C. R. Chimie 5 (2002) 577–590

S1631074802014170/FLA

Total synthesis of asymmetric flavonoids: the development and applications of ^{13}C-labelling

Bastien Nay[†], Valérie Arnaudinaud, Joseph Vercauteren*[‡]

Laboratoire de pharmacognosie, EA 491, faculté de pharmacie, université Victor Segalen, Bordeaux 2, 146, rue Léo-Saignat, 33076 Bordeaux, France

Received 1 July 2002; accepted 1 October 2002

Abstract – This article compiles our results in the field of flavonoid chemistry with the aim to synthesise isotopically labelled products. Two strategies ($C_6 + C_3–C_6$ vs $C_6–C_2 + C_1–C_6$) and some organometallic (Pd^0, Mo^{IV}) couplings were explored to build the $C_6–C_3–C_6$ flavonoid skeleton. Following this work, the gram scale has been reached in addition to the asymmetry of the targeted natural flavan-3-ols, i.e. (+)-catechin, (–)-epicatechin, and (–)-procyanidin B3. These characteristics were necessary in the event of using these molecules for pharmacokinetic and metabolic studies in human beings. ***To cite this article: B. Nay et al., C. R. Chimie 5 (2002) 577–590*** © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

flavonoids / total synthesis / ^{13}C-labelling / catechin / resolution / procyanidins / bioavailability

Résumé – Synthèse totale de flavonoïdes asymétriques : développements et applications du marquage au carbone 13. Cet article fait le point sur les résultats que nous avons obtenus en étudiant la chimie des flavonoïdes, dans le but de synthétiser des dérivés marqués isotopiquement. Deux stratégies ($C_6 + C_3–C_6$ et $C_6–C_2 + C_1–C_6$) et des couplages organométalliques (Pd^0, Mo^{IV}) ont été explorés pour construire le squelette flavonoïde en $C_6–C_3–C_6$. À la suite de cette étude, l'échelle du gramme a été atteinte ainsi que l'asymétrie des flavan-3-ols naturels ciblés : la (+)-catéchine, la (–)-épicatéchine et la (–)-proanthocyanidine B3. Ces caractéristiques étaient nécessaires en vue de l'utilisation de ces molécules dans des études pharmacocinétiques et métaboliques chez l'homme. ***Pour citer cet article : B. Nay et al., C. R. Chimie 5 (2002) 577–590*** © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

flavonoïdes / synthèse totale / marquage au ^{13}C / catéchine / dédoublement / proanthocyanidines / biodisponibilité

1. Introduction

Considering the means of defence and communication that the evolution gave to plants, flavonoids are among the more important chemical classes. They appeared early, 500 millions years ago, with some green algae, and result from the fusion of two biogenetic pathways, i.e. the cinnamate and the ancient polyketide route. They have then seemed to get more and more complexity with plant evolution [1]. If we consider (subjectively indeed) that evolved characters are beneficial to life, we can then attribute to flavonoids a large biological importance, not only for the vegetal kingdom itself, but also for animals. In fact, flavonoids are increasingly suspected of being responsible for the longer life expectancy of populations with well-balanced and healthy diets. Such a diet usually contains a large part of fruits and vegetables as well as beverages from vegetal origin, particularly tea and red wine, which make it rich in flavonoids and in other polyphenols [2, 3]. This nutritious phenomenon has often been referred to as the 'French paradox' [4].

* *Correspondence and reprints.*
E-mail addresses: bastien_nay@yahoo.fr (B. Nay), joseph.vercauteren@pharma.univ-mtp1.fr (J. Vercauteren).
† Present address: Department of Organic Chemistry (Lab. B9), University of Nottingham, University Park, NG7 2RD, Nottingham, UK.
‡ Laboratoire de Pharmacologie, 15, avenue Charles Flahault, BP 14491, Université Montpellier 1, 34093 Montpellier cedex 5, France.

Beyond their 'vitamin P' activity, which has been known for a long time [5, 6], many other biological properties have been discovered. Because one of the main physicochemical features of flavonoids is their powerful anti-oxidant and radical scavenger properties, they may be involved in defences against oxidative stress [7, 8] and ageing processes in cells that are responsible for degenerative diseases (cardio-vascular diseases [9–11], cancer [12] and even Alzheimer disease [13]). It has been demonstrated in vitro that such compounds inhibit LDL (Low Density Lipoproteins) oxidation [9], lipoperoxidation and the inflammatory response [11, 13]. Moreover, chelation of iron by flavonoids in cells may prevent the Fenton reaction, responsible for $HO^{\bullet}$ free radical formation [14]. Besides this experimental data, epidemiological studies have comforted the role of flavonoids in the prevention of many degenerative diseases [3–4, 15–17].

Nevertheless, flavonoids would only be able to produce such activities in vivo if they were absorbed through the intestinal wall after ingestion. There is little experimental data about their absorption and their metabolism, especially in the conditions of a normal meal [18, 19]. Although some studies have shown that flavonoids are bioavailable, even though as degradation metabolites [20], the results are still quite variable, depending on the authors. In order to obtain more precise information about the natural bioavailability of flavonoids (which occurs after a normal meal), we decided to use isotopically labelled compounds. These could be detected by isotopic mass spectrometry and quantified independently of compounds naturally present in the diet. We chose carbon-13 as a good tracer within the skeleton of our molecules and possibly of their metabolites. The flavan-3-ol derivatives (+)-catechin **1**, (–)-epicatechin **2** and (–)-procyanidin B3 **3** (Fig. 1) were chosen as good representatives of flavonoids that are present in a typical healthy diet. This review focuses on the synthetic venture that was undertaken to produce such ^{13}C-labelled compounds in a gram scale and in an asymmetric manner, describing all the chemical routes that were explored during this work.

2. General structure of flavonoids

Flavonoids are polyphenolic natural products. Their structures include two phenolic rings (A and B), linked by a small chain of three carbons (Fig. 1). Usually, this linkage is part of the ring C fused to the first aromatic ring A to form a benzopyran heterocycle. Each of the three cycles of this skeleton can be diversely oxidised, giving rise to different classes of flavonoids. The more distinctive characteristic of each of these classes is the oxidation level of the oxygenated heterocycle C. This heterocycle in the highly

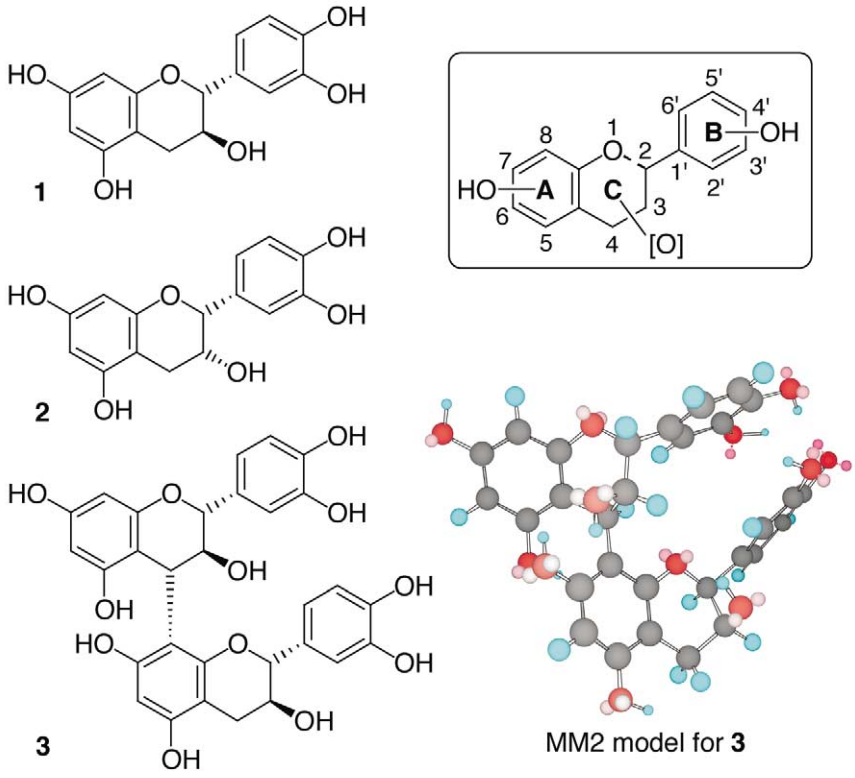


Fig. 1. General structure of flavonoids (in frame) and compounds of interest: (+)-catechin **1**, (–)-epicatechin **2**, (–)-procyanidin B3 **3**.

oxidised species (3-hydroxyflavones, anthocyanidins) has a planar geometry, associated with highly conjugated unsaturation, which is also responsible for their visible character (from yellow to violet). For the classes with a saturated heterocycle, such as the flavan-3-ols, of interest in this paper, some chirality arises, due to 1 to 3 possible stereocentres.

3. Synthetic strategies

The C_6–C_3–C_6 flavonoid skeleton can be accessed following two of the more obvious retrosynthetic analyses (Fig. 2) [21].

On the one hand (Fig. 2a), it is possible to unravel the structure into a C_6 phenolic and a C_3–C_6 cinnamic synthon that could either be a carboxylic acid, an aldehyde or an alcohol (Fig. 3). The process involved either a retro-acylation (into a cinnamic acid **4**, route A) or a retro-allylation (into a cinnamaldehyde **5**, route B, or a cinnamic alcohol **6**, route C), respectively. Each oxidation state of these precursors could be valuable in the specific synthesis of different classes of flavonoid intermediates (e.g., chalcones **7**, 2-phenyl-2*H*-chromenes **8** or cinnamylphenols **9**, respectively). Usually, these reactions are not simple and the alternative strategy is often preferred.

In this second strategy (Fig. 2b), the flavonoid skeleton is unravelled into a C_6–C_2 acetophenone part and a C_1–C_6 benzaldehyde unit, thanks to a retro-crotonisation operation. This route is used more frequently in the literature and is the result of the easy accessibility of the starting materials (acetophenones and benzaldehydes).

4. The contribution of organometallic chemistry to the $C_6 + C_3$-C_6 strategy

Among the two possible strategies of synthesis of the C_6–C_3–C_6 flavonoid skeleton, the $C_6 + C_3$–C_6 'biomimetic' type has often been neglected for the benefit of the C_6–$C_2 + C_1$–C_6 one. Indeed, it involves the difficult arylation step of a cinnamic moiety by a phenolic nucleophile. Some examples have been reported in the literature [22–24], but they always displayed side-reactions or poor yields when using either a very nucleophilic synthon such as phloroglucinol ring or an electrophilic caffeic acid derivative, and yet both could be turned to good account in an easy access to flavan precursors bearing the hydroxylation pattern of catechin.

As shown in Fig. 3, we can envisage the synthesis of the C_6–C_3–C_6 skeleton thanks to an allylic substitution of a suitable leaving group on a cinnamyl derivative by a phenolic nucleophile (routes B and C). Allylic substitutions are well described in the literature, particularly in organometallic catalysis. Palladium and molybdenum catalysts are often used in such reactions, so we decided to explore their chemistry to reach potential precursors in the synthesis of flavonoids.

4.1. Palladium(0)-catalysed allylic substitutions

This reaction [25], often referred to as the Tsuji–Trost reaction (Fig. 4) [26, 27], was chosen to substitute cinnamyl acetate **10** by a phenol, even though aromatic nucleophiles are rare in this kind of substitution [28, 29].

All phenols were used under their native form, the protected derivatives being unreactive. Reagents are listed in Figs. 5 and 6. It is noticeable that cinnamaldehyde diacetyl acylal **16** [30] contains two leaving groups, thus being a promising electrophile in front of the doubly nucleophile phloroglucinol **13**.

Results for the coupling reaction are shown in Table 1 [25]. It is noteworthy that simple phenols (entries 1–3) react through their oxygen to form ether

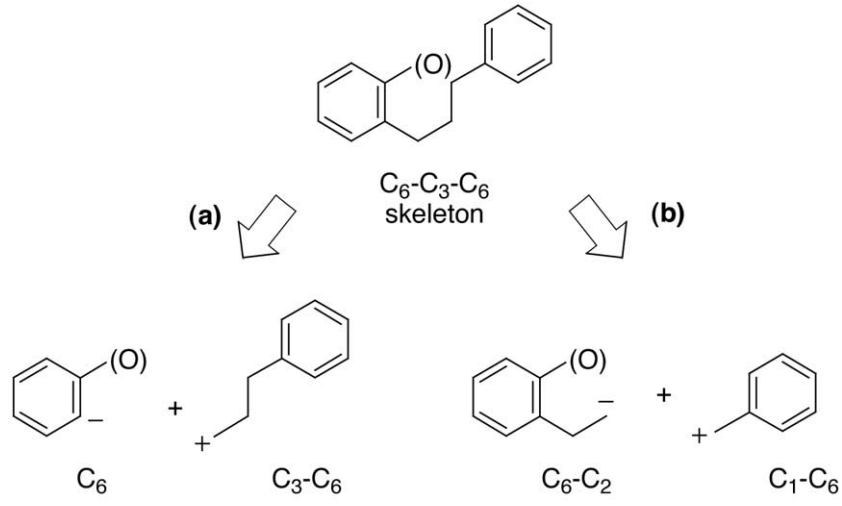


Fig. 2. Retrosynthetic analysis: $C_6 + C_3$–C_6 strategy (**a**) and C_6–$C_2 + C_1$–C_6 strategy (**b**).

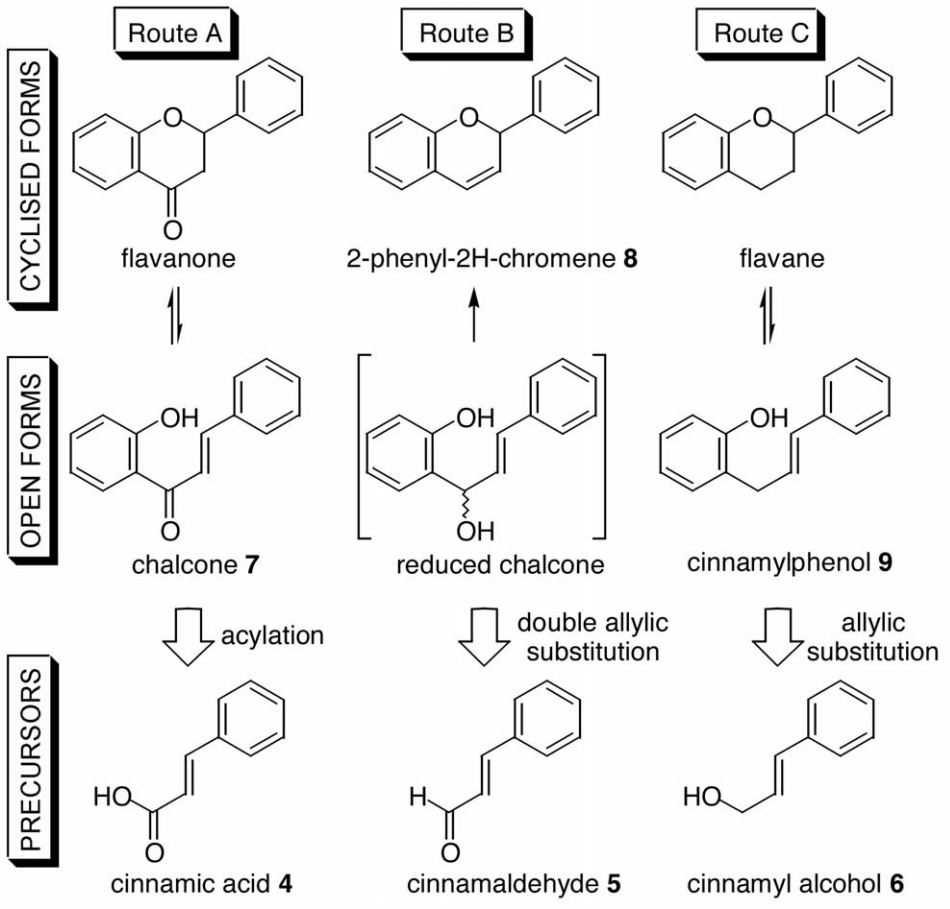


Fig. 3. Three alternative routes in the C_6–C_3–C_6 strategy.

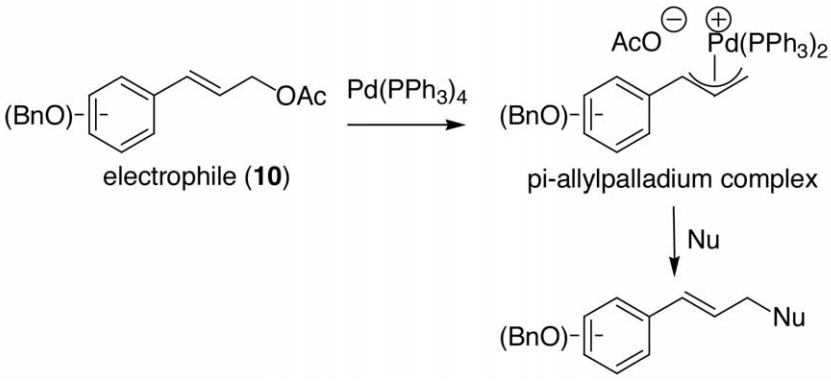


Fig. 4. The Tsuji–Trost reaction applied to cinnamyl acetates **10**.

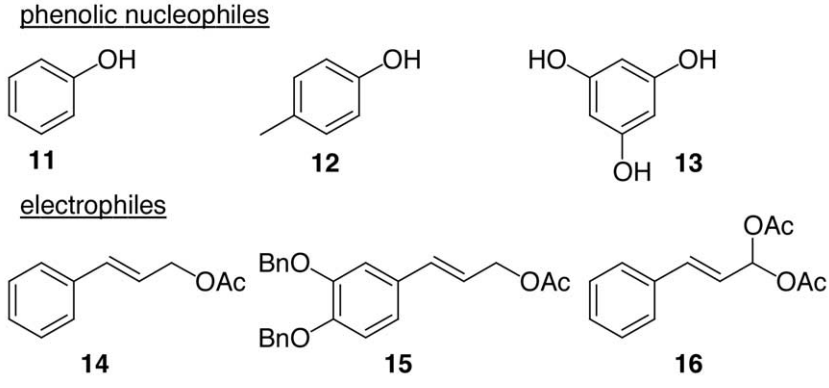


Fig. 5. Substrates for the Tsuji–Trost reaction.

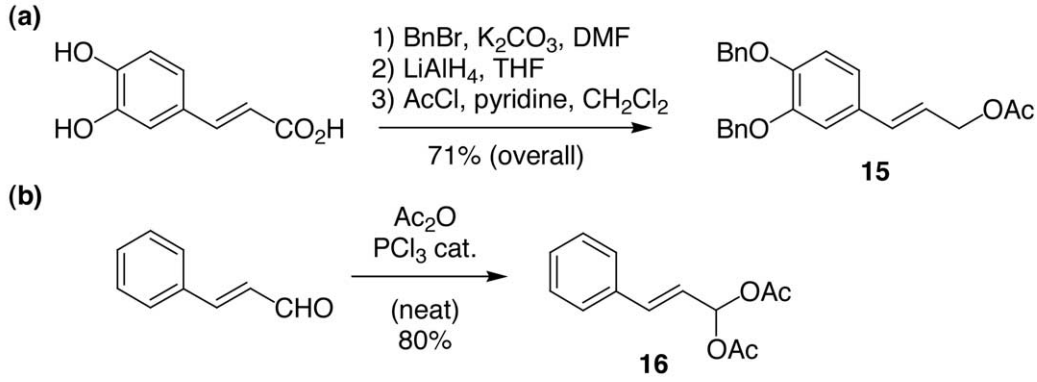
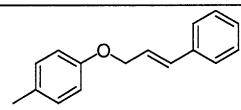
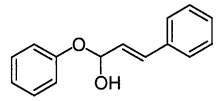
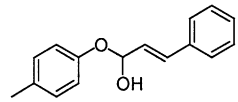
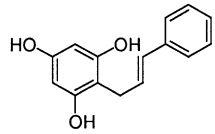
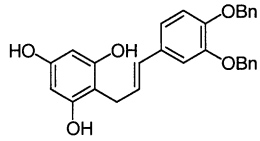
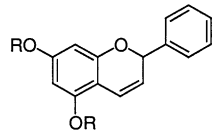


Fig. 6. Synthesis of cinnamyl substrates **15** (**a**) and **16** (**b**).

Table 1. Results of palladium-catalysed couplings between phenols and cinnamyl acetates (all reactions performed in THF with 10 mol% $Pd(PPh_3)_4$ in the presence of K_2CO_3 except for **13**).

entry	nucleophile	electrophile	product	Yield (%)
1	**12**	**14**	 (**17**)	66
2	**11**	**16**	 (**18**)	88
3	**12**	**16**	 (**19**)	94
4	**13**	**14**	 (**20**)	30
5	**13**	**15**	 (**21**)	17
6	**13**	**16**	 (**22**)	18[a]

[a] Yield after methylation in situ using MeI/K_2CO_3/DMF (R = Me).

17 or acetals **18**–**19** with cinnamyl acetate **14** or acylal **16**, respectively. On the contrary, phloroglucinol **13** reacts through its nucleophilic carbons in a C–C bond formation, giving the flavonoid skeleton **20**–**22** (entries 4–6), in spite of disappointing yields.

A particular interest was found when reacting **13** with acylal **16** (entry 6). This reaction gave a chromene **22**, both acetate groups of **16** being substituted, firstly by the aromatic ring and secondly by the phenolic oxygen in a cyclisation step. Possible mechanism is described in Fig. 7a. The yield was low and product **23** very unstable, even in its more manipulable methylated form (**22**). This was explained by the ability of this 5,7,8a-trihydroxylated chromene to form the highly stabilised allylic cation **24** [31] and/or quinone methide **25** [25] (Fig. 7b). Owing to this instability and to the low yields, this route was not considered for a total synthesis of labelled flavonoids.

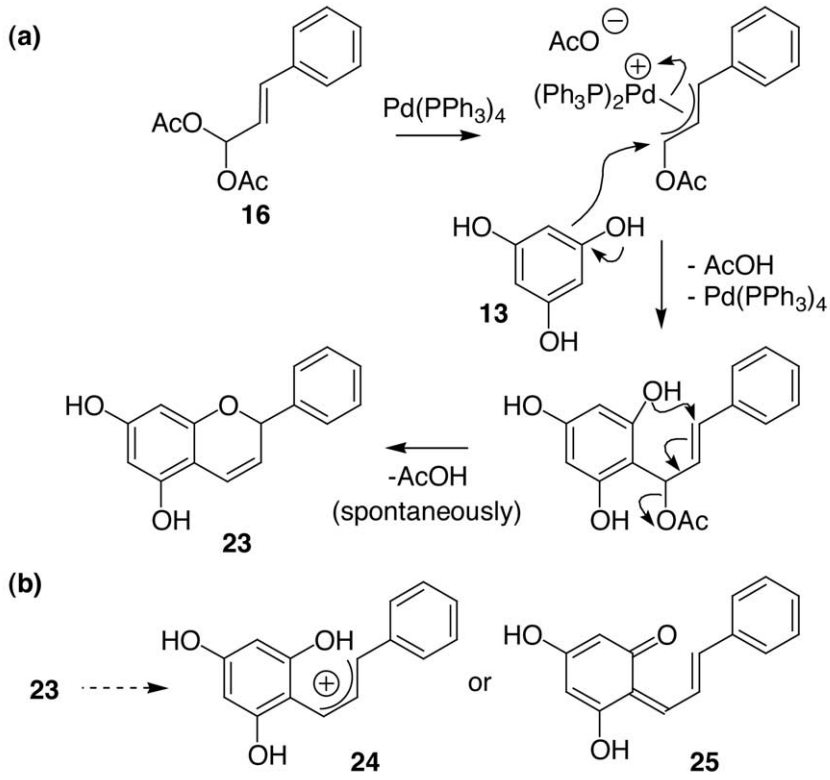


Fig. 7. Mechanism of the formation of chromene **23** (**a**) and rationalisation of its instability (**b**).

4.2. Molybdenum(IV)-catalysed allylic substitutions

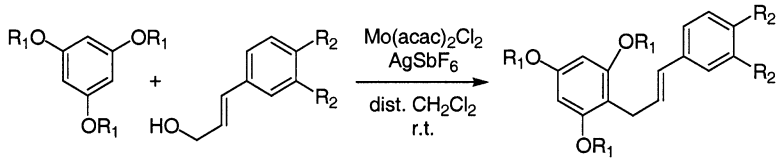
Based on the work of Malkov et al. [32], we used the catalyst $Mo^{IV}(acac)_2(SbF_6)_2$ to substitute cinnamyl alcohol by phloroglucinol derivatives [33]. This mild Lewis acid catalyst is able to generate smoothly an allylic cation from the corresponding alcohol. Moreover, it is noteworthy that phloroglucinol was expected to be more nucleophilic than simple phenols and that the allylic cation from 3,4-dibenzyloxycinnamyl alcohol could be stabilised by the *para* alkoxy group.

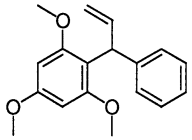
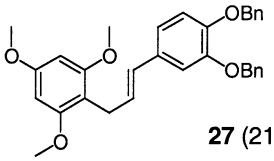
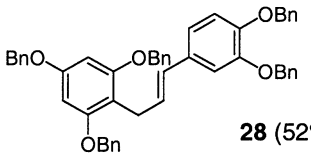
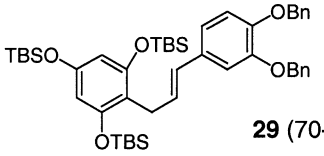
Results are shown in Table 2. Using simple phenol derivatives, only poor yields and regioselectivities were obtained (not shown). However, phloroglucinol derivatives were more reactive. Free or acetylated phloroglucinol reactions with cinnamyl alcohol yielded only complex mixtures (entries 1 and 2). Phloroglucinol trimethyl ether furnished 30% of the unexpected product **26** from S_N2' substitution (a neoflavonoid skeleton was then built up, entry 3), but when reacted with 3,4-dibenzyloxycinnamyl alcohol (entry 4), the S_N2 product **27** was isolated as the major adduct (21% yield). This indicates the possible influence of the substitution pattern of the cinnamic part on the outcome of the reaction.

Phloroglucinol tribenzyl ether gave good yields of the expected product **28** (52%, entry 5), which bears all the oxygens of the catechin series. Finally, phloroglucinol tris[*t*-butyl(dimethyl)silyl] ether provided product **29** in 70–80% yield (entry 6), which is the best yield of adducts obtained in this study.

Compound **28** was later subjected to asymmetric dihydroxylation. According to Sharpless rules for the use of AD-Mix reagents (Fig. 8a) [34, 35], product **30** from AD-Mix β oxidation (86% yield) should have occurred with the central hydroxyl group consistent with the (–)-epicatechin configuration (Fig. 8b) [36, 37]. An enantiomeric excess of 50% was found by NMR. This low selectivity was explained by steric hindrance resulting from the conformation of the phloroglucinol ring, which would be perpendicular to the double bond to be oxidised (Fig. 8a). Palladium-catalysed hydrogenolysis in methanol and in the presence of K_2CO_3 furnished 1,3-diarylpropan-1,2-diol **31** in 56% yield. Unfortunately, all attempts to cyclise this highly hydroxylated product into **1** or **2** under acidic or basic conditions failed.

Table 2. Molybdenum(IV)-catalysed couplings of phenols to cinnamyl alcohols: results.



Entry	R_1	R_2	Products (yield, %)
1	OH	H	inseparable mixture
2	OAc	H	inseparable mixture
3	OCH_3	H	 **26** (30%) + many unidentified products
4	OCH_3	OBn	 **27** (21%) + many unidentified side products
5	OBn	OBn	 **28** (52%)
6	OTBS	OBn	 **29** (70-80%)

5. Application of the $C_6 + C_3–C_6$ strategy to the synthesis of an isotopically labelled chalcone intermediate: first route to racemic 4-[^{13}C]catechin

Besides the studies of Pd- and Mo-catalysed allylic substitutions, the acylation of protected phloroglucinol by a cinnamic acid to get a chalcone was developed (Fig. 3, route A) [38].

Phloroglucinol tribenzyl ether **32** can be obtained by two different methods (Fig. 9). The first consists of direct benzylation of phloroglucinol **13**, using benzyl bromide in the presence of K_2CO_3 and in DMSO. The yield was poor, although better than that of the same reaction in DMF, which gave many C-benzylated products. The second method was developed by Kawamoto et al. [39] and gave good yields of **32** via triacetate **33** without C-benzylation. Therefore, this method was later chosen in order to obtain large quantities of **32**.

The cinnamic acid derivative, 3,4-dibenzyloxy-cinnamic acid **38** from the caffeic acid family, was synthesised from K^{13}CN via 1-[^{13}C]acetonitrile **34**, which was added to 3,4-dibenzyloxybenzaldehyde **35** (Fig. 10). The alcohol **36** was then transformed into acid **38** by dehydration and hydrolysis.

The acylation of **32** by acid **38** was performed using a mixed anhydride formed in situ in the presence of trifluoroacetic anhydride (TFAA) [40]. The yield was rather satisfying if we consider the difficulties that usually arise with other methods during the acylation of the very nucleophilic phloroglucinol derivatives [23]. This reaction provided chalcone **39**, which was selectively monodeprotected by $TiCl_4$ to give **40**. However, a small amount of the undesired

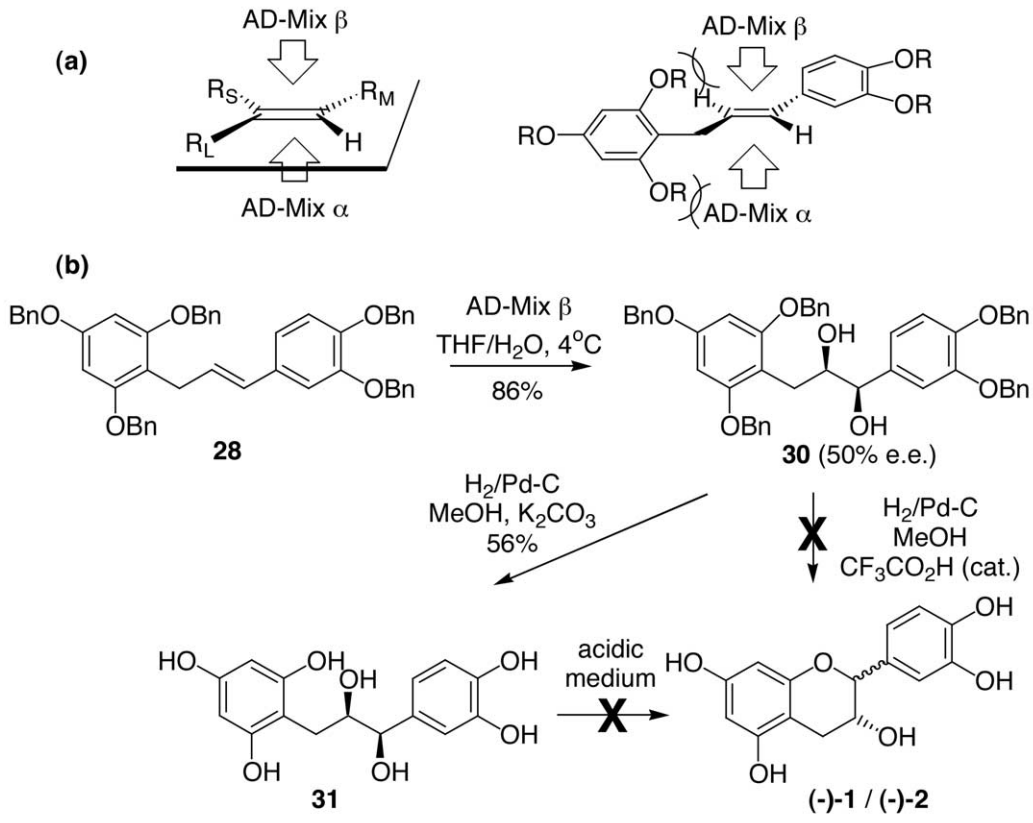


Fig. 8. Predicting rules for asymmetric dihydroxylation of 1,3-diarylpropenes (**a**) and application to **28** (**b**).

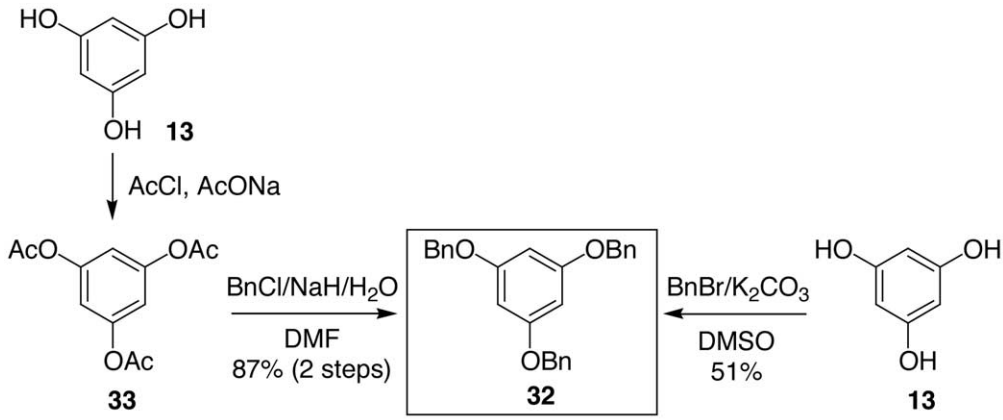


Fig. 9. Synthesis of phloroglucinol tribenzyl ether **32**.

C-benzylated product **41** was formed during this deprotection step by displacement of the benzyl group.

Reduction of hydroxychalcone **40** using $NaBH_4$ followed by BF_3–OEt_2 catalysed ring closing provided chromene **42** (Clark–Lewis method [41, 42], Fig. 11). Due to its instability, it was readily dihydroxylated by OsO_4 to give **43** in a diastereoselective manner. Flavan-3,4-diol **43**, a common derivative in flavonoid chemistry, was deoxygenated on labelled carbon 4, furnishing tetrabenzyl ether **44** of racemic labelled catechin, which was deprotected by palladium-catalysed hydrogenation.

The $C_6 + C_3$-C_6 strategy allowed us to perform the total synthesis of 40 mg of racemic 4-[^{13}C]catechin 1 [38]. Nevertheless, we did not consider it convenient enough for a large-scale application and we explored the other strategy as follows.

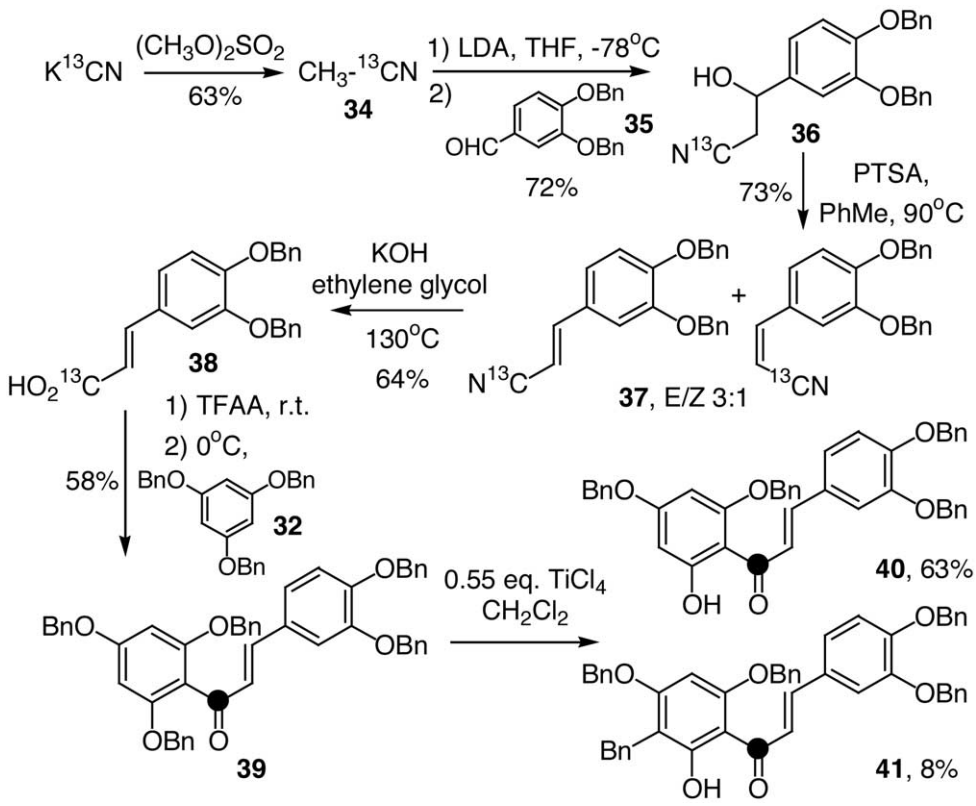


Fig. 10. Synthesis of ^{13}C-labelled chalcones from $K^{13}CN$ using the $C_6 + C_3–C_6$ strategy (the black point figures the carbon 13).

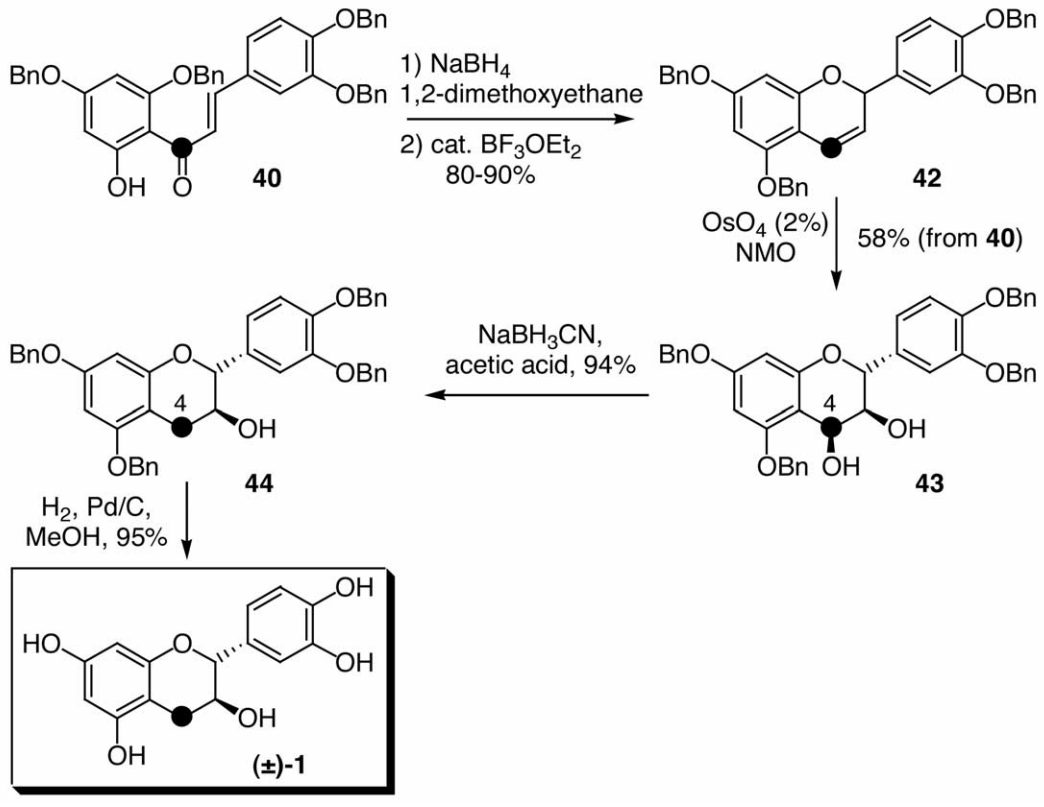


Fig. 11. Synthesis of racemic ^{13}C-catechin **1** from chalcone **40**: the Clark–Lewis method.

6. Application of the C_6–C_2 + C_1–C_6 strategy to the synthesis of an isotopically labelled chalcone intermediate: first access to ^{13}C-labelled (–)-procyanidin B3

As explained above (Fig. 2), this alternative route consists in the crotonisation of an acetophenone with a benzaldehyde [43]. In our case, ^{13}C-labelled phloroacetophenone derivative **45** was obtained by mean of the acylation of **32**, using 1-[^{13}C]acetic acid, which was transiently activated with TFAA [40] (Fig. 12). Compound **45** was then selectively deprotected by $TiCl_4$ as for chalcone **39** [42], giving acetophenone **47**, which reacted with benzaldehyde **35**, affording chalcone **40** in high yield.

At this step, the synthesis was joining the route previously described in Fig. 11 to yield flavan-3,4-diol **43**. This diol is an interesting precursor of catechin dimers [44]. Indeed, it possesses a good electrophilic carbon on position 4, which can be activated by the use of a Brønsted or a Lewis acid, $TiCl_4$ in the present case, to form the corresponding stabilised benzylic cation. This electrophilic intermediate was trapped by (2*R*,3*S*)-tetra-*O*-benzylcatechin **48**, derived from unlabelled natural (+)-catechin **1** and which reacts specifically through its nucleophilic C-8 in the used conditions (Fig. 13). Since diol **43** was racemic and facial selectivity was not complete, four dimeric isomers were obtained from this condensation (two pairs of diastereoisomers): **49** (6%), **50** (13%) and a mixture of two inseparable dimers **51** + **52** (65%, 1:1 ratio) including benzylated dimer B3 (**52**). Hydrogenolysis of **51** + **52** gave the corresponding procyanidins (**53** and **3**, 30% yield each from diol **43**) and small amounts of catechin due to the reduction of interflavanolic linkage 4*C*-8*D*.

By this way, 33 mg of (–)-4*C*-[^{13}C]procyanidin B3 dimer **3** were obtained [43]. In comparison with the C_6 + C_3–C_6 route, the C_6–C_2 + C_1–C_6 strategy proved to be more convenient, shorter and with better yield. It was later chosen for a gram-scale synthesis. However, the use of racemic derivative diol **43** gave rise to troublesome purification (four stereoisomers), which was particularly unwelcome for preparation on a gram-scale. Furthermore, the use of labelled racemic catechin **(±)-1** for studies in human beings was not tolerable, as the non-natural enantiomer (**(–)-1**) is poorly known on a pharmacological point of view. Therefore, we should synthesise enantiomerically pure derivatives to get rid of these problems. Such requirements were overcome as described in the next part.

7. Catechin, epicatechin, dimer B3: the gram-scale synthesis of optically pure derivatives

7.1. About the asymmetric synthesis of catechin derivatives

Numerous attempts have been made to synthesise catechin derivatives in an asymmetric manner, but all of them failed in our hands. The efficient and outstanding methods developed by Ferreira [36, 37], Kozikowski [31] or Chan [45] were not suitable for the present synthesis. Our failure in epoxidation and dihydroxylation of chalcones **39** and **40** or flavene **42**, and asymmetric reduction and cyclisation of chalcone **40** were attributed to the unfavourable electronic properties of the double bond of interest. Indeed, it is strongly influenced by the vicinity of phloroglucinol ring and the multiple resonance forms it is able to induce. Especially in the case of flavene **42**, no chirality could be detected after reactions in asymmetric conditions. The one eventually formed would be shat-

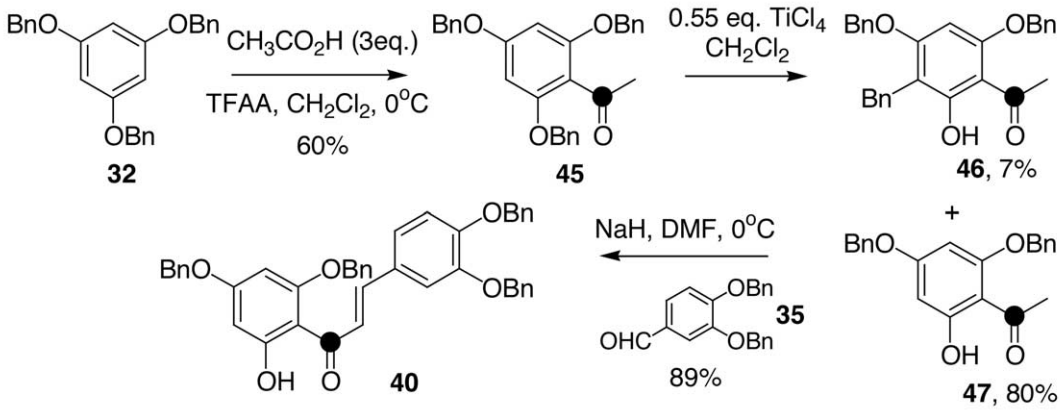


Fig. 12. Synthesis of ^{13}C-chalcone **40** using the C_6–C_2 + C_1–C_6 strategy.

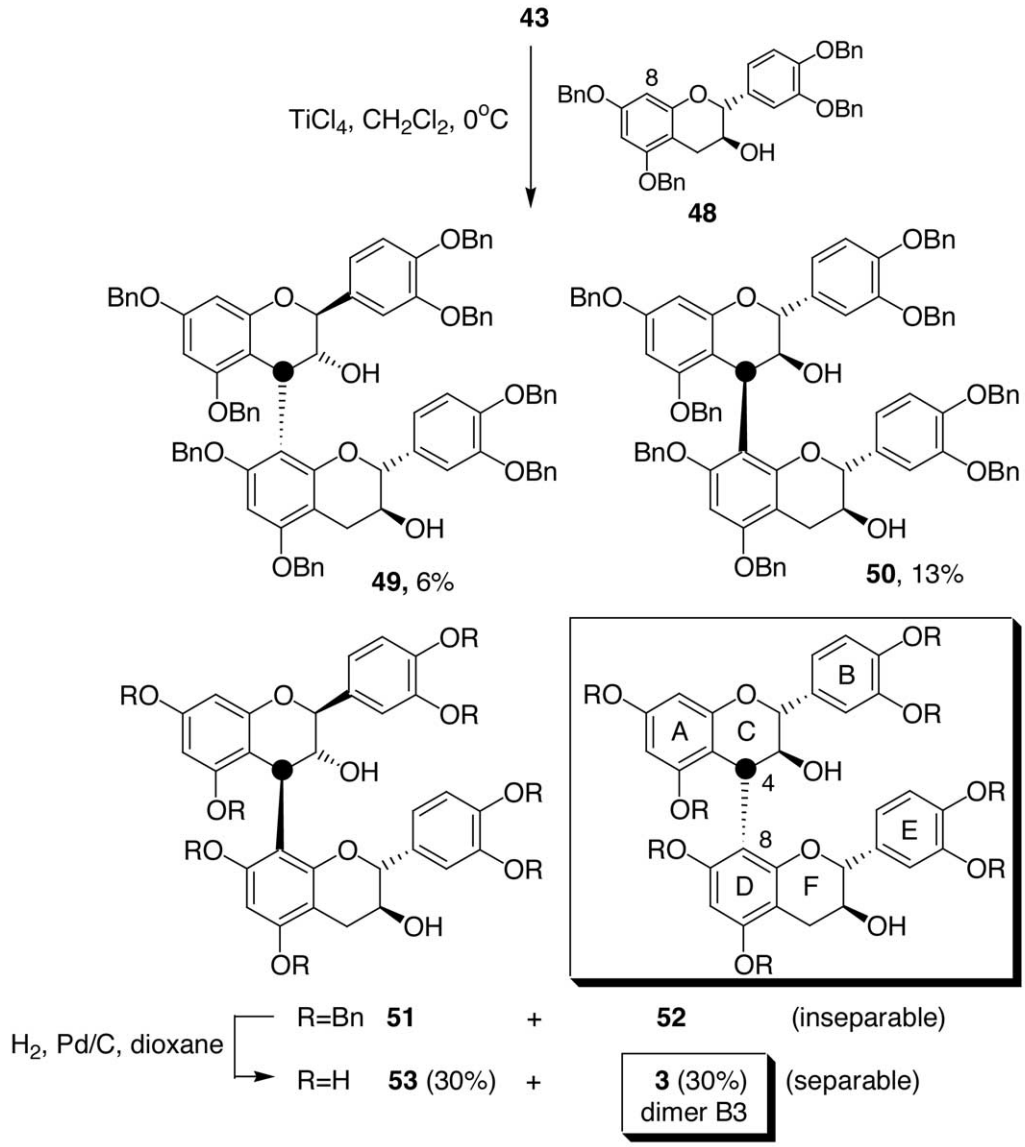


Fig. 13. Synthesis of dimer B3 (**3**) from racemic diol **43**.

tered by equilibrium of **42** with the open forms **54** [31] and/or **55** [25] (Fig. 14).

7.2. Resolution of racemic catechin derivatives

To circumvent the difficulties experienced in asymmetric synthesis, we rather chose to investigate some resolution processes on our racemic compounds. We found that coupling racemic benzylated catechin **44** to a chiral derivative (Fig. 15) provided an easy access to enantiomerically pure products [46].

Benzylated catechin **44** was first esterified with commercially available (–)-(1*S*)-camphanyl chloride **56**, giving two stereoisomeric compounds in a 1:1 ratio. Although these products were distinguished by analytical HPLC, they were neither separable by column chromatography nor by preparative HPLC. Alternatively, compound **44** was esterified by chiral tartaric acid derivative **L-58** (Fig. 16), which was easily synthesised from commercially available dibenzoyl-L-tartaric acid **L-57** (Fig. 15b). Here again, no satisfactory chromatographic separation was found for a preparative purpose, even by HPLC. However, we could enjoy the fact that an abandoned solution (hexane/dichloromethane 4:1) was found crystallised after three days. Analysis of the crystals and comparison with those reproduced from natural (+)-catechin showed that the natural enantiomer of benzylated catechin **44** esterified by the acid **L-58** had crystallised. Therefore, from the solution of **(–)-59** and **(–)-60** (1:1

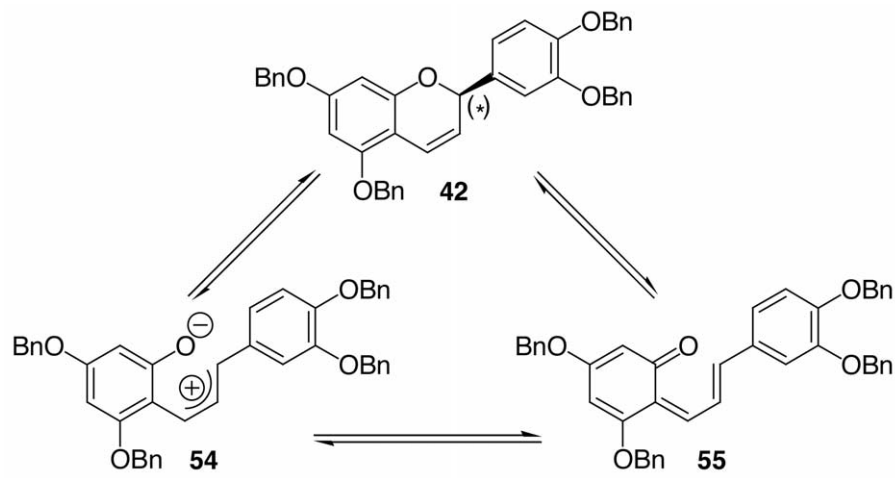


Fig. 14. Rationalisation for the difficult use of **42** in asymmetric synthesis of flavonoids.

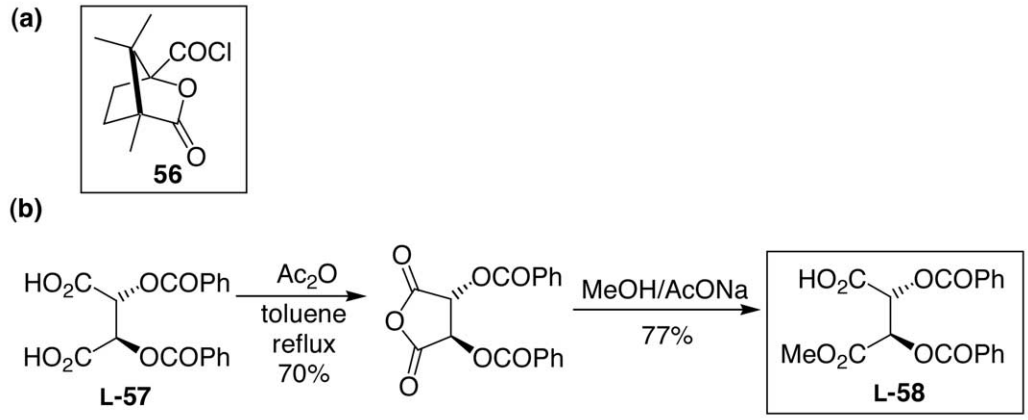


Fig. 15. Chiral reagents chosen for the resolution of **44**.

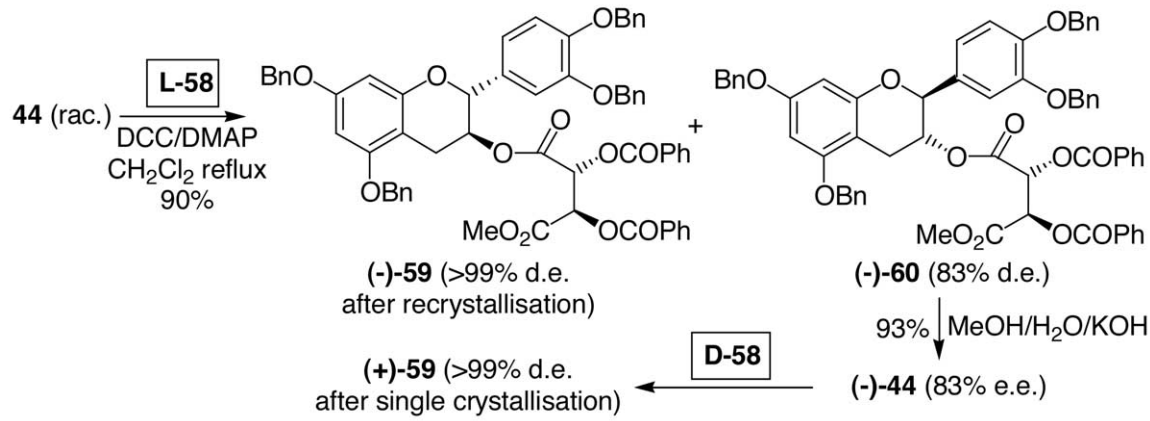


Fig. 16. Resolution process of **44** using tartaric acids **L-58** and **D-58**.

ratio) in hexane/dichloromethane 4:1, **(–)-59** was able to crystallise selectively and with > 99% d.e. after recrystallisation from the same solvent. The other isomer **(–)-60** did not crystallise and gave colourless oil, with 70–83% d.e., upon concentration of mother liquids.

After hydrolysis of **(–)-59** and hydrogenolysis, (+)-catechin **1** was obtained in more than 99% e.e. [46] and displayed all the spectrometric and optical data of the natural product. This method provided an efficient way of preparation of (+)-catechin and proved to be applicable on a large scale.

7.3. Gram-scale synthesis of (+)-4-[^{13}C]catechin and (–)-4-[^{13}C]epicatechin

Based on the results obtained when applying the C_6–C_2 + C_1–C_6 strategy [43] and the resolution of racemic catechin [46], we have been able to perform the gram-scale synthesis of asymmetric [^{13}C]flavan-3-ols with the aim of their use in biomedical studies [47].

Labelled racemic compound **44** (25 g) was synthesised according to Fig. 12 and 13, starting from 117 g of **32**. Application of the resolution process outlined in Fig. 16 allowed us to obtain each enantiomer of **44** with > 99% enantiomeric excess. Particularly, the antipodes (non natural configuration) of (+)-catechin derivatives were attained after hydrolysis of oily ester **(–)-60** (83% e.e.) and then esterification with the antipodal D-tartaric acid **D-58**, reaching crystallisable ester **(+)-59** of (–)-catechin.

We took benefit of both enantiomers to synthesise labelled catechin **1** and epicatechin **2** with natural configurations. (+)-[^{13}C]Catechin tetrabenzyl ether **(+)-44** yielded 3.6 g of (+)-4-[^{13}C]catechin **(+)-1** in >99% e.e. after deprotection (Fig. 17a). (–)-4-[^{13}C]Epicatechin **(–)-2** (1.3 g, >99% e.e.) could be obtained from deprotection of **(–)-44** to (–)-4-[^{13}C]catechin **(–)-1** and then epimerisation at carbon 2 in Na_3PO_4 buffer (pH 11.4), taking care of the strict absence of oxygen during this reaction (Fig. 17b). The gram-scale synthesis of labelled flavan-3-ols (about 7 g altogether) has been performed in 8% global yield from starting material **32**.

7.4. Gram-scale synthesis of 4*C*-[^{13}C]procyanidin B3

As shown in section 6, the synthesis of dimers on a gram-scale was compromised by the use of racemic diol **43**, which caused difficult purification due to the four stereoisomers formed. After reducing diol **43** to **44** and by mean of resolution, enantiomerically pure methylated diol **(+)-61** could be obtained after reoxidation of (+)-catechin derivative **(+)-44** on benzylic carbon 4 (Fig. 18). Then **(+)-61** was condensed to (+)-catechin tetrabenzyl ether **48** in the presence of $TiCl_4$, providing two stereoisomers, **50** and **52**, instead

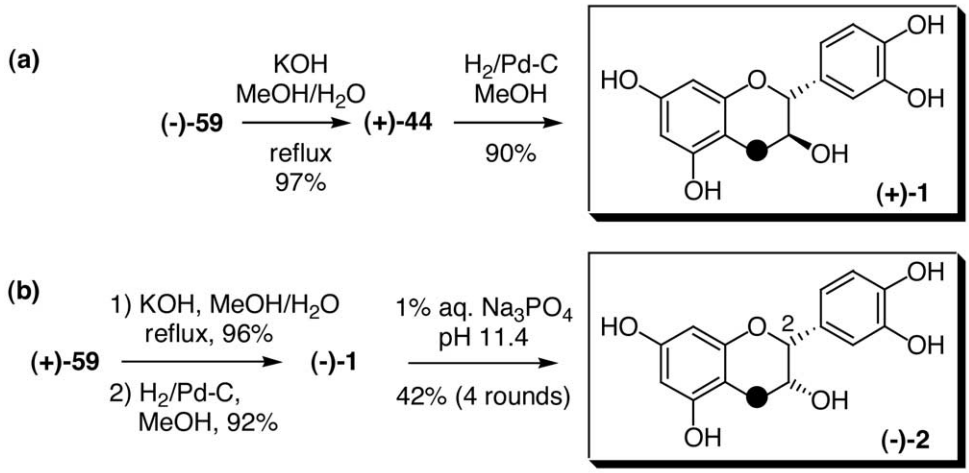


Fig. 17. Access to optically pure labelled catechin **1** and epicatechin **2**.

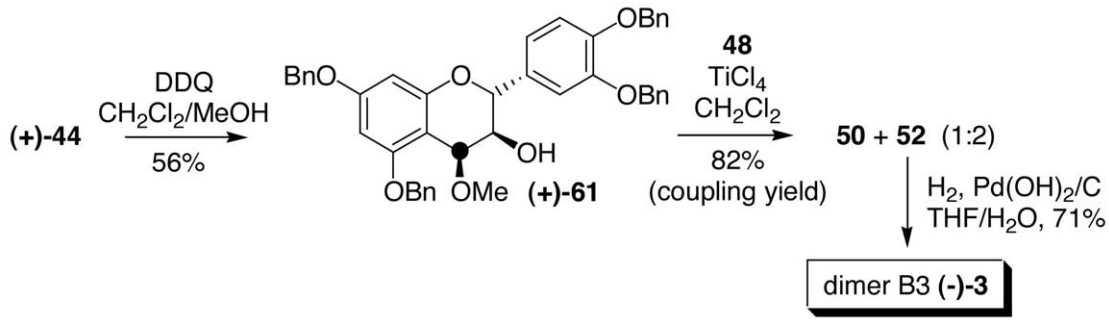


Fig. 18. Access to gram amounts of (–)-4*C*-[^{13}C]procyanidin B3 (**3**).

of four. Consequently, purification was easily carried out before deprotection and the yield was not lowered by stereoisomeric dilution. This approach allowed us to complete the synthesis of 1.5 g of stereomerically pure (–)-4*C*-[^{13}C]procyanidin B3 **(–)-3** [48].

8. Conclusion

The gram-scale synthesis of ^{13}C-labelled (+)-catechin **1**, (–)-epicatechin **2** [47] and (–)-procyanidin B3 **3** [48] has been successfully achieved using the C_6–C_2 + C_1–C_6 strategy [43] and an efficient method of resolution of racemic benzylated catechin **44** [46]. Thanks to their carbon 13 that does not have the toxicity of its radioactive isotopes, these natural products of the food are now used in vivo in pharmacokinetic studies in human beings. Natural amounts have been incorporated in red wine and swallowed at mealtime. Carbon-13 can serve to trace the compounds and their metabolites using isotopic mass spectrometry without interfering with unlabelled flavonoids of the diet, in order to quantify the data.

This study should let us know how much of the food flavonoids pass through the intestinal wall after a meal. By the end, we should be able to see if these polyphenolic compounds reach the required blood concentration to fully exert their biological activities, thus confirming (or refuting) their involvement in such epidemiological observations, as low incidence of degenerative diseases, or French paradox, in some populations.

Acknowledgements. We thank the 'Région Aquitaine' (France) and the French MRT for financial supports. B.N. acknowledges receipt of a scholarship from the MRT. We are particularly grateful to Miss Amanda Royle for her kind help concerning English language.

References

[1] T. Swain, in: J.B. Harborne, T.J. Mabry, H. Mabry (Eds.), The Flavonoids, Chapman and Hall, London, UK, 1975 p. 1096.
[2] C. Remésy, C. Manach, C. Demigne, O. Texier, F. Regerat, in: J. Vercauteren, C. Chèze, J. Triaud (Eds.), Polyphenol 96, 18th International Conference on Polyphenols, INRA Éditions, (France), 1996, p. 251.
[3] G.L. Hertog, E.J.M. Feskens, P.C.H. Hollman, M.B. Katan, D. Kromhout, The Lancet 342 (1993) 1007.
[4] S. Renaud, M. De Lorgeril, The Lancet 339 (1992) 1523.
[5] A. Szent-Györgyi, Oxydation, Energy Transfer and Vitamins, Nobel Lecture, 11 December 1937.
[6] P.C.H. Hollman, M.B. Katan, Biomed. Pharmacother. 51 (1997) 305.
[7] T. Tsuda, Y. Kato, T. Osawa, FEBS Lett. 484 (2000) 207.
[8] R. Rodrigo, G. Rivera, Free Radic. Biol. Med. 33 (2002) 409.
[9] E. Frankel, J. Kanner, J. German, E. Parks, J. Kinsella, The Lancet 341 (1993) 454.
[10] D.F. Fitzpatrick, S.L. Hirschfield, R.G. Coffey, Am. J. Physiol. 265 (1993) H774.
[11] R.M. Facino, M. Carini, G. Aldini, Life Sci. 64 (1999) 627.
[12] J. Jankun, S.H. Selman, R. Swiercz, Nature 387 (1997) 561.
[13] B. Draczynska-Lusiak, A. Doung, A. Sun, Mol. Chem. Neuropathol. 33 (1998) 139.
[14] A. Puppo, Phytochemistry 31 (1992) 85.
[15] M.H. Criqui, B.L. Ringel, The Lancet 344 (1994) 1719.
[16] G. Block, B. Patterson, A. Subrazr, Nutr. Cancer 18 (1992) 1.
[17] S.F. Asad, S. Singh, A. Ahmad, S.M. Hadi, Med. Sci. Res. 26 (1998) 723.
[18] N.P. Das, Biochem. Pharmacol. 20 (1971) 3435.
[19] J.L. Donovan, J.R. Bell, S. Kasim-Karakas, J.B. German, R.L. Walzem, R.J. Hansen, A.L. Waterhouse, J. Nutr. 129 (1999) 1662.
[20] A.R. Rechner, G. Kuhnle, P. Bremner, G.P. Hubbard, K.P. Moore, C.A. Rice-Evans, Free Radic. Biol. Med. 33 (2002) 220.
[21] H. Wagner, L. Farkas, in: J.B. Harborne, T.J. Mabry, H. Mabry (Eds.), The Flavonoids, Chapman and Hall, London, UK, 1975, p. 127.
[22] G.P. Schiemenz, U. Schmidt, Liebigs Ann. Chem. (1976) 1514.
[23] G.P. Schiemenz, U. Schmidt, Liebigs Ann. Chem. (1982) 1509.
[24] L. Jurd, Tetrahedron 25 (1969) 1407.
[25] B. Nay, J.F. Peyrat, J. Vercauteren, Eur. J. Org. Chem. (1999) 2231.
[26] J. Tsuji, Pure Appl. Chem. 54 (1982) 197.
[27] B.M. Trost, L. Weber, P.E. Strege, T.J. Fullerton, T.J. Dietsche, J. Am. Chem. Soc. 100 (1978) 3416.
[28] G. Balavoine, G. Bram, F. Guibe, Nouv. J. Chim. 3 (1977) 207.
[29] C. Goux, M. Massacret, P. Lhoste, D. Sinou, Organometallics 14 (1995) 4585.
[30] J.K. Michie, J.A. Miller, Synthesis (1981) 824.
[31] W. Tückmantel, A.P. Kozikowski, L.J. Romanczyk Jr, J. Am. Chem. Soc. 121 (1999) 12073.
[32] A.V. Malkov, P. Spoor, V. Vinader, P. Kocovsky, J. Org. Chem. 64 (1999) 5308.
[33] B. Nay, M. Collet, M. Lebon, C. Chaze, J. Vercauteren, Tetrahedron Lett. 43 (2002) 2675.
[34] K.B. Sharpless, W. Amberg, Y.L. Bennani, G.A. Crispino, J. Hartung, K. Jeong, H. Kwong, K. Morikawa, Z. Wang, D. Xu, X. Zhang, J. Org. Chem. 57 (1992) 2768.
[35] Y. Ogino, H. Chen, H.L. Kwong, K.B. Sharpless, Tetrahedron Lett. 32 (1991) 3965.
[36] H. Van Rensburg, P.S. Van Heerden, D. Ferreira, J. Chem. Soc. Perkin Trans. 1 (1997) 3415.
[37] H. Van Rensburg, P.S. Van Heerden, B.C.B. Bezuidenhoudt, D. Ferreira, Tetrahedron Lett. 38 (1997) 3089.
[38] B. Nay, V. Arnaudinaud, J.F. Peyrat, A. Nuhrich, G. Deffieux, J.-M. Mérillon, J. Vercauteren, Eur, J. Org. Chem. (2000) 1279.
[39] H. Kawamoto, F. Nakatsubo, K. Murakami, Synth. Commun. 26 (1996) 531.
[40] J.M. Tedder, Chem. Rev. (1955) 787.
[41] J.W. Clark-Lewis, D.C. Skingle, Aust. J. Chem. 20 (1967) 2169.
[42] H. Kawamoto, F. Nakatsubo, K. Murakami, J. Wood Chem. Technol. 9 (1989) 35.
[43] V. Arnaudinaud, B. Nay, A. Nurich, G. Deffieux, J.-M. Mérillon, J.P. Monti, J. Vercauteren, Tetrahedron Lett. 42 (2001) 1279.
[44] (a) H. Kawamoto, F. Nakatsubo, K. Murakami, Mokuzai Gakkaishi 37 (1991) 488; (b) H. Kawamoto, F. Nakatsubo, K. Murakami, Chem. Abstr. 115 (1991) 279643y.
[45] L. Li, T.H. Chan, Org. Lett. 3 (2001) 739.
[46] B. Nay, J.P. Monti, A. Nuhrich, G. Deffieux, J.-M. Mérillon, J. Vercauteren, Tetrahedron Lett. 41 (2000) 9049.
[47] B. Nay, V. Arnaudinaud, J. Vercauteren, Eur. J. Org. Chem. (2001) 2379.
[48] V. Arnaudinaud, B. Nay, S. Vergé, A. Nurich, G. Deffieux, J.-M. Mérillon, J.P. Monti, J. Vercauteren, Tetrahedron Lett. 42 (2001) 5669.