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Synthesis of modified proanthocyanidins: introduction of acyl substituents at C-8 of catechin. Selective synthesis of a C-4 → O → C-3 ether-linked procyanidin-like dimer

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Abstract—The regioselective introduction of substituents at C-8 of (+)-catechin is described, leading to the synthesis of several catechin derivatives with various substitution patterns to be used for the further synthesis of modified proanthocyanidins. Thereafter, a new 3-O-4 ether-linked procyanidin-like derivative was synthesized. Its formation was selectively achieved through $TiCl_4$-catalyzed condensation of 4-(2-hydroxyethoxy)tetra-*O*-benzyl catechin with the 8-trifluoroacetyl adduct of tetra-*O*-benzyl catechin.

Proanthocyanidins are known as condensed or non-hydrolyzable tannins.[1–3] Many biological activities, and mainly a powerful free-radical scavenging[4–6] activity, have been reported for flavonoids, and their investigation is now increasingly important. The flavan-3-ols' protective effects on diseases involving oxidative stress like cancers,[7,8] cardiovascular,[9,10] and neurodegenerative[11] diseases have often been attributed to their antioxidative properties. Most of the described proanthocyanidins (procyanidins **1**) involve oligomerization of catechin derivatives **2a–d** (Fig. 1). However, proanthocyanidins may be linked either through carbon–carbon and/or carbon–oxygen bonds. While the natural occurrence of dimeric procyanidins with a C–C interflavanyl linkage is well documented,[12–14] little is known about proanthocyanidin possessing an ether bond only as interflavanyl linkage. Natural compounds exhibiting this ether interflavanyl bond are relatively rare but some have been reported in species of Acacia heartwood and are not derived from catechins but from epioritin-4-ol. The first single ether-linked (C-4 → O → C-4) dimeric promelacacinidins were indeed identified in *A. melanoxylon*[15] and were followed by similar bis-teracacinidins,[16] and the first (C-4 → O → C-3) ether-linked bis-teracacinidins[17] from *A. galpinii*. Recently both C-4 → O → C-4 and C-4 → O → C-3 ether-linked dimers were also isolated from *A. coffra*.[18]

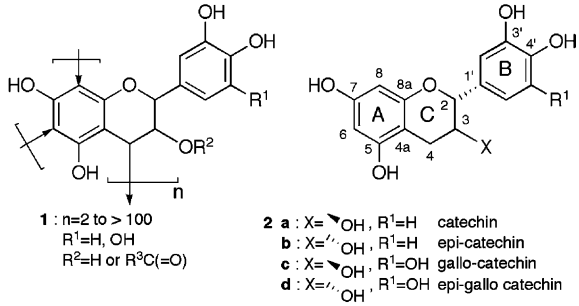

Figure 1.

In an ongoing program aimed at the synthesis of modified proanthocyanidins, we were interested in the preparation of modified catechin derivatives involving introduction of substituents either at C-6 and/or C-8. The goal of this work was thereafter to investigate the influence of the substitution pattern on ring A in the behavior of these compounds in the well documented Lewis acid catalyzed synthesis of natural occurring

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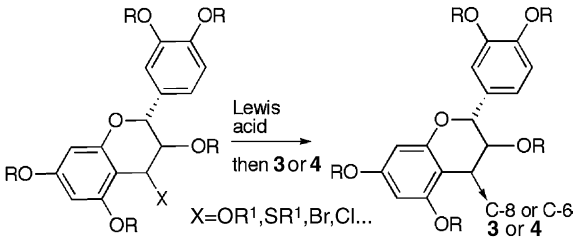


Scheme 1.

procyanidins (Scheme 1). Our starting materials, namely tetra-*O*-benzyl catechin **3** and penta-*O*-benzyl catechin **4**, have been sequentially prepared through classical procedures (NaH, BnBr, DMF) with, respectively, 52% and 49% yield in our hands from commercially available (+)-catechin **2a** (Scheme 2).

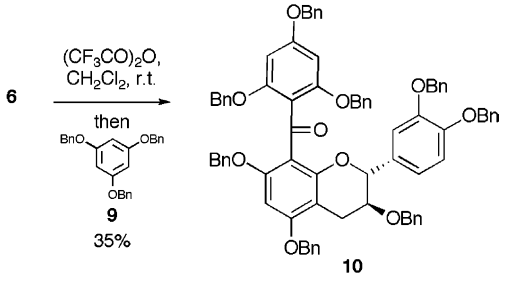
The classical Vilsmeier reaction effected on perbenzylated catechin **4** led to the clean formation of a unique isolated regioisomer **5**. The determination of the regiochemistry of the reaction was not achieved at this step and **5** was directly oxidized to the corresponding benzoic acid **6**. The substitution at C-8 was thereafter unambiguously established through NMR spectroscopy (HSQC/HMBC derived long range ^{1}H–^{13}C correlations).

Our next goal thereafter was to use other electrophiles for similar electrophilic additions reactions. Surprisingly, the trifluoro acetic anhydride mediated acylation[19] of catechin derivatives **3** and **4** by carboxylic acid did not succeed, even when performed with highly reactive benzoic acids. The only reaction products were in fact the products of the direct Friedel–Crafts condensation of trifluoroacetic anhydride, namely catechin derivatives **7** and **8**. After optimization, the same reaction, performed with only trifluoroacetic anhydride in dichloromethane, led to the formation of **7** (64% after hydrolysis of its C-3 trifluoro acetate obtained as primary product of the reaction) and **8** (42%) starting from **4** and **3**, respectively (Scheme 2). Similar NMR observations as for **6** were used to confirm the regiochemistry of these reactions.

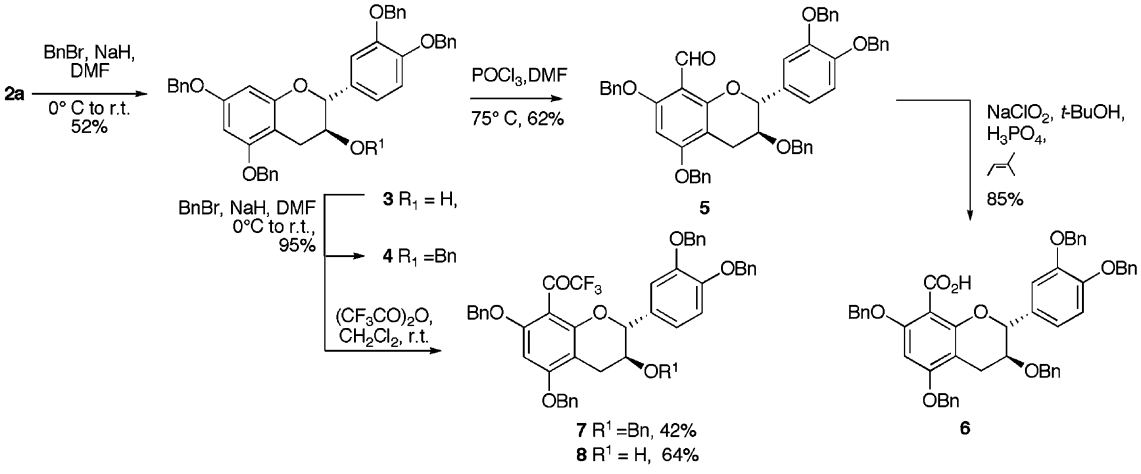
However, hence having in hand the carboxylic acid derivative of catechin **6**, we turned to its use as the carboxylic acid in the trifluoroacetic anhydride mediated acylation of aromatic compounds. Thereby, the reaction of **6** with tri-*O*-benzylphloroglucinol **9** in the presence of trifluoroacetic anhydride led to the exclusive formation of the benzophenone derivative **10** in 35% yield and, in this case, no trifluoroacetyl phloroglucinol was observed. This behavioral discrepancy between phloroglucinol and pentabenzyl catechin **4** as electrophile acceptor in this type of reaction is probably due more to steric than electronic effects.

In order to investigate the influence of an electron withdrawing group at C-8 on the behavior of catechin as nucleophile in the Lewis acid catalyzed flavanol coupling, we decided to turn to the use of compound **8** as nucleophile in this model reaction Scheme 3.

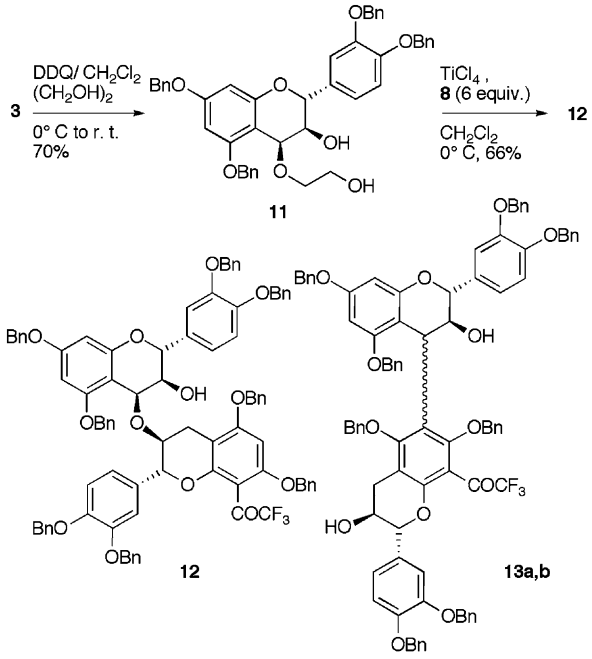
Indeed, this compound still exhibits now two potential nucleophilic sites: C-6 and (C-3)OH and no carboxylic function, which should interfere in this Lewis acid mediated reaction. Albeit C-4 → C-6 interflavanyl bond formation has been generally observed as a minor pathway in the already described flavanol coupling reactions involving C-8 non-substituted catechin derived subunits, the C-4 → O → C-3 ether bond formation has, to our knowledge, never been reported as the main reaction pathway in the catechin series but only as traces without complete elucidation of stereochemical features.[20]



Scheme 3.



Scheme 2.



Scheme 4.

After several attempts under various conditions, the use of $TiCl_4$ using the 4-(2-hydroxyethyloxy)flavan-3-ol **11** was found to be the most satisfactory. Derivative **11** was prepared by DDQ benzylic oxidation of tetra-*O*-benzyl catechin **3** according to previously published data.[20,21] The coupling reaction with C-8 substituted catechin **8** (Scheme 4) was thereafter monitored by CCM and HPLC and quenched after complete disappearance of the starting material. Among the formed products, compound **12** was further purified as the major product of the reaction. Its structure was established through UV, LC-ESI–MS, LC-ESI–MS–MS, and NMR analysis.

The UV spectrum of compound **12** was exhibiting similar absorption maxima (285 and 305 nm) to that of **8** indicating that the original flavanic structure with the $COCF_3$ group was retained. The mass spectrum obtained in the positive ion mode (Fig. 2) showed signals at *m*/*z*: 1395.97, 1413.0, 1417.96, and 1433.92 amu corresponding, respectively, to MH^+, MNH_4^+, MNa^+ and MK^+ indicating a molecular weight of 1394 amu in agreement with a dimeric structure. The remaining problem was, however, to establish the linkage type.

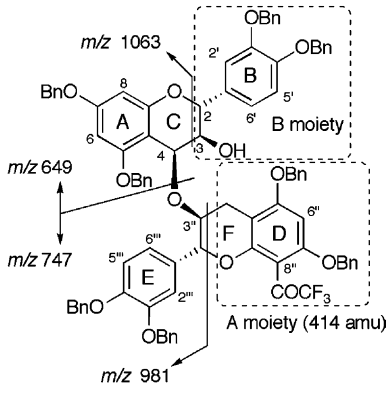


Figure 2.

In addition to the signals indicated above, the mass spectrum of compound **12** also showed signals at *m*/*z*: 747.82 and 649.8 amu corresponding to the fission of the bond between both constitutive units. Among the other observed signals, two of them were located at *m*/*z*: 1063 and 981.5 amu and were also observed in the spectrum obtained through positive MS–MS fragmentation of the signal located at *m*/*z*: 1395.97 amu and corresponding to the MH^+ ion. The signal observed at *m*/*z*: 1063.6 amu was obviously attributed to the characteristic retro Diels–Alder (RDA) fragmentation, corresponding to the $[MH^+-332]$ ion as was observed for compound **12** through a loss of the B moiety. The second fragmentation observed at *m*/*z*: 981 amu corresponds in fact to the $[MH^+-414]$ ion, meaning a loss of the A moiety of compound **12** unit and corresponding to another RDA fragmentation. The occurrence of this fission indicated the presence of the A moiety in the structure of compound **12**. In other words, this means that the isolated compound is not a C-4 → C-6 dimer since only one RDA fragmentation corresponding to the $[MH^+-332]$ ion would be possible in this case. The possible linkage is thus expected to occur via the 3″ position of the F ring or eventually the 2‴, 5‴, or 6‴ positions of the ring E.

In the chemical shift region for aromatic proton, the ^1H NMR spectrum[22] showed two doublets (*J* 1.8 Hz) integrating one proton each located at 6.12 and 6.27 ppm and a singlet integrating one proton located at 6.20 ppm. The first doublets were assigned to H-6 and H-8 protons of the A ring while the singlet was assigned to H-6″ of the D ring. This indicated that the interflavanyl linkage did not involve the D ring, thus confirming the conclusion of the MS observations. Neither did it involve the E ring since the three corresponding CH resonances were observed in ^{13}C and DEPT NMR spectra.

On another hand, the presence of two distinct catechin proton systems was clearly demonstrated in the proton spectrum. This was established through 1D ^1H and 2D ^1H–^1H COSY NMR spectra, which showed the presence of two spin systems corresponding to the two catechin units. The first AA′MX one, including signals at 2.61, 2.78, 4.49, and 4.99 ppm could be readily assigned to the H-4″ (α and β), H-3″ and H-2″ of F terminal unit ring. In the HETCOR spectra, these aliphatic protons correlate with carbons located at 27.15, 74.59, and 84.57 ppm.

Furthermore, in conjunction with the absence of a doubly benzylic methylene proton characteristic of a C-4 → C-6/C-8 linkage and taking into account the dimeric structure of the compound as supported by MS analysis, the NMR data collectively indicated a dimeric structure with an interflavanyl ether bond connecting the two heterocyclic C and F rings. Indeed, a second AMX spin system was observed in the proton spectrum including resonances at 3.83, 5.06, and 4.85 ppm, which

were assigned to the H-3, H-4, and H-2 of the extension C ring catechin system. These protons were correlating with carbon resonances appearing at 74.03, 70.29, and 80.82 ppm, respectively. The furthest upfield carbon and proton chemical shifts were in agreement with the presence of an oxygen atom on the corresponding carbon atom. This was also confirmed by comparison of the chemical shifts of the H-4 and H-3 resonances of both the C and the F rings with those of their precursors. In addition, the HETCOR spectra showed correlations between the B and E ring protons and their corresponding carbons, which were thus unambiguously assigned.

A (4-O-3) mode of linkage was concluded to occur between the two flavan-3-ol units. Moreover, coupling constants for the AMX spin system of the C-ring protons ($J_{3,4}$ = 3.2 Hz) indicated a 3,4 *cis* relative configuration for this ring, that is a 4β linkage between both flavanol units. The complete stereoselectivity of the reaction remains, however, to be explained and should presumably be due to a participation of the hydroxy group at C-3 of **6**. However, its involvement in the stereochemical course of the reaction cannot be, in our case, related to the formation of a protonated epoxide similar to that reported by Ferreira and co-workers[18] in a work dealing with the dimerization of epioritin-4-ol derivatives.

Indeed, the stereochemical outcome of the reaction in our case should be rather more consistent with a chelation process of the Lewis acid by both hydroxy groups of **11** and **8**, therefore inducing the approach of the nucleophile from the β face of **11**. The possible participation of the oxygen atoms of the ethylene glycol moiety of **11**, in such a chelation process, thereby inducing a quasi-concerted process has also to be considered.

In order to verify the presence of other dimeric structures the mixture was explored by HPLC coupled to mass spectrometry detection operating in the positive ion mode. An extracted ion current chromatogram recorded at *m*/*z*: 1395 and 1412 amu and corresponding to a dimeric structure molecular weight showed the presence, in addition to compound **12**, of a minor compound, which may well be the carbon–carbon coupled dimer **13** but which was, however, less predominant compared to the ether linked one.

The almost exclusive, high yielding formation, in these conditions, of this novel ether-linked procyanidin as main compound rather than its carbon–carbon C-4 → C-6 coupled analogue reflects the importance of the electronic features in the formation of flavan-3-ol dimers. Indeed, the poor nucleophilicity of the A ring in the monomeric precursor **8** has to be related to the presence of the $COCF_3$ group, hence permitting alternative nucleophilic sites of the molecule to participate in the interflavanyl bond formation.

These results form the starting point of a new methodology for the management of the regiochemical features related to the dimerization reaction of flavan-3-ol monomers. Further results will be reported in due course.

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22. ^{1}H NMR (500 MHz) and ^{13}C NMR (125.7 MHz) spectral data ($CDCl_3$, 298 °K) for compound **4** (resonances of the benzyl groups are not mentioned).

	^{1}H δ (ppm) *J* (Hz)	^{13}C δ (ppm)		^{1}H δ (ppm) *J* (Hz)	^{13}C δ (ppm) $J_{C\text{-}F}$
2	4.85 (d, 10)	80.82			
3	3.83 (m, $J_{3,4}$ = 3.2)	74.03	4″	2.61 (dd, 16.7, 4.4)	27.15
4	5.06 (m)	70.29		2.78 (dd, 16.7, 6.6)	
6	6.27 (d, 2.2)	97.61	6″	6.20 (s)	95.15
8	6.12 (d, 2.2)	98.84	8″		109.60
4a		106.78	4a″		106.62
8a		160.99	8a″		158.79
5		162.72	5″		165.29
7		165.59	7″		161.88
1′		136.70	1‴		136.15
2′	7.08 (d, 1.8)	118.57	2‴	6.78 (d, 1.3)	116.35
3′		153.66	3‴		153.4
4′		153.59	4‴		152.94
5′	7.01 (d, 8.2)	119.07	5‴	6.80 (d, 8.2)	118.57
6′	7.00 (dd, 8.2, 1.8)	125.33	6‴	6.63 (dd, 8.2, 1.3)	123.51
2″	4.99 (m)	84.57	CO		188.89, 38
3″	4.49 (m)	74.59	CF_3		115.67, 292