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Total synthesis of isotopically labelled flavonoids. Part 3:† ^{13}C-labelled (−)-procyanidin B3 from 1-[^{13}C]-acetic acid

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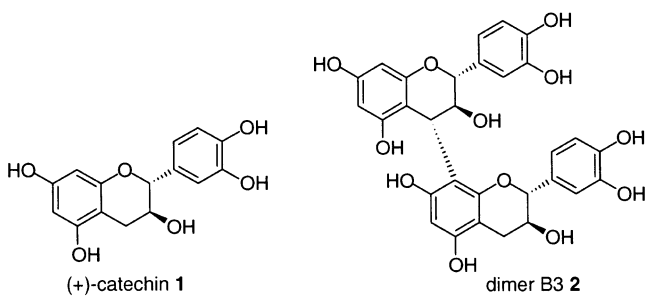
Abstract—The regioselectively ^{13}C-labelled (−)-procyanidin B3 was prepared in eight steps from 1-[^{13}C]-acetic acid.

Epidemiological studies have established strong correlations between wine drinking and lower risks of cardiovascular,[2] cancer[3] or Alzheimer[4] diseases. Because red wines are particularly rich in flavonoids, some authors have considered that they could be responsible for these observations. Indeed, many of these molecules, in vitro, demonstrate important biological properties (anti-atherosclerotic,[5] anti-thrombotic,[6] anti-inflammatory[7] and anti-carcinogenic[8]). But this opinion is not shared unanimously and, on the contrary, other authors have shown that some wine flavonoids even promote atherosclerosis.[9]

There is yet almost no direct proof of their gut resorption and their metabolism in humans is poorly known. Information on these issues could be obtained by using non-radioactive isotopically labelled compounds, detectable in human biological fluids by NMR as well as by mass spectrometry. Several reports dealt with the synthesis of deuteriated flavonoids such as catechin **1** or its dimer procyanidin B3 **2** (Scheme 1),[10,11] but their ^{2}H NMR signals lack sensitivity. Moreover, in our group, [^{13}C]-biolabelling of anthocyanidins and stilbenoids was made by *Vitis vinifera* cell cultures.[12,13] As large amounts of ^{13}C-labelled procyanidins (the most abundant flavonoids in wine) were not attainable by this method, we built them up by total synthesis. This letter reports the first total synthesis of [^{13}C]-labelled natural (−)-procyanidin **2**, by coupling a C_6–C_2 labelled acetophenone (from 1-[^{13}C]-acetic acid) with a C_1–C_6 benzaldehyde unit. This method allowed us to improve the yields and to decrease the number of steps to reach the pivotal intermediate chalcone **7**, with respect to our previous synthesis of racemic ^{13}C-labelled catechin, from K^{13}CN.[1]

Benzylated [^{13}C]-phloroacetophenone **4** (Scheme 2) was synthesised (60% yield) from phloroglucinol tribenzyl ether **3** (obtained by the Kawamoto method[14]) and activated 1-[^{13}C]-acetic acid by formation of a mixed anhydride upon treatment with trifluoroacetic anhydride (TFAA). Selective deprotection of **4** by $TiCl_4$ gave phloroacetophenone 2,4-dibenzylether **5** (80%). Crotonisation of **5** with 3,4-dibenzyloxybenzaldehyde **6** in the presence of NaH led to the tetrabenzylated chalcone **7** (89%). Flavan-3,4-diol **9** was obtained in 58% yield in three steps from **7** by the Clark–Lewis method:[15] borohydride reduction of **7** and Lewis acid catalysed cyclization into racemic flavene **8**, which was then directly transformed into the glycol **9** by an osmium-catalysed dihydroxylation with high diastereoselectivity. As expected, **8** was revealed to be too labile a compound to be purified.



Scheme 1.

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† For Part 2, see Ref. 1.

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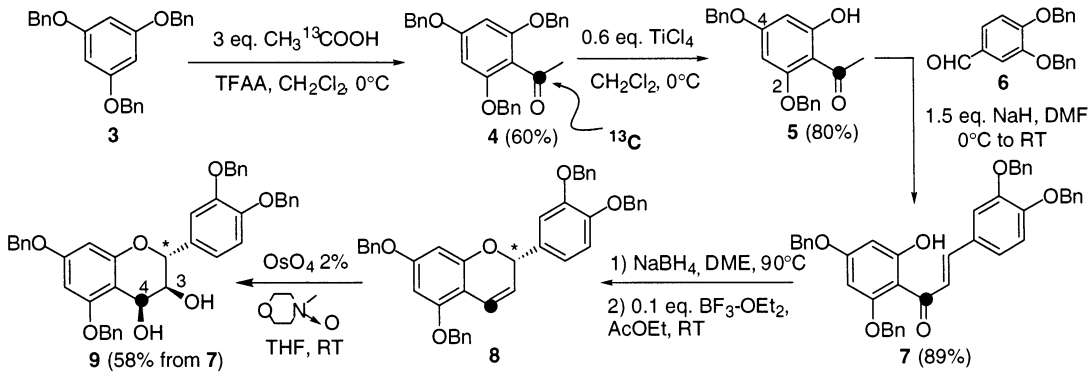
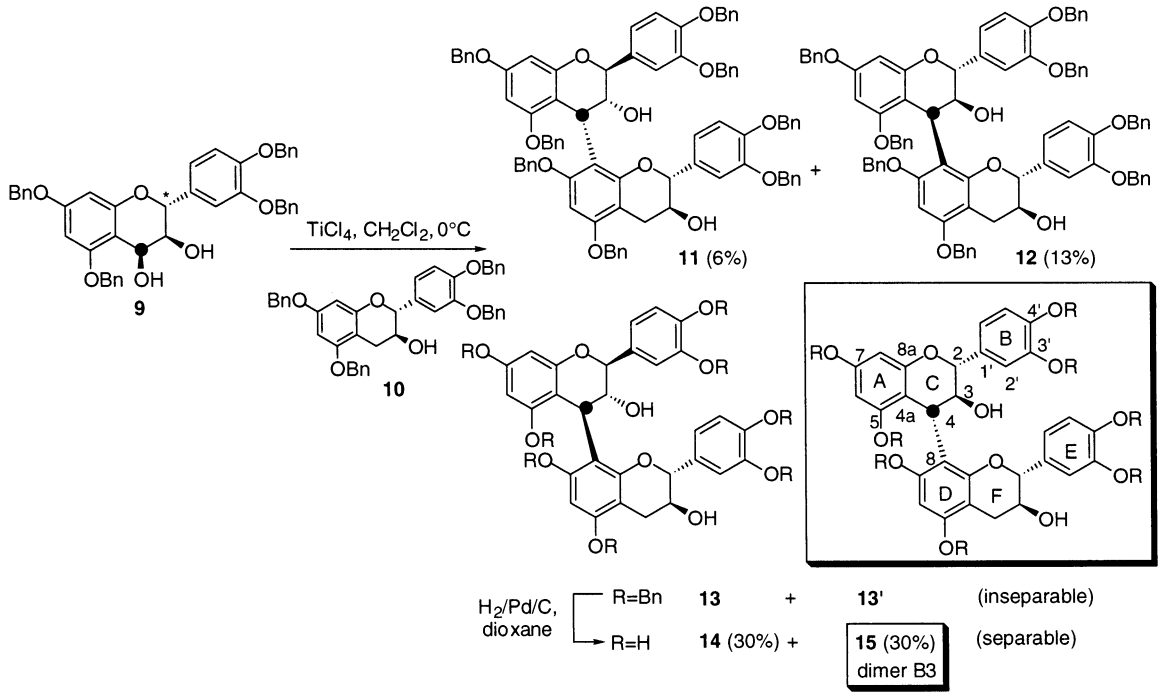


Scheme 2. Synthesis of labelled racemic flavan-3,4-diol **9** from phloroglucinol tribenzyl ether **3**.



Scheme 3. Synthesis of labelled procyanidin B3 **15** from (±)-flavan-3,4-diol **9**.

All attempts at enantioselective synthesis of **9** were unsuccessful: neither Sharpless asymmetric epoxidation[16] (of the very unstable allylic alcohol issued from the reduction of **7**), nor asymmetric dihydroxylation by AD-mix reagents[17] (of **7** or **8**) were possible.

The $TiCl_4$-catalysed condensation[18] of **9** with optically pure (2*R*,3*S*)-tetrabenzyloxycatechin **10**, prepared as reported by Kawamoto et al.,[18] yielded four diastereomeric dimers: **11** (6%) and **12** (13%) which possess unnatural absolute configuration at the C-ring chiral centres, along with a mixture of two inseparable dimers **13**+**13′** in a 1:1 ratio (65%), including benzylated procyanidin B3 (Scheme 3). Compounds **11** and **12** were characterised by 1D and 2D NMR and by comparison to the corresponding unlabelled products.[11] Hydrogenolysis of **13**+**13′** with H_2–Pd/C gave the corresponding procyanidins (**14** and **15**) and small amounts of catechin (8%), resulting of the reduction of the interflavanolic linkage.

Successive separation by chromatography on Sephadex LH-20 and reversed phase (C18) preparative HPLC, provided the 4*C*-[^{13}C]-procyanidin B3 dimer **15** (20% from **9**, $[\alpha]_D = -202$ (*c* 1, EtOH); lit.[11] −210 (*c* 0.1, EtOH) and **14** (20% from **9**, $[\alpha]_D = +142$ (*c* 1, EtOH). The NMR data matched in all respects those reported in the literature,[11] but for the signals of proton 4*C*-H (coupling constant of 130 Hz) and carbons C-3*C*, C-4a*A*, C-4*C*, C-8*D* altered by the presence of ^{13}C-labelling.

^{13}C-labelled dimer B3 **15** was synthesised in eight steps from phloroglucinol tribenzyl ether **3** and 1-[^{13}C]-acetic acid, in 6% overall yield from **3**, based on the (C_6–C_2+C_1–C_6)-type strategy. As both 2-[^{13}C]- and 1,2-[^{13}C]$_2$-acetic acid are commercially available, they offer further possibilities for other regioselective labelling of procyanidins, if needed. Combining this method to the recently described resolution of racemic flavonoid skeleton,[19] that allow us to get rid of the numerous

diastereomeric dimers, the production of large amounts (5–10 g) of labelled dimeric compounds is now under progress. Labelled procyanidins will be used in pharmacokinetic studies in human beings, to assess their gut absorption and their metabolism when delivered at mealtime.

Supplementary material

Expansion of a water-presaturated ^{1}H NMR (4.20–4.60 ppm) and full ^{13}C NMR spectra of ^{13}C-labelled procyanidin B3 **15**, showing the large coupling constants due to labelled carbon.

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