Gram-Scale Production and Applications of Optically Pure ^{13}C-Labelled (+)-Catechin and (−)-Epicatechin[‡]

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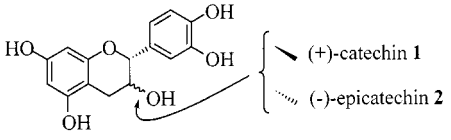
In this paper gram-scale asymmetric total syntheses of pure (+)-4-[^{13}C]catechin (**1**) and (–)-4-[^{13}C]epicatechin (**2**) are described. Labelling was introduced through acylation of a phloroglucinol derivative **4** by 1-[^{13}C]acetic acid, after activation by TFAA. Condensation of the resulting C_6–C_2 acetophenone building block **6** with a C_1–C_6 benzaldehyde unit **9** provided the C_6–C_3–C_6 flavonoid skeleton, with benzyl ethers as phenol protecting groups. Asymmetry was introduced by an inexpensive and very efficient resolution process, performed at the penultimate step towards naturally occurring flavan-3-ols, by using tartaric acid derivatives. Both enantiomers of 4-[^{13}C]catechin were obtained with a high level of enantiomeric purity, especially **(+)-1** of the natural series. The other enantiomer **(–)-1** was a valuable precursor of natural (–)-4-[^{13}C]epicatechin by epimerisation at C-2. These two natural flavanols are thus obtained for the first time in large quantities in optically pure form as labelled compounds and will be useful tools for biological studies using NMR or isotopic mass spectrometry in forthcoming experiments.

Introduction

As we have already presented in previous papers,[1,2] it would be very useful to dispose of ^{13}C-labelled catechin and epicatechin (**1** and **2**, Scheme 1) to assess the question of their resorption and to increase the knowledge of their metabolism in humans. Introduction of ^{13}C labelling requires the total synthesis of the skeleton, as described in earlier syntheses (monomer[1] and dimer B3[3]). In addition, the problems posed by the stereochemistry at C-2 and C-3 must be overcome,[4] as has been achieved, for example, by chemical resolution of unlabelled racemic catechin with an L-tartrate derivative.[2]



Scheme 1

This paper describes the first total synthesis of ^{13}C-labelled and optically pure natural (+)-catechin (**1**) simultaneously. The total synthesis of the biologically interesting (−)-epicatechin (**2**) incorporating ^{13}C labelling, could not be obtained in an optically pure form through *ent*-catechin, as expected from catechin L-tartrate.[2] We also report herein, the first total synthesis of ^{13}C-labelled (−)-epicatechin (**2**) with 99% *ee* thanks to the D-tartrate derivatives. After saponification, the pure (−)-catechin was epimerized at C-2 to (−)-**2** in 50% yield. Therefore, these labelled compounds, obtained in usable amounts and for the first time in enantiomerically pure native forms (free phenolic groups), will definitely allow us to assess their fate in humans.

Results and Discussion

Choice of Synthetic Strategy

Flavonoid synthesis refers to two possible routes. The first route consists of coupling a C_6 phenolic unit to a C_3−C_6 cinnamic moiety to form the C_6−C_3−C_6 skeleton. We recently applied this to the synthesis of racemic 4-[^{13}C]catechin (40 mg).[1] The more efficient and convenient second strategy was applicable on a larger scale, consisting in the crotonization reaction between a C_6−C_2 acetophenone and a C_1−C_6 benzaldehyde to form a chalcone. This formally allowed us to synthesize ^{13}C-labelled (−)-procyanidin B3, a dimer of catechin (30 mg).[3] We applied it herein to the production of gram amounts of catechins.

Phenol Protections

Benzyl protecting groups are commonly used in phenol chemistry. A difficulty that often arises during benzylation of nucleophilic polyphenols such as phloroglucinol is the undesirable *C*-alkylation in addition to *O*-alkylation. As phloroglucinol was our starting material, this problem was avoided by using the method of Kawamoto et al.,[5] through phloroglucinol triacetate (Scheme 2): 40 g of dried phloroglucinol (**3**) was acetylated before benzylation, giving 106 g of tri-*O*-benzylphloroglucinol (**4**) (87%, 2 steps). Addition-

[‡] Total Synthesis of Isotopically Labelled Flavonoids, 4. − Part 3: Ref.[3]

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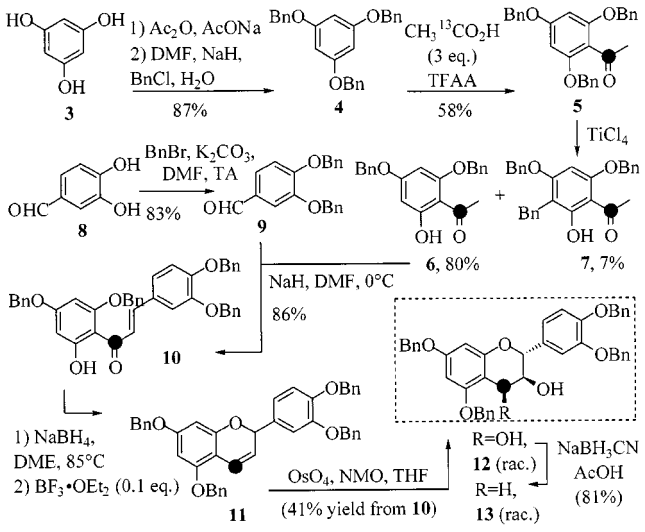
ally, 58 g of 3,4-bis(benzyloxy)benzaldehyde (**9**) was produced from 30 g of 3,4-dihydroxybenzaldehyde (**8**) (83%).



Scheme 2. Total synthesis of labelled racemic compound **13**

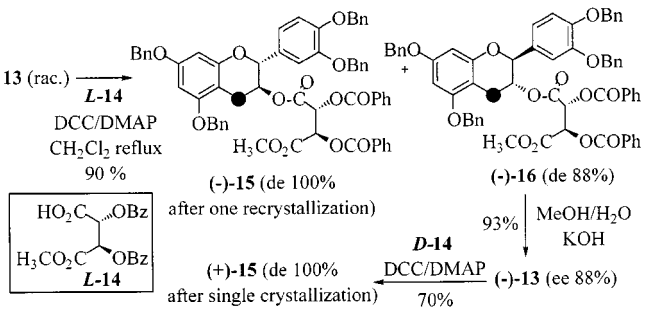
Synthesis of Labelled Phloroacetophenone Derivatives

Tri-*O*-benzylphloroacetophenone (**5**), labelled at the carbonyl group, was synthesized by acylating **4** (117 g) with 1-[^{13}C]acetic acid (99% ^{13}C enrichment) in the presence of trifluoroacetic anhydride (Scheme 2),[3,6,7] giving 75 g of pure **5** after silica gel column chromatography (58% from **4**). Selective deprotection of **5** was achieved by the action of $TiCl_4$, yielding 47 g of pure **6** (80%) after purification from the *C*-benzylated by-product **7** (7%).

Synthesis of Labelled Intermediates Chalcone 10 and Racemic Catechin 13

Crotonization of **6** (47 g) and **9** (45 g) led to chalcone **10** (75 g) in 86% yield after crystallization (Scheme 2). Reduction of **10** with $NaBH_4$ and cyclization in the presence of $BF_3{\cdot}OEt_2$ (Clark−Lewis method)[8,9] led to the labile 2*H*-chromene **11**. These steps were particularly delicate, probably due to the ability of **11** to form an allylic cation[10] as well as a stabilized methylenequinone by Claisen rearrangement.[11] No purification was carried out on **11** which was immediately oxidized by an osmium-catalyzed diastereoselective dihydroxylation, providing 31 g of racemic 4-[^{13}C]flavan-3,4-diol **12** from 74 g of **10** (41% yield after crystallization). Deoxygenation at C-4 was carried out with $NaBH_3CN$,[1,12] giving 24.3 g of racemic 4-[^{13}C]catechin tetrabenzyl ether **13** (81% yield).

Numerous attempts were made to make the synthesis of **13** asymmetric. However, stereoselective reduction of **10** was not possible, only a racemic mixture of **11** was observed in all cases {NMR analysis in the presence of Pr(hfc)$_3$ in [D$_6$]benzene}. Moreover, neither epoxidation nor enantioselective dihydroxylation of **10** and **11** were successful. Therefore, we finally turned to chemical resolution of **13**.

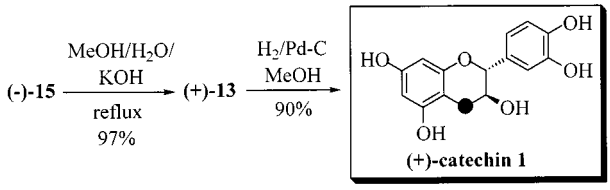


Scheme 3. Resolution process of compound **13**

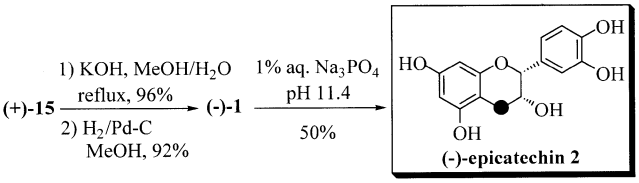
Resolution of 13 and Subsequent Synthesis of Labelled (+)- and (−)-Catechin

Recently, we described that coupling **13** (*rac*) to a derivative of L-tartaric acid provided an efficient method of synthesizing optically pure native catechins.[2] Esterification (Scheme 3) of the free 3-hydroxy group of **13** with dibenzoyltartaric acid monomethyl ester (***L*-14**) in the presence of DCC and DMAP gave a mixture of diastereomers **15** and **16** (35 g, 92%) which were inseparable by preparative HPLC. One, **(−)-15** from the (+)-catechin series, crystallized slowly but efficiently in hexane/dichloromethane (3:1) in a diastereomerically pure form (14.6 g after recrystallization in the same solvent, *de* > 99%). The other, **(−)-16** from the nonnatural (−)-catechin series (20 g, *de* = 88%), remained in solution, making the separation successful. A diastereomerically pure product from this last series was obtained by hydrolysis of **(−)-16** to **(−)-13** (*de* = 88%) followed by esterification by ***D*-14**, yielding 11.5 g of pure **(+)-15** after column chromatography and crystallization (*de* > 99%).

Employing this method, we could prepare **(−)-15** and **(+)-15** separately in optically pure forms. Both enantiomers were subjected to hydrolysis and phenol deprotection (Scheme 4 and Scheme 5) to give the two optically pure (*ee* > 99%, checked by HPLC analysis on β-cyclodextrin bonded column) ^{13}C-labelled catechins **1** [3.60 g of **(+)-1** from



Scheme 4. Synthesis of optically pure labelled (+)-catechin (**1**)



Scheme 5. Synthesis of labelled (−)-epicatechin (**2**)

(−)-15 and 2.91 g of **(−)-1** from **(+)-15**]. Figure 1a shows the 4-H section of the ^{1}H NMR spectrum of **(+)-1**. It displays the large coupling constants of 130 Hz within the ddd observed at δ = 2.50 and 2.85 for each proton directly bound to labelled C-4.

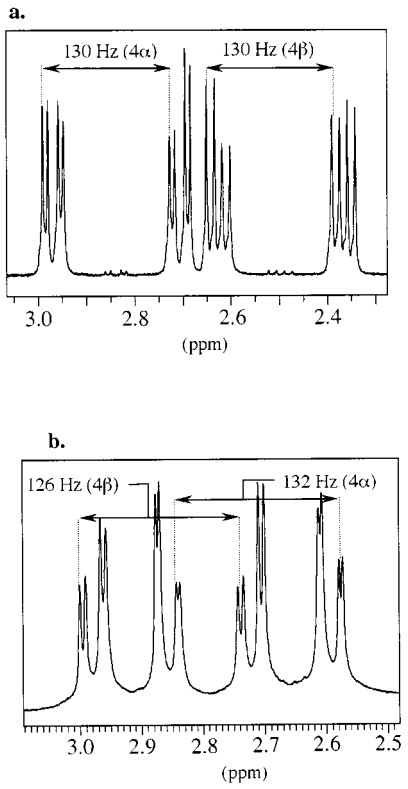


Figure 1. Expansion of ^{1}H NMR spectra (500 MHz) showing signals of protons 4-Hα and 4-Hβ of (+)-[4-^{13}C]catechin (a) and (−)-[4-^{13}C]epicatechin (b) with their broad characteristic coupling constant ^1J(4-H,^{13}C-4)

Epimerisation of (−)-4-[^{13}C]Catechin into Natural (−)-4-[^{13}C]Epicatechin

It is well known that catechin (**1**) can undergo epimerisation at C-2 to form *ent*-epicatechin (**2**), through reversible opening of ring C in basic medium.[13−15] We found more straightforward practical conditions than those of Foo and Porter[15] to synthesize **(−)-2** from **(−)-1**, using 1% (w/v) aq. Na_3PO_4 (pH = 11.4) under nitrogen. This reaction led to an equilibrium mixture of **(−)-1** and (−)-4-[^{13}C]epicatechin [**(−)-2**] in an approximate 3:1 ratio after 20 h at room temperature (Scheme 5); **(−)-2** was purified by chromatography on Sephadex LH-20 and **(−)-1** could be recycled sequentially four times to obtain 1.23 g of enantiomerically pure **(−)-2** (*ee* > 99%, checked by HPLC on β-cyclodextrin), as well as 434 mg of recovered **(−)-1**. Figure 1b shows a part of the ^{1}H NMR spectrum of **(−)-2** with the large coupling constants (126 and 132 Hz) of the two 4-H bound to the labelled carbon atom.

Conclusion

The total synthesis of gram amounts of ^{13}C-labelled natural (+)-catechin [**(+)-1**] and (−)-epicatechin [**(−)-2**] has been completed in 6.2% overall yield (as asymmetric flavan-3-ols) from phloroglucinol (**3**). Therefore, we significantly improved the yields in comparison with the other strategy (C_3 + C_3−C_6)[1] and furthermore, this route is asymmetric. Indeed, an efficient resolution of racemic benzylated catechin was used as the key step, allowing us to obtain the two antipodal series in high optical purity. In particular, the (−)-epicatechin series was synthesized for the first time in its native labelled form. Nonracemic compounds are absolutely necessary to carry out future biological studies, including pharmacokinetic studies in humans, ^{13}C being very useful in this work as an isotopic tracer.

Moreover, the gram-scale synthesis of ^{13}C-labelled procyanidin dimer B3 is underway. Therefore, after a preliminary study showing that racemic flavan-3,4-diol **12** cannot be used in such a large-scale synthesis,[3] it is now conceivable that we could oxidize labelled **(+)-13** at C-4, as a source of optically pure diol **12**, the precursor of procyanidolic oligomers.[10,16]

Experimental Section

General Remarks: $CH_3{}^{13}COOH$ was purchased from Euriso-top (Gif-sur-Yvette, France), with a 99% ^{13}C enrichment. All other commercial materials were used without further purification. All reactions were performed under nitrogen and monitored by TLC. Compounds **4**,[5] **9** (reaction was performed at room temp., 83% yield),[17] *L*-**14** and *D*-**14**[2] were prepared according to the literature. Crystallizations were performed as often as possible. − All ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AMX-500 spectrometer at 500.13 and 125.73 MHz, respectively (proton decoupling mode for carbon). − ^{1}H NMR spectra were referenced to the signal at δ = 7.27 of residual $CHCl_3$ or to CHD_2OD at δ = 3.31. − ^{13}C NMR spectra were referenced to signals of $CDCl_3$ (δ = 77.0) or of CD_3OD (δ = 49.1) according to the solvent used. − UV/Vis spectra were recorded using a Hitachi U2000 spectrometer. − FT-IR spectra were recorded with a Bomem MB100 spectrometer. − Low- and high-resolution mass spectra were obtained with Finnigan MAT TSQ 700 and Micromass (UK) ZAB2-SEQ spectrometers, respectively. − Elemental analyses were measured by the Service Central d'Analyses du CNRS (69390 Vernaison, France). − Optical rotations were measured with a Perkin−Elmer 141 polarimeter equipped with a sodium lamp (589 nm). − Analytical TLC was performed on Merck silica gel 60 F254 plates, and column chromatography using the indicated solvents on silica gel 60 Å 70−200 μm (SDS, 13124 PEYPIN, France). − Melting points were obtained with a Tottoli apparatus and are uncorrected.

[^{13}CO]-1-[2,4,6-Tris(benzyloxy)phenyl]ethanone (5): $CH_3{}^{13}COOH$ (21.9 mL, 379 mmol) and TFAA (64 mL, 454 mmol) were stirred for 5 min at room temp. Phloroglucinol tribenzyl ether (**4**) (50 g, 126 mmol) was dissolved in CH_2Cl_2 (200 mL) and then added at 0 °C. After 1.5 h, the deep purple solution was diluted with AcOEt and poured into saturated aq. $NaHCO_3$ solution. The organic layer was washed with brine, dried with Na_2SO_4 and concentrated. The crude extract was purified by silica gel column chromatography,

eluent CH_2Cl_2/cyclohexane (6:4, v/v), to give 30 g of **5** (55% yield from **4**). 75 g of **5** was produced from 106 g of **4** (64%). Pale yellow oil. − UV/Vis (MeOH): λ_{max} = 267 nm. − IR (thin film): $\tilde{\nu}$ = 1694 cm^{-1} (C=O). − ^1H NMR ($CDCl_3$): δ = 2.50 (d, *J* = 6.3 Hz, 3 H, α-H), 5.01 (s, 2 H, 4-O$CH_2C_6H_5$), 5.06 (s, 4 H, 2- and 6-O$CH_2C_6H_5$), 6.27 (d, *J* = 1.0 Hz, 2 H, 3-H, 5-H), 7.36−7.41 (m, 15 H, 3 $OCH_2C_6H_5$). − ^{13}C NMR ($CDCl_3$): δ = 32.3 (d, *J* = 47.0 Hz, C-α), 70.3 (4-O$CH_2C_6H_5$), 70.7 (2- and 6-O$CH_2C_6H_5$), 93.7 (C-3, C-5), 115.4 (d, *J* = 55.0 Hz, C-1), 127.1, 127.4, 127.9, 128.1, 128.5, 128.6 ($OCH_2C_6H_5$ *ortho*/*meta*/*para*), 136.4 (4-$OCH_2C_6H_5$ *ipso*), 136.5 (2- and 6-$OCH_2C_6H_5$ *ipso*), 157.2 (C-2, C-6), 161.1 (C-4), 201.2 (labelled CO). − HRMS (EI): *m*/*z* calcd. for $^{13}CC_{28}H_{26}O_4$ 439.1859; found 439.1864.

[^{13}CO]-1-[2,4-Bis(benzyloxy)-6-hydroxyphenyl]ethanone (6): $TiCl_4$ (1 M in CH_2Cl_2, 102 mL, 102 mmol) was added dropwise at 0 °C within 1 h to a solution of **5** (74.7 g, 170.1 mmol) in CH_2Cl_2 (1.1 L). After 160 min of stirring, the mixture was slowly poured into aqueous saturated $NaHCO_3$, washed with water and brine, dried with Na_2SO_4 and concentrated. The residue was crystallized from ether, and the mother liquors were purified by column chromatography on silica gel (eluent CH_2Cl_2/cyclohexane, 6:4), providing 47.1 g of **6** (80%) after elution of 5.1 g of by-product **7** (7%).

6: White crystals. − M.p. 104 °C (ref.[9] 103 °C). − UV/Vis (MeOH): λ_{max} = 287 nm. − IR (KBr): $\tilde{\nu}$ = 1604 cm^{-1} (C=O). − ^1H NMR ($CDCl_3$): δ = 2.57 (d, *J* = 6.1 Hz, 3 H, H-α), 5.06, 5.07 (4 H, 4- and 6-O$CH_2C_6H_5$), 6.12 (d, *J* = 2.2 Hz, 1 H, 5-H), 6.19 (d, *J* = 2.2 Hz, 1 H, 3-H), 7.38−7.43 (m, 10 H, 2 $OCH_2C_6H_5$), 14.02 (s, 1 H, 2-OH). − ^{13}C NMR ($CDCl_3$): δ = 33.2 (d, *J* = 43.0 Hz, C-α), 70.2, 71.1 (4- and 6-O$CH_2C_6H_5$), 92.3 (C-5), 94.8 (C-3), 106.3 (d, *J* = 57.0 Hz, C-1), 127.6, 127.9, 128.3, 128.4, 128.7, 128.7 ($OCH_2C_6H_5$ *ortho*/*meta*/*para*), 135.6, 135.9 ($OCH_2C_6H_5$ *ipso*), 162.0 (C-6), 165.1 (C-4), 167.5 (C-2), 203.1 (labelled CO). − MS (EI, 70 eV): *m*/*z* (%) = 349 (30) $[M]^+$, 91 (100). − $^{13}C_1C_{21}H_{20}O_4$ (349.4): calcd. C 75.91, H 5.77; found C 75.65, H 5.76.

7: White crystals. − M.p. 117 °C. − UV/Vis (MeOH): λ_{max} = 287 nm. − IR (thin film): $\tilde{\nu}$ = 1621 cm^{-1} (C=O). − ^1H NMR ($CDCl_3$): δ = 2.60 (d, *J* = 6.1 Hz, 3 H, α-H), 4.05 (s, 2 H, 3-C$H_2C_6H_5$), 5.08 (s, 2 H, 6-O$CH_2C_6H_5$), 5.12 (s, 2 H, 4-O$CH_2C_6H_5$), 6.11 (s, 1 H, 5-H), 7.25−7.43 (m, 15 H, 3 $OCH_2C_6H_5$), 14.11 (s, 1 H, 2-OH). − ^{13}C NMR ($CDCl_3$): δ = 28.1 (3-$CH_2C_6H_5$), 33.3 (d, *J* = 43.0 Hz, C-α), 70.1 (4-O$CH_2C_6H_5$), 71.0 (6-O$CH_2C_6H_5$), 88.7 (C-5), 106.4 (d, *J* = 56.7 Hz, C-1), 110.2 (C-3), 125.4, 127.2, 127.8, 128.0, 128.1, 128.4, 128.6, 128.7, 128.8 ($CH_2C_6H_5$ *ortho*/*meta*/*para*), 135.8 (6-$OCH_2C_6H_5$ *ipso*), 136.2 (4-$OCH_2C_6H_5$ *ipso*), 141.6 (3-$CH_2C_6H_5$ *ipso*), 161.0 (C-6), 162.4 (C-4), 164.0 (C-2), 203.3 (labelled CO). − MS (EI, 70 eV): *m*/*z* (%) = 439 (10) $[M]^+$, 91 (100). − $^{13}C_1C_{28}H_{26}O_4$ (439.5): calcd. C 79.48, H 5.96; found C 79.11, H 5.87.

[^{13}CO]-1-[2,4-Bis(benzyloxy)-6-hydroxyphenyl]-3-[3,4-bis(benzyloxy)phenyl]propenone (10): To a stirred solution of **6** (47 g, 135 mmol) in DMF (800 mL), NaH (60%), dispersed in mineral oil (22 g, 540 mmol) was added and then **9** (45 g, 142 mmol) in DMF (200 mL) was added dropwise over a period of 15 min at 0 °C. After stirring for 2 h at room temp., the reaction was carefully quenched with water, DMF was evaporated under vacuum, and the residue was dissolved in dichloromethane (800 mL) before washing with water and brine. The extract was concentrated and the residue was crystallized from ether to give **10** as yellow crystals (75 g, 86% yield). − M.p. 137−138 °C (ref.[9] 140 °C). − Spectroscopic data as described previously.[1] Alterations due to labelling: − ^1H NMR ($CDCl_3$): δ = 7.68 (dd, *J* = 15.5, 6.2 Hz, β-H), 7.78 (dd, *J* = 15.5, 5.1 Hz, α-H). − ^{13}C NMR ($CDCl_3$): δ = 106.6 (d, *J* = 58 Hz, C-1′), 125.9 (d, *J* = 55 Hz, C-α), 129.0 (d, *J* = 6.1 Hz, C-1), 142.7 (C-β), 192.6 (labelled CO).

[4−^{13}C]-5,7-Dibenzyloxy-2-[3,4-bis(benzyloxy)phenyl]-2*H*-chromene (11): According to Kawamoto et al.,[9] compound **10** (20 g, 30.7 mmol) was dissolved in 1,2-dimethoxyethane (300 mL) at 85 °C and then $NaBH_4$ (1.2 g, 31.5 mmol) was added. After 5 min at 85 °C, the mixture was cooled, diluted with ethyl acetate and washed 3 times with brine. The organic layer was dried with sodium sulfate before adding a solution of $BF_3{\cdot}OEt_2$ (380 μL) in CH_2Cl_2 (5 mL). After 20 min of stirring at room temp., the solution was washed 3 times with brine, dried with sodium sulfate and concentrated to give an orange resin (19.8 g) that was not purified due to the instability of product **11**. − Data as described previously.[1] Alterations due to labelling: − ^1H NMR ($CDCl_3$): δ = 6.88 (ddd, *J* = 166, 9.9, 1.7 Hz, 4-H). − ^{13}C NMR ($CDCl_3$): δ = 105.0 (d, *J* = 53 Hz, C-4a), 118.9 (labelled C-4), 119.7 (broad s, C-3). − MS (EI, 70 eV): *m*/*z* (%) = 633 $[M^+]$ (40), 542 (30), 91 (100).

2,3-*trans*-3,4-*cis*-[4−^{13}C]-5,7-Dibenzyloxy-2-[3,4-bis(benzyloxy)phenyl]chroman-3,4-diol (12): According to Kawamoto et al.,[9] a solution of **11** (19.8 g) in THF (240 mL) was added to a mixture of *N*-methylmorpholine *N*-oxide (4.6 g, 34 mmol), water (12 mL), THF (160 mL) and OsO_4 (0.5 mmol, 6.2 mL of a 2.5 wt-% solution in 2-methyl-2-propanol, purchased from Aldrich (ref. 20,886-8). After 6 h at room temp., the pale yellow precipitating mixture was dissolved in CH_2Cl_2 (500 mL) and washed with an aqueous solution of $Na_2S_2O_3$ (10 g/100 mL), water and brine. Racemic compound **12** was crystallized from ether after dissolution in a minimum CH_2Cl_2. Yield: 10.7 g (52% from **10**). We obtained 31 g of **12** from 74 g of **10** (41% yield) in several runs via **11**. − Data as described previously.[1,9] − M.p. 175 °C. − Alterations due to labelling: − ^1H NMR ($CDCl_3$): δ = 5.09 (dd, *J* = 152.0, 3.9 Hz, 4-H). − ^{13}C NMR ($CDCl_3$): δ = 61.5 (labelled C-4), 70.2 (d, *J* = 34 Hz, C-3), 105.2 (d, *J* = 48 Hz, C-4a). − HRMS (FAB+, nitrobenzyl alcohol): *m*/*z* calcd. for $^{13}CC_{42}H_{38}O_7Li$ [M + Li] 674.2811; found 674.2868.

(±)-[4−^{13}C]-5,7-Dibenzyloxy-2-[3,4-bis(benzyloxy)phenyl]chroman-3-ol (13): According to Brown and Fuller,[12] $NaBH_3CN$ (44 g, 698 mmol) was carefully added to a suspension of **12** (31 g) in acetic acid (1.5 L). After 2 d, the acetic acid was evaporated and the residue was dissolved in CH_2Cl_2 before washing with saturated aqueous $NaHCO_3$, water, and brine. The crude extract containing **13** and residual **12** was purified by centrifugal chromatography on silica gel (Merck, ref. 1.07749), eluent CH_2Cl_2/AcOEt (97:3), giving 19.55 g of racemic **13**. The remaining **12** (7.93 g) was recycled and resubmitted to reduction, giving 6.52 g of **13** after purification. As some impurities remained in **13** (26.1 g in total), it was purified once more by silica gel column chromatography (eluent CH_2Cl_2) to give 24.4 g of **13** (80% yield). Data as described previously.[1,10,18] Alterations due to labelling: − ^1H NMR ($CDCl_3$): δ = 2.68 (ddd, *J* = 130.5, 16.5, 8.8 Hz, axial 4β-H), 3.13 (ddd, *J* = 133.2, 16.5, 5.6 Hz, equatorial 4α-H). − ^{13}C NMR ($CDCl_3$): δ = 27.6 (labelled C-4), 68.1 (d, *J* = 36 Hz, C-3), 102.3 (d, *J* = 44 Hz, C-4a).

Resolution of (±)-13: A solution of **(±)-13** (24.3 g, 37.4 mmol) in CH_2Cl_2 (400 mL) was refluxed for 2.5 h in the presence of the L-tartaric acid derivative ***L*-14** (27.8 g, 74.8 mmol), DCC (15.4 g, 74.8 mmol), and 4-DMAP (228 mg, 1.87 mmol). After cooling and filtering the mixture, the CH_2Cl_2 layer was washed with water, brine, and dried with Na_2SO_4. The crude extract was purified by silica gel column chromatography (eluent CH_2Cl_2/hexane, 9:1), fur-

nishing 35 g of a mixture of inseparable **(−)-15** and **(−)-16**. **(−)-15** was crystallized from hexane/CH_2Cl_2 (3:1), giving 14.6 g of white crystals after one recrystallization from the same solvent (83% of expected, *de* > 99% based on silica gel HPLC, eluent hexane/CH_2Cl_2, 85:15, 1 mL/min), and 20 g of diastereomerically enriched **(−)-16** (*de* = 88%). Hydrolysis of **(−)-15** in MeOH/H_2O/KOH (270 mL/30 mL/3 g) led to a precipitate of enantiomerically pure **(+)-13** that was extracted with CH_2Cl_2 and washed with water and brine and then concentrated. **(+)-13** was used without further purification (97% yield for hydrolysis, *ee* > 99%). The same procedure for **(−)-16** led to enantiomerically enriched **(−)-13** (12.1 g, 93% yield, *ee* = 88%) that was esterified with D-tartaric acid derivative ***D*-14** (13.9 g, 37.3 mmol) by the above-mentioned method to give **(+)-15** after crystallization in an optically pure form (11.5 g, 70% of expected, *de* > 99%) as for **(−)-15**. Hydrolysis of **(+)-15** gave optically pure **(−)-13** (7.1 g, 96% yield, *ee* > 99%).

(−)-15: $[\alpha]_D^{20} = -35$ (*c* = 1, CH_2Cl_2). − M.p. 150 °C. − UV/Vis (CH_3OH): λ_{max} = 274 nm. − IR (KBr): $\tilde{\nu}$ = 3063, 3032, 2939, 2870, 1746 (s), 1619, 1594, 1513, 1498, 1452, 1439, 1378, 1259 (s), 1180, 1124 (s), 1019, 809, 742, 705 cm^{-1}. − ^1H NMR ($CDCl_3$): δ = 2.62 (ddd, *J* = 4.8, 17.0, 132.0 Hz, 4-H_a), 2.69 (ddd, *J* = 4.9, 17.0, 134.0 Hz, 4-H_b), 3.73 (s, CH_3 ester), 4.73 and 4.83 (2 d, *J* = 11.9 Hz, 5-OCH_aH_bC$_6$H$_5$), 5.00 (s, 7-OCH_2C$_6$H$_5$), 5.05 and 5.12 (2 s, 3′- and 4′-OCH_2C$_6$H$_5$), 5.13 (d, *J* = 3.5 Hz, 2-H), 5.38 (large dd, *J* = 4.8, 11.0 Hz, 3-H), 5.97 (m, 2′′- and 3′′-H), 6.09 (d, *J* = 2.2 Hz, 6-H), 6.21 (d, *J* = 2.2 Hz, 8-H), 6.79 (dd, *J* = 1.9, 8.4 Hz, 6′-H), 6.86 (d, *J* = 8.4 Hz, 5′-H), 6.91 (d, *J* = 1.9 Hz, 2′-H), 7.22−7.48 (m, 24 H, 4 OCH$_2$C$_6$*H*$_5$ and 2 OCOC$_5$*H*$_6$ *meta*), 7.53 and 7.58 (2 m, 2 H, 2 OCOC$_5$*H*$_6$ *para*), 8.02 and 8.06 (2 m, 4 H, 2 OCOC$_5$*H*$_6$ *ortho*). − ^{13}C NMR ($CDCl_3$): δ = 22.3 (labelled C-4), 52.9 (*C*H$_3$ ester), 69.6 (5-O*C*H$_2$C$_6$H$_5$), 70.1 (7-O*C*H$_2$C$_6$H$_5$), 71.1 (d, *J* = 46 Hz, C-3), 71.3 (C-2′′ and C-3′′), 71.4 (3′- and 4′-O*C*H$_2$C$_6$H$_5$), 77.2 (C-2), 93.8 (C-6), 94.3 (C-8), 100.3 (C-4a), 112.9 (C-2′), 115.1 (C-5′), 119.4 (C-6′), 126.9, 127.2, 127.4, 127.5, 127.7, 127.9, 128.3, 128.4, 128.5, 128.6 (OCH$_2$*C*$_6$H$_5$ *ortho*/*meta*/*para,* and OCO*C*$_6$H$_5$ *meta*), 128.5 and 128.6 (OCO*C*$_6$H$_5$ *ipso*), 130.0 and 130.1 (OCO*C*$_6$H$_5$ *ortho*), 130.7 (C-1′), 133.5 and 133.6 (OCO*C*$_6$H$_5$ *para*), 136.8 (5-OCH$_2$*C*$_6$H$_5$ *ipso*), 136.9 (7-OCH$_2$*C*$_6$H$_5$ *ipso*), 137.0 and 137.2 (3′- and 4′-OCH$_2$*C*$_6$H$_5$ *ipso*), 149.1 (C-3′, C-4′), 154.3 (C-8a), 157.4 (C-5), 158.9 (C-7), 164.9 (3′′-O*C*OC$_6$H$_5$), 165.0 (2′′-O*C*OC$_6$H$_5$), 165.2 (C-1′′), 166.3 (C-4′′). − MS (FAB+, nitrobenzyl alcohol): *m*/*z* (%) = 1006 [MH^+] (100), 634 (50), 542 (20). − $^{13}C_1C_{61}H_{52}O_{13}$ (1006.1): calcd. C 74.12, H 5.21; found C 74.14, H 5.09.

(−)-16: $[\alpha]_D^{20} = -29$ (*c* = 1, CH_2Cl_2). − UV/Vis (CH_3OH): λ_{max} = 275 nm. − IR (film): $\tilde{\nu}$ = 3063, 3032, 2932, 2862, 1767, 1734 (s), 1618, 1594, 1510, 1498, 1452, 1438, 1376, 1249 (s), 1122 (s), 1025, 734, 698 cm^{-1}. − ^1H NMR ($CDCl_3$): δ = 2.85 (dd, *J* = 5.0, 132 Hz, two 4-H), 3.75 (s, CH_3 ester), 4.96, 5.01 and 5.08 (2 d, *J* = 12 Hz, and s, respectively, 3′- and 4′-OCH_2C$_6$H$_5$), 5.02 and 5.03 (5- and 7-OCH_2C$_6$H$_5$), 5.08 (d, *J* = 11.4 Hz, 2-H), 5.42 (dd, *J* = 5.0, 11.4 Hz, 3-H), 5.79 (d, *J* = 2.9 Hz, 2′′-H), 6.99 (d, *J* = 2.9 Hz, 3′′-H), 6.16 (d, *J* = 2.2 Hz, 8-H), 6.27 (d, *J* = 2.2 Hz, 6-H), 6.72 (dd, *J* = 1.9, 8.7 Hz, 6′-H), 6.76 (d, *J* = 8.7 Hz, 5′-H), 6.84 (d, *J* = 1.9 Hz, 2′-H), 7.26−7.50 (m, 24 H, 4 OCH$_2$C$_6$*H*$_5$ and 2 OCOC$_6$*H*$_5$ *meta*), 7.53 and 7.62 (2 m, 2 OCOC$_6$*H*$_5$ *para*), 8.10 (m, 2 OCOC$_6$*H*$_5$ *ortho*). − ^{13}C NMR ($CDCl_3$): δ = 22.8 (labelled C-4), 52.9 (*C*H$_3$ ester), 70.0 and 70.1 (5- and 7-O*C*H$_2$C$_6$H$_5$), 71.0, 71.1, 71.4 and 71.5 (C-3, C-2′′, C-3′′, 3′- and 4′-O*C*H$_2$C$_6$H$_5$), 76.9 (C-2), 93.9 (C-8), 94.5 (C-6), 100.5 (d, *J* = 43 Hz, C-4a), 112.8 (C-2′), 114.7 (C-5′), 119.2 (C-6′), 127.1, 127.2, 127.3, 127.6, 127.7, 127.9, 128.0, 128.3, 128.4, 128.5, 128.6, 133.5, 133.6 (OCH$_2$*C*$_6$H$_5$ *ortho*/*meta*/*para* and OCO*C*$_6$H$_5$ *ortho*/*meta*/*para*), 128.6 and 128.7 (2 OCO*C*$_6$H$_5$ *ipso*), 130.5 (C-1′), 136.8 (5-OCH$_2$*C*$_6$H$_5$ *ipso*), 136.9 (7-OCH$_2$*C*$_6$H$_5$ *ipso*), 137.1 and 137.3 (3′- and 4′-OCH$_2$*C*$_6$H$_5$ *ipso*), 148.9 and 149.0 (C-3′ and C-4′), 154.4 (C-8a), 157.5 (C-5), 159.0 (C-7), 164.8 (3′′-O*C*OC$_6$H$_5$), 165.1 (2′′-O*C*OC$_6$H$_5$), 165.3 (C-1′′), 166.3 (C-4′′). − MS (FAB+, nitrobenzyl alcohol): *m*/*z* (%) = 1006 [MH^+] (75), 634 (100), 542 (70). − $^{13}C_1C_{61}H_{52}O_{13}$ (1006.1): calcd. C 74.12, H 5.21; found C 73.78, H 5.29.

(+)-15: $[\alpha]_D^{20} = +35$ (*c* = 1, CH_2Cl_2). − M.p. 150 °C. − Same spectroscopic data as **(−)-15**. − $^{13}C_1C_{61}H_{52}O_{13}$ (1006.1): calcd. C 74.12, H 5.21; found C 74.22, H 5.20.

(+)-13: $[\alpha]_D^{20} = +1.5$ (*c* = 1, CH_2Cl_2; ref.[18] ca. 0, *c* = 3.3, $CHCl_3$). Data as previously described [cf. compound **(±)-13**].

(−)-13: $[\alpha]_D^{20} = -1.5$ (*c* = 1, CH_2Cl_2). Data as previously described [cf. compound **(±)-13**].

(+)- and (−)-[4−^{13}C]Catechin (1): A suspension of **(+)-13** (7.9 g) in MeOH (120 mL) was submitted to hydrogenolysis at room temp. in the presence of palladium (790 mg) on activated carbon (Aldrich, ref. 20,569-9), under hydrogen. After 6 h, the methanol was evaporated and the residue was dissolved in ethyl acetate before filtration through Celite and concentration. The crude extract (4.15 g) was purified by chromatography on Sephadex LH-20 (water/ethanol, 85:15, 5 mL/min), yielding 3.60 g of pure **(+)-1** (90% yield). The same procedure was applied to produce 2.91 g of **(−)-1** from 7.12 g of **(−)-13** (92% yield).

(+)-1: *ee* > 99% based on HPLC analysis on a chiral Cyclobond® I (β-cyclodextrin bonded) column (1 mL/min, eluent H_2O/MeOH, 85:15 from 0 to 10 min, linear gradient from 85:15 at 10 min to 50:50 at 60 min). − $[\alpha]_D^{20} = +16$ (*c* = 1, water/acetone, 1:1; ref.[13] +15.4). − M.p. 205−210 °C (dec.). − UV/Vis (MeOH): λ_{max} = 279 nm. − ^1H NMR (CD_3OD): δ = 2.51 (ddd, *J* = 130.0, 16.2, 8.0 Hz, 4β-H), 2.84 (ddd, *J* = 130.0, 16.2, 5.4 Hz, 4α-H), 3.98 (m, 3-H), 4.57 (dd, *J* = 7.4, 3.3 Hz, 2-H), 5.87 (d, *J* = 2.2 Hz, 8-H), 5.94 (d, *J* = 2.2 Hz, 6-H), 6.72 (dd, *J* = 8.1, 2.0 Hz, 6′-H), 6.76 (d, *J* = 8.1 Hz, 5′-H), 6.84 (d, *J* = 2.0 Hz, 2′-H). − ^{13}C NMR (CD_3OD): δ = 28.4 (labelled C-4), 68.8 (d, *J* = 37 Hz, C-3), 82.7 (C-2), 95.6 (C-6), 96.4 (C-8), 100.9 (d, *J* = 43 Hz, C-4a), 115.3 (C-2′), 116.2 (C-5′), 120.1 (C-6′), 132.2 (C-1′), 146.2 (C-3′, C-4′), 156.8 (d, *J* = 5 Hz, C-8a), 157.5 (C-7), 157.7 (d, *J* = 6 Hz, C-5). − HRMS (FAB+): *m*/*z* calcd. for [$^{13}C^{12}C_{14}H_{14}O_6$+H] 292.0902; found 292.0901. − $^{13}C_1C_{14}H_{16}O_7$,H_2O (309.3): calcd. C 58.57, H 5.21; found C 58.65, H 5.26.

(−)-1: *ee* > 99%. − $[\alpha]_D^{20} = -16$ (*c* = 1, water/acetone, 1:1). − Same spectroscopic data as **(+)-1**. − HRMS (FAB+): *m*/*z* calcd. for [$^{13}C^{12}C_{14}H_{14}O_6$ + H] 292.0902; found 292.0909.

(−)-[4−^{13}C]Epicatechin (2): Compound **(−)-1** (2.1 g) was dissolved in a solution of Na_3PO_4 (2 g) in distilled water at pH = 11.4, in a flask purged with nitrogen. After 20 h at room temp., HPLC analysis showed a mixture of 24% epicatechin and 76% catechin (RP18, gradient from H_2O/MeOH/TFA, 85:15:0.0025 to 20:80:0.0025 within 1 h, detection at 280 nm). The solution was then acidified to pH = 6.5 with aq. 1 N HCl, and extracted 5 times with ethyl acetate. The combined organic extracts were dried with Na_2SO_4 and concentrated. The crude extract was purified by chromatography on Sephadex LH-20 (water/ethanol, 85:15, 5 mL/min), yielding pure (−)-**2** in the first fractions [386 mg, 18% from starting **(−)-1**] and recovered **(−)-1** (1.45 g, 69%) which was epimerized once more. In this way, we obtained 1.23 g of **(−)-2** from 2.9 g of **(−)-1** [50% yield based on recovered **(−)-1**] while 434 mg of **(−)-1**

were recovered. According to the *ee* of **(−)-1**, the *ee* of (−)-**2** was > 99% and was checked by HPLC analysis on a β-cyclodextrin-bonded column (1 mL/min, eluent H_2O/MeOH, 96:4). − $[\alpha]_D^{20}$ = −56 (*c* = 1, acetone/water, 1:1; ref.[14] −60). − M.p. 230−235 °C (dec.). − UV/Vis (MeOH): λ_{max} = 279 nm. − ^{1}H NMR (CD_3OD): δ = 2.72 (ddd, *J* = 132.1, 16.7, 2.8 Hz, 4α-H), 2.85 (ddd, *J* = 126.4, 16.7, 4.6 Hz, 4β-H), 4.17 (m, 3-H), 4.81 (large s, 2-H), 5.91 (d, *J* = 2.2 Hz, 8-H), 5.94 (d, *J* = 2.2 Hz, 6-H), 6.75 (d, *J* = 8.1 Hz, 5′-H), 6.79 (dd, *J* = 8.1, 2.0 Hz, 6′-H), 6.97 (d, *J* = 2.0 Hz, 2′-H). − ^{13}C NMR (CD_3OD) δ = 31.7 (labelled C-4), 70.0 (d, *J* = 36 Hz, C-3), 82.4 (C-2), 98.4, 99.0 (C-8 and C-6), 102.6 (d, *J* = 44 Hz, C-4a), 117.8 (C-6′), 118.4 (C-5′), 121.9 (C-2′), 134.9 (C-1′), 148.2, 148.3 (C-3′ and C-4′), 159.9 (C-8a), 160.2 (C-7), 160.5 (C-5). − HRMS (FAB+): *m*/*z* calcd. for [$^{13}C^{12}C_{14}H_{14}O_6$+H] 292.0902; found 292.0903.

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