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COMMUNICATION

Unified approach to catechin hetero-oligomers: first total synthesis of trimer EZ–EG–CA isolated from *Ziziphus jujuba*†

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A catechin hetero-trimer isolated from *Ziziphus jujuba* has been synthesized. Among three constituent monomers, (−)-epiafzelechin and (−)-epigallocatechin were prepared by *de novo* synthesis. Trimer formation relied on the unified approach to oligomers based on the bromo-capping and the orthogonal activation, reaching the reported structure of the natural product.

Oligomeric proanthocyanidins (flavan-3-ol oligomers) found in woody and herbaceous plants are related to traditional folk medicines for treating inflammation, viral diseases, and high blood pressure.[1] The plant extracts are generally composed of diverse catechin monomers with different oxygenation patterns and their oligomers with varying degrees of oligomerization. Among the oligomers, *hetero-oligomers* composed of different monomers are the key contributor to molecular diversity that may serve as potential sources of bioactive compounds.

However, exploration into such a "proanthocyanidin library" has been limited by the difficulty in isolating individual compounds even by modern separation methods.[2] The characterization is also difficult. *NMR analysis* is hampered by severe peak overlap and broadening as well as the rotameric issues, and *X-ray crystallography* is ineffective by the generally low crystallinity of the polyphenols.[3]

As a case study, let us focus on natural hetero-trimer **1** composed of (−)-epiafzelechin (EZ, **2**), (−)-epigallocatechin (EG, **3**), and (+)-catechin (CA, **4**), which was isolated from the bark of *Ziziphus jujuba* (Fig. 1).[4]

Although the sequence EZ–EG–CA was proposed based on the degradation study by thiolysis[5] (Fig. 2), which identifies the monomer composition (EZ, EG, and CA) and the terminal unit as CA, other information on the connectivity and stereo- and regiochemistry of the interflavan bonds becomes elusive. Thus, room remains for the possible sequence EG–EZ–CA differing in the connectivity, which features the critical lack of general means of structural assignment on this class of natural products.

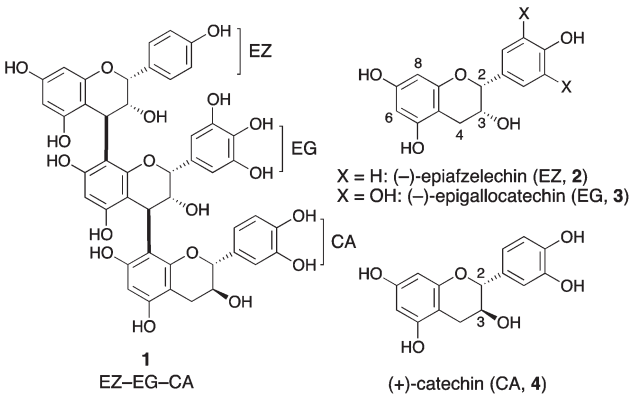

Fig. 1 Catechin hetero-trimer **1** and the constituent monomers.

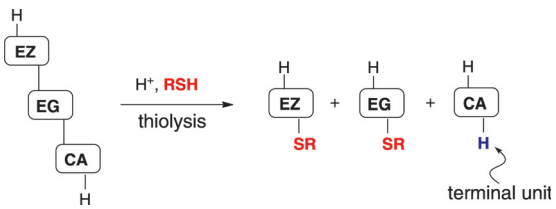

Fig. 2 Thiolysis of hetero-trimer **1**.

Organic synthesis has promising potential in this regard, providing samples useful for analytical standards and biological studies. However, despite numerous reports on *hetero-oligomers*,[6] their syntheses have rarely been achieved[7] for two reasons: (1) scarce availability of monomers: except for (+)-catechin and to a lesser extent (−)-epicatechin, other congeners are virtually unavailable; (2) lack of viable methods for connecting the interflavan bonds.

This communication reports the first total synthesis and structure confirmation of hetero-trimer **1**, featuring a unified approach to such catechin hetero-oligomers. Fig. 3 is a cartoon to show three stages of the approach: (1) *de novo* synthesis of the constituent monomers, (2) suitable functionalization of the monomers (bromo-capping[8] for suppressing non-selective oligomerization and installation of the leaving groups[9] ready for the coupling), and (3) oligomer formation by the orthogonal activation.[8]

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†Electronic supplementary information (ESI) available: Experimental procedures for the preparation and spectral data of all compounds. See DOI: 10.1039/c2ob26337h

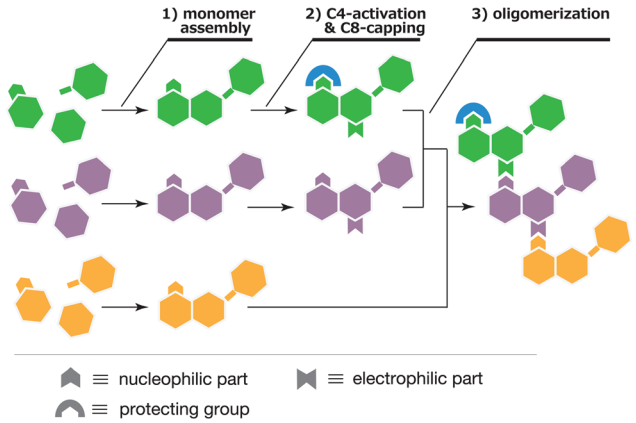


Fig. 3 Three stages for the assembly of hetero-trimer.

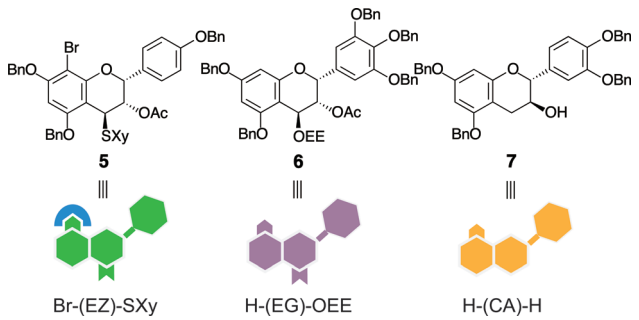


Fig. 4 Three constituting monomers.

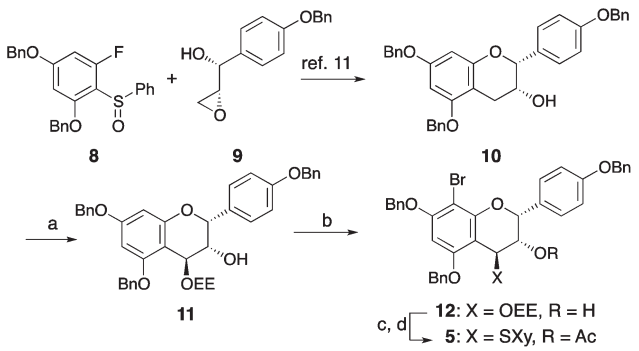
Three constituent monomers (Fig. 4), designated as Br-(EZ)-SXy **5**, H-(EG)-OEE **6**, and H-(CA)-H **7**,[10] were prepared in the following manner, respectively (see Schemes 1 and 2).

Synthesis of the epiafzelechin derivative **5** started with assembly of two building blocks **8** and **9** by the protocol previously reported.[11,12] Epiafzelechin derivative **10**, thus prepared, was treated with DDQ[13] in the presence of 2-ethoxyethanol[13c] to give H-(EZ)-OEE **11** in 72% yield.[14] A bromine atom was introduced at the C(8)-position in **11** by treatment with *N*-bromosuccinimide (NBS), giving Br-(EZ)-OEE **12** in 71% yield. The C(4) leaving group of **12** was replaced by an arylthio group by treatment with 2,6-dimethylbenzenethiol[15] (XySH) and $BF_3{\cdot}OEt_2$, and subsequent acetylation gave Br-(EZ)-SXy **5** (91% yield, two steps from **12**).

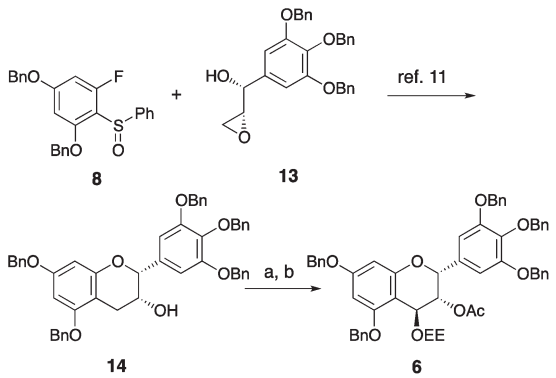
In the same manner, the epigallocatechin unit **6** was prepared *via* **14**, which was assembled from building blocks **8** and **13**. Exposure of **14** to DDQ oxidation followed by acetylation furnished H-(EG)-OEE **6** in 62% yield (Scheme 2).

Catechin unit **7** was prepared by a modified Kawamoto protocol[16] from commercial (+)-catechin (**4**).

Having three building blocks **5**, **6** and **7** in hand, synthesis of trimer Br-(EZ)-(EG)-(CA)-H **16** was put into practice by using the orthogonal activation method (Scheme 3). The electrophilic unit Br-(EZ)-SXy **5** was selectively activated by *N*-iodosuccinimide (NIS) in the presence of the nucleophilic unit H-(EG)-OEE **6**, giving dimer Br-(EZ)-(EG)-OEE **15** in 91% yield. The stereo- and regiochemistry of the newly-formed interflavan linkage was assigned as 4β → 8 by the 2D NMR analyses (see **15A**).[17]



Scheme 1 Epiafzelechin unit **5**; (a) 2-ethoxyethanol, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ), toluene, room temp., 23 h (72%); (b) NBS, CH_2Cl_2, −15 → −8 °C over 3 h (71%); (c) XySH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −78 → −55 °C over 3.5 h (96%); (d) Ac_2O, *N*,*N*-dimethylaminopyridine (DMAP), pyridine, CH_2Cl_2, room temp., 2 h (95%).

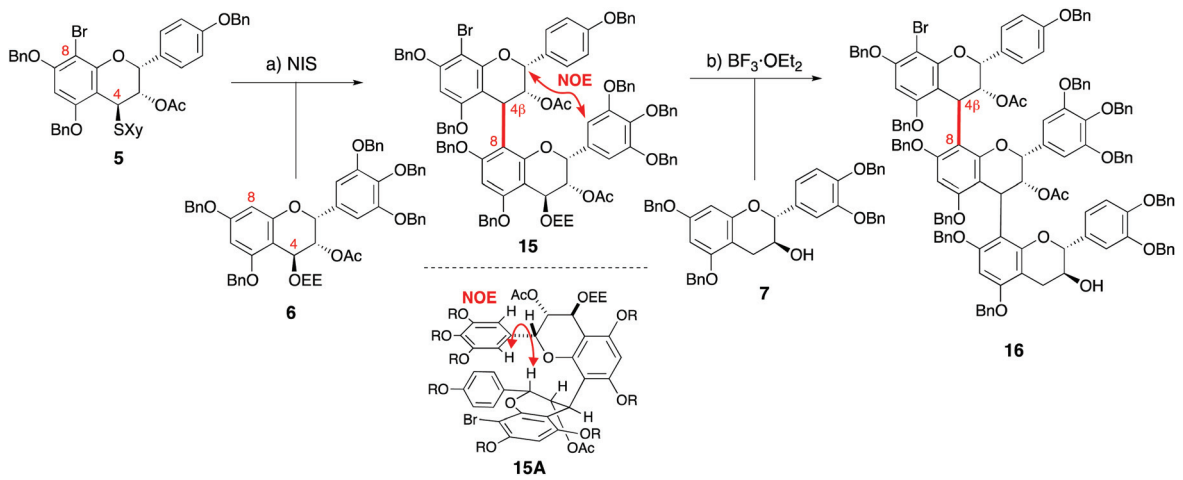


Scheme 2 Epigallocatechin unit **6**; (a) 2-ethoxyethanol, DDQ, toluene, room temp., 12 h; (b) Ac_2O, DMAP, pyridine, CH_2Cl_2, room temp., 12 h (62%, 2 steps).

The dimer **15** with a C(4)-oxy leaving group was activated by $BF_3{\cdot}OEt_2$ in the presence of the catechin unit **7**, affording the targeted trimer **16** in 92% yield. An issue arose, however, in assigning the stereo- and regiochemistries of the second interflavan bond. The NMR analysis of trimer **16** was hampered by the presence of three rotamers (65 : 20 : 15 in $CDCl_3$, 27 °C) by the restricted rotation around two interflavan bonds on the NMR time scale (500 MHz).

For addressing the stereostructure of trimer **16**, the reversed order of couplings was carried out, *i.e.*, starting with connecting the lower units, EG and CA (Fig. 5, *upward assembly*). The hope was that one could address the stereochemistry of the lower interflavan bond at the stage of the dimeric unit. Were the two samples of **16** identical, interpolation would allow the regio- and stereochemistry of two interflavan bonds.

The *upward assembly* (Scheme 4) started with the formation of dimer **17** in 74% yield by subjecting H-(EG)-OEE **6** and H-(CA)-H **7** to the $BF_3{\cdot}OEt_2$-promoted conditions. In order to suppress the self-condensation of **6**, the reaction partner **7** was used in excess (3.0 equiv.). The connectivity and stereochemistry of the interflavan bond in **17** was verified as H-(EG)-|4β → 8|-(CA)-H by extensive 2D NMR analyses (see **17B**).



Scheme 3 Downward assembly (a) molar ratio: **5**/**6**/NIS = 1.0 : 1.5 : 1.3, MS4A, CH_2Cl_2, −78 → 0 °C over 25 min, then 0 °C, 11 h (91%); (b) molar ratio: **15**/**7**/BF_3·OEt_2 = 1.0 : 1.3 : 1.9, CH_2Cl_2, −78 → −30 °C over 1.3 h (92%).

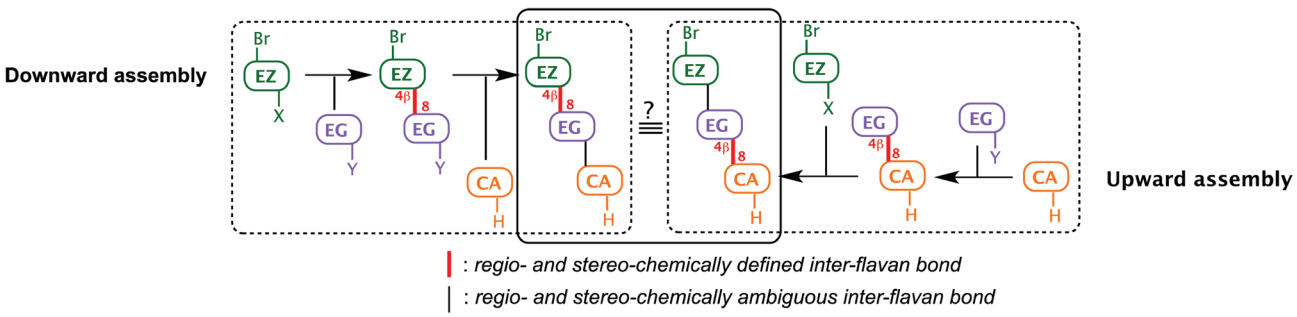
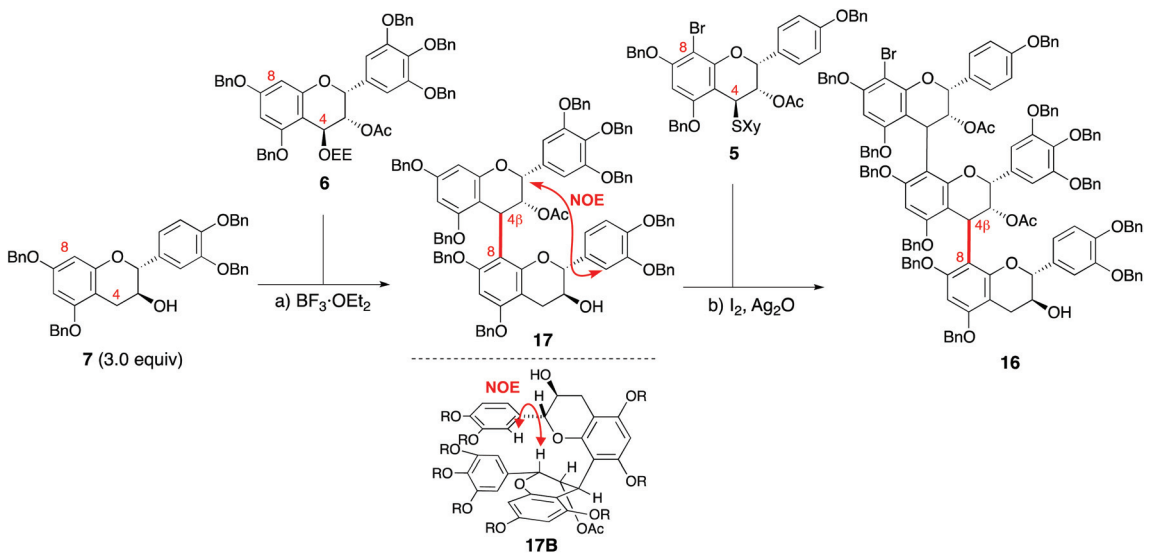


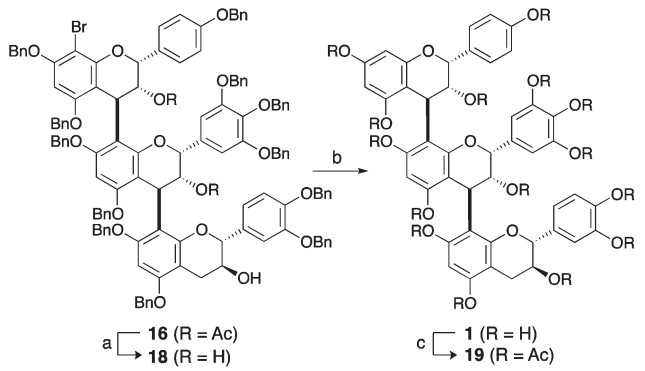
Fig. 5 Interpolative approach for stereochemical assignment of hetero-trimer **16**.



Scheme 4 Upward assembly (a) molar ratio: **6**/**7**/BF_3·OEt_2 = 1.0 : 3.0 : 2.7, CH_2Cl_2, −78 → −25 °C over 40 min (74%); (b) molar ratio: **17**/**5**/I_2/Ag_2O = 1.5 : 1.0 : 3.2 : 1.6, MS4A, CH_2Cl_2, −78 → 0 °C over 1 h (76%).

Condensation of dimer **17** with Br-(EZ)-SXy **5** under the soft activation conditions (I_2 and Ag_2O) smoothly gave trimer **16** in 76% yield, amenable to comparison.

Indeed, the samples of trimer **16**, obtained by two independent routes, fully coincided upon analyses by NMR, MALDI-TOF MS, TLC (several different solvent systems), and HPLC,



Scheme 5 (a) NaOMe, MeOH, CH_2Cl_2 (1 : 4) (94%); (b) H_2, ASCA-2® (5% $Pd(OH)_2$/C), THF, MeOH, H_2O (4 : 4 : 1), room temp., 45 min; (c) Ac_2O, pyridine, DMAP, 0 °C, 1 h (63%, 2 steps).

confirming the structure as Br-(EZ)-|4β → 8|-(EG)-|4β → 8|-(CA)-H.

Scheme 5 shows final removal of the protecting groups. The acetyl protecting groups in **16** were detached by exposure to sodium methoxide to give Br-(EZ)-(EG)-(CA)-H **18** in 94% yield. Hydrogenolysis of **18** [H_2, ASCA-2® (5% $Pd(OH)_2$/C),[18] THF, MeOH, H_2O] and lyophilization afforded trimer EZ–EG–CA **1** {70% yield, $[\alpha]_D^{30}$ +52 (*c* 1.3, acetone–H_2O = 1 : 1), *lit.* $[\alpha]_D^{22}$ +58 (*c* 1.0, acetone–H_2O = 1 : 1)[4]} as an off-white powder. The ^{13}C chemical shifts (acetone-d_6–D_2O = 1 : 1) were in good accordance with the literature data.[4] For further characterization, product **1** was converted to peracetate **19** (63% yield from **18**), where the structure was reconfirmed by MALDI-TOF mass spectrometry.

In summary, the first total synthesis of catechin hetero-trimer **1** has been achieved, demonstrating the power of the unified strategy in providing various catechin oligomers. Further work along these lines is in progress.

Acknowledgements

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10 As arbitrary abbreviations for flavan monomers and its oligomers, we use the following representations: the constituent monomers of **5**–**7** are expressed as in Br-(EZ)-SXy, H-(EG)-OEE, H-(CA)-H, respectively, where Br stands for a C8 bromo group of an electrophilic unit, OEE for the C4-ethoxyethoxy group in an electrophilic unit. Hetero-trimer **1** is expressed as H-(EZ)-(EG)-(CA)-H, where the top H stands for a C8 hydrogen of the top epiafzelechin unit, the tail H for the C4 methylene group in the terminal catechin unit.

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14 The stereochemistry of the new stereogenic centre in compound **5** was determined by NOE experiments. For details, see the ESI.† In contrast, the stereochemistries of compounds **6** and **11** were deduced from the precedent works. See ref. 13*b*, and (*a*) A. P. Kozikowski, W. Tückmantel and C. George, *J. Org. Chem.*, 2000, **65**, 5371; (*b*) A. Saito, Y. Mizushina, H. Ikawa, H. Yoshida, Y. Doi, A. Tanaka and N. Nakajima, *Bioorg. Med. Chem.*, 2005, **13**, 2759.

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