Studies in Polyphenol Chemistry and Bioactivity. 1. Preparation of Building Blocks from (+)-Catechin. Procyanidin Formation. Synthesis of the Cancer Cell Growth Inhibitor, 3-*O*-Galloyl-(2*R*,3*R*)-epicatechin-4β,8-[3-*O*-galloyl-(2*R*,3*R*)-epicatechin]

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Abstract: A project has been initiated to synthesize proanthocyanidin oligomers found in cocoa. Natural, readily available (+)-catechin was transformed into 5,7,3′,4′-tetra-*O*-benzyl-(−)-epicatechin (**14**) by (a) benzylation of the phenolic oxygens; (b) oxidation of the 3-alcohol to ketone by the Dess−Martin periodinane; and (c) reduction with lithium tri-*sec*-butylborohydride (L-Selectride) in the presence of LiBr. The additive diminishes the extent of ketone enolization while maintaining a stereoselectivity of ≥200:1. Oxidation of **14** with DDQ was performed best from the standpoint of product purification if ethylene glycol was used as the nucleophilic trapping agent. The resulting ether **19** was condensed with **14** using $TiCl_4$ to give a good yield of benzyl-protected epicatechin-4β,8-epicatechin (octa-*O*-benzylprocyanidin B_2, **20**) as the sole dimeric product. Hydrogenolysis of **20** yielded procyanidin B_2 in the first enantiospecific synthesis of this natural product which employs protected intermediates and thereby allows the necessary product separation after the condensation step to be performed on nonpolar, nonsensitive intermediates. Acylation of **20** with tri-*O*-benzylgalloyl chloride followed by hydrogenolysis gave access to the title bis-gallate (**24**). This constitutes the first synthesis of this natural product, a compound notable for its PKC-inhibitory and anticancer activity.

Introduction

Polyphenols are an important class of natural products.[1] They are ubiquitous in the plant kingdom and thus enter the human diet[2] and are found in traditional herbal medicines.[3] As a consequence of their multiple polar functionality, they interact strongly but often unselectively with proteins, frequently resulting in the precipitation of insoluble protein−polyphenol complexes.[4] This reaction is the basis of the tanning process of leather, for which reason certain classes of polyphenols are commonly referred to as tannins. In addition, polyphenols readily react with one-electron oxidants, resulting in powerful free-radical scavenging (antioxidant) activity,[5] and they complex Fe^{2+} which is an initiator of radical formation.[6] On the other hand, certain polyphenols may under certain conditions exhibit the opposite (prooxidant) effect.[7] Cell damage by free radicals is an important cause of various diseases. While protein binding and radical scavenging are widespread properties among

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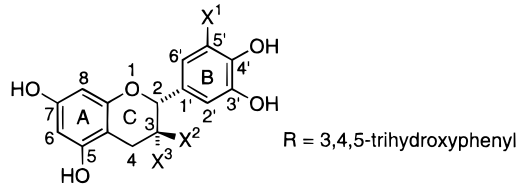

1	X^1 = H	X^2 = OH	X^3 = H	catechin
2	X^1 = H	X^2 = H	X^3 = OH	epicatechin
3	X^1 = H	X^2 = H	X^3 = OCOR	epicatechin 3-gallate
4	X^1 = OH	X^2 = H	X^3 = OH	epigallocatechin
5	X^1 = OH	X^2 = H	X^3 = OCOR	epigallocatechin 3-gallate

Figure 1. Structures and atom numbering of catechin and related natural products.

polyphenols, little information was available until recently as to whether polyphenols also participate in selective interactions with enzymes or receptors. It has now, however, been shown that polyphenols are indeed capable of such selective interactions. A variety of eukaryote protein kinases including PKC are inhibited by *Ribes nigrum* condensed tannin with IC_{50} values ranging from 9 nM to 16 μM, and the IC_{50} values for three out of four kinases vary by an order of magnitude for different condensed tannin preparations.[8] Specific intermolecular NOE cross-peaks are found in solutions containing both (+)-catechin (**1**) (Figure 1) and any of several small peptides, indicating the existence of well-defined binding interactions.[9] Rough binding data have been obtained for 20 phenolic compounds at 16 receptors, and activities ranging from a high level of binding to no binding at all have been observed both for certain receptors when combined with different polyphenols and for certain polyphenols when combined with different receptors.[10] Numerous other biological activities have been reported for polyphenols; for example, they inhibit viral reverse transcriptase,[11] inhibit the replication of HIV 1 in vitro,[12] reduce the risk of heart disease,[13] suppress ulcer formation,[14] are antimutagenic,[15] neuroprotective,[16] antiinflammatory,[17] antibacterial,[18] and hypotensive,[19] and inhibit the growth of cancer cells.[5b,20] Epigallocatechin gallate (**5**), a polyphenol abundant in green tea, inhibits the growth of human PC-3 and LNCaP 104-R prostate tumor cells and of human MCF-7 mammary cancer cells in nude mice while the structurally related compounds, epicatechin gallate (**3**) and epigallocatechin (**4**), are inactive.[21] A number of polyphenols and crude plant extracts containing such compounds inhibit tumor promotion (or biochemical markers thereof) by 12-*O*-tetradecanoylphorbol-13-acetate in mouse epidermis in vivo.[22] Additional mechanisms of action besides radical scavenging or nonspecific protein binding are believed to be operative in the anticancer activity of at least some compounds. The antitumor polyphenol coriariin A stimulates the secretion of interleukin-1β and tumor necrosis factor-α, and the latter protein has been proposed to be responsible for the observed activity.[23] Epigallocatechin and epigallocatechin gallate have been shown not only to inhibit leukemia cell growth but to induce apoptosis.[24] Epigallocatechin gallate inhibits urokinase, an enzyme crucial for cancer growth.[25] These and related findings explain the increasing popularity of polyphenol preparations, such as extracts of green tea, grape seeds, and pine bark, as dietary supplements, even though the investigation of absorption and bioavailability has lagged epidemiological studies

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and in vitro experiments,[26] and the economic importance of polyphenols for health-related products can no longer be dismissed.

One major class of polyphenolic natural products, the hydrolyzable tannins, are derived from carbohydrates by esterification with tannic acid or more complex polyhydroxylated aromatic carboxylic acids. The second important group, and the one on which our current interest is focused, is derived from flavan-3-ols bearing one or several additional hydroxyl groups on their aromatic rings. Eleven different hydroxylation patterns of the A and B rings have been found in nature.[27] Flavan-3-ols are biosynthetically derived from (2*S*)-phenylalanine via flavan-3,4-diols.[28] These latter intermediates readily form a highly stabilized carbenium ion (or quinone methide)[29] in position 4 which attacks the A ring of a flavan-3-ol in what is essentially a Friedel−Crafts alkylation process, forming an interflavan bond.[30] This process can be repeated once or several times, resulting in chain-type oligomers which together with the dimers are known as nonhydrolyzable tannins, condensed tannins, or proanthocyanidins. The structural complexity of these compounds rapidly increases with their chain length as a consequence of different hydroxylation patterns and C-3 stereochemistry in the monomer units and different regio- and stereochemistries of the interflavan linkages, as well as additional structural modifications. In addition, chain branching may occur by alkylation of a monomer unit in both its 6- and 8-positions. The isolation of pure compounds from natural sources thus becomes increasingly difficult with increasing degree of oligomerization. Degradation by thiolysis[31] permits identification of the underlying building blocks but the task of elucidating the position and stereochemistry of the interflavan linkages is nontrivial. Both of these factors have resulted in few defined oligomers above the tetramer level being described in the literature. Proanthocyanidins and their parent monomers also occur naturally in the form of a variety of derivatives, for example, glycosides or esters with hydroxylated aromatic carboxylic acids, such as gallic or hexahydroxydiphenic acid. An example is known in which a gallate derivative exhibits anticancer activity not found in the parent polyphenol.[21]

Among the proanthocyanidins, two subtypes, the procyanidins (5,7,3′,4′-hydroxylation) and prodelphinidins (5,7,3′,4′,5′-hydroxylation), are widespread in human foodstuffs,[2,31a,32] e.g., cocoa.[33−35] Cocoa procyanidins consist predominantly of epicatechin building blocks,[34d] and oligomers up to the size of the decamer have been identified.[34g] From the pentamer on, these oligomers exhibit growth inhibitory activity against various cancer cell lines.[35] To confirm the structures assigned to these compounds, we have embarked on a program to synthesize epicatechin oligomers of defined structure for comparison with material purified from cocoa and to determine structure−activity relationships in various in vitro and ultimately in vivo models of anticancer activity.

The synthetic challenge posed by proanthocyanidins is related to the difficulty in controlling the interflavan regio- and stereochemistry, as well as the sensitivity of the nonprotected compounds to acids,[36,37] bases,[1v,36,38] and oxidizing agents.[39] The condensation between flavan-3-ols and 4-substituted, electrophilic flavans has traditionally been performed without the use of phenol protecting groups in a mildly acidic medium[1v,31b,38,40] or recently with $AgBF_4$ for SBn as the 4-substituent.[41] The products are mixtures of regio- and sometimes stereoisomers, as well as higher oligomers despite the application of an excess of the nucleophilic building block. They have usually been separated by gel chromatography on Sephadex LH-20,[31a] a tedious process that requires a considerable investment of time to develop for each particular separation task because of the nonavailability of fast analytical tools such as HPLC columns or TLC plates for this adsorbent. In addition, optically pure, nonprotected 4-substituted catechins and epicatechins are not readily available, being prepared by reduction of the expensive natural product, (+)-taxifolin (the 4-ketone),[42] or by in situ degradation[38a,40a,b] or thiolytic degradation[40a] of natural proanthocyanidin oligomer fractions for which commercial sources are difficult to identify or nonexisting.

It is therefore not surprising that efforts were undertaken prior to our studies to address the possibility of building oligomeric proanthocyanidins from protected building blocks. As an additional incentive, protection of the phenolic but not of the alcoholic hydroxyls would permit the regioselective elaboration of derivatives such as 3-esters and -glycosides, as has been done in the case of catechin using acetyl protecting groups.[43] An

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(40) (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) Roux, D. G.; Ferreira, D. *Fortschr. Chem. Org. Naturst.* **1982**, 47−76. (d) Delcour, J. A.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1711. (e) Fonknechten, G.; Moll, M.; Cagniant, D.; Kirsch, G.; Muller, J. F. *J. Inst. Brewing* **1983**, *89*, 424. (f) Steenkamp, J. A.; Ferreira, D.; Roux, D. G.; Hull, W. E. *J. Chem. Soc., Perkin Trans. 1* **1983**, 23. (g) Delcour, J. A.; Vercruysse, S. A. R. *J. Inst. Brew.* **1986**, *92*, 244. (h) Delcour, J. A. *Farm. Tijdschr. Belg.* **1989**, *66*, 7.

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

(42) (a) Swain, T. *Chem. Ind.* **1954**, 1144. (b) Porter, L. J.; Foo, L. Y. *Phytochemistry* **1982**, *21*, 2947.

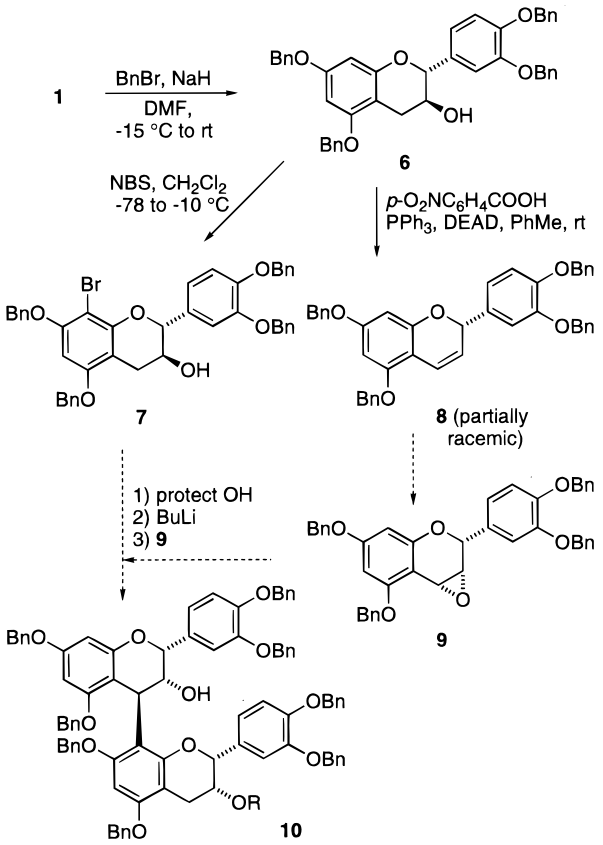
(43) Weinges, K.; Seiler, D. *Liebigs Ann. Chem.* **1968**, *714*, 193.

interesting approach has been reported in which the 8-bromo derivative of 3-*O*-benzyl-5,7,3′,4′-tetra-*O*-methylcatechin was subjected to halogen−lithium exchange and reacted with an *O*-methylated 4-ketone,[44] thus ensuring complete regiocontrol, but the choice of methyl as *O*-blocking group in this case precludes the final obtention of the free dimer. The remaining published work has made use of the above-described electrophilic substitution process with inclusion of phenol protecting groups on one or both of the reaction partners.[45,46] In fact, all of the key reactions—protection, activation of the 4-position, interflavan bond formation, and deprotection—have been demonstrated in the literature, as will be discussed below. To the best of our knowledge, however, none of this work has resulted in the synthesis of nonprotected, natural proanthocyanidins from stereochemically pure, readily available precursors.[46] We were attracted to this approach by the ready availability of (−)-epicatechin and, even more so, (+)-catechin as starting materials, and by the prospect of being able to perform the foreseeable product separations after the condensation step on nonpolar intermediates which are more comfortable to handle for the typical organic chemist than the polar, sensitive final products.

Results and Discussion

Phenol Protection. Our previous experience with the synthesis of polar organic compounds, such as amino acids and inositol phosphates, suggested the use of benzyl as the phenol protecting group, which can be removed in the final step by a hydrogenolysis reaction that is usually clean and efficient. This choice is corroborated by a literature report indicating that several other commonly used phenol protecting groups (methoxymethyl, benzyloxymethyl, tosyl, benzenesulfonyl) could not be removed from the final products in a similar scheme.[45a] Whereas the phenolic hydroxyls of catechin are readily *O*-methylated (Me_2SO_4, K_2CO_3, acetone, reflux),[47] the analogous alkylation with benzyl bromide was first reported in the literature to give only traces of the desired product.[43] Others have subsequently improved the yield to 45% using modified conditions (BnBr, K_2CO_3, DMF[45c] or NaH, BnBr, DMF[48]) (Scheme 1). As in the related case of phloroglucinol,[45a] *C*-benzylation competes with *O*-benzylation,[43] and under different conditions, mono- and di-*C*-benzyl derivatives with free hydroxyl groups have been prepared from catechin by direct alkylation with benzyl bromide.[49] In view of the large amount of byproducts, we considered it prudent to let a rough separation by column chromatography precede the crystallization step, and we have consistently obtained yields in the 20% range, and occasionally somewhat higher, of 5,7,3′,4′-tetra-*O*-benzylcatechin (**6**), which was at least 97% pure by HPLC.[50] While the percentage of yield is far from impressive, the starting material is available at a moderate price, and 35 g batches of **6** are readily prepared in a 2 L flask.

Scheme 1



The analogous benzylation of epicatechin (**2**) was less successful, giving a product of lower purity even after recrystallization. Since epicatechin is, in addition, considerably more expensive than catechin, it was decided to start from catechin and to invert the stereochemistry at C-3 subsequently.

Catechin is known to undergo quite readily a base-catalyzed epimerization at C-2 to form *ent*-epicatechin through reversible opening of ring C via a B-ring quinone methide intermediate.[38a] No diastereomeric material was seen by NMR in our crystallized tetra-*O*-benzylcatechin. We went farther and also checked for the presence of the enantiomer by forming diastereomeric esters with both Mosher acid chlorides (see the Supporting Information). While both esters exhibited sharp and distinct signals in their ^{19}F and ^{1}H NMR spectra, no cross-contamination could be detected. We thus conclude that our starting material is of 99% ee or better. It appears that quinone methide formation is not important under aprotic conditions and/or in the presence of a sufficiently reactive electrophile.

We have repeatedly made use in this investigation of the 8-bromo derivative **7** which is readily prepared in 99% regioselectivity (^{1}H NMR) and high yield by reacting **6** with 1 equiv of recrystallized NBS[49] in CH_2Cl_2 at low temperature. This

(44) Weinges, K.; Perner, J.; Marx, H.-D. *Chem. Ber.* **1970**, *103*, 2344.

(45) (a) Kawamoto, H.; Nakatsubo, F.; Murakami, K. *J. Wood Chem. Technol.* **1989**, *9*, 35. (b) Kawamoto, H.; Nakatsubo, F.; Murakami, K. *J. Wood Chem. Technol.* **1990**, *10*, 59. (c) Kawamoto, H.; Nakatsubo, F.; Murakami, K. *Mokuzai Gakkaishi* **1991**, *37*, 488; *Chem. Abstr.* **1991**, *115*, 279643y. (d) Kawamoto, H.; Nakatsubo, F.; Murakami, K. *Mokuzai Gakkaishi* **1991**, *37*, 741; *Chem. Abstr.* **1992**, *117*, 69641m.

(46) A partially racemic flavandiol building block analogous to **19** but with 2,3-trans stereochemistry has been obtained by benzylation of (+)-taxifolin followed by $NaBH_4$ reduction, and condensed with **6** and **14**: Pierre, M.-C.; Chèze, C.; Vercauteren, J. *Tetrahedron Lett.* **1997**, *38*, 5639. The partial racemization is a consequence of reversible O−C2 cleavage with formation of an unsaturated system that may be a quinone methide as assumed by these authors, but which could also exist as an enone tautomer. It should be noted that catechin does not epimerize under these benzylation conditions (ref 45c), indicating that some mode of stabilization of the reactive intermediate is of importance here. Also, the concomitant inversion at C-3 together with that at C-2 necessitates that C-3 becomes olefinic at some point during the course of the reaction, if not during the ring cleavage step itself, then in a preceding or subsequent enolization event.

(47) (a) Kostanecki, St. v.; Tambor, J. *Ber. Dtsch. Chem. Ges.* **1902**, *35*, 1867. (b) Mehta, P. P.; Whalley, W. B. *J. Chem. Soc.* **1963**, 5327.

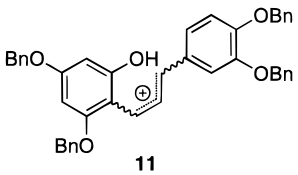
(48) Miura, S.; Midorikawa, T.; Awata, N. *Radioisotopes* **1983**, *32*, 225; *Chem. Abstr.* **1983**, *99*, 158183r.

(49) Ballenegger, M. E.; Rimbault, C. G.; Albert, A. I.; Weith, A. J.; Courbat, P.; Tyson, R. G.; Palmer, D. R.; Thompson, D. G. Eur. Pat. 0096007, July 29, 1987; *Chem. Abstr.* **1984**, *100*, 209512w.

(50) The tetrabenzyl ethers of 6- and 8-benzyl- and 6,8-dibenzylcatechin were identified in the mother liquor, and tribenzyl ethers with and without a *C*-benzyl group were isolated from more polar fractions eluted after the tetrabenzyl ethers: Kozikowski, A. P.; Tückmantel, W. Unpublished.

method is superior to the one using pyridinium tribromide[51,52] which in our hands gave impure reaction mixtures containing small amounts of compounds in which benzyl groups appear to have been oxidized to benzoate.

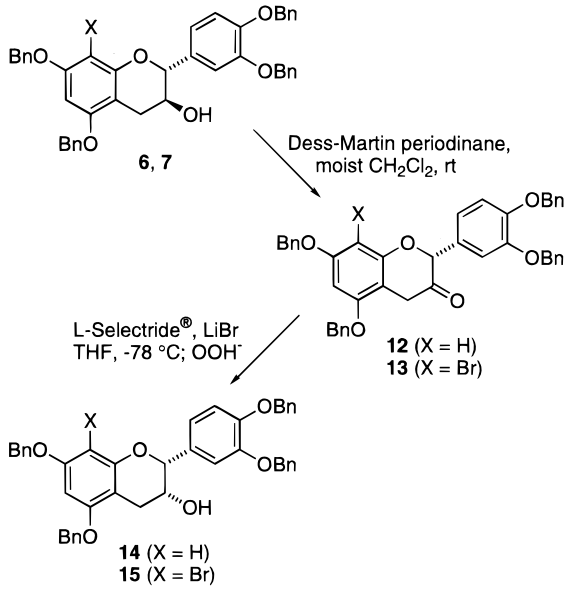
Initial Attempts at an Inherently Regioselective Approach. Our initial synthetic plan did not employ the well-documented Lewis acid-mediated aromatic alkylation by 4-hydroxyflavans, the regioselectivity of which could not be predicted with certainty, but instead attempted to impose regioselectivity by using a 6- or 8-lithiated protected flavan as the nucleophile, as the Weinges group had done before. The electrophilic component could then, of course, not be an alcohol. Weinges et al. used a ketone but then an additional hydroxyl group originated in position 4 which had subsequently to be removed. We envisaged instead the use of the epoxide **9** (or a cyclic ester of an analogous diol) (Scheme 1). Epoxides similar to **9** but having a lower degree of oxygenation in their A ring have been prepared or invoked as reaction intermediates.[53] Olefin **8** could serve as a precursor to **9**. The compound is known but is formed as a racemate on treatment of the mesylate of **6** with base.[45c] During first attempts at inverting the C-3 stereochemistry of **7**, we subjected the compound to Mitsunobu conditions[54] and obtained the olefin **8** in 87% yield. Omission of the acid resulted in a 70% yield of **8** which exhibited an $[\alpha]_D$ of $-85°$. To our disappointment, however, a subsequent preparation gave a product with an $[\alpha]_D$ of only $-61°$, a clear indication that racemization still occurred to a significant degree even under these very mild and essentially neutral conditions. This finding disqualified **8** as an intermediate in a controlled procyanidin synthesis and prompted us to return to the conventional electrophilic substitution approach. In hindsight, the lability of **8** is not really surprising since a highly stabilized, achiral carbenium ion **11** may form under acid or surface catalysis. Equally, the envisaged epoxide intermediate **9** might well prove to be unattainable on any route[55] because of a collusion toward its destabilization of epoxide ring strain and carbenium ion stabilization by the three oxygen substituents on ring A.



11

Alcohol Inversion. Since the Mitsunobu reaction failed to effect the required inversion at C-3, attention was turned to an oxidation−reduction sequence. The low-yielding oxidation of **6** with DMSO/Ac_2O has been described[48] but gave at best traces of the ketone in our hands. Others have reported that the oxidation of hepta-*O*-methylprocyanidin A_2 gave a moderate yield of a monoketone under modified Oppenauer conditions (fluorenone, $KOCMe_3$), but they obtained complex mixtures with CrO_3 and with activated DMSO.[56] We recovered starting material upon exposure of **7** to pyridinium dichromate (DMF, room temperature) or to *N*-methylmorpholine-*N*-oxide/catalytic tetrapropylammonium perruthenate (MS 4 Å, CH_2Cl_2, room temperature). On the other hand, reaction of **6** or **7** with the Dess−Martin periodinane[57] in moist CH_2Cl_2[58] gave reproducibly yields of 90% or better of the corresponding ketones **12**, **13** (Scheme 2). The halogen-free ketone **12** crystallizes readily from $CHCl_3/Et_2O$ and can be stored in crystalline form at -20 °C for extended time periods.

Scheme 2



The reduction of **12** has previously been performed with $NaBD_4$ ($CHCl_3$/MeOH, room temperature) to give a 67:33 ratio of epicatechin and catechin stereoisomers **6** and **14**, respectively.[48] We found that DIBAL-H (applied to **13**) gives an improved but still unsatisfactory stereoselection (Table 1). A bulky hydride source could be expected to exhibit a better selectivity toward attack from the less hindered β face. Gratifyingly, the use of lithium tri-*sec*-butylborohydride (L-Selectride) in THF resulted in the exclusive ($\geq$200:1, ^{1}H NMR) formation of the desired 3α-isomer. The yield, however, was only moderate. We noticed that the thin layer chromatogram of the reaction mixture did not further change upon addition of an excess of the reagent, with some starting material apparently remaining unreacted. This suggested that a fraction of the starting material underwent enolization rather than reduction, with the enolate being hydrolyzed back to the ketone by silica gel. Upon alkaline H_2O_2 workup, however, the enolate was destroyed, and the 3α-alcohol **15** was obtained as the sole product.[59] The situation is reminiscent of the addition of Grignard and organolithium reagents to ketones, in which case enolization can usually be suppressed or at least diminished by transmetalation to an organocerium reagent with anhydrous $CeCl_3$.[60] The following experiments were performed on the bromine-free ketone **12**. When $CeCl_3$ (1 equiv in relation to L-Selectride) was included in the reaction mixture, an improved yield of the 3α-alcohol **14** was obtained without sacrifice in stereoselectivity. Unfortunately, when the amount of reagent was reduced from 3 to 1.5 equiv in a larger scale experiment,

(51) Engel, D. W.; Hattingh, M.; Hundt, H. K. L.; Roux, D. G. *J. Chem. Soc., Chem. Commun.* **1978**, 695.

(52) Hundt, H. K. L.; Roux, D. G. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1227.

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(54) Martin, S. F.; Dodge, J. A. *Tetrahedron Lett.* **1991**, *32*, 3017.

(55) Dimethyldioxirane, the reagent most likely to epoxidize **8** without subsequent epoxide opening, can be expected to attack predominantly from the less hindered face, resulting in 3β,4β, i.e., catechin, stereochemistry.

(56) Nonaka, G.; Morimoto, S.; Kinjo, J.; Nohara, T.; Nishioka, I. *Chem. Pharm. Bull.* **1987**, *35*, 149.

(57) (a) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155. (b) Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, *58*, 2899.

(58) Meyer, S. D.; Schreiber, S. L. *J. Org. Chem.* **1994**, *59*, 7549.

(59) For the preparation of **15** on this route or alternatively by bromination of **14** with NBS, see the Supporting Information.

(60) Imamoto, T.; Kusumoto, T.; Tawarayama, Y.; Sugiura, Y.; Mita, T.; Hatanaka, Y.; Yokoyama, M. *J. Org. Chem.* **1984**, *49*, 3904.

Table 1. Effect of Additives on the Reduction of Ketones **12** and **13**[a]

compd	reagent/equiv	additive/equiv	scale (mmol)	cis/trans[b]	crude yield (%)	recrystd yield (%)	ketone/alcohol[c]
13	DIBAH/3	none	0.06	88:12	83[d]		
	LiBH(*s*-Bu)$_3$/3	none	0.82	≥200:1	55	47	
10	LiBH(*s*-Bu)$_3$/3	$ZnCl_2$/1.5	0.09	94:6	80		
	LiBH(*s*-Bu)$_3$/3	$ZnCl_2$/3	0.08	53:47	96		
	LiBH(*s*-Bu)$_3$/3	$CeCl_3$/3.1	0.07	≥200:1	77		
	LiBH(*s*-Bu)$_3$/1.5	$CeCl_3$/1.5	4.5	≥200:1		56	
	$LiBHEt_3$/3	none	0.06	83:17	68		
	LiBH(*s*-Bu)$_3$/1.5	none	0.07	≥200:1			19:81
	LiBH(*s*-Bu)$_3$/1.5	$CeCl_3$/1.5	0.13	≥200:1			19:81
	LiBH(*s*-Bu)$_3$/1.5	$CeCl_3$/1.5, LiBr/6	0.10	≥200:1			5:95
	LiBH(*s*-Bu)$_3$/1.5	LiBr/6	0.09	≥200:1			8:92
	LiBH(*s*-Bu)$_3$/1.3	LiBr/5.2	77	≥200:1		81	
	LiBH(*s*-Bu)$_3$/1.5	*n*-Bu_4NCl/3	0.11	200:1	81		

[a] All reactions in THF (DIBAH reduction in CH_2Cl_2/hexane) at −78 °C. Borohydride reductions were followed by an oxidative workup with $NaOH/H_2O_2$. [b] By ^1H NMR. [c] For entries in this column, the oxidative workup was omitted. [d] Besides 9% of unreacted **13** (by ^1H NMR).

the yield returned to near its original value. One factor that may be involved in this decrease is the presence of residual water in supposedly anhydrous $CeCl_3$ which may destroy part of the reducing agent. We also noticed that all or part of the $CeCl_3$ remained undissolved throughout the course of the reaction, thus challenging the assumption of the formation of a new reagent on which this experiment was based in the first place. Substituting soluble $ZnCl_2$ for $CeCl_3$, a steep improvement in yield resulted as the amount of additive was increased from 0.5 to 1 equiv relative to the hydride, but the loss in stereoselectivity was equally pronounced. An influence of additives (ZnI_2, $MgBr_2$, Ti(O^{i}Pr)$_4$, or TiCl(O^{i}Pr)$_3$) on the stereoselectivity of an L-Selectride reduction has previously been observed.[61] We eventually also replaced L-Selectride by the less bulky lithium triethylborohydride, reasoning that lowered steric hindrance might favor reduction over enolization, and observed indeed a marginally improved yield, but again accompanied by a serious loss in stereoselectivity.

At this point, we wondered whether $CeCl_3$ could be solubilized in THF through addition of lithium halides in the same manner as observed for some transition metal halides. In this series of experiments, the oxidative workup was omitted, allowing the ratio of alcohol and unreacted ketone to be determined by NMR, although the presence of boron impurities in the crude products prevented the determination of yields. When **12** was reduced with 1.5 equiv of L-Selectride, a 4:1 ratio of alcohol and starting ketone resulted. Inclusion of 1.5 equiv of $CeCl_3$ and 6 equiv of anhydrous LiBr improved this ratio to 20:1. By visual appearance, we nevertheless observed that no or little $CeCl_3$ had dissolved. Concluding that at least some of the effect was due to LiBr rather than $CeCl_3$, we used 6 equiv of LiBr alone as the additive and obtained an alcohol/ketone ratio of 11:1. This protocol was scaled up including an oxidative workup and afforded reproducibly 5,7,3′,4′-tetra-*O*-benzylepicatechin (**14**) in a reasonable 81−82% yield on an up to 77 mmol scale. That no racemization had occurred at the ketone stage was demonstrated by the preparation of both Mosher esters from **14** which showed no cross-contamination, and we estimate the chemical and optical purity of our material to be at least 98%.

We are not aware of previous observations of an effect of nontransmetalating salt additives on the product distribution in borohydride reductions of ketones but there are reports in the literature of such effects on the related addition of Grignard reagents to aldehydes and ketones. It has recently been shown that the addition of isopropylmagnesium bromide to an aldehyde susceptible to enolization was greatly improved upon adding an equimolar amount (with respect to the Grignard reagent) of hexaethylguanidinium bromide.[62] These authors quoted older work[63] that reported an improvement in the ratio of direct addition vs β-hydride transfer in the reaction of diisopropyl ketone with *n*-PrMgBr. Among the successful additives were LiBr and $LiClO_4$ as well as several tetra-*n*-butylammonium salts, most notably the chloride. When **12** was reduced with 1.5 equiv of L-Selectride in the presence of 3 equiv of dried *n*-Bu_4NCl, **14** was formed in 81% yield and a stereoselectivity of 200:1. This result demonstrates that the Lewis acidity of LiBr is not the deciding factor in modifying the outcome of the reaction, but what exactly the salt additive does is not clear. A recent investigation[64] concludes that the much-used ionic reaction medium $LiClO_4$/ether functions as a weak Lewis acid in its acceleration of Diels−Alder reactions, but this finding does not have to be applicable to other types of reactions or to different ionic solutions. From the preparative point of view, it appears that the use of nontransmetalating salt additives in hydride reductions would deserve further investigation.

Oxidation at C-4. Three reagents have been employed in the literature to oxidize *O*-alkylated catechins and epicatechins, namely $K_2S_2O_8$/catalytic $CuSO_4$,[65] lead(IV) acetate,[45c,66] and DDQ.[67] Using the first-listed reagent, we observed that the starting material was not or little soluble in the reaction medium, aqueous CH_3CN, and that a complex mixture of unidentified products was eventually formed. Reaction of **15** or its 3-*O*-acetyl derivative with lead tetraacetate gave, after acetate saponification (NaOMe, MeOH/THF), meager yields of impure 3,4-diol. On the other hand, a much cleaner reaction was observed between **14** and DDQ in $CHCl_3$/MeOH. Over several hours at room temperature, a polar product emerged while a TLC spot with the same mobility as the starting material persisted even after 9 h. Isolation of both components gave a 21% yield of the

(61) Tanis, S. P.; Chuang, Y.-H.; Head, D. B. *J. Org. Chem.* **1988**, *53*, 4929.

(62) Schlama, T.; Baati, R.; Gouverneur, V.; Valleix, A.; Falck, J. R.; Mioskowski, C. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2085.

(63) Chastrette, M.; Amouroux, R. *Bull. Soc. Chim. Fr.* **1970**, 4348.

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(66) (a) Bokadia, M. M.; Brown, B. R.; Cummings, W. *J. Chem. Soc.* **1960**, 3308. (b) Betts, M. J.; Brown, B. R.; Shaw, M. R. *J. Chem. Soc. (C)* **1969**, 1178.

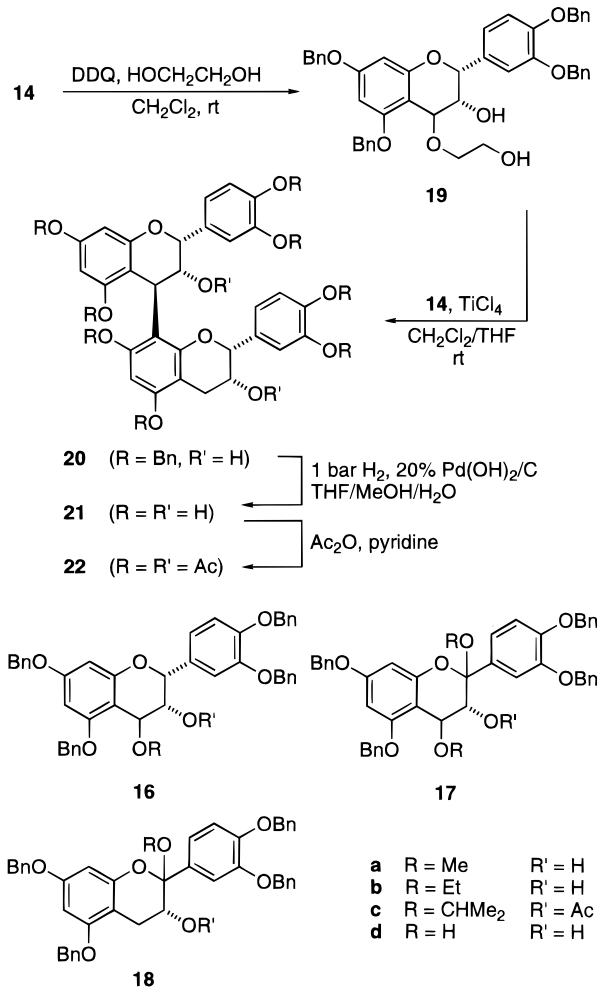
(67) (a) Steenkamp, J. A.; Ferreira, D.; Roux, D. G. *Tetrahedron Lett.* **1985**, *26*, 3045. (b) Steenkamp, J. A.; Mouton, C. H. L.; Ferreira, D. *Tetrahedron* **1991**, *47*, 6705.

4-methoxy derivative **16a**, which had the same chromatographic mobility as the starting material under a variety of conditions, and 29% of a single, polar 2,4-dimethoxy derivative **17a** of unknown stereochemistry. Since the presence of bis-oxidized material indicated that the reaction should be terminated promptly after consumption of the starting material, and our inability to monitor the initial oxidation of **14** to **16a** rendered this task difficult, 2-propanol was substituted for MeOH in the hope that this would result in a lower polarity of the 4-alkoxy derivative. This measure fulfilled its purpose, and after completion of the reaction, an 87% crude yield of the oxidation product was obtained. This material, however, proved very impure. An analogous result was obtained using the 3-acetate as starting material. In this case, we obtained small amounts of pure or enriched byproducts by preparative HPLC, and identified these as the 2,4-diisopropoxy-, the 2-isopropoxy-, and the 4-ethoxy derivatives, **17c**, **18c**, and **16b**, respectively. The latter undoubtedly arose from the ethanol present in chloroform as a stabilizer, and $CHCl_3$ was henceforth replaced with CH_2Cl_2. Coming to the conclusion that reducing the polarity of the alcohol nucleophile had made things worse by rendering the dialkoxylation product, the formation of a small amount of which apparently cannot be avoided even if the reaction is stopped in a timely manner, barely separable from the desired product, the opposite strategy was pursued next. Exposure of **14** to DDQ in THF/water 10:1 (THF was used here to obtain a homogeneous solution) gave 33% of the 3,4-diol **16d** together with 44% of recovered **14**, both somewhat impure. Better results were obtained using ethylene glycol in CH_2Cl_2[68] as the nucleophile, giving a 52% isolated yield of **19** on a 20 mmol scale. All impurities detected by us[69] including the presumed 2-alkoxylated regioisomer are readily removed by column chromatography, and the product itself could be crystallized and is in this form stable to extended storage at −20 °C.[70]

We leave the question of stereochemistry at C-4 open since $J_{3,4}$ is not a realiable indicator of stereochemistry in the epicatechin series (see below). Specifically, the small, unresolved coupling between 2-H and 3-H requires that these two protons be not far from orthogonal, leaving for the dihedral angles between 3-H and 4-H in the 4α and 4*β* stereoisomers relatively small but still overlapping ranges. Mechanistic arguments favor 4*β*-stereochemistry as discussed below for the epicatechin 4,8-dimer.

Condensation Step: Reaction of 14 with 19. A protocol exists for the electrophilic alkylation of *O*-protected polyphenols with flavan-3,4-diols[45] and was found applicable to **19** as well (Scheme 3). The nucleophilic component **14** was employed in a 4-fold excess to decrease the extent of higher oligomer

Scheme 3



formation that result from repeated attack of the electrophile on already formed smaller oligomers. Nevertheless, when the reactants were exposed to 2 equiv of $TiCl_4$ in a solvent mixture of CH_2Cl_2 and THF at room temperature, a mixture of a single dimer **20**, two trimers, and a tetramer were obtained in yields of 53, 22, 4.5, and 1.5%, respectively. The trimers and the tetramer are still under study and will be the subjects of a separate publication. The formation of single stereoisomers in the reaction of epicatechin-derived flavan-3,4-diols has been commonly observed in the literature, and 4*β* (i.e., 3,4-trans) stereochemistry has generally been assigned to these products.[71] This makes sense from a mechanistic point of view if backside shielding of the 4-carbenium ion by the 3α-hydroxyl is effective during the alkylation reaction, and also represents a reaction on the less congested face of the pyran ring opposite from the 2-aryl substituent. Characteristically, in the catechin series where these two effects are opposed to each other, mixtures of 4α and 4*β* stereoisomers may be obtained.[45c] We did, however, feel disconcerted by the fact that the assignment of interflavan stereochemistry in the literature is based on ^{1}H NMR coupling constants and chiroptical methods,[72,73] both techniques of limited reliability in conformationally flexible systems. Only if the magnitude of $J_{3,4}$ demonstrates a (nearly) trans-diaxial relation-

(68) We initially overlooked the fact that ethylene glycol is poorly soluble in CH_2Cl_2, but the reaction works fine anyway.

(69) HPLC separation of the column forerun resulted in isolation or enrichment of residual starting material and of byproducts to which the following structures have tentatively been assigned: the 2-alkoxy derivative; a product containing two epicatechin moieties linked by an ethylenedioxy group, most likely both in their 4-positions; and a product of 2-fold oxidation in positions 2,4 or 4,4 which are spanned by a single ethylenedioxy group.

(70) We also changed the workup protocol, which aims at reducing or otherwise destroying residual DDQ and at neutralizing $DDQH_2$, so as to permit the isolation of prepurified product by filtration over a short silica gel column that would be readily penetrated by the large amounts of DDQ and $DDQH_2$ present. The original procedure used an excess of $NaBH_4$, a rather hazardous operation on larger reaction scales. We were able to replace this scavenging agent with Amberlite IRA-400 (OH^- form) but remained dissatisfied because of the large amount of resin needed. Eventually, it was found that a slight excess of 4-(dimethylamino)pyridine (DMAP) serves well for this purpose. The formation of solid, dark-colored molecular complexes between DDQ and the three isomeric aminopyridines has been reported: Tosi, G.; Bruni, P.; Cardellini, L. *Gazz. Chim. Ital.* **1984**, *114*, 125.

(71) According to ref 1t (p 92), only a single compound, a natural product, with 2,3-cis-3,4-cis stereochemistry is known.

(72) For a characteristic example, see: Kolodziej, H. *Phytochemistry* **1986**, *25*, 1209.

(73) Reliance on circular dichroism as a source of errors is documented in: Hundt, A. F.; Burger, J. F. W.; Steynberg, J. P.; Steenkamp, J. A.; Ferreira, D. *Tetrahedron Lett.* **1990**, *31*, 5073.

ship between the coupling protons, as is often the case for catechin derivatives, can a safe conclusion regarding the interflavan stereochemistry be drawn. To complicate the situation, it has been shown that catechin units in proanthocyanidin oligomers may adopt an alternative C-ring conformation that results in small, unresolved $J_{2,3}$ and $J_{3,4}$ despite trans,trans disposition of the substituents.[74] For epicatechin derivatives, 3-H and 4-H have an equatorial−axial or equatorial−equatorial relationship, and these two alternatives cannot be distinguished with certainty by coupling constants. Under these circumstances, an X-ray structure analysis of **20** or a derivative thereof would be highly desirable. We were, unfortunately, unable to crystallize **20** or several of its derivatives,[75] and we are not aware of a crystal structure analysis for any dimeric proanthocyanidin[76] other than rigidified so-called A-type proanthocyanidins.[77] As a consequence, a traditional oxidative degradation approach has been pursued and has resulted in the transformation of an *O*-alkylated derivative of **21** into (*R*)-2,4-diphenylbutyric acid in which the carboxyl group is derived from the A^2 ring, and the phenyl groups are derived from the A^1 and B^1 rings. This work confirms the hitherto hypothetical β stereochemistry of the interflavan linkage in **21** and will be published in due course.

The 4,8-regiochemistry of **20** follows from that of its deprotection product **21** (see below). Preferred attack of an electrophile at the 8- vs the 6-position of a tetra-*O*-alkylcatechin has previously been demonstrated by X-ray structure analysis of the reaction product, 8-bromo-5,7,3′,4′-tetra-*O*-methylcatechin.[51] The inference of regiochemistry from the small chemical shift differences between 6-H and 8-H in 8- and 6-substituted compounds[52,72,78] is, to the contrary, unreliable, and the assignment of resonances to 6-H and 8-H has been subjected to revision.[79,80] As there is little electronic difference between positions 6 and 8, their differential reactivity is apparently based on the lower degree of steric hindrance exerted by the tied-back alkoxy group of which the pyran oxygen is a part, compared to the flexible alkoxy groups at C-5 and C-7. It therefore stands to reason that this effect is even more pronounced in the *O*-benzyl series compared with the *O*-methyl series. The alkylation of tetra-*O*-methylcatechin with *o*- or *p*-hydroxybenzyl alcohol (smaller electrophiles than **19**) at elevated temperatures resulted, however, in a mixture of 6- and 8-substituted products.[81] Others have previously assigned 4,8-regiochemistry to condensation products derived from **6** and **14**.[45c,46] While the exclusive formation of the 4,8-dimer in this reaction is a welcome result as far as the purification of the product is concerned, it also raises the question of how to prepare the 4,6-dimer. In a first unsuccessful attempt, **19** was reacted with **15**, which is prevented from reacting at the 8-position by its bromo substituent, but no dimer was isolated.[82] This result conforms to the preceding reasoning but may also reflect the inductive deactivation of the A ring by the Br substituent. That the situation may not be entirely so simple is pointed out by the fact that two trimers were obtained in the condensation reaction, one of which therefore must violate the provided stereochemical or regiochemical rationale, or both; unless, of course, these compounds turn out to be isolatable rotamers.

The issue of rotational isomerism around the interflavan bond (or around several interflavan bonds in the case of higher oligomers, with an associated exponential increase in the number of possible rotamers)[31a,40a,b,83] introduces a significant complication in working with these compounds. At present, there is no indication (e.g., by HPLC) that **20** or its derivatives exist in the form of rotamers isolatable on the laboratory time scale (if the two trimers are not such a case). On the NMR time scale, however, two sets of sharp signals for the two rotamers can be distinguished. The ^{1}H NMR spectra at 300 MHz of *O*-protected dimers usually remain interpretable (outside their benzyl regions) if a COSY experiment is enlisted for help, but for higher oligomers, product characterization will predominantly have to depend on HPLC and mass spectrometry.

The free epicatechin 4β,8-dimer (**21**, procyanidin B_2)[31a,38a,84,85] was uneventfully prepared from its benzylated precursor by hydrogenolysis. Only one set each of ^{1}H and ^{13}C NMR signals is observed, but the ^{1}H signals are close to the coalescence point at room temperature, and part of the ^{13}C signals are strongly broadened as well. The 4,8-regiochemistry of natural procyanidin B_2 has recently been rigorously proven by ^{1}H−^{13}C NMR correlation (HMBC).[80] The decaacetate **22** derived from our material exhibits ^{1}H and ^{13}C NMR spectra which are in good agreement with those reported by these authors for the decaacetate of the natural product. Selected ^{1}H NMR signals published for **22** derived from natural material[85a,86] agree also with ours, and appropriate ^{13}C NMR signals of our product agree with selected data published earlier for the decaacetate of natural[87] and synthetic material.[38a]

Galloylation of the Dimer and Biological Activity of the Bisgallate. A recent report[21] disclosing the anticancer activity of epigallocatechin 3-gallate (**5**) in a system in which epigal-

(74) Balas, L.; Vercauteren, J.; Laguerre, M. *Magn. Reson. Chem.* **1995**, *33*, 85.

(75) A variety of esters and urethanes, as well as two monobromides, the 3,3-dideoxy derivative, and the diketone, have been investigated by us. One group of researchers reported crystallization of the peracetate (ref 34a) but others described the compound as amorphous (ref 84).

(76) The free dimeric proanthocyanidin, fisetinidol-4,8-catechin (fisetinidol is the 5-deoxy analogue of catechin), has been crystallized, but an X-ray diffraction analysis failed to yield its structure as a consequence of conformational disorder: unpublished work quoted by R. W. Hemingway in *Polyphenols 96* (proceedings of the conference in Bordeaux, France, July 15−18, 1996); Vercauteren, J.; Chèze, C.; Triaud, J., Eds.; Editions INRA, Paris, 1998; pp 81−103.

(77) van Rooyen, P. H.; Redelinghuys, H. J. P. *S. Afr. J. Chem.* **1983**, *36*, 49.

(78) A summary of the truly bewildering sole reliance, until quite recently, on questionable empirical techniques for regiochemical assignment is given in: Balas, L.; Vercauteren, J. *Magn. Reson. Chem.* **1994**, *32*, 386.

(79) (a) Kiehlmann, E.; Tracey, A. S. *Can. J. Chem.* **1986**, *64*, 1998. (b) Kiehlmann, E.; Lehto, N.; Cherniwchan, D. *Can. J. Chem.* **1988**, *66*, 2431. (c) De Bruyne, T.; Pieters, L. A. C.; Dommisse, R. A.; Kolodziej, H.; Wray, V.; Domke, T.; Vlietinck, A. J. *Phytochemistry* **1996** *43*, 265. (d) Hemingway, R. W.; Tobiason, F. L.; McGraw, G. W.; Steynberg, J. P. *Magn. Reson. Chem.* **1996**, *34*, 424.

(80) Khan, M. L.; Haslam, E.; Williamson, M. P. *Magn. Reson. Chem.* **1997**, *35*, 854.

(81) McGraw, G. W.; Hemingway, R. W. *J. Chem. Soc., Perkin Trans. 1* **1982**, 973. The authors assigned product regiochemistries solely based on chemical shifts, rather than correlating their 8-substituted products with 8-bromotetra-*O*-methylcatechin the X-ray structure of which had been reported by that time (ref 51).

(82) Unpublished experiment with A. Hoepping. The related use of 6-iodocatechin as a nucleophilic component in coupling reactions with the aim of imposing regioselectivity has been described: Young, D. A.; Cronjé, A.; Botes, A. L.; Ferreira, D.; Roux, D. J. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2521. Other authors, however, have subsequently challenged both the regioisomeric nature of the starting material as well as its direct involvement in the interflavan bond forming process: ref 79b.

(83) (a) Bergmann, W. R.; Barkley, M. D.; Hemingway, R. W.; Mattice, W. L. *J. Am. Chem. Soc.* **1987**, *109*, 6614. (b) Hatano, T.; Hemingway, R. W. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1035.

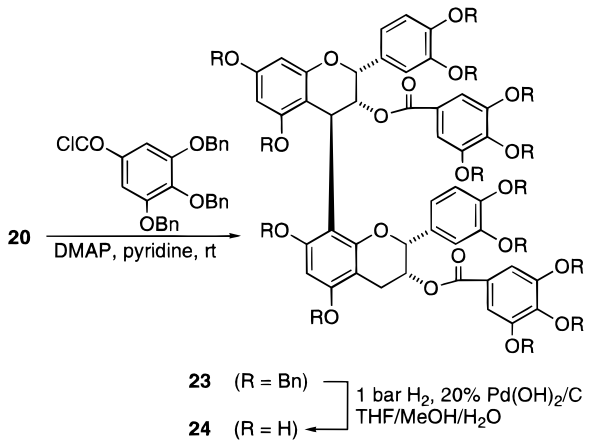
(84) Weinges, K.; Kaltenhäuser, W.; Marx, H.-D.; Nader, E.; Nader, F.; Perner, J.; Seiler, D. *Liebigs Ann. Chem.* **1968**, *711*, 184.

(85) (a) Weinges, K.; Göritz, K.; Nader, F. *Liebigs Ann. Chem.* **1968**, *715*, 164. (b) Hsu, F.-L.; Chen, H.-F. *Planta Med.* **1993**, *59*, 405. (c) The enantiomer has also been isolated: delle Monache, F.; Ferrari, F.; Marini Bettòlo, G. B. *Gazz. Chim. Ital.* **1971**, *101*, 387.

(86) (a) Langhammer, L.; Rauwald, H.-W.; Schulze, G. *Arch. Pharm. (Weinheim)* **1981**, *114*, 424. (b) Engelshowe, R. *Planta Med.* **1983**, *49*, 170.

(87) Porter, L. J.; Newman, R. H.; Foo, L. Y.; Wong, H. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1217.

Scheme 4



locatechin (**4**) is inactive prompted us to synthesize the 3,3-bisgallate **24** derived from **21**, which has not previously been obtained synthetically (Scheme 4). Tri-*O*-benzylgallic acid[88] was converted in situ into its chloride, and **20** was esterified with this reagent in pyridine solution in the presence of DMAP, furnishing the diester **23** in near-quantitative yield. Hydrogenolysis of **23** over 20% $Pd(OH)_2$/C gave the deprotected bisgallate **24** in 90% yield as a hydrate without need for chromatographic purification (5.3 equiv of water per bisgallate molecule by combustion analysis of a batch lyophilized from water). This compound has previously been isolated from natural sources.[34e,39b,89,90] Our ^{1}H NMR spectrum, while not highly characteristic because of line broadening, is in reasonable agreement with that of ref 90a (which was acquired at 100 MHz), except for the omission without comment of the B^1 and B^2 ring protons from the literature spectrum.

3-*O*-Galloylepicatechin is equally readily prepared from **14**.

The bisgallate **24** has been isolated from grape seeds, wine, rhubarb, and cocoa and thus is taken up with the human diet. Compound **24** is a low-micromolar inhibitor of protein kinase C[91] and an inhibitor of ACE (angiotensin-converting enzyme).[34e] It increases the lifespan of mice inoculated with sarcoma-180 and is more active in this model than **5** but less active than several oligomeric ellagitannins.[20d] It also exhibits weak cytotoxicity against RPMI-7951 human melanoma cells while being inactive against several other human cancer cell lines.[20f] Recently, **24** has been shown to inhibit the growth of several human breast cancer cell lines in a concentration range of 50−100 mg/L,[92] comparable to compound **5** which has generated considerable attention because of its anticancer activity.

Conclusion and Perspectives

A convenient, partially optimized procedure has been developed for the preparation of the electrophilic building block **19** which permits the stereoselective assembly of epicatechin oligomers in which all phenolic hydroxyl groups are protected as benzyl ethers. The only dimer formed in the $TiCl_4$-mediated condensation reaction between **14** and **19** is the 4β,8-isomer. Resubjection of smaller oligomers to the reaction conditions should enable us to generate mixtures of larger oligomers, the complexity of which is increased in a stepwise manner (all products contain the common partial structure of the starting oligomer) and thus hopefully will remain manageable with present-day purification techniques. While the preparation of these compounds and the rigorous elucidation of their structure will require additional effort, a natural product exhibiting significant activity against cancer cells has been obtained at this early project stage by esterifying the alcoholic hydroxyl groups of **20** with protected gallic acid, followed by hydrogenolysis.

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Supporting Information Available: Paragraphs on nomenclature and the occurrence of proanthocyanidins in human foodstuffs; Experimental Section (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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