_{4,2} = 2.0$ Hz, H-2), 5.79 (d, 1H, $J_{1'',2''ax} = 1.9$ Hz, H-1″), 5.47 (ddd, 1H, $J_{2''eq,3''} = 5.0$ Hz, $J_{3'',2''ax} = 10.1$ Hz, H-3″), 5.04 (t, 1H, $J_{3'',4''} = J_{4'',5''} = 9.6$ Hz, H-4″), 4.25 (dd, 1H, $J_{5'',6''a} = 4.8$ Hz, $J_{6''a,b} = 12.0$ Hz, H-6″a), 4.09–4.00 (m, 2H, H-5″, H-6″b), 2.47 (dd, 1H, $J_{2''ax,2''eq} = 13.3$ Hz, $J_{2''eq,3''} = 5.1$ Hz, H-2″eq), 2.06 (s, 3H, CH_3, −OAc), 2.05 (s, 3H, CH_3, −OAc), 2.03–1.95 (m, 4H, H-2″ax, −OAc). ^{13}C NMR (MeOD) δ 171.0, 170.6, 170.3 (C=O, OAc), 158.3 (C-3, C-1), 155.7 (C-4′), 139.5 (C-5), 131.9 (C-1′), 127.4, 127.3 (C-8′, C-7′), 127.2 (C-2′, C-6′), 116.5 (C-3′, C-5′), 104.6 (C-4, C-6), 101.6 (C-2), 95.2 (C-1″), 69.2 (C-4″), 68.8 (C-3″), 68.5 (C-5″), 62.1 (C-6″), 34.5 (C-2″), 19.4, 19.2, 19.2 (CH_3, OAc).

3-(3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*arabino*-hexopyranosyloxy)-5-[(*E*)-(4-hydroxyphenyl)ethenyl]phenol (8)
Reaction of glycal **3** with resveratrol afforded compound **8**, isolated in 22 % yield (0.195 g), as a yellowish oil. LC-MS data (high pH): RT: 0.99, [M+H] = 443.00 *m/z*, [M+Na] = 465.00 *m/z*, [M − H] = 441.00 *m/z*; R_f ($CHCl_3$/EtOH 95:5) = 0.40; ^{1}H NMR ($CDCl_3$) δ 7.30 (d, 2H, $J_{2',3'} = J_{5',6'} = 8.6$ Hz, H-2′, H-6′), 6.90 (d, 1H, $J_{7',8'} = 16.2$ Hz, H-7′), 6.81 (d, 2H, H-3′, H-5′), 6.74 (d, 1H, H-8′), 6.71 (br s, 1H, H-4), 6.61 (br s, 1H, H-6), 6.48 (br s, 1H, H-2), 5.57 (d, 1H, $J_{1'',2''ax} = 2.8$ Hz, H-1″), 5.47 (ddd, 1H, $J_{2''eq,3''} = 5.3$ Hz, $J_{3'',2''ax} = 10.4$ Hz, H-3″), 4.83 (t, 1H, $J_{3'',4''} = J_{4'',5''} = 9.6$ Hz, H-4″), 3.96 (qd, 1H, $J_{5'',6''} = 6.3$ Hz, H-5″), 2.42 (dd, 1H, $J_{2''ax,2''eq} = 13.0$ Hz, H-2″eq), 2.06 (s, 3H, −CH_3, OAc), 2.05 (s, 3H, −CH_3, OAc), 1.92 (ddd, 1H, H-2″ax), 1.14 (d, 3H, H-6″); ^{13}C NMR ($CDCl_3$) δ 171.3, 170.8 (C=O, OAc), 157.6 (C-3), 157.1 (C-1), 155.8 (C-4′), 140.1 (C-5), 129.6 (C-1′), 129.1 (C-7′), 128.1 (C-2′, C-6′), 125.9 (C-8′), 115.6 (C-3′, C-5′), 107.3 (C-6), 106.6 (C-4), 102.6 (C-2), 95.0 (C-1″), 74.7 (C-4″), 69.2 (C-3″), 66.6 (C-5″), 35.2 (C-2″), 21.1, 20.9 (CH_3, OAc), 18.2 (C-6″); HRMS Calcd. [$C_{24}H_{27}O_8$] 443.1700; Found 443.1695 (error 1.2 ppm).

5-{[(*E*)-4-(3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*arabino*-hexopyranosyloxy)phenyl]ethenyl}benzene-1,3-diol (9)
Reaction of glycal **3** with resveratrol afforded compound **8**, isolated in 8 % yield (0.071 g), as a yellowish oil. LC-MS data (high pH): RT: 0.99, [M+H] = 443.00 *m/z*, [M+Na] = 465.00 *m/z*, [M − H] = 441.00 *m/z*; R_f ($CHCl_3$/

EtOH 95:5) = 0.31; ^{1}H NMR ($CDCl_3$) δ 7.37 (d, 2H, $J_{2',3'} = J_{5',6'} = 8.7$ Hz, H-2′, H-6′), 7.01 (d, 2H, H-3′, H-5′), 6.94 (d, 1H, $J_{7',8'} = 16.2$ Hz, H-7′), 6.79 (d, 1H, H-8′), 6.54 (d, 2H, $J_{6,2} = J_{4,2} = 2.2$ Hz, H-4, H-6), 6.28 (br t, 1H, H-2), 5.59 (d, 1H, $J_{1'',2''ax} = 2.5$ Hz, H-1″), 5.47 (ddd, 1H, $J_{2''eq,3''} = 5.3$ Hz, $J_{2''ax,3''} = 10.4$ Hz, H-3″), 4.83 (t, 1H, $J_{3'',4''} = J_{4'',5''} = 9.8$ Hz, H-4″), 3.94 (qd, 1H, $J_{5'',6''} = 6.3$ Hz, H-5″), 2.42 (dd, 1H, $J_{2''ax,2''eq} = 12.9$ Hz, H-2″eq), 2.06 (s, 3H, $-CH_3$, OAc), 2.05 (s, 3H, $-CH_3$, OAc), 1.92 (br td, 1H, $J_{2''ax,3''} = 3.5$ Hz, H-2″ax), 1.14 (d, 3H, H-6″); ^{13}C NMR ($CDCl_3$) δ 171.3, 170.8 (C=O, OAc), 158.3 (C-3, C-1), 155.7 (C-4′), 139.5 (C-5), 131.9 (C-1′), 127.4, 127.3 (C-8′, C-7′), 127.2 (C-2′, C-6′), 116.5 (C-3′, C-5′), 104.6 (C-4, C-6), 101.6 (C-2), 95.0 (C-1″), 74.7 (C-4″), 69.2 (C-3″), 66.6 (C-5″), 35.2 (C-2″), 21.1, 20.9 (CH_3, OAc), 18.2 (C-6″). HRMS: Calcd. [$C_{24}H_{27}O_8$] 443.1700; Found 443.1695 (error 1.2 ppm).

Deacetylation

Glycosides **4**, **5**, **8** and **9** were deacetylated according to the Zemplén procedure [30]. The crude final compounds were obtained in quantitative yield, with chromatographic purity from 72 to 92 %. Purer fractions (>98 %CP) were obtained by purification either with flash chromatography or with preparative HPLC, using an elution method equivalent to the low pH method described above.

3-(2-deoxy-α-D-*arabino*-hexopyranosyloxy)-5-[(*E*)-(4- hydroxyphenyl)ethenyl]phenol (6)

Deacetylation of compound **4** (0.200 g, 0.4 mmol) afforded compound **6** in quantitative yield, with 92 % CP (0.158 g). LC-MS data (low pH): RT: 0.69, [M + Na] = 397.00 *m/z*, [M − H] = 373.00 *m/z*; $[\alpha]_D^{20} = +148$ (*c*0.5, MeOH); ^{1}H NMR (MeOD) δ 7.35 (d, 2H, $J_{2',3'} = J_{5',6'} = 8.5$ Hz, H-2′, H-6′), 6.99 (d, 1H, $J_{7',8'} = 16.3$ Hz, H-7′), 6.84 (d, 1H, H-8′), 6.79–6.75 (m, 3H, H-3′, H-5′, H-4), 6.61 (br t, 1H, $J_{2,6} = J_{4,6} = 1.7$ Hz, H-6), 6.44 (br t, 1H, $J_{4,2} = J_{2,6} = 2.1$ Hz, H-2), 5.64 (d, 1H, $J_{1'',2''ax} = 2.6$ Hz, H-1″), 4.04 (ddd, 1H, $J_{2''eq,3''} = 5.0$ Hz, $J_{2''ax,3''} = 12.9$ Hz, $J_{3'',4''} = 10.3$ Hz, H-3″), 3.75 (br d, 2H, $J_{5'',6''} = 5.4$ Hz, H-6″), 3.63 (td, 1H, $J_{4'',5''} = 9.8$ Hz, H-5″), 3.41 (t, 1H, H-4″), 2.26 (dd, 1H, $J_{2''ax,2''eq} = 13.2$ Hz, $J_{2''eq,3''} = 5.0$ Hz, H-2″eq), 1.78 (td, 1H, $J_{2''ax,3''} = J_{2''ax,2''eq} = 12.6$ Hz, H-2″ax); ^{13}C NMR (MeOD) δ 159.5 (C-3, C-1), 158.4 (C-4′), 140.9 (C-5), 130.3 (C-1′), 129.8 (C-7′), 128.9 (C-2′, C-6′), 126.7 (C-8′), 116.5 (C-3′, C-5′), 107.9 (C-6), 107.1 (C-4), 104.1 (C-2), 97.5 (C-1″), 74.7 (C-5″), 72.9 (C-4″), 69.8 (C-3″), 62.6 (C-6″), 38.9 (C-2″). HRMS: Calcd. [$C_{20}H_{22}NaO_7$] 497.1258; Found 497.1258 (error 3.2 ppm).

5-{[(*E*)-4-(2-deoxy-α-D-*arabino*-hexopyranosyloxy)phenyl]ethenyl}benzene-1,3-diol (7)

Deacetylation of compound **5** (0.031 g, 0.06 mmol) afforded compound **7** in quantitative yield, with 72 % CP (0.026 mg). Further purification by flash chromatography eluted with $CHCl_3 \rightarrow CHCl_3$/EtOH 85:15 afforded the title compound with 100 %CP as a colourless oil. LC-MS data (low pH): RT: 0.66, [M + H] = 375.00 *m/z*, [M + Na] = 397.00 *m/z*, [M − H] = 373.00 *m/z*; $[\alpha]_D^{20} = +123$ (*c*0.9, MeOH); ^{1}H NMR (MeOD) δ 7.43 (d, 2H, $J_{2',3'} = J_{5',6'} = 8.6$ Hz, H-2′, H-6′), 7.08 (d, 2H, H-3′, H-5′), 6.97 (d, 1H, $J_{7',8'} = 16.4$ Hz, H-7′), 6.86 (d, 1H, H-8′), 6.43, 6.42 (each singlet, 2H, H-4, H-6), 6.17 (br s, 1H, H-2), 5.67 (d, 1H, $J_{1'',2''ax} = 2.5$ Hz, H-1″), 4.04 (ddd, 1H, $J_{2''eq,3''} = 5.0$ Hz, $J_{2''ax,3''} = 12.9$ Hz, $J_{3'',4''} = 10.1$ Hz, H-3″), 3.73 (br d, 2H, $J_{5'',6''} = 5.4$ Hz, H-6″a, H-6″b), 3.61 (td, 1H, $J_{4'',5''} = 10.0$ Hz, H-5″), 3.39 (t, 1H, H-4″), 2.28 (dd, 1H, $J_{2''ax,2''eq} = 13.3$ Hz, $J_{2''eq,3''} = 5.1$ Hz, H-2″eq), 1.79 (td, 1H, $J_{2''ax,3''} = J_{2''ax,2''eq} = 12.5$ Hz, H-2″ax). ^{13}C NMR (MeOD) δ 160.5 (C-3, C-1), 157.7 (C-4′), 140.8 (C-5), 132.9 (C-1′), 128.7 (C-7′), 128.6 (C-8′), 128.5 (C-2′, C-6′), 117.9 (C-3′, C-5′), 105.9 (C-4, C-6), 103.5 (C-2), 97.6 (C-1″), 74.8 (C-5″), 72.9 (C-4″), 69.7 (C-3″), 62.5 (C-6″), 38.9 (C-2″). HRMS: Calcd. [$C_{20}H_{22}NaO_7$] 497.1258; Found 497.1239 (error 4.6 ppm).

3-(2,6-dideoxy-α-L-*arabino*-hexopyranosyloxy)-5-[(*E*)-(4-hydroxyphenyl)ethenyl]phenol (10)

Deacetylation of compound **8** (0.190 g, 0.44 mmol) afforded compound **10** in quantitative yield, with 97 % CP (0.158 g). Further purification by preparative HPLC (low pH method) afforded the title compound with 100 % CP as a colourless oil. LC-MS data (low pH): RT: 0.78, [M + H] = 359.00 *m/z*, [M + Na] = 381.00 *m/z*, [M − H] = 357.00 *m/z*; $[\alpha]_D^{20} = -52$ (*c*0.6, MeOH); ^{1}H NMR (MeOH) δ 7.34 (d, 2H, $J_{2',3'} = J_{5',6'} = 8.6$ Hz, H-2′, H-6′),

6.98 (d, 1H, $J_{7',8'}$ = 16.3 Hz, H-7′), 6.81 (d, 1H, H-8′), 6.75 (d, 2H, H-3′, H-5′), 6.67 (br s, 1H, H-4), 6.59 (br s, 1H, H-6), 6.40 (br s, 1H, H-2), 5.59 (d, 1H, $J_{1'',2''ax}$ = 2.2 Hz, H-1″), 3.97 (ddd, 1H, $J_{3'',4''}$ = 8.9 Hz, H-3″), 3.70 (qd, 1H, $J_{4'',5''}$ = 9.4 Hz, $J_{5'',6''}$ = 6.2 Hz, H-5″), 3.04 (t, 1H, H-4″), 2.27 (dd, 1H, $J_{2''ax,2''eq}$ = 13.2 Hz, $J_{2''eq,3''}$ = 5 Hz, H-2″eq), 1.77 (td, 1H, $J_{2''ax,3''} = J_{2''ax,2''eq}$ = 12.7 Hz, H-2″ax), 1.23 (d, 3H, H-6″); ^{13}C NMR δ 159.7 (C-3, C-1), 157.7 (C-4′), 141.4 (C-5), 129.8 (C-1′), 129.1 (C-7′), 128.8 (C-2′, C-6′), 128.1 (C-8′), 116.9 (C-3′, C-5′), 106.6 (C-4), 104.0 (C-2), 97.1 (C-1″), 78.8 (C-4″), 70.1 (C-5″), 69.6 (C-3″), 39.2 (C-2″), 18.2 (C-6″); HRMS: Calcd. [$C_{20}H_{22}NaO_6$] 381.1309; Found 381.1300 (error 2.3 ppm).

5-{[(*E*)-4-(2,6-dideoxy-α-L-*arabino*-hexopyranosyloxy)phenyl]ethenyl}benzene-1,3-diol (11)

Deacetylation of compound **9** (0.071 g, 0.016 mmol) afforded compound **11** in quantitative yield, with 75 %CP (0.058 g). Further purification by preparative HPLC (low pH method) afforded the title compound with 100 %CP as a yellowish oil. LC-MS data (low pH): RT: 0.76, [M + H] = 359.00 *m/z*, [M + Na] = 381.00 *m/z*, [M − H] = 357.00 *m/z*; $[\alpha]_D^{20}$ = −93 (*c*0.9, MeOH); ^{1}H NMR (MeOD) δ 7.44 (d, 2H, $J_{2',3'} = J_{5',6'}$ = 8.7 Hz, H-2′, H-6′), 7.04 (d, 2H, H-3′, H-5′), 6.99 (d, 1H, $J_{7',8'}$ = 16.3 Hz, H-7′), 6.87 (d, 1H, H-8′), 6.47, 6.46 (each br s, 2H, H-4, H-6), 6.17 (t, 1H, $J_{4,2} = J_{2,6}$ = 2.1 Hz, H-2), 5.52 (d, 1H, $J_{1'',2''ax}$ = 2.7 Hz, H-1″), 3.98 (td, 1H, $J_{2''eq,3''}$ = 5.2 Hz, $J_{3'',2''ax} = J_{3'',4''}$ = 9.2 Hz, H-3″), 3.66 (qd, 1H, $J_{4'',5''}$ = 9.2 Hz, $J_{5'',6''}$ = 6.2 Hz, H-5″), 3.04 (t, 1H, H-4″), 2.27 (ddd, 1H, $J_{2''ax,2''eq}$ = 13.2 Hz, $J_{1'',2''eq}$ = 0.7 Hz, H-2″eq), 1.79 (br td, 1H, $J_{2''ax,3}$ = 3.4 Hz, H-2″ax), 1.21 (d, 3H, H-6″); ^{13}C NMR (MeOH) δ 159.7 (C-3, C-1), 157.7 (C-4′), 141.0 (C-5), 132.7 (C-1′), 128.9 (C-7′), 128.6 (C-2′, C-6′), 128.3 (C-8′), 117.7 (C-3′, C-5′), 105.9 (C-2), 97.1 (C-1″), 78.8 (C-4″), 70.1 (C-5″), 69.5 (C-3″), 39.1 (C-2″), 18.2 (C-6″); HRMS: Calcd. [$C_{20}H_{23}O_6$] 359.1489; Found 359.1492 (error −0.9 ppm).

(*E*)-3-[3,4-Bis(*tert*-butyldimethylsilyloxy)phenyl]prop-2-enoic acid (13)

In a round bottom flask equipped with a magnetic stirrer, TBDMSCl (3.011 g, 0.020 mol, 3.6 equiv.) and imidazole (2.834 g, 0.042 mol, 7.5 equiv.) were added to a solution of caffeic acid (1 g, 5.55 mmol) in DMF (5 mL), and the reaction mixture was stirred at room temperature for 4 h. The reaction was quenched by adding purified water (50 mL), and extracted with DCM (4 × 25 mL). The combined organic phases were dried with anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography, eluted with hex/EtOAc 5:1 → 3:1, affording the title compound as fine needles in 71 % yield (1.62 g). Rf = 0.42 (Hex/EtOAc 2:1); m.p. = 154.3–155.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.68 (d, 1H, $J_{3,2}$ = 15.8 Hz, H-3), 7.06–7.03 (m, 2H, H-2′, H-6′), 6.84 (br d, $J_{5',6'}$ = 8.5 Hz, H-5′), 6.25 (d, 1H, H-2), 1.00 (s, 9H, *tert*-butyl), 0.99 (s, 9H, *tert*-butyl), 0.22, 0.22 (two singlets, each 6H, −CH_3); ^{13}C NMR ($CDCl_3$) δ 171.2 (C-1), 149.9 (C-3), 147.3, 147.0 (C-3′, C-4′), 127.6 (C-1′), 122.7 (C-6′), 121.2 (C-5′), 120.6 (C-2′), 114.6 (C-2), 25.9, 25.8 (−CH_3, *tert*-butyl), 18.5, 18.4 (Cq, *tert*-butyl), −4.0, −4.1 (−CH_3). HRMS: Calcd. [$C_{21}H_{36}O_4Si_2$] 409.2225; Found 409.2217 (error 1.9 ppm).

2-Hydroxyethyl (*E*)-3-[3,4-bis(*tert*-butyldimethylsilyloxy)phenyl]prop-2-enoate (14)

In a round bottom flask equipped with a magnetic stirrer, to a solution of compound **13** (0.500 g, 1.22 mmol) in DCM (2 mL). DMF (2 mL), DMAP (7.5 mg, 0.061 mmol) and ethyleneglycol (0.3 mL, 5.36 mmol, 4.4 equiv.) were added and the solution was cooled to 0 °C. *N*,*N*-dicyclohexylcarbodiimide (DCC, 0.278 g, 1.34 mmol, 1.1 equiv.) was added in one portion, stirred for 5 min at 0 °C and 20 h at room temperature. Formed urea was filtered off, and the filtrate was evaporated under reduced pressure. The resulting residue was purified by column chromatography, eluted with hex/EtOAc 6:1, affording the title compound as a colorless oil in 54 % yield (0.300 g). Rf = 0.83 (Hex/EtOAc 4:1); ^{1}H NMR ($CDCl_3$) δ 7.60 (d, 1H, $J_{2,3}$ = 16.0 Hz, H-3), 7.02–6.98 (m, 2H, H-2′, H-6′), 6.80 (br d, $J_{5',6'}$ = 8.5 Hz, H-5′), 6.26 (d, 1H, H-2), 4.35–4.31 (m, 2H, H-1‴), 3.91–3.87 (m, 2H, H-2‴), 0.99, 0.99 (two singlets, each 9H, *tert*-butyl), 0.20, 0.21 (two singlets, each 6H, −CH_3). ^{13}C NMR ($CDCl_3$) δ 167.7 (C-1), 149.6, 147.2 (C-3′, C-4′), 145.5 (C-3), 127.8 (C-1′), 122.5 (C-2′), 121.2 (C-5′), 120.4 (C-6′), 115.1 (C-2), 66.1 (C-1‴), 61.3 (C-2‴), 25.8, 25.8 (−CH_3, *tert*-butyl), 18.4, 18.3 (Cq, *tert*-butyl), −4.1, −4.2 (−CH_3). HRMS: Calcd. [$C_{23}H_{41}O_5Si_2$] 453.2487; Found 453.2479 (error 1.8 ppm).

1,5-anhydro-3,4-di-*O*-*tert*-butyldimetylsilyl-2,6-dideoxy-L-*arabino*-hex-1-enitol (15)

To a solution of 3,4-di-*O*-acetyl-1,5-anhydro-2,6-dideoxy-D-*arabino*-hex-1-enitol (1.0 g, 4.9 mmol) in MeOH (10 mL), NaOMe (0.050 g, 0.92 mmol, 0.2 equiv.) was added under stirring. After 1 h at room temperature, methanol was evaporated under reduced pressure, and the residue was re-suspended in DMF (40 mL). The solution was cooled to 0 °C, and imidazole (2.54 g, 37.3 mmol, 8 equiv.) and *tert*-butyldimethylsilyl chloride (6.6 g, 43.8 mmol, 9 equiv.) were added. After 5 min at 0 °C, the reaction was stirred at room temperature for 42 h. The reaction mixture was poured into purified water (150 mL), and extracted with EtOAc (3×150 mL). The organic layers were combined and washed with a saturated solution of $NaHCO_3$ (150 mL) and water (150 mL), and then dried with anhydrous $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography eluted with Hex → Hex/EtOAc 95:5, affording the title compound in 71 % yield (0.329 g) as a colorless liquid. R_f (Hex/EtOAc 9:1) = 0.87; $[\alpha]_D^{20}$ = +146 (*c*1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 6.29 (d, 1H, $J_{1,2}$ = 6.1 Hz, H-1), 4.70–4.65 (m, 1 H, H-2), 4.08–4.12 (m, 1H, H-3), 3.95 (br q, 1H, $J_{5,6}$ = 6.6 Hz, H-5), 3.58 (br t, 1H, $J_{4,5}$ = 5.9 Hz H-4), 1.34 (d, 3H, H-6), 0.92 (br s, 18H, *tert*-butyl), 0.14 (br s, 3H, –Me), 0.12 (br s, 9H, –Me); ^{13}C NMR ($CDCl_3$) δ 143.4 (C-1), 102.9 (C-2), 75.2 (C-3), 74.7 (C-4), 69.4 (C-5), 26.0, 25.9 (–CH_3, *tert*-butyl), 18.1, 18.0, 17.2 (Cq, *tert*-butyl), –3.6, –3.9, –4.1, –4.2 (brd s, 9H, –CH_3); HRMS: Calcd. [$C_{18}H_{30}NaO_3Si_2$] 381.2252; Found 381.2253 (error –0.3 ppm).

(3,4-Di-*O*-*tert*-butyldimethylsilyl-2,6-dideoxy-α/β-L-*arabino*-hexopyranosyloxy)ethyl (*E*)-3-[3,4-bis(*tert*-butyldimetylsilyloxy)phenyl]prop-2-enoate (16a,b)

A solution of glycal **15** (0.149 g, 0.42 mmol) in DCM (0.5 mL) was added to a solution of **14** (0.255 g, 0.56 mmol, 1.3 equiv.) in THF (2 mL). Then, TPHB (0.0141 g, 0.041 mmol, 0.1 equiv.) was added and the solution was heated to 35 °C for 5 h. The reaction mixture was neutralized with a saturated solution of $NaHCO_3$ (2×40 mL), and extracted with DCM (2×40 mL). The organic layers were combined, dried with anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The resulting residue was purified by column chromatography eluted with hex → hex/EtOAc 20:1, affording the title compound as an anomeric mixture (α/β 2.3:1) in 45 % yield (0.150 g) as a colourless oil. R_f (hex/EtOAc 30:1) = 0.3; $[\alpha]_D^{20}$ = –21 (*c*1, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.58 (d, 1H, $J_{3,2}$ = 15.9 Hz, H-3β), 7.57 (d, 1H, $J_{3,2}$ = 15.9 Hz, H-3α), 7.03–6.98 (m, 4H, H-2′α,β, H-6′α,β), 6.81 (d, 1H, $J_{5',6'}$ = 9.0 Hz, H-5′α,β), 6.28 (d, 1H, H-2β), 6.25 (d, 1H, H-2α), 4.83 (d, 1H, $J_{1'',2''ax}$ = 2.3 Hz, H-1″α), 4.50 (dd, 1H, $J_{1'',2''ax}$ = 9.8 Hz, $J_{1'',2''eq}$ = 1.7 Hz, H-1″β), 4.44–4.27 (m, 4H, H-1‴α,β) 4.08 (ddd, 1H, H-2‴aβ), 3.92 (ddd, 1H, $J_{3'',4''}$ = 8.4 Hz, $J_{2''ax,3''}$ = 11.9 Hz, $J_{2''eq,3''}$ = 4.8 Hz, H-3″α), 3.84 (ddd, 1H, H-2‴aα), 3.77 (ddd, 1H, H-2‴bβ), 3.71–3.59 (m, 3H, H-2‴bα, H-5″α, H-3″β), 3.23 (qd,1H, $J_{5'',6''}$ = 6.0 Hz, $J_{4'',5''}$ = 8.4 Hz, H-5″β), 3.14 (t, 2H, $J_{3'',4''}$ = $J_{4'',5''}$ = 8.4 Hz, H-4″α,β), 2.15 (dd, 1H, $J_{2''eq,3''}$ = 4.8 Hz, $J_{2''ax,2''eq}$ = 12.7 Hz, H-2″eqβ), 2.08 (dd, 1H, $J_{2''eq,3''}$ = 4.8 Hz, $J_{2''ax,2''eq}$ = 13.2 Hz, H-2″eqα), 1.69–1.60 (m, 2H, H-2″axα,β), 1.27 (d, 3H, $J_{5'',6''}$ = 6.2 Hz, H-6″β), 1.22 (d, 3H, $J_{5'',6''}$ = 6.3 Hz, H-6″α), 0.99, 0.99, 0.89, 0.87 (four singlets, each 18H, *tert*-butyl, OTBDMS, α/β), 0.22, 0.21, 0.09, 0.07 (four singlets, each 12H, Me, OTBDMS, α/β); ^{13}C NMR ($CDCl_3$) δ 169.1 (C-1), 149.6 (C-3), 147.1, 146.8 (C-3′, C-4′), 127.7 (C-1′), 123.0 (C-6′), 116.5 (C-5′), 115.1 (C-2), 114.9 (C-2′), 101.1 (C-1″α), 98.7 (C-1″β), 78.9 (C-4″α), 78.5 (C-4″β), 73.4 (C-5″β), 72.1 (C-3″β), 69.6 (C-5″α), 69.3 (C-3″α), 68.1 (C-1‴α), 66.3 (C-1‴β), 64.6 (C-2‴α), 61.5 (C-2‴β), 40.5 (C-2″β), 39.1 (C-2″α), 18.2 (C-6″ α/β). HRMS: Calcd. [$C_{41}H_{79}O_8Si_4$] 811.4847; Found 811.4816 (error –3.8 ppm).

(2,6-Dideoxy-α/β-L-*arabino*-hexopyranosyloxy)ethyl (*E*)-3-(3,4-dihydroxyphenyl) prop-2-enoate (17a,b)

A 1 M solution of TBAF in THF (2.6 mL, 2.6 mmol, 16.2 equiv.) was added to a solution of **16a,b** (0.130 g, 0.160 mmol) in THF (2 mL). After 14 h at room temperature, the solution was neutralized with saturated ammonium chloride aqueous solution (20 mL) and extracted with EtOAc (4×25 mL). The organic layers were combined, dried with anhydrous $MgSO_4$, filtered and evaporated to dryness. This residue was purified by column chromatography eluted with EtOAc/ACN 9:1, to give the title compound (anomeric mix α/β 3.8:1) as colourless oil in 52 % yield (0.030 g). R_f (EtOAc/ACN 9:1) = 0.3; $[\alpha]_D^{20}$ = –21 (*c*1, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.58 (d, 1H, $J_{3,2}$ = 16.0 Hz, H-3αβ), 7.07 (br s, 1H, $J_{2',3'}$ = 1.9 Hz, H-2′αβ), 6.97 (br d, 1H, $J_{5',6'}$ = 8.2 Hz, H-6′αβ), 6.80 (d, 1H, H-5′αβ), 6.30 (d, 1H, H-2αβ), 4.90 (br s, 1H, H-1″α), 4.63–4.59 (m, 2H, H-1‴aβ, H-1″β),

4.38 – 4.27 (m, 3H, H-1‴a,bα, H-1‴bβ), 4.08–4.02 (m, 1H, H-2‴aβ), 3.90–3.75 (m, 3H, H-2‴aα, H-2‴bβ, H-3″α), 3.70–3.62 (m, 2H, H-2‴bα, H-5″α), 3.59–3.48 (m, 1H, H-3″β), 3.25 (qd,1H, $J_{5'',6''}$ = 6.3 Hz, $J_{4'',5''}$ = 9.1 Hz, H-5″β), 2.97 (t, 1H, $J_{3'',4''} = J_{4'',5''}$ = 9.1 Hz, H-4″α), 2.93 (t, 1H, $J_{3'',4''} = J_{4'',5''}$ = 9.1 Hz, H-4″β), 2.20–2.14 (m, 1H, H-2″eqβ), 2.08 (dd, 1H, $J_{2''eq,3''}$ = 4.7 Hz, $J_{2''eq,2''ax}$ = 12.7 Hz, H-2″eqα), 1.62 (td, 1H, $J_{2''ax,3''}$ = 12.2 Hz, $J_{1'',2''ax}$ = 1.9 Hz, H-2″axα), 1.50 (d, 1H, $J_{2ax'',2eq''}$ = 12.4 Hz, H-2″axβ), 1.29 (d, 3H, $J_{5'',6''}$ = 6.0 Hz, H-6″β), 1.24 (d, 3H, $J_{5'',6''}$ = 6.0 Hz, H-6″α); ^{13}C NMR (MeOH) δ 167.7 (C-1), 148.1 (C-4′), 145.3 (C-3′), 146.8 (C-3), 126.6 (C-1′), 115.1 (C-5′), 121.6 (C-6′), 113.7, 113.5 (C-2, C-2′), 99.8 (C-1″β), 97.3 (C-1″α), 77.5 (C-4″α), 77.0 (C-4″β), 72.0 (C-5″β), 70.8 (C-3″β), 69.6 (C-5″α), 69.3 (C-3″α), 67.9 (C-1‴α), 66.7 (C-1‴β), 63.3 (C-2‴β), 63.2 (C-2‴α), 39.2 (C-2″β), 37.7 (C-2″α), 16.8 (C-6″ α/β); HRMS: Calcd. [$C_{17}H_{22}NaO_8$] 377.1207; Found 377.1225 (error –4.7 ppm).

Log P determination

The *in-silico* prediction tool ALOGPS [31, 32] was used to estimate the octanol-water partition coefficients (Log P) of the compounds. Depending on these values, the compounds were classified into three categories: hydrophilic compounds (Log P below zero), moderately lipophilic compounds (Log P between zero and one) and lipophilic compounds (Log P above one). For each category, two different ratios (volume of octan-1-ol to volume of buffer) were defined as experimental parameters (Table 3).

Equal amounts of phosphate buffer (0.1 M, pH 7.4) and octan-1-ol were mixed and shaken vigorously for 5 min to saturate the phases. The mixture was left until separation of the two phases, and the buffer was retrieved. Stock solutions of the test compounds were diluted with buffer to a concentration of 1 µM. For each compound, three determinations per octan-1-ol:buffer ratio were performed in different wells of a 96-well plate. The respective volumes of buffer containing analyte (1 µM) were pipetted to the wells and covered by saturated octan-1-ol according to the chosen volume ratio. The plate was sealed with aluminum foil, shaken (1350 rpm, 25 °C, 2 h) on a Heidolph Titramax 1000 plate-shaker (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) and centrifuged (2000 rpm, 25 °C, 5 min, 5804 R Eppendorf centrifuge, Hamburg, Germany). The aqueous phase was transferred to a 96-well plate for analysis by liquid chromatography-mass spectrometry (LCMS, see below).

The Log P coefficients were calculated from the octan-1-ol:buffer ratio (o:b), the initial concentration of the analyte in buffer ($1\,\mu M$), and the concentration of the analyte in buffer (c_B) with Equation 1:

$$\mathrm{Log\,P} = \mathrm{Log}\left(\frac{1\,\mu M - c_B}{c_B} \times \frac{1}{o:b}\right) \tag{1}$$

The average of the three Log P values per octan-1-ol:buffer ratio was calculated. If the two means obtained for a compound did not differ by more than 0.1 units, the results were accepted.

PAMPA

Effective permeability (Log Pe) was determined in a 96-well format test by a PAMPA Explorer Permeability Assay from Pion Inc. For each compound, measurements were performed at pH 7.4 in quadruplicate. Four wells of a deep well plate were filled with 650 µL of PRISMA HT universal buffer, adjusted to pH 7.4 by adding

Table 3: Compound classification based on estimated Log P-values.

Compound type	Log P	Ratios (octan-1-ol:buffer)
Hydrophilic	<0	30:140, 40:130
Moderately lipophilic	0–1	70:110, 110:70
Lipophilic	>1	3:180, 4:180

the requested amount of NaOH (0.5 M). Samples (150 µL) were withdrawn from each well to determine the blank spectra by UV/Vis-spectroscopy (190–500 nm, SpectraMax 190, Molecular Devices, Silicon Valley, CA, USA). Then, analyte dissolved in DMSO (10 mM) was added to the remaining buffer to yield 50 µM solutions. To exclude precipitation, the optical density (OD) was measured at 650 nm, and solutions exceeding OD 0.01 were filtrated. Afterwards, samples (150 µL) were withdrawn to determine the reference spectra. Further 200 µL were transferred to each well of the donor plate of the PAMPA sandwich (Pion, P/N 110 163). The filter membranes at the bottom of the acceptor plate were infused with 5 µL of GIT-0 Lipid Solution and 200 µL of Acceptor Sink Buffer were filled into each acceptor well. The sandwich was assembled, placed in the Gut-BoxTM, and left undisturbed for 16 h. Then, it was disassembled and samples (150 µL) were transferred from each donor and acceptor well to UV-plates for determination of the UV/Vis spectra. Effective permeability (Log Pe) was calculated from the compound flux deduced from the spectra, the filter area, and the initial sample concentration in the donor well with the aid of the PAMPA Explorer Software (Pion, version 3.5).

LC-MS measurements

Analyses were performed using a 1100/1200 Series HPLC System coupled to a 6410 Triple Quadrupole mass detector (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with electrospray ionization. The system was controlled with the Agilent MassHunter Workstation Data Acquisition software (version B.01.04). The column used was an AtlantisR T3 C18 column (2.1×50 mm) with a 3 µm-particle size (Waters Corp., Milford, MA, USA). The mobile phase consisted of eluent A: 10 mM ammonium acetate, pH 5.0 in 95:5, H_2O:MeCN; and eluent B: MeCN containing 0.1 % formic acid. The flow rate was maintained at 0.6 mL/min. The gradient was ramped up from 95 % A/5 % B to 5 % A/95 % B over 1 min, and then hold at 5 % A/95 % B for 0.1 min. The system was then brought back to 95 % A/5 % B, resulting in a total duration of 4 min. MS parameters such as fragmentor voltage, collision energy, polarity were optimized individually for each drug, and the molecular ion was followed for each compound in the multiple reaction monitoring mode. The concentrations of the analytes were quantified by the Agilent Mass Hunter Quantitative Analysis software (version B.01.04).

Neuroprotective assays

Neuroprotective assays in human neuroblastoma (SHSY-5Y) cells. SHSY-5Y cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Life Technologies) containing 10 % fetal bovine serum (FBS, Biochrom GmbH) and 1 % Penicillin-Streptomycin (Gibco, Life Technologies) at 37 °C, 5 % CO_2. For the neuroprotective activity assay, undifferentiated SGSY-5Y cells were plated onto 96-well flat-bottomed microtiter plates at a density of 1×104 cells/well in DMEM supplemented with 2 % FBS and preincubated for 24 h at 37 °C, 5 % CO_2. Compounds (stored as 10 mM solutions in DMSO at –20 °C) were then added to achieve a final concentration of 50 µM and, after 30 min, cells were incubated in the presence or absence of 100 µM of H_2O_2 (Sigma–Aldrich, dissolved to 10 mM in 0.9 % NaCl aqueous solution immediately prior to the assay) overnight at 37 °C, 5 % CO_2. The final DMSO percentage was 0.5 %. After 24 h, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (20 µL) solution in PBS (Gibco, Life Technologies) (5 mg/mL) was added to each well and the plates were further incubated for 4 h at 37 °C, followed by the addition of DMSO (200 µL) to each well in order to dissolve the resulting insoluble dye crystals. After 2 h incubating at 37 °C, the optical density (OD) at 540 nm (with a 620 nm reference filter) was measured in an Amersham Biosciences Biotrak II Plate Reader. The percentage of MTT reduction was determined according to Eq. (2). All experiments were performed in triplicate and results are presented as means ± standard error. Differences between experimental conditions were compared for statistical significance by one-way ANOVA followed by a Tukey's post-test – an analysis carried out using GraphPad Prism Software (LA Jolla, CA, USA). Differences were considered significant when $P < 0.05$. In order to exclude direct MTT reduction, compounds were also tested in the absence of cells, using the same experimental conditions above described.

$$\text{MTT Reduction (\% of Control)} = \left[\frac{OD_{sample} - OD_{medium}}{OD_{cell\ control} - OD_{medium}}\right] \times 100 \quad (2)$$

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