DOI: 10.1002/ejoc.200800886

First Total Synthesis of ^{14}C-Labeled Procyanidin B2 – A Milestone Toward Understanding Cocoa Polyphenol Metabolism

Florian Viton,[a] Cyrille Landreau,[b] David Rustidge,[b] Fabien Robert,[a] Gary Williamson,[a] and Denis Barron*[a]

Keywords: Natural products / Polyphenols / Radiochemistry / Total synthesis

The idea that foods consumed for pure pleasure could provide health benefits received much recognition in the recent years. Among these foods, cocoa and dark chocolate are particularly rich in procyanidins, one of the major dietary families of polyphenols. We developed the first asymmetric total synthesis of procyanidin B2 and applied it to the preparation of a regioselectively radiolabeled ^{14}C-analogue, which will be used to strengthen our knowledge on the metabolism of procyanidins.

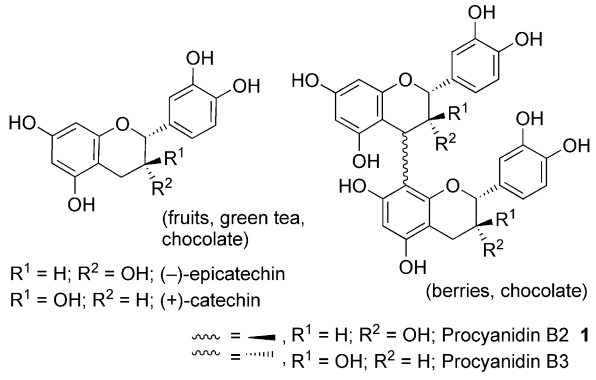
(© Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, Germany, 2008)

Introduction

The appealing idea that foods commonly consumed for pure pleasure (such as dark chocolate) could also bring tangible benefits for health is now generally recognised and supported by solid scientific evidence. Flavan-3-ols and their oligomeric procyanidin forms (Scheme 1) represent one of the major dietary families of polyphenols; fresh fruits, tea, cocoa and dark chocolate are particularly rich in procyanidins.[1] During the last few decades, many in vitro and in vivo studies have shown the beneficial effects of procyanidins on health.[2] However, their absorption and metabolism is still not fully understood and some aspects are still controversial.[3]

Radiolabeled compounds have proven invaluable in metabolism studies for tracing parent compounds and their metabolites. Therefore, to support our continuous efforts in studying polyphenols metabolism, we decided to undertake the preparation of radiolabeled procyanidin B2 [^{14}C]-**1**, one of the major procyanidin present in cocoa and chocolate.

Isotopically labeled flavan-3-ols (mono and several oligomeric forms) have been prepared, either by biolabeling (^{14}C),[4] or by total synthesis (^{13}C),[5] and hemi-synthesis (^{2}H).[6] However, the former approach is generally associated with tedious purifications, poor selectivity of the labeling and limited specific activities. On the other hand, compounds prepared by the latter approach lack the sensitivity of radioactive isotopes, which greatly facilitate the detection and positive identification of trace amounts.[4c] Moreover, the synthetic approaches of isotopically labeled flavan-3-ols reported so far involved the resolution of racemic mixtures,[5] thus hampering their synthetic efficiency.



Scheme 1. Selected dietary flavan-3-ols and oligomers.

We report here the first asymmetric total synthesis of procyanidin B2 [(–)-epicatechin-(4β–8)-(–)-epicatechin] and its application to the preparation of a regioselectively radiolabeled ^{14}C-analogue.

Results and Discussion

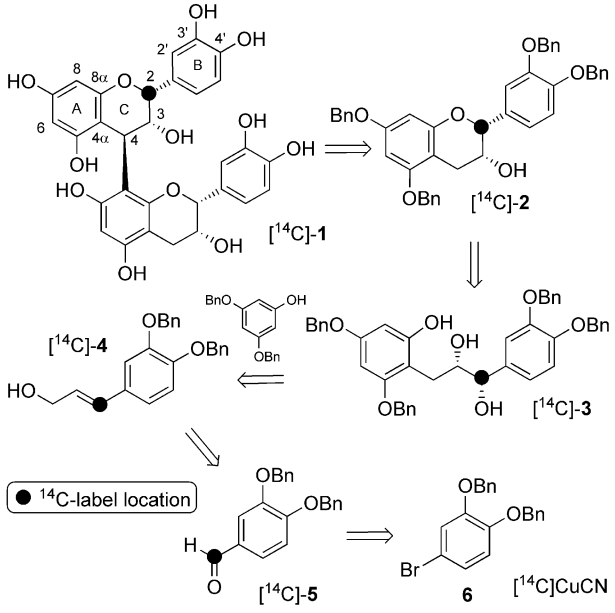
Previous syntheses of procyanidins were based on the coupling of readily available flavan-3-ol monomers.[7] However, this strategy was not compatible with the introduction of a ^{14}C-label in the target molecule. Proton/tritium exchange from a labile site of a natural precursor was also discarded so as not to compromise the stability of the target molecule during biological assays.[8]

[a] Nestlé Research Center,
P. O. Box 44, 1000 Lausanne 26, Switzerland
Fax: +41-21-785-8554
E-mail: denis.barron@rdls.nestle.com
[b] Selcia Ltd., Fyfield Business & Research Park,
Fyfield Road, Ongar, Essex, CM5 0GS, UK

Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

 © 2008 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

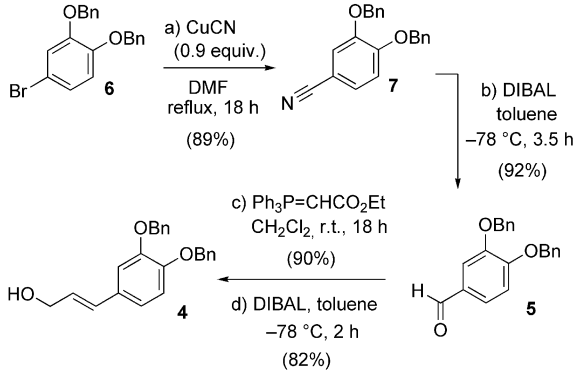
Our retrosynthetic analysis of procyanidin B2 is shown in Scheme 2. In terms of a radiolabeling strategy, preliminary work led us to consider position 2 of the upper C-ring moiety as the most feasible position to introduce the radioactive label. Thus, the target ^{14}C-labeled molecule was envisioned to arise from the heterologous coupling of radiolabeled/non-labeled (–)-epicatechin functionalized units. This coupling step is known to require an excess of one of the two units, which precluded labeling of both top and bottom moieties of procyanidin B2. In our synthetic approach, radiolabeled (–)-epicatechin derivative [^{14}C]-**2** would be obtained from the stereoselective intramolecular cyclization of [^{14}C]-**3**.[5,9] In turn, the condensation of cinnamyl alcohol [^{14}C]-**4** with a C6 phenolic unit to form the required C6–C3–C6 skeleton, followed by Sharpless asymmetric dihydroxylation, would afford [^{14}C]-**3**. Introduction of the ^{14}C-label would occur through the reaction of aryl bromide **6** with ^{14}C-radiolabeled copper cyanide.



Scheme 2. Retrosynthetic analysis of ^{14}C-labeled procyanidin B2 [^{14}C]-**1**.

Our work started with securing an efficient and reliable synthetic pathway, compatible with the constraints of a radioactive synthesis. Scheme 3 presents the synthetic approach for cinnamyl alcohol **4**, starting from aryl bromide **6**.[10]

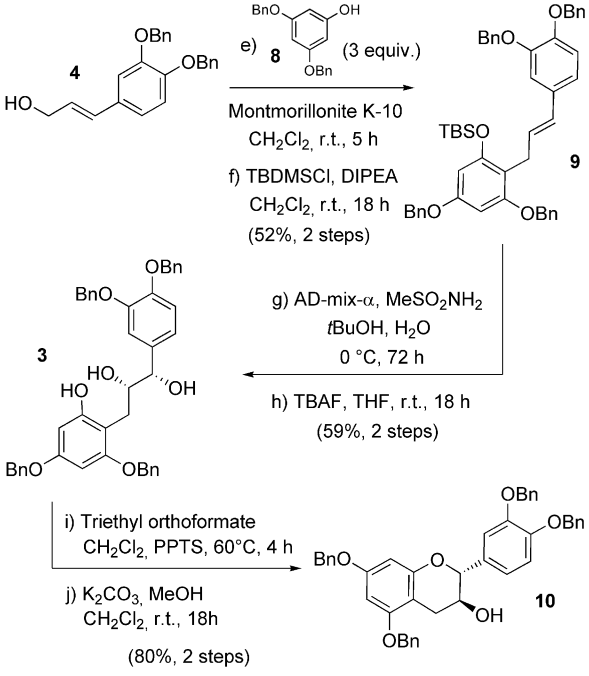
Introduction of the ^{14}C-label was originally foreseen through the palladium coupling of **6** and potassium [^{14}C] cyanide, a cheap and reliable source of carbon-14. However, the electronically rich aryl group in **6** proved a poor substrate in this reaction. This prompted us to consider the direct condensation of **6** and CuCN and we were pleased to find that the required nitrile **7** formed in high yield (89%). **7** was then submitted to reduction with DIBAL, allowing us to prepare aldehyde **5** in excellent yield (92%). Stereoselective Wittig condensation (affording almost exclusively the



Scheme 3. Synthesis of cinnamyl alcohol intermediate **4**.

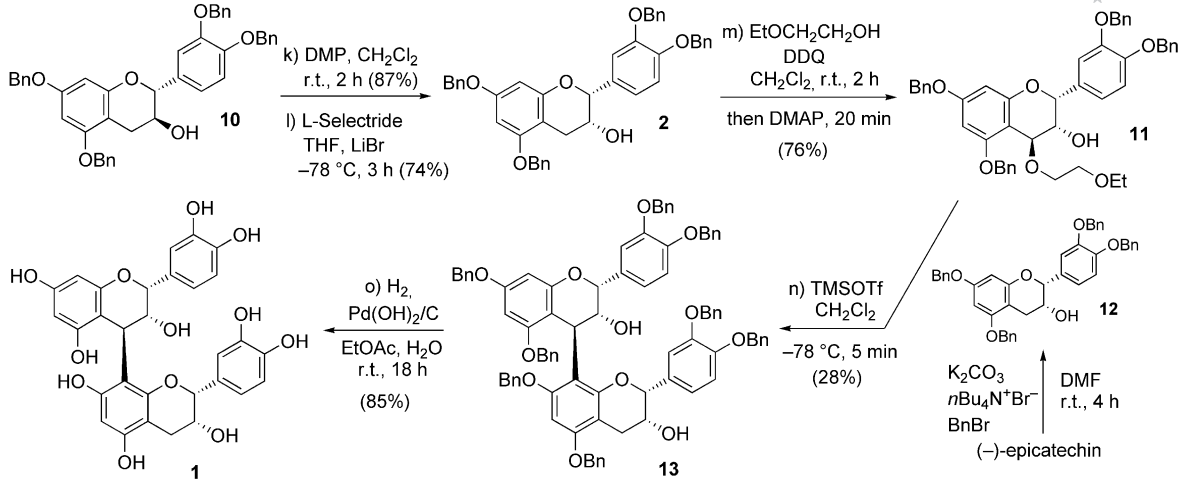
trans isomer) followed by reduction with DIBAL at –78 °C gave the cinnamyl alcohol intermediate **4** in 74% overall yield.

The elaborated *trans*-alkene **9** having the necessary C6–C3–C6 skeleton was then prepared from **4** as shown in Scheme 4. In the synthetic route we selected, this coupling step of C6–C3 and C6 units was generally limiting due to "over-alkylation" of the C6 phenolic unit. This potential issue of coupling alcohol **4** with 1,3-di-*O*-benzylphloroglucinol (**8**)[11] in the presence of montmorillonite K-10 was overcome by ensuring that the phloroglucinol was permanently present in excess in the reaction medium. Thus, we were pleased to find that, when using 3 equiv. of **8** and performing a slow addition of the alcohol **4**, the condensation proceeded cleanly and with a satisfactory conversion, affording **9** after silylation with TBDMSCl (52% overall yield from **4** to **9**). This material was then converted into benzylated (+)-catechin **10** using the sequence described by Wan et al.[9d] Thus, Sharpless asymmetric dihydroxylation of **9**



Scheme 4. Synthesis of benzylated (+)-catechin **10**.

 © 2008 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



Scheme 5. Synthesis of procyanidin B2 (**1**).

using AD-mix-α followed by deprotection with TBAF afforded diol **3** in 59% yield. Cyclization to benzylated (+)-catechin **10** was achieved in 80% yield using triethyl orthoformate in the presence of PPTS followed by methanolysis. This cyclization proceeded cleanly with inversion of the stereochemistry at carbon C-2, the free phenolic hydroxy group reacting with the intermediate cyclic orthoacetate.[9d] Our material was positively compared by ^{1}H NMR and TLC to another batch of **10**, separately prepared by benzylation of commercially available (+)-catechin. Benzylated (+)-catechin **10** was then converted into the corresponding (–)-epicatechin derivative **2** by Dess–Martin oxidation to give the corresponding ketone (87% yield) which was reduced with L-Selectride® at low temperature to provide **2**[9d,12] in 74% yield (Scheme 5). Further confirmation of the structure and stereochemistry was achieved by submitting our material to hydrogenation and comparing it to an authentic sample of (–)-epicatechin by ^{1}H NMR and chiral HPLC.[13] The chiral purity of our synthetic (–)-epicatechin was assessed to be >92%.

Having secured benzylated (–)-epicatechin **2** in 12 steps and 9.5% overall yield from aryl bromide **6** and with excellent chiral enrichment, we turned our efforts towards the preparation of the dimer procyanidin B2 (Scheme 5). Treating **2** with DDQ in the presence of 2-ethoxyethanol led to the activated monomer **11**[14] in 76% yield. Condensation of **11** with 4 equiv. of benzylated (–)-epicatechin **12** [prepared from commercially available (–)-epicatechin] using the conditions of Saito et al.[7j,14] led to dimer **13** in 28% yield, after purification by reverse phase preparative HPLC.[15] Finally, debenzylation of **13** into procyanidin B2 (**1**) was achieved by hydrogenation with palladium hydroxide,[7d] in 85% yield.

At this point, we felt sufficiently confident in our synthetic pathway to undertake the preparation of the ^{14}C-analogue labeled at position 2, without the need for further optimizations. [^{14}C]CuCN was prepared from 524 mCi of [^{14}C]KCN,[16] and used as the radioactive precursor in the radiolabeled synthesis of [^{14}C]-**1**. In the synthesis of radioactive procyanidin B2, results comparable to the synthesis of non-labeled procyanidin B2 **1** were obtained (Table 1). The only noticeable difference concerned the preparation of [^{14}C]-**2** by the reduction of the corresponding ketone with L-Selectride® (Table 1, step l), which proceeded with modest yield; fortunately this was favourably compensated by two high yielding steps (Table 1, steps i + j). Thus, radiolabeled benzylated (–)-epicatechin [^{14}C]-**2** was obtained in 12 "hot" steps and 8.5% overall yield from [^{14}C]KCN.

Table 1. Syntheses of procyanidin B2 **1** vs. ^{14}C-radiolabeled procyanidin B2 [^{14}C]-**1**.

Reaction Step	Synthesis of **1** Products	% Yield	Synthesis of [^{14}C]-**1** Products	% Yield
a	**7**	89	[^{14}C]-**7**	80[a]
b	**5**	92	[^{14}C]-**5**	94
c	–	90	–	97
d	**4**	82	[^{14}C]-**4**	85
e + f	**9**	52	[^{14}C]-**9**	53
g + h	**3**	59	[^{14}C]-**3**	72
i + j	**10**	80	[^{14}C]-**10**	94
k	–	87	–	87
l	**2**	74	[^{14}C]-**2**	44
m	**11**	76	[^{14}C]-**11**	68
n	**13**	28	[^{14}C]-**13**	37
o	**1**	85[b]	[^{14}C]-**1**	85[b]
Global[c]		2.0		2.2

[a] Overall yield (2 steps from [^{14}C]KCN). [b] Performed on small scale – product not isolated, see main text. [c] Overall yield (14 steps, **7** to **13**).

Finally, the last steps of the radioactive synthesis [electrophilic activation and coupling of (–)-epicatechin monomers] afforded 11.6 mCi (at 55.2 mCi/mmol) of benzylated procyanidin B2 [^{14}C]-**13**. Small amounts of radiolabeled procyanidin B2 [^{14}C]-**1** were obtained after hydrogenation and minor work-up in the form of diluted aqueous solutions, which exhibited satisfactory purity and stability ([^{14}C]-**1** stable under N_2 at 4 °C in the dark for at least two days).

 © 2008 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Thus, the radioactive material was kept in the protected form [^{14}C]-**13** for batch to batch deprotections prior to use in biological assays.

Conclusions

We have developed the first asymmetric total synthesis of procyanidin B2 and applied this synthetic route to the preparation of a regioselectively ^{14}C-radiolabeled analogue. The ^{14}C-labeled procyanidin B2 will be used to improve our knowledge of the metabolism of procyanidins.

Experimental Section

General: All commercially available reagents were used without further purification. All anhydrous solvents were obtained from Acros or Aldrich. Organic solutions were concentrated under reduced pressure on a Heidolf VV Microrotary evaporator using a water bath. Chromatographic purification of products was accomplished using forced-flow chromatography on Fluorochem Davisil 40–63u 60A silica gel or IST Isolute 100 g, 50 g or 20 g Flash SI silica cartridges. Thin-layer chromatography (TLC) was performed with Macherey–Nagel Alugram SIL G/UV$_{254}$ 0.25 mm silica gel plates. Reverse-phase TLC was performed with Merck 60 RP-18 F$_{254}$S 0.25 mm silica gel plates. [^{14}C]Labeled compounds were co-eluted side by side with their corresponding unlabeled counterparts. Thin-layer chromatography was analysed by autoradiography using a Packard Instant Imager Model A2024. ^{1}H NMR spectra were recorded with Bruker ARX300 and DPX400 spectrometers. Chemical shifts (δ) are reported from tetramethylsilane with the solvent resonance as the internal standard ($CDCl_3$: δ = 7.26 ppm). Chemical shifts and coupling constants are reported in ppm and Hz, respectively. Multiplicity is reported as follow: s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet. Mass spectral analysis was performed with a Micromass Platform II single quadrupole mass spectrometer. High performance Liquid Chromatography (HPLC) was carried out using a Jasco LC-UV system with a B-RAM radiodetector. Preparative High Performance Liquid Chromatography (Prep. HPLC) was carried out using a Gilson LC-UV system. Radioactive concentration was performed by liquid scintillation counting using a Packard Tricarb 2100TR Liquid Scintillation Counter.

"Cold" Route Development. 1,2-Bis(benzyloxy)-4-bromobenzene (6): Compound **6** was prepared from 4-bromoveratrole according to the literature by deprotection with boron tribromide followed by benzylation.[17] 4-Bromoveratrole (14.5 g, 66.7 mmol) was diluted under nitrogen with anhydrous CH_2Cl_2 (100 mL) in a three-neck reaction flask and then cooled to –78 °C. Boron tribromide (***Caution:*** BBr_3 is moisture-sensitive, corrosive, and highly toxic) (1.0 M in CH_2Cl_2, 100 mL, 100 mmol, 1.5 equiv.) was slowly introduced through a dropping funnel. After the addition was complete, the brown reaction mixture was stirred at room temperature for 72 h. The solution was chilled to 0 °C and water (100 mL) was added slowly. The residue was hydrolyzed with a minimum amount of 10% aqueous NaOH (20 mL). The resulting solution was acidified (to pH 1) with concd. hydrochloric acid and extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were washed with water (70 mL) and brine (70 mL), then dried with anhydrous $MgSO_4$ and concentrated in vacuo to afford bromo-3,4-dihydroxybenzene as a grey solid (8.77 g, 70%). The product was used in the next step without purification (only one spot by TLC, isohexane/Et_2O, 1:1, R_f 4-bromoveratrole = 0.76, R_f bromo-3,4-dihydroxybenzene = 0.71). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.03 (d, J = 2.2 Hz, 1 H), 6.93 (dd, J = 8.4, J = 2.2 Hz, 1 H), 6.74 (d, J = 8.4 Hz, 1 H), 5.12 (s, 1 H), 4.99 (s, 1 H) ppm. A stirred solution of crude 4-bromo-1,2-dihydroxybenzene (8.77 g, 46.4 mmol) in acetone (220 mL) was treated with K_2CO_3 (19.25 g, 139.2 mmol, 3 equiv.), and benzyl bromide (17 mL, 139.2 mmol, 3 equiv.). The mixture was heated at reflux temperature for 3 h, cooled to room temperature, filtered and the solid pellet was thoroughly washed with acetone. The combined filtrate and washings were evaporated to afford a residue, which was dissolved in Et_2O (220 mL), washed with brine (100 mL), dried with $MgSO_4$, filtered and evaporated to give a yellow oil. This oil was purified by column chromatography, eluting with isohexane/Et_2O (8:2) to give **6** as a white solid (8.35 g, 48%). TLC isohexane/Et_2O, 1:1: R_f of the bromo-dihydroxybenzene = 0.71, R_f of the bis(benzyloxy)bromobenzene = 0.83. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.46–7.30 (m, 10 H), 7.07 (d, J = 2.1 Hz, 1 H), 6.99 (dd, J = 8.5, 2.1 Hz, 1 H), 6.80 (d, J = 8.5 Hz, 1 H), 5.12 (s, 4 H) ppm.

3,4-Bis(benzyloxy)benzonitrile (7): Copper cyanide (***Caution:*** CuCN is toxic) (64.3 mg, 0.717 mmol) was added to a solution of **6** (291.5 mg, 0.789 mmol, 1.1 equiv.) in anhydrous DMF (5 mL) under nitrogen. The resulting solution was stirred at 160 °C for 18 h to leave a cream suspension. The cooled reaction mixture was diluted with ethyl acetate (10 mL) then washed with 2% aqueous ferric chloride (5 mL), water (5 mL), 0.8% w/w aqueous sodium metabisulfite (5 mL), water (5 mL) and saturated brine (2 mL). Each wash was subsequently back extracted with ethyl acetate. The organic phase was dried with $MgSO_4$, filtered and the solvents evaporated under vacuum. The residue was diluted with CH_2Cl_2 (5 mL + 2 × 3 mL) and loaded on the top of a 20 g silica cartridge pre-wetted with isohexane. Elution using isohexane/Et_2O (100:0 to 60:40) gave **7** as a white solid (202 mg, 89%). TLC isohexane/Et_2O, 1:1: R_f of 1,2-bis(benzyloxy)-4-bromobenzene = 0.83, R_f of 3,4-bis(benzyloxy)benzonitrile = 0.61. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.46–7.30 (m, 10 H), 7.22 (dd, J = 8.4, 2.0 Hz, 1 H), 7.14 (d, J = 2.0 Hz, 1 H), 6.94 (d, J = 8.4 Hz, 1 H), 5.22 (s, 2 H), 5.16 (s, 2 H) ppm.

3,4-Bis(benzyloxy)benzaldehyde (5): 3,4-Bis(benzyloxy)benzonitrile (**7**, 188.7 mg, 0.598 mmol) was dissolved in anhydrous toluene (10 mL) under nitrogen and this solution was then cooled to –78 °C. DIBAL (1.0 M in toluene, 1.2 equiv., 0.718 mmol, 0.72 mL) was added dropwise maintaining the inner temperature below –70 °C. After stirring for 3 h at –78 °C, a small aliquot was partitioned between water and EtOAc. TLC analysis (isohexane/EtOAc, 7:3, R_f of 3,4-bis(benzyloxy)benzonitrile = 0.75, R_f of 3,4-bis(benzyloxy)benzaldehyde = 0.65) revealed that the reaction had not gone to completion. Further DIBAL (0.5 mL) was added dropwise and the reaction was stirred at –78 °C for an extra 2 h, after which TLC analysis showed the reaction to be complete. The reaction mixture was poured (***Caution:*** the reaction is exothermic) into a mixture of 10% aqueous acetic acid (13 mL) and ice (≈ 10 g). The phases were separated and the aq layer was extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine (20 mL) and dried ($MgSO_4$). The solvents were evaporated at reduced pressure and the crude product was purified by column chromatography [20 g silica cartridge pre-wetted with isohexane, loading with CH_2Cl_2 (2 mL + 2 × 1 mL), elution with isohexane/EtOAc (100:0 to 80:20)], affording **5** (175.8 mg, 92%) as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 9.81 (s, 1 H), 7.50–7.30 (m, 12 H), 7.02 (d, J = 8.2 Hz, 1 H), 5.26 (s, 2 H), 5.22 (s, 2 H) ppm.

***(E)*-3,4-Bis(benzyloxy)cinnamyl Alcohol (4):** (Ethoxycarbonylmethylene)triphenylphosphorane (7 g, 20.1 mmol) was added to a

 © 2008 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

solution of **5** (6.38 g, 20.04 mmol) in anhydrous CH_2Cl_2 (60 mL). After stirring at room temperature for 16 h, TLC (isohexane/EtOAc, 8:2, R_f of 3,4-bis(benzyloxy)benzaldehyde = 0.31, R_f of the ester = 0.45) showed that some starting aldehyde remained. Further (ethoxycarbonylmethylene)triphenylphosphorane (1.4 g, 4 mmol, 0.2 equiv.) was added and stirring was continued for 8 h. After checking by TLC that the reaction had gone to completion, the mixture was directly loaded onto the top of a SiO_2 column and purified by column chromatography [175 g SiO_2, elution with isohexane/EtOAc (100:0 to 40:60)]. The desired fractions were concentrated to dryness to give ethyl (*E*)-3,4-bis(benzyloxy)cinnamate in ca. 90% yield (7.10 g). ^1H NMR ($CDCl_3$, 300 MHz): δ = 7.57 (d, J = 15.9 Hz, 1 H), 7.50–7.29 (m, 10 H), 7.12 (d, J = 1.6 Hz, 1 H), 7.07 (dd, J = 8.4, 1.6 Hz, 1 H), 6.92 (d, J = 8.4 Hz, 1 H), 6.24 (d, J = 15.9 Hz, 1 H), 5.20 (s, 2 H), 5.18 (s, 2 H), 4.25 (q, J = 7.1 Hz, 2 H), 1.33 (t, J = 7.1 Hz, 3 H) ppm. To a solution of ethyl (*E*)-3,4-bis(benzyloxy)cinnamate (4.23 g, 10.9 mmol) in anhydrous toluene (60 mL) at –78 °C under nitrogen was added dropwise DIBAL (26.2 mL, 1.0 M solution in toluene, 26.2 mmol, 2.4 equiv.), the temperature being maintained below –70 °C. The clear yellow solution was stirred at –78 °C for 2 h then a small aliquot was partitioned between water and ethyl acetate. TLC analysis (isohexane/EtOAc, 8:2, R_f ester = 0.35, R_f alcohol = 0.06) showed complete consumption of the starting ester. The reaction mixture was then poured onto a mixture of saturated aqueous NH_4Cl and ice. The phases were separated and the aqueous phase was extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with brine (100 mL), dried ($MgSO_4$) and concentrated to dryness. The residue was purified by column chromatography [175 g SiO_2, elution with isohexane/EtOAc (100:0 to 40:60)]. The desired fractions were concentrated to dryness to give **4** in 82% yield. ^1H NMR ($CDCl_3$, 300 MHz): δ = 7.48–7.27 (m, 10 H), 7.02 (s, 1 H), 6.92–6.86 (m, 2 H), 6.50 (d, J = 15.7 Hz, 1 H), 6.18 (dt, J = 15.7, 5.8 Hz, 1 H), 5.17 (s, 2 H), 5.16 (s, 2 H), 4.28 (dd, J = 5.8, 1.3 Hz, 2 H), 1.37 (t, J = 1.3 Hz, 1 H) ppm.

(*E*)-3-[2,4-Bis(benzyloxy)-6-(*tert*-butyldimethylsilyloxy)phenyl]-1-[3,4-bis(benzyloxy)phenyl]propene (9): 3,5-Dibenzyloxyphenol (**8**) was prepared following an adaptation of the literature.[11] All the operations were conducted under argon; *n*-butanethiol (2.16 g, 5.44 mmol, 1.0 equiv.) was added dropwise to a suspension of sodium hydride (60% dispersion in mineral oil; 414 mg, 10.34 mmol, 1.90 equiv.) in 10.0 mL anhydrous DMF at 0 °C. After 1 h 30 stirring at 0 °C, a solution of 1,3,5-tri(*O*-benzyloxy)phloroglucinol (1.10 mL, 9.52 mmol, 1.89 equiv.) in 10.0 mL anhydrous DMF was added dropwise to the reaction mixture, which was then transferred into an oil bath and stirred at 150 °C overnight (TLC hexane/EtOAc, 80:20, R_f of 1,3,5-tri-*O*-benzylphloroglucinol = 0.72, R_f of 1,3-di-*O*-benzylphloroglucinol = 0.23). The reaction mixture was then cooled to room temperature, diluted with 10 mL EtOAc, extracted with 10 mL HCl 1 N, washed with 4 × 10 mL water, dried with Na_2SO4 and finally evaporated to dryness under vacuum to afford 2.98 g of orange oily residue. This residue was diluted with 2 mL EtOAc and 4 mL hexane and purified by column chromatography on silica gel (hexane/EtOAc, 100:0 to 50:50) to afford **8** as a white powder in 84% yield (1.40 g, 4.56 mmol). ^1H NMR ($CDCl_3$, 360 MHz): δ = 7.40–7.28 (m, 10 H), 6.23 (t, J = 2.0 Hz, 1 H), 6.09 (d, J = 2.0 Hz, 2 H), 5.12 (br. s, 1 H), 4.97 (s, 4 H) ppm. To a well-stirred mixture of 3,5-bis(benzyloxy)phenol (**8**, 960 mg, 3.13 mmol, 3 equiv.) in anhydrous CH_2Cl_2 (40 mL) was added montmorillonite K-10 (960 mg) at room temperature and under nitrogen. Shortly after, a solution of (*E*)-3,4-bis(benzyloxy)cinnamyl alcohol (**4**, 362 mg, 1.04 mmol) in anhydrous CH_2Cl_2 (20 mL) was added dropwise over 30 min. The resulting purple mixture was stirred at room temperature overnight (TLC isohexane/EtOAc, 1:1, R_f of the alcohol = 0.35, R_f of 3,5-bis(benzyloxy)phenol = 0.56, R_f of **9** = 0.54), then filtered through a pad of Celite, which was rinsed with EtOAc (4 × 15 mL). After evaporation, the residue was purified by column chromatography on silica gel (isohexane/EtOAc, 100:0 to 60:40) to afford a mixture of the desired adduct and 3,5-bis(benzyloxy)phenol. This mixture was diluted with anhydrous CH_2Cl_2 (13 mL) under nitrogen then DIPEA (2 mL, ca. 6 equiv.) and TBDMSCl (685 mg, 2.4 equiv.) were successively added and the reaction mixture was stirred at room temperature overnight. After checking by TLC that the reaction had gone to completion (isohexane/CH_2Cl_2, 1:1, R_f adduct = 0.03, R_f silylated adduct = 0.45), the mixture was evaporated to dryness and purified by column chromatography (loading with isohexane and a minimum of CH_2Cl_2, eluting with isohexane/CH_2Cl_2, 100:0 to 20:80) to afford propene **9** as a colorless oil in 59% yield (464.4 mg). ^1H NMR ($CDCl_3$, 300 MHz): δ = 7.48–7.28 (m, 20 H), 6.91 (d, J = 1.8 Hz, 1 H), 6.83 (d, J = 8.2 Hz, 1 H), 6.76 (dd, J = 8.2, 1.8 Hz, 1 H), 6.25–6.05 (m, 2 H, concealed), 6.28 (d, J = 2.3 Hz, 1 H), 6.10 (d, J = 2.3 Hz, 1 H), 5.12 (s, 2 H), 5.10 (s, 2 H), 5.01 (s, 2 H), 5.00 (s, 2 H), 3.47 (d, J = 6.0 Hz, 2 H), 0.99 (s, 9 H), 0.18 (s, 6 H) ppm.

(1*S*,2*S*)-3-[2,4-Bis(benzyloxy)-6-hydroxyphenyl]-1-[3,4-bis(benzyloxy)phenyl]propane-1,2-diol (3): AD-mix-α (12.51 g) and methanesulfonamide (853 mg) were dissolved in a solvent mixture of *t*BuOH (20 mL) and water (20 mL). The resulting mixture was stirred at room temperature for 5 min, then cooled to 0 °C and a solution of **9**, (1.33 g, 1.78 mmol) in CH_2Cl_2 (10 mL) was added. After stirring for 72 h at 0 °C, TLC (isohexane/EtOAc, 8:2, R_f of **9** = 0.83, R_f of the diol = 0.31) showed that the reaction was complete. A 10% $Na_2S_2O_3$ aqueous solution (20 mL) was added to quench the reaction. After stirring for 15 min, the mixture was filtered through a pad of Celite, which was further rinsed with EtOAc (4 × 20 mL). The phases were separated and the aqueous layer was further extracted with EtOAc (4 × 30 mL). The organic phases were combined, dried ($MgSO_4$) and the solvents evaporated. The residue was purified by column chromatography (50 g silica cartridge, loading with 2 × 5 mL CH_2Cl_2, eluting with isohexane/EtOAc, 100:0 to 40:60). The desired fractions were concentrated to dryness (760 mg) then dissolved in anhydrous THF (10 mL) and TBAF (2.1 mL, 1.0 M in THF) was added. The resulting mixture was stirred at room temperature for 4 h, after which time TLC analysis showed complete deprotection of the material (isohexane/EtOAc, 1:1, R_f of the silylated diol = 0.82, R_f of the triol = 0.52). Saturated aqueous $NaHCO_3$ solution was added and the mixture was extracted with EtOAc. The organic layers were combined, dried ($MgSO_4$) and the solvents evaporated. The residue was purified by flash chromatography on silica gel ($CHCl_3$/EtOAc, 100:0 to 95:5) to give **3** as a white solid (620 mg, 52%). ^1H NMR ($CDCl_3$, 300 MHz): δ = 7.47–7.25 (m, 18 H), 7.11 (d, J = 6.9 Hz, 2 H), 6.92 (d, J = 1.5 Hz, 1 H), 6.80 (AB, J = 8 Hz, 2 H), 6.27 (d, J = 2.2 Hz, 1 H), 6.19 (d, J = 2.2 Hz, 1 H), 5.10 (AB, J = 12.2 Hz, 2 H), 5.07 (s, 2 H), 5.00 (s, 2 H), 4.85 (AB, J = 11.8 Hz, 2 H), 4.44 (d, J = 6.8 Hz, 1 H), 3.97–3.93 (m, 1 H), 2.91 (A of *AB*, J = 14.5, 3.6 Hz, 1 H), 2.72 (B of AB, J = 14.5, 8.2 Hz, 1 H) ppm.

5,7,3′,4′-Tetra-*O*-benzylcatechin (10): Triethyl orthoformate (230 μL) was added to a suspension of diol **3** (218.5 mg, 0.327 mmol) in 1,2-dichloroethane (5.3 mL) was added, followed by PPTS (50 mg). The mixture was stirred at room temperature for 20 min and the solid dissolved. The mixture was heated to 60 °C for 5 h until TLC showed the reaction was complete. After evaporation of the solvent, the residue was redissolved in DME (2.1 mL) and MeOH (2.1 mL), K_2CO_3 (35 mg) was added, and the mixture was stirred at room temperature overnight. The solvent was evapo-

 © 2008 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

rated, and the residue was purified by flash chromatography on silica gel (isohexane/EtOAc, 100:0 to 75:25) to afford the desired product **10** as white solid (180 mg, 85%). TLC isohexane/EtOAc, 75:25, R_f of 5,7,3′,4′-tetra-*O*-benzylcatechin = 0.27. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.47–7.25 (m, 20 H), 7.03 (s, 1 H), 6.96 (s, 2 H), 6.27, 6.21 (ABq, J = 2.2 Hz, 2 H), 5.18 (s, 2 H), 5.17 (narrow ABq, concealed, 2 H), 5.03 (s, 2 H), 4.99 (s, 2 H), 4.63 (d, J = 8.2 Hz, 1 H), 4.03–3.97 (m, 1 H), 3.11, 2.65 (ABq, J = 16.4, both parts d with J = 5.7 and 8.8 Hz, 2 H), 1.58 (d, J = 3.7 Hz, 1 H) ppm.

5,7,3′,4′-Tetra-*O*-benzylepicatechin (2): Dess–Martin periodinane (≈ 2 mL, 0.3 M in CH_2Cl_2, 0.608 mmol, 2.2 equiv.) was added in one batch to a stirred solution of **10** (180 mg, 0.277 mmol) in anhydrous CH_2Cl_2 (10 mL) under nnitrogen. The mixture was stirred at room temperature for about 2 h until TLC (isohexane/EtOAc, 2:1, R_f of 5,7,3′,4′-tetra-*O*-benzylcatechin = 0.4, R_f of the ketone = 0.54) showed the absence of starting material. Subsequently, saturated aqueous $NaHCO_3$ solution (4.2 mL) and 10% aqueous $Na_2S_2O_3$ solution (4.2 mL) were added to quench the reaction. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$) and the solvents evaporated. The residue was quickly purified by filtration through a 10 g silica cartridge (eluting with $CHCl_3$/EtOAc, 9:1) to afford the desired (2*R*)-5,7,3′,4′-tetrakis(benzyloxy)flavan-3-one as a yellow oil (157 mg, 87%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.45–7.26 (m, 20 H), 6.96 (s, 1 H), 6.88, 6.86 (ABq, J = 8, B part d with J = 1.5 Hz, 2 H), 6.35 (narrow ABq, 2 H), 5.24 (s, 1 H), 5.14 (s, 2 H), 5.10 (narrow ABq, 2 H), 5.02 (s, 2 H), 5.02 (s, 2 H), 3.61, 3.45 (ABq, J = 21.5 Hz, 2 H) ppm. Under nitrogen, the ketone (157 mg, 0.242 mmol) was dissolved in dry THF (5 mL), and the solution was cooled to –78 °C. Then L-Selectride® (380 µL, 1.0 M solution in THF, 0.38 mmol, 1.6 equiv.) was added dropwise. The resulting solution was stirred at –78 °C overnight. When TLC showed the reaction was complete, saturated $NaHCO_3$ aqueous solution (≈ 5 mL) was added to quench the reaction. The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic phases were dried ($MgSO_4$) and the solvents evaporated. The residue was purified by flash chromatography (20 g silica cartridge, eluting with isohexane/EtOAc, 100:0 to 80:20) to give **2** in 74% yield (116.8 mg). TLC isohexane/EtOAc, 2:1, R_f of the ketone = 0.54, R_f of 5,7,3′,4′-tetra-*O*-benzylepicatechin = 0.42. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.48–7.25 (m, 20 H), 7.15 (s, 1 H), 7.00, 6.97 (ABq, J = 8.5 Hz, A part d with J = 1.5 Hz, 2 H), 6.27 (s, 2 H), 5.19 (s, 2 H), 5.18 (s, 2 H), 5.03 (s, 2 H), 5.02 (s, 2 H), 4.92 (s, 1 H), 4.22 (br. s, 1 H), 3.00, 2.92 (ABq, J = 17.5 Hz, both parts d with J = 1.5 and 4 Hz, resp., 2 H), 1.64 (d, J = 5.5 Hz, 1 H). Part of this material was submitted to hydrogenation to provide a batch of "synthetic" (–)-epicatechin, which chiral purity was assessed to be >92% by HPLC [HPLC conditions were adapted from the literature:[13] Cyclobond I-2000 RSP 250 × 4.6 mm column; mobile phase A: 50 mM NaH_2PO_4 at pH 3, mobile phase B: 80% acetonitrile in 30 mM NaH_2PO_4 at pH 3; flow 1 mL/min; linear gradient from 10.0 to 13.5% over 45 min, then increasing to 45% B at 70 min; detection at 210 nm; authentic samples of (–)-epicatechin and (+/–)-catechin from Biochemika were used as references].

5,7,3′,4′-Tetra-*O*-benzyl-4-(2-ethoxyethoxy)epicatechin (11): 2-Ethoxyethanol (110 µL, 1.14 mmol, 6 equiv.) and then all at once with good stirring DDQ (86.3 mg, 0.38 mmol, 2 equiv.) was added to a solution of **2** (124 mg, 0.19 mmol) in anhydrous CH_2Cl_2 (5 mL) at room temperature. A black-green color appeared instantaneously. After 2 h of vigorous stirring at room temperature under nitrogen, DMAP (52 mg, 0.42 mmol, 2.2 equiv.) was added and stirring was continued for 10 min at room temperature. The dark precipitate was eliminated by filtration through a Pasteur pipette fitted with cotton wool and rinsed with CH_2Cl_2 (3 × 5 mL). The combined organic layers were directly loaded on the top of a 20 g silica cartridge, which was then eluted with isohexane/EtOAc (100:0 to 40:60) to afford compound **11** in 76% yield (108 mg). TLC isohexane/EtOAc, 7:3, R_f of 5,7,3′,4′-tetra-*O*-benzyl-4-(2-ethoxyethoxy)epicatechin = 0.45, R_f of 5,7,3′,4′-tetra-*O*-benzylepicatechin = 0.52. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.49–7.26 (m, 20 H), 7.19 (d, J = 1.5 Hz, 1 H), 7.07, 7.00 (ABq, J = 8.2 Hz, A part d with J = 1.5 Hz, 2 H), 6.29, 6.27 (ABq, J = 2.2 Hz, 2 H), 5.20 (s, 2 H), 5.20 (s, 1 H, concealed), 5.19 (s, 2 H), 5.03 (s, 2 H), 5.09, 5.02 (ABq, partially concealed, J = 11.5 Hz, 2 H), 4.61 (d, J = 2.9 Hz, 1 H), 4.04 (br., 1 H), 3.83–3.78 (m, 2 H), 3.53–3.41 (m, 4 H), 1.55 (d, J = 6.9 Hz, 1 H), 1.17 (t, J = 7.1 Hz, 3 H).

8-(5,7,3′,4′-Tetra-*O*-benzylepicatechin-4β-yl)(5,7,3′,4′-tetra-*O*-benzylepicatechin) (13): TMSOTf (68 µL, 0.376 mmol, 1 equiv.) was added dropwise to a solution of 5,7,3′,4′-tetra-*O*-benzylepicatechin **12** (979 mg, 1.504 mmol, 4 equiv.) and 5,7,3′,4′-tetra-*O*-benzyl-4-(2-ethoxy-ethoxy)epicatechin **11** (277.8 mg, 0.376 mmol) in 34 mL of anhydrous CH_2Cl_2 at –78 °C. The resulting solution was stirred at –78 °C for 5 min then the reaction was terminated by addition of saturated aqueous $KHCO_3$ (0.6 mL). The CH_2Cl_2 phase was washed with water (3 × 56 mL), dried ($MgSO_4$), filtered and concentrated to dryness. The residue was purified by column chromatography (loading with CH_2Cl_2, 20 g silica cartridge prewetted with isohexane, elution with isohexane/EtOAc, 100:0 to 50:50). Fractions containing **13** were concentrated and further purified by preparative HPLC [HPLC conditions adapted from the literature,[7k] using a Phenomenex Gemini C18 column 10µ, 110A, 250 × 21–20 mm. Conditions: at 20 mL/min; 80 to 100% CH_3CN in water (gradient over 25 min) then CH_3CN. UV 280 nm; retention time of the dimer: ca. 25.6 min]. Pure fractions were concentrated, filtered through a 2 g silica cartridge eluting with EtOAc, and concentrated to dryness to afford **13** as a white foam (139 mg, 28% yield). TLC isohexane/EtOAc, 7:3, R_f of 5,7,3′,4′-tetra-*O*-benzyl-4-(2-ethoxyethoxy)epicatechin = 0.43, R_f of 5,7,3′,4′-tetra-*O*-benzylepicatechin = 0.51, R_f of 5,7,3′,4′-tetra-*O*-benzylepicatechin-4β,8-(5,7,3′,4′-tetra-*O*-benzylepicatechin) = 0.34. ^{1}H NMR (selection; 2 rotamers, approx. 3:1, MR = major, mr = minor rotamer) ($CDCl_3$, 300 MHz): δ = 6.80, 6.44 (ABq, MR, J = 8, B part d with J = 1.5 Hz, 2 H), 6.36 (s, 1 H, MR), 6.25, 6.09 (ABq, mr, J = 2.5 Hz, 2 H), 6.20 (s, 1 H, mr), 6.02, 5.72 (ABq, MR, J = 2 Hz, 2 H), 5.55 (s, 1 H, MR), 5.29 (s, 1 H, mr), 4.66, 4.38 (ABq, mr, J = 12 Hz, 2 H), 4.63, 4.45 (ABq, MR, J = 11 Hz, 2 H), 4.32 (m, 1 H, mr), 3.95 (br. d, mr, J = 6 Hz, 1 H), 3.86 (m, 1 H, MR), 3.09–2.84 (m, MR + mr, 2 H), 1.80 (d, MR, J = 6 Hz, 1 H), 1.71 (d, mr, J = 6.5 Hz, 1 H), 1.47 (d, MR, J = 4 Hz, 1 H), 1.30 (d, mr, J = 6.5 Hz, 1 H) ppm.

Procyanidin B2 (1): Epicatechin derivative **13** (50 mg, 0.0385 mmol) was dissolved in ethyl acetate (5 mL). Water (5 mL) was added, followed by palladium hydroxide over carbon (15 mg), and the biphasic mixture was hydrogenated (1 atm hydrogen) overnight at room temperature. Reverse phase TLC analysis (H_2O/MeOH, 1:1 + 1% AcOH) showed completion of the reaction. The reaction mixture was filtered through a sintered funnel fitted with a glass fiber prefilter and concentrated to a 4 mL aqueous solution of **1**. This material was compared to an authentic sample of procyanidin B2 (obtained from Fluka) by reverse-phase TLC (H_2O/MeOH, 1:1 + 1% AcOH).

Synthesis of [2-^{14}C]Procyanidin B2. Preparation of Copper [^{14}C]Cyanide: Potassium [^{14}C]cyanide (630 mg, 524 mCi, 9.42 mmol at

 © 2008 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

55.64 mCi/mmol) was dissolved in water (22.2 mL) at room temperature. A solution of sodium sulfite (645 mg, 0.54 equiv.) in water (22.2 mL) was added dropwise, followed by a solution of copper(II) sulfate (2518 mg, 1.07 equiv.) in water (29 mL). This gave a white solid precipitate. After 10 min stirring, a solution of sodium hydroxide (175.6 mg, 0.465 equiv.) in water (4.6 mL) was added dropwise and stirring was continued for a further 20 min. The solids were allowed to settle and the pale blue liquors were pipetted from the solid precipitate. The solid was washed with water (2 × 30 mL) and acetone (3 × 30 mL), by stirring, allowing the solids to settle and pipetting off the liquors. Any remaining solvent was evaporated under vacuum to give copper [^{14}C]cyanide as a white solid which was stored overnight under vacuum over dried silica gel.

3,4-Bis(benzyloxy)benzo[^{14}C]nitrile ([^{14}C]-7): Compound **6** (3.83 g, 10.37 mmol, 1.1 equiv.) was added to a suspension of copper [^{14}C]-cyanide (524 mCi, 9.42 mmol) in anhydrous DMF (40 mL) and the reaction was heated under an nitrogen at 160 °C overnight. The cooled cream suspension was diluted with EtOAc (80 mL) then washed with 2% aqueous ferric chloride (40 mL), water (40 mL), 0.8% w/w aqueous sodium metabisulfite (40 mL), water (40 mL) and saturated brine (20 mL). Each wash was subsequently back extracted with EtOAc. The combined organic phases were dried with $MgSO_4$, filtered and the solvents evaporated under vacuum. The residue (95.1% pure by radioTLC using as eluant isohexane/diethyl ether, 1:1) was diluted with CH_2Cl_2 (10 mL + 2 × 5 mL) and loaded on the top of a 100 g silica cartridge pre-wetted with isohexane. Elution using isohexane/diethyl ether (100:0 to 70:30) gave batch I (best fractions) and batch II (fractions of lesser purity). The latter was repurified using a 20 g silica cartridge pre-wetted with isohexane and eluting using isohexane/diethyl ether (100:0 to 80:20). The desired fractions were combined with batch I and concentrated to dryness to give [^{14}C]-**7** as a white solid (2.40 g, 7.63 mmol, 424 mCi, 80%). The radiopurity of this material was assessed to be 99.8% by TLC/autoradiography (isohexane/diethyl ether, 1:1).

3,4-Bis(benzyloxy)[CO-^{14}C]benzaldehyde ([^{14}C]-5): [^{14}C]-**7** (2.40 g, 7.63 mmol, 424 mCi) was dissolved in anhydrous toluene (40 mL) under nitrogen and the solution was cooled to –78 °C. DIBAL (1.0 M in toluene, 9.2 mL, 9.2 mmol, 1.2 equiv.) was added dropwise maintaining the inner temperature below –70 °C. After stirring for 2.5 h at –78 °C, a small aliquot was partitioned between water and EtOAc. TLC analysis (isohexane/EtOAc, 7:3) revealed that the reaction had not gone to completion (81% of the aldehyde, 16% of the nitrile). Further DIBAL (2 mL) was added dropwise and the reaction was stirred at –78 °C for an extra 1 h, after which TLC analysis showed completion of the reaction (88.9% of the aldehyde, 5.8% of the nitrile and 4.7% of a product due to over reduction). The reaction mixture was poured (CAUTION: exothermic) into a mixture of 10% aqueous acetic acid (130 mL) and ice (≈ 100 g), stirred for 30 min and then filtered through Celite. The Celite was further rinsed with EtOAc. The phases were separated and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with brine (50 mL) and dried ($MgSO_4$). The solvents were evaporated at reduced pressure and the crude product was purified by column chromatography [100 g silica cartridge pre-wetted with isohexane, loading with CH_2Cl_2 (5 mL + 2 × 3 mL), elution with isohexane/EtOAc (100:0 to 80:20)], affording 3,4-dibenzyloxy[CO-^{14}C]benzaldehyde [^{14}C]-**5** (2.30 g, 7.22 mmol, 401 mCi, 94%) as a white solid. The radiopurity of this material was assessed to be 99.7% by TLC/autoradiography (isohexane/EtOAc, 7:3).

(*E*)-3-[3,4-Bis(benzyloxy)phenyl][3-^{14}C]prop-2-en-1-ol ([^{14}C]-4): (Ethoxycarbonylmethylene)triphenylphosphorane (3.02 g, 8.66 mmol, 1.2 equiv.) was added to a solution of [^{14}C]-**5** (2.30 g, 7.22 mmol, 401 mCi) in anhydrous CH_2Cl_2 (40 mL). After stirring at room temperature overnight, TLC (isohexane/EtOAc, 8:2) showed near completion of the reaction (6.5% of the aldehyde, 90.5% of the desired *trans* ester, 2.6% of the undesired *cis* ester). The mixture was poured on the top of a 100 g silica cartridge pre-wetted with isohexane, which was then eluted with isohexane/EtOAc (100:0 to 40:60). The desired fractions were evaporated at reduced pressure and the residue was further purified using another 100 g silica cartridge [loading with ca. 5 mL CH_2Cl_2, elution with isohexane/EtOAc (100:0 to 60:40)]. The desired fractions were concentrated to dryness to give the desired ester in ca. 97% yield (2.72 g, 7.01 mmol, 390 mCi). The radiopurity of this material was assessed to be 96.3% by TLC/autoradiography (isohexane/EtOAc, 8:2, 3.1% of the aldehyde, 0.5% of the *cis* ester). To a solution of this ester (2.72 g, 7.01 mmol, 390 mCi) in anhydrous toluene (40 mL) at –78 °C under nitrogen was added dropwise DIBAL (1.0 M solution in toluene, 17 mL, 16.83 mmol, 2.4 equiv.), the temperature being maintained below –70 °C. The clear yellow solution was stirred at –78 °C for 2 h and then a small aliquot was partitioned between water and EtOAc. TLC analysis (isohexane/EtOAc, 7:3) showed complete consumption of the starting ester (radiopurity of [^{14}C]-**4** was assessed to be 96.5%). The reaction mixture was then poured onto a mixture of saturated aqueous NH_4Cl and ice (≈ 100 mL), stirred for 30 min then filtered through Celite. The Celite was further rinsed with EtOAc. The phases were separated and the aqueous phase was extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with brine (100 mL), dried ($MgSO_4$) and concentrated to dryness. The residue was purified by column chromatography [100 g silica cartridge, elution with isohexane/EtOAc (100:0 to 40:60)]. The desired fractions were concentrated to dryness to give alcohol [^{14}C]-**4** in 85% yield (2.07 g, 5.98 mmol, 332 mCi). The radiopurity of this material was assessed to be 98.9% by TLC/autoradiography (isohexane/EtOAc, 1:1).

(*E*)-3-[2,4-Bis(benzyloxy)-6-(*tert*-butyldimethylsilyloxy)phenyl]-1-[3,4-bis(benzyloxy)phenyl][1-^{14}C]propene ([^{14}C]-9): Montmorillonite K-10 (5.5 g) was added to a well-stirred mixture of **8** (5.5 g, 17.94 mmol, 3 equiv.) in anhydrous CH_2Cl_2 (230 mL) was added at room temperature and under nitrogen. A solution of alcohol [^{14}C]-**4** (2.07 g, 5.98 mmol, 332 mCi) in anhydrous CH_2Cl_2 (120 mL) was then added dropwise over 1.5 h. The resulting purple mixture was stirred at room temperature for 3.5 h, after which TLC showed complete consumption of the starting alcohol [^{14}C]-**4** (isohexane/EtOAc, 1:1, 77.6% of the desired adduct, 5.7% of an impurity running in front, 12.6% of another impurity running behind). The mixture was filtered through a pad of Celite, which was rinsed with EtOAc. After evaporation, the residue was purified by column chromatography on silica gel [400 g of silica pre-wetted with isohexane, loading with CH_2Cl_2 (10 mL + 2 × 5 mL), eluting with isohexane/EtOAc, 100:0 to 60:40] to afford a mixture of the desired adduct and unreacted **8** (5.04 g). The radiopurity of this material was assessed to be 90.5% by TLC (isohexane/EtOAc, 1:1, 6.0% of an impurity running in front, 3.3% of another impurity running behind). The mixture was diluted with anhydrous CH_2Cl_2 (80 mL) under nitrogen then *N*,*N*-diisopropylethylamine (9 mL, 48 mmol, 8 equiv.) and *tert*-butyldimethylsilyl chloride (2.89 g, 19.2 mmol, 3.2 equiv.) were successively added and the reaction mixture was stirred at room temperature overnight. The next morning, TLC analyses revealed the presence of compound [^{14}C]-**9** (in 72.3% when eluting with isohexane/ CH_2Cl_2 1:1 and in 75.1% when eluting with isohexane/EtOAc, 1:1). Further *tert*-butyldimethylsilyl chloride (1 g) was added and stirring was continued for 3 h. TLC analyses showed no improvement (74.5% of [^{14}C]-**9** when eluting with

 © 2008 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

isohexane/CH_2Cl_2, 1:1, 76.9% with isohexane/EtOAc, 1:1). The mixture was poured on the top of a 100 g silica cartridge, which was then eluted with CH_2Cl_2. The desired fractions were evaporated to dryness and the residue was purified by column chromatography (100 g silica cartridge, loading with a minimum of CH_2Cl_2, eluting with isohexane/CH_2Cl_2, 100:0 to 20:80) to afford the corresponding silylated alkene [^{14}C]-**9** as a colorless oil in 53% yield over two steps (2.38 g, 3.18 mmol, 177 mCi). The radiopurity of this material was assessed to be 99.4% by TLC/autoradiography (isohexane/CH_2Cl_2, 1:1).

(1*S*,2*S*)-3-[2,4-Bis(benzyloxy)-6-hydroxyphenyl]-1-[3,4-bis(benzyloxy)phenyl][1-^{14}C]propane-1,2-diol ([^{14}C]-3): Silylated alkene [^{14}C]-**9** (2.38 g, 3.18 mmol, 177 mCi) was dissolved in a solvent mixture of *tert*-butyl alcohol (40 mL), water (40 mL) and CH_2Cl_2 (20 mL). The resulting mixture was cooled to 0–4 °C then methanesulfonamide (601 mg) was added, followed by AD-mix-α (8.95 g). Stirring was continued at this temperature for 24 h. TLC (isohexane/EtOAc, 8:2) revealed that the mixture contained ca. 35% of the desired diol and ca. 62% of the starting alkene [^{14}C]-**9**. Further methanesulfonamide (308 mg) and AD-mix-α (4.48 g) were added and stirring was continued at 0–4 °C. After 24 h, TLC (isohexane/EtOAc, 8:2) showed ca. 67% conversion into the desired diol and ca. 27% of the starting material [^{14}C]-**9**. Further methanesulfonamide (308 mg) and AD-mix-α (4.48 g) were added and stirring was continued at 0–4 °C. After 24 h, TLC (isohexane/EtOAc, 8:2) showed near completion of the reaction (≈ 88% of diol, ≈ 2.7% of an unidentified side product, ≈ 4.5% of the starting material [^{14}C]-**9**). A 10% $Na_2S_2O_3$ aqueous solution (30 mL) was added to quench the reaction. After stirring for 15 min, the mixture was filtered through a pad of Celite, which was further rinsed with EtOAc (4 × 40 mL). The phases were separated and the aqueous layer was further extracted with EtOAc (4 × 60 mL). The organic phases were combined, dried ($MgSO_4$) and the solvents evaporated. The residue was purified by column chromatography (100 g silica cartridge, loading with 3 × 5 mL CH_2Cl_2, eluting with isohexane/EtOAc, 100:0 to 40:60) to give the desired diol as a colourless oil. The radiopurity of this material was assessed to be 98.7% by TLC/autoradiography (isohexane/EtOAc, 8:2). This material was dissolved in anhydrous THF (45 mL) under nitrogen at room temperature and then tetrabutylammonium fluoride (1.0 M in THF, 9 mL, 2.8 equiv.) was added. The resulting mixture was stirred at room temperature overnight, after which time TLC analysis showed complete deprotection of the material ($CHCl_3$/EtOAc, 1:1, 96.1%). Saturated aqueous $NaHCO_3$ solution (50 mL) was added and the mixture was extracted with EtOAc (4 × 50 mL). The organic layers were combined, dried ($MgSO_4$) and the solvents evaporated. The residue was triturated with isohexane (40 mL) then the white solid was collected by filtration. The mother liquor was concentrated to dryness and purified by flash chromatography on silica gel (20 g silica cartridge, $CHCl_3$/EtOAc, 1:1 to 0:1) to give further material. The combined batches were further dried to give triol [^{14}C]-**3** as a white solid in 72% yield over two steps (1.537 g, 2.3 mmol, 128 mCi). The radiopurity of this material was assessed to be >96.1% by TLC/autoradiography ($CHCl_3$/EtOAc, 1:1).

5,7,3′,4′-Tetra-*O*-benzyl[2-^{14}C]catechin ([^{14}C]-10): Triethyl orthoformate (1.62 mL) was added to a suspension of triol [^{14}C]-**3** (1.537 g, 2.3 mmol, 128 mCi) in anhydrous 1,2-dichloroethane (40 mL), followed by pyridinium *p*-toluenesulfonate (352 mg). The mixture was stirred at room temperature for 20 min and the solid dissolved. The mixture was then heated to 60 °C for 5 h. A small aliquot was quenched with methanol and potassium carbonate and TLC analysis of the resulting sample showed the reaction was complete (>81% of 5,7,3′,4′-tetra-*O*-benzyl[2-^{14}C]catechin ^{14}C-**10**). After evaporation of the solvent, the residue was suspended in 1,2-dimethoxyethane (15 mL) and methanol (15 mL), potassium carbonate (246 mg) was added, and the mixture was stirred at room temperature overnight. The solvent was evaporated, and the residue was triturated with CH_2Cl_2 (≈ 10 mL + 2 × 5 mL), slurried onto the top of a 100 g silica cartridge, which was then eluted with isohexane/EtOAc (100:0 to 0:100, 5,7,3′,4′-tetra-*O*-benzyl[2-^{14}C]-catechin ^{14}C-**10** crystallizes on the column). The best fractions were concentrated to dryness. The fractions of lesser purity were concentrated as another batch, which was further purified using a 20 g silica cartridge eluting with isohexane/EtOAc (100:0 to 70:30). The best fractions were combined and concentrated to dryness to afford 5,7,3′,4′-tetra-*O*-benzyl[2-^{14}C]catechin [^{14}C]-**10** as a white solid (1.40 g, 2.16 mmol, 120 mCi, 94%). Radiopurity of this material was estimated to be >83% by TLC/autoradiography analysis (isohexane/EtOAc, 3:1, the compound streaks on TLC).

5,7,3′,4′-Tetra-*O*-benzyl[2-^{14}C]epicatechin ([^{14}C]-2): Dess–Martin periodinane (0.3 M in CH_2Cl_2, ≈ 14 mL, 2.2 equiv.) was added in one batch to a stirred solution of 5,7,3′,4′-tetra-*O*-benzyl[2-^{14}C]-catechin [^{14}C]-**10** (1.40 g, 2.16 mmol, 120 mCi) in anhydrous CH_2Cl_2 (65 mL) under nitrogen. The mixture was stirred at room temperature for about 2 h until TLC (isohexane/EtOAc, 2:1) showed the absence of starting material. Subsequently, saturated aqueous $NaHCO_3$ solution (30 mL) and 10% $Na_2S_2O_3$ aqueous solution (30 mL) were added to quench the reaction. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 60 mL). The combined organic phases were dried ($MgSO_4$) and the solvents evaporated. The residue was quickly purified by filtration through a 100 g silica cartridge (eluting with $CHCl_3$/EtOAc, 9:1). The desired fractions were concentrated to dryness to afford the desired (2*R*)-5,7,3′,4′-tetrakis(benzyloxy)[2-^{14}C]flavan-3-one as a yellow solid in ca. 87% yield (1.22 g, 1.89 mmol, 105 mCi). Radiopurity of this material was estimated to be >83% by TLC/autoradiography analysis (isohexane/EtOAc, 2:1). Under nitrogen, (2*R*)-5,7,3′,4′-tetrakis(benzyloxy)[2-^{14}C]flavan-3-one and lithium bromide (821 mg, 9.45 mmol, 5 equiv.) were dissolved in dry THF (40 mL). The solution was cooled to –78 °C then L-Selectride® (1.0 M solution in THF, 3.8 mL, 3.78 mmol, 2 equiv.) was added dropwise over 1 h. The resulting solution was stirred at –78 °C for further 2 h, after which TLC showed the reaction was complete (>81% of [^{14}C]-2, isohexane/EtOAc, 2:1). Saturated $NaHCO_3$ aqueous solution (40 mL) was cautiously added to quench the reaction (evolution of hydrogen). The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 40 mL). The combined organic phases were dried ($MgSO_4$) and the solvents evaporated. The residue was purified by flash chromatography (100 g silica cartridge, eluting with $CHCl_3$/EtOAc, 100:0 to 12:1). The desired fractions were evaporated and further purified using 100 g silica cartridges, eluting this time with isohexane/EtOAc (100:0 to 80:20). The desired fractions were concentrated to dryness to give 5,7,3′,4′-tetra-*O*-benzyl[2-^{14}C]epicatechin [^{14}C]-**2** in 44% yield (541 mg, 0.832 mmol, 46 mCi). Radiopurity of this material was estimated to be >93% by TLC/autoradiography analysis (isohexane/EtOAc, 2:1).

5,7,3′,4′-Tetra-*O*-benzyl-4-(2-ethoxyethoxy)[2-^{14}C]epicatechin ([^{14}C]-11): 2-Ethoxyethanol (0.49 mL, 5 mmol, 6 equiv.) and then all at once with good stirring 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (378 mg, 1.67 mmol, 2 equiv.) was added to a solution of 5,7,3′,4′-tetra-*O*-benzyl[2-^{14}C]epicatechin [^{14}C]-**2** (541 mg, 0.832 mmol, 46 mCi) in anhydrous CH_2Cl_2 (16 mL) at room temperature. A black-green color appeared instantaneously. After 2 h of vigorous stirring at room temperature under nitrogen, powdered DMAP (214 mg, 1.75 mmol, 2.1 equiv.) was added and stirring was

 © 2008 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

continued for 10 min at room temperature. The dark precipitate was eliminated by filtration and rinsed with CH_2Cl_2 (3 × 5 mL). The combined organic layers were directly loaded on the top of a 100 g silica cartridge, which was then eluted with isohexane/EtOAc (100:0 to 40:60) to afford [^{14}C]-**11** in 68% yield (418 mg, 0.566 mmol, 31 mCi). Radiopurity of this material was estimated to be >95% by TLC/autoradiography analysis (isohexane/EtOAc, 1:1).

[^{14}C]-13: TMSOTf (102 μL, 0.566 mmol, 1 equiv.) was added dropwise to a solution of 5,7,3′,4′-tetra-*O*-benzylepicatechin **12** (1.48 g, 2.27 mmol, 4 equiv.) and 5,7,3′,4′-tetra-*O*-benzyl-4-(2-ethoxyethoxy)[2-^{14}C]epicatechin [^{14}C]-**11** (418 mg, 0.566 mmol, 31 mCi) in anhydrous CH_2Cl_2 (51 mL) at –78 °C under nitrogen. The resulting solution was stirred at –78 °C for 5 min, then the reaction was terminated by addition of saturated aqueous potassium hydrogen carbonate (0.9 mL). The CH_2Cl_2 phase was washed with water (3 × 60 mL), each wash was back extracted with CH_2Cl_2 (10 mL). The combined organics were dried ($MgSO_4$), filtered and concentrated to dryness. The residue was purified by column chromatography (loading with a minimum of CH_2Cl_2, 100 g silica cartridge pre-wetted with isohexane, elution with isohexane/EtOAc, 100:0 to 50:50). Fractions containing the desired adduct were concentrated and the residue was further purified by column chromatography to give a foam (505 mg, ≈ 70–80% pure by NMR and TLC). This material was further purified by preparative HPLC [HPLC conditions adapted from the literature,[7k] using a Phenomenex Gemini C18 column 10μ, 110A, 250 × 21–20 mm. Conditions: at 20 mL/min; 80 to 100% CH_3CN in water (gradient over 25 min) then CH_3CN. UV 280 nm; retention time of the dimer: ≈ 25.6 min]. Pure fractions were concentrated, filtered through a 1 g silica cartridge eluting with EtOAc, and concentrated to dryness to afford [^{14}C]-**13** as a colorless oil (247.1 mg, 0.21 mmol, 11.6 mCi, ≈ 37% yield). Purity of this material was estimated to be ca. 99% by HPLC, NMR and TLC analyses.

[2-^{14}C]Procyanidin B2 ([^{14}C]-1): A first attempt to deprotect part of this batch of [^{14}C]-**13** into [^{14}C]-**1** failed due to the instability of the latter at high specific activity (55 mCi/mmol) and led to a fast degrading material. Hence the remaining material [^{14}C]-**13** was stored at –78 °C until needed, then was repurified and diluted with HPLC purified non-labeled **13** to give a stock batch of [^{14}C]-**13** (716.7 mg, 0.551 mmol) with a lower specific activity of 6.53 mCi/mmol. Some of this material was later further diluted with HPLC purified non-labeled 5,7,3′,4′-tetra-*O*-benzylepicatechin-4β,8-(5,7,3′,4′-tetra-*O*-benzylepicatechin) **13** to provide the batches of [^{14}C]-**13** destined for deprotection. [^{14}C]-**13** (859.4 mg, 0.6613 mmol at 1.34 mCi/mmol, 0.886 mCi) was dissolved in EtOAc (10 mL). Water (10 mL) was added, followed by palladium hydroxide over carbon (120 mg), and the biphasic mixture was hydrogenated (1 atm hydrogen) overnight at room temperature. Reverse phase TLC analysis (H_2O/MeOH, 1:1 + 1% AcOH) showed completion of the reaction. The reaction mixture was filtered through a sintered funnel fitted with a glass fiber prefilter and concentrated to a 4.25 mL aqueous solution of [^{14}C]-**1**. This material was compared to an authentic sample of procyanidin B2 (obtained from Fluka) by reverse phase TLC/autoradiography (H_2O/MeOH, 1:1 + 1% AcOH, 95.6% radiopurity), HPLC (96.1% radiochemical purity, 98.8% chemical purity by UV-HPLC) and LC-MS. The specific activity of the organic material [^{14}C]-**1** was assumed to be that of precursor [^{14}C]-**13** (1.34 mCi/mmol) with a calculated molecular weight of 578.57 at that specific activity. Radioactive concentration of the aqueous solution was measured to be 6.69 MBq/mL (0.18 mCi/mL), thus the 4.25 mL sample was calculated to contain ca. 326 mg (0.564 mmol) of [2-^{14}C]procyanidin B2 [^{14}C]-**1** (85% yield). The material was kept in a sealed vial under nitrogen and kept in the fridge and in the dark, and was used within a few days in biological assays.

Supporting Information (see also the footnote on the first page of this article): NMR, HPLC and radio-TLC of compounds are provided as well as MS spectra of compounds **1** and [^{14}C]-**1**.

Acknowledgments

We would like to thank the European Union (6th Framework project "FLAVO") for partial support of this research work.

[1] a) P. M. Aron, J. A. Kennedy, *Mol. Nutr. Food Res.* **2008**, *52*, 79–104; b) C. Manach, A. Scalbert, C. Morand, C. Remesy, L. Jimenez, *Am. J. Clin. Nutr.* **2004**, *79*, 727–747.
[2] a) G. Williamson, C. Manach, *Am. J. Clin. Nutr.* **2005**, *81*, 243S–255S; b) C. Manach, G. Williamson, C. Morand, A. Scalbert, C. Remesy, *Am. J. Clin. Nutr.* **2005**, *81*, 230S–242S.
[3] M. Hu, *Mol. Pharmaceutics* **2007**, *4*, 803–806.
[4] a) J. Valls, T. Richard, F. Trotin, J. P. Monti, J. M. Merillon, X. Vitrac, *Food Chem.* **2007**, *105*, 879–882; b) S. Deprez, I. Mila, A. Scalbert, *J. Agric. Food Chem.* **1999**, *47*, 4219–4230 and ref. therein; c) S. Deprez, A. Scalbert, in *Plant Polyphenols 2: Chemistry, Biology, Pharmacology, Ecology*, Basic Life Sciences, vol. 66 (Eds.: G. G. Gross, R. W. Hemingway, T. Yoshida), Kluwer Academic/Plenum Publishers, New York, **1999**, pp. 357–370.
[5] a) B. Nay, V. Arnaudinaud, J. Vercauteren, *Eur. J. Org. Chem.* **2001**, 2379–2384; b) V. Arnaudinaud, B. Nay, S. Verge, A. Nuhrich, G. Deffieux, J. M. Merillon, J. P. Monti, J. Vercauteren, *Tetrahedron Lett.* **2001**, *42*, 5669–5671; c) V. Arnaudinaud, B. Nay, A. Nuhrich, G. Deffieux, J. M. Merillon, J. P. Monti, J. Vercauteren, *Tetrahedron Lett.* **2001**, *42*, 1279–1281; d) B. Nay, V. Arnaudinaud, J. F. Peyrat, A. Nuhrich, G. Deffieux, J. M. Merillon, J. Vercauteren, *Eur. J. Org. Chem.* **2000**, 1279–1283.
[6] M. C. Pierre, C. Cheze, J. Vercauteren, *Tetrahedron Lett.* **1997**, *38*, 5639–5642.
[7] For reviews, see: a) N. E. Es-Safi, S. Ghidouche, P. H. Ducrot, *Molecules* **2007**, *12*, 2228–2258; b) D. Ferreira, D. Slade, *Nat. Prod. Rep.* **2002**, *19*, 517–541; For recent examples, see: c) P. K. Sharma, A. Kolchinski, H. A. Shea, J. J. Nair, Y. Gou, L. J. Romanczyk Jr., H. H. Schmitz, *Org. Process Res. Dev.* **2007**, *11*, 422–430; d) L. Romanczyk, P. K. Sharma, A. G. Kolchinski, H. A. Shea, Y. Gou (Mars Inc.), WO2007005248, **2007**; e) Y. Mohri, M. Sagehashi, T. Yamada, Y. Hattori, K. Morimura, T. Kamo, M. Hirota, H. Makabe, *Tetrahedron Lett.* **2007**, *48*, 5891–5894; f) C. J. Hayes, B. P. Whittaker, S. A. Watson, A. M. Grabowska, *J. Org. Chem.* **2006**, *71*, 9701–9712; g) H. Sakuda, A. Saito, Y. Mizushina, H. Yoshida, A. Tanaka, N. Nakajima, *Heterocycles* **2006**, *67*, 175–188; h) I. Tarascou, K. Barathieu, Y. Andre, I. Pianet, E. J. Dufourc, E. Fouquet, *Eur. J. Org. Chem.* **2006**, 5367–5377; i) A. P. Kozikowski, W. Tueckmantel, L. J. Romanczyk, M. Xingquan (Mars Inc.), US2005020512, **2005**; j) A. Saito, Y. Mizushina, H. Ikawa, H. Yoshida, Y. Doi, A. Tanaka, N. Nakajima, *Bioorg. Med. Chem.* **2005**, *13*, 2759–2771; k) A. P. Kozikowski, W. Tueckmantel, G. Boettcher, L. J. Romanczyk Jr., *J. Org. Chem.* **2003**, *68*, 1641–1658.
[8] S. Deprez, S. Buffnoir, A. Scalbert, C. Rolando, *Analusis* **1997**, *25*, M43–M46.
[9] For selected examples of catechins asymmetric total syntheses, see: a) T. Higuchi, K. Ohmori, K. Suzuki, *Chem. Lett.* **2006**, *35*, 1006–1007; b) M. Kitade, Y. Ohno, H. Tanaka, T. Takahashi, *Synlett* **2006**, 2827–2829; c) J. C. Anderson, C. Headley, P. D. Stapleton, P. W. Taylor, *Tetrahedron* **2005**, *61*, 7703–7711; d) S. B. Wan, D. Chen, Q. P. Dou, T. H. Chan, *Bioorg. Med.*

 © 2008 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Chem. **2004**, *12*, 3521–3527; e) S. S. Jew, D. Y. Lim, S. Y. Bae, H. A. Kim, J. H. Kim, J. Lee, H. G. Park, *Tetrahedron: Asymmetry* **2002**, *13*, 715–720; f) L. Li, T. H. Chan, *Org. Lett.* **2001**, *3*, 739–741; g) H. van Rensburg, P. S. van Heerden, D. Ferreira, *J. Chem. Soc. Perkin Trans. 1* **1997**, 3415–3422.

[10] Aryl bromide **6** was prepared by demethylation of commercially available 4-bromoveratrole with boron tribromide, followed by benzylation; see Exp. Sect. for details.

[11] Adapted from: S. B. Wan, K. R. Landis-Piwowar, D. J. Kuhn, D. Chen, Q. P. Dou, T. H. Chan, *Bioorg. Med. Chem.* **2005**, *13*, 2177–2185; see Exp. Sect.

[12] W. Tueckmantel, A. P. Kozikowski, L. J. Romanczyk, *J. Am. Chem. Soc.* **1999**, *121*, 12073–12081.

[13] For chiral HPLC conditions, see: J. L. Donovan, V. Crespy, M. Oliveira, K. A. Cooper, B. B. Gibson, G. Williamson, *Free Radical Res.* **2006**, *40*, 1029–1034.

[14] a) A. Saito, N. Nakajima, A. Tanaka, M. Ubukata, *Heterocycles* **2003**, *61*, 287–298; b) A. Saito, N. Nakajima, A. Tanaka, M. Ubukata, *Tetrahedron* **2002**, *58*, 7829–7837.

[15] HPLC conditions adapted from ref. [7k]; see Exp. Sect.

[16] Copper [^{14}C]cyanide was prepared using a Selcia procedure in 90% yield; see Exp. Sect.

[17] Y.-S. Ding, C.-Y. Shiue, J. S. Fowler, A. P. Wolf, A. Plenevaux, *J. Fluorine Chem.* **1990**, *48*, 189–205.

Received: September 10, 2008
Published Online: November 4, 2008

 © 2008 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim