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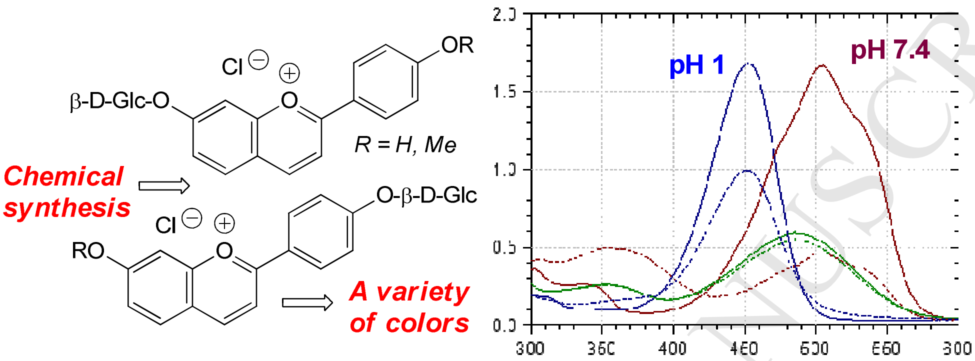
Graphical Abstract



A simple synthesis of 3-deoxyanthocyanidins and their O-glucosides

Sheiraz Al Bittar, Nathalie Mora, Michèle Loonis, Olivier Dangles*

University of Avignon, INRA, UMR408, 84000 Avignon, France

Corresponding author. Tel.: +33 490 14 44 46.

E-mail address: olivier.dangles@univ-avignon.fr (O. Dangles)

ABSTRACT

This work deals with the chemical synthesis of simple analogs of anthocyanins, the main class of water-soluble natural pigments. Flavylium ions with hydroxyl, methoxyl and β-D-glucopyranosyloxyl substituents at positions 4' and 7 have been prepared by straightforward chemical procedures. Moreover, the two 3-deoxyanthocyanidins of red sorghum apigeninidin (4',5,7-trihydroxyflavylium) and luteolinidin (3',4',5,7-tetrahydroxyflavylium) were synthesized in a one-step protocol. Attempts to synthesize luteolinidin O-β-D-glucosides resulted in a mixture of the 5-O- and 7-O-regioisomers in low yield. A preliminary study of the 4'-β-D-glucopyranosyloxy-7-hydroxyflavylium and 7-β-D-glucopyranosyloxy-4'-hydroxy-flavylium ions shows that simply changing the glucosidation site can profoundly affect the color intensity and stability.

Keywords: Anthocyanin; 3-Deoxyanthocyanidin; Glycoside; Synthesis; Sorghum; Color

1. Introduction

For centuries, food colorants have been used to attract the consumer's eye, to reproduce color lost during processing and to mask the heterogeneity of food formulations.[1] Chemistry has provided the food industry with diverse, cheap, and stable synthetic colorants. However, during the last decades, the safety of these synthetic colorants has been questioned. For instance, attention deficit hyperactivity disorder (ADHD) was observed in children having consumed red drinks supplemented with azo dyes[2] and products including these synthetic colorants have now to issue specific warnings on their packaging. Overall, natural colorants enjoy a much more positive image to consumers than artificial ones and the search for stable natural colorants remains a current challenge.

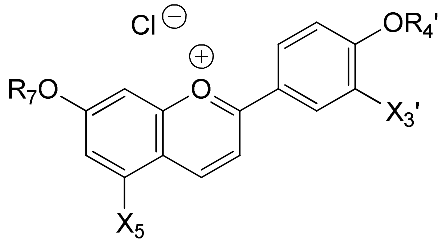
Anthocyanins are the most important group of water-soluble plant pigments. Stored in cell vacuoles, they are responsible for most of the red, purple and blue colors found in the plant kingdom.[3] Anthocyanins are generally found in Nature as anthocyanidin O-glycosides and the anthocyanidin aglycone is typically represented by its major colored form in acidic conditions, *i.e*. the 3,4',5,7-tetrahydroxyflavylium ion (pelargonidin) and common parent ions with additional OH or OMe groups at C3' and/or C5'. Among these 6 chromophores, anthocyanidins having a 3',4'-dihydroxy substitution (*e.g*., cyanidin as the simplest) are especially important from two viewpoints: they can bind hard metal ions (Al^{3+}, Fe^{3+}, Mg^{2+}) to form deeply colored chelates, one of the main mechanisms for natural color variation. They are good electron donors (as, more generally, polyphenols and flavonoids bearing catechol nuclei) and thus potential antioxidants.[4,5]

While each anthocyanidin OH group can be potentially glycosylated in anthocyanins, glycosidation at C3-OH suffers no exception. Indeed, a free C3-OH is associated with a high chemical instability (except in highly acidic conditions, pH < 2) and the corresponding C-ring is rapidly cleaved to yield a mixture of simple colorless phenols. Hence, hydrolysis of the glycosidic bond at C3-OH, either chemically during thermal processing, or enzymatically by human or bacterial glycosidases, is rapidly followed by irreversible loss of the typical chromophore.[6,7] This is one of the main mechanisms underlying the well-known low chemical stability and apparent bioavailability of anthocyanins.

Although much less common than anthocyanins, 3-deoxyanthocyanidins (3-DAs) and their O-glycosides have been found in high concentration in some food sources, especially red sorghum.[8,9] The lack of OH group at C3 makes these pigments much less sensitive to water addition at C2,[10,11] which reversibly leads to the colorless hemiketal and chalcone forms, and also more resistant to irreversible chemical degradation.[12] Thus, in mildly acidic to neutral conditions, 3-DAs express more intense and more stable colors than common anthocyanins. On the other hand, the applications of 3-DAs as food colorants could be limited by their low water solubility, which can be markedly improved by conjugation to D-glucose. Red sorghum actually also contains 3-DA glucosides, such as luteolinidin 5-O-β-D-glucoside.[13]

Extraction of anthocyanins from various natural sources typically affords complex extracts combining anthocyanins, colorless polyphenols and other hydrophilic plant components, such as sugars and acids. Hence, the purification of these extracts for the preparation of anthocyanins at the gram scale remains challenging and time-consuming. In this respect, the simple chemical synthesis of anthocyanins and simpler analogs remains interesting, if not for direct industrial application, at least for facilitating the evaluation of their potential as natural colorants for foods and cosmetic products, and as nonessential micronutrients, whose bioavailability and bioactivity are extensively studied.[14,15]

In this present work, we report on the efficient chemical synthesis of 3-DAs and their O-β-D-glucosides (Scheme 1), including the characteristic red sorghum pigments apigeninidin (APN, 4',5,7-trihydroxyflavylium) and luteolinidin (LTN, 3',4',5,7-tetrahydroxyflavylium), as well as a series of simpler analogs. After careful optimization of reaction conditions (time, temperature, solvent composition…), the chemical synthesis of APN and LTN could be achieved in an essentially one-step procedure, *i.e.* via a much simpler route than previously described in the literature.[16,17] A preliminary investigation of the coloring properties of two 3-DA glucosides is also reported, showing the large influence of the sugar position on the resulting color and its stability.



Pigment	$X_{3'}$	$R_{4'}$	X_5	R_7	Yield (%) [a])
P1[18]	OH	H	H	H	56
P2[18]	OH	H	H	β-D-Glc	75 [b])
P3	H	H	H	β-D-Glc	42 [b])
P4	H	Me	H	β-D-Glc	23 [b])
P5	H	β-D-Glc	H	H	77
P6	H	β-D-Glc	H	Me	77
P7	H	H	H	H	45
P8	H	H	H	Me	90
P9	H	Me	H	H	85
APN	H	H	OH	H	34
LTN	OH	H	OH	H	65

[a]) Yield of isolated pigment in the final (chromophore-building) condensation step

[b]) Yield also includes the step of sugar deprotection

Scheme 1. Pigments synthesized in this work (except **P1** and **P2**[18])

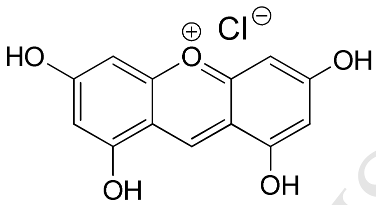
2. Results and discussion

The chemical synthesis 3-DAs and analogs has already been described over the last 3 - 4 decades.[16-19] The simplest route typically involves the acid-catalyzed aldol condensation of a 2-hydroxybenzaldehyde and an acetophenone bearing additional OH, OMe or O-glycosyl substituents. Using gaseous HCl bubbled into the cooled solution of both reagents in ethylacetate, the corresponding flavylium chloride precipitates and is recovered by simple filtration. Alternatively, 3-DAs were prepared by reduction of the corresponding flavones[20] and from condensation between cinnamaldehyde derivatives and phloroglucinol.[21] Finally, 3-deoxyanthocyanidins can also be prepared by condensation between phenol derivatives and arylethynylketones.[22,23]

As natural anthocyanins all possess a 5,7-dihydroxy substitution (A-ring), aldol condensations involving 2,4,6-trihydroxybenzaldehyde are of particular interest. Unfortunately, 2,4,6-trihydroxybenzaldehyde is poorly soluble in AcOEt and prone to acid-catalyzed dimerization (possibly, oligomerization) by electrophilic aromatic substitution, leading to yellow – orange xanthylium pigments. To circumvent these disadvantages, less simple routes have been devised, some still based on 2,4,6-trihydroxybenzaldehyde but requiring its preliminary partial O-acylation to improve its solubility in weakly polar solvent (convenient for the precipitation of the flavylium salts) and decrease the nucleophilic character of its aromatic ring.[17,24] In this work, special attention has been devoted to optimizing the direct condensation of (unprotected) 2,4,6-trihydroxybenzaldehyde with an acetophenone to ensure a reasonable yield of flavylium while keeping at a minimum the side-reaction leading to xanthylium pigments. Important factors in the optimization are the solvent composition (ensuring both 2,4,6-trihydroxybenzaldehyde solubility and flavylium precipitation), the temperature, the reagent concentration and the proton donor. For the synthesis of the red sorghum pigments APN and LTN, AcOEt / MeOH (2:1) mixtures were used and gaseous HCl was in situ generated by reaction between MeOH and TMSCl added in controlled excess to a solution of both reagents in a closed flask cooled to 0°C. In such conditions, LTN was formed in 65% yield and isolated after 30 min by simple filtration. LTN was contaminated by small quantities (*ca*. 5%) of 2,4,6,8-tetrahydroxyxanthylium chloride (Scheme 2) characterized by UPLC-MS: t_R = 4.2 min, λ_{max} = 440 nm, m/z = 244.9 (M^+) and by a very simple spectrum ^{1}H-NMR (in CD_3OD): δ (ppm) = 6.0 (2H, *s*, H_1, H_9), 6.4 (2H, *s*, H_3, H_7), 9.5

(1H, *s*, H_4).[25] Its formation from 2,4,6-trihydroxybenzaldehyde is not fully clear as it involves a decarbonylation step (CO loss). Solubilization in EtOH and precipitation by AcOEt addition afforded pure LTN.

Remarkably, a similar procedure yielded APN devoid of xanthylium pigment and only contaminated by low amounts of unreacted 2,4,6-trihydroxybenzaldehyde. Repeating the reaction over 60 min with 2 equiv. of 4-hydroxyacetophenone gave pure APN after purification in EtOH / AcOEt.

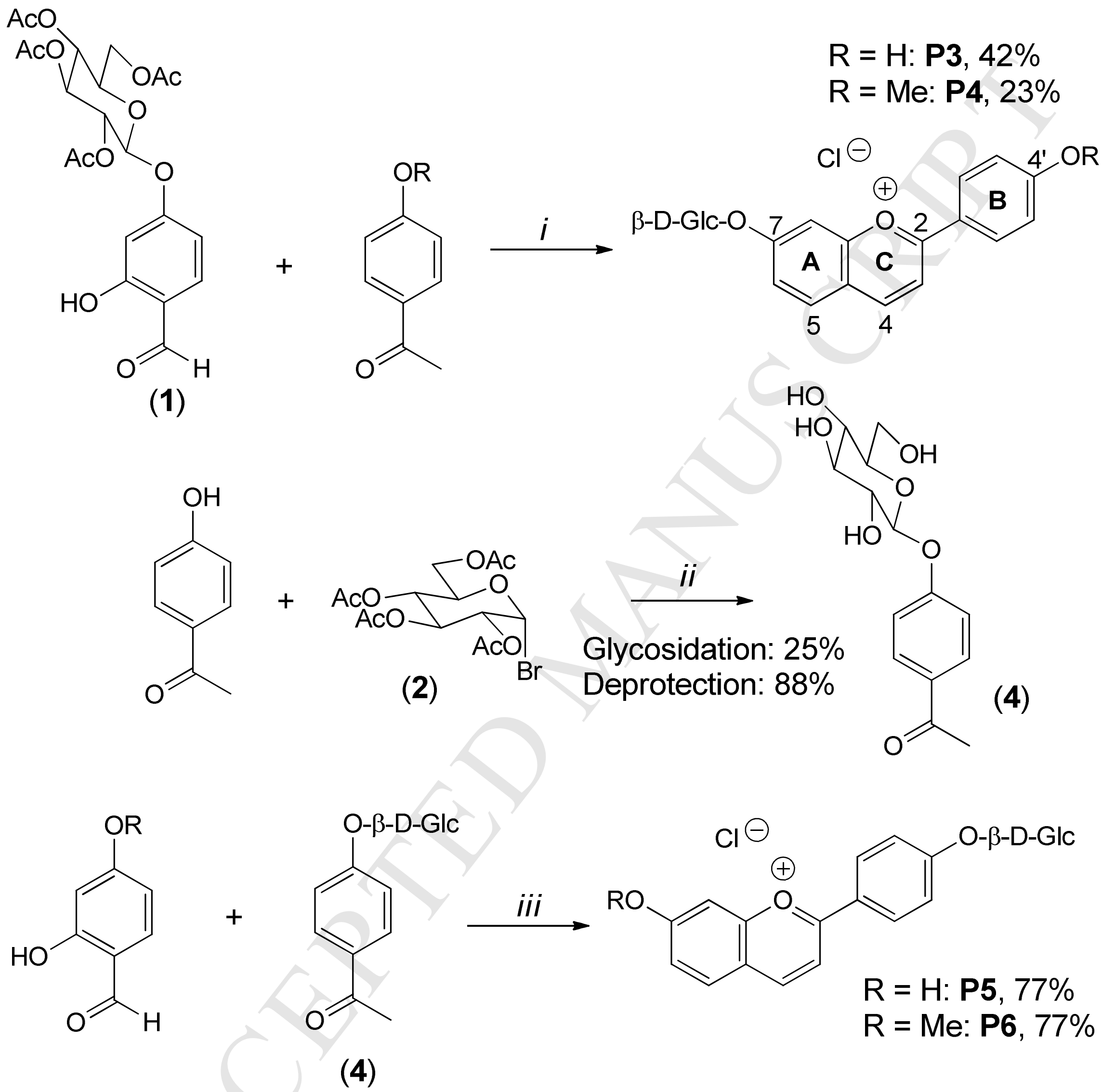


Scheme 2. Structure of 2,4,6,8-tetrahydroxyxanthylium chloride.

Based on this improved procedure, a series of flavylium ions was efficiently prepared with the typical OH and O-β-D-Glc substituents of natural anthocyanins at positions 4' and 7. Although much less common at those positions, the corresponding O-methylethers were also synthesized. The chemical synthesis of aglycones **P7**, **P8** and **P9** was achieved in AcOEt / MeOH (1:1). As neither 2,4-dihydroxybenzaldehyde nor 2-hydroxy-4-methoxybenzaldehyde is prone to competing dimerization, the aldol condensation was prolonged over 3 days at 4°C to ensure total flavylium precipitation and a maximal yield (up to 90% for **P8**).

As for the glucosides, a preliminary glucosidation step of 2,4-dihydroxybenzaldehyde and 4-hydroxyacetophenone was required (Scheme 3). With the former, satisfactory yields were obtained using phase transfer conditions in a 1:1 mixture of saturated aqueous K_2CO_3 (pH 12) and CH_2Cl_2 using 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide as the glucosyl donor.[18] Due to the strong hydrogen bond between the carbonyl group and C2-OH, the glucosidation of 2,4-dihydroxybenzaldehyde was regioselective and took place at C4-OH. Such strongly alkaline conditions did not allow us to convert 4-hydroxyacetophenone into its glucoside, possibly because of competing enolate formation.

A less alkaline aqueous phase ($NaHCO_3$ / KCl (1:1), pH 8.7) was thus used[19] and the glucoside was obtained, although in low yield.



Scheme 3. Chemical synthesis of pigments: (*i*) TMSCl, AcOEt, then MeONa, MeOH, then aq. HCl. (*ii*) tris(2-(2-methoxyethoxy)ethyl)amine in CH_2Cl_2 / 1 M aq. $NaHCO_3$, 1 M aq. KCl, then MeONa, MeOH. (*iii*) TMSCl, AcOEt / MeOH.

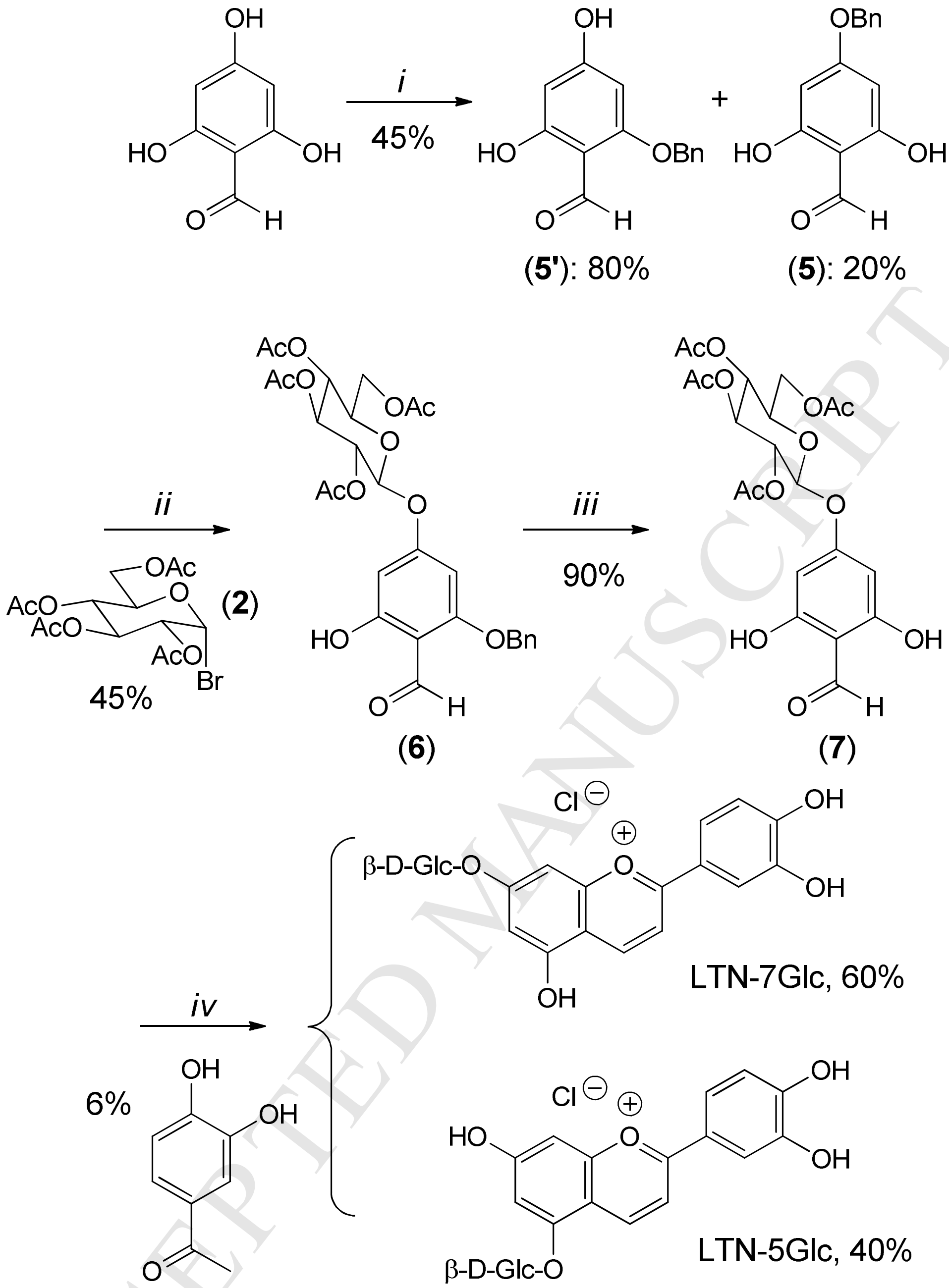
To prepare pigments **P3** and **P4**, aldol condensation was achieved in AcOEt / MeOH (4:1) in the presence of TMSCl (10 equiv.) and the mixture kept 3 days at -18°C to minimize possible deglycosidation. A next step of glucose deacetylation was carried out using MeONa in excess (8 equiv.) as part of the reagent is consumed by reversible addition to the pyrylium ring and/or phenolate formation. After acidification, **P3** and **P4** were obtained in moderate over all yields (42% and 23%, respectively).

Actually, the final step (pigment deacetylation) is rather difficult to monitor and push to completion. It also requires strongly alkaline conditions and subsequent acidification and concentration that may be damaging to the pigment. Hence, to prepare pigments **P5** and **P6**, another route was tested in which deacetylation was carried out prior to condensation. For the condensation step, solvent conditions have to be tuned so as to ensure a sufficient solubility for the deprotected glucoside (4-O-β-D-glucopyranosyloxyacetophenone **4**) while still permitting the precipitation of the flavylium ions in the medium. Thus, **3** (protected **4**) was first deacetylated in good yield, then successfully condensed with 2,4-dihydroxybenzaldehyde or 2-hydroxy-4-methoxybenzaldehyde in MeOH / AcOEt (2:1) (Scheme 3).

Red sorghum contains not only 3-DAs but also some of their glucosides, in particular luteolinidin 5-O-β-glucoside (LTN-5Glc).[13] To ensure glucosidation of 2,4,6-trihydroxybenzaldehyde at C2-OH and also lower its hydrophilic character and thus favor its transfer into the organic phase, selective protection at C4-OH was attempted. Benzoylation with benzoylchloride / *t*-BuOK in THF at 0°C afforded an inseparable mixture of 2- and 4-regioisomers along with the dibenzoylated product. Unfortunately, the subsequent step of glucosidation failed because of insufficient stability of the benzoyl groups in the phase transfer conditions. Benzylation with benzyl bromide / K_2CO_3 in DMF turned out to take place mainly at C2-OH, yielding a 4:1 inseparable mixture of 2-benzyloxy-4,6-dihydroxybenzaldehyde and 4-benzyloxy-2,6-dihydroxybenzaldehyde (Scheme 4). It may be speculated that the phenolate anion resulting from deprotonation at C4-OH is stabilized through extensive conjugation with the CHO group while its tautomer formed by deprotonation at C2-OH could bear a larger negative charge on its O-atom, making it more nucleophilic.

Glucosidation of the benzylether regioisomers led to pure 2-benzyloxy-6-hydroxy-4-(2’,3’,4’,6’-tetra-O-acetyl-β-D-glucopyranosyloxy)-benzaldehyde (**6**) in acceptable yield, which suggests that the minor 4-benzyloxy-2,6-dihydroxybenzaldehyde is unreactive in the phase transfer reaction (confirmed by isolation and independent attempt of glycosidation). Then, hydrogenolysis was carried out in soft conditions (30 min, 2 bars of H_2) to avoid aldehyde reduction. Aldol condensation with 3,4-dihydroxyacetophenone gave 7-

(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-3',4',5-trihydroxyflavylium in acceptable yield (ca. 60%). Unfortunately, the final deprotection step turned out to be slow and incomplete even in strongly alkaline conditions (excess MeONa in MeOH or KOH in MeOH/H_2O (1:1)[24]). A possible explanation would be that all phenolic groups are deprotonated in those conditions, so that the nucleophilic attack of methoxide or hydroxide anions onto the acetate groups is impeded by electrostatic repulsion (especially with the nearby C5-O^- group). Overall, despite prolonged reaction time and repeated deprotection, partially protected pigments remained in solution and had to be removed by chromatography on TSK gel. Given these difficulties, the overall yield of the condensation – deacetylation – purification sequence is very disappointing (< 10%).

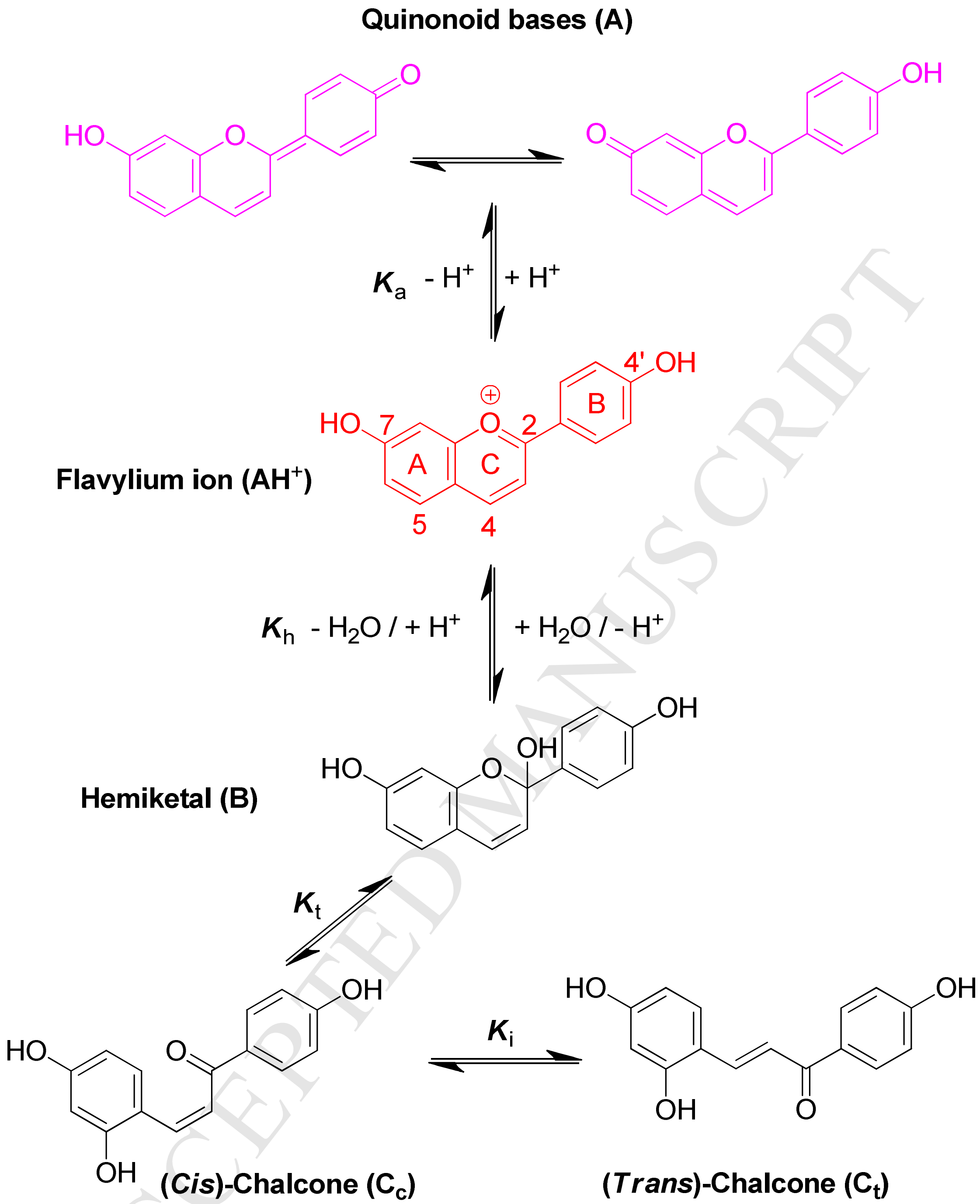


Scheme 4. Chemical synthesis of luteolinidin glucosides: (*i*) BnBr (1 equiv.), K_2CO_3, DMF (*ii*) tris(2-(2-methoxyethoxy)ethyl)amine in CH_2Cl_2 / 1 M aq. $NaHCO_3$, 1 M aq. KCl. (*iii*) H_2, Pd/C, THF - MeOH. (*iv*) TMSCl, AcOEt, then MeONa, MeOH, then aq. HCl.

The alternative route (deprotection followed by condensation) was also tested. Deacetylation of **7** actually gave 2,6-dihydroxy-4-β-D-glucopyranosyloxybenzaldehyde. Unfortunately, this glucoside was insoluble in most organic solvents and the final condensation could not be carried out.

Although obtained in low yield, the LTN O-glucoside could be isolated for full structural characterization. Surprisingly, UPLC-MS analysis of the deprotected product revealed that it actually was a *ca*. 3:2 mixture of LTN-7-Glc and the naturally occurring LTN-5-Glc. The peak assignment was confirmed by co-injection with a sample of pure LTN-7Glc obtained by reduction[20] of the corresponding flavone luteolin7-O-β-D-glucoside. Both glucosides were characterized by NMR in comparison with the literature.[13] To our knowledge, this unexpected isomerization in alkaline conditions has no equivalent in the literature.

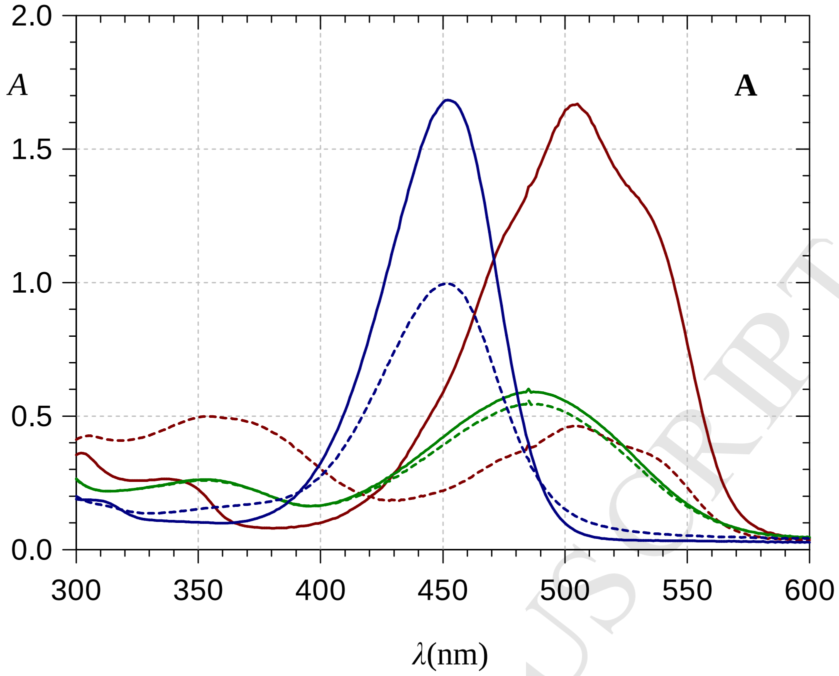
Hydroxylated flavylium ions (AH^+, typically, red) undergo two competitive pathways in mildly acidic solution featuring the natural medium of anthocyanins,[11,27] *i.e*. the vacuole of plant cells: the first one is fast proton loss and concomitant formation of purple quinonoid bases (A), the second one is the much slower reversible addition of water at the electrophilic C2 center with concomitant proton loss and formation of a colorless hemiketal (B) in fast equilibrium with a *cis*-chalcone (C_c), itself in slow equilibrium with the corresponding *trans*-chalcone (C_t) (Scheme 5).



Scheme 5. Structural transformations of flavylium ions in mildly acidic aqueous solution.

With 3-DAs, B and C_c are only transient non-accumulating intermediates and C_t comes up as the only significant colorless form.[18,28]. A simplified scheme can thus be proposed in which A and C_t are respectively the kinetic and thermodynamic products of the concurrent transformations of AH^+ in mildly acidic solution. The set of flavylium ions synthesized in this work offers a good opportunity to investigate the influence on these competitive pathways of the typical substituents of anthocyanins at C4'-OH and C7-OH, in particular the β-D-glucosyl group, and their consequences in terms of color expression and stability. This point will be briefly exemplified with **P3** and **P5**.

When a small volume of pigment solution in acidified MeOH (100% flavylium form) is diluted into a pH 7.4 phosphate buffer, the corresponding quinonoid base is instantaneously formed. **P3** and **P5** bearing only one OH group, a single deprotonation is observed (no further proton loss leading to anionic bases) and a single base is formed (instead of 2 or 3 possible tautomers as with natural anthocyanins). As the first pK_a of flavylium ions is typically in the range 4 - 5,[11] the proton loss can be assumed quantitative. Thus, the spectra recorded immediately after dilution (Fig. 1A) can be ascribed to pure A forms resulting from proton loss at C4'-OH (A4', **P3**) or at C7-OH (A7, **P5**). Interestingly, while both flavylium ions display almost the same λ_{max} in the visible range (*ca*. 450 nm), the visible spectrum of the **P3** base (λ_{max} = 505 nm) is shifted by 20 nm to longer wavelengths, compared to the **P5** base (λ_{max} = 485 nm). Moreover, the higher molar absorption coefficient of the **P3** *vs*. **P5** flavylium ion (*ca*. +70%) is translated and amplified (almost a factor 3) into the corresponding bases. Therefore, although proton loss reduces the HOMO – LUMO gap in both pigments, this decrease and thus the bathochromic shift are larger when deprotonation occurs at C4'-OH. Moreover, the HOMO – LUMO match, which must be mainly responsible for the visible absorption, is better in **P3** than in **P5**, regardless of the acid - base form.



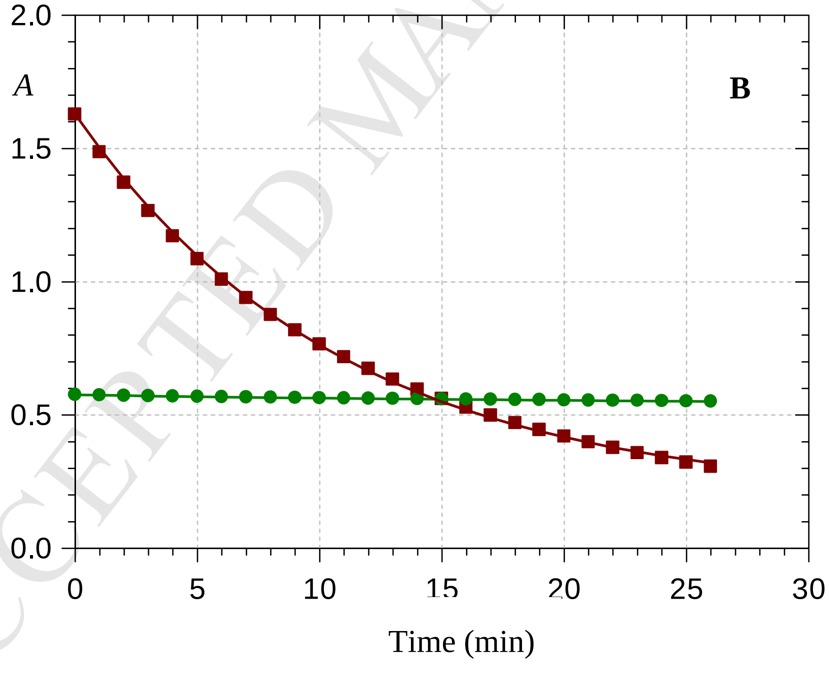


Fig. 1. A: UV-visible spectra (pigment concentration = 50 µM). a) Quinonoid bases (pH 7.4 phosphate buffer, 25°C): **P3** (—) and **P5** (—) immediately after dilution in the buffer, **P3** (---) and **P5** (---) after 30 min. b) Flavylium ions (0.1 M HCl, 25°C): **P3** (—), **P5** (---). **B:** decay of the visible absorbance of the bases at λ_{max}. **P3** (■, detection at 505 nm), **P5** (●, detection at 485 nm). Solid lines result from curve-fitting assuming apparent first-order kinetics: k_{obs}(**P3**) = 15.4 (± 0.2) x10^{-4} s^{-1}, k_{obs}(**P5**) = 19.4 (± 0.2) x10^{-6} s^{-1}, r = 0.9998.

Remarkably, the advantage of **P3** over **P5** in terms of color variation and intensity is not translated in terms of color stability in neutral conditions. Indeed, while the visible spectrum of the **P5** base remains essentially unchanged over 30 min (half-life of **P5** base ≈ 10h based on the observed first-order decay, Fig. 1B), the one of **P3** is markedly weakened (half-life of **P3** base ≈ 7.5 min) with concomitant appearance of a new absorption band around 360 nm featuring the **P3** *trans*-chalcone (Fig. 1A). Thus, **P5** appears as exceptionally resistant to water addition and subsequent fading in neutral conditions.

The color loss features the overall conversion of the colored forms into the *trans*-chalcone. Its rate is governed by the slow step of chalcone *cis* – *trans* isomerization. Hence, the observed rate constant of fading can be written as Eqn. (1), in which the fraction represents the percentage of *cis*-chalcone at pseudo-equilibrium with the colored forms and hemiketal:[27]

$$k_{obs} = k_i \frac{K_h K_t}{[H^+] + K_a + K_h(1+K_t)} + k_{-i} \tag{1}$$

With the 3',4'-dihydroxy-7-O-β-D-glucopyranosyloxyflavylium ion (**P2**), which is closely related to **P3**, the following values were estimated for the different parameters:[28] $K_h = 2.5\text{x}10^{-5}$ M, $K_a = 1.3\text{x}10^{-5}$ M, $K_t = 3.3$, $k_i = 1.2\text{x}10^{-3}$ s^{-1}, $k_{-i} = 1.6\text{x}10^{-5}$ s^{-1}($K_i = k_i / k_{-i} = 75$). At pH 7.4, one has: $[H^+] << K_a + K_h(1 + K_t)$. Moreover, C_t is much more stable than C_c ($k_i << k_{-i}$). Hence, Eqn. (1) can be simplified to give Eqn. (2):

$$k_{obs} = k_i \frac{K_t}{1 + K_t + \frac{K_a}{K_h}} \tag{2}$$

From Eqn. (2), one obtains: $k_{obs} = 8.2\text{x}10^{-4}$ s^{-1}. This value is actually close to our estimate for **P3**: $k_{obs} = 15.4\ (\pm\ 0.2)\ \text{x}10^{-4}$ s^{-1}. More work is needed to unveil the cause for the much higher stability of **P5**. As there is no reason for K_t and K_h to be dramatically different for **P3** and **P5** or for the *cis-trans* chalcone isomerization barrier to be higher for **P5** than for **P3**, it may be speculated that the very slow fading of **P5** stems from a much higher K_a value due to proton loss being much more favorable from C7-OH than from C4'-OH. Consistently, the pK_a values of the 4'-hydroxyflavylium and 7-hydroxyflavylium ions were reported to be 5.5 and 3.55, respectively.[11] In other words, in the competition between proton loss and water addition, the quinonoid base of **P5** is probably both the thermodynamic and kinetic product of the flavylium ion ($K_a > K_h$) and the concurrent route leading to the colorless forms is strongly inhibited.

3. Conclusion

Efficient syntheses have been devised for the red sorghum pigments luteolinidin and apigeninidin, as well as for a series of flavylium ions substituted by the typical OH, OMe and O-β-D-Glc groups of naturally occurring anthocyanins. Due to their high water solubility, the glucosides are convenient analogs of natural pigments for systematic investigations of the impact of substitution on color. Based on the preliminary study of two regioisomers, it seems clear that the glucosidation site has a considerable influence on the resulting color and its stability in aqueous solution. The detailed physico-chemical investigation of these pigments is under way.

4. Experimental

4.1. Materials and instruments

All starting materials were obtained from commercial suppliers and were used without purification. 4'-Hydroxyacetophenone, 4'-methoxyacetophenone, 2,4-dihydroxy-benzaldehyde, 2-hydroxy-4-methoxybenzaldehyde and chlorotrimethylsilane were purchased from Aldrich-Sigma (France). 2,4,6-Trihydroxybenzaldehyde was purchased from Extrasynthese (France). 3',4'-Dihydroxyacetophenone and 4-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyloxy)-2-hydroxybenzaldehyde (**1**) were prepared as already reported.[18] TLC analyses were performed on silica gel 60 F254 or C-18 silica gel F254s. Detection was achieved by UV light (254 nm) and by charring after exposure to a 5%

H_2SO_4 solution in EtOH. Purifications of intermediates were performed by column chromatography on silica gel 60 (40-63 µm, from Merck). Pigments were purified by elution on C18 silica gel cartridges (bond elut from Varian).

^{1}H- and ^{13}C-NMR spectra were recorded on an Ascend^{TM}400 Bruker apparatus at 400.18 MHz (^{1}H) or 100.62 MHz (^{13}C). Chemical shifts (δ) are in ppm relative to tetramethylsilane using the deuterium signal of the solvent ($CDCl_3$, CD_3OD) for calibration. ^{1}H-^{1}H coupling constants (J) are in Hz. ^{13}C-NMR signals of the glucosylated pigments were assigned from their HSQC spectra.

UPLC-MS analyses were performed on an Acquity Ultra Performance LC™ apparatus from Waters, equipped with an UV-visible diode array detector (DAD) and coupled with a Bruker Daltonics HCT ultra ion trap mass spectrometer monitoring in the positive electrospray ionization (ESI) mode. Separation was conducted on a 1.7 µm (2.1 - 50 mm) Acquity UPLC BEH C18 column thermostated at 30°C. Mass spectra were generated in the Ultrascan mode in the *m/z* range 100 - 1000. The ion source parameters were: nebulizer pressure = 50 psi, capillary voltage = 2 kV, drying gas flow = 10 L min^{-1}, drying gas temperature = 365°C. The mobile phase consisted of H_2O / HCO_2H (99:1, v/v) (eluent A) and MeCN / H_2O (60:40) + 1% HCO_2H (eluent B) at a flow rate of 0.5 mL min^{-1}. The elution program was: 5 - 20% B (0 - 5 min), 20 - 100% B (5 - 10 min), 100 - 5% B (10 - 11 min), 5% B (11 - 14 min).

HR-MS analysis was carried out on Qstar Elite mass spectrometer (Applied Biosystems SCIEX, Foster City, CA, USA). Mass detection was performed in the positive ESI mode.

UV-Vis absorption spectra were recorded on an Agilent 8453 diode array spectrometer equipped with a magnetically stirred quartz cell (optical path length = 1 cm). The temperature in the cell was controlled by means of a water-thermostated bath at 25°C.

4.2 Chemical synthesis

These syntheses prolong our previous work[18] about the chemical synthesis of 3’,4’,7-trihydroxyflavylium chloride (**P1**) and 3’,4’-dihydroxy-7-O-β-D-glucopyranosyloxyflavylium chloride (**P2**).

4.2.1. 4'-Hydroxy-7-O-β-D-glucopyranosyloxyflavylium chloride (**P3**)

Equimolar amounts (1 mmol) of **1** and 4-hydroxacetophenone were dissolved in 5 mL of AcOEt / MeOH (4/1 v/v) and cooled to 0°C. After addition of TMSCl (0.8 mL, 10 equiv.), the solution was stirred for 10 min and kept for 3 days at -18°C. The pigment was precipitated by adding an access AcOEt. After filtration, the solid was dissolved in MeOH (20 mL) under Ar and MeONa (8 equiv.) was added until pH 9 (wet pH paper). After stirring for 3h at room temperature, 1 M HCl was added (until pH 1). The mixture was kept for 12h at 4°C for complete cyclization, then concentrated under reduced pressure. The residue was dissolved in 0.01 M HCl and loaded on a C18 cartridge. After elution with 100 mL of 0.01 M HCl to remove contaminating NaCl, the compound was eluted with 0.2 M HCl in MeOH. After evaporation, the solid was crystallized in MeOH / AcOEt to give **P3** as a dark red powder. Yield = 42%, R_f = 0.18 (BAW, *i.e.* n-BuOH / AcOH / H_2O, 3/2/1, v/v/v). ^{1}H-NMR (CD_3OD / TFA-d, 95/5): δ = 9.13 (1H, *d*, *J* = 8.8, H_4), 8.51 (2H, *d*, *J* = 8.7, $H_{2'}$, $H_{6'}$), 8.45 (1H, *d*, *J* = 8.8, H_3), 8.20 (1H, *d*, *J* = 8.6, H_5), 7.90 (1H, *d*, *J* = 2.2, H_8), 7.60 (1H, *dd*, *J* = 8.6 and 2.2, H_6), 7.12 (2H, *d*, *J* = 8.7, $H_{3'}$, $H_{5'}$), 5.34 (1H, *d*, *J* = 7.1, $H_{1''}$), 3.98 (1H, broad *d*, *J* = 9.8, $H_{6''B}$), 3.71 - 3.76 (2H, *m*, $H_{5''}$, $H_{6''A}$), 3.55-3.60 (2H, *m*, $H_{2''}$, $H_{3''}$), 3.45 (1H, broad *t*, *J* = 9.4, $H_{4''}$). ^{13}C-NMR (CD_3OD / TFA-d, 95/5): δ = 175.1 (C_2), 169.0 (C_7), 167.3 (C_9), 159.3 ($C_{4'}$), 154.6 (C_4), 134.9 ($C_{2'}$, $C_{6'}$), 133.1 (C_5), 122.5 (C_6), 121.1 ($C_{1'}$), 121.0 (C_{10}), 118.3($C_{3'}$, $C_{5'}$), 115.3 (C_3), 105.0 (C_8), 101.9 ($C_{1''}$), 78.8 ($C_{5''}$), 77.8 ($C_{3''}$), 74.6 ($C_{2''}$), 71.3 ($C_{4''}$), 62.6 ($C_{6''}$). UV/VIS (0.01M HCl in MeOH): ε(465 nm) = 42800 M^{-1} cm^{-1}. The purity of **P3** was checked by UPLC-DAD-MS, t_R = 2.4 min, λ_{max} = 455 nm, *m*/*z* (M^+) = 400.9.

4.2.2. 4'-Methoxy-7-O-β-D-glucopyranosyloxyflavylium chloride (**P4**)

Equimolar amounts (1 mmol) of **1** and 4-methoxyacetophenone were dissolved in 6 ml of AcOEt/MeOH (1/1, v/v) and cooled to 0°C. After addition of TMSCl (0.8 mL, 10 equiv.), the mixture was stirred for 30 min and kept for 3 days at -18°C. The pigment was precipitated by adding an access of AcOEt. After filtration, the same deacetylation procedure as for **P3** was carried out. **P4** was obtained as a dark red powder after crystallization in MeOH / AcOEt / Et_2O. Yield = 23%, R_f = 0.37 (BAW). ^{1}H-NMR (CD_3OD / TFA-d, 95/5): δ = 9.22 (1H, *d*, *J* = 8.8, H_4), 8.60 (2H, *d*, *J* = 8.8, $H_{2'}$, $H_{6'}$), 8.45

(1H, *d*, J = 8.8, H_3), 8.53 (1H, *d*, J = 8.8, H_5), 7.96 (1H, *d*, J = 2.2, H_8), 7.65 (1H, *dd*, J = 8.8 and 2.2, H_6), 7.31 (2H, *d*, J =8.8, $H_{3'}$, $H_{5'}$), 5.35 (1H, *d*, J = 7.3, $H_{1''}$), 4.04 (3H, s, OCH_3), 3.98 (1H, broad *d*, J = 10.0, $H_{6''B}$), 3.68 - 3.75 (2H, *m*, $H_{5''}$, $H_{6''A}$), 3.53 - 3.60 (2H, *m*, $H_{2''}$, $H_{3''}$), 3.45 (1H, *t*, J = 9.0, $H_{4''}$). ^{13}C-NMR (CD_3OD / TFA-d, 95/5): δ = 175.1 (C_2), 169.4 (C_7), 167.7 (C_9), 159.7 ($C_{4'}$), 155.4 (C_4), 133.3 ($C_{2'}$, $C_{6'}$), 133.2 (C_5), 122.9 (C_6), 122.5 ($C_{1'}$), 121.4 (C_{10}), 117.3, 117.1 ($C_{3'}$, $C_{5'}$), 115.4 (C_3), 105.0 (C_8), 102.0 ($C_{1''}$), 78.8 ($C_{5''}$), 77.9 ($C_{3''}$), 74.6 ($C_{2''}$), 71.2($C_{4''}$), 62.6 ($C_{6''}$), 57.0 (OMe). UV/VIS (0.01 M HCl in MeOH): ε(458 nm) = 35700 M^{-1} cm^{-1}. The purity of **P4** was checked by UPLC-DAD-MS, t_R = 3.8 min, λ_{max} = 455 nm, m/z (M^+) = 414.9.

4.2.3. 4-(2',3',4',6'-Tetra-O-acetyl-β-D-glucopyranosyloxy)acetophenone (**3**)

A solution of **2** (1.68 g, 4.1 mmol) in distilled CH_2Cl_2 (5 mL) was added to a solution of 4-hydroxyacetophenone (1.49 g, 1.5 equiv.) and tris(2-(2-methoxyethoxy)ethyl)amine (3.52 mL, 1.5 equiv.) in 1 M $NaHCO_3$ / 1 M KCl (1:1) (pH 9, 5 mL). The mixture was refluxed for 72h. After addition of H_2O (10 mL) and extraction with CH_2Cl_2 (3x10 mL), the combined organic phases were successively washed with 1 M HCl (2x10 mL), then with H_2O (2x10 mL), dried over Na_2SO_4 and concentrated. The syrupy residue was purified on silica gel using cHex/ AcOEt (8/2 to 7.5/2.5, then 7/3, v/v). Finally, crystallization in MeOH / H_2O gave **3** as white crystals. Yield = 25%, R_f = 0.21 (cHex / AcOEt, 6/4, v/v). ^{1}H-NMR ($CDCl_3$): δ = 7.87 (2H, *d*, J = 8.8, $H_{2'}$, $H_{6'}$), 6.96 (2H, *d*, J = 8.8, $H_{3'}$, $H_{5'}$), 5.24 (2H, *m*, $H_{3''}$, $H_{4''}$), 5.12 (2H, *m*, $H_{1''}$, $H_{2''}$), 4.22 (1H, *dd*, J = 12.3 and 5.4, $H_{6''B}$), 4.10 (1H, *dd*, J = 12.3 and 2.3,$H_{6''A}$), 3.85 (1H, *m*, $H_{5''}$), 2.50 (3H, *s*, OCH_3), 1.99 (12H, broad *s*, 4Ac). ^{13}C-NMR ($CDCl_3$): δ = 196.6 (C=O), 160.2 ($C_{1'}$), 132.5 ($C_{4'}$), 130.5($C_{2'}$, $C_{6'}$), 116.3($C_{3'}$, $C_{5'}$), 98.2 ($C_{1''}$), 72.6 ($C_{3''}$), 72.3 ($C_{5''}$), 71.1 ($C_{2''}$), 68.2 ($C_{4''}$), 61.9 ($C_{6''}$), 20.7 (OMe), 20.6 (Me of Ac).

4.2.4. 4-O-β-D-Glucopyranosyloxyacetophenone (**4**)

Compound **3** (320 mg, 0.69 mmol) was dissolved in dry MeOH (10 mL) and treated with a catalytic amount of MeONa (pH 8-9 using wet pH paper). After stirring for 4 h, total deacetylation was confirmed by TLC (cHex / AcOEt, 3/2, v/v).The mixture was then acidified with ion exchange resin (Amberlite IRC_{50}, H^+ form) to pH 1-2 (wet pH paper), filtered and evaporated. White crystals were obtained after crystallization in MeOH / Et_2O. Yield = 88%, R_f = 0.88 (cHex / AcOEt, 6/4, v/v). ^{1}H NMR (CD_3OD): δ = 8.98 (2H, *d*, J =

8.9, $H_{2'}$, $H_{6'}$), 7.17 (2H, *d*, *J* = 8.9, $H_{3'}$,$H_{5'}$), 5.03 (1H, *d*, *J* = 7.5, $H_{1''}$), 3.90 (1H, *dd*, *J* = 12.0 and 2.2, $H_{6''B}$), 3.70 (1H, *dd*, *J* = 12.0 and 5.6, $H_{6''A}$), 3.48 - 3.50 (3H, *m*,$H_{2''}$, $H_{3''}$,$H_{5''}$), 3.40 (1H, *dd*, *J* = 5.4 and 2.0, $H_{4''}$), 2.56 (3H, *s*, CH_3). ^{13}C-NMR (CD_3OD): δ = 199.4 (C=O), 163.1 ($C_{1'}$), 132.7 ($C_{4'}$), 131.6 ($C_{2'}$, $C_{6'}$), 117.3 ($C_{3'}$, $C_{5'}$), 101.6 ($C_{1''}$), 78.3 ($C_{5''}$), 77.3 ($C_{3''}$), 74.8 ($C_{2''}$), 71.3 ($C_{4''}$), 62.5 ($C_{6''}$), 49.5 (OMe), 26.5 (Me of Ac).

4.2.5. 4'-O-β-D-Glucopyranosyloxy-7-hydroxyflavylium chloride (**P5**)

Equimolar amounts (0.5 mmol) of **4** and 2,4-dihydroxybenzaldehyde were dissolved in 15 mL of AcOEt / MeOH (1/2, v/v). After addition of TMSCl (1.6 mL, 20 equiv.), the solution was stirred for 30 min at room temperature. The pigment was precipitated by adding AcOEt and isolated as a red powder after filtration. Yield = 77%, R_f = 0.14 (BAW). ^{1}H-NMR (CD_3OD / TFA-d, 95/5): δ = 9.15 (1H, *d*, *J*= 8.9, H_4), 8.48 (2H, *d*, *J* = 9.1, $H_{2'}$, $H_{6'}$), 8.39 (1H, d, *J* = 8.9, H_3), 8.19 (1H, *d*, *J* = 8.9, H_5), 7.54 (1H, *d*, *J* = 2.2, H_8), 7.42 (1H, *dd*, *J* = 8.9 and 2.2, H_6), 7.40 (2H, *d*, *J* = 9.1, $H_{3'}$, $H_{5'}$), 5.17 (1H, *d*, *J* = 7.2, $H_{1''}$), 3.93 (1H, *dd*, *J* = 12.0 and 2.2, $H_{6''B}$), 3.72 (1H, *dd*, *J* = 12.0 and 5.8, $H_{6''A}$), 3.55-3.57 (3H, *m*, $H_{2''}$, $H_{3''}$, $H_{5''}$), 3.45 (1H, *t*, *J* = 9.2, $H_{4''}$).^{13}C-NMR (CD_3OD / TFA-d, 95/5): δ = 173.4 (C_2), 171.0 (C_7), 165.9 (C_9), 160.9 ($C_{4'}$), 155.4 (C_4), 134.2 (C_5), 133.1 ($C_{2'}$, $C_{6'}$), 124.1 ($C_{1'}$), 123.0 (C_6), 120.8 (C_{10}), 119.1 ($C_{3'}$, $C_{5'}$), 113.7 (C_3), 103.7 (C_8), 101.5 ($C_{1''}$), 78.6 ($C_{5''}$), 77.9 ($C_{3''}$), 74.7 ($C_{2''}$), 71.2 ($C_{4''}$), 62.5 ($C_{6''}$). UV/VIS (0.01 M HCl in MeOH), ε(460 nm) = 37600 M^{-1} cm^{-1}. The purity of **P5** was checked by UPLC-DAD-MS: t_R = 2.6 min, λ_{max}= 450 nm, *m/z* (M^+) = 400.9.

4.2.6. 4'-O-β-D-Glucopyranosyloxy-7-methoxyflavylium chloride (**P6**)

Equimolar amounts (1 mmol) of **4** and 2-hydroxy-4-methoxybenzaldehyde were dissolved in 15 mL of AcOEt / MeOH (1/2, v/v). After addition of TMSCl (20 equiv.), the mixture was stirred for 30 min at room temperature. **P6** as a dark red powder was obtained by simple filtration. Yield = 77%, R_f = 0.29 (BAW). ^{1}H-NMR (CD_3OD / TFA-d, 95/5): δ= 9.22 (1H, *d*, *J*= 8.7, H_4), 8.55 (2H, *dd*, *J* = 9.1, $H_{2'}$, $H_{6'}$), 8.50 (1H, *d*, *J*= 8.7, H_3), 8.22 (1H, *d*, *J* = 8.9, H_5), 7.88 (1H, *d*, *J* = 2.2, H_8), 7.56 (1H, *dd*, *J* = 8.9 and 2.2, H_6), 7.42 (2H, *d*, *J* = 9.1, $H_{3'}$, $H_{5'}$), 5.18 (1H, *d*, *J* = 7.4, $H_{1''}$), 4.17 (3H, OCH_3), 3.92 (1H, *dd*, *J* = 12.0 and 2.2, $H_{6''B}$), 3.71 (1H, *dd*, *J* = 12.0 and 5.9, $H_{6''A}$), 3.56 - 3.60 (3H, *m*, $H_{2''}$, $H_{3''}$, $H_{5''}$), 3.53 (1H, *t*, J = 9.2, $H_{4''}$). ^{13}C-NMR (CD_3OD / TFA-d, 95/5): δ = 174.1 (C_2), 171.2 (C_7), 166.2 (C_9), 160.9 ($C_{4'}$), 155.7 (C_4), 133.5 (C_5), 133.2 ($C_{2'}$, $C_{6'}$), 124.0 ($C_{1'}$), 123.1 (C_6), 121.3 (C_{10}), 119.2 ($C_{3'}$, $C_{5'}$), 114.8 (C_3), 101.6 (C_8), 101.5 ($C_{1''}$), 78.6 ($C_{5''}$), 77.9 ($C_{3''}$), 74.7 ($C_{2''}$), 71.2 ($C_{4''}$), 62.5 ($C_{6''}$), 58.1 (OMe). UV/VIS (0.01 M HCl in MeOH): ε(455 nm) = 25400 M^{-1} cm^{-1}. The purity of **P6** was checked by UPLC-DAD-MS: t_R = 3.4 min, λ_{max} = 450 nm, *m/z* (M^+) = 414.9.

4.2.7. 4',7-Dihydroxyflavylium chloride (**P7**)

Equimolar amounts (1 mmol) of 2,4-dihydroxybenzaldehyde and 4-hydroxyacetophenone were dissolved in 5 mL of AcOEt / MeOH (4/1, v/v). After cooling to 0°C, TMSCl (10 equiv.) was added. The mixture was stirred for 10 min, then kept at 4°C for 3 days. Precipitation of pigment was achieved by adding AcOEt. After filtration, **P7** was obtained as a dark red powder. Yield = 45%, R_f = 0.52 (ABFW, *i.e*. AcOH / n-BuOH / HCO_2H / H_2O, 20/2/1/1, v/v/v). ^{1}H-NMR (CD_3OD / TFA-d, 95/5): δ = 9.04 (1H, *d*, J = 8.8, H_4), 8.42 (2H, *dd*, J = 8.9 and 2.0, $H_{2'}$, $H_{6'}$), 8.30 (1H, *d*, J = 8.8, H_3), 8.13 (1H, *d*, J = 8.9, H_5), 7.49 (1H, *d*, J = 1.9, H_8), 7.40 (1H, broad *d*, J = 8.9, H_6), 7.10 (2H, *dd*, J = 8.9 and 2.0, $H_{3'}$, $H_{5'}$). ^{13}C-NMR (CD_3OD / TFA-d, 95/5): δ = 173.8 (C_2), 170.2 (C_7), 168.2 (C_9), 160.2 ($C_{4'}$), 154.4 (C_4), 134 ($C_{2'}$, $C_{6'}$), 133.9 (C_5), 122.3 (C_6), 121.2 ($C_{1'}$), 120.0 (C_{10}), 118.6 ($C_{3'}$, $C_{5'}$), 113.4 (C_3), 103.7 (C_8). UV/VIS (0.01 M HCl in MeOH): ε(470 nm) = 59700 M^{-1} cm^{-1}.The purity of **P7** was checked by UPLC-DAD-MS: t_R = 4.8 min, λ_{max} = 465 nm, *m/z* (M^+) = 238.7.

4.2.8. 7-Hydroxy-4'-methoxyflavylium chloride (**P8**)

Equimolar amounts (1 mmol) of 2,4-dihydroxybenzaldehyde and 4-methoxyacetophenone were reacted in the same conditions as for **P7**. **P8** was obtained as a dark red powder. Yield = 90%, R_f = 0.18 (EBFW). ^{1}H-NMR (CD_3OD / TFA-d, 95/5): δ = 9.10 (1H, *d*, J = 8.7, H_4), 8.47 (2H, *dd*, J = 7.6 and 2.2, $H_{2'}$, $H_{6'}$), 8.35 (1H, *d*, J = 8.7, H_3), 8.15 (1H, *d*, J = 9.0, H_5), 7.51 (1H, *d*, J = 2.2, H_8), 7.42 (1H, *dd*, J = 9.0 and 2.2, H_6), 7.25 (2H, *dd*, J = 7.6 and 2.0, $H_{3'}$, $H_{5'}$), 3.99 (3H, OCH_3). ^{13}C-NMR (CD_3OD / TFA-d, 95/5): δ = 173.5 (C_2), 170.7 (C_7), 168.6 (C_9), 160.5 ($C_{4'}$), 154.9 (C_4), 134.0 (C_5), 133.4 ($C_{2'}$, $C_{6'}$), 122.7 (C_6), 122.5 ($C_{1'}$), 120.4 (C_{10}), 117.1 ($C_{3'}$, $C_{5'}$), 113.5 (C_3), 103.7 (C_8), 56.8 (OCH_3).

UV/VIS (0.01 M HCl in MeOH): ε(467 nm) = 57500 M^{-1} cm^{-1}. The purity of **P8** was checked by UPLC-DAD-MS: t_R = 5.9 min, λ_{max} = 465 nm, m/z (M^+) = 252.7.

4.2.9. 4'-Hydroxy-7-methoxyflavylium chloride (**P9**)

Equimolar amounts (1 mmol) of 2-hydroxy-4-methoxybenzaldehyde and 4-hydroxyacetophenone were reacted in the same conditions as for **P7**. **P9** was obtained as a dark red powder. Yield = 85%, R_f = 0.18 (EBFW). ^{1}H-NMR (CD_3OD / TFA-d, 95/5): δ = 9.10 (1H, *d*, *J* = 8.8, H_4), 8.46 (2H, *dd*, *J* = 7.5 and 2.0, $H_{2'}$, $H_{6'}$), 8.39 (1H, *d*, *J* = 8.8, H_3), 8.15 (1H, *d*, *J* = 9.0, H_5), 7.8 (1H, *d*, *J* = 2.2, H_8), 7.49 (1H, broad *d*, *J* = 9.0, H_6), 7.1 (2H, *dd*, *J* = 7.5 and 2.0, $H_{3'}$, $H_{5'}$), 4.15 (3H, *s*, OCH_3). ^{13}C-NMR (CD_3OD / TFA-d, 95/5): δ = 174.3 (C_2), 170.4 (C_7), 168.6 (C_9), 160.2 ($C_{4'}$), 154.4 (C_4), 134.4 ($C_{2'}$, $C_{6'}$), 133 (C_5), 121.1 ($C_{1'}$), 122.3 (C_6), 120.5 (C_{10}), 118.8 ($C_{3'}$, $C_{5'}$), 114.4 (C_3), 101.5 (C_8), 58.0 (OMe). UV/VIS (0.01 M HCl in MeOH): ε(467 nm) = 50400 M^{-1} cm^{-1}. The purity of **P9** was checked by UPLC-DAD-MS: t_R= 6.1 min, λ_{max} = 465 nm, m/z (M^+) = 252.7.

4.2.10. 4',5,7-Trihydroxyflavylium chloride (apigeninidin)

A solution of 2,4,6-trihydroxybenzaldehyde (1 mmol) and 4-hydroxyacetophenone (2 equiv.) in 3 mL of AcOEt / MeOH (2/1, v/v) was cooled to 0°C and TMSCl (20 equiv.) was added. After 60 min of stirring, complete precipitation of APN was achieved. Crystallization in MeOH / AcOEt afforded APN as a red powder. Yield = 34%, R_f = 0.83 (BAW). ^{1}H-NMR (CD_3OD / TFA-d, 95/5): δ = 9.10 (1H, *d*, *J* = 8.7, H_4), 8.33 (2H, *dd*, *J* = 8.9 and 2.0, $H_{2'}$, $H_{6'}$), 8.05 (1H, *d*, *J* = 8.7, H_3), 7.08 (2H, *dd*, *J* = 8.9 and 2.0, $H_{3'}$, $H_{5'}$), 6.95 (1H, *d*, *J* = 2.1, H_8), 6.66 (1H, *d*, *J* = 2.1, H_6). ^{13}C-NMR (CD_3OD / TFA-d, 95/5): δ = 172.9 (C_2), 172.3 (C_7), 167.3 (C_9), 160.4 (C_5), 160.1 ($C_{4'}$), 149.6 (C_4), 133.2 ($C_{2'}$, $C_{6'}$), 121.4 ($C_{1'}$), 118.4 ($C_{3'}$, $C_{5'}$), 113.8 (C_{10}), 110.4 (C_6), 103.1 (C_3), 96.1 (C_8). UV/VIS (0.01 M HCl in MeOH): ε(480 nm) = 38400 M^{-1} cm^{-1}.The purity of APN was checked by UPLC-DAD-MS: t_R = 5.7 min, λ_{max} = 475 nm, m/z (M^+) = 254.7. HRMS-ESI: m/z(M^+) calcd = 255.0652, found = 255.0651.

4.2.11. 3',4',5,7-Tetrahydroxyflavylium chloride (luteolinidin)

Equimolar amounts (1 mmol) of 2,4,6-trihydroxybenzaldehyde and 3,4-dihydroxyacetophenone were dissolved in 3 mL of AcOEt / MeOH (2/1, v/v). After cooling to 0°C, TMSCl (20 equiv.) was added and the solution stirred for 30 min at 0°C.

LTN was totally precipitated by adding AcOEt. After filtration, LTN was isolated as a red powder (yield = 65%). To remove the yellow xanthylium contaminant (*ca*. 5%), LTN was crystallized in EtOH / AcOEt. R_f = 0.83 (BAW). ^{1}H-NMR (CD_3OD / TFA-d, 95/5): δ = 9.02 (1H, *d*, *J* = 8.7, H_4), 7.97 (1H, *d*, *J* = 8.7, H_3), 7.88 (1H, *dd*, *J* = 8.7 and 2.3, $H_{6'}$), 7.73 (1H, *d*, *J* = 2.3, $H_{2'}$), 7.04 (1H, d, *J* = 8.7, $H_{5'}$), 6.89 (1H, *d*, *J* = 2.1, H_8), 6.62 (1H, *d*, *J* = 2.1, H_6). ^{13}C-NMR (CD_3OD / TFA-d, 95/5): δ = 173.6 (C_2), 173.1 (C_7), 161.3 (C_9), 160.8 (C_5), 157.2 ($C_{4'}$), 149.9 (C_4), 149.1 ($C_{3'}$), 126.1 ($C_{6'}$), 122.6 ($C_{1'}$), 118.7 ($C_{5'}$), 116.8 ($C_{2'}$), 114.5 (C_{10}), 111.4 (C_6), 104.0 (C_3), 96.9 (C_8). UV/VIS (0.01 M HCl in MeOH): ε(500 nm) = 24000 M^{-1} cm^{-1}. The purity of LTN was checked by UPLC-DAD-MS: t_R = 4.9 min, λ_{max} = 485 nm, *m*/*z* (M^+) = 270.7. HRMS-ESI: *m/z*(M^+) calcd=271.0601, found = 271.0603.

4.2.12. 4-Benzyloxy-2,6-dihydroxybenzaldehyde (**5**) *and 2-benzyloxy-4,6-dihydroxy-benzaldehyde* (**5’**)

A solution of 2,4,6-trihydroxybenzaldehyde (3 g, 19.5 mmol) and dry K_2CO_3 (2.69 g, 1 equiv.) in 20 mL of anhydrous DMF was cooled to 0°C and stirred for 15 min. Then, benzyl bromide (2.31 mL, 1 equiv.) was added drop-wise and the mixture was kept at 0°C under vigorous stirring during 2.5h. Glacial 0.1 M HCl (100 mL) was added and the aqueous phase extracted with AcOEt (3×100 mL). The combined organic phases were dried over Na_2SO_4, then concentrated. The crude product was purified by column chromatography on silica gel (cHex / AcOEt, 7/3, v/v) to afford the mixture of two regioisomers **5** and **5’** (1/4 molar ratio) as a white amorphous powder. Total yield = 45%, R_f = 0.57 (cHex / AcOEt, 6/4, v/v). **5**. ^{1}H-NMR(CD_3OD): δ = 10.05 (1H, *s*, CHO), 7.41 (5H, *m*, Ph), 5.97 (2H, *s*, H_3, H_5), 5.09 (2H, *s*, CH_2). ^{13}C-NMR (CD_3OD): δ = 191.1 (CHO), 167.5 (C_2, C_6), 163.5 (C_4), 136.2 ($1C_{Ph}$), 128.3, 128.2, 127.2 ($5C_{Ph}$), 95.1 (C_3, C_5), 70.2 (CH_2). **5’**. ^{1}H-NMR (CD_3OD): δ = 10.08 (1H, *s*, CHO), 7.41 (5H, *m*, Ph), 6.01 (1H, *d*, *J* = 2.0, H_5), 5.86 (1H, *d*, *J* = 2.0, H_3), 5.07 (2H, *s*, CH_2). ^{13}C-NMR (CD_3OD): δ = 191.9 (CHO), 165.9 (C_6), 164.2 (C_4), 163.5 (C_2), 136.3 ($1C_{Ph}$), 128.2, 127.9, 127.8 ($5C_{Ph}$), 105.6 (C_1), 93.3 (C_5), 91.8 (C_3), 69.8 (CH_2).

4.2.13. 2-Benzyloxy-6-hydroxy-4-(2’,3’,4’,6’-tetra-O-acetyl-β-D-glucopyranosyloxy)-benzaldehyde (**6**)

A solution of tetra-*O*-acetyl-α-D-glucopyranosylbromide (**2**, 2.03 g, 1.5 equiv.) in distilled CH_2Cl_2 (10 mL) was added to a solution of **5** and **5’** (1 g, 3.28 mmol) and tris[2-(2-methoxyethoxy)ethyl]amine (TMEA) (1.58 mL, 1.5 equiv.) in a saturated aqueous K_2CO_3 solution (10 mL). The mixture was refluxed for 18 h. After the same treatment as for **3**, the syrupy residue was purified on silica gel (cHex / AcOEt, 65/35, v/v), then crystallized in CH_2Cl_2 / Et_2O to afford **6** as white crystals. Yield = 49%, R_f = 0.52 (cHex / AcOEt, 6/4, v/v). ^{1}H-NMR($CDCl_3$): δ = 12.36 (1H, *s*, OH), 10.19 (2H, *s*, CHO), 7.39 (5H, *m*, Ph), 6.59 (1H, *d*, *J* = 2.2, H_5), 6.54 (1H, *d*, *J* = 2.2, H_3), 5.10 (2H, *s*, CH_2), 5.06-5.28 (4H, *m*, $H_{1'}$, $H_{2'}$, $H_{3'}$, $H_{4'}$), 4.15 - 4.22 (2H, 2*dd*, *J* = 12.1 and 6.1, *J* = 12.1 and 2.1,$H_{6'A}$, $H_{6'B}$), 3.87 (1H, *m*, $H_{5'}$), 2.02 - 2.07 (12H, *m*, 4Ac). ^{13}C-NMR ($CDCl_3$): δ = 192.5 (CHO), 169.6-170.2 (4 C=O), 166.1 (C_2), 165.9 (C_6), 164.3 (C_4), 135.5 ($1C_{Ph}$), 128.8, 128.5, 128.1, 127.9, 127.1 ($5C_{Ph}$), 107.3 (C_1), 95.7 ($C_{1'}$), 97.5 (C_5), 93.3 (C_3), 72.6 ($C_{3'}$), 72.4 ($C_{5'}$), 70.9 ($C_{2'}$), 70.7 (CH_2), 68.2 ($C_{4'}$), 61.9 ($C_{6'}$), 20.6 (4Me).

4.2.14. 2,6-Dihydroxy-4-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyloxy)benzaldehyde (**7**)

Compound **6** (1 g, 1.74 mmol) was dissolved in 10 mL of THF / MeOH (4/1, v/v) and 10% Pd on activated charcoal (0.124 g, 0.12 mmol) was added. Debenzylation was carried out in a hydrogenation reactor under 2 bars of H_2 for 30 min. The mixture was diluted in AcOEt (30 mL), filtered over celite, concentrated and purified on silica gel (AcOEt / cHex, 45/55, v/v). After crystallization in AcOEt / n-hexane, **7** was obtained as white crystals. Yield = 90%, R_f = 0.27 (cHex / AcOEt, 6/4, v/v). ^{1}H-NMR ($CDCl_3$): δ = 12.36 (2H, *s*, OH), 10.19 (1H, *s*, CHO), 6.01 (2H, *s*, H_3, H_5), 5.28 - 5.33 (2H, *m*, $H_{3'}$, $H_{4'}$), 5.13 - 5.20 (2H, *m*, $H_{1'}$, $H_{2'}$), 4.18 - 4.33 (2H, 2*dd*, *J* = 11.0 and 5.7, 11.0 and 2.0, $H_{6'A}$, $H_{6'B}$), 3.92 (1H, *m*, $H_{5'}$), 2.07 - 2.13 (12H, *m*, 4Ac). ^{13}C-NMR ($CDCl_3$): δ = 192.5 (CHO), 169.8 - 171.3 (4C=O), 164.2 (C_2, C_4, C_6), 106.8 (C_1), 97.4 ($C_{1'}$), 95.6 (C_3, C_5), 72.6 ($C_{3'}$), 72.3 ($C_{5'}$), 71.0 ($C_{2'}$), 68.3 ($C_{4'}$), 62.0 ($C_{6'}$), 20.6 (4Me).

2.2.15. 7-(2'',3'',4'',6''-Tetra-O-acetyl-β-D-glucopyranosyloxy)-3',4',5-trihydroxy-flavylium chloride (**8**)

Condensation between equimolar quantities (1 mmol) of **7** and 3,4-dihydroxyacetophenone in the presence of TMSCl (20 equiv.) was achieved in 5 mL of AcOEt / MeOH (4/1, v/v). After 30 min at 0°C, **8** was isolated by filtration as a red powder. Yield = 60%. ^{1}H-NMR (CD_3OD): δ = 9.13 (1H, *d*, *J* = 8.6, H_4), 8.20 (1H, *d*, *J* = 8.6, H_3), 8.00 (1H, *d*, *J* = 8.9 and 1.9, $H_{6'}$), 7.84 (1H, *d*, *J* = 1.9, $H_{2'}$), 7.31 (1H, broad *s*, H_8), 7.06 (1H, *d*, *J* = 8.9, $H_{5'}$), 6.73 (1H, broad *s*, H_6), 5.73 (1H, *d*, *J* = 7.8, $H_{1''}$), 5.46 (1H, *t*, *J* = 9.3, $H_{3''}$), 5.27 (1H, *dd*, *J* = 9.3 and 7.8, $H_{2''}$), 5.18 (1H, *t*, *J* = 7.8, $H_{4''}$), 4.22 - 4.36 (3H, *m*, $H_{5''}$, $H_{6''A}$, $H_{6''B}$), 2.07 - 2.13 (12H, *m*, 4 Me).

2.2.16. 5-(β-D-glucopyranosyloxy)-3',4',7-trihydroxyflavylium chloride (LTN-5Glc) and 7-(β-D-glucopyranosyloxy)-3',4',5-trihydroxyflavylium chloride (LTN-7Glc).

The same deacetylation procedure as for **P3** was carried out. UPLC-MS analysis of the red powder obtained showed a mixture of two deprotected isomers and several less polar partially acetylated pigments. Purification over TSK gel with elution by 1% aqueous HCl afforded the two inseparable deprotected isomers. Overall yield (deprotection + purification) = 10%. The purity of the LTN-Glc mixture was checked by UPLC-DAD-MS. LTN-7Glc (60%): t_R = 1.85 min, λ_{max} = 485 nm, m/z (M^+) = 433.1. LTN-5Glc (40%): t_R = 1.90 min, λ_{max} = 485 nm, m/z (M^+) = 433.1. LTN-7Glc (major). ^{1}H-NMR (CD_3OD / TFA-d, 95/5): δ = 9.15 (1H, *d*, *J* = 8.8, H_4), 8.10 (1H, *d*, *J* = 8.8, H_3), 7.91 (1H, *dd*, *J* = 9.2 and 2.2, $H_{6'}$), 7.74 (1H, *d*, *J* = 2.2, $H_{2'}$), 7.06 (1H, broad *s*, H_8), 7.01 (1H, *d*, *J* = 9.2, $H_{5'}$), 7.00 (1H, *d*, *J* = 1.5, H_6), 5.18 (1H, *d*, *J* = 7.2, $H_{1''}$), 3.98 (1H, broad *d*, *J* = 11.9, $H_{6''B}$), 3.76 (1H, *dd*, *J* = 11.9 and 5.4, $H_{6''A}$), 3.64 (1H, *m*, $H_{5''}$), 3.51 - 3.61 (2H, *m*, $H_{2''}$,$H_{3''}$), 3.43 (1H, *t*, *J* = 9.4, $H_{4''}$). ^{13}C-NMR (CD_3OD / TFA-d, 95/5): δ = 171.4 (C_2), 169.1 (C_7), 158.1 (C_9), 157.5 (C_5), 157.1 ($C_{4'}$), 149.1 (C_4), 148.4 ($C_{3'}$), 126.0 ($C_{6'}$), 121.5 ($C_{1'}$), 117.8 ($C_{5'}$), 116.2 ($C_{2'}$), 113.5 (C_{10}), 111.9 (C_3), 105.0 (C_6), 101.8 ($C_{1''}$), 98.1 (C_8), 78.7 ($C_{3''}$), 77.8 ($C_{5''}$), 74.6 ($C_{2''}$), 71.2 ($C_{4''}$), 62.5 ($C_{6''}$). LTN-5Glc (minor). ^{1}H-NMR (CD_3OD / TFA-d, 95/5): δ = 9.09 (1H, *d*, *J* = 8.8, H_4), 8.14 (1H, *d*, *J* = 8.8, H_3), 7.96 (1H, *dd*, *J* = 9.2 and 2.2, $H_{6'}$), 7.79 (1H, *d*, *J* = 2.2, $H_{2'}$), 7.28 (1H, broad *s*, H_8), 7.03 (1H, *d*, *J* = 9.2, $H_{5'}$), 6.90 (1H, *d*, *J* = 1.5, H_6), 5.24 (1H, *d*, *J* = 7.2, $H_{1''}$), 3.98 (1H, broad *d*, *J* = 11.9, $H_{6''B}$), 3.76 (1H, *dd*, *J* = 11.9 and 5.4, $H_{6''A}$), 3.64 (1H, *m*, $H_{5''}$), 3.51 - 3.61 (2H, *m*, $H_{2''}$,$H_{3''}$), 3.43 (1H, *t*, *J* = 9.4, $H_{4''}$). ^{13}C-NMR (CD_3OD / TFA-d, 95/5): δ = 173.3 (C_7), 171.4 (C_2), 159.6 (C_5), 158.1 (C_9), 157.1 ($C_{4'}$), 149.5 (C_4), 149.0 ($C_{3'}$), 126.5 ($C_{6'}$), 121.5 ($C_{1'}$), 118.0 ($C_{5'}$), 116.5 ($C_{2'}$), 114.0 (C_{10}), 112.7 (C_3), 103.8 (C_6), 101.8 ($C_{1''}$), 97.7 (C_8), 78.7 ($C_{3''}$), 77.7 ($C_{5''}$), 74.6 ($C_{2''}$), 71.1 ($C_{4''}$), 62.4 ($C_{6''}$).

5. Supplementary Information

^{1}H-NMR and UPLC-DAD-MS analyses of all pigments.

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A simple synthesis of 3-deoxyanthocyanidins and their O-glucosides

Sheiraz Al Bittar, Nathalie Mora, Michèle Loonis, Olivier Dangles*

Highlights

- Synthesis of flavylium ions with OH, OMe and β-D-GlcO groups at positions 4' and 7
- Sorghum 3-deoxyanthocyanidins prepared in one-step
- Synthesis of a mixture of 5- and 7-O-β-D-glucosides of luteolinidin
- Glucose at C4'-OH or C7-OH causes huge differences in color intensity and stability