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# Deuterium Labeled Procyanidin Syntheses

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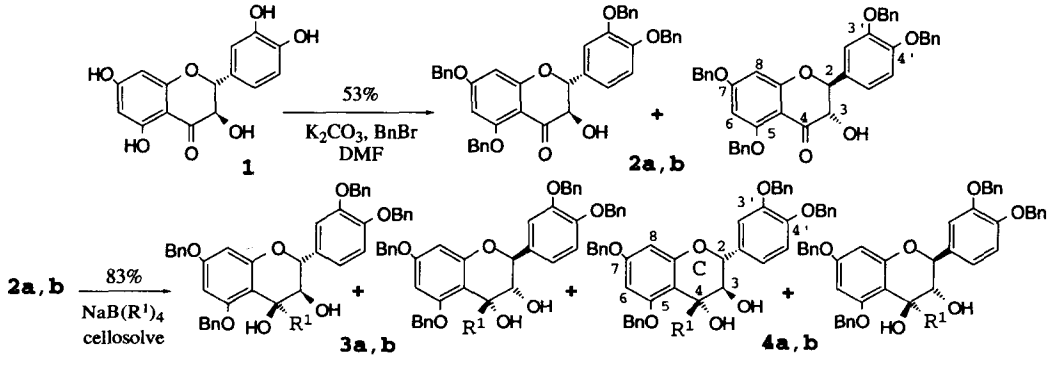
**Abstract** : Deuterium-labeled procyanidins have been prepared by hemisynthesis from taxifolin in order to investigate their metabolism in human. The structures of the desired deuterated natural compounds B3 **10D** ($R^1$=D, $R^2$=H) and B4 **13D** ($R^1$=D, $R^2$=H) were proven by spectroscopic and physical properties means, including $^2H$ NMR spectrum. By-products with unatural absolute configuration at some centers were also formed along the process and were characterised.


Epidemiological studies[1] suggest that moderate consumption of red wine may lower the incidence of cardiovascular diseases. Compared with other alcoholic beverages, red wine is rich in polyphenolic compounds : anthocyanins and procyanidins[2] are abundant, other polyphenols[3, 4] isolated most recently are present in lower yield. *In vitro* studies have proven the antioxidative[5] and radical scavenging[2] efficiency of procyanidins in inhibiting lipoperoxidation[6], the antihypertensive[7] and anticollagenolytic[8] properties, putatively explaining their biological effects. Curiously, little is known about polyphenol metabolism[8] in human, in particular the bioavailability of procyanidins of the B series has never been established. Our aim was to identify these compounds in human fluids after a dietary level intake. For the purpose of developing qualitative and quantitative analytical tools, deuterated natural procyanidins were required.

We synthesised [4C-$^2H_1$]-procyanidin B3 **10D** ($R^1$=D, $R^2$=H) and B4 **13D** ($R^1$=D, $R^2$=H) *via* four step reactions in 8% and 19% overall yields respectively, starting from (+)-taxifoliol **1**. In order to be able to establish relative configuration at C4 (by measuring coupling constants) in intermediate and final products bearing a deuterium at this position, we first synthesised the complete series of unlabeled compounds ($R^1$=H).

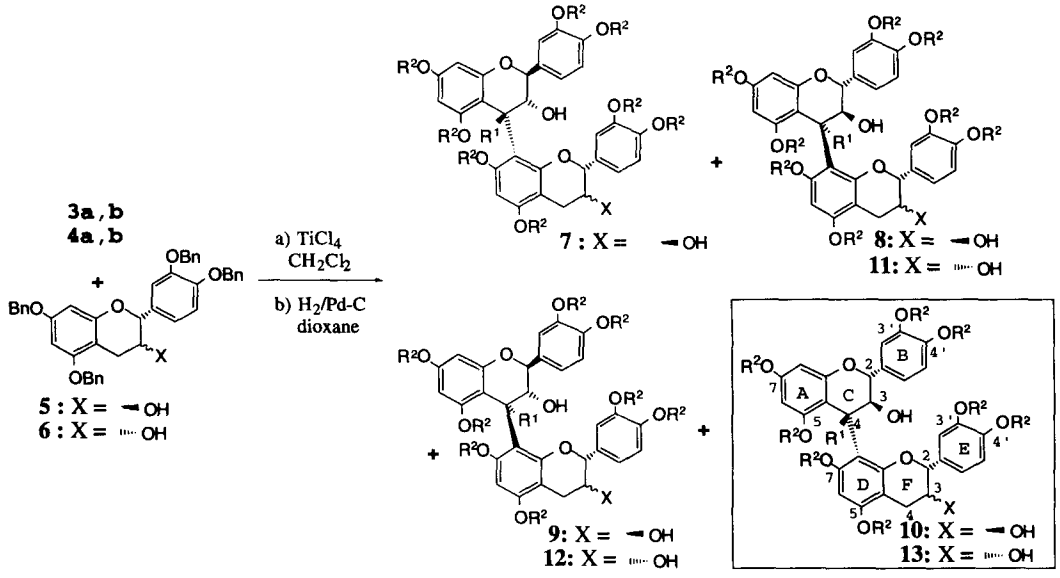
During protection step ($K_2CO_3$, BnBr), (+)-taxifoliol **1** partly racemised to afford an unseparable mixture of **2a,b** (scheme1).




Scheme 1

Indeed, the $^1H$ NMR spectrum of compound **2a,b** taken in the presence of Eu(hfc)$_3$ (0.5 eq.) showed two signals in a 3:7 ratio, at 7.13 and 7.09 ppm, for the H-6 proton, substantiating both C2, C3 epimerisation, through quinone methide formed in a basic medium. However, the positive optical rotation ($[\alpha]_D^{25}$ = 21, c = 1, $H_2O$/acetone-v/v) of taxifoliol derived from **2a,b** by hydrogenolysis with $H_2$/Pd-C is still in favour of a 2*R*,3*S*

absolute configuration for predominant enantiomer. Compound **2a,b** was converted upon reduction with $NaBH_4$, into *cis*-glycol **3a,b** ($R^1$=H) and *trans*-glycol **4a,b** ($R^1$=H), in a 1:1 ratio, evaluated after separation on $SiO_2$ (hexane/$CH_2Cl_2$). Dimeric products **7H-10H**, and **11H-13H** ($R^1$=H, $R^2$=Bn) were prepared by titanium tetrachloride catalysed (TTC) condensation of diols **3a,b** and **4a,b** with optically pure (2*R*,3*S*)-tetra-*O*-benzylcatechin **5** and (2*R*,3*R*)-tetra-*O*-benzylepicatechin **6** respectively (scheme 2), under the same conditions as those described by Kawamoto[9] ($CH_2Cl_2$, 0°C, **5** 5 eq., $TiCl_4$ 1 eq.). Diols **3a,b** and **4a,b** were not separated since Kawamoto reported that TTC condensation of pure enantiomer of (2*R*,3*S*,4*S*)-diol **3** with (2*R*,3*S*)-tetra-*O*-benzylcatechin **5** afforded a mixture of dimeric procyanidins, consisting of 3,4-*cis* and 3,4-*trans* configuration for upper unit in a 2:3 ratio.




**Unlabeled series** : **7H**, ... , **13H** : $R^1$=H
**Deuterated series** : **7D**, ... , **13D** : $R^1$=D

Scheme 2

Relative configurations of carbons C-2C, C-3C and C-4C and interflavanoid linkage of compounds **11H-13H** ($R^1$=H, $R^2$=Bn), obtained in the 8:25:67 ratio after chromatography on $SiO_2$ ($CH_2Cl_2$), were characterised by 2D NMR correlation spectroscopies[10]. As Kawamoto[9] also stated, we observed a [4→8] interflavanoid linkage in every of the formed compounds of the two series **7-10** and **11-13**.

Procyanidin B4 **13H** ($R^1$=$R^2$=H) ($[\alpha]_D^{25}$ = -181, c = 0.16, ethanol ; Lit. $[\alpha]_{578}^{20}$ = 193.5, c = 1, ethanol[11]), its diastereomers **12H**[12] and **11H**[13] ($R^1$=$R^2$=H) were obtained after hydrogenolysis of the corresponding dimeric compounds **13H-11H** ($R^1$=H, $R^2$=Bn), in 44%, 17% and 5% yield respectively from **2a,b**.

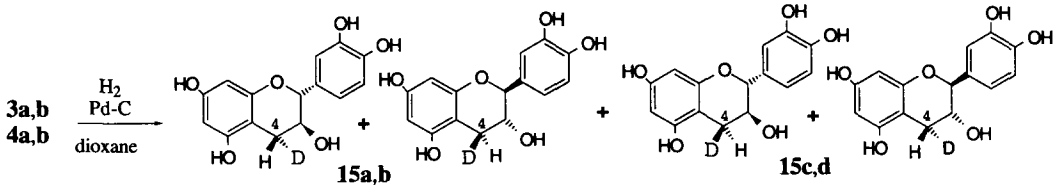
**Synthesis of labeled compounds :**

The same procedure conducted with $NaBD_4$ afforded [4C-$^2H_1$]-procyanidin B4 **13D**[14] ($R^1$=D, $R^2$=H), the structure of which was established by NMR and mass spectroscopies. The absence at 4.41 ppm of H-4C signal in the $^1H$ NMR spectrum, and the appearance of a singlet at this chemical shift in the $^2H$ NMR spectrum, confirmed the exchange of the H-4C by a deuterium.

These hemisyntheses were reproduced in presence of (2*R*,3*S*)-3',4',5,7-tetra-*O*-benzylcatechin **6** to afford [4C-$^2H_1$]-procyanidin B3 **10D** ($R^1$=D, $R^2$=H). Crude mixtures of TTC condensations were

chromatographed ($SiO_2$, hexane/$CH_2Cl_2$) and yielded pure **7D** ($R^1$=D, $R^2$=Bn, 4%), **8D** ($R^1$=D, $R^2$=Bn, 20%) and unseparable **9D**, **10D** ($R^1$=D, $R^2$=Bn, 60%). Compounds **7D** and **8D** ($R^1$=D, $R^2$=Bn) were identified by 1D and 2D NMR and by comparison with the corresponding unlabeled products, **7H**[15] and **8H**[16] ($R^1$=H, $R^2$=Bn). Debenzylation of mixture **9D**, **10D** ($R^1$=D, $R^2$=Bn) allowed purification of native procyanidins by preparative C18 HPLC furnishing [4C-$^2H_1$]-procyanidin B3 **10D** ($R^1$=D, $R^2$=H) and its diastereomer **9D**[17] ($R^1$=D, $R^2$=H) in the 7:3 ratio. Structure of [4C-$^2H_1$]-procyanidin B3 **10D**[18] ($R^1$=D, $R^2$=H) was corroborated by $^2H$ NMR spectrum and comparison of the $^1H$ NMR spectrum with the one of undeuterated compound **10H** ($R^1$=$R^2$=H), which proved to be identical with that already described in our laboratories[10].

Deuterated (+)- and (-)-catechin, were also prepared in a 3:1 ratio with 46% yield from glycols **3**,**4**. As hydrogenolysis was realised on 1:1 mixture of [4-$^2H_1$]-*cis* **3a**,**b** and *trans*-glycols **4a**,**b**, [4α-$^2H_1$]-catechin **15a**,**b** and [4β-$^2H_1$]-catechin **15c**,**d**[19] were coprecipitated (scheme 3). On the $^1H$ NMR spectrum, two signals integrated for 0.5 H each at 2.66 and 3.04 ppm, corresponding to the chemical shifts of H-4β and H-4α.




Scheme 3

To understand the metabolism of procyanidins is crucial for an accurate assessment of their effects on human health. To confirm indications drawn from epidemiological studies and *in vitro* findings, more information is required on the absorption of procyanidins and their pharmacokinetic properties. Deuterated procyanidins B3 and B4 will be precious tools for such experimental human studies on the bioavailability of these substances.

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12. Data for **12H** ($R^1$=$R^2$=H): FAB$^+$-MS (NBA) : m/z 579 $[M+H]^+$ ; $^1$H NMR (500 MHz, $D_2O$) δ ppm 2.68 (dl, J=17.1, H-4βF), 2.89 (dd, J=17.1, 4.2, H-4αF), 4.19 (m, $w_{\frac{1}{2}}$ = 10 Hz, H-3F), 4.37 (s, H-2F), 4.41 (d, J=9.5, H-2C), 4.56 (d, J=9.5, H-3C), 4.75 (s, H-4C), 5.51 and 5.83 (2H, s, H-6A and H-8A), 6.16 (s, H-6D), 6.78 (d, J=8.3, H-6'E), 6.85 (s, H-2'E), 6.86 (d, J=8.3, H-5'E), 6.9 (d, J=8.3, H-6'B), 6.95 (d, J=8.3, H-5'B), 7.04 (s, H-2'B) ; $[\alpha]_D^{25}$ = 107, c = 0.8, EtOH.

13. Data for **11H** ($R^1$=$R^2$=H): FAB$^+$-MS (NBA) : m/z 579 $[M+H]^+$ ; $^1$H NMR (500 MHz, $CD_3COCD_3$) δ ppm 2.98 (dl, J=16.7, H-4βF), 3.08 (dd, J=16.7, 4.6, H-4αF), 4.35 (m, $w_{\frac{1}{2}}$ = 14 Hz, H-3F), 4.52 (m, $w_{\frac{1}{2}}$ = 21 Hz, H-3C), 5 (d, J=4.8, H-4C), 5.02 (d, J=9.8, H-2F), 5.31 (s, H-2C), 6.02 and 6,13 (2H, s, H-6A and H-8A), 6.19 (s, H-6D), 6.85 (d, J=8, H-6'), 6.89 (2H, d, J=8, H-6', H-5'), 6.94 (d, J=8, H-5'), 7 (2H, s, H-2') ; ($R^1$=$R^2$=Bn) : $[\alpha]_D^{25}$ = - 103, c = 0.78, $CHCl_3$.

14. Data for **13D** ($R^1$=D, $R^2$=H): FAB$^+$-MS (glycerol) : m/z 580 ($[M+H]^+$, 100%), 581 (14%) ; $^1$H NMR (500 MHz, $H_2O$) δ ppm 2.69 (dl, J=17.2, H-4βF), 2.9 (dd, J=17.2, 5.1, H-4αF), 4.17 (ml, $w_{\frac{1}{2}}$ = 9 Hz, H-3F), 4.29 (d, J=9.9, H-3C), 4.48 (d, J=9.9, H-2C), 4.9 (s, H-2F), 5.97 (s, H-8A), 6.04 (s, H-6A), 6.17 (s, H-6D), 6.46 (dd, J=8.3, 2, H-6'E), 6.5 (dd, J=8.2, 2, H-6'B), 6.7 (d, J=2, H-2'E), 6.71 (d, J=2, H-2'B), 6.79 (d, J=8.2, H-5'B), 6.83 (d, J=8.3, H-5'E) ; $^2$H NMR (76 MHz, $H_2O$) δ ppm 4.41 ($^2$H-4C).

15. Data for **7H** ($R^1$=H, $R^2$=Bn): FAB$^+$-MS (NBA) : m/z 1299 $[M+H]^+$ ; $^1$H NMR (500 MHz, $C_6D_6$ ; x and y speak for the two conformers) δ ppm 3.1 (dd, J=16.3, 9.5, H-4βFx), 3.17 (dd, J=16.6, 9.1, H-4βFy), 3.59 (dd, J=16.6, 6.2, H-4αFy), 3.61 (dd, J=16.3, 6, H-4αFx), 3.96 (m, H-3Fx), 4.06 (m, H-3Fy), 4.57 (m, H-3Cxy), 4.6 (d, J=8.8, H-2Fx), 4.84 (d, J=8.5, H-2Fy), 5,59 (d, J=6,1, H-4Cx), 5.62 (d, J=9, H-2Cy), 5.78 (d J=6.2, H-4Cy), 5.87 (d, J=8.5, H-2Cx), 6.3 (d, J=2, H-8Ay), 6.55 (d, J=2, H-6Ax, H-6Ay), 6.6 (d, J=2, H-8Ax), 6.45 (s, H-6Dx), 6.5 (s, H-6Dy), 6.85 (dd, J=8.2, 2, H-6'Ey), 6.9, (d, J=2, H-2'Ey), 6.97 (d, J=8.2, H-5'Ey), 7.01 (m, H-5'Bx, H-5'Ex), 7,04 (d, J=8.3, H-5'By), 7.11 (dd, J=8.2, 2, H-6'Ex), 7,2-7.63 (m, H-5'Bxy, H-6'Bxy, H-2'Bxy, H-2'Ex and aromatic protons of benzyl groups), 4.69-5.2 (benzylic protons) ; $[\alpha]_D^{25}$ = - 123, c = 0.3, $CHCl_3$.

16. Data for **8H** ($R^1$=H, $R^2$=Bn): FAB$^+$-MS (NBA) : m/z 1299 $[M+H]^+$ ; $^1$H NMR (500 MHz, $C_6D_6$ ; x and y speak for the two conformers) δ ppm 3.07 (dd, J=16.3, 9.1, H-4βFx), 3.08 (dd, J=16.7, 9.2, H-4βFy), 3,55 (dd, J=16.3, 5.5, H-4αFx), 3,64 (dd, J=16.7, 6.2, H-4αFy), 4 (td, J=9.1, 5.5, H-3Fx), 4.09 (td, J=9.2, 6.2, H-3Fy), 4.19 (d, J=9.2, H-2Fy), 4.5 (dd, J=8.4, 6.1, H-3Cx), 4.58 (dd, J=9.1, 6, H-3Cy), 4.88 (H-2Fx), 5.62 (d, J=6.1, H-4Cx), 5.67 (d, J=6, H-4Cy), 5.82 (d, J=8.4, H-2Cx), 5.86 (d, J=9.1, H-2Cy), 6.06 (d, J=2.1, H-8Ay), 6.58 (d, J=2.1, H-8Ax), 6.46 (d, J=2.1, H-6Ay), 6.56 (d, J=2.1, H-6Ax), 6.45 (s, H-6Dx), 6.50 (s, H-6Dy), 6.9 (m, H-6'Ey, H-5'Ex), 6,97 (d, J=8.1, H-5'Ey), 6.99 (d, J=8.3, H-5'Bx), 7.04 (d, J=8.4, H-5'By), 7.11 (dd, J=8.1, 1.7, H-6'Ex), 7.15-7.66 (m, H-6'Bxy, H-2'Bxy, H-2'Exy and aromatic protons of benzyl groups), 4.66-5.44 (benzylic protons) ; $[\alpha]_D^{25}$ = 109, c = 1.5, $CHCl_3$ ; Lit. $[\alpha]_D^{30}$ = 106.8, c = 1.2, $CHCl_3$[9].

17. Data for **9D** ($R^1$=D, $R^2$=H): FAB$^+$-MS (NBA) : m/z 580 $[M+H]^+$ ; $^1$H NMR (500 MHz, $H_2O$) δ ppm 2.49 (dd, J=16, 9.1, H-4βF), 3.06 (dd, J=16, 5.9, H-4αF), 4.03 (d, J=8.7, H-2F), 4.14 (m, H-3F), 4.32 (d, J=9.7, H-3C), 4.49 (d, J=9.7, H-2C), 5.51, 5.94, 6.19 (s, H-8A, H-6A, H-6D), 6.65 (d, J=6.5, H-6'E), 6.67 (d, J=6.5, H-6'B), 6.77 (s, H-2'E), 6.82 (s, H-2'B), 6.86 (d, J=6.5, H-5'E/B) ; $^2$H NMR (76 MHz, $H_2O$) δ ppm 4.39 ($^2$H-4C).

18. Data for **10D** ($R^1$=D, $R^2$=H): FAB$^+$-MS (glycerol) : m/z 580 ($[M+H]^+$, 100%), 581 (17%) ; $^1$H NMR (500 MHz, $H_2O$) δ ppm 2.54 (dd, J=16, 8.5, H-4βF), 2.89 (dd, J=16, 5.6, H-4αF), 3.96 (m, H-3F), 4.34 (d, J=9.9, H-3C), 4.44 (d, J=9.9, H-2C), 4.62 (d, J=8, H-2F), 5.78 (d, J=2,3, H-8A), 6.06 (d, J=2.3, H-6A), 6.21 (s, H-6D), 6.52 (d, J=8.2, H-6'E), 6.66 (s, H-2'E), 6.67 (dd, J=8.2, 1.6, H-6'B), 6.86 (d, J=1.6, H-2'B), 6.89 (d, J=8.2, H-5'E), 6.92 (d, J=8.2, H-5'B) ; $^2$H NMR (76 MHz, $H_2O$) δ ppm 4.38 ($^2$H-4C) ; ($R^1$=$R^2$=H), $[\alpha]_D^{25}$ = - 210, c = 0.1, EtOH ; Lit. $[\alpha]_{578}^{20}$ = - 244.7, c = 2, EtOH[11].

19. Data for **15a-d**: FAB$^+$-MS (NBA) : 292 ($[M+H]^+$, 100%), 293 (17%) ; $^1$H NMR (500 MHz, $CH_3COCH_3$) δ ppm 2.66 (0.5 H, d, J=8.5, H-4β), 3.04 (0.5 H, d, J=5.4, H-4α), 4.1 (1H, m, H-3), 4.7 (1H, d, J=7.8, H-2), 6.01 (s, H-8), 6.16 (s, H-6), 6,89 (dd, J=8, 2, H-6'), 6,93 (d, J=8, H-5'), 7.03 (d, J=2, H-2') ; $^2$H NMR (76 MHz, $CH_3COCH_3$) δ ppm 2.66 ($^2$H-4β), 3,04 ($^2$H-4α).

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