Coupling Deoxy Sugars to Polyphenols: Neuroprotection and Bioavailability

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The state of the art:

Stilbenes, particularly resveratrol, are amongst the best documented and emblematic neuroprotective natural products. In addition to the well-known antioxidant and anti-inflammatory activities, this compound inhibits Aβ oligomeric cytotoxicity and reduces neuronal cell death [1, 2]. On the other hand, rosmarinic acid, the active principle of the neuroprotective plant *Salvia sclareoides*, prevents amyloid aggregation, reducing also a number of other events underlying AD pathology [3-5]. Interestingly, methyl caffeate itself, a sub structural unit of rosmarinic acid, reduces significantly Aβ oligomeric cytotoxicity and promotes disaggregation of Aβ oligomers [5].

The problem:

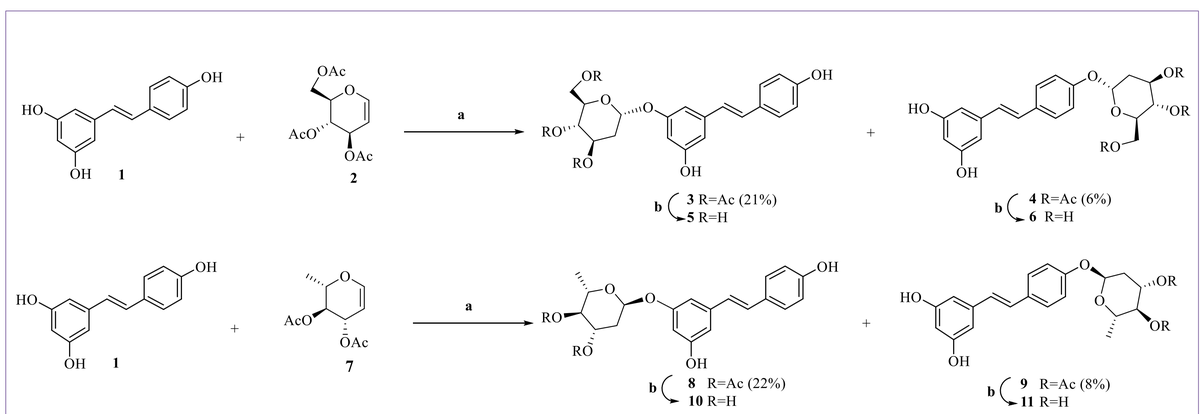
Resveratrol bioavailability in humans is less than 1% [6, 7]. Also, methyl caffeate has low bioavailability [5]. These compounds' water-insolubility limits their further pharmacological exploitation.

The solution:

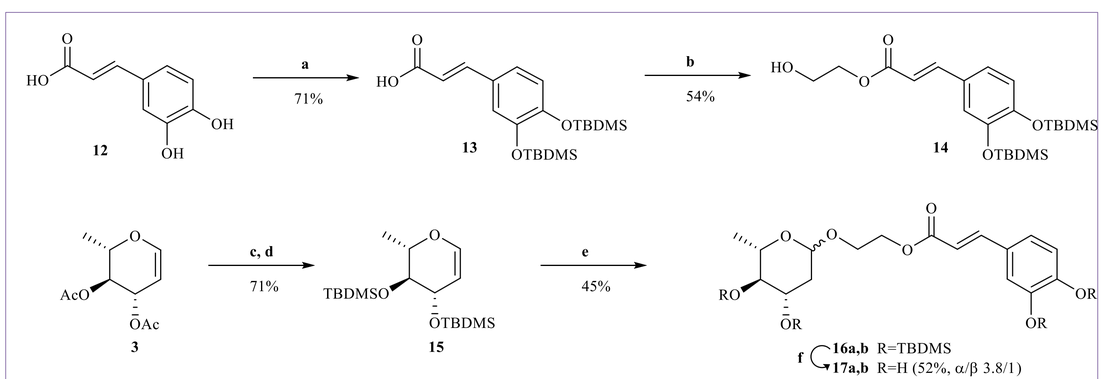
These findings encouraged us to investigate neuroprotective principles' glycosylation, using glycals as glycosyl donors to afford new resveratrol and caffeic acid ester 2-deoxyglycosides, envisioning more effective and bioavailable compounds.

The synthesis:

Resveratrol 3-*O*- and 4'-*O*-glycosides **3**, **4**, **8** and **9** were prepared by reaction of resveratrol with the appropriate acetyl protected glycal (**2** and **7**, respectively), in the presence of triphenylphosphane hydrobromide (TPHB), a catalyst known to afford almost exclusively 2-deoxyglycosylation instead of the well known Ferrier products, obtained starting from acylglycals with Lewis and Brønsted catalysts. Separation of 3-*O*- and 4'-*O*-glycosides followed by Zemplén deprotection afforded glycosides **5**, **6**, **10** and **11** in 21%, 6%, 22% and 8%, respectively. On the other hand, for the synthesis of the caffeic acid ester, the aromatic hydroxy groups of caffeic acid were protected with a *tert*-butyldimethylsilyl (TBDMS) group, followed by Steglich esterification. With TPDPS or TMS protection, no further developments were achieved. Coupling of **15** with **14** was once again accessed with TPHB as catalyst, to give **17a/b** as an anomeric mixture α/β in ratio 3:1 in 52% yield.



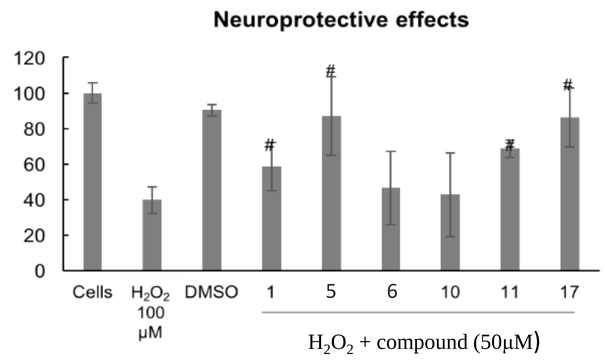
Scheme 1. Synthesis of resveratrol 2-deoxyglycosides. a) TPHB, THF, 40 ºC, 48 h; b) NaOMe, MeOH



Scheme 2. Synthesis of the caffeic acid esters **17a,b**. a) TBMDSCl, DCM; b) Ethylene glycol, DCC, DMAP, DCM; c) NaOMe, MeOH, d)TBMDSCl, imidazole, DMF; e) TPHB, THF; f) TBAF, THF.

The neruroprotective assays and drugability evaluation:

Hydrogen peroxide overproduction causing oxidative stress in neuroblastoma cells (SH-SY5Y) was used to access the neuroprotective effects of glycosides **5**, **6**, **10**, **11** and **17**.



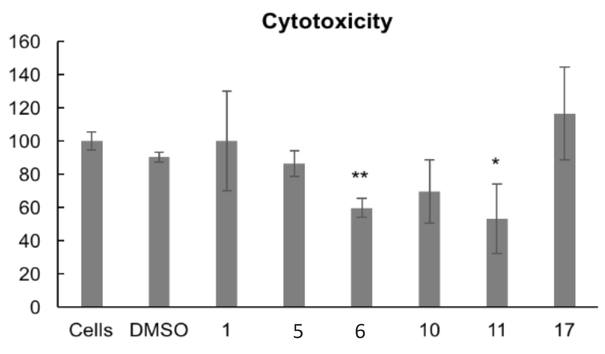


Figure 1. Neuroprotective and cytotoxic effects of synthesized compounds in neuroblastoma (SH-SY5Y) cells. * = significantly different when compared to cells control (p-value < 0.05); # = significantly different when compared to hydrogen peroxide controls (p-value < 0.05). Results are expressed as the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

Incubation of cells with 100 µM of H_2O_2 led to a cell viability decrease of ca. 60%. Incubating the cells with both hydrogen peroxide (100 µM) and resveratrol glycosides **5** and **11** resulted in a statistically significant increase in the percentage of viable cells (figure 1). Caffeic acid ester **17** showed the best neuroprotection activity, maintaining cellular viability similar to that of the control. By accessing glycosides' toxicity in neuroblastoma cells, only compounds **6** and **11** led to a statistically different loss of viability at 50 µM, remaining above 50% in all cases. The toxicity of the two most promising compounds was also assessed in Caco-2 and in HepG2 cell lines, and they were not toxic at all concentrations tested (0.1 - 100µM). When evaluating the drugability of the glycosides, Log $D_{7.4}$ values indicated a moderate lipophilicity, essential to their bioavailability and blood-brain barrier penetration. Indeed, Log D values close to 2 have been established as ideal for BBB penetration [8, 9]. The best permeability results were shown by 2,6-dideoxy-*arabino*-hexopyranosides **10** and **11** (L-series).

Table 1. Physicochemical properties and intestinal wall permeability of lead candidates.

Compound	HBA	HBD	log $D_{7.4}$	PAMPA (-log *Pe*)/cms^{-1})
1	0	3	n.d.[a]	3.97 ± 0.02
5	2	5	1.748 ± 0.074	10.00 ± 0.00
6	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 a/b	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.

Conclusion:

Our results show a good improvement in compounds' efficiency. Indeed, resveratrol glycosides **5** and **11** were more effective at protecting neuronal cells from peroxide-induced cytotoxicity than resveratrol itself. In addition, caffeic acid ester **17** showed the best neuroprotection activity. Coefficient partition measurements demonstrated the moderate lipophilicity of resveratrol glycosides, with log D values typical of CNS drug and ideal for BBB penetration, while passive permeation assessed by PAMPA revealed that our glycosides from the L series were more effective to permeate the intestinal barrier than our glycosides from the D series.

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