VIP Oligocatechins

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Integrated Synthetic Strategy for Higher Catechin Oligomers**

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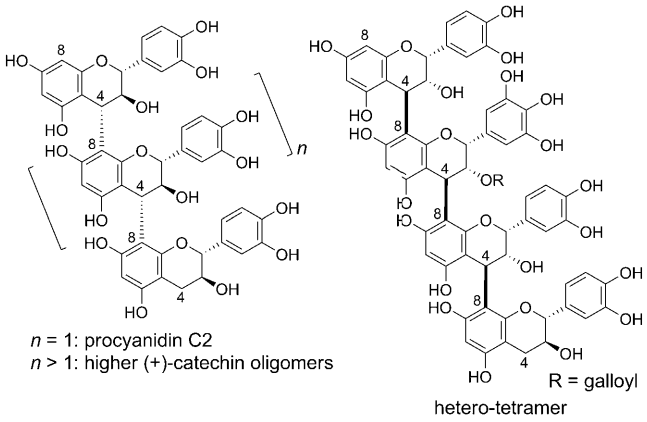
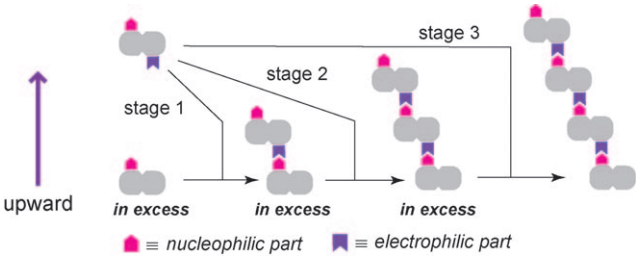
Considerable attention has recently been focused on procyanidins (oligomeric catechins) for their potential bioactivities.[1] The pioneering work of Tückmantel, Kozikowski, et al.



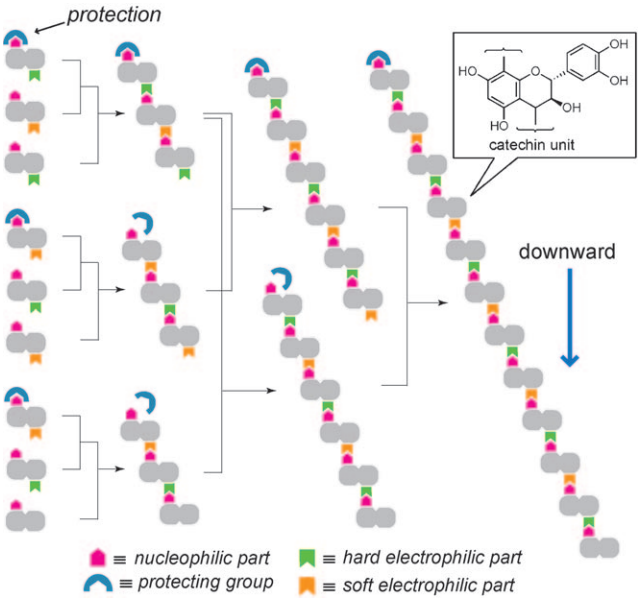
on the cocoa-derived epicatechin linear oligomers showed that there was a tendency for the higher degree of oligomerization to lead to enhanced bioactivities.[2]

In principle, such oligomers are synthetically accessible through repeated Friedel–Crafts reactions of catechin units by exploiting the inherent C8 nucleophilicity and the C4 electrophilicity.[3] An essential issue, however, is the ubiquity of these dual polar reactivities in the starting materials as well as the products. Scheme 1 illustrates the issue in the conventional formation of the key interflavan bonds executed in an upward direction. A key disadvantage is that one needs to use larger substrates in excess to avoid the multiple coupling.

Recently, we developed a promising approach that relies on two key innovations (Scheme 2):[4] 1) The capping of the top of the chain with Br suppresses the C8 nucleophilicity, thus enabling the downward extension of catechin units. Significantly, it provided a clear division of roles between the nucleophilic and electrophilic reaction partners, thus enabling their equimolar coupling, even for combining oligomers.



Scheme 1. Conventional upward-extension approach.



Scheme 2. Downward-extension approach by orthogonal and block coupling.

2) Orthogonal activation,[5] that is, application of alternate "hard→soft→hard…" activation modes, which enabled rapid block oligomerization.

Herein we demonstrate competence of the strategy by selective synthesis of higher oligomers up to the tetracosamer.

The key for the orthogonal activation sequence was the choice in the C4 leaving groups (Scheme 3). We selected the ethoxyethoxy (OEE) group for hard activation[6] and the 2,6-xylylthio (SXy) group for soft activation. The latter choice was made to suppress the side reaction (see **A** in Scheme 3) when phenylthio was employed for the activation.[7] Of additional note here is the choice of the acetyl protection for the C3 hydroxy groups, thus securing the α stereochemistry at the coupling stages, and use of [D_7]benzyl (Bn*) groups, for simplifying ^{1}H NMR analysis.

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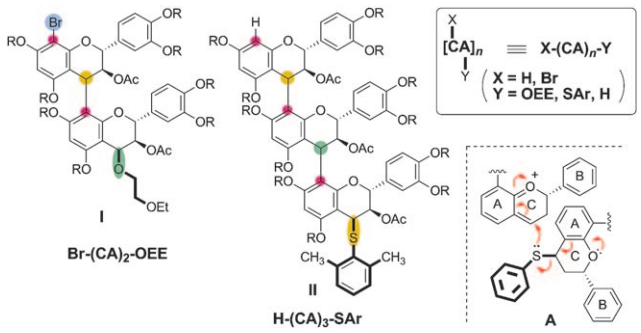
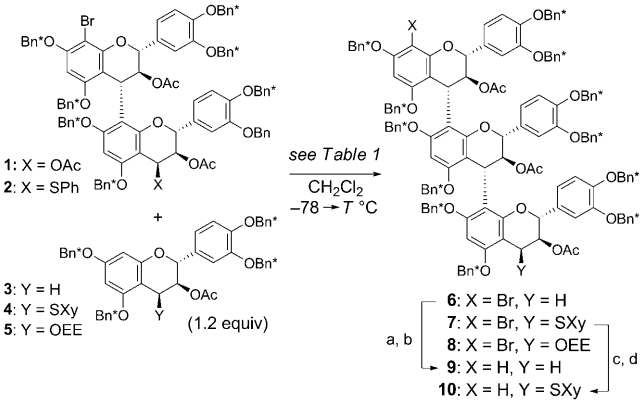


Scheme 3. The C4 leaving groups and designation. OEE = 2-ethoxyethoxy.

As arbitrary abbreviations for the linear catechin oligomers, we use the following representations in the text: structure **I** is expressed as in Br-$(CA)_2$-OEE, where Br stands for a C8 bromo group of the top catechin unit, $(CA)_2$ for two catechin units, and OEE for the C4 OEE group in the terminal catechin unit.

For the block synthesis of higher oligomers, trimers were chosen as the basic building blocks, and preparation of suitably functionalized trimers was carried out (Scheme 4). Table 1 summarizes the coupling of bromo-capped dimers **1**[8] and **2**[9] with monomers **3**, **4**, **5**[10] ({2 + 1} coupling) with and without a terminal activatable group. Importantly, bromo-capped substrates **1** and **2** nicely allowed the equimolar



Scheme 4. Trimer building blocks. a) $LiAlH_4$, THF, RT, 5.4 h; b) Ac_2O, DMAP, pyr, RT, 1.5 h (87%, 2 steps); c) $LiAlH_4$, THF, RT, 2 h. d) Ac_2O, DMAP, pyr, RT, 1 h (81%, 2 steps). Bn = benzyl, Bn* = [D_7]benzyl, DMAP = *N*,*N*-dimethylaminopyridine, pyr = pyridine, SXy = 2,6-xylylthio, THF = tetrahydrofuran.

Table 1: The {2 + 1} couplings.

Run	X	Y	Activator	*T* [°C]	*t* [h]	Product	Yield [%]	α/β[c]
1	OAc	H	$BF_3{\cdot}OEt_2$[a]	−30	0.7	**6**	93	95:5
2	OAc	SXy	$BF_3{\cdot}OEt_2$[a]	−35	1.0	**7**	96	97:3
3	SPh	H	NIS[b]	−5	1.4	**6**	84	95:5
4	SPh	OEE	NIS[b]	−15	1.4	**8**	62	95:5

[a] 1.1 equiv. [b] 1.2 equiv of NIS in the presence of 4 Å M.S. (1 g mmol^{-1}). [c] Separable by preparative TLC methods; see the Supporting Information. M.S. = molecular sieves.

coupling with H-$(CA)_1$-H (**3**, 1.2 equiv relative to **1** or **2**) upon $BF_3{\cdot}OEt_2$ or NIS activation, thus giving Br-$(CA)_3$-H (**6**) in high yield with good stereoselectivity (α/β = 95:5; runs 1, 3). Furthermore, the orthogonal combination was possible, that is, Br-$(CA)_2$-OAc (**1**) was selectively activated by $BF_3{\cdot}OEt_2$, allowing coupling with H-$(CA)_1$-SXy (**4**) to give Br-$(CA)_3$-SXy (**7**) (α/β = 97:3; run 2), whereas NIS activated Br-$(CA)_2$-SPh (**2**) to couple with H-$(CA)_1$-OEE (**5**) to give Br-$(CA)_3$-OEE (**8**) (α/β = 95:5; run 4). It should be noted that all the trimeric building blocks, **6**–**8**, were diastereomerically pure after removing the minor isomers by preparative thin layer chromatography (see the Supporting Information).

Nucleophilic trimers H-$(CA)_3$-H (**9**) and H-$(CA)_3$-SXy (**10**) were prepared by debromination ($LiAlH_4$) of **6** and **7**, respectively. Partial reductive deacetylation occurred, but these by-products were converted into **9** and **10** with acetic anhydride.

Scheme 5 illustrates syntheses of the hexamers by the {3+3} coupling. For activation of Br-$(CA)_3$-SXy (**7**), AgOTf proved to be the reagent of choice, enabling smooth coupling with a nucleophilic trimer H-$(CA)_3$-H (**9**) to give Br-$(CA)_6$-H (**11**) as a diastereomerically pure foam in 74% yield[11] [run 1, Table 2; MALDI-TOF MS (2,5-dihydroxybenzoic acid

Table 2: The {3 + 3} coupling of the trimers.

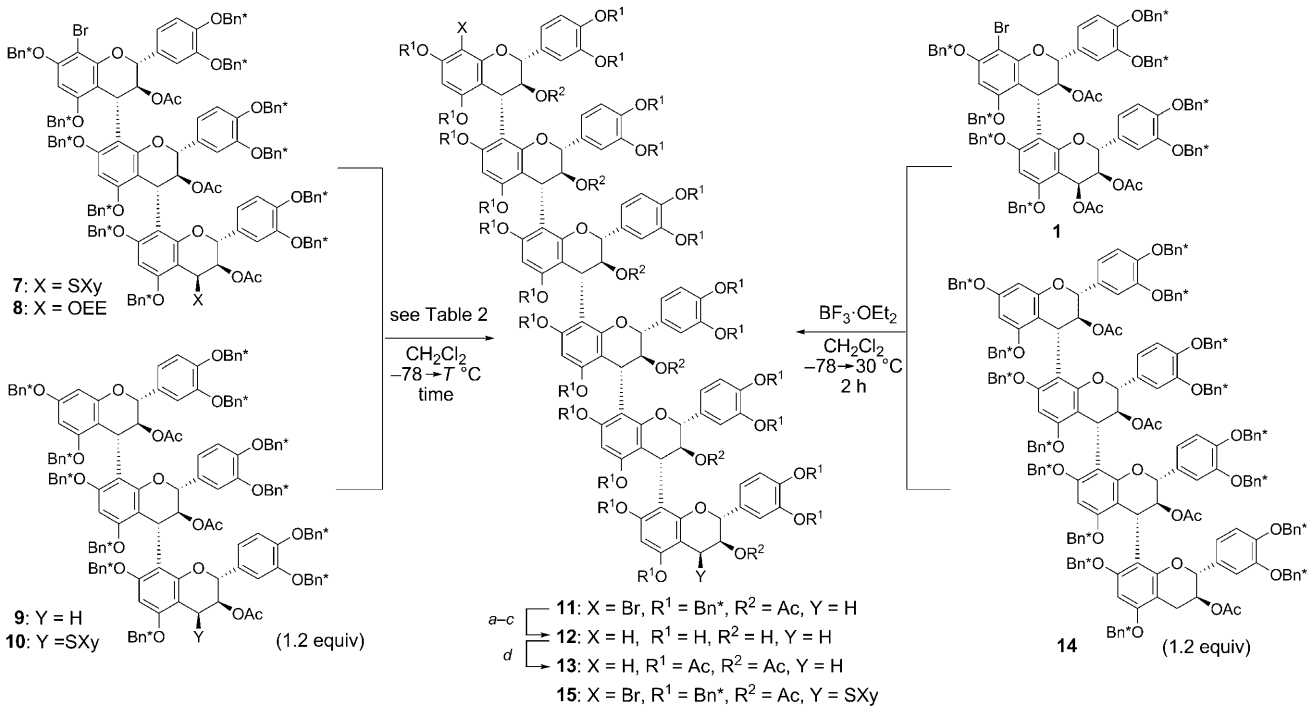
Run	X	Y	Activator	*T* [°C]	*t* [h]	Product	Yield [%]
1	SXy	H	AgOTf[a]	−15	14	**11**	74
2	OEE	H	$BF_3{\cdot}OEt_2$[b]	−35	0.7	**11**	85
3	OEE	SXy	$BF_3{\cdot}OEt_2$[c]	−30	1	**15**	89

[a] 3.2 equiv in the presence of 4 Å M.S. (1 g mmol^{-1}). [b] 4.0 equiv [c] 7.4 equiv.

(DHB) matrix) *m/z* 4416.6 ($[M+Na]^+$ calcd for $^{12}C_{267}{}^{13}C_3{}^{1}H_{63}{}^{2}D_{166}{}^{81}Br{}^{16}O_{42}{}^{23}Na$: 4416.5)]. Likewise, Br-$(CA)_3$-OEE (**8**) was activated by $BF_3{\cdot}OEt_2$ in the presence of **9** to give **11** in 85% yield (run 2).[11]

To confirm the formation of hexamer **11**, an independent synthesis was carried out with the {2 + 4} combination using structurally defined building blocks **1** and **14**,[12] thereby sharing full α stereochemistry of the interflavan linkages. Thus, coupling of Br-$(CA)_2$-OAc (**1**) with H-$(CA)_4$-H (**14**) was promoted by $BF_3{\cdot}OEt_2$ to give a 92% yield of hexamer **11**, which was indistinguishable from the samples of **11** listed in runs 1 and 2 of Table 2.[13]

Having obtained various oligomers, ranging from dimers to hexamers, we examined removal of the protecting groups, including the bromine atom. The major obstacle was that interflavan bonds were prone to cleavage under reductive conditions once the phenols were liberated. To get around this difficulty, we opted to remove the three types of protecting groups in three separate steps. As the most challenging test case, we describe here the deprotection of Br-$(CA)_6$-H (**11**; Scheme 5). Debromination was achieved upon treatment of bromide **11** with $LiAlH_4$ (THF, RT, 12 h). Some acetyl groups remained intact, but were completely removed by exposure to base (KOH aq./1,4-dioxane/ethanol (1:2:2), 90 °C, 5 h). Finally, benzyl groups were removed by hydrogenolysis over



Scheme 5. Hexamer building blocks. a) $LiAlH_4$, THF, RT, 12 h; b) 50 wt% KOH aq., 1,4-dioxane, ethanol, 90 °C, 5 h (76%, 2 steps); c) H_2, cat. ASCA-2, THF, MeOH, H_2O, RT, 3 h (46%); d) Ac_2O, pyridine, 25 °C.

ASCA-2 (5% $Pd(OH)_2$ on carbon;[14] THF/MeOH/H_2O (2:2:1), RT, 3 h) to give free catechin hexamer **12**. For coping with high susceptibility of deprotected compounds to air oxidation, an effective isolation procedure was devised: 1) careful filtration under an inert atmosphere, 2) precipitation (CH_3CN) and membrane filtration under an inert atmosphere to give almost pure compound **12** as a white powder, and 3) further purification by the preparative HPLC methods (Inertsil WP300 Diol) to afford pure hexamer **12** in 46% yield as a white powder [MALDI-TOF MS (DHB matrix) *m/z* 1753.15 ($[M+Na]^+$ calcd for $^{12}C_{90}{}^{1}H_{74}{}^{16}O_{36}{}^{23}Na$: 1753.40)]. For characterization purposes, a small sample of **12** was subjected to acetylation to give the corresponding peracetate [MALDI-TOF MS (DHB matrix) *m/z* 3014.9 ($[M+Na]^+$ calcd for $^{12}C_{149}{}^{13}C_1{}^{1}H_{134}{}^{16}O_{66}{}^{23}Na$: 3014.7)].

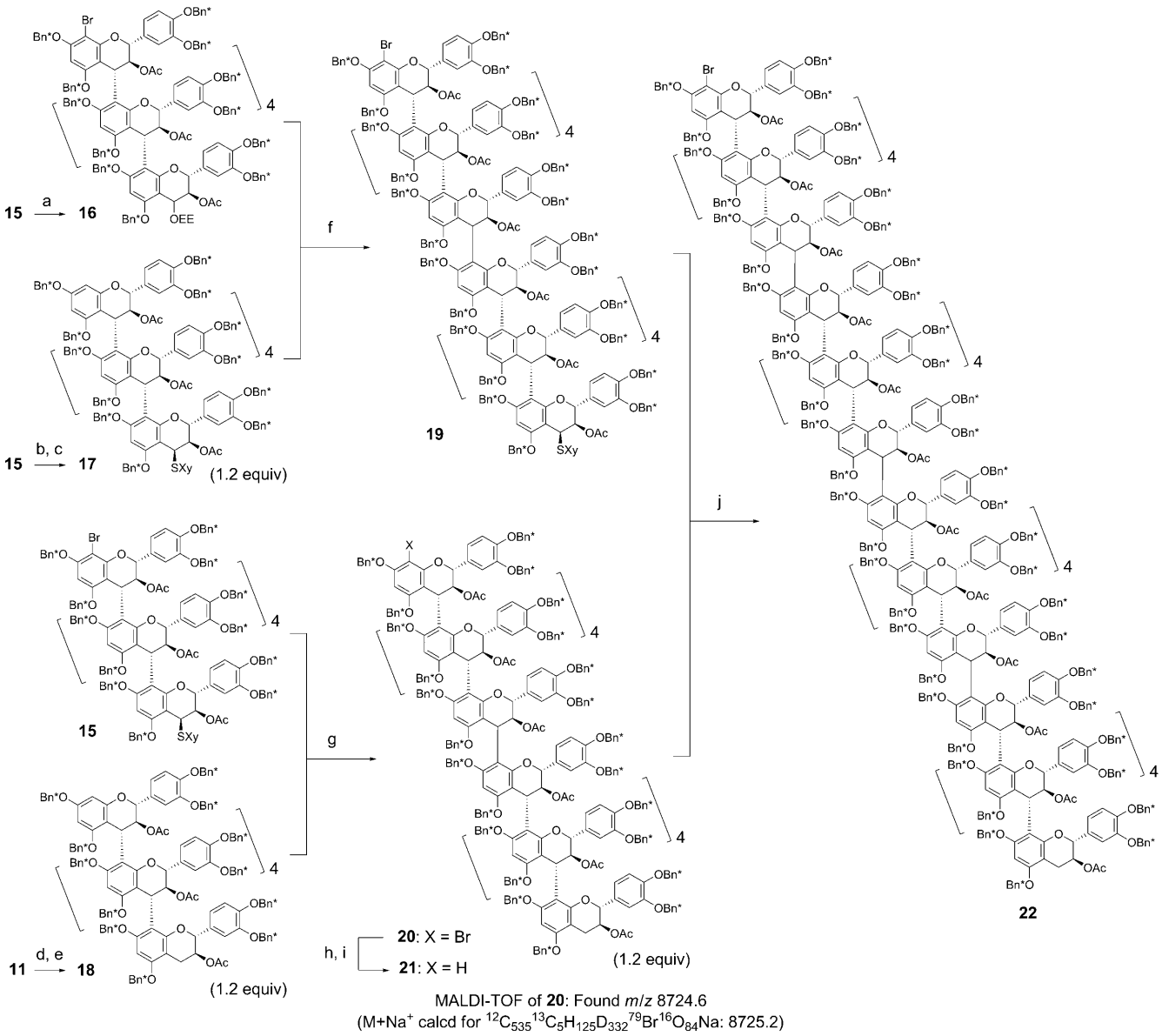
To test the scope of the block synthesis, we examined the synthesis of even larger oligomers. An initial question was the accessibility of the activatable hexameric building blocks. Pleasingly, equimolar orthogonal coupling of trimers was again possible; the treatment of a mixture of Br-$(CA)_3$-OEE (**8**) and H-$(CA)_3$-SXy (**10**) with $BF_3{\cdot}OEt_2$ allowed selective formation of Br-$(CA)_6$-SXy (**15**) in 89% yield (run 3, Table 2).

Scheme 6 outlines the higher-oligomer synthesis directed at tetracosamer **22**. Among four hexamer building blocks, two were prepared from Br-$(CA)_6$-SXy (**15**), a versatile precursor. Br-$(CA)_6$-OEE (**16**) was obtained quantitatively by switching the leaving group (SXy→OEE) through treatment of **15** with I_2 and Ag_2O in the presence of 2-ethoxyethanol. Two debrominated hexamers, H-$(CA)_6$-SXy (**17**) and H-$(CA)_6$-H (**18**), were prepared by debromination of **15** and **11**, respectively, with $LiAlH_4$. Some missing acetyl groups were recovered by the acetylation as before.

En route to the tetracosamer, these hexamer building blocks were combined to give two dodecamers, **19** and **20** (Scheme 6). Upon treatment with $BF_3{\cdot}OEt_2$, Br-$(CA)_6$-OEE (**16**, MW = 4483) was selectively activated and underwent attack by H-$(CA)_6$-SXy (**17**, MW = 4452) to afford an activatable dodecamer, Br-$(CA)_{12}$-SXy (**19**, MW = 8845), in 88% yield. As for the other {6 + 6} coupling, the combination of I_2 and Ag_2O proved to be effective to allow clean union of Br-$(CA)_6$-SXy (**15**, MW = 4531) and H-$(CA)_6$-H (**18**, MW = 4316), thus giving terminal dodecamer Br-$(CA)_{12}$-H (**20**, MW = 8708) in 82% yield. Debromination and acetylation of **20** afforded the nucleophilic dodecamer H-$(CA)_{12}$-H (**21**, MW = 8629).

Now the stage was set for addressing the accessibility to tetracosamer by the {12 + 12} coupling. Pleasingly, upon exposure of a mixture of Br-$(CA)_{12}$-SXy (**19**, MW = 8845) and H-$(CA)_{12}$-H (**21**, MW = 8629) to the combined mixture of I_2 and Ag_2O, the desired linear oligomer, Br-$(CA)_{24}$-H (**22**, MW = 17336) was cleanly obtained in 80% yield.[15] Particularly significant is that combination of two large substrates is possible by employing essentially equimolar quantities (1:1.2 molar ratio) of the coupling partners to give **22** with a single molecular-weight dispersion, clearly indicating the efficacy of the integrated strategy for assembling linear catechin oligomers.

^1H NMR analysis rapidly became difficult with the increased chain length of oligomers containing multiple oxygen atoms (Figure 1; see also the Supporting Information). Additional complications come from the atropisomerism resulting from the hindered rotation around the interflavan bonds, which is slow on the ^1H NMR time scale (500 MHz). However, interesting observations were made:



Scheme 6. Block synthesis of tetracosamer **22** (unless otherwise noted, the reactions were performed at ambient temperatures). a) 2-ethoxyethanol, I_2, Ag_2O, CH_2Cl_2, 4 Å M.S., −78 °C, 2 h. (99%, $\alpha/\beta = 81{:}19$); b) $LiAlH_4$, 7 h; c) Ac_2O, DMAP, pyr, 18.5 h (69%, 2 steps); d) $LiAlH_4$, THF, 3 h. e) Ac_2O, DMAP, pyr, 1 h (90%, 2 steps); f) $BF_3{\cdot}OEt_2$, CH_2Cl_2, −78 → −25 °C, 1 h (88%); g) I_2, Ag_2O, 4 Å M.S., CH_2Cl_2, −78 °C, 1 h (82%); h) $LiAlH_4$, THF, 10.5 h; i) Ac_2O, DMAP, pyr, 10.5 h (90%, 2 steps); j) I_2, Ag_2O, 4 Å M.S., CH_2Cl_2, −78 °C, 2 h (80%).

1) oligomers larger than a dodecamer showed much simpler spectra and 2) the proton signals of the internal units gradually converge with slight broadening as the number of catechin units increases. Notably, chemical shifts for the C2 benzylic protons of the internal catechin units appeared upfield (ca. $\delta = 2.9$ ppm) in comparison with a typical benzylic proton attached to an oxygen atom. The tendency suggests dominance of higher-order structures that we assume to be helical. Molecular modeling suggested anisotropic effects of the proximal benzene rings in the C5 benzyl groups of the upper catechin units when the local conformation between two consecutive catechin units adopt a right-handed helix (**B′**; Scheme 7).

In conclusion, rapid assembly of higher linear catechin oligomers has become possible through block synthesis based on an orthogonal strategy. The bromo-capped electrophilic

Scheme 7. Local conformation around interflavan bonds.

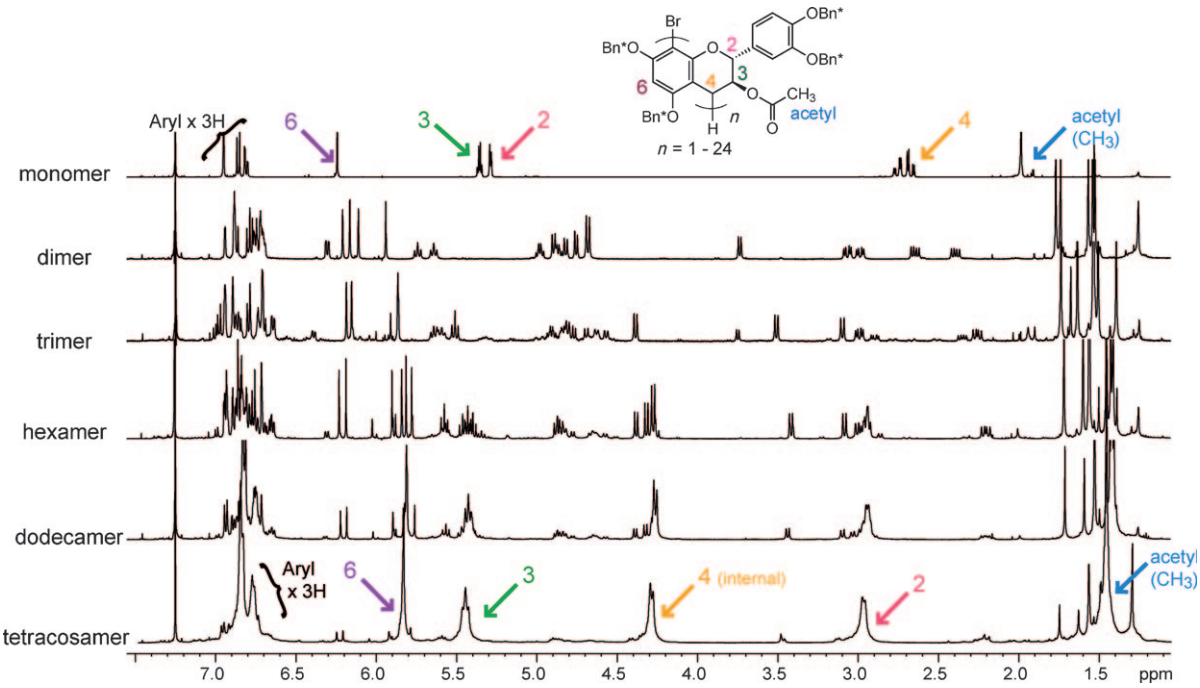


Figure 1. Stacked ^{1}H NMR spectra of protected oligomers (500 MHz, $CDCl_3$, 300 K).

units were subjected to equimolar coupling even with larger nucleophilic oligomeric substrates (up to a dodecamer), thus providing efficient entries into controlled oligomer formation. Efficacy of the strategy was demonstrated by the synthesis of the tetracosamer **22** having a single molecular-weight dispersion. Formation and deprotection of higher oligomers are now in progress in our laboratory.

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[6] Monomeric C4 acetate proved to be less reactive as a nucleophilic building block for higher oligomer synthesis, due presumably to the decreased electron density at its C8-position by inductive electron-withdrawal. For the ethoxyethoxy group in hard activation, see Ref. [3d].

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[8] Br-$(CA)_2$-OAc (**1**) was prepared by combining Br-$(CA)_1$-SPh and H-$(CA)_1$-OAc promoted by NIS (83% yield, $\alpha/\beta = 95{:}5$). For details, see the Supporting Information.

[9] Br-$(CA)_2$-SPh (**2**) was prepared by combining Br-$(CA)_1$-OAc and H-$(CA)_1$-SPh in the presence of $BF_3{\cdot}OEt_2$ (95% yield, $\alpha/\beta =$ 94:6). For details, see the Supporting Information.

[10] For preparation of monomers **3**–**5**; see the Supporting Information.

[11] ^{1}H NMR analysis of crude reaction mixture suggested a mixture of diastereomers. However, after purification, a single diastereomer was isolated; see the Supporting Information.

[12] H-$(CA)_4$-H (**14**) was prepared by combining Br-$(CA)_2$-OEE and H-$(CA)_2$-H with $BF_3{\cdot}OEt_2$ and then debromination ($LiAlH_4$) and O acetylation (63% yield).

[13] These results imply that a new α linkage could be formed at the coupling stage. The viability was clearly shown for assembling the linear structures. In spite of the large molecules with many functionalities, excellent selectivity/reactivity persisted for regiochemistry at C4 and C8.

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[15] Diffusion coefficients (D) of Br-$(CA)_6$-H (**11**), Br-$(CA)_{12}$-H (**20**), and Br-$(CA)_{24}$-H (**22**) were measured by the diffusion-ordered NMR experiments ($CDCl_3$, 300 K). The values (D) were estimated as follows; **11**: $(3.36 \pm 0.19)\ 10^{-10}\ m^2 s^{-1}$, **20**: $(2.43 \pm 0.12)\ 10^{-10}\ m^2 s^{-1}$, **22**: $(1.95 \pm 0.07)\ 10^{-10}\ m^2 s^{-1}$, which were well correlated to the order of molecular sizes.

