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Studies in Polyphenol Chemistry and Bioactivity. 4.[1] Synthesis of Trimeric, Tetrameric, Pentameric, and Higher Oligomeric Epicatechin-Derived Procyanidins Having All-4β,8-Interflavan Connectivity and Their Inhibition of Cancer Cell Growth through Cell Cycle Arrest[1]

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We report an improved synthesis of bis(5,7,3′,4′-tetra-*O*-benzyl)epicatechin 4β,8-dimer (**3**) from 5,7,3′,4′-tetra-*O*-benzylepicatechin (**1**) and 5,7,3′,4′-tetra-*O*-benzyl-4-(2-hydroxyethoxy)epicatechin (**2**) by replacing the previously employed Lewis acid, titanium tetrachloride, with the clay mineral Bentonite K-10. Under the same conditions, the benzyl-protected all-4β,8-trimer, -tetramer, and -pentamer were obtained regioselectively from their lower homologues, albeit in rapidly decreasing yields. Reaction of **2** with an organoaluminum thiolate generated from 2-mercaptobenzothiazole and trimethylaluminum followed by acetylation produced 3-*O*-acetyl-4-[(2-benzothiazolyl)thio]-5,7,3′,4′-tetra-*O*-benzylepicatechin (**12**). Medium-sized protected oligomers with 4β,8-interflavan linkages are obtained in improved yields by using this compound as the electrophile and silver tetrafluoroborate as activator and are isolated by reversed-phase HPLC. Their deprotection by ester saponification followed by hydrogenolysis yielded the free procyanidins, which were characterized as their peracetates. The synthetic procyanidins are identical by normal-phase HPLC with fractions isolated from cocoa. The principle of chain extension by two members was demonstrated using a dimeric electrophile obtained by self-condensation of compound **12**. Both the synthetic and natural pentamer **32** inhibit the growth of several breast cancer cell lines. Using the MDA MB 231 line, it was established that this outcome is based on the induction of cell cycle arrest in the G_0/G_1 phase. Subsequent cell death is more likely necrotic rather than apoptotic. Control experiments demonstrate that the polyphenol itself, rather than hydrogen peroxide potentially formed by its autoxidation, is the causative agent.

Introduction

Condensed tannins (proanthocyanidins) are widespread in the plant kingdom, form part of the human diet, and display multiple biological activities that render them significant to health.[1a,2,3] We have undertaken a program aimed at synthesizing condensed tannins previously isolated from cocoa[4] and reported on the condensation of 5,7,3′,4′-tetra-*O*-benzylepicatechin (**1**) with 5,7,3′,4′-tetra-*O*-benzyl-4-(2-hydroxyethoxy)epicatechin (**2**) (Chart 1, eq 1),[1] which leads to the 4β,8-dimer **3** together with higher oligomers in yields that decrease with their molecular mass. The investigation of these byproducts was deferred in favor of the more urgent task to unequivocally establish the stereochemical nature of the interflavan bond in the dimer **3**, a problem that has eluded chemists relying solely on instrumental analytical

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(1) (a) Part 1: Tückmantel, W.; Kozikowski, A. P.; Romanczyk, L. L., Jr. *J. Am. Chem. Soc.* **1999**, *121*, 12073. See here for a general introduction of plant polyphenols, especially condensed tannins. (b) Part 3: Kozikowski, A. P.; Tückmantel, W.; Hu, Y. *J. Org. Chem.* **2001**, *66*, 1287.

(2) Recent reviews and monographs: (a) Polyphenols, Wine and Health. In *Proceedings of the Phytochemical Society of Europe*, Bordeaux, France, 14−16 April 1999; Chèze, C., Vercauteren, J., Verpoorte, R., Eds; Kluwer Academic Publishers: Dordrecht, 2001. (b) *Plant Polyphenols 2. Chemistry, Biology, Pharmacology, Ecology;* Gross, G. G., Hemingway, R. W., Yoshida, T., Eds; Kluwer Academic/Plenum Publishers: New York, 2000. (c) Merken, H. M.; Beecher, G. R. *J. Agric. Food Chem.* **2000**, *48*, 577. (d) Harborne, J. B.; Williams, C. A. *Phytochemistry* **2000**, *55*, 481. (e) Ferreira, D.; Li, X.-C. *Nat. Prod. Rep.* **2000**, 193.

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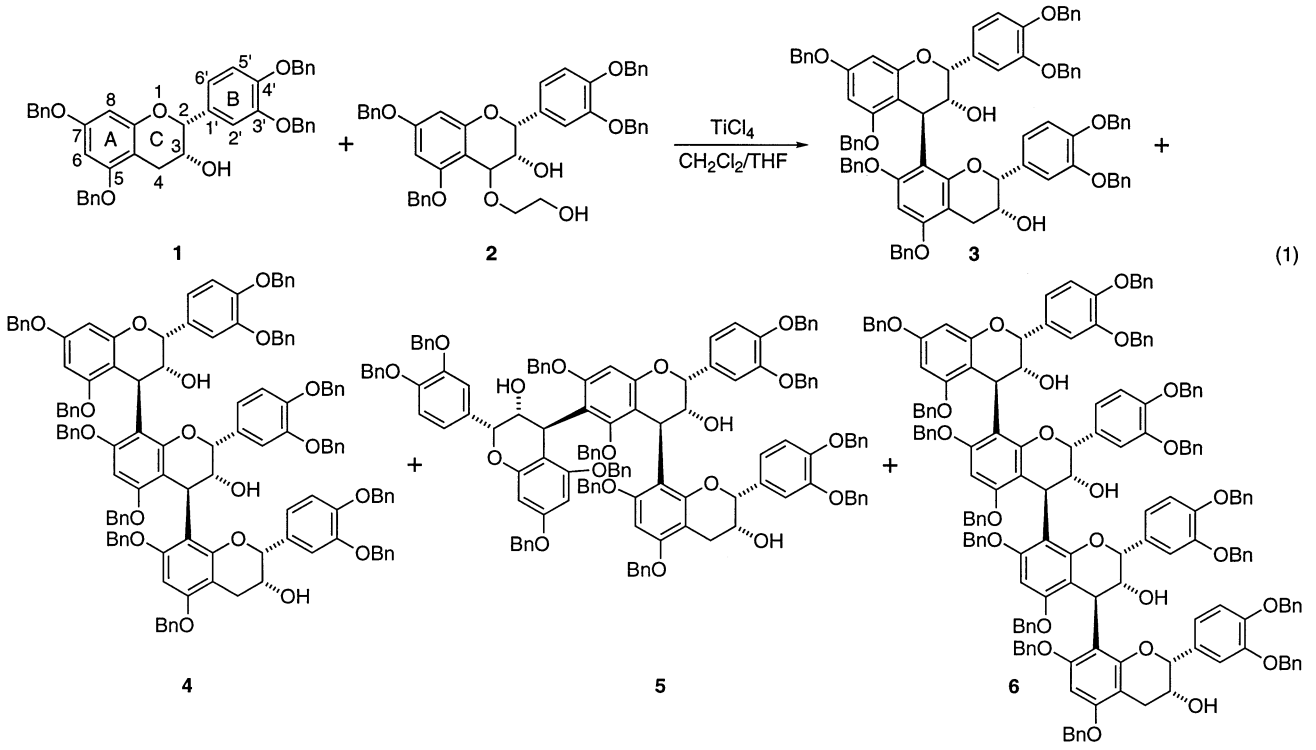
CHART 1



methods and that we recently solved through synthesis of a specifically protected derivative and its subsequent degradation.[5] After this issue had been settled, we turned our attention to the higher epicatechin oligomers. The isolation and characterization of these compounds, together with improved methods for their synthesis and a brief investigation of their anticancer activity, are the subject of the present paper.

Results

$TiCl_4$-Mediated Chain Extension. The predominant larger oligomers isolated from the reported[1a] condensation of compounds **1** and **2** in the presence of $TiCl_4$ have been identified as the 4β,8:4β,8-trimer **4**, the 4β,6:4β,8-trimer **5**, and the all-4β,8-tetramer **6** (Chart 1, eq 1). Trace amounts of even larger oligomers and possibly additional regioisomers are present in the crude product mixture but were not further investigated. The same products were obtained starting from the epicatechin dimer **3** rather than the monomer **1**. The separation of the product mixture was initially performed by normal-phase HPLC on silica gel with ethyl acetate/hexane mixtures as eluent, but we later found that much sharper

(3) See the following selected recent references. Lowering of plasma cholesterol levels: (a) Bursill, C.; Roach, P. D.; Bottema, C. D. K.; Pal, S. *J. Agric. Food Chem.* **2001**, *49*, 5639. Inhibition of squalene epoxidase: (b) Abe, I.; Seki, T.; Umehara, K.; Miyase, T.; Noguchi, H.; Sakakibara, J.; Ono, T. *Biochem. Biophys. Res. Commun.* **2000**, *268*, 767. Inhibition of endothelin-1 synthesis: (c) Corder, R.; Douthwaite, J. A.; Lees, D. M.; Khan, N. Q.; Viseu dos Santos, A. C.; Wood, E. G.; Carrier, M. J. *Nature* **2001**, *414*, 863. Antiallergic activity: (d) Fujimura, Y.; Tachibana, H.; Yamada, K. *J. Agric. Food Chem.* **2001**, *49*, 2527. Neuroprotection: (e) Levites, Y.; Weinreb, O.; Maor, G.; Youdim, M. B.; Mandel, S. *J. Neurochem.* **2001**, *78*, 1073. (f) Shimada, Y.; Goto, H.; Shibahara, N.; Sakakibara, I.; Sasaki, H.; Terasawa, K. *Am. J. Chin. Med.* **2001**, *29*, 173. Anti-inflammatory activity: (g) Surh, Y.; Chun, K.; Han, S. S.; Keum, Y.; Park, K.; Lee, S. S. *Mutation Res.* **2001**, *480*−*481*, 243. Inhibition of metabolic activation of procarcinogens: (h) Muto, S.; Fujita, K.; Yamazaki, Y.; Kamataki, T. *Mutation Res.* **2001**, *479*, 197. Induction of apoptosis: (i) Saeki, K.; Hayakawa, S.; Isemura, M.; Miyase, T. *Phytochemistry* **2000**, *53*, 391. (j) Smith, D. M.; Dou, Q. P. *Int. J. Mol. Med.* **2001**, *7*, 645. (k) Hayakawa, S.; Saeki, K.; Sazuka, M.; Suzuki, Y.; Shoji, Y.; Ohta, T.; Kaji, K.; Yuo, A.; Isemura, M. *Biochem. Biophys. Res. Commun.* **2001**, *285*, 1102. Reversion of multidrug resistance: (l) Zhu, A.; Wang, X.; Guo, Z. *Nucl. Med. Biol.* **2001**, *28*, 735. Modulation of gene expression: (m) Okabe, S.; Fujimoto, N.; Sueoka, N.; Suganuma, M.; Fujiki, H. *Biol. Pharm. Bull.* **2001**, *24*, 883. Inhibition of phenol sulfotransferase: (n) Isozaki, T.; Tamura, H. *Biol. Pharm. Bull.* **2001**, *24*, 1076. Modification of arachidonic acid metabolism: (o) Hong, J.; Smith, T. J.; Ho, C.-T.; August, D. A.; Yang, C. S. *Biochem. Pharmacol.* **2001**, *62*, 1175. Inhibition of the Ras-MAP kinase signaling pathway: (p) Chung, J. Y.; Park, J. O.; Phyu, H.; Dong, Z.; Yang, C. S. *FASEB J.* **2001**, *15*, 2022. Review of mechanisms of activity: (q) Nijveldt, R. J.; van Nood, E.; van Hoorn, D. E. C.; Boelens, P. G.; van Norren, K.; Leeuwen, P. A. M. *Am. J. Clin. Nutr.* **2001**, *74*, 418.

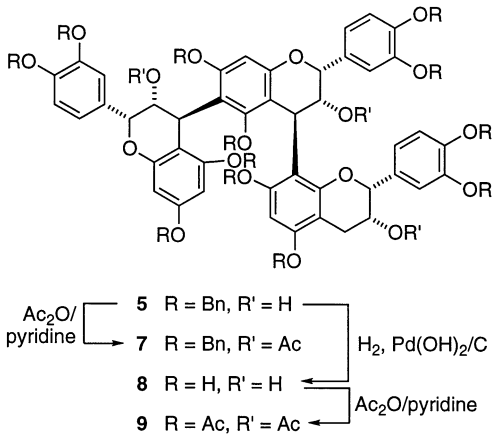
(4) (a) Forsyth, W. G. C.; Roberts, J. B. *Biochem. J.* **1960**, *74*, 374. (b) Quesnel, V. C. *Phytochemistry* **1968**, *7*, 1583. (c) Jalal, M. A. F.; Collin, H. A. *Phytochemistry* **1977**, *16*, 1377. (d) Porter, L. J.; Ma, Z.; Chan, B. G. *Phytochemistry* **1991**, *30*, 1657. (e) Rigaud, J.; Escribano-Bailon, M. T.; Prieur, C.; Souquet, J.-M.; Cheynier, V. *J. Chromatogr.* **1993**, *654*, 255. (f) Romanczyk, L. J., Jr.; Hammerstone, J. F., Jr.; Buck, M. M. US Patent 5,554,645, Sep 10, 1996. (g) Romanczyk, L. J., Jr.; Hammerstone, J. F., Jr.; Buck, M. M.; Post, L. S.; Cipolla, G. G.; McClelland, C. A.; Mundt, J. A.; Schmitz, H. H. PCT Int. Appl. WO 9736,597, Oct 9, 1997; *Chem. Abstr.* **1997**, *127*, 322787. (h) Romanczyk, L. J., Jr.; Hammerstone, J. F., Jr.; Buck, M. M.; Post, L. S.; Cipolla, G. G.; McClelland, C. A.; Mundt, J. A.; Schmitz, H. H. PCT Int. Appl. WO 9736,497, Oct. 9, 1997; *Chem. Abstr.* **1997**, *127*, 330680. (i) Romanczyk, L. J., Jr.; Hammerstone, J. F., Jr.; Buck, M. M. US Patent 5,712,305, Jan 27, 1998. (j) Lee, M. C.; Chang, Y. Y.; Yim, M. H. *Han'guk Nonghwa Hakhoechi* **1998**, *41*, 110−7; *Chem. Abstr.* **1998**, *129*, 207072. (k) Sanbongi, C.; Osakabe, N.; Natsume, M.; Takizawa, T.; Gomi, S.; Osawa, T. *J. Agric. Food Chem.* **1998**, *46*, 454. (l) Hammerstone, J. F.; Lazarus, S. A.; Mitchell, A. E.; Rucker, R.; Schmitz, H. H. *J. Agric. Food Chem.* **1999**, *47*, 490. (m) De Bruyne, T.; Pieters, L.; Witvrouw, M.; De Clercq, E.; Vanden Berghe, D.; Vlietinck, A. J. *J. Nat. Prod.* **1999**, *62*, 954. (n) Wollgast, J.; Pallaroni, L.; Agazzi, M.-E.; Anklam, E. *J. Chromatogr. A* **2001**, *926*, 211.

(5) Kozikowski, A. P.; Tückmantel, W.; George, C. *J. Org. Chem.* **2000**, *65*, 5371−5381.

peaks are obtained on C_8 or C_{18} columns with an acetonitrile−water gradient. On silica gel, retention increases with molecular size, presumably because of the increasing number of alcohol and ether functions. Paradoxically, the same elution sequence is observed on reverse phases. It appears that these adsorbents discriminate predominantly by the number of the nonpolar benzyl groups, which also increases with the degree of oligomerization. The couple of regioisomers **4** and **5**, however, behaves as expected: whereas compound **4** elutes before **5** on silica gel, the opposite sequence is observed on reverse phases.

Compounds **4** and **6** are identical with products obtained by alternative protocols and will be discussed below. Compound **5** was additionally transformed into its triacetate **7**, a type of derivative that was also routinely employed in the preparation of the all-4β,8 oligomers. Since compound **5** is obtained by way of the dimer **3**, it ought to contain the 4β,8-interflavan linkage present in that material. The additional interflavan linkage can then originate from any of the three unsubstituted positions of its A rings. With structure **4** already being assigned to another product, only **5** and the "branched" trimer (containing simultaneously a 4,6- and a 4,8-interflavan bond to the "bottom" A ring) remain. As in the case of other benzylated intermediates, compound **5** was deprotected hydrogenolytically, and the resulting free polyphenol **8** was acetylated with an excess of acetic anhydride and pyridine to yield the acetate **9** (Scheme 1). The ^{1}H NMR spectrum of compound **9**

SCHEME 1



demonstrated the presence of a major component exhibiting sharp signals and of one or more minor components exhibiting broad signals. The major component proved identical to the peracetate prepared from natural epicatechin 4β,6:4β,8-trimer.[6] While it is commonly observed that NMR spectra of proanthocyanidins and their derivatives are complicated by the presence of several rotamers that interconvert slowly on the NMR time scale, ref 6 explicitly states that the compound consists of a single rotamer. Regrettably, therefore, we must conclude that our preparation contains unknown contaminants; a result the more surprising since all three compounds **5**, **7**, and **9** appear homogeneous by HPLC. It is interesting to note that the position 6 of the top ring of the dimer **3** displays significant nucleophilic reactivity in the present chain extension reaction, whereas we have never found the 4,6-dimer in reactions starting from the monomer **1**.

The formation of regioisomers and possibly other types of byproducts constitutes a serious drawback of the $TiCl_4$-mediated chain extension reaction, not only in terms of yield. Even though the all-4β,8-trimer and -tetramer were isolated in pure form, the same could not automatically be expected for larger oligomers, for which the number of possible isomers, and thus contaminants, grows rapidly. One potential way of dealing with this problem would be to carefully purify the chain-extended intermediate after each step to ensure that all products are at least derived from a single isomer of the starting material. Upon reaction of the electrophile **2** with 2 equiv of the trimer **4** (2 equiv of $TiCl_4$, CH_2Cl_2/THF 9:11, 0 °C, 15 min, then rt, 140 min), we observed, however, not only the formation of the tetramer, pentamer, and small amounts of higher oligomers, but also a degradation of the trimer to the monomer and dimer, which then returned into the chain-extension reaction, giving rise to regioisomeric oligomers such as small amounts of the 4β,6:4β,8-trimer **5**. While the reaction conditions were not optimized, we felt that the occurrence of chain degradation was of such fundamental importance that a search for a different approach was warranted. Chain degradation of nonprotected polymeric proanthocyanidins in the presence of protic acids has previously been observed by others, and the resulting electrophilic species have to some extent been utilized in the construction of oligomers.[7]

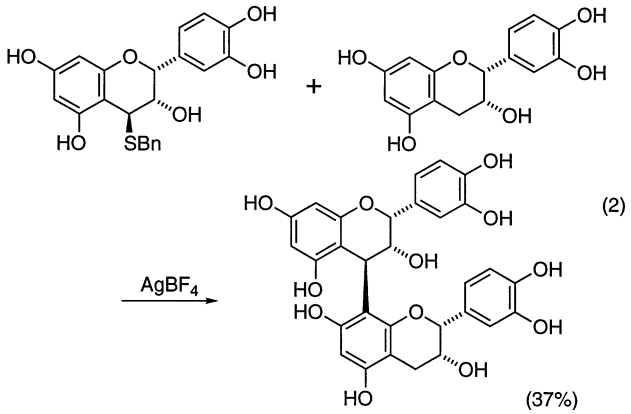
Chain Extension Mediated by Bentonite K-10. A serendipitous discovery led the way. Stirring of the starting materials **1** (4 equiv) and **2** in CH_2Cl_2 with the commercial acidic clay mineral, Bentonite K-10, resulted in the almost exclusive formation of the 4β,8-dimer **3** in a 90% isolated yield together with small amounts of the 4β,8:4β,8-trimer **4**; no 4,6-linked material was observed. The surprisingly high reactivity differential under these conditions between monomer **1** and dimer **3** that allows most of the dimer to survive without entering into further chain extension means, of course, that application of the same protocol to the extension of the dimer to the trimer could be expected to proceed less efficiently. Indeed, reaction of the dimer **3** (3 equiv) with the electrophile **2** yielded only 40% of the 4β,8:4β,8-trimer **4** together with 13% of the all-4β,8-tetramer **6**. The poorer ratio of singly to doubly chain-extended product certainly in part reflects the lower excess of nucleophilic component used here (a consequence of its limited availability) in comparison with the same reaction involving reactants **1** and **2**, but probably also a lower reactivity differential between trimer and dimer than between monomer and dimer. Still, the reactivity of the trimer and tetramer is so much reduced that their chain extension by this protocol, while yielding small amounts of the desired tetramer **6** and pentamer, is impractical. From an operational point of view, it is also worthwhile to note that the cleanness of the reaction permitted for the first time to achieve at least a partial separation of the

(6) Hör, M.; Heinrich, M.; Rimpler, H. *Phytochemistry* **1996**, *42*, 109.

(7) (a) Fletcher, A.; Porter, L. J.; Haslam, E.; Gupta, R. K. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1628. (b) Botha, J. J.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1235. (c) Foo, L. Y.; Porter, L. J. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1535.

monomer from the dimer and even of the dimer from the trimer by column chromatography, thus significantly reducing the amount of material that needs to be put through HPLC purification. Together with a simplified isolation procedure for the electrophilic building block **2**,[8] it is now possible using this protocol to obtain several grams of the intermediates **3** and **4** with a reasonable amount of effort.

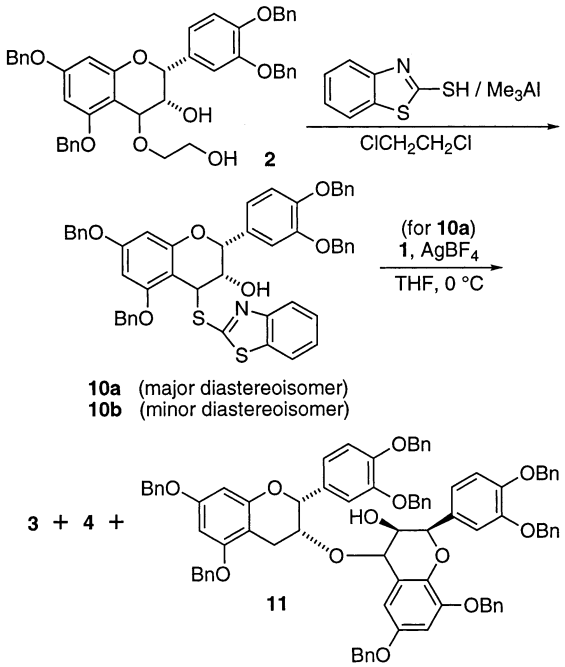
Chain Extension Employing the 4-[(2-Benzothiazolyl)thio] Derivative. Since oligomers larger than the tetramer remained poorly or not accessible, we remained interested in alternative chain extension protocols. In this context, the activation of 4-(benzylthio)catechin and -epicatechin by dimethyl(methylthio)sulfonium tetrafluoroborate or, preferably, by silver tetrafluoroborate came to our attention (eq 2).[9] The reaction has considerable



(2)

similarity with the activation of another type of reactive sulfides, namely α-alkoxyalkyl sulfides (especially thioglycosides), by various classes of electrophiles.[10] The particular virtue of this protocol resides in the exclusive formation of 4,8-interflavan linkages even for nonprotected substrates, which would otherwise yield mixtures of 4,6- and 4,8-linked products. On the other hand, the noxious nature of benzyl mercaptan involved in the preparation of the starting material was considered a serious drawback by us. We therefore decided from the beginning to replace this reagent with a nonvolatile, odorless heterocyclic thiol and found that 2-mercaptobenzothiazole is suitable for this purpose, although during one particular transformation a side reaction caused by the nucleophilicity of the nitrogen atom was encountered (see below). Furthermore, we intended to perform this chemistry on benzyl-protected intermediates as we had done before, both because of the easier handling and better stability of these compounds and because of the poor accessibility of nonprotected, 4-substituted epicatechins. The 4-(2-hydroxyethoxy) derivative **2** was selected as the starting material. Its reaction with an organoaluminum thiolate prepared in situ from 2-mercaptobenzothiazole and trimethylaluminum[11] furnished the 4-thioether as a mixture of two stereoisomers

SCHEME 2



(**10a**,**b**) which were isolated in 67 and 2.5% yield, respectively, by fractional crystallization and column chromatography (Scheme 2). Only the major isomer was used for subsequent reactions. To our delight, addition of $AgBF_4$ to a solution of **1** and **10a** in THF resulted in the formation of the 4β,8-dimer **3** (56%) and the (4β,8)$_2$-trimer **4** (14%) together with recovered monomer **1** after normal-phase HPLC separation. The recovered monomer proved impure; further separation by reversed-phase HPLC yielded 5% of a material to which we assign the structure of a 3-*O*-4-dimer **11** on the basis of its ^{1}H NMR spectrum, which displayed two AB quartets for two pairs of A-ring protons together with a single OH proton and a single 4-methylene group. The formation of similar dimers mediated by $AgBF_4$ has been reported for nonprotected flavan-3,4-diols containing a 7,8-dioxygenated A ring,[12] which is less nucleophilic than the present 6,8-dioxygenated one, and related compounds have also been isolated from natural sources.[13]

To avoid the undesired intervention of the 3-hydroxyl group in the chain elongation process, this group was protected in both the electrophilic and the nucleophilic reaction partner (in this case, the dimer **3**) by acetylation (Scheme 3). The yields were near-quantitative. When a solution of $AgBF_4$ in THF was added to a THF solution of the resulting acetates **12** and **13** (molar ratio 1:3.6) at 0 °C, the expected trimer **16** and tetramer **17** were formed, but only in 34% and 4% yield, respectively. Chain elongation proceeds so slowly in this case that adventitious water successfully competes with the flavanoid

(8) See the Supporting Information

(9) Steynberg, P. J.; Nel, R. J. J.; van Rensburg, H.; Bezuidenhoudt, B. C. B.; Ferreira, D. *Tetrahedron* **1998**, *54*, 8153.

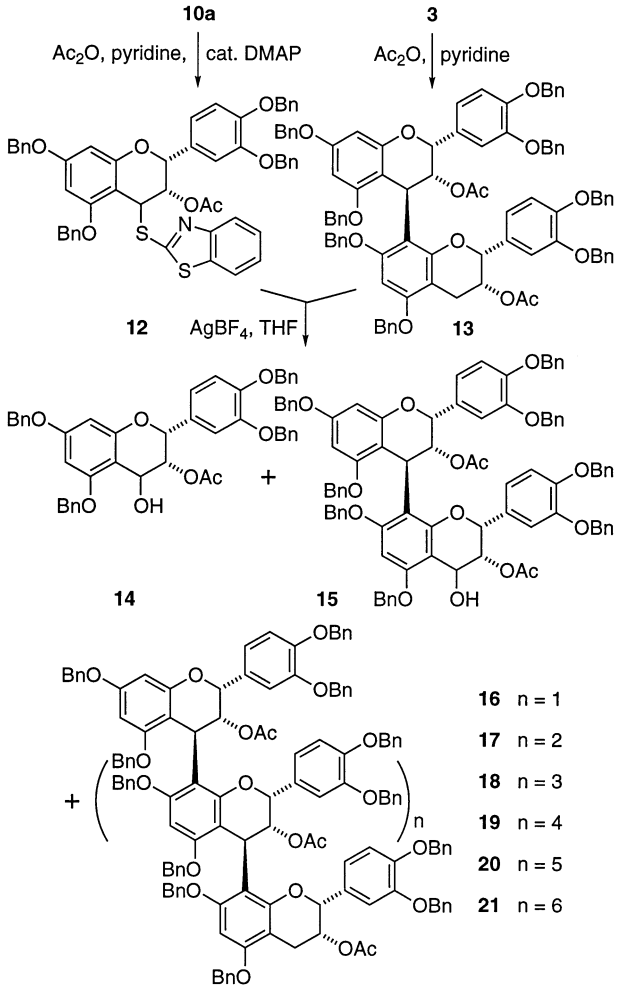
(10) (a) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503. (b) Garegg, P. J. *Adv. Carbohydr. Chem. Biochem.* **1997**, *52*, 179. (c) Marcune, B. F.; Karady, S.; Dolling, U.-H.; Novak, T. J. *J. Org. Chem.* **1999**, *64*, 2446.

(11) The replacement of an allylic methoxyl group by phenylthio with a reagent derived from thiophenol and diisobutylaluminum chloride has been reported: Dzhemilev, U. M.; Ibragimov, A. G.; Morozov, A. B.; Tolstikov, G. A. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1988**, 2645; *Chem. Abstr.* **1989**, *110*, 153414c.

(12) Bennie, L.; Malan, E.; Coetzee, J.; Ferreira, D. *Phytochemistry* **2000**, *53*, 785.

(13) Bennie, L.; Coetzee, J.; Malan, E.; Ferreira, D. *Phytochemistry* **2001**, *57*, 1023 and references quoted therein.

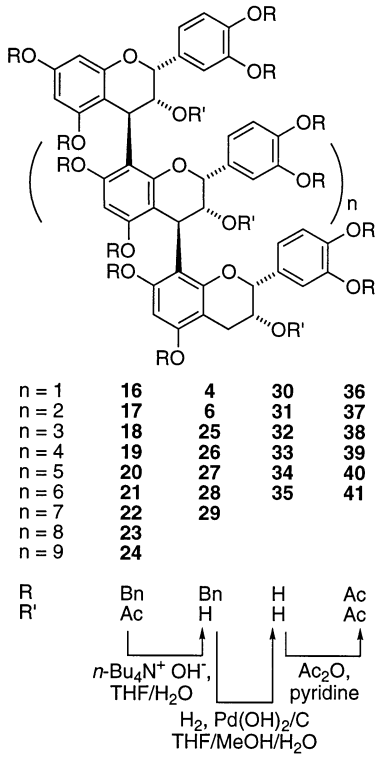
SCHEME 3



nucleophile, for the major product of the reaction (44% yield) was the 4-hydroxy monomer **14**. In addition, 4% of the 4-hydroxy dimer **15** was also isolated, pointing to self-condensation of the thioether **12** followed by either chain elongation (to yield **17**) or hydrolysis. An attempt to improve yields by stirring the reactants with powdered molecular sieves prior to the addition of $AgBF_4$ was ineffective. Since the major source of moisture in this reaction is probably the $AgBF_4$, a very hygroscopic reagent, it appeared logical to dry it and the dimer with molecular sieves first and to add compound **12** subsequently. From this experiment, however, both organic reactants were recovered unchanged! It thus appears that the silver salt is adsorbed on the molecular sieves and in this form is no longer capable of reacting with the thioether **12**. This result remains puzzling. Inclusion of molecular sieves in the reaction mixture is a common practice in glycoside synthesis, and it has actually been reported that $AgNO_3$ adsorbed on MS 4 Å is an activator of glycosyl halides.[14] Fortunately, simple vacuum-drying of the $AgBF_4$ immediately before the reaction was subsequently found to reduce the hydrolysis of **12** to an acceptable level, at least for gram-scale runs. Under these conditions, and with a **12**/**13** molar ratio of 1:2.5, a series of oligomers spanning from the trimer **16** to the octamer

(14) Garegg, P. J.; Ossowski, P. *Acta Chem. Scand. B* **1983**, *37*, 249.

SCHEME 4



21 were isolated in a combined yield of 91%. The reaction is exceptionally clean; a chromatogram of the reaction mixture is shown in Figure 1. No 4,6-linked products were found. Similar results were obtained in the reactions of **12** with the trimer **16** and with the tetramer **17**. From the latter reaction, oligomers up to the undecamer **24** could be isolated by reversed-phase HPLC if ethyl acetate was admixed to the acetonitrile in the final step of the gradient. This nonpolar solvent permitted recovery of the highly retained larger oligomers but also eluted significant amounts of aliphatic impurities which subsequently had to be removed by additional HPLC steps, thus reducing the total product recovery.[15]

All of the benzyl ether−acetates up to the nonamer (compounds **16**−**22**) were deacetylated in near-quantitative yield with 40% aqueous tetra-*n*-butylammonium hydroxide in THF (Scheme 4). This base was chosen because of its good solubility in the relatively nonpolar reaction medium that is required by the lipophilicity of the starting materials. The ^{1}H NMR spectra of the resulting benzyl ethers **4**, **6**, and **25**−**29** display signals of two major rotamers together with trace amounts of additional rotamers that increase as the oligomer chain grows. We are confident that these minor components are rotamers rather than regioisomers because similar signals are absent from the spectra of the precursor acetates. Samples of the benzyl ethers prepared in $CDCl_3$ exhibit well-resolved, characteristic signals for the OH protons in the δ 1.8−1.1 region.

(15) In all of these reactions, the yields of oligomers decrease with the degree of oligomerization, as should be expected. However, this decrease is not uniform. Especially the dimer **20** reproducibly furnishes a percentage yield of the tetramer **22** which comes fairly close to that of the trimer **21**. A weaker effect of the same kind is found for the tetramer as the starting material, but not for the trimer. There does not appear to be a simple explanation for these discrepancies.

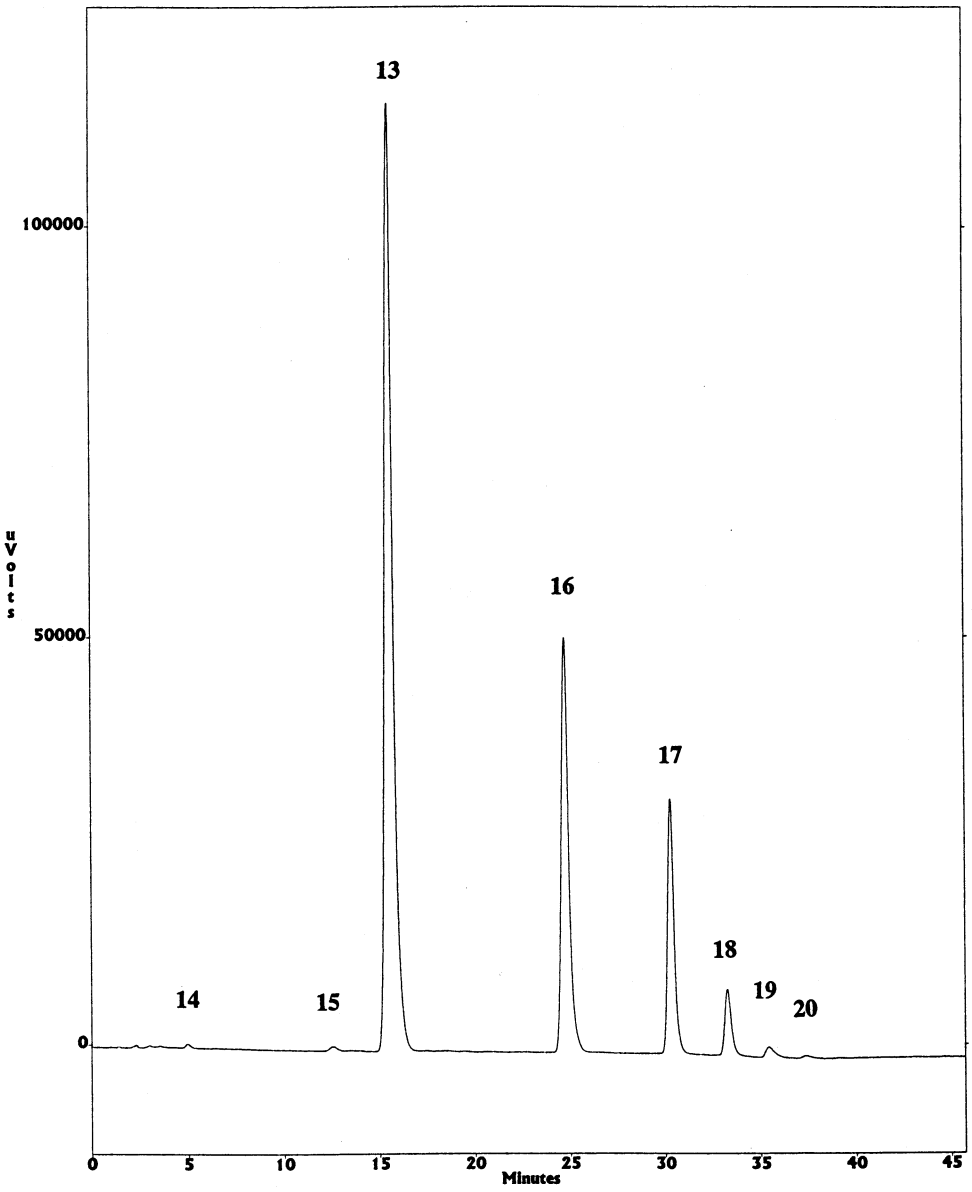


FIGURE 1. $AgBF_4$-mediated reaction between compounds **12** and **13**: chromatogram of the reaction mixture after filtration over silica gel. Conditions: Hewlett-Packard RP-8, 200 × 4.6 mm, flow rate 1.0 mL/min, UV detection at 280 nm; 0−30 min, 80 to 100% CH_3CN in H_2O, then CH_3CN.

Deprotection and Characterization. The benzyl ethers **4**, **6**, and **25**−**28** (trimer through octamer) were deprotected by hydrogenolysis over Pearlman's catalyst to yield the free polyphenols **30**−**35**. We found it advisable to perform this reaction in bicarbonate-washed glassware, as partial fragmentation to lower oligomers was occasionally observed without this precaution, quite probably as a consequence of an acidic reaction of the glass surface of the reaction flask.[16] To obtain a readily soluble polyphenol, it was furthermore found necessary, as similarly reported by others,[17] to dilute the filtered solution of the crude product with water (in place of dioxane used in ref 17), to evaporate only partially so as to remove most of the organic solvents, and to lyophilize the residual solution. If the crude polyphenol solutions are directly evaporated to dryness, partially insoluble materials result, indicating that some decomposition has occurred. Combustion analyses show that the lyophilized products contain 1.3−2 equiv of water per epicatechin moiety.

The synthetic trimer **30**, tetramer **31**, and pentamer **32** were compared against the natural products purified

(16) An alternative explanation, namely the actual hydrogenolysis of interflavan bonds, appears unlikely since several authors have reported that this reaction, when performed intentionally, requires elevated temperatures and hydrogen pressures: (a) Nisi, D.; Panizzi, L. *Gazz. Chim. Ital.* **1966**, *96*, 803. (b) Jacques, D.; Haslam, E.; Bedford, G. R.; Greatbanks, D. *J. Chem. Soc., Perkin Trans. 1* **1974**, 2663. (c) Foo, L. Y. *Phytochemistry* **1982**, *21*, 1741. It is, to the contrary, quite conceivable that these reported reactions proceed via initial solvolytic cleavage of the interflavan bond followed by hydrogenolytic removal of the resulting 4-substituent. Acidic glass surfaces may also be involved in reported cases of partial fragmentation of benzylated dimeric procyanidins: (d) Jacques, D.; Haslam, E.; Bedford, G. R.; Greatbanks, D. *J. Chem. Soc., Perkin Trans. 1* **1974**, 2663. (e) Arnaudinaud, V.; Nay, B.; Nuhrich, A.; Deffieux, G.; Mérillon, J.-M.; Monti, J.-P.; Vercauteren, J. *Tetrahedron Lett.* **2001**, *42*, 1279.

(17) Yoneda, S.; Kawamoto, H.; Nakatsubo, F. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1025.

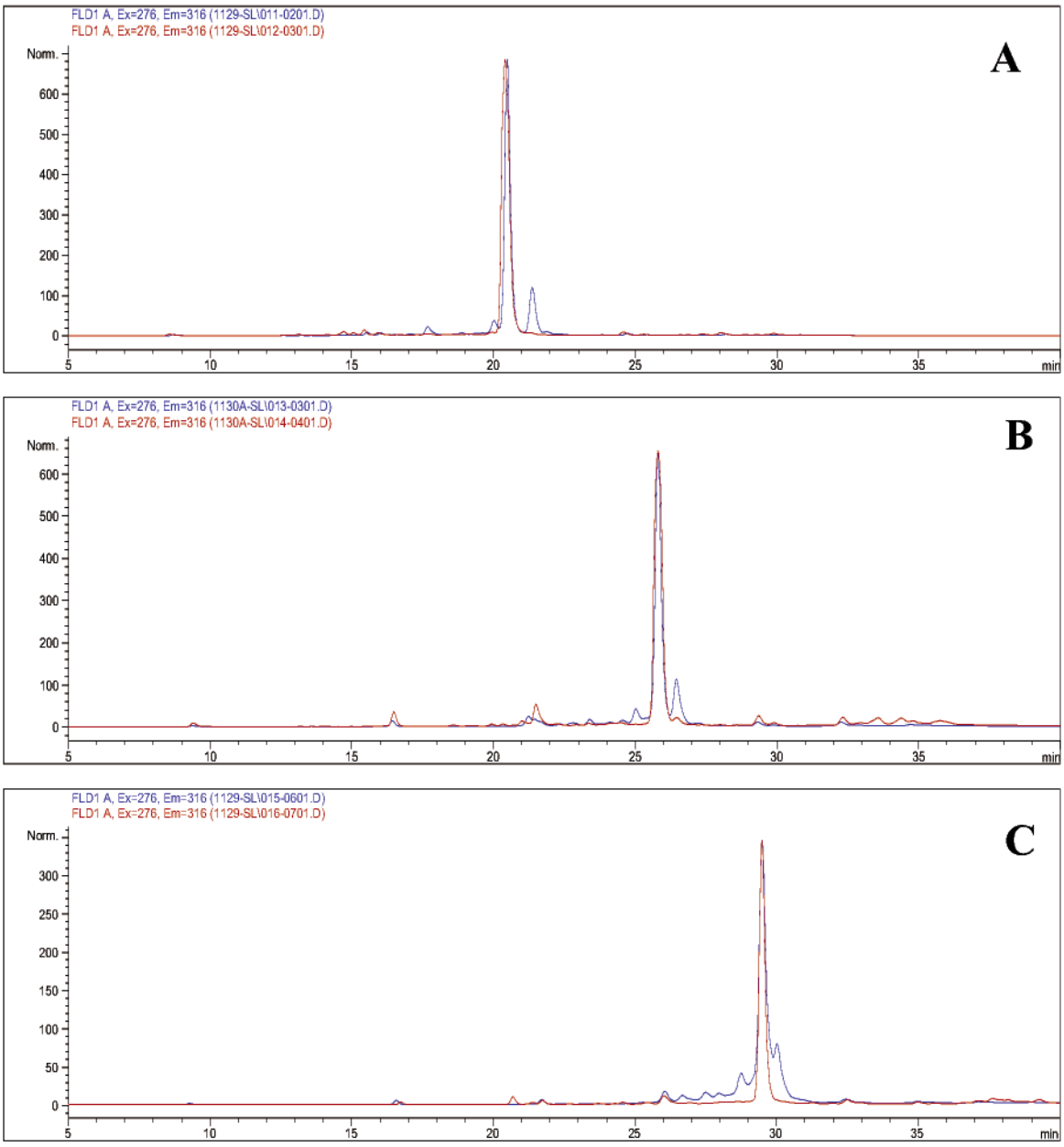


FIGURE 2. Comparison HPLC traces of purified natural (blue) and synthetic (red) procyanidin oligomers: A, **30**; B, **31**; C, **32**. For conditions, see the Supporting Information.

from *Theobroma cacao*[4] by normal-phase[4e,l] HPLC analysis (Figure 2). Purities ranging from 94 to 96% were observed for the synthetic procyanidins, which were 2−4% higher than those obtained for the natural procyanidins. The t_R's of the synthetic procyanidins matched those observed for the natural products, thus confirming epicatechin 4β,8 regio- and stereochemistry in cocoa. All of the natural procyanidins purified from cocoa showed impurity peaks preceding and following the main peak. Scanning these regions by HPLC/MS revealed no change in the $[M]^+$ or $[M + Na]^+$ ions, indicating that these minor impurities are isomers of the major oligomer. These minor impurities may contribute to in vitro and in vivo activities reported in the literature and potentially confound structure−activity relationships based on natural material only. As a precaution, both natural and synthetic samples of procyanidins were therefore used in the biological assays.

Since free polyphenols are inherently poorly amenable to purification because of their oxygen and acid sensitivity (acid being required as a solvent additive to reduce peak tailing during HPLC), and their NMR spectra are anyway uncharacteristic because of severe line broadening, we preferred to characterize these compounds as their peracetates **36**−**41**. In an attempt to avoid acid-induced interflavan bond cleavage during this reaction, we increased the amount of pyridine in the Ac_2O/pyridine reagent from one (the usual amount) to two volumes relative to Ac_2O, but as a result, impurities emerged that eluted immediately before the peracetates and could not be removed on a preparative scale. The ^{1}H NMR spectra of the peracetates exhibit sharp signals for two rotamers

(in a 2:1 ratio for the trimer **14** and in a 3:2 ratio for all higher homologues) and are, up to the heptamer or octamer, quite suitable for compound identification. Especially the acetate region serves as a useful "fingerprint". As the oligomer chain grows, the chemical shift differences between analogous protons of epicatechin units in the inner positions of the chain become eventually insufficient at 300 MHz, resulting in the growth of uncharacteristic signal clusters without the appearance of well-separated new signals. We believe that these spectra can be useful for future reference, and we have included suitable sections thereof in the Supporting Information. ^{13}C NMR spectra have been acquired for oligomers up to the hexamer, beyond which insufficient amounts of material were available, and have also been included in the Supporting Information. Regrettably, some of the literature work is limited to partial thiolytic degradation as a tool of structural assignment. While this technique is sound[18] as long as the structure of the smaller fragments can be established with certainty, it fails to provide reference data for future workers who reisolate or synthesize the compound in question. Only for the trimer **14**[6,19f] and the tetramer **15**[6] have ^{1}H NMR spectra been published, and these are in good agreement with ours. In the case of the tetramer **12**, partial thiolysis[6] has established 4β,8-regio- and -stereochemistry for the "upper" interflavan linkage whereas the "lower" portion of the molecule is identical with the trimer **11**. This compound, in turn, has also been subjected to partial thiolysis, and both interflavan linkages have been identified as 4β,8.[6] Since, therefore, in the course of the present chain extension process the first three interflavan linkages formed are exclusively of the 4β,8-type, we conclude that the same must be true for the additional interflavan linkages present in the larger oligomers. Compounds **30**−**33** are natural products widespread in various groups of vascular plants.[19] The reduced frequency with which the larger oligomers have been reported in the literature probably reflects, at least in part, their poor extraction and the difficulty of their purification, rather than their uncommon occurrence.

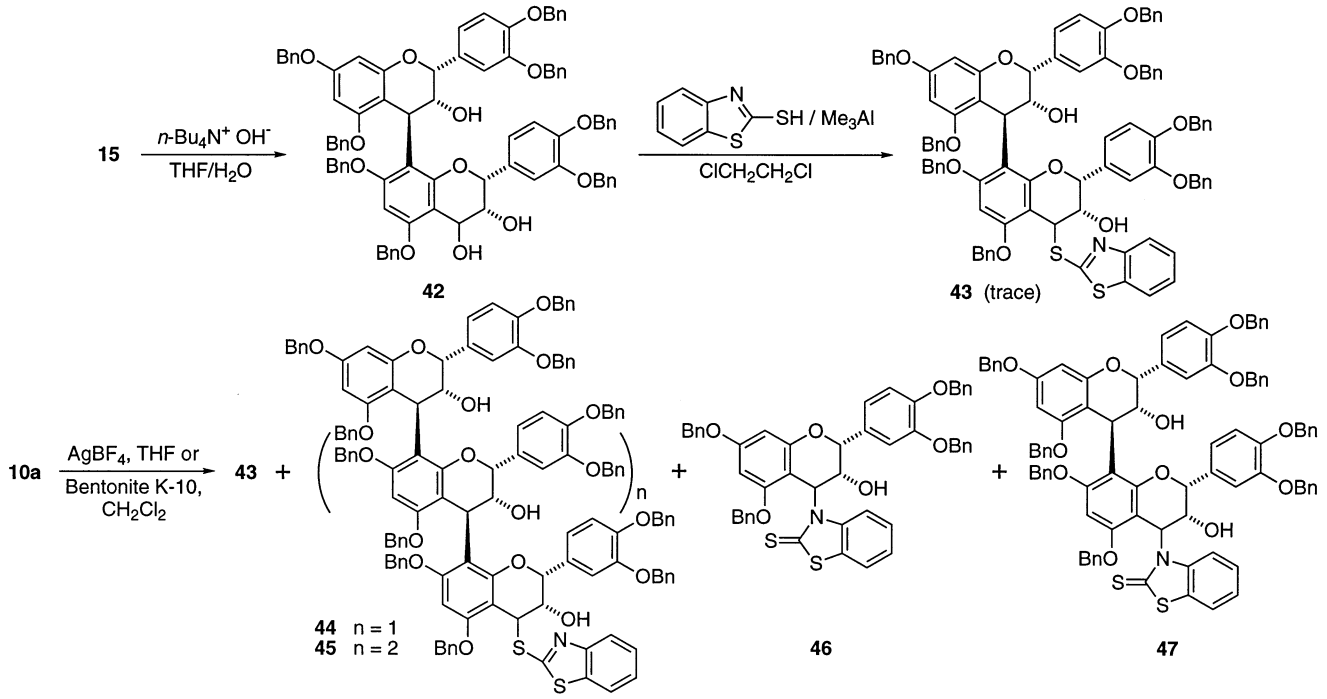
Chain Extension by Two Members. The HPLC separation of oligomer mixtures would be much improved if a given oligomer could be homologated in increments of two epicatechin units rather than just one. It was therefore of interest to search for a synthetic approach to a protected 4β,8-dimer bearing a (2-benzothiazolyl)-thio substituent in the 4-position of its bottom epicatechin unit. The direct oxidation of a methyl/acetyl-protected dimeric procyanidin with DDQ occurs in the doubly activated 4-position of its *top* flavanol moiety.[20] A small amount of the 4-hydroxylated dimer **15** was available as a byproduct, but extension of the organoaluminum thiolate protocol to the free triol **42** obtained by acetate saponification yielded but traces of the thioether **43** (Scheme 5). As an alternative, we envisaged a cross-coupling between the 4-(2-hydroxyethoxy)monomer **2** and the 4-[(2-benzothiazolyl)thio]monomer **10a**, either under acid catalysis for activation of the reactant **2** or under the action of $AgBF_4$ for activation of **10a**. These attempts largely met with failure. However, we found that compound **10a** self-condenses under the action of $AgBF_4$ or, surprisingly, Bentonite K-10 to yield fairly complex mixtures from which small amounts of the 4-[(2-benzothiazolyl)thio]-substituted dimer **43**, trimer **44**, and presumably the tetramer **45** were isolated together with the rearranged monomer **46** and rearranged dimer **47**. The migration of the flavanoid moiety from sulfur to nitrogen was confirmed for compound **46** by the observation of a ^{13}C NMR signal at δ 190.3 assignable to the thiocarbonyl carbon atom. The related silver ion-induced migration of a reactive alkyl group from sulfur to nitrogen has been documented for the case of 2-[(methoxymethyl)-thio]pyridine.[10c] We suspected that the complexity of the above reaction mixtures was in part due to the formation of 4-*O*-3-linked oligomers similar to compound **11**, and applied the same remedy used in that case, namely acetylation. $AgBF_4$-induced self-condensation of compound **12** resulted in low yields of the 4-[(2-benzothiazolyl)thio]-substituted oligomers **48** (dimer, 14% yield) through **50** (tetramer) (eq 3). Together with these products, and in considerable quantities because of the small reaction scale, the 4-hydroxylated oligomers **14**, **15**, **51**

(18) Side reactions occurring during exhaustive thiolysis (McGraw, G. W.; Steynberg, J. P.; Hemingway, R. W. *Tetrahedron Lett.* **1992**, *34*, 987) have led to the questioning of all structures established by thiolytic methods (Balas, L.; Vercauteren, J. *Magn. Reson. Chem.* **1994**, *32*, 386). The latter authors go far beyond the conclusions of the former, who solely reject the *quantitative* interpretation of *exhaustive* thiolysis experiments. The "sequencing" of condensed tannins takes place under the milder conditions of *partial* thiolysis. There is at present no indication that the reported side reaction occurs to any significant extent under these conditions. Furthermore, even if this reaction takes place and the sequence of events postulated by McGraw et al. is entirely reversible, and therefore re-addition of the substituted phloroglucinol fragment could initiate a return of the reactants to either a 4,6- or 4,8-linked 4-(benzylthio)oligomer, steric factors would favor the former over the latter. The finding of 4,8-linkage in the case of compounds **11** and **12** (ref 6) leaves thus no doubt that it is genuine. Furthermore, as no 4,6-linked fragments are formed from these compounds, the finding of 4,6-linkage in other cases should be considered genuine as well. Finally, if re-addition of the substituted phloroglucinol fragment did actually occur, so would the addition of any nonhindered phenolic hydroxyl group of other components present in the reaction mixture, resulting in a complex mixture of products rather than just the isomerized 4-(benzylthio)oligomer. It is, of course, assumed that all major fragments, rather than just a few arbitrarily selected components of complex mixtures, have been identified in these experiments.

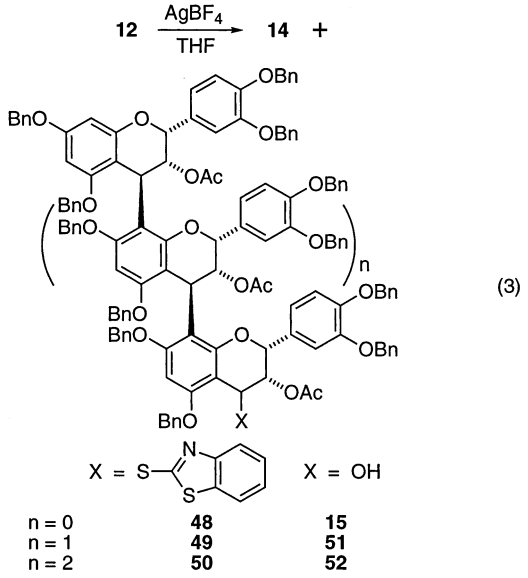
(19) Isolation from (a) cocoa (**30**−**33**): see ref 4. (b) *Pinus taeda* (loblolly pine) phloem (**30**): Hemingway, R. W.; Foo, L. Y.; Porter, L. J. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1209. (c) *Cinchona succirubra* bark (**30**): Nonaka, G.; Kawahara, O.; Nishioka, I. *Chem. Pharm. Bull.* **1982**, *30*, 4277. (d), (e) *Crataegus* (hawthorn) fruit (**30**, **31**): Kolodziej, H.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. 1* **1984**, 343. Rohr, G. E.; Meier, B.; Sticher, O. *J. Chromatogr. A* **1999**, *835*, 59. (f) *Nelia meyeri* leaves (**30**): Kolodziej, H. *Phytochemistry* **1984**, *23*, 1745. (g) *Kandelia candel* bark (**30**): Hsu, F.-L.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull.* **1985**, *33*, 3142. (h) *Dioscorea cirrhosa* (yam) tubers (**30**, **31**): Hsu, F.-L.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull.* **1985**, *33*, 3293. (i) *Cinnamomum cassia* bark (**30**−**33**): Morimoto, S.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull.* **1986**, *34*, 633. (j) *Rhaphiolepis umbellata* bark (**30**−**32**): Ezaki-Furuichi, E.; Nonaka, G.; Nishioka, I.; Hayashi, K. *Agric. Biol. Chem.* **1986**, *50*, 2061. (k), (l) *Pseudotsuga menziesii* (Douglas fir) bark (**30**−**32**): Foo, L. Y.; Karchesy, J. J. *Phytochemistry* **1989**, *28*, 1743. Foo, L. Y.; Karchesy, J. J. *Phytochemistry* **1991**, *30*, 667. (m), (n) Grape seeds (**30**, **31**): Ricardo da Silva, J. M.; Rigaud, J.; Cheynier, V.; Cheminat, A.; Moutounet, M. *Phytochemistry* **1991**, *30*, 1259. Santos-Buelga, C.; Francia-Aricha, E. M.; Escribano-Bailón, M. T. *Food Chem.* **1995**, *53*, 197. (o), (p), (q) Apples (**30**): Pérez-Ilzarbe, F. J.; Martínez, V.; Hernández, T.; Estrella, I. *J. Liquid Chromatogr.* **1992**, *15*, 637. Kanda, T.; Shoji, T.; Ohnishi-Kameyama, M.; Nagata, T. *J. Chromatogr. A* **1999**, *855*, 181. Gomis, D. B.; Palomino, N. F.; Alonso, J. J. M. *Anal. Chim. Acta* **2001**, *426*, 111. (r) *Guazuma ulmifolia bark* (**30**, **31**): ref 6. (s) almond fruit flesh (**30**, **31**): de Pascual-Teresa, S.; Gutiérrez-Fernandez, Y.; Rivas-Gonzalo, J. C.; Santos-Buelga, C. *Phytochem. Anal.* **1998**, *9*, 21. (t) *Hypericum perforatum* herb (**30**): Ploss, O.; Petereit, F.; Nahrstedt, A. *Pharmazie* **2001**, *56*, 509.

(20) Steenkamp, J. A.; Mouton, C. H. L.; Ferreira, D. *Tetrahedron* **1991**, *47*, 6705.

SCHEME 5



(trimer), and **52** (tetramer) were also formed. Reaction of compound **48** with the tetramer **17** in the presence of $AgBF_4$ finally resulted in the formation of the expected hexamer **19** in 12% yield together with the byproducts **21** (octamer), **15**, and **52**. We have thus demonstrated the principle that chain elongation can be performed in increments of two flavanol units. While the present yields are impractical, they should improve considerably upon scale-up because of the diminished impact of adventitious moisture. The procedure can be of potential use for the chain-elongation of the larger protected epicatechin oligomers as these compounds far exceed the building block **12** in value.



(3)

Anticancer Activity. The proanthocyanidins have attracted a great deal of recent attention in the fields of nutrition, medicine, and health due to their wide range of potentially significant biological activities.[3] There is a growing body of evidence suggesting that these compounds act as potent antioxidants in vitro, ex vivo, and in vivo and may thus alter the pathophysiology of imbalances or perturbations of free radical and/or oxidatively driven processes in many diseases or directly interfere with many cellular processes.[21] Our interest is focused in the cancer area where initial observations[4f−h] showed procyanidin-rich fractions from cocoa to elicit in vitro growth inhibition in several human cancer cell lines. The development of improved analytical and preparative HPLC methods[4e,l] has allowed us to obtain quantities of purified oligomers which were screened against certain human breast cancer cell lines together with our synthetic products (up to the pentamer). Cytotoxic effects were clearly associated with high molecular weight oligomers (pentamer and higher) at the 100 μg/mL dose level (Figure 3). This effect was also observed in MCF-7, MDA MB 231, and MDA 435 human breast cancer cell lines treated in a similar fashion (data not shown). Curiously, no activity was observed for the dimer, trimer, and tetramer, which others have reported to show activity on small cell lung and colorectal cancer cell lines.[22] Cytotoxicity thus increases sharply with the degree of oligomerization.

Several studies have demonstrated an association between cell cycle regulation and cancer.[23] The cell cycle machinery regulates cell proliferation, and dysregulated

(21) Nijveldt, R. J.; van Nood, E.; van Hoorn, D. E. C.; Boelens, P. G.; van Norren, K.; van Leeuwen, P. A. M. *Am. J. Clin. Nutr.* **2001**, *74*, 418.

(22) (a) Kolodziej, H.; Haberland, C.; Woerdenbag, H. J.; Konings, A. W. T. *Phytotherapy Res.* **1995**, *9*, 410. (b) Gali, H. U.; Perchellet, E. M.; Gao, X. M.; Karchesy, J. J.; Perchellet, J. P. *Planta Med.* **1994**, *60*, 235.

(23) Hajduch, M.; Havlieek, L.; Vesely, J.; Novotny, R.; Mihal, V.; Strand, M. *Adv. Exp. Med. Biol.* **1999**, *457*, 341.

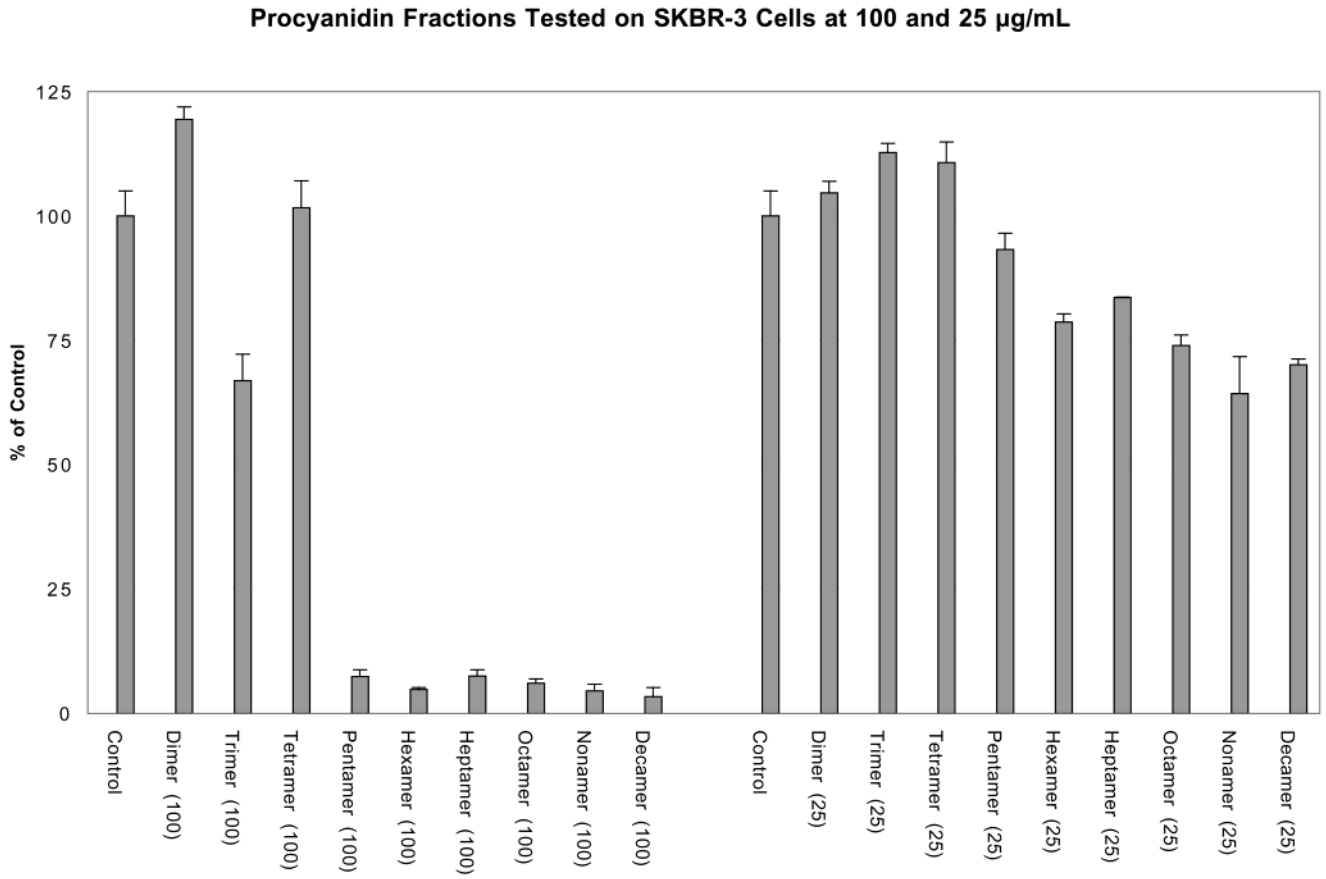


FIGURE 3. Cytotoxicity of procyanidin fractions purified from cocoa on human breast cancer cell line SKBR-3 at 100 and 25 μg/mL.

TABLE 1. Cell Cycle Analysis of MDA MB 231 Human Breast Cancer Cells Treated with Oligomeric Procyanidins Purified from Cocoa

	% G_0/G_1	% S	% G_2/M
control	36.69	23.39	39.92
vehicle	38.26	22.43	39.30
dimer (200 μg/mL; 24 h)	38.13	22.43	39.45
control	42.28	35.61	22.12
vehicle	43.60	34.10	22.30
trimer (200 μg/mL; 24 h)	43.22	35.98	20.80
control	40.33	36.25	23.42
vehicle	43.71	34.42	21.87
tetramer (200 μg/mL; 24 h)	51.46	28.25	20.30
control	38.33	21.05	40.61
vehicle	37.84	21.39	40.77
pentamer (200 μg/mL; 24 h)	66.03	17.23	16.67
pentamer (200 μg/mL; 48 h)	88.31	6.07	5.62

TABLE 2. Comparison Cell Cycle Analysis of MDA MB 231 Human Breast Cancer Cells Treated with Natural versus Synthetic Oligomeric Procyanidins

	% G_0/G_1	% S	% G_2/M
control	28.65	49.28	22.06
vehicle	27.19	49.61	23.2
natural trimer (200 μg/mL; 24 h)	28.46	48.49	23.05
synthetic trimer (**30**) (200 μg/mL; 24 h)	26.98	49.57	23.45
natural tetramer (200 μg/mL; 24 h)	36.82	43.37	19.02
synthetic tetramer (**31**) (200 μg/mL; 24 h)	43.49	39.39	17.03
natural pentamer (200 μg/mL; 24 h)	45.99	38.25	15.76
synthetic pentamer (**32**) (200 μg/mL; 24 h)	64.15	23.36	12.49

cellular proliferation is a hallmark of cancer.[24] Many synthetic cell cycle inhibitors such as flavopiridol, olomoucine, roscovitine, and puvalanol B are viewed as a new generation of anticancer drugs, and some are being investigated in clinical trials.[25] Cell cycle analysis of procyanidin-treated MDA MB 231 cells clearly showed a G_0/G_1 arrest by the pentamer, no effect by the dimer or trimer, and only a slight effect by the tetramer (Table 1). The increase in G_0/G_1 was accompanied with a decrease of cell numbers in the S phase and in G_2/M. The pentamer-caused G_0/G_1 arrest was reversible in cells treated up to 8 h and irreversible after a 24 h treatment. No difference in activity was observed between natural and synthetic trimer **30**; an approximately 15% increase in G_0/G_1 arrest was seen for synthetic tetramer **31** compared to natural material, and an approximately 30% increase for synthetic vs natural pentamer **32** (Table 2). These differences can be attributed to the higher purities

(24) (a) Paggi, M. G.; Baldi, A.; Bonetto, F. Giordano, A. *J. Cell Biochem.* **1996**, *62*, 418. (b) McDonald, E. R., III; El Deiry, W. S. *Int. J. Oncol.* **2000**, *16*, 871. (c) Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57.

(25) (a) Buolamwini, J. K. *Curr. Pharm. Des.* **2000**, *6*, 379. (b) Stadler, W. M.; Volgelzang, N. J.; Amato, R.; Sosman, J.; Taber, D.; Liebowitz, D.; Vokes, E. E. *J. Clin. Oncol.* **2000**, *18*, 371. (c) Senderowicz, A. M.; Headlee, D.; Stinson, S. F.; Lush, R. M.; Kalil, N.; Villalba, L.; Hill, K.; Steinberg, S. M.; Figg, W. D.; Tompkins, A.; Arbuck, S. G.; Sausville, E. A. *J. Clin. Oncol.* **1998**, *16*, 2986.

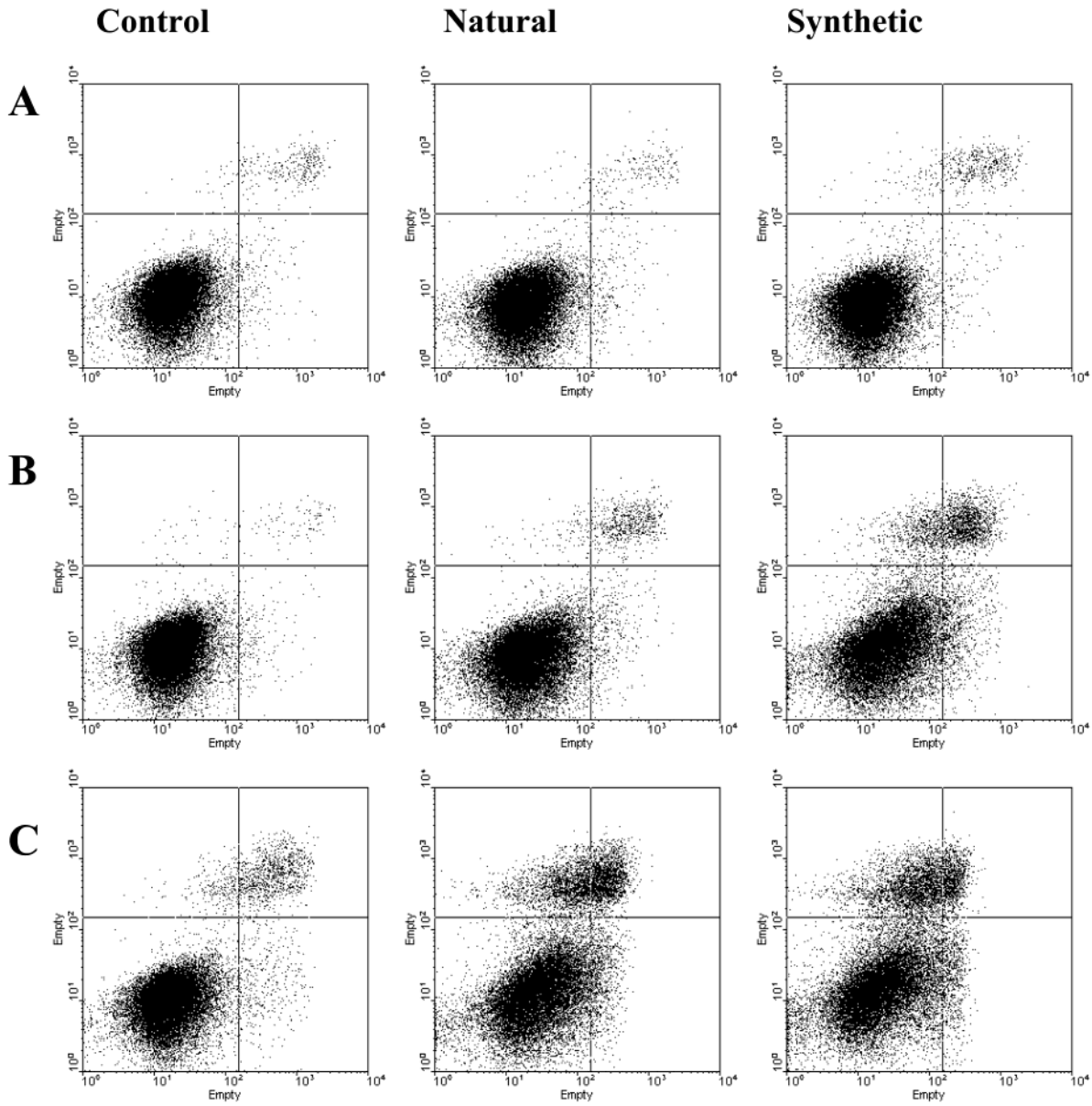


FIGURE 4. Flow cytometry of procyanidin treated MDA MD 231 human breast cancer cells using Annexin V-FITC and propidium iodide (control versus 24 h treatment with 200 μg/mL of oligomer): A, **30**; B, **31**; C, **32**. Lower left quadrant: viable cells. Lower right quadrant: early apoptotic events. Upper right quadrant: late apoptotic events. Upper left quadrant: nonviable cells.

of the synthetic procyanidins (see above). Cell cycle arrest induced by monomeric catechins, especially epigallocatechin gallate (EGCG), has previously been observed by others.[26]

Cell cycle arrest may result in cell death, which was indeed observed in the present experiments. The manner of cell death (apoptosis or necrosis) was investigated by the annexin V-fluorescein isothiocyanate (FITC) assay using Trevigen's TACS Annexin V-FITC kit. Cell cycle analysis of MDA MB 231 cells treated with natural and synthetic oligomers **30**, **31**, and **32** is shown in Figure 4. Cells treated with both natural and synthetic procyanidins showed similar profiles, and increases in cell populations in the upper right quadrant were observed as the oligomer size increased. This quadrant represents annexin V positive cells that also take up propidium iodide. These cells are considered to be in either late apoptosis or in necrosis. The absence of a distinct cell population in the lower right quadrant (cells associated with early apoptotic events) in the case of pentamer-treated cells suggests a necrotic pathway to cell death possibly due to a direct interaction with the cell membrane leading to damage, cell crisis, and eventual death. However, this conclusion will require additional investigations for verification. We note that a number of other polyphenols, especially the important green tea constituent, EGCG, have been demonstrated to induce apoptosis.[3i−k,26a]

A recent report has shown H_2O_2 to be artifactually produced in vitro by several different polyphenolic com-

(26) (a) Ahmad, N.; Feyes, D. K.; Nieminen, A.-L.; Agarwal, R.; Mukhtar, H. *J. Natl. Cancer Inst.* **1997**, *89*, 1881. (b) Liang, Y. C.; Lin-Shiau, S.-Y.; Chen, C. F.; Lin, J. K. *J. Cell Biochem.* **1999**, *75*, 1. (c) Ahmad, N.; Cheng, P.; Mukhtar, H. *Biochem. Biophys. Res. Commun.* **2000**, *275*, 328. (d) Gupta, S.; Ahmad, N.; Nieminen, A.-L.; Mukhtar, H. *Toxicol. Appl. Pharmacol.* **2000**, *164*, 82. (e) Tan, X.; Hu, D.; Li, S.; Han, Y.; Zhang, Y.; Zhou, D. *Cancer Lett.* **2000**, *158*, 1.

TABLE 3. Cell Cycle Analysis of MDA MB 231 Treated Cells

	% G_0/G_1	% S	% G_2/M
control	33.14	44.06	22.81
vehicle	36.44	41.63	21.94
100 μM H_2O_2; 24 h	20.32	44.92	34.76
100 μM H_2O_2 + catalase; 24 h	35.20	42.93	21.86
100 μM H_2O_2 + heat inactivated catalase; 24 h	20.27	45.48	34.25
control	29.87	46.21	23.92
vehicle	30.28	47.25	22.47
32 (200 μg/mL); 24 h	44.94	38.01	17.05
32 (200 μg/mL) + catalase; 24 h	41.23	39.65	20.12
32 (200 μg/mL) + heat inactivated catalase; 24 h	42.89	39.43	17.68
32 (200 μg/mL) + 100 μM H_2O_2; 24 h	42.67	18.63	38.71
32 (200 μg/mL) + 100 μM H_2O_2 + catalase; 24 h	48.20	31.12	20.68
32 (200 μg/mL) + 100 μM H_2O_2 + heat inactivated catalase; 24 h	39.47	23.39	37.14

pounds and to be reponsible for causing a variety of biological activities.[27] Our results (Table 3) indicate that if H_2O_2 were present at the levels reported in the literature, it would produce a shift in the cell cycle to G_2/M with a decrease in G_0/G_1. The addition of catalase abrogated these effects, causing a shift in the cell cycle back to control values. The addition of catalase alone to pentamer-treated cells produced no conclusive change in the cell cycle attributable to H_2O_2, i.e., the typical G_0/G_1 arrest caused by the pentamer remained essentially unchanged. To eliminate the possibility that the pentamer might inhibit catalase activity, H_2O_2 was added to pentamer treated cells in the presence and absence of catalase. The addition of H_2O_2 to pentamer-treated cells led to an increase in G_0/G_1 and G_2/M at the expense of cells in the S phase. Catalase addition caused a shift back to the G_0/G_1 arrest typical of pentamer-treated cells, and heat-inactivated catalase had no effect. We conclude that the G_0/G_1 arrest is directly caused by the pentamer and not by H_2O_2.

Collectively, these results confirm the cytotoxicity to human breast cancer cell lines by an epicatechin pentamer purified from cocoa and assigned the structure **32**. This procyanidin causes a G_0/G_1 arrest in MDA MB 231 cells that is independent of any effects caused by H_2O_2. An increase in annexin V and propidium iodide positive cells suggests that pentamer-treated cells quickly enter into a necrotic phase of cell death. However, additional efforts are required to elucidate the molecular mechanisms by which **32** exerts its effects in vitro and to conclusively determine whether apoptosis or necrosis is the pathway of cell death.

Experimental Section

Pearlman's catalyst (20% $Pd(OH)_2/C$) contained up to 50% H_2O. For other chemicals, see ref 1a. ^1H and ^{13}C NMR spectra were acquired at nominal frequencies of 300 and 75 MHz, respectively, in $CDCl_3$ unless specified otherwise. ^1H NMR spectra are referenced to internal TMS; ^{13}C NMR spectra to internal TMS if so marked, otherwise to the $CDCl_3$ signal (δ 77.00). Combustion analyses: Micro-Analysis, Inc. (Wilmington, DE). Column chromatography (CC): silica gel 60, particle size 63−200 μm. TLC: silica gel 60 with fluorescent indicator, layer thickness 250 μm; visualization by UV light (254 nm) or with alkaline $KMnO_4$ solution. HPLC: column A, Hewlett-Packard RP-8, 200 × 4.6 mm, at 1.0 mL/min; column B, Waters μBondapak C_{18}, 300 × 7.8 mm, at 2.8 mL/min; column C, Waters μBondapak C_{18}, 300 × 19 mm; column D, Waters μBondapak C_{18}, 300 × 30 mm, at 42 mL/min; column E, Whatman Partisil 10, 500 × 9.4 mm, at 5.0 mL/min; column F, Whatman Partisil 10, 500 × 22 mm, at 26 mL/min. Detection by UV absorption at 280 nm. Retention times vary substantially depending on column history and other subtle circumstances. They are quoted solely for orientation and should not be employed for product identification without comparison to an authentic reference sample.

HPLC Analysis of Procyanidins. Normal-phase separations were performed on a 250 × 4.6 mm Phenomenex 5 μm Prodigy column. The detector was a fluorescence detector operating at λ_{ex} = 276 nm and λ_{em} = 316 nm. The ternary mobile phase consisted of (A) dichloromethane, (B) methanol and (C) acetic acid/water (1:1 v/v). Separations were effected by a series of linear gradients of B into A with a constant 4% C at a flow rate of 1 mL/min as follows: 0−30 min, 14.0−28.4% B in A; 30−50 min, 28.4−38.0% B in A; 50−51 min, 38.0−86.0% B in A; 51−56 min, 86.0% B in A isocratic.

HPLC/MS Analysis of Procyanidins. HPLC/MS analyses of natural and synthetic procyanidins were performed on an HPLC system as described above which was interfaced to mass selective detector equipped with an API-ES ionization chamber. Ionization reagents were added via a tee in the eluent stream just prior to the mass spectrometer. Conditions for analysis in the positive ion mode included the introduction of 0.05 M sodium chloride at a flow rate of 0.05 mL/min to assist ionization, a capillary voltage of 3.5 kV, a fragmentor voltage of 100 V, a nebulizing pressure of 25 psig, and a drying gas temperature of 350 °C. Scans were performed over a mass range of *m*/*z* 100−3000 at 1.96 s per cycle.

Condensation of 1 with 2 Catalyzed by Bentonite K-10. Bis(5,7,3′,4′-tetra-*O*-benzyl)epicatechin 4β,8-Dimer (3). To a solution/suspension of 9.26 g (14.2 mmol, 4 equiv) of compound **1** and 5.0 g of Bentonite K-10 in 115 mL of anhydrous CH_2Cl_2 was added with ice cooling, stirring, and exclusion of moisture within 2.5 h 2.53 g (3.56 mmol) of compound **2** in 35 mL of anhydrous CH_2Cl_2. The bath temperature rose to +6 °C at the end of the addition. Stirring in the bath was continued for 1 h, during which time the temperature rose to +12 °C. The clay was filtered off with suction over Celite, and the solids were washed with 2 × 50 mL of CH_2Cl_2. Twenty milliliters of toluene was added, and the solution was evaporated to a small volume. The residue was chromatographed on silica gel (60 × 5 cm) with EtOAc/$CHCl_3$/hexane 1:14:14. Initially, 5.95 g of unreacted **1** was eluted, followed by 4.01 g of monomer/dimer mixed fractions and 1.15 g of pure (98% by HPLC) dimer **3**. The last traces of the dimer together with the trimer were eluted as a mixed fraction (0.27 g) with a solvent ratio of 1:7:7.

The mixed fractions were each dissolved in CH_3CN and separated by preparative HPLC (column D; 0−30 min, 80 to 100% CH_3CN in H_2O, then CH_3CN); the retention times for the dimer and trimer were 23.3 and 30.1 min, respectively. After combination of appropriate fractions, evaporation, and

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drying in vacuo, the following yields were obtained: **1**, 6.89 g (74% recovery); **3**, 4.26 g (92%); **4**, 74 mg (2%). Title compound: ^{13}C NMR ($CDCl_3$, TMS) δ 158.34, 158.07, 157.91, 157.07, 156.83, 156.56, 156.49, 155.89, 155.53, 155.07, 154.44, 152.83, 149.17, 149.01, 148.92, 148.66, 148.60, 148.40, 148.18, 137.40, 137.38, 137.30, 137.28, 137.22, 137.17, 137.01, 136.97, 132.61, 132.43, 131.18, 131.14, 128.6−126.6, 119.96, 119.79, 118.79, 118.65, 115.02, 114.89, 114.35, 114.05, 113.52, 112.93, 112.46, 111.58, 111.17, 104.45, 102.29, 101.76, 94.34, 93.96, 93.33, 93.15, 92.93, 91.52, 78.84, 78.07, 75.63, 72.41, 72.14, 71.48, 71.35, 71.22, 70.81, 70.48, 69.92, 69.86, 69.78, 69.47, 69.05, 66.50, 65.15, 35.90, 35.78, 28.74, 28.61. Other data have been published.[1a]

In another run (3.17 mM in **2**), an essentially complete separation of monomer and dimer and of dimer and trimer was achieved during column chromatography, with only the trimer and the very dilute tail of the dimer requiring purification by HPLC. The following yields were obtained: **1**, 6.20 g (75% recovery); **3**, 3.63 g (88%); **4**, 0.15 g (5%).

Condensation of 3 with 2 Catalyzed by Bentonite K-10. To a solution/suspension of 5.60 g (4.31 mmol, 3 equiv) of compound **3** and 2.04 g of Bentonite K-10 in 45 mL of anhydrous CH_2Cl_2 was added with ice cooling, stirring, and exclusion of moisture within 110 min 1.02 g (1.44 mmol) of compound **2** in 15 mL of anhydrous CH_2Cl_2. The bath temperature rose to +6 °C at the end of the addition. Stirring in the bath was continued for 1 h, during which time the temperature rose to +12 °C. The clay was filtered off with suction over Celite, and the solids were washed with 4 × 25 mL of EtOAc. The combined solutions were evaporated. Attempted separation by column chromatography on silica gel (56 × 5 cm) with EtOAc/$CHCl_3$/hexane 1:10:10 failed to separate the dimer and the trimer; subsequent elution with a solvent ratio of 1:7:7 gave 0.50 g of a fraction consisting mostly of tetramer together with residual trimer. The dimer/trimer fraction was again subjected to column chromatography on silica gel (55 × 5 cm), this time starting with EtOAc/$CHCl_3$/hexane 1:14:14. After elution with 20 L of this mixture, the solvent ratio was switched to 1:12:12 (5 L), then 1:10:10, resulting in the recovery of 4.40 g of the dimer. Further elution with a mixing ratio of 1:8:8 gave 1.04 g of crude trimer (purity 90% by HPLC).

The crude trimer and the trimer/tetramer mixture were each dissolved in CH_3CN and separated by preparative HPLC (column D; 0−30 min, 80 to 100% CH_3CN in H_2O, then CH_3CN); the retention times for the dimer, trimer, and tetramer were 22.5 (22.7), 30.1 (30.8), and 33.9 min, respectively. After combination of appropriate fractions, evaporation, and drying in vacuo, the following yields were obtained: **3**, 4.43 g (79% recovery); **4**, 1.13 g (40%); **6**, 0.24 g (13%).

4-[(2-Benzothiazolyl)thio]-5,7,3′,4′-tetra-*O*-benzylepicatechin (10a,b). *Caution!* Although 2-mercaptobenzothiazole is odorless, small quantities of malodorous (but not very volatile) 2-(benzylthio)benzothiazole are formed in this reaction, which should therefore be conducted in a well-ventilated fume hood. To a solution of 6.5 g (39 mmol) of 2-mercaptobenzothiazole in 40 mL of 1,2-dichloroethane [HPLC grade, filtered over basic alumina (activity I) immediately before use] was added dropwise in 10 min under N_2 with ice cooling and stirring 19.5 mL of trimethylaluminum solution (2.0 M in toluene). The resulting amber solution was stirred at 0 °C for 15 min, then a solution of 5.56 g (7.82 mmol) of compound **2** in 60 mL of 1,2-dichloroethane (pretreated as above) was added dropwise in 20 min. The orange-colored reaction mixture was stirred at room temperature for 5 h, then cooled in an ice bath, and a solution of 22.6 g (80 mmol) of potassium sodium tartrate tetrahydrate in 90 mL of H_2O and 100 mL of 2.5 M aqueous NaOH was added dropwise (very cautiously at first; gas evolution!). Methylene chloride (100 mL) was added, and the phases were separated. The organic phase was washed with 2 × 100 mL of 2.5 M aqueous NaOH and dried over Na_2SO_4. After evaporation to a small volume, the residue was chromatographed on a short SiO_2 column with EtOAc/toluene 1:19 (until the beginning elution of product), then 1:9. The eluate was evaporated to yield an oil, which soon turned into a light-yellow solid. This material was dissolved in 30 mL of hot EtOAc, 90 mL of 1-chlorobutane was added, and the solution was seeded and set aside for crystallization first at room temperature, then at −20 °C. The precipitate was isolated by suction filtration, washed with 2 × 20 mL of cold 1-chlorobutane, and dried in vacuo to afford 3.50 g of the predominant diastereoisomer. Chromatography of the mother liquor (SiO_2, EtOAc/CH_2Cl_2/hexane 1:18:11 to 2:18:11) followed by crystallization from EtOAc/1-chlorobutane yielded an additional 0.78 g of the major isomer (together 4.28 g, 67%) and 0.16 g (2.5%) of the less polar minor isomer. Major diastereoisomer **10a**: mp 160−161 °C (from EtOAc/1-chlorobutane); $[\alpha]_D$ +106, $[\alpha]_{546}$ +133 (EtOAc, *c* 10.6 g L^{-1}); ^{1}H NMR ($CDCl_3$) δ 7.89 (ddd, 1 H, J = 8, 1.2, 0.7 Hz), 7.78 (ddd, 1 H, J = 8, 1.2, 0.7 Hz), 7.47−7.20 (m, 19 H), 7.17 (d, 1 H, J = 2 Hz), 7.12−7.00 (m, 4 H), 6.95 (B part of an ABq, 1 H, J = 8.5 Hz), 6.30, 6.29 (ABq, 2 H, J = 2 Hz), 5.46 (d, 1 H, J = 2 Hz), 5.42 (s, 1 H), 5.17 (s, 2 H), 5.16 (s, 2 H), 5.10, 5.05 (ABq, 2 H, J = 12 Hz), 5.03 (s, 2 H), 4.40 (ddd, 1 H, J = 6, 2.5, 1 Hz), 2.00 (d, 1 H, J = 5.5 Hz); ^{13}C NMR ($CDCl_3$) δ 165.00, 160.67, 158.76, 155.95, 153.16, 148.96, 148.88, 137.17, 137.07, 136.53, 136.47, 135.29, 130.76, 128.61, 128.45, 128.37, 128.16, 128.09, 127.75, 127.56, 127.49, 127.46, 127.21, 126.57, 126.06, 124.41, 121.83, 120.97, 119.65, 114.91, 113.58, 98.34, 94.48, 75.13, 71.32, 71.22, 70.78, 70.13, 69.86, 44.43. Anal. Calcd for $C_{50}H_{41}NO_6S_2$: C, 73.60; H, 5.06; N, 1.72. Found: C, 73.92; H, 4.75; N, 1.74. Minor diastereoisomer **10b**: see the Supporting Information.

3-*O*-Acetyl-4-[(2-benzothiazolyl)thio]-5,7,3′,4′-tetra-*O*-benzylepicatechin (12). To a solution of 3.50 g (4.29 mmol) of **10a** and 53 mg (0.43 mmol) of 4-(dimethylamino)pyridine in 12 mL of anhydrous pyridine was added all at once 2.0 mL (21.5 mmol) of acetic anhydride. The reaction mixture was kept at room temperature in a closed flask for 50 h. Ice and 150 mL of 5% aqueous HCl were added, and the product was extracted into 100 + 20 mL of CH_2Cl_2. The combined organic phases were washed with 100 mL of H_2O and 2 × 50 mL of 10% aqueous NaOH; after each washing, the aqueous phase was back-extracted with 20 mL of CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and evaporated, and the residue was taken up in a small volume of toluene and filtered over SiO_2 with EtOAc/hexane 1:3. Evaporation and drying in vacuo yielded 3.58 g (97%) of the acetate as a yellowish foam: $[\alpha]_D$ +91.7, $[\alpha]_{546}$ +115 (EtOAc, *c* 13.2 g L^{-1}); ^{1}H NMR ($CDCl_3$) δ 7.90 (d, 1 H, J = 8 Hz), 7.77 (d, 1 H, J = 8 Hz), 7.46−7.22 (m, 19 H), 7.11 (d, 1 H, J = 2 Hz), 7.09−7.00 (m, 3 H), 6.99, 6.91 (ABq, 2 H, J = 8.5 Hz, A part d with J = 2 Hz), 6.31, 6.30 (ABq, 2 H, J = 2.5 Hz), 5.63 (dd, 1 H, J = 2.5, 1.2 Hz), 5.55 (s, 1 H), 5.31 (d, 1 H, J = 2 Hz), 5.17, 5.12 (ABq, 2 H, J = 12 Hz), 5.14 (s, 2 H), 5.10, 5.05 (ABq, 2 H, J not readable because of overlap), 5.07, 5.02 (ABq, 2 H, J = 11.5 Hz), 1.84 (s, 3 H); ^{13}C NMR ($CDCl_3$, TMS) δ 169.08, 164.07, 160.69, 158.31, 156.03, 153.22, 148.92, 148.89, 137.18, 137.16, 136.53, 136.31, 135.62, 130.29, 128.67, 128.45, 128.24, 128.19, 127.78, 127.65, 127.43, 127.31, 126.87, 126.10, 124.50, 122.16, 121.01, 119.80, 114.97, 113.51, 98.50, 94.46, 94.30, 74.13, 71.44, 71.23, 70.74, 70.19, 70.13, 42.59, 20.84. Anal. Calcd for $C_{52}H_{43}NO_7S_2$: C, 72.79; H, 5.05; N, 1.63. Found: C, 73.01; H, 4.79; N, 1.61.

Bis(3-*O*-acetyl-5,7,3′,4′-tetra-*O*-benzyl)epicatechin 4β,8-Dimer (13). A solution of 1.5 mL (16 mmol) of acetic anhydride in 4 mL of anhydrous pyridine was added all at once to 3.69 g (2.84 mmol) of **3**. The mixture was occasionally swirled until all starting material dissolved and then allowed to stand in a closed flask at room temperature for 99 h. The reaction was terminated by addition of 30 mL of EtOAc and 2 mL of MeOH and allowed to stand at room temperature for 1.5 h. Another 20 mL of EtOAc was added, and then the solution was washed with 200 mL of 0.5 M aqueous H_3PO_4. The aqueous layer was back-extracted with 50 mL of EtOAc, and the combined organic

phases were dried over $MgSO_4$. After evaporation, the residue was taken up in a small volume of toluene and chromatographed on a short SiO_2 column with EtOAc/hexane (1:9, then 1:3, then 1:1). Evaporation and drying in vacuo yielded 3.82 g (97%) of the acetate as a colorless foam: $[\alpha]_D$ +44.9, $[\alpha]_{546}$ +54.6 (EtOAc, *c* 14.2 g L^{-1}); ^1H NMR ($CDCl_3$; two rotamers, 86:14) major rotamer and shared multiplets δ 7.47−6.82 (m, 44 H), 6.75, 6.41 (ABq, 2 H, J = 8 Hz, B part d with J = 1.5 Hz), 6.31 (s, 1 H), 6.02 (d, 1 H, J = 2 Hz), 5.70 (s, 1 H), 5.62 (d, 1 H, J = 2 Hz), 5.46 (t, 1 H, J = 1.5 Hz), 5.12 (s, 2 H), 5.10−4.89 (m, 11 H), 4.80 (s, 2 H), 4.77 (s, 1 H), 4.63, 4.46 (ABq, 2 H, J = 11 Hz), 4.08 (s, 1 H), 2.99, 2.90 (ABq, 2 H, J = 18.5 Hz, A part d with J = 5 Hz), 1.72 (s, 3 H), 1.68 (s, 3 H); conspicuous signals of minor rotamer δ 6.21 (br, 1 H), 6.17 (s, 1 H), 6.11 (br, 1 H), 5.44 (br, 1 H), 5.34 (s, 2 H), 4.85 (s, 2 H), 4.61, 4.34 (ABq, 2 H, J = 12 Hz), 1.43 (s, 3 H), 1.31 (s, 3 H); ^{13}C NMR ($CDCl_3$, TMS; major rotamer only) δ 170.10, 169.10, 158.01, 156.35, 155.96, 155.46, 154.50, 149.17, 148.85, 148.42, 148.24, 137.36−137.23, 136.96, 132.35, 130.74, 128.7−127.1, 126.56, 119.84, 119.31, 114.60, 113.78, 113.59, 112.33, 110.58, 104.43, 101.66, 93.76, 92.89, 91.19, 77.96, 71.46, 71.27, 71.20, 70.51, 69.95, 69.69, 69.57, 69.09, 68.34, 33.20, 26.95, 20.89, 20.80. Anal. Calcd for $C_{90}H_{78}O_{14}$: C, 78.13; H, 5.68. Found: C, 78.22; H, 5.43.

Reaction of 12 with 13. An 0.80 g (4.1 mmol) sample of $AgBF_4$ was dried in the reaction flask at 100 °C in an oil pump vacuum with exclusion of light for 1.5 h. After cooling, the vacuum was broken with N_2, and a solution of 5.66 g (4.09 mmol) of **13** in 60 mL of anhydrous THF was added all at once. The flask was placed in an ice bath under dim light, and a solution of 1.40 g (1.64 mmol) of **12** in 30 mL of anhydrous THF was added dropwise in 70 min with stirring. The reaction mixture turned yellow, and a turbidity eventually appeared. Stirring at 0 °C was continued for 40 min, during which time period the reaction mixture turned into a milky, whitish suspension. Triethylamine (1.1 mL, 8 mmol) was added, the mixture was evaporated to near dryness, and the residue was filtered over a short SiO_2 column with EtOAc/hexane 1:1. The eluate was evaporated, and the crude product was analyzed by HPLC (column A; 0−30 min, 80 to 100% CH_3CN in H_2O, then CH_3CN). The following peaks were observed (assignment/area%): t_R 5.0 (4-OH-monomer, 0.15), 12.6 (4-OH-dimer, 0.25), 15.6 (dimer, 59.4), 24.8 (trimer, 23.4), 30.3 (tetramer, 12.5), 33.3 (pentamer, 3.2), 35.4 (hexamer, 0.8), 37.3 (heptamer, 0.1), 39.1 min (octamer, 0.02). A partial separation was achieved by column chromatography on SiO_2 (38 × 9 cm). Initial elution with 25 L of EtOAc/$CHCl_3$/hexane 1:10:9 did not result in product recovery (this stage is, however, essential for achieving separation). Another 25 L of EtOAc/$CHCl_3$/hexane 1:11:8 eluted 4.01 g of the dimer (71% recovery; pure by HPLC). A fraction (1.72 g) consisting of trimer, tetramer, and some pentamer was eluted with 20 L of EtOAc/$CHCl_3$/hexane 1:12:7; finally, the column was stripped with EtOAc/$CHCl_3$/hexane 2:12:7 to give 0.87 g of a fraction consisting mostly of the larger oligomers. The latter two fractions were taken up in CH_3CN and separated in several portions by prep. HPLC (column D; 0−30 min, 80−100% CH_3CN in H_2O, then CH_3CN), and appropriate fractions were pooled and dried in vacuo to obtain the oligomers as colorless films or foams. The retention times and yields relative to **12** for the trimer through octamer were 31.9 min (1.46 g, 43%), 36.0 min (755 mg, 33%), 39.6 min (204 mg, 11%), 45.0 min (45 mg, 2.6%), 52.8 min (13.8 mg, 0.9%), and 64.1 min (5.2 mg, 0.3%), respectively; for **15**, 22.2 min (13.4 mg, 1.2%). The 4-OH-monomer **14** was not recovered from the SiO_2 column, probably because of its high polarity. The total mass balance relative to **12** was 92%.

Reaction of 12 with 16. The reaction was conducted analogously, using 0.41 g (2.1 mmol) of $AgBF_4$, 4.40 g (2.12 mmol) of **16**, and 729 mg (850 μmol) of **12**. After filtration over SiO_2 with EtOAc/hexane 1:1, the crude product was taken up in CH_3CN and separated in several portions by preparative HPLC as above to yield the following products: **14** (31 mg, 5%); **15** (34 mg, 6%); trimer (3.07 g, 70% recovery); tetramer (1.47 g, 62%); pentamer (221 mg, 15%); hexamer (57 mg, 5%); heptamer (25.2 mg, 2%); octamer (10.8 mg, 1%). Total mass balance relative to **12**: 96%.

Reaction of 12 with 17. The reaction was conducted analogously, using 0.34 g (1.75 mmol) of $AgBF_4$, 4.77 g (1.73 mmol) of **17**, and 592 mg (690 μmol) of **12**. After filtration over SiO_2 with EtOAc/hexane 1:1, the crude product was subjected in several portions to a preliminary separation by preparative HPLC (column D; 0−30 min, 80 to 100% CH_3CN in H_2O; 30−38 min, CH_3CN; 38−65 min, 10% EtOAc in CH_3CN) to yield the following products: **14** (t_R 11.0 min; 19 mg, 4%); **15** (22.2 min; 47 mg, 10%); tetramer (36.0 min; 3.56 g, 74% recovery); pentamer (39.4 min; 1.03 g); hexamer (43.6 min; 260 mg); heptamer (46.0 min; 86 mg); octamer (48.9 min; 41 mg); nonamer (52.2 min; 22 mg); decamer (56.2 min; 13.5 mg); undecamer (61.4 min; 8.2 mg). All products from the pentamer on required additional purification because of peak tailing, which led to a contamination with lower oligomers that increased with the degree of oligomerization, and because of increasing contamination with unidentified aliphatic material from the nonpolar solvent and/or column. For sample preparation, a small percentage of THF had to be added to the CH_3CN from the heptamer on because of limited solubility in CH_3CN alone. For the pentamer through nonamer, the additional purification was performed on column D (0−30 min, 80 to 100% CH_3CN in H_2O, then CH_3CN), For the decamer and undecamer, column B was used in combination with the same gradient. The nonamer, decamer, and undecamer still contained excessive amounts of aliphatic impurities after this treatment and were subjected to a third HPLC purification on column A using the same gradient. The following yields of pure products (97% or better by HPLC) were obtained: pentamer, 987 mg (41%); hexamer, 226 mg (16%); heptamer, 68 mg (6.1%); octamer, 26 mg (2.7%); nonamer, 11.5 mg (1.3%); decamer, 6.5 mg (0.8%); undecamer, 2.5 mg (0.3%). Total mass balance relative to **12**: 82%.

Tris(3-*O*-acetyl-5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4β,8)$_2$-Trimer (16). This product was also obtained in 97% yield from 412 mg (211 μmol) of **4** and 1 mL each of acetic anhydride and pyridine in a similar manner as described for the acetylation of **3**. Colorless foam; $[\alpha]_D$ +108, $[\alpha]_{546}$ +131 (EtOAc, *c* 19.3 g L^{-1}); ^1H NMR ($CDCl_3$; three rotamers, 60:29:11; selection) δ 6.30 (s), 5.94 (narrow m), 5.87 (s), 5.81 (s), 5.63 (d, 1 H, J = 1.5 Hz), 5.53 (s) (these are the 6 most intense signals in the range of δ 6.6−5.2), 3.11−2.88 (m), 1.72 (s, major + third rotamer), 1.67 (s, third rotamer), 1.55 (third rotamer), 1.45 (major rotamer), 1.36 (second rotamer), 1.27 (major + second rotamer), 1.17 (second rotamer); ^{13}C NMR ($CDCl_3$, TMS; only intense signals below δ 145 and above δ 40) δ 170.18, 169.16, 168.60, 158.05, 156.62, 155.96, 155.81, 155.73, 155.69, 155.62, 155.27, 154.58, 152.65, 149.22, 148.89, 148.56, 148.38, 148.24, 148.13, 34.50, 32.99, 25.76, 20.85, 20.60, 20.31; acetate methyl groups of the second isomer: δ 20.41, 20.21 (× 2); of the third isomer: δ 20.93, 20.80, 20.75. Anal. Calcd for $C_{135}H_{116}O_{21}$: C, 78.17; H, 5.64. Found: C, 78.23 H, 5.58.

Tetrakis(3-*O*-acetyl-5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4β,8)$_3$-Tetramer (17). This product was also obtained in 95% yield from 145 mg (55.8 μmol) of **6** and 0.25 mL each of acetic anhydride and pyridine in a similar manner as described for the acetylation of **3**. Colorless foam: $[\alpha]_D$ +122, $[\alpha]_{546}$ +148 (EtOAc, *c* 11.7 g L^{-1}); ^1H NMR ($CDCl_3$; two rotamers, 74:26; selection) δ 6.50, 6.48, 6.31, 6.22, 6.19, 6.00, 5.97, 5.85, 5.78, 5.76, 5.70, 5.57, 5.56, 5.50, 5.44, 5.43 (multiplicities unknown, all signals reported as s), 3.09-2.84 (m), 1.72 (s, major rotamer), 1.38 (s, minor rotamer), 1.31 (s, major rotamer), 1.24 (s, major rotamer), 1.19 (s, both rotamers), 1.12 (s, minor rotamer), 1.08 (s, minor rotamer); ^{13}C NMR ($CDCl_3$, TMS; only intense signals below δ 145 and above δ 40) δ 170.15, 169.13, 168.98, 168.70, 157.97, 156.51, 156.03, 155.99, 155.70, 155.62, 155.54, 155.36, 155.16, 154.49, 153.28, 152.84, 149.22, 148.92, 148.56, 148.48, 148.37, 148.21, 147.65, 147.51,

34.59, 32.90, 32.13, 25.41, 20.85, 20.34, 20.26 (× 2). Anal. Calcd for $C_{180}H_{154}O_{28}$: C, 78.19; H, 5.61. Found: C, 78.09; H, 5.88.

Pentakis(3-*O*-acetyl-5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4*β*,8)$_4$-Pentamer (18). Colorless foam: $[\alpha]_D$ +124, $[\alpha]_{546}$ +151 (EtOAc, *c* 11.9 g L^{-1}); ^{1}H NMR ($CDCl_3$; two rotamers, 72:28; selection) δ 6.59, 6.56, 6.54, 6.47, 6.44, 6.42, 6.40, 6.33, 6.26, 6.24, 5.96, 5.93, 5.87, 5.83, 5.80, 5.66, 5.57 (d, J = 2 Hz), 5.52 (d, 1 H, J = 1.5 Hz), 5.47, 5.26, 5.21 (multiplicities unknown except for the two narrow d, all other signals reported as s), 3.09-2.86 (m), 1.72 (s, major rotamer), 1.39 (s, minor rotamer), 1.36 (s, major rotamer), 1.20 (s, minor rotamer), 1.15 (s, × 2, major rotamer), 1.10 (s, minor rotamer), 1.08 (s, minor rotamer), 1.06 (s, minor rotamer), 1.03 (s, major rotamer); ^{13}C NMR ($CDCl_3$, TMS; only intense signals below δ 145 and above δ 40) δ 170.13, 169.15, 169.12, 168.89, 168.67, 157.97, 156.53, 156.20, 155.91 (× 2 or × 3), 155.60, 155.46, 155.30, 155.12, 155.03, 154.41, 153.71, 153.20, 153.02, 149.21, 148.90, 148.74, 148.54, 148.36, 148.15, 148.07, 147.82, 147.47, 147.38, 34.75, 32.91, 31.98, 31.72, 25.33, 20.84, 20.40, 20.27, 20.13, 19.95. Anal. Calcd for $C_{225}H_{192}O_{35}$: C, 78.20; H, 5.60. Found: C, 77.89; H, 5.51.

Hexakis(3-*O*-acetyl-5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4*β*,8)$_5$-Hexamer (19). Colorless foam: $[\alpha]_D$ +122, $[\alpha]_{546}$ +147 (EtOAc, *c* 15.4 g L^{-1}); ^{1}H NMR ($CDCl_3$; at least two rotamers, ratio not determinable because of signal overlap; selection) δ 6.66, 6.63, 6.61−6.51, 6.50, 6.47, 6.39, 6.37, 6.31, 6.27, 6.24, 5.98, 5.94, 5.88−5.79, 5.67, 5.61, 5.57 (d, J = 2 Hz), 5.52 (d, J = 1.5 Hz), 5.46, 5.21 (multiplicities unknown except for the two narrow d, all other signals reported as s), 3.09-2.86 (m), 1.71 (s, major rotamer), 1.60 (s, minor rotamer), 1.38 (s, major rotamer), 1.20 (s, minor rotamer), 1.15, 1.10, 1.07, 1.00 (each s, major rotamer); ^{13}C NMR ($CDCl_3$, TMS; only intense signals below δ 145 and above δ 40) δ 170.14, 169.10, 168.99, 168.88, 168.63, 157.96, 156.52, 156.21, 155.87, 155.85, 155.63, 155.58, 155.51, 155.29, 155.21, 155.17, 155.11, 154.41, 153.94, 153.63, 153.19, 153.07, 149.20, 148.89, 148.69, 148.51, 148.41, 148.37, 148.14, 148.05, 147.86, 147.66, 147.47, 147.36, 34.71, 32.89, 31.97, 31.79, 31.57, 25.35, 20.83, 20.43, 20.21, 20.13, 20.00, 19.96. Anal. Calcd for $C_{270}H_{230}O_{42}$: C, 78.20; H, 5.59. Found: C, 78.33; H, 5.38.

Heptakis(3-*O*-acetyl-5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4*β*,8)$_6$-Heptamer (20). Colorless foam: $[\alpha]_D$ +120, $[\alpha]_{546}$ +145 (EtOAc, *c* 10.2 g L^{-1}); ^{1}H NMR ($CDCl_3$; at least two rotamers, ratio not determinable because of signal overlap; selection) δ 6.68, 6.65, 6.58, 6.55, 6.53, 6.50, 6.47, 6.44, 6.41, 6.38, 6.31, 6.26, 6.23, 6.10, 5.98, 5.95, 5.88−5.83, 5.82, 5.78, 5.68, 5.62−5.58, 5.56 (d, J = 2 Hz), 5.54−5.50, 5.47 (multiplicities unknown except for the narrow d, all other signals reported as s), 3.08-2.87 (m), 1.71, 1.38 (each s, major rotamer), 1.20 (s, minor rotamer), 1.16, 1.10, 1.09 (each s, major rotamer), 1.08 (s, minor rotamer), 1.04 (s, major rotamer), 1.01, 1.00 (each s, minor rotamer), 0.95 (major rotamer); ^{13}C NMR ($CDCl_3$, TMS; only intense signals below δ 145 and above δ 40) δ 170.14, 169.12, 169.00, 168.93, 168.66, 157.99, 156.54, 156.45, 155.91, 155.87, 155.7−155.45, 155.35, 155.31, 155.24, 155.13, 154.44, 154.00, 153.92, 153.67, 153.22, 153.09, 149.22, 148.91, 148.69, 148.56, 148.43, 148.16, 148.08, 147.93, 147.8−147.65, 147.50, 147.40, 34.74, 32.94, 31.99, 31.85, 31.69, 31.60, 29.68, 25.40, 20.83, 20.43, 20.23, 20.16, 20.06, 20.03, 19.92.

Octakis(3-*O*-acetyl-5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4*β*,8)$_7$-Octamer (21). Colorless foam: $[\alpha]_D$ +119, $[\alpha]_{546}$ +144 (EtOAc, *c* 8.5 g L^{-1}); ^{1}H NMR ($CDCl_3$; at least two rotamers, ratio not determinable because of signal overlap; selection) δ 6.67, 6.65, 6.60−6.51, 6.48, 6.45, 6.37, 6.31, 6.24, 6.22, 6.09, 6.06, 6.04, 5.98, 5.94, 5.88−5.82, 5.78, 5.66, 5.62−5.56, 5.54−5.50, 5.46 (multiplicities unknown, all signals reported as s), 3.08-2.87 (m), 1.71, 1.38 (each s, major rotamer), 1.20 (s, minor rotamer), 1.15, 1.101, 1.098 (each s, major rotamer), 1.07 (s, minor rotamer), 1.06 (s, major rotamer), 1.02, 1.00 (each s, minor rotamer), 0.98, 0.95 (each s, major rotamer). Anal. Calcd for $C_{360}H_{306}O_{56}$: C, 78.21; H, 5.58. Found: C, 77.79; H, 5.53.

Nonakis(3-*O*-acetyl-5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4*β*,8)$_8$-Nonamer (22). Colorless foam: $[\alpha]_D$ +117, $[\alpha]_{546}$ +142 (EtOAc, *c* 9.2 g L^{-1}); ^{1}H NMR ($CDCl_3$; at least two rotamers, ratio not determinable because of signal overlap; selection) δ 6.67, 6.64, 6.58, 6.56, 6.53, 6.47, 6.45, 6.41, 6.38, 6.36, 6.30, 6.24, 6.22, 6.08, 6.05, 5.98, 5.87, 5.85, 5.83, 5.78, 5.66, 5.60, 5.56 (d, J = 2 Hz), 5.54−5.50, 5.45 (multiplicities unknown except for the narrow d, all other signals reported as s), 3.08-2.87 (m), 1.71, 1.38 (each s, major rotamer), 1.20 (s, minor rotamer), 1.15, 1.10 (× 2), 1.07 (each s, major rotamer), 1.02 (s, minor rotamer), 1.01 (s, major rotamer), 1.00 (minor rotamer), 0.98, 0.95 (each s, major rotamer).

Decakis(3-*O*-acetyl-5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4*β*,8)$_9$-Decamer (23). Colorless foam: $[\alpha]_D$ +114, $[\alpha]_{546}$ +139 (EtOAc, *c* 3.6 g L^{-1}); ^{1}H NMR ($CDCl_3$; at least two rotamers, ratio not determinable because of signal overlap; selection) δ 3.09−2.87 (m), 1.71, 1.38 (each s, major rotamer), 1.20 (s, minor rotamer), 1.15, 1.10 (× 2), 1.07, 1.01 (× 2), 0.98, 0.95 (each s, major rotamer).

Undecakis(3-*O*-acetyl-5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4*β*,8)$_{10}$-Undecamer (24). Colorless foam: $[\alpha]_D$ +108, $[\alpha]_{546}$ +130 (EtOAc, *c* 1.1 g L^{-1}); ^{1}H NMR ($CDCl_3$; at least two rotamers, ratio not determinable because of signal overlap; selection) δ 3.08−2.87 (m), 1.71, 1.38 (each s, major rotamer), 1.20 (s, minor rotamer), 1.15, 1.10 (× 2), 1.07, 1.01 (× 3), 0.98, 0.94 (each s, major rotamer).

Tris(5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4*β*,8)$_2$-Trimer (4). Preparation by hydrolysis of **16**: To a solution of 1.54 g (742 μmol) of **16** in 30 mL of THF was added all at once 5.8 mL (8.9 mmol) of 40% aqueous tetra-*n*-butylammonium hydroxide. The reaction mixture was allowed to stand at room temperature in a closed flask for 94 h, then partially evaporated to remove THF. The residue was diluted with 20 mL of H_2O, the product was extracted into 2 × 20 mL of EtOAc, and the combined organic phases were washed with 10 mL of brine and evaporated. Filtration over a short SiO_2 column with EtOAc yielded, after evaporation and drying in vacuo, 1.44 g (99%) of the product as a colorless foam: $[\alpha]_D$ +72.1, $[\alpha]_{546}$ +86.5 (EtOAc, *c* 6.9 g L^{-1}); ^{1}H NMR ($CDCl_3$; two major rotamers, 55:45; selection) δ 6.45 (dd, 1 H, J = 2, 8.5 Hz), 6.39 (d, 1 H, J = 8.5 Hz), 6.35 (s, 1 H), 6.27 (d, 1 H, J = 2 Hz), 6.18 (s, 1 H), 6.14 (dd, 1 H, J = 2, 8.5 Hz), 6.07 (d, 1 H, J = 2 Hz), 5.95 (d, 1 H, J = 2.5 Hz), 5.90 (s, 1 H), 5.85 (s, 1 H), 5.73 (s, 1 H), 5.70 (d, 1 H, J = 2 Hz), 5.50 (s, 1 H), 3.96 (br d, 1 H, J = 5.5 Hz), 3.64 (br d, 1 H, J = 4 Hz), 3.07−2.78 (m, 2 H, containing B part of an ABq(d) at δ 2.83; J_{AB} = 17.5 Hz, J_d = 4.5 Hz), 1.81 (d, 1 H, J = 6 Hz), 1.69 (d, 1 H, J = 6 Hz), 1.45 (d, 1 H, J = 7.5 Hz), 1.20 (d, 1 H, J = 8 Hz), 1.19 (d, 1 H, J = 8 Hz); ^{13}C NMR ($CDCl_3$, TMS; only intense signals below δ 145 and above δ 40) δ 158.29, 158.09, 158.00, 157.40, 156.70, 156.43, 156.33, 156.17, 156.09, 156.04, 155.97, 155.66, 155.44, 155.24, 154.67, 153.10, 152.83, 152.41, 148.18, 148.96, 148.93, 148.88, 148.76, 148.72, 148.66, 148.36, 148.24, 148.17, 148.04, 36.54, 36.15, 35.44, 28.61, 28.55. Anal. Calcd for $C_{129}H_{110}O_{18}$: C, 79.53; H, 5.69. Found: C, 79.76; H, 6.03.

Tetrakis(5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4*β*,8)$_3$-Tetramer (6). Preparation by hydrolysis of **17**: Reaction of 1.59 g (573 μmol) of **17** with 5.6 mL (8.6 mmol) of 40% aqueous tetra-*n*-butylammonium hydroxide in 29 mL of THF for 96 h as described for the trimer yielded 1.45 g (97%) of **6** as a colorless foam: $[\alpha]_D$ +85.9, $[\alpha]_{546}$ +104 (EtOAc, *c* 12.0 g L^{-1}); ^{1}H NMR ($CDCl_3$; two major rotamers in comparable amounts; selection) δ 6.43−6.35 (m, 4 H), 6.33 (d, 1 H, J = 8.5 Hz), 6.27 (d, 1 H, J = 2 Hz), 6.23 (s, 1 H), 6.14 (dd, 1 H, J = 1.5, 8.5 Hz), 6.09−6.00 (m, 3 H), 5.92−5.87 (m, 3 H), 5.86 (d, 1 H, J = 2 Hz), 5.74 (s, 2 H), 5.63 (d, 1 H, J = 1.5 Hz), 5.53 (s, 1 H), 5.43 (d, 1 H, J = 8 Hz), 5.39−5.37 (m, 2 H), 5.20 (narrow m, 1 H), 3.65 (d, 1 H, J = 3.5 Hz), 2.98−2.80 (m, 2 H), 1.79 (d, 1 H, J = 6 Hz), 1.59 (d, 1 H, J = 6 Hz), 1.43 (d, 1 H, J = 7 Hz), 1.37 (d, 1 H, J = 8 Hz), 1.34 (d, 1 H, J = 7 Hz), 1.14 (d, 1 H, J = 7.5 Hz), 1.05 (d, 1 H, J = 8 Hz); ^{13}C NMR ($CDCl_3$, TMS; only intense signals below δ 145 and above δ 40) δ 158.28, 158.03,

158.01, 157.31, 156.67, 156.56, 156.37, 156.29, 156.15, 156.11, 156.03, 155.96, 155.69, 155.63, 155.35, 155.17, 154.74, 153.22, 153.12, 152.78, 152.75, 149.22, 149.01, 148.93, 148.87, 148.84, 148.82, 148.78, 148.75, 148.53, 148.32, 148.24, 148.09, 148.04, 147.72, 147.63, 36.84, 36.28, 35.61, 35.39, 35.12, 29.64, 28.46. Anal. Calcd for $C_{172}H_{146}O_{24}$: C, 79.55; H, 5.67. Found: C, 79.71; H, 5.43.

Pentakis(5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4*β*,8)$_4$-Pentamer (25). Reaction of 1.81 g (524 μmol) of **18** with 6.9 mL (10.5 mmol) of 40% aqueous tetra-*n*-butylammonium hydroxide in 35 mL of THF for 118 h as described for the trimer yielded 1.45 g (97%) of **25** as a colorless foam. The analytical sample was further purified by preparative HPLC (column B; 0−30 min, 80−100% CH_3CN/H_2O, then CH_3CN; t_R 33.4 min): $[\alpha]_D$ +100, $[\alpha]_{546}$ +121 (EtOAc, *c* 13 g L^{-1}); ^{1}H NMR ($CDCl_3$; apparent multiplicities; weak signals of minor rotamers omitted) δ 7.53 (d, 2 H, J = 7 Hz), 7.5−6.6, 6.57 (s), 6.55 (s), 6.51 (s), 6.49 (s), 6.45 (s), 6.43 (s), 6.39 (s), 6.36 (s), 6.35 (s), 6.334 (s), 6.325 (s), 6.31 (s), 6.28 (s), 6.27 (s), 6.23 (s), 6.17 (d, J = 2 Hz), 6.14 (d, J = 1.5 Hz), 6.08 (s), 6.07 (s), 6.04 (s), 5.95 (s), 5.94 (s), 5.88 (s), 5.87 (d, J = 2 Hz), 5.75 (s), 5.71 (s), 5.63 (d, J = 2 Hz), 5.51 (s), 5.48 (s), 5.43 (s), 5.40 (s), 5.27 (s), 5.22 (s), 5.20 (d, J = 2.5 Hz), 5.17 (d, J = 2 Hz), 5.15−3.95, 3.89 (br d, J = 7 Hz), 3.66 (d, J = 4.5 Hz), 3.0−2.8 (m), 1.80 (d, J = 6 Hz), 1.59 (d, J = 6 Hz), 1.42−1.38, 1.37 (d, J = 7 Hz), 1.32, 1.30 (2 overlapping d), 1.24 (d, J = 7 Hz), 1.14 (d, J = 8 Hz), 1.09 (d, J = 8 Hz); ^{13}C NMR ($CDCl_3$, TMS; only intense signals below δ 145 and above δ 40) δ 158.28, 158.03, 158.01, 157.33, 156.67, 156.58, 156.39, 156.34, 156.27, 156.15, 156.12, 155.99, 155.92, 155.71, 155.44, 155.37, 155.15, 154.77, 153.24, 153.10, 153.03, 152.94, 152.78, 152.72, 149.21, 148.97, 148.93, 148.84, 148.75, 148.67, 148.51, 148.29, 148.26, 148.24, 148.03, 147.82, 147.72, 147.62, 37.00, 36.29, 35.69, 35.43, 35.32, 35.26, 35.08, 34.94, 28.43. Anal. Calcd for $C_{215}H_{182}O_{30}$: C, 79.56; H, 5.65. Found: C, 79.31; H, 5.68.

Hexakis(5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4*β*,8)$_5$-Hexamer (26). Reaction of 486 mg (117 μmol) of **19** with 1.5 mL (2.3 mmol) of 40% aqueous tetra-*n*-butylammonium hydroxide in 8 mL of THF for 101 h as described for the trimer yielded 455 mg (100%) of **26** as a colorless glass which was further purified by preparative HPLC as described for compound **25**: t_R 35.7 min; $[\alpha]_D$ +102, $[\alpha]_{546}$ +123 (EtOAc, *c* 12.2 g L^{-1}); ^{1}H NMR ($CDCl_3$; apparent multiplicities; weak signals of minor rotamers omitted) δ 7.52 (d, J = 7 Hz), 7.46−6.63, 6.61 (s), 6.52 (s), 6.50 (s), 6.44−6.38, 6.36 (s), 6.34 (s), 6.31 (s), 6.23 (s), 6.07 (s), 6.04 (s), 5.96 (s), 5.93 (s), 5.91 (s), 5.90 (s), 5.87 (d, J = 2 Hz), 5.84 (s), 5.76 (s), 5.75 (s), 5.63 (d, J = 2 Hz), 5.49 (s), 5.47 (s), 5.44 (s), 5.41 (s), 5.40 (s), 5.37 (s), 5.27 (s), 5.22−3.87, 3.66 (dd, J = 1.5, 4.5 Hz), 2.96−2.82, 1.80 (d, J = 6 Hz), 1.59 (d, J = 6 Hz), 1.40 (d, J = 5 Hz), 1.34 (d, J = 7 Hz), 1.32−1.20, 1.13 (d, J = 8 Hz), 1.11 (d, J = 8 Hz); ^{13}C NMR ($CDCl_3$, TMS; only intense signals below δ 145 and above δ 40) δ 158.26, 158.03, 157.99, 157.31, 156.66, 156.56, 156.36, 156.20−155.88, 155.83, 155.70, 155.39, 155.37, 155.13, 154.78, 153.25, 153.22, 153.14, 153.10, 153.02, 152.96, 152.74, 149.20, 148.98, 148.78, 148.73, 148.66, 148.50, 148.25, 148.01, 147.93, 147.81, 147.76, 147.73, 147.63, 37.01, 36.29, 35.69, 35.50−35.00, 28.42. Anal. Calcd for $C_{258}H_{218}O_{36}$: C, 79.57; H, 5.64. Found: C, 79.59; H, 5.57.

Heptakis(5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4*β*,8)$_6$-Heptamer (27). Reaction of 126 mg (26.1 μmol) of **20** with 0.34 mL (0.52 mmol) of 40% aqueous tetra-*n*-butylammonium hydroxide in 1.8 mL of THF for 94 h as described for the trimer yielded 118 mg (100%) of the product as a colorless foam: $[\alpha]_D$ +105, $[\alpha]_{546}$ +127 (EtOAc, *c* 8.0 g L^{-1}); ^{1}H NMR ($CDCl_3$; apparent multiplicities; selection) δ 6.53 (s), 6.51 (s), 6.43−6.36, 6.33 (s), 6.31 (s), 6.28−6.14, 6.07 (s), 6.05−6.02, 5.95 (s), 5.92(s), 5.90−5.85, 5.76 (s), 5.75 (s), 5.63 (d, J = 2 Hz), 5.48 (s), 5.40 (s), 5.38 (s), 5.27 (s), 2.95−2.82 (m), 1.81 (d, J = 6 Hz), 1.62 (d, J = 6 Hz), 1.45−1.40, 1.36 (d, J = 7 Hz), 1.33−1.20, 1.15 (d, J = 8 Hz); ^{13}C NMR ($CDCl_3$, TMS; only intense signals below δ 145 and above δ 40) δ 158.29, 158.05, 158.02, 157.34, 156.68, 156.58, 156.40, 156.34, 156.28, 156.16, 156.12, 156.04−155.92, 155.83, 155.72, 155.42, 155.39, 155.16, 154.81, 153.33, 153.28, 153.25, 153.17, 153.12, 153.09, 152.78, 149.23, 149.00−148.82, 148.75, 148.69, 148.53, 148.33, 148.27, 148.05, 148.01, 147.85, 147.80−147.74, 147.70, 147.66, 37.03, 36.31, 35.71, 35.47−35.21, 35.13, 28.46. Anal. Calcd for $C_{301}H_{254}O_{42}$: C, 79.57; H, 5.64. Found: C, 79.50; H, 5.44.

Octakis(5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4*β*,8)$_7$-Octamer (28). Reaction of 41.2 mg (26.1 μmol) of **21** with 0.10 mL (0.15 mmol) of 40% aqueous tetra-*n*-butylammonium hydroxide in 0.5 mL of THF for 126 h as described for the trimer yielded 39.4 mg (102%) of the product as a colorless foam: $[\alpha]_D$ +109, $[\alpha]_{546}$ +131 (EtOAc, *c* 10.8 g L^{-1}).

Nonakis(5,7,3′,4′-tetra-*O*-benzyl)epicatechin (4*β*,8)$_8$-Nonamer (29). Reaction of 17.9 mg (2.88 μmol) of **22** with 47 μL (72 μmol) of 40% aqueous tetra-*n*-butylammonium hydroxide in 0.3 mL of THF for 134 h as described for the trimer yielded 16.8 mg (100%) of the product as a colorless foam: $[\alpha]_D$ +110, $[\alpha]_{546}$ +133 (EtOAc, *c* 7.8 g L^{-1}). Anal. Calcd for $C_{387}H_{326}O_{54}$: C, 79.58; H, 5.63. Found: C, 79.79; H, 5.34.

Epicatechin (4*β*,8)$_2$-Trimer (30). To a solution of 64.3 mg (33.0 μmol) of **4** in 5 mL of THF were added 5 mL of MeOH, 0.25 mL of H_2O, and 57 mg of 20% $Pd(OH)_2/C$. The mixture was stirred under 1 bar of H_2 for 80 min and filtered over cotton. The filtration residue was washed with 2 × 10 mL of MeOH. The combined filtrates were evaporated, and the residue was taken up in 10 mL of HPLC-grade H_2O. The solution was filtered and lyophilized to yield 32.4 mg (101%) of **30**·6H_2O as a fluffy, amorphous, off-white solid: $[\alpha]_D$ +70.4, $[\alpha]_{546}$ +84.4 (MeOH, *c* 2.2 g L^{-1}); (ref 4c: $[\alpha]_D$ +75.2, acetone, *c* 8.7 g L^{-1}; ref 4d: $[\alpha]_{578}$ +90, MeOH, *c* 2 g L^{-1}; ref 6: $[\alpha]_D$ +76.4, acetone, *c* 8.6 g L^{-1}; ref 19b: $[\alpha]_{578}$ +92, H_2O, *c* 1.9 g L^{-1}; ref 19k: $[\alpha]_D$ +80, MeOH, *c* 1.6 g L^{-1}); ^{13}C NMR (CD_3OD, TMS; δ 60−85 region only) δ 79.73, 77.08, 73.47, 72.94, 66.84; MS (API/ES) *m/z* 865.0 (calcd for $[M - H]^-$ 865.2). Anal. Calcd for $C_{45}H_{38}O_{18}$·6H_2O: C, 55.44; H, 5.17. Found: C, 55.71; H, 5.07.

Epicatechin (4*β*,8)$_3$-Tetramer (31). To a solution of 56 mg (21.6 μmol) of **6** in 4 mL of THF were added 4 mL of MeOH, 0.2 mL of H_2O, and 47 mg of 20% $Pd(OH)_2/C$. The mixture was stirred under 1 bar of H_2 for 75 min and filtered over cotton. The filtration residue was washed with 2 × 5 mL of MeOH. The combined filtrates were diluted with 5 mL of HPLC-grade H_2O and partially evaporated to remove organic solvents. After dilution with another 10 mL of HPLC-grade H_2O, the solution was filtered and lyophilized to yield 24.4 mg (89%) of **31**·6H_2O as a fluffy, amorphous, off-white solid: $[\alpha]_D$ +93.3, $[\alpha]_{546}$ +114 (MeOH, *c* 9.3 g L^{-1}) (ref 4d: $[\alpha]_{578}$ +73.2, MeOH, *c* 3.7 g L^{-1}; ref 4j: $[\alpha]_D$ +59.8, acetone, *c* 12 g L^{-1}; ref 6: $[\alpha]_D$ +109.5, acetone, *c* 12.3 g L^{-1}; ref 19i: $[\alpha]_D$ +89.2, acetone, *c* 9 g L^{-1}; ref 19l: $[\alpha]_D$ +81, MeOH, *c* 1.1 g L^{-1}); MS (API/ES) *m/z* 1153.3 (calcd for $[M-H]^-$: 1153.3). Anal. Calcd for $C_{60}H_{50}O_{24}$·6H_2O: C, 56.96; H, 5.10. Found: C, 56.98; H, 4.83.

Epicatechin (4*β*,8)$_4$-Pentamer (32). To a solution of 76 mg (23.4 μmol) of **25** in 4 mL THF were added 4 mL of MeOH, 0.2 mL of H_2O, and 60 mg of 20% $Pd(OH)_2/C$. The mixture was stirred under 1 bar of H_2 for 2 h and filtered over cotton. The filtration residue was washed with MeOH, and the combined filtrates were partially evaporated to remove organic solvents. The residue was diluted with 10 mL of HPLC-grade H_2O, filtered, and lyophilized to produce 34.8 mg of the title compound as a fluffy, amorphous, off-white solid: $[\alpha]_D$ +116, $[\alpha]_{546}$ +140 (MeOH, *c* 8.3 g L^{-1}) (ref 4d: $[\alpha]_{578}$ +96, MeOH, *c* 1 g L^{-1}; ref 19i: $[\alpha]_D$ +102.1, acetone, *c* 10 g L^{-1}; ref 19l: $[\alpha]_D$ +102, MeOH, *c* 1.2 g L^{-1}); MS (API/ES) *m/z* 1441.4 (calcd for $[M - H]^-$ 1441.3). Anal. Calcd for $C_{75}H_{62}O_{30}$·7.5H_2O: C, 57.07; H, 4.92. Found: C, 56.99; H, 4.79.

Epicatechin (4*β*,8)$_5$-Hexamer (33). To a solution of 92.3 mg (23.7 μmol) of **26** in 8 mL of THF were added 8 mL of MeOH, 0.4 mL of H_2O, and 169 mg of 20% $Pd(OH)_2/C$. The mixture was stirred under 1 bar of H_2 for 50 min and filtered

over cotton. The filtration residue was washed with MeOH, and the combined filtrates were partially evaporated after addition of 10 mL of HPLC-grade H_2O. The residue was diluted with another 20 mL of HPLC-grade H_2O, filtered, and lyophilized to produce 47.4 mg of the title compound as a fluffy, amorphous, off-white solid: $[\alpha]_D$ +123, $[\alpha]_{546}$ +149 (MeOH, *c* 8.6 g L^{-1}); MS (API/ES) *m/z* 1730.3 (calcd for $[M - H]^-$ 1729.4). Anal. Calcd for $C_{90}H_{74}O_{36}\cdot 9.2H_2O$: C, 56.98; H, 4.91. Found: C, 56.89; H, 4.61.

Epicatechin (4*β*,8)$_6$-Heptamer (34). To a solution of 87.5 mg (19.3 μmol) of **27** in 8 mL of THF were added 8 mL of MeOH, 0.4 mL of H_2O, and 111 mg of 20% $Pd(OH)_2/C$. The mixture was stirred under 1 bar of H_2 for 1 h and filtered over cotton. The filtration residue was washed with MeOH, and the combined filtrates were partially evaporated after addition of 10 mL of HPLC-grade H_2O. The residue was diluted with another 10 mL of HPLC-grade H_2O, filtered, and lyophilized to produce 39.3 mg of the title compound as a fluffy, amorphous, off-white solid: $[\alpha]_D$ +134, $[\alpha]_{546}$ +164 (MeOH, *c* 9.6 g L^{-1}). Anal. Calcd for $C_{105}H_{86}O_{42}\cdot 10H_2O$: C, 57.33; H, 4.86. Found: C, 57.49; H, 4.80

Epicatechin (4*β*,8)$_7$-Octamer (35). To a solution of 35.7 mg (6.88 μmol) of **28** in 3 mL of THF were added 3 mL of MeOH, 0.15 mL of H_2O, and 57 mg of 20% $Pd(OH)_2/C$. The mixture was stirred under 1 bar of H_2 for 55 min and filtered over cotton. The filtration residue was washed with MeOH, and the combined filtrates were partially evaporated after addition of 10 mL of HPLC-grade H_2O. The residue was diluted with another 10 mL of HPLC-grade H_2O, filtered, and lyophilized to produce 17.1 mg of the title compound as a fluffy, amorphous, off-white solid: $[\alpha]_D$ +148, $[\alpha]_{546}$ +180 (MeOH, *c* 5.2 g L^{-1}). Anal. Calcd for $C_{120}H_{98}O_{48}\cdot 10.7H_2O$: C, 57.66; H, 4.77. Found: C, 57.68; H, 4.79.

Per-*O*-acetylepicatechin (4*β*,8)$_2$-Trimer (36). A mixture of 0.25 mL of pyridine and 0.25 mL of acetic anhydride was added to 30.6 mg (35.3 μmol) of **30**. The reaction mixture was stirred at room temperature for 27 h, 20 mL of saturated aqueous $NaHCO_3$ solution was added, and the product was extracted into 3 × 10 mL of EtOAc. The organic layers were washed with 30 mL of H_2O, 20 mL of 0.5 M H_3PO_4, 10 mL of H_2O, and 10 mL of saturated aqueous $NaHCO_3$ solution, and dried over $MgSO_4$. After filtration over a short SiO_2 column with EtOAc/hexane 85:15, the crude product was purified by preparative HPLC (column E, EtOAc/hexane 85:15); t_R 12.2 min. Evaporation and drying in vacuo yielded 28.6 mg (54%) of the peracetate as a colorless glass: $[\alpha]_D$ +57.0, $[\alpha]_{546}$ +69.8 (EtOAc, *c* 9.2 g L^{-1}); $[\alpha]_D$ +55.3, $[\alpha]_{546}$ +67.7 (acetone, *c* 10.4 g L^{-1}) (ref 19b: $[\alpha]_{578}$ +51.6, acetone, *c* 3.8 g L^{-1}); ^{1}H NMR ($CDCl_3$; two rotamers, ratio 2:1; acetate signals only) δ 2.38, 2.32 (× 2), 2.30, 2.30−2.25, 2.23, 2.218 (× 2), 2.215 (× 2), 2.164, 2.158, 2.02 (× 2), 1.95 (× 3), 1.89, 1.88 (× 4), 1.82 (× 2), 1.79 (× 2), 1.71, 1.55 (× 2), 1.50, 1.38 (× 2) (The complete ^{1}H NMR spectrum has been reported: refs 6, 19f); ^{13}C NMR ($CDCl_3$, TMS; only signals below δ 145 and above δ 40) δ 170.10, 170.06, 169.94, 169.07, 169.00, 168.80, 168.77, 168.68, 168.54, 168.40, 168.35, 168.29, 168.14, 168.07, 168.01, 167.99, 167.94, 167.90, 167.87, 167.80, 167.76, 167.68, 167.61, 167.57, 167.43, 155.76, 154.88, 154.06, 151.86, 151.84, 151.73, 149.88, 149.84, 149.78, 148.79, 148.58, 148.49, 148.29, 147.96, 147.58, 147.17, 146.89, 35.61, 35.04, 34.36, 32.96, 26.70, 26.41, 21.16, 21.05, 20.97, 20.76, 20.72−20.56, 20.54, 20.40, 20.26, 20.21, 20.13, 20.11, 20.05, 19.78, 19.72, 19.48. Anal. Calcd for $C_{75}H_{68}O_{33}$: C, 60.16; H, 4.58. Found: C, 59.80; H, 4.41.

Per-*O*-acetylepicatechin (4*β*,8)$_3$-Tetramer (37). A 102 mg (39.2 μmol) sample of **6** was deprotected as described above. The crude product solution was evaporated to dryness, and a mixture of 0.3 mL of pyridine and 0.3 mL of acetic anhydride was added to the residue. The reaction mixture was kept in a closed flask at room temperature for 41 h, and then 1 mL of CH_2Cl_2 was added followed by 1 mL of MeOH. After another 80 min at room temperature, 20 mL of 0.5 M H_3PO_4 was added, and the product was extracted into 3 × 20 mL of EtOAc. The combined organic phases were washed with 30 mL of H_2O and 20 mL of saturated aqueous $NaHCO_3$ solution and dried over $MgSO_4$. The evaporation residue was prepurified by filtration over SiO_2 with EtOAc and then subjected to preparative HPLC (column D; 0−45 min, 40 to 80% CH_3CN in H_2O); t_R 30.0 min. Evaporation and drying in vacuo yielded 40.6 mg (52%) of the peracetate as a glass: $[\alpha]_D$ +82.2, $[\alpha]_{546}$ +101 (EtOAc, *c* 18.7 g L^{-1}); ^{1}H NMR ($CDCl_3$; two rotamers, ratio 3:2; acetate signals only) δ 2.38, 2.32, 2.29, 2.28−2.21, 2.18, 2.17, 2.04, 1.991 (× 2), 1.988, 1.96, 1.94, 1.91, 1.88, 1.87 (× 2), 1.86, 1.85, 1.79, 1.77, 1.64, 1.52, 1.44, 1.433, 1.428, 1.35 (The complete ^{1}H NMR spectrum has been reported: ref 6); ^{13}C NMR ($CDCl_3$, TMS; only signals below δ 145 and above δ 40) δ 170.12, 169.91, 169.13, 169.01, 168.89, 168.79, 168.69, 168.50, 168.40, 168.36, 168.30, 168.28, 168.16, 168.10, 168.08−168.00, 167.98, 167.94, 167.89, 167.87−167.77, 167.74, 167.71, 167.59, 155.84, 154.89, 154.05, 151.88, 151.85, 151.81, 151.65, 151.56, 149.91, 149.87, 149.83, 148.93, 148.72, 148.59, 148.52, 148.46, 147.99, 147.91, 147.44, 147.35, 147.25, 147.13, 35.70, 35.25, 35.17, 34.25, 32.91, 26.33, 21.18, 21.03, 20.94, 20.76, 20.74, 20.72−20.50, 20.45, 20.42, 20.30, 20.18, 20.16, 20.09, 20.03, 19.97, 19.65, 19.58, 19.50, 19.32.

Per-*O*-acetylepicatechin (4*β*,8)$_4$-Pentamer (38). A mixture of 0.25 mL of pyridine and 0.25 mL of acetic anhydride was added to 47.4 mg (32.8 μmol) of **32**. The reaction mixture was kept in a closed flask at room temperature for 50 h, and then 1.5 mL of CH_2Cl_2 was added followed by 0.5 mL of MeOH. After another 1 h at room temperature, 10 mL of 0.5 M H_3PO_4 was added, and the product was extracted into 2 × 10 mL of EtOAc. The combined organic phases were washed with 10 mL of H_2O and 10 mL of saturated aqueous $NaHCO_3$ solution and dried over $MgSO_4$. The evaporation residue was prepurified by filtration over SiO_2 with EtOAc and then subjected to preparative HPLC (column B; 0−30 min, 40−80% CH_3CN in H_2O, then 80%); t_R 30.5 min. Evaporation and drying in vacuo yielded 37.6 mg (46%) of the peracetate as a glass: $[\alpha]_D$ +106, $[\alpha]_{546}$ +129 (EtOAc, *c* 11.3 g L^{-1}); ^{1}H NMR ($CDCl_3$; two rotamers, ratio 3:2; acetate signals only) δ 2.38, 2.32, 2.31, 2.29−2.21, 2.18, 2.17, 2.04, 2.00, 1.99−1.96, 1.94, 1.92, 1.90, 1.88, 1.87, 1.83, 1.80, 1.79, 1.78, 1.70, 1.53, 1.43, 1.42, 1.41, 1.39, 1.38, 1.33; ^{13}C NMR ($CDCl_3$, TMS; only signals below δ 145 and above δ 40) δ 170.12, 170.08, 169.93, 169.15, 169.00, 168.96, 168.85, 168.70, 168.60−168.44, 168.40, 168.32, 168.26, 168.18, 168.12, 168.10−167.98, 167.93−167.79, 167.72, 167.66, 167.63, 155.86, 154.89, 154.05, 151.90−151.74, 151.72−151.62, 149.91, 149.87, 149.82, 148.90, 148.84, 148.76−148.42, 147.99, 147.93, 147.87, 147.48, 147.41, 147.36, 147.27, 147.22, 147.16, 147.02, 146.96, 35.56, 35.41, 35.11, 34.24, 32.91, 26.35, 21.18, 21.04, 20.96, 20.76, 20.70−20.52, 20.48, 20.43, 20.32−20.02, 19.69, 19.51, 19.34, 19.28. Anal. Calcd for $C_{125}H_{112}O_{55}$: C, 60.19; H, 4.53. Found: C, 60.07; H, 4.35.

Per-*O*-acetylepicatechin (4*β*,8)$_5$-Hexamer (39). A mixture of 0.27 mL of pyridine and 0.27 mL of acetic anhydride was added to 39.5 mg of **33**. The reaction mixture was kept in a closed flask at room temperature for 52 h, and then 2 mL of EtOAc was added followed by 1 mL of MeOH. After another 80 min at room temperature, 20 mL of 0.5 M H_3PO_4 was added, and the product was extracted into 3 × 10 mL of EtOAc. The combined organic phases were washed with 20 mL of H_2O and 10 mL of saturated aqueous $NaHCO_3$ solution. The evaporation residue was prepurified by filtration over SiO_2 with EtOAc and then subjected to preparative HPLC (column D; 0−30 min, 40−80% CH_3CN in H_2O, then 80%); t_R 32.5 min. Evaporation and drying in vacuo yielded 34.4 mg (58% over 2 steps) of the peracetate as a glass: $[\alpha]_D$ +126, $[\alpha]_{546}$ +153 (EtOAc, *c* 5.5 g L^{-1}); ^{1}H NMR ($CDCl_3$; two rotamers, ratio 3:2; acetate signals only) δ 2.38, 2.32, 2.30−2.26, 2.25, 2.24, 2.235−2.21, 2.18, 2.17, 2.04, 2.01, 1.99, 1.98−1.97, 1.96, 1.95, 1.93, 1.925, 1.895, 1.89, 1.88, 1.875, 1.86, 1.835, 1.83, 1.785, 1.78, 1.71, 1.435, 1.43, 1.38, 1.37, 1.365; ^{13}C NMR ($CDCl_3$, TMS; only signals below δ 145 and above δ 40) δ 170.12, 170.08, 169.95, 169.14, 169.01, 168.96, 168.90, 168.82, 168.71, 168.59, 168.54, 168.47, 168.39,

168.32, 168.25, 168.20, 168.12, 168.06, 168.01, 167.95, 167.87, 167.82, 167.77−167.67, 167.63, 155.86, 154.90, 154.06, 151.88, 151.82, 151.79, 151.76, 151.66, 149.90, 149.86, 149.82, 148.86, 148.75−148.68, 148.65, 148.60, 148.51, 147.98, 147.93, 147.47, 147.43, 147.28, 147.05, 35.6−35.0, 34.25, 32.91, 26.36, 21.18, 21.05, 20.97, 20.78, 20.74−20.52, 20.48, 20.43, 20.29, 20.24−20.02, 19.68, 19.57−19.50, 19.35, 19.29. Anal. Calcd for $C_{150}H_{134}O_{66}$: C, 60.20; H, 4.51. Found: C, 60.44; H, 4.20.

Per-*O*-acetylepicatechin (4*β*,8)$_6$-Heptamer (40). A mixture of 0.25 mL of pyridine and 0.25 mL of acetic anhydride was added to 34.8 mg of **34**. The reaction mixture was kept in a closed flask at room temperature for 44 h, and then 1 mL of EtOAc was added followed by 0.5 mL of MeOH. After another 1.5 h at room temperature, 20 mL of 0.5 M H_3PO_4 was added, and the product was extracted into 3 × 10 mL of EtOAc. The combined organic phases were washed with 20 mL of H_2O and 10 mL of saturated aqueous $NaHCO_3$ solution. The evaporation residue was prepurified by filtration over SiO_2 with EtOAc and then subjected to preparative HPLC (column D; 0−30 min, 40−80% CH_3CN in H_2O, then 80%); t_R 29.6 min. Evaporation and drying in vacuo yielded 24.6 mg (41% over two steps) of the peracetate as a glass: $[\alpha]_D$ +140, $[\alpha]_{546}$ +170 (EtOAc, *c* 11.4 g L^{-1}); ^{1}H NMR ($CDCl_3$; two rotamers, ratio 3:2; acetate signals only) δ 2.38, 2.32, 2.30−2.22, 2.18, 2.17, 2.04, 2.00, 1.99−1.96, 1.93, 1.92, 1.91−1.87, 1.83, 1.825, 1.785, 1.78, 1.71, 1.435, 1.43, 1.39, 1.38, 1.37, 1.36, 1.34, 1.33.

Per-*O*-acetylepicatechin (4*β*,8)$_7$-Octamer (41). A mixture of 0.1 mL of pyridine and 0.1 mL of acetic anhydride was added to 14.5 mg of **35**. The reaction mixture was kept in a closed flask at room temperature for 44 h, and then 0.5 mL of EtOAc was added followed by 0.25 mL of MeOH. After another 70 min at room temperature, the solution was evaporated, and the evaporation residue was prepurified by filtration with EtOAc over a short SiO_2 column to the top of which a small amount of *p*-toluenesulfonic acid had been applied (as a solution in EtOAc + a minimum volume of MeOH). The crude product was subjected to preparative HPLC (column D; 0−30 min, 40 to 80% CH_3CN in H_2O, then 80%); t_R 33.6 min. Evaporation and drying in vacuo yielded 7.9 mg (34% over 2 steps) of the peracetate as a glass: $[\alpha]_D$ +150, $[\alpha]_{546}$ +184 (EtOAc, *c* 4.0 g L^{-1}); ^{1}H NMR ($CDCl_3$; two rotamers, ratio 3:2; acetate signals only) δ 2.38, 2.32, 2.30−2.21, 2.18, 2.17, 2.04, 2.01−1.96, 1.93, 1.92, 1.91−1.87, 1.83, 1.825, 1.79, 1.78, 1.71, 1.53, 1.44−1.42, 1.39, 1.38, 1.37, 1.36, 1.35, 1.33.

Cell Lines. The human breast cancer cell lines MCF-7, SKBR-3, MDA 435, and MDA MB 231 were obtained from the Lombardi Cancer Center Cell Culture Core Facility at Georgetown University Medical Center. The MDA MB 231 cell line is *P53* defective, *ER* negative, and constitutively expresses *K-ras*. Cells were cultured in T-75s in IMEM medium supplemented with 10% FBS in a humidified 5% CO_2 atmosphere at 37 °C.

Cytotoxicity Assay. Cytotoxicity assays were performed on several human breast cancer cell lines treated with test compounds in a 96-well microtiter plate format using the microculture tetrazolium assay[28] modified for use with crystal violet rather than MTT. Briefly, (1−2) × 10^3 cells were added per well and allowed to culture in a humidified, 5% CO_2 atmosphere until they reached approximately 50% confluence. Sterile filtered test compounds were added at various concentrations, and the plates were allowed to culture for an additional 12−36 h. The growth medium was then removed, and each well was washed twice with 200 μL each of pH 7.4 PBS. After washing, 50 μL of filtered crystal violet solution (2.5 g/125 mL of methanol + 375 mL of H_2O) was added. At the end of 5 min, the crystal violet was removed, and the plate was washed three times with H_2O. Plates were allowed to dry, and the crystal violet stained cells were resolubilized in 100 μL of 0.1 M sodium citrate in ethanol/H_2O (1:1, v/v). At the end of 1 h, the plates were scanned at 570 nm (ref 405 nm) with a microtiter plate reader, and the data were recorded with the SOFTMAX software program. The average of three readings was taken for each blank, control, vehicle, and test concentration for statistical data manipulation.

Flow Cytometry. MBA MB 231 cells were cultured as described above until they reached approximately 50% confluence. Sterile filtered test compound or catalase adjusted to 100 U/mL or heat-inactivated catalase (solution immersed in boiling water for 15 min) or H_2O_2 was then added, and the cells were allowed to culture for an additional 24 h. The cells were then trypsinized and counted, and 1.5 × 10^6 cells were taken for cell cycle analysis by the Vindelov method.[29] Analyses were performed by the Lombardi Cancer Center Flow Cytometry Core Facility at Georgetown University Medical Center.

Annexin V-FITC. The annexin V-FITC assay was performed on procyanidin-treated MDA MB 231 cells using the TACS Annexin V-FITC kit according to the manufacturer's procedure.

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Supporting Information Available: Preparation/characterization of compounds **2**, **5**, **9**, **10b**, **11**, **14**, **15**, **42-44**, and **46-52**; IR spectra; selections from the ^{1}H NMR spectra of compounds **37-41**; selections from the ^{13}C NMR spectra of compounds **36-39** (pdf). This material is available free of charge via the Internet at http://pubs.acs.org.

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