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Syntheses of doubly linked proanthocyanidins using free flavan units as nucleophiles: insight into the origin of the high regioselectivity of annulation†

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A synthesis method of doubly linked flavan dimers is reported *via* the acid-promoted annulation reaction using nascent catechins, (+)-catechin or (−)-epicatechin, as a dianionic partner and an ethylenedioxy-bridged flavan as a dicationic partner. Procyanidins A1 and A2 were synthesized. On the high regioselectivity of the annulation reactions, model experiments and computational studies were carried out.

Oligomeric proanthocyanidins (OPA) are widespread in nature, featuring extreme molecular diversity.[1] One of the elements that generates their diversity is the connectivity of the monomeric flavan units. In addition to the OPAs connected through a single interflavan bond, doubly linked OPAs have also been identified featuring a dioxabicyclo[3.3.1]nonane skeleton. Representatives are procyanidin A1 (**1**), composed of epicatechin and catechin, and also procyanidin A2 (**2**), composed of two epicatechins (Fig. 1).[2]

Although potential biological activities of the doubly-linked OPAs have been reported,[3] further studies have been hampered by scarcity of their samples. This situation could be changed by organic synthesis, making pure samples available, although several synthesis challenges need to be solved.

Previously, we reported the total synthesis of procyanidin A2 (**2**) (Scheme 1).[4a] The double linkage was formed by using 2,4-ethylenedioxy flavan derivative **3** as an equivalent of dication synthon **I** and phenol **4**[5] as that of a dianion synthon **II**. Among the two oxy-leaving groups in **3**, the C4 one is initially activated by a Lewis acid, generating the C4-cation, that is captured by the C8 nucleophilic carbon center in **4** with a rigorous regioselectivity to form intermediate **III**. The subsequent generation of the C2-cation in **III** allows the oxy-cyclization by the C7-phenol to give the annulated product **5**.

Two notable features in substrates **3** and **4** are: (1) the dioxy derivative **3** is bromo-capped at its C8 position to tame the nucleophilicity for avoiding the potential self-reactions,[4] and (2) the nucleophilic substrate **4** is selectively protected with the free C7-phenol, which was prepared by our *de novo* protocol.[5]

For expanding the scope of the approach, we decided to study two substrate modifications. Firstly, we conceived of the idea of using catechin and epicatechin as the commercially available starting materials. Since the selective protection of the phenols in those compounds seemed tedious, we envisaged to use them in their free forms, although the regioselectivity may be problematic, because the C6/C8 nucleophilic sites in the A-ring have similar reactivities (blue and yellow in **I**, Fig. 2).

Secondly, one could expect a higher nucleophilicity of **I** at the A-ring moiety from the extra free phenol (red, Fig. 2) than its protected congener **II**. Given the case, we came to a notion that the C8-bromo capping as in the dioxy-flavan unit may not be necessary.

Some additional concerns were: (1) poor solubility of free flavans in common organic solvents, and (2) high sensitivity of free flavans toward oxidants and other conditions.[6]

We report here realization of these ideas to open an efficient access to doubly linked OPAs, as demonstrated by the achievement of concise total syntheses of procyanidin A1 (**1**) and A2 (**2**). On the high regioselectivity of the annulation reac-

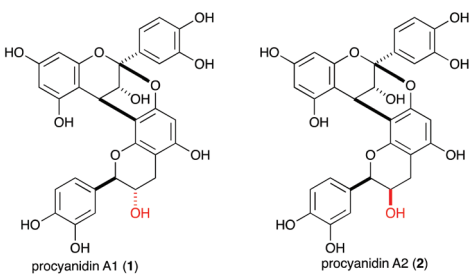

Fig. 1 Doubly-linked proanthocyanidin dimers.

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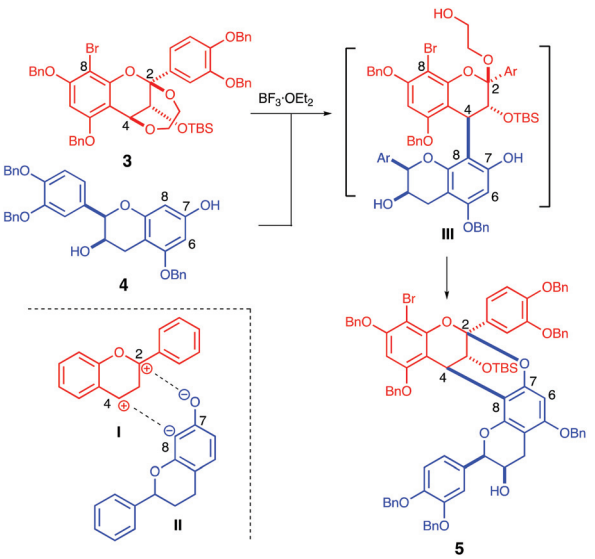


Scheme 1 Catechin annulation.

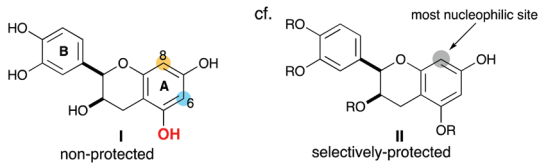
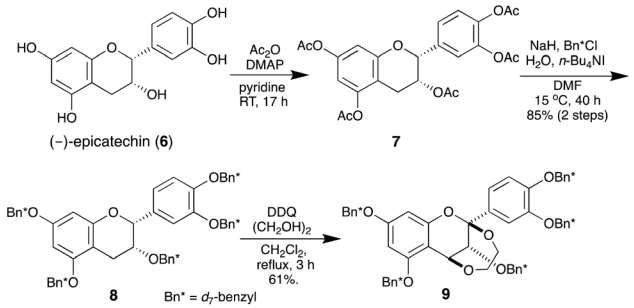


Fig. 2 Nucleophilic flavan units.

tions, model experiments and computational studies have been carried out.

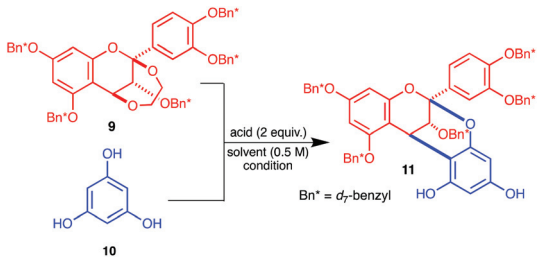
Scheme 2 shows the preparation of the top flavan unit **9**. For the ease of the ^{1}H NMR spectra analysis, all hydroxy groups in (−)-epicatechin (**6**) were protected as d_7-benzyl (Bn*) ethers.[4b,d] According to Kawamoto's protocol,[7] pentaacetate **7** was subjected to *in situ* hydrolysis and concurrent benzylation of the resultant phenolates, giving ether **8**. Heating of **8**, ethylene glycol (1.5 equiv.) and DDQ (4.0 equiv.) in refluxing CH_2Cl_2 gave dioxy-flavan **9**, ready for the annulation study.

As a feasibility study, we examined the reaction of dioxy-flavan unit **9** and phloroglucinol (**10**) as a nucleophile for modelling the reactivity of the A-ring in free flavan units. An objective was to find out suitable reaction media, because the two reaction partners have quite different solubility trends. While dioxy-flavan unit **9** is lipophilic, free catechins are poorly soluble in common non-protic solvents. Thus, we started with ethanol as the medium to test the model reaction, and the results are summarized in Table 1.



Scheme 2 Synthesis of top flavan unit **9**.

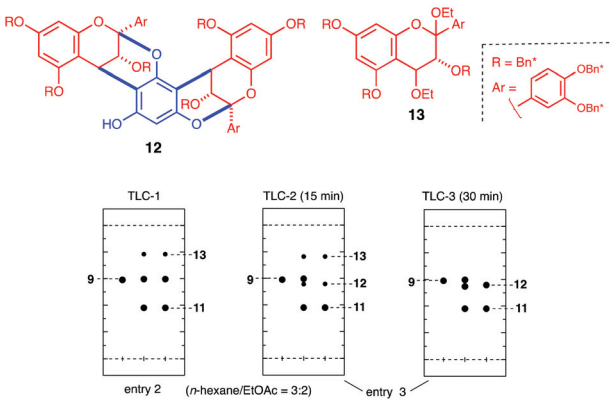
Table 1 Model study



Run	Acid	Solvent	Conditions	**11**/%	**12**/%
1	PPTS	EtOH	RT, 24 h	—	—
2	PPTS	EtOH	Reflux, 5 min	49[a]	—
3	PPTS	EtOH	Reflux, 0.5 h	50	24
4	CSA	EtOH	RT, 24 h	65[b]	14
5	CSA	EtOH, dioxane[d]	RT, 24 h	84	7
6[c]	CSA	EtOH, dioxane[d]	RT, 24 h	98	1

[a] Compound **13** was obtained in 12% yield, and compound **9** was recovered in 36% yield. [b] Compound **13** was obtained in 16% yield. [c] Molar ratio of **9**/**10** was 1.0/1.5. [d] v/v = 1.

To a mixture of dioxy-flavan unit **9** and **10** (1 : 1 molar ratio) in ethanol was added 2 equivalents of pyridinium *p*-toluenesulfonate (PPTS).[8] At room temperature, no reaction occurred even after 24 h, resulting in a complete recovery of the starting materials (entry 1). However, upon refluxing, the reaction proceeded rapidly as suggested by TLC-1 after 5 min (entry 2). Chromatographic purification gave the desired product **11** in 49% yield, and the starting material **9** was recovered in 36% yield. In addition, a non-polar product was also isolated, which proved to be 2,4-diethoxy derivative **13** [9] (12% yield). We reasoned that this is a solvolytic product, which could be reactivated to serve as a dication equivalent to take part in the annulation.



Upon conducting the same reaction for a slightly longer time (30 min), dioxy-flavan unit **9** was fully consumed (TLC-2),

giving the desired product **11** in 50% yield (entry 3). The 2,4-diethoxy derivative **13** was no longer obtained. However, a side product was obtained, which was identified as the bis-annulated product **12** in 24% yield, arising from the iterated annulation reaction of the product **11** with **9** and/or **13**.

To suppress this second annulation, the reaction conditions were screened. When PPTS was replaced by camphorsulfonic acid (CSA), a stronger acid, the reaction proceeded at room temperature (entry 4). The yield of the desired product **11** was improved to 65%, although the formation of **12** still persisted. During this reaction, we noted that the reaction mixture became turbid, for which we suspected that the 2,4-diethoxy intermediate **13** was poorly soluble in ethanol at room temperature.[10,11]

In contrast, the use of 1,4-dioxane as a co-solvent (EtOH, 1,4-dioxane v/v = 1) led to a clear solution, improving the ratio of **11** over **12** (12 : 1) (entry 5). Increased loading of **10** (1.5 equiv.) significantly improved the yield of **11** (98% yield) with a minimal formation of the bis-annulated product **12** (entry 6).[12]

Notably, in all the reactions stated above, we never observed any self-reaction product of the dioxy-flavan unit **9**. This promising data supported our expectancy that the non-bromo capped substrate **9** would work, and we hoped it would apply for real systems as well.

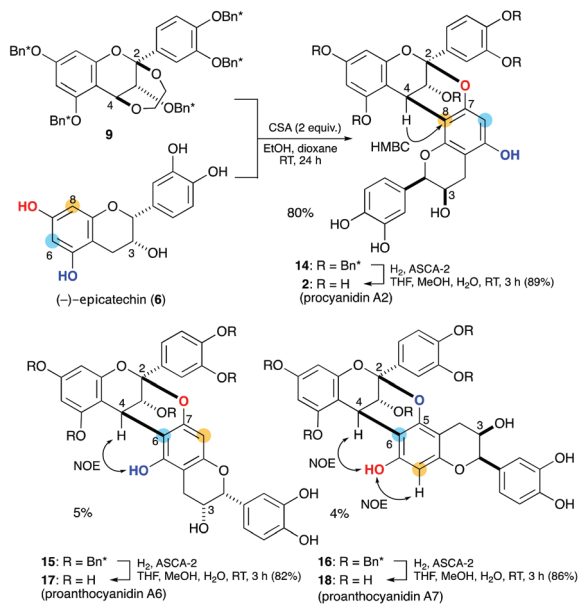
With these promising results in hand, we turned our attention to the annulation of dioxy-flavan unit **9** with (−)-epicatechin (**6**). In a solvent mixture of ethanol and 1,4-dioxane (v/v = 1), free flavan **6** (1.5 equiv.) was allowed to react with dioxy-flavan **9** in the presence of CSA (Scheme 3). After separation by preparative TLC [CH_2Cl_2, EtOAc (7/3)], the major annulated product **14** (R_f = 0.2) was obtained in 80% yield. The connectivity of **14** was proven by a diagnostic HMBC correlation between H4 and C8, confirming it as the C8 linked isomer (C2 → *O* → C7, C4 → C8).[13] The reaction predominantly occurred at the C8 and C7–O positions in **6**.

The TLC purification also gave a small amount of a less polar component (R_f = 0.3), which proved to be a mixture of two compounds. Further purification by another preparative TLC [CH_2Cl_2, EtOAc (7/3), two runs] gave **15** (R_f = 0.28, 5% yield) and **16** (R_f = 0.33, 4% yield). The less polar isomer was assigned as **16** (C2 → *O* → C5, C4 → C6) by diagnostic NOEs observed between H4/C7-phenol and H8/C7-phenol. On the other hand, the more polar isomer **15**, having a connectivity as C2 → *O* → C7, C4 → C6, by the fact an NOE correlation was solely observed between H4/C7-phenol, but not between H8/C7-phenol.[13]

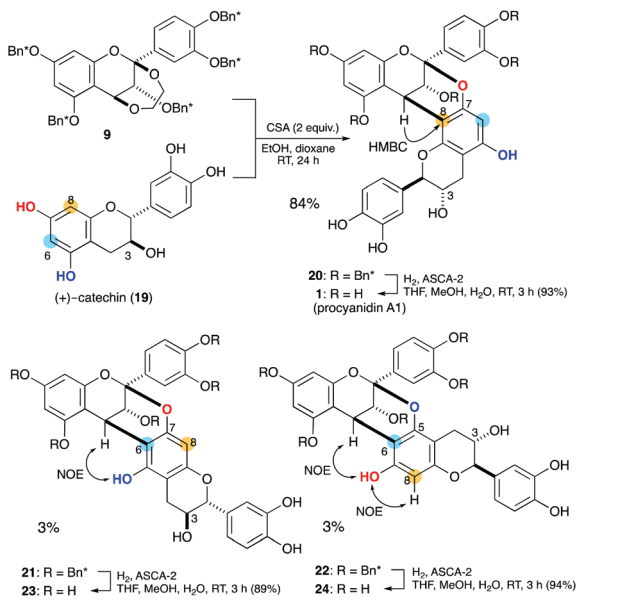
By following our previously established conditions,[4] all the benzyl groups in **14** were removed by hydrogenolysis [H_2 (1 atm), ASCA-2®,[14] MeOH, THF, H_2O (2/2/1), 3 h]. Anaerobic filtration (argon) and removal of volatile materials followed by lyophilization gave procyanidin A2 (**2**) (almost pure as assessed by ^{1}H NMR). Further purification by reverse phase preparative HPLC [Mightysil RP-GP II, 20 mm φ × 250 mm, MeOH, H_2O (35/65) containing 0.1% TFA] and lyophilization gave **2** as a snow-white amorphous solid. The spectral data (^{1}H and ^{13}C NMR, IR, HRMS) of the final product **2** were in full agreement with our previously reported data of the natural product.[4a] Similarly, the deprotection of other regioisomeric products **15** and **16** was carried out under hydrogenolytic conditions, giving proanthocyanidin A6 (**17**)[15] and proanthocyanidin A7 (**18**)[15a,16] in 82% and 86% yield, respectively.

To test the generality of the present annulation method, we next examined the synthesis of procyanidin A1 (**1**), a heterodimer composed of (−)-epicatechin (**6**) as an upper flavan unit and (+)-catechin (**19**) as a lower flavan unit (Scheme 4).

By the protocol described in Scheme 3, stirring a solution of flavan **9** with **19** in the presence of CSA in a mixed solvent



Scheme 3 Annulation with (−)-epicatechin and synthesis of procyanidin A2.



Scheme 4 Annulation with (+)-catechin and synthesis of procyanidin A1.

of ethanol and 1,4-dioxane (v/v = 1) smoothly gave the annulation product **20** as the major product in 84% yield along with a mixture of two minor regioisomers **21** and **22** in 8% combined yield. The connectivity (2β → *O* → 7, 4β → 8) in **20** was verified by a diagnostic HMBC correlation between H4 and C8.[13]

Further separation of the minor regioisomers by preparative TLC [CH_2Cl_2, EtOAc (7/3), two runs] gave **21** and **22** in 3% yield each, which were characterized by NOE studies.

Finally, dimer **20** was subjected to hydrogenolysis [H_2 (1 atm), ASCA-2®, MeOH, THF, H_2O (2/2/1), 3 h], giving procyanidin A1 (**1**). All physical data of the synthetic compound fully matched with those of the reported natural product.[2a] Moreover, other regioisomers **21** and **22** were deprotected by the same hydrogenolysis, giving a natural proanthocyanidin **23** [17] (no trivial name) and its regioisomer **24** in 89% and 94% yield, respectively. The latter isomer **24** is a new compound, which has not yet been isolated from nature.

The OPA syntheses described above feature high regioselectivity of the annulation, which is outlined in Fig. 3. Formation of the major product, **14** or **20**, could be traced back to the predominant formation of the C4–C8 bond between two flavan units **I** and **II**, forming the major intermediate **III**, which unequivocally undergoes oxycyclization by the C7-phenol, giving the major product **V**. The minor pathway starts with the C6 center in **II** to generate intermediate **IV**, which entails two optional oxycyclizations to give almost equal amounts of the cyclized products **VI** and **VII** in an essentially non-selective manner.

Concerning the mechanistic basis for the regioselectivity at the key annulation stage, we raised a question on the origin of the C8/C6-regioselectivity. To address the preference of the C8 position over the C6 position in the initial C–C bond formation, we carried out a model study with a "simplified" nucleophile **28**, removing the B-ring and the C3 hydroxy group from a general flavan unit. Thus, the model substrate **28** was synthesized based on our reported method (Scheme 5).[5] The regioselective lithiation of **25** (*n*-BuLi, THF, −78 °C, 1 h) and subsequent reaction with oxetane in the presence of $BF_3{\cdot}OEt_2$ [18] gave alcohol **26**, which was cyclized by treatment with NaH (DMF, 80 °C, 2 h), cleanly affording pyran **27** in 72% yield. The simultaneous removal of benzyl groups by hydrogenolysis [H_2 (1 atm), ASCA-2®] gave phenol **28**.

Having model nucleophile **28** in hand, we conducted the annulation with di-oxy flavan **9** under previously optimized conditions. The reaction proceeded smoothly, giving the anticipated C8-regioisomer **29** as a major product (86% yield), along with the C6-regioisomers, **30** and **31** in 4% yield, respectively (Scheme 6).[13] This observation concluded that the presence of an aryl ring (catechol) at C2 and the hydroxy group at C3 on the pyran ring has essentially no effect on the C8/C6-regioselectivity in the dimerization.

The question was now focused to the relative reactivities of the C8 and the C6 centers toward electrophiles. The high regioselectivities of the annulation originate from the site selectivity of the nucleophilic free catechin unit, C8 > C6, implying the relevance to the relative natural abundance of the C8-linked oligomers in comparison with the C6-linked congeners.[19]

To address the origin of C8/C6-regioselectivity, the HOMO coefficients of **28** at the C6 and the C8 positions were estimated by DFT calculation, which was not fruitful, as no obvious difference was observed. By contrast, DFT calculation

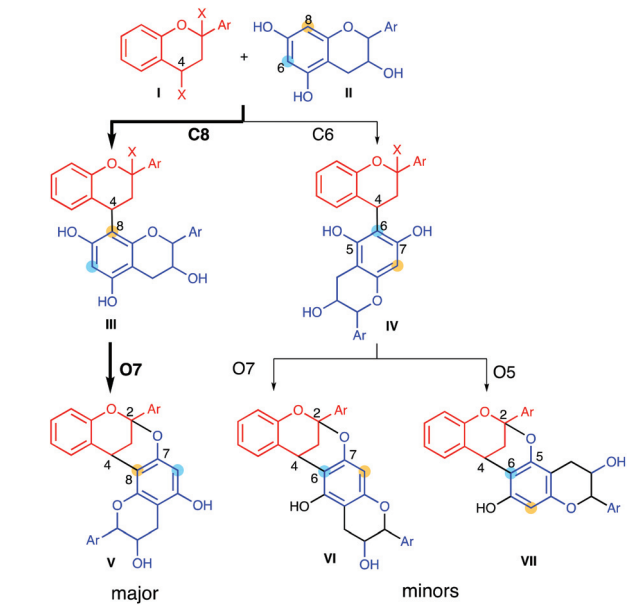
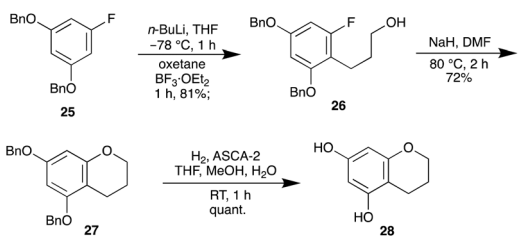
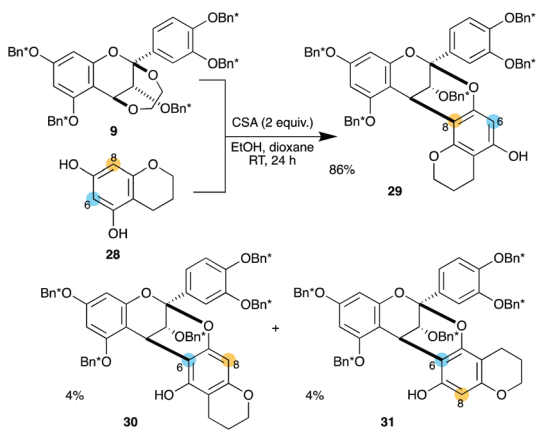


Fig. 3 Rationale for the regioselective annulation.



Scheme 5 Synthesis of model substrate **28**.



Scheme 6 Annulation with model nucleophilic partner **28**.

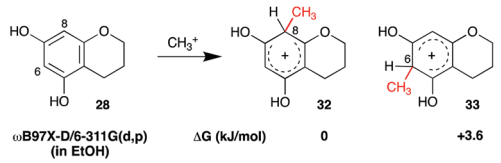


Fig. 4 DFT calculations on the Wheland intermediate.

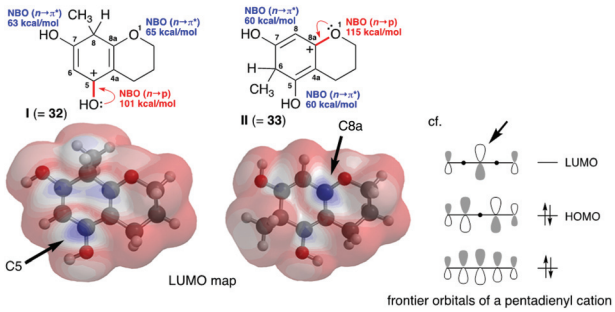


Fig. 5 Origin of C8 regioselectivity.

on the putative Wheland intermediates **32** and **33**, generated by the reaction on the model substrate **28** with "CH_3^+" suggested that the C8-methyl intermediate **32** is energetically preferred over the C6-counterpart **33**, in line with the preferred C8 substitution rather than the C6 substitution (Fig. 4).

To gain further insight, we addressed LUMO maps[20a] and the NBOs[20b] of **32** and **33** (Fig. 5). The LUMO maps identified C5 for **32** and C8a for **33** as the most electron-deficient sites, which correspond to the *para* positions to the methyl-bonded carbons. The NBO analyses showed both of these sites accept significant electron donation from the adjacent oxygen atoms (see C5–O5 for **32** and C8a–O1 for **33**), which stand in contrast to the weaker interactions of other C–O bonds.

Implication from these aspects is that both **32** and **33** are best expressed by canonical structures **I** and **II**, in which one could find a common local structure of a 1,3,5-trioxy-substituted pentadienyl cation, sharing a localized cation at a central carbon center that is stabilized not only by the two C=C bonds, but also by a strong n → p conjugation.

Although the electronic and orbital parameters are virtually the same in **I** and **II**, we noticed a notable difference in the molecular shape. Namely, the bicyclic skeleton in **II** is sizably distorted, presumably for paying the cost of localizing a C=C bond at the *exo* position (C4a–C5) to the pyran ring, while the shape of **I** appears to be free from such constraints. We believe this is the origin of the energy difference of **32** and **33**, which in turn would explain the C8/C6-regioslectivity.[21]

In conclusion, we have demonstrated the direct syntheses of the doubly linked OPAs, procyanidins A1 (**1**) and A2 (**2**) in five steps from commercially available starting materials. This approach significantly simplifies the synthetic procedure, sidestepping the selective protection of synthetically indistinguishable phenols on the nucleophilic flavan unit, and it could be employed for the synthesis of various doubly linked proanthocyanidins. Model experiments and computational studies validated the high regiochemical selectivity in annulation reactions. Biological studies using synthetic dimers are currently in progress.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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9 Obtained as a single diastereomer, albeit stereochemistries unassigned.
10 A pure sample of diethoxy flavan **13** was prepared in 93% yield from **9** (CSA, EtOH, 1,4-dioxane, RT, 16 h). Indeed, it was found to be sparingly soluble in ethanol, and soluble in 1,4-dioxane.
11 Re-exposure of **13** with **10** to the same acidic conditions gave the annulation products **11** and **12**.
12 Reversal of the ratio of **9**/**10** to (1.5 : 1) drastically increased the formation of the bis-annulated product **12** over **11**.
13 See the ESI for spectral details.†
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20 (*a*) Calculated by Spartan'18 program. For details, see the ESI;†; (*b*) Calculated by Gaussian16 program. For details, see the ESI.†
21 Details will be reported in due course.

