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ENANTIOSELECTIVE SYNTHESIS AND PROTEASOME INHIBITION OF A-RING ANALOGS OF (-)-*EPI*GALLOCATECHIN GALLATE (EGCG), THE ACTIVE INGREDIENT OF GREEN TEA EXTRACT[1]

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Abstract – The A-ring analog compound **8** of (-)-EGCG (**1**), the active ingredient of green tea, and compound **9**, analog of the natural catechin **6** from *C. salvifolius*, as well as their enantiomers **10** and **11** have been synthesized enantioselectively. They show proteasomal inhibitory activities under cell free conditions, at a potency lower than that of (-)-EGCG. The results suggest that the hydroxyl groups in A-ring of (-)-EGCG play a role in enhancing the activity. The acetylated derivatives of these compounds, **12**-**15**, show cytotoxic activities and proteasomal inhibition after cellular intake, presumably acting as pro-drugs.

INTRODUCTION

Tea, produced from the plant *Camellia sinensis*, has been a popular beverage in China and Japan for many years and is now consumed by 2/3 of the world's population. Green tea, which does not undergo fermentation in the harvesting, contains catechins: (-)-epigallocatechin-3-gallate (EGCG, **1**), (-)-epigallocatechin (EGC, **2**), (-)-epicatechin-3-gallate (ECG, **3**) and (-)-epicatechin (EC, **4**) (Figure 1). Together, they constitute about 30-40% of the water-soluble compounds in brewed green tea.[1] Of these, EGCG is by far the most abundant and accounts for more than half of the catechins. Regular drinking of

[1] This article is part of a Special Issue dedicated to Prof. Dr. Ryoji Noyori on the occasion of his 70th birthday.

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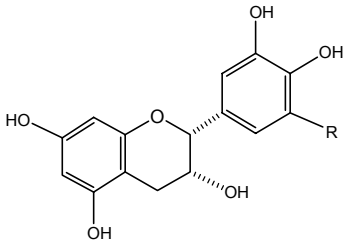
green tea has been associated with many beneficial effects,[2] one of which is reduced risk of cancer.[3,4] In the past decade, the cancer-preventive effects of green tea and its main constituent (-)-EGCG have been confirmed by epidemiological,[5,6] cell culture,[7] and animal studies.[8-11] However, the molecular mechanisms for the anti-cancer activities are not well understood.[4]



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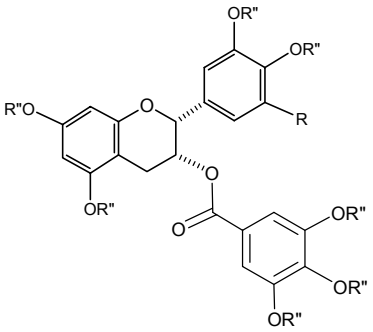
4:, R=H; EC



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3: R=H, R"=H, ECG
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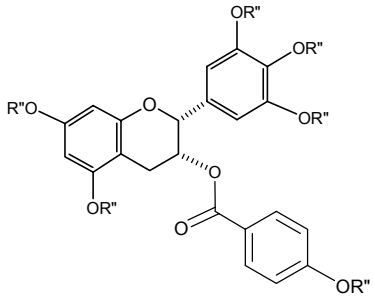

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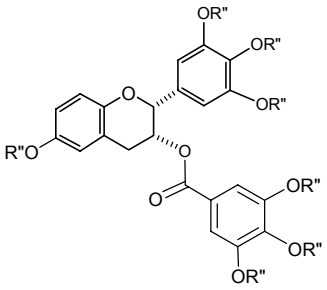
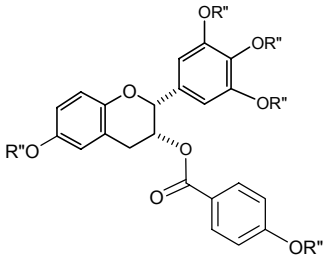
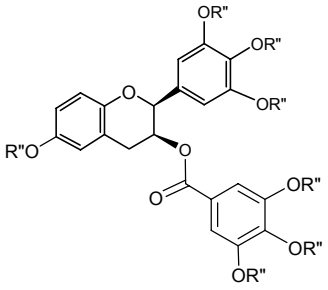
7: R"=Ac



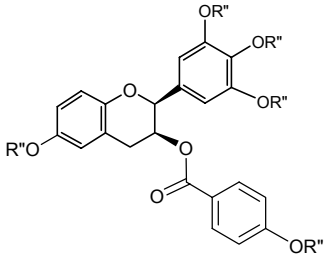
8: R"=H
12: R"=Ac



9: R"=H
13: R"=Ac



10: R"=H
14: R"=Ac



11: R"=H
15: R"=Ac

Figure 1

Recently, we showed that (-)-EGCG potently and specifically inhibited the chymotrypsin-like activity of the proteasome *in vitro* (IC_{50}=86-194 nM) and this may be a key mechanism accounting for the anti-cancer activities of green tea.[12] The eukaryotic proteasome is a large multi-catalytic, multi-subunit protease complex having at least three distinct protease activities: the chymotrypsin-like (within β5 subunit), trypsin-like (within β2 subunit) and peptidyl-glutamyl peptide-hydrolyzing-like (PGPH- or caspase-like; within β1 subunit) activities.[13] Inhibition of the chymotrypsin-like, but not the trypsin-like activity of the proteasome was found to be associated with induction of tumor cell apoptosis.[14] The discovery of new proteasome inhibitors with little or no toxicity is considered highly desirable in

anticancer therapy.[15] Our structure-activity studies on proteasome inhibition using natural and synthetic EGCG analogs showed a number of interesting features: (a) the carbonyl function is essential in the inhibitory activity[12]; (b) synthetic (+)-EGCG, the enantiomer of the natural (-)-EGCG, showed nearly equal potency[16]; (c) the ester oxygen at C-3 can be replaced by the NH isostere with little reduced activity[17] and (d) decreasing the number of –OH groups from either the B- or D- ring leads to diminished proteasome inhibitory activity *in vitro.*[18, 19] A rational model has been proposed with *in silico* docking studies. The results indicate that (-)-EGCG and the active analogs predictably bind to the *N*-terminal threonine (Thr) of the proteasomal chymotrypsin β-5 subunit active site.[17] This orientation is suitable for nucleophilic attack by the hydroxyl group of Thr 1 to the carbonyl carbon of (-)-EGCG, thus deactivating the proteasomal chymotrypsin-like activity.[17] In order to further understand the structure-activity of proteasome inhibition by these compounds, we are interested in examining the modification of the A-ring OH substitutions in EGCG.

Another known issue in the potential application of EGCG as an anti-cancer agent is its poor bioavailability[20] which may be due to poor cellular uptake as well of instability of EGCG in alkaline or neutral solutions.[21, 22] To improve the bioavailability of EGCG, we recently proposed the use of pro-drug EGCG octaacetate (pro-EGCG, **5**) which was more stable to alkaline and more readily taken up intracellularly than EGCG.[23] While the pro-drug **5** was inactive *in vitro* as an inhibitor of the proteasome, it was intracellularly converted into EGCG that inhibited the proteasome and induced apoptosis in cancer cells.[11, 24] More importantly, the pro-drug **5** showed a significant inhibition of cancer growth compared to EGCG itself *in vivo* for either breast cancer animal model[11] or androgen-independent prostate cancer animal model.[25]

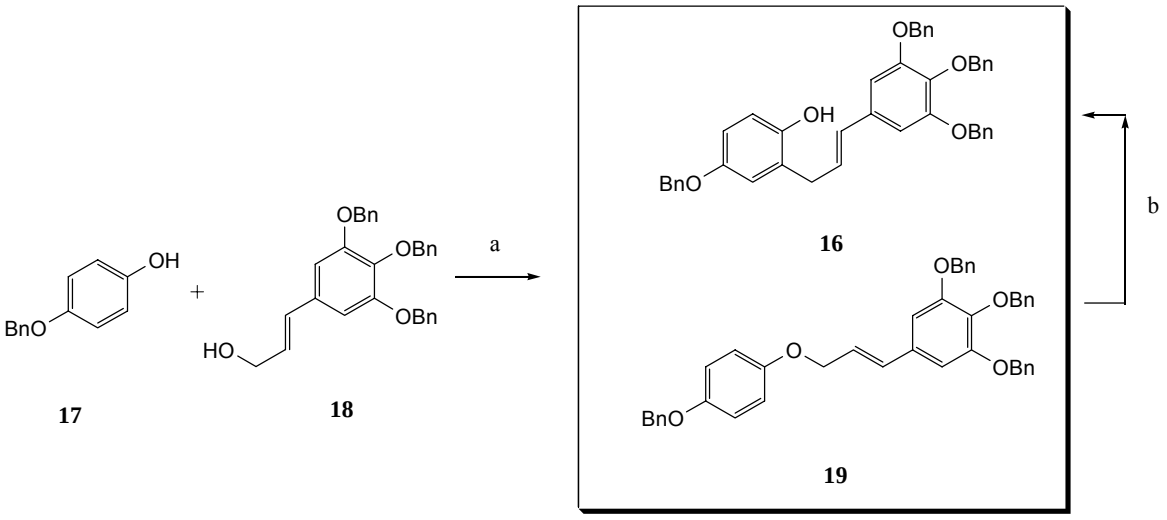
Finally, the natural compound (-)-EGC p-hydroxybenzoate (**6**), though not found in green tea extract, was isolated from the species *Cistus salvifolius.*[26] We recently showed that while compound **6** is not as active as (-)-EGCG in proteasome inhibition *in vitro*, its acetate derivative **7** nevertheless showed intracellular proteasome inhibition and cytotoxic activities comparable to the pro-drug **5**.[27] The origin for the enhanced activity of **7** is not clear but metabolic methylation which suppresses the proteasome inhibition of green tea polyphenols may be a contributing factor.[28]

With the above considerations in mind, we undertook to synthesize the analogs **8** and **9**, th**e**ir enantiomers **10** an**d 11** as well as their acetates **12-15** (Figure 1). Their proteasome inhibitory and cytotoxic activities were examined in comparison with EGCG (**1**) and the pro-drug **5**.

RESULTS AND DISCUSSION

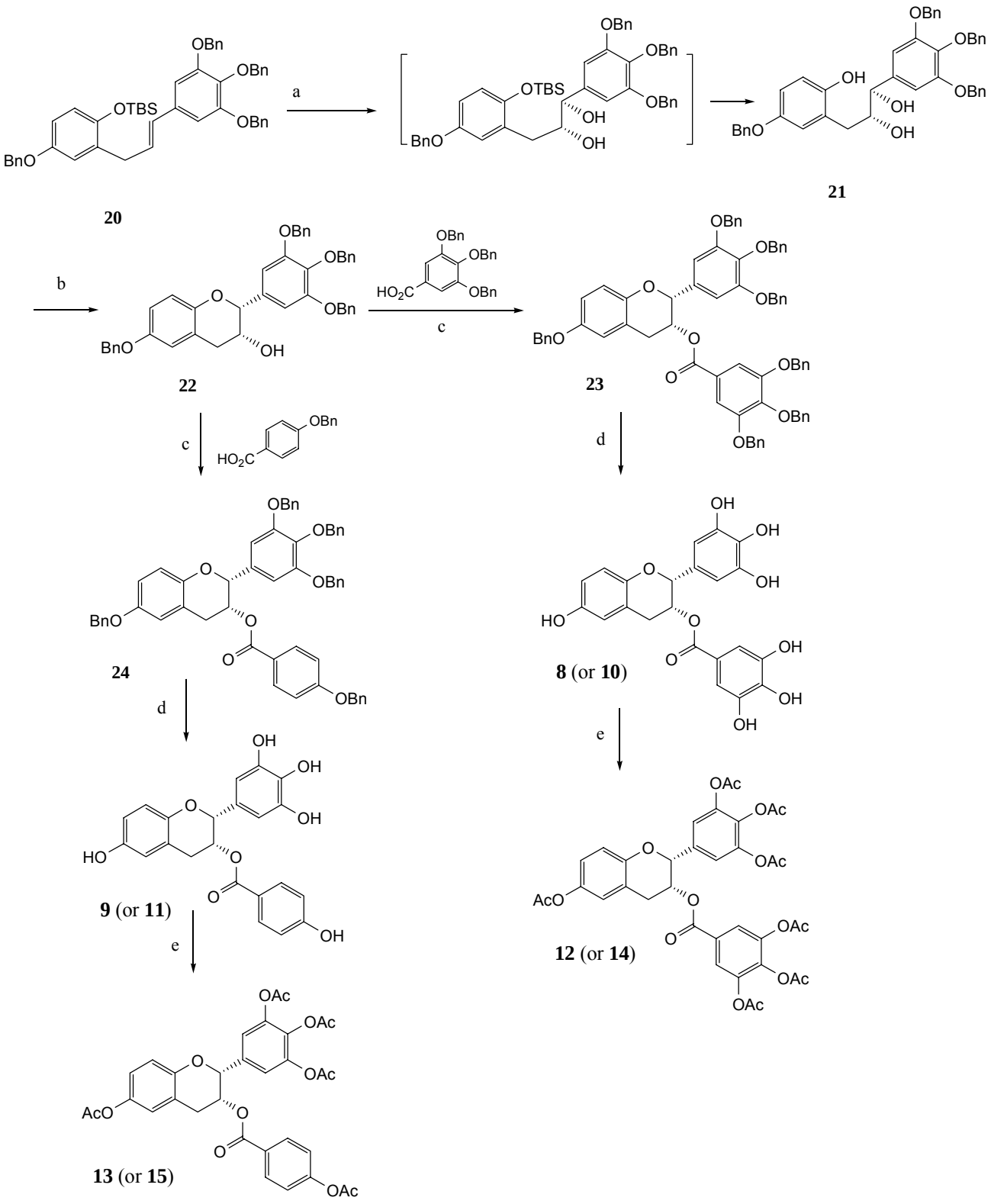
Chemical synthesis.

In 2001, we reported the first total enantioselective synthesis of EGCG the first step of which was a Friedel-Crafts alkylation of a substituted phenol with an arylpropenol to construct the carbon skeleton.[29] Using similar approach, Friedel-Crafts alkylation of 4-benzyloxyphenol (**17**) with 3-[3',4',5'-tris*(ben*zyloxy)phenylprop-2-enol (**18**) to give the *C*-alkylated product **16** was examined. However, under all conditions examined, the reaction gave a complex mixture with only small amount of **16**. On the other hand, under Mitsunobu conditions using triphenylphosphine and di-*iso*propyl azodicarboxylate as the condensation agents, compounds **17** and **18** reacted to give cleanly the *C*- and *O*-alkylated products **16** and **19**, albeit with **16** as the minor product. Fortunately, treatment with trimethylaluminum in toluene converted **19** to the *C*-alkylated compound cleanly, giving an acceptable overall yield of the desired **16** (Scheme 1).



Scheme 1. (a) Ph_3P, $(NCO_2iPr)_2$, toluene, -15 ^oC, 30 min; (b) $AlMe_3$, toluene, rt, 30 min

Sharpless asymmetric dihydroxylation of compound **16** directly did not give clean reaction as we had found previously for similar compounds.[29] Protection of the hydroxyl group of **16** with *t*-butyldimethylsilyl moiety gave the protected compound **20** which was cleanly dihydroxylated under the standard Sharpless conditions followed by desilylation to give the diol **21** (Scheme 2). Cyclization of **21** using previously established conditions[29] gave the cyclic compound **22**. When AD-mix-β was used in the dihydroxylation step, compound **22** has the *2R, 3R*-configuration based on the optical rotation observed and in line with similar compounds.[29] Acylation of either enantiomers of **22** with the appropriate benzoic acids *via* the acid chlorides gave the corresponding esters **23** and **24**. Catalytic hydrogenolysis removed the benzyl protecting groups to give compounds **8**, **9**, **10** and **11** respectively. Acetylation of compounds **8-11** gave the peracetates **12-15**.



Scheme 2. (a) AD-mix-β, $MeSO_2NH_2$, *t*-BuOH/H_2O, 0°C, 48h; (b) (i) $HC(OEt)_3$, pyridine·TsOH; (ii) AcBr; (iii) K_2CO_3, MeOH; (c) acid chloride, DMAP; (d) H_2, $Pd(OH)_2$; (e) Ac_2O, pyridine.

Biological Studies.

Anti-proliferative effect. We first investigated the effect of the four newly synthesized unprotected EGCG analogs **8-11** (Scheme 3) on HL60 leukaemia cell growth. By using an MTS assay, we found that 24 h treatment with analogs **8-11** at 50 μM had almost no effect on HL60 proliferation (Figure 2). However, in the same experiment, when used at the same concentration, acetylated compounds **12** and **14** inhibited about 60% of HL60 cell proliferation (Figure 2) and compounds **13** and **15** were shown to be less effective by inhibiting about 35% HL60 proliferation (Figure 2).

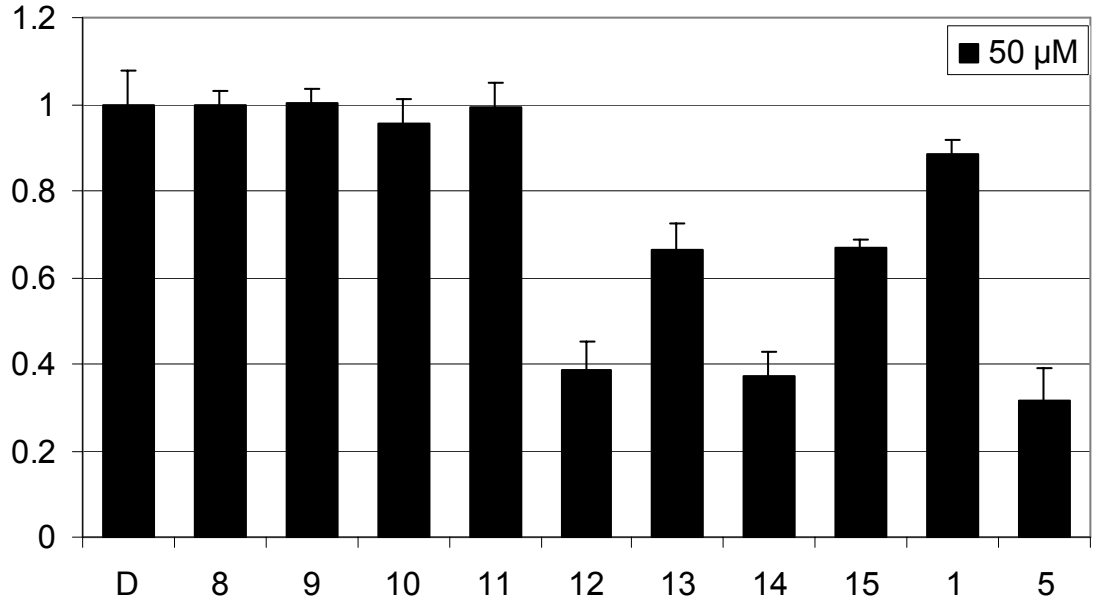


Figure 2. Antiproliferative effect of synthetic protected and unprotected EGCG analogs. HL60 cells were treated for 24 h with compounds **8**, **9**, **10**, **11**, **12**, **13**, **14**, **15**, (-)-EGCG (**1**) and **5** at 50 μM. At the end of the treatment, 20 μL of MTS solution was added to the wells and incubated for 2 to 4 h. The presence of the bioreduced colored formazan product was measured as described in Experimental. DMSO (D) was used as a control.

Inhibition of chymotrypsin (CT)-like activity of a purified 20S proteasome. The four synthetic EGCG analogs **8-11** were tested for their SAR in comparison with natural (-)-EGCG against the chymotrypsin-like activity of a purified 20S proteasome (Table 1). Compounds **8** and **10**, with three hydroxyl groups on the D ring, showed similar proteasome inhibitory potency with IC_{50} values 6.5 and 2.3 μM, respectively. Compounds **9** and **11** that contain only one hydroxyl group on the same D ring were found to be much less potent with IC_{50} values 23.3 and 22.2 μM, respectively. The results are interesting in several respects. Consistent with previous observation that the unnatural enantiomer of EGCG with *2S, 3S*-configuration is more or equally potent in proteasome inhibition as the natural (-)-EGCG with *2R,*

3R-configuration, the *2S, 3S*-compound **10** is more potent than the *2R, 3R*-enantiomer **8**. Secondly, compounds **8** and **10** are more potent than compounds **9** and **11**. This is also consistent with previous observation that the hydroxyl groups on the D ring are important for the proteasomal inhibitory potency and that increased number of hydroxyl groups on the D ring enhances the potency of these EGCG analogs to inhibit the proteasome (also compare EGCG with compound **6** in Table 1). Finally, EGCG is more potent than compounds **8** and **10** and compound **6** is more potent than either compound **9** or **11**. This suggests that the hydroxyl groups on the A ring are also important for the proteasomal inhibitory potency.

Table 1. Inhibition of chymotrypsin-like activity of purified 20S proteasome

Compound	IC50 (μM)
(-)-EGCG	0.4±0.06
8	6.5±0.08
9	23.3±0.3
10	2.3±0.07
11	22.2±0.4
6	6.6±0.7[a]

[a]Data from reference 30

Inhibition of proteasomal activity in intact tumor cells. After we showed that all four un-acetylated compounds **8**-**11** could inhibit the proteasome under cell free conditions, we wanted to test if these compounds and their *O*-acetyl analogs **12**-**15** exhibit proteasomal inhibition in intact tumor cells. For that reason, leukaemia HL60 cells were treated with 50 μM of each compound for 4 and 24 h and proteins extracted from the cells were used for various assays. We found that compound **9** was unable to inhibit the proteasomal CT-like activity, while compounds **8**, **10** and **11** showed only minimal proteasomal inhibition, at both time points (Figure 3). This suggests that, similar to (-)-EGCG, un-acetylated EGCG analogs **8**-**11** are less bioavailable within the cellular environment.[23,11] However, their *O*-acetyl protected analogs **12**-**15** exhibited much higher inhibitory effect. Compounds **12** and **14** inhibited almost 40% of the proteasomal CT-like activity after 4 h of treatment and caused about 80% inhibition after 24 h of treatment (Figure 3). *O*-Acetyl compound **15** inhibited only 20% of the CT-like activity after 4 h and about 40% after 24 h of treatment and compound **13** was even less potent by inducing 10% inhibition after 4 h and ~25% after 24 h (Figure 3). As a comparison, similar to other un-acetylated compounds, compound **1** had only a minimal effect on the proteasomal activity, while compound **5** induced 60% and

84% inhibition after 4 and 24 h of treatment, respectively (Figure 3). It has been shown that cellular esterases are necessary for conversion of *O*-acetyl compounds into the de-acetylated and active forms.[11,24] Therefore, the finding that compounds **12** and **14** are highly effective in intact tumor cells is consistent with the finding that their unprotected, active forms, compounds **8** and **10**, are highly effective against the purified 20S proteasome (Figure 3 and Table 1).

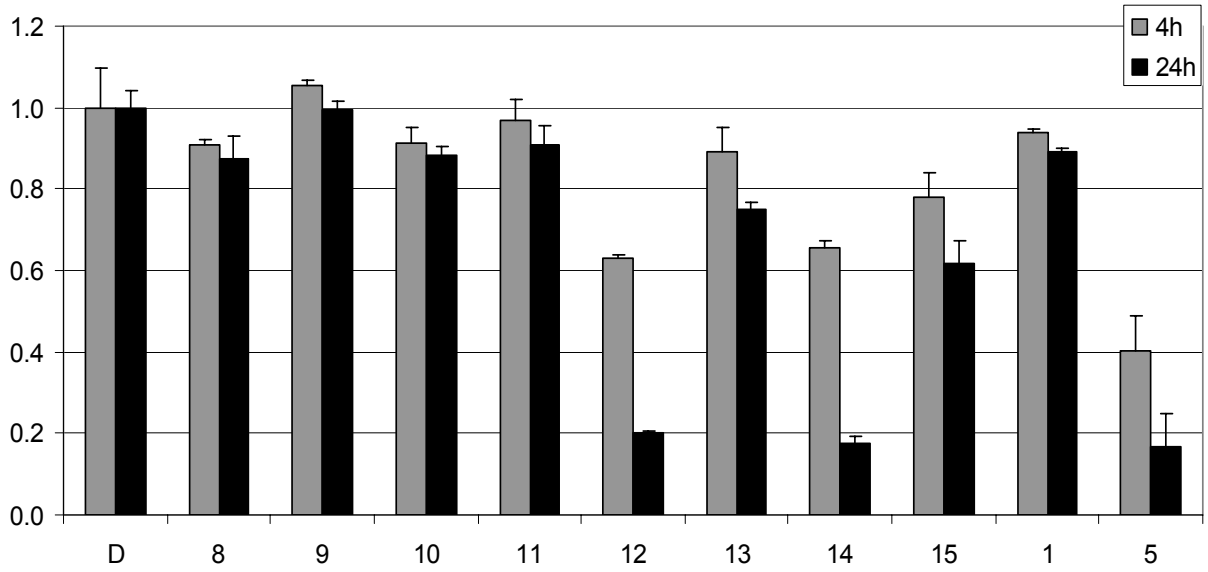


Figure 3. *O*-Acetylated EGCG analogs are potent proteasome inhibitors in cultured tumor cells. HL60 cells were treated with 50 μM of each compound for 4 and 24 h, harvested and analyzed for the chymotrypsin-like activity, as described in Experimental. DMSO (D), (-)-EGCG (**1**) and **5** were used as a control.

It has been shown that inhibition of the proteasomal CT-like activity is causing accumulation of ubiquitinated proteins and proteasome target proteins, such as IκB-α and Bax.[31] We found that all four un-acetylated compounds (**8**, **9**, **10**, and **11**) slightly increased the level of ubiquitinated proteins without affecting the level of IκB-α and Bax proteins after 4 h of treatment (Figure 4A). However, after the 24 h, some accumulation of these two proteasome target proteins was detected (Figure 4B). On the other hand, *O*-Acetyl analogs (**12**, **13**, **14**, and **15**) induced higher levels of ubiquitinated proteins at both time points (Figure 4A & B). Additionally, an ubiquitinated form of IκBα protein appeared after the treatment with compounds **12** and **14** at both 4 and 24 h (Figure 4A & B). Although these compounds did not induce significant accumulation of Bax protein after 4 h, ubiquitinated form of Bax protein was detected at the end of 24 h treatment (Figure 4B). In the same experiment, the effect of EGCG (**1**) was similar to that of other un-acetylated compounds (Figure 4A & B), while the effect of compound **5** was a little stronger than the effect of the most potent compound **14** (Figure 4C).

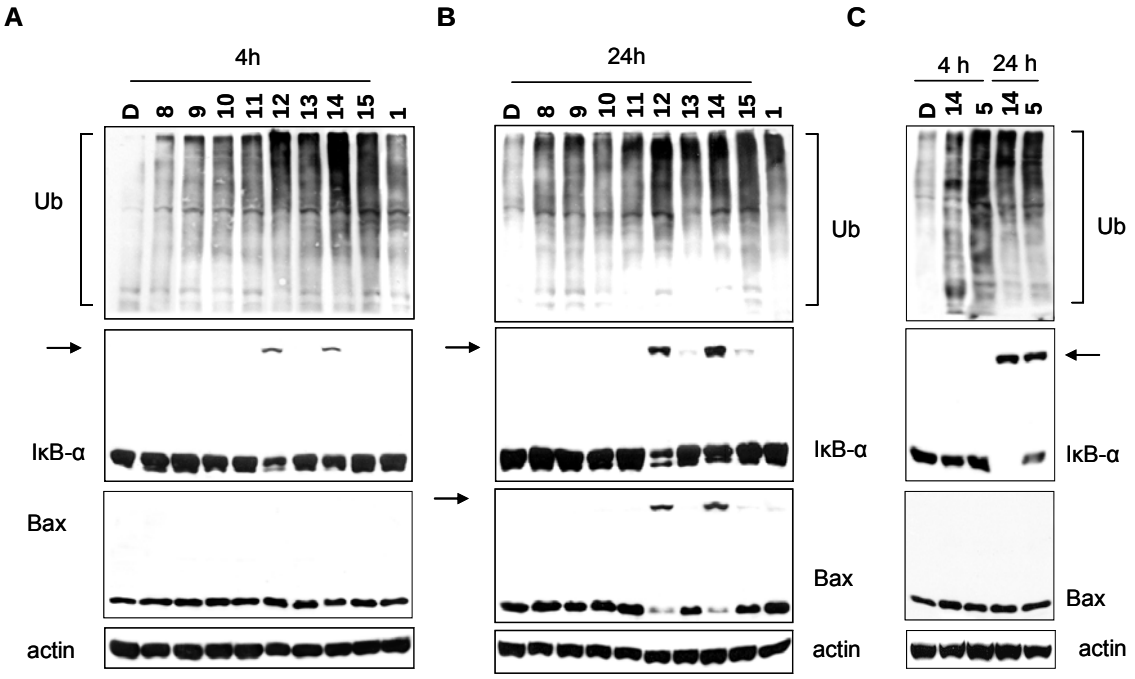


Figure 4. *O*-Acetyl protected EGCG analogs accumulate proteasome target proteins in cultured tumor cells. Western blot analysis of cell extract from HL60 cells treated with 50 μM of unprotected and *O*-acetyl protected EGCG analogs for 4 h (A) and 24 h (B) using specific antibodies to Ubiquitin, IκB-α, Bax, and actin. (-)-EGCG (**1**) was used as a control. (C) Western blot analysis of HL60 cells treated for 4 and 24 h with 50 μM of **14** and **5** using specific antibodies to Ubiquitin, IκB-α, Bax, and actin. Actin was used as a loading control.

Proteasomal inhibition induced by *O*-acetyl compounds **12-15** is associated with apoptosis induction. It has been documented that inhibition of the proteasomal CT-like activity induces apoptosis in various cancer cell lines.[31] In the same experiment, apoptotic morphological changes (shrunken cells and characteristic apoptotic blebbing) were detected after 4 and 24 h treatment with protected compounds **12** and **14**, and to some extent with compound **15** (not shown). To determine whether the observed cell death was representative of apoptosis, aliquots of the cell extracts from the same experiment were used to measure caspase-3 activation and poly(ADP-Ribose)polymerase (PARP) cleavage. After 4 h treatment, compounds **12** and **14** induced a 10.2- and 11-fold increase in caspase-3 activity, respectively (Figure 5A). Some caspase-3 activation was also detected in the cells treated with the same compounds for 24 h, 4.5-fold (compound **12**) and 3.7-fold (compound **14**) (Figure 5A). Compound **15** also induced caspase-3 activation, 4.8- and 2.6-fold after 4 and 24 h, respectively (Figure 5A). Similar to the proteasome inhibitory activity, apoptosis-inducing effect of compound **1** was similar to that of un-acetylated compounds, while compound **5** was more potent than other tested acetylated compounds (Figure 5A). Moreover, Western blot analysis showed that only in the cells treated with compounds **12** and **14** (and compound **5** used for comparison), apoptosis-specific p85 cleaved PARP fragment was generated after 4 h (Figure 5B) and then additionally cleaved to a p65 cleaved PARP fragment at the end of 24 h treatment

(Figure 5B). In the cells treated with compound **15** for 24 h, some p85 and p65 cleaved PARP fragments were also detected (Figure 5B). These data suggest that the *O*-acetyl analogs are more potent than their un-acetylated counterparts in inhibiting the proteasome and inducing the apoptosis.

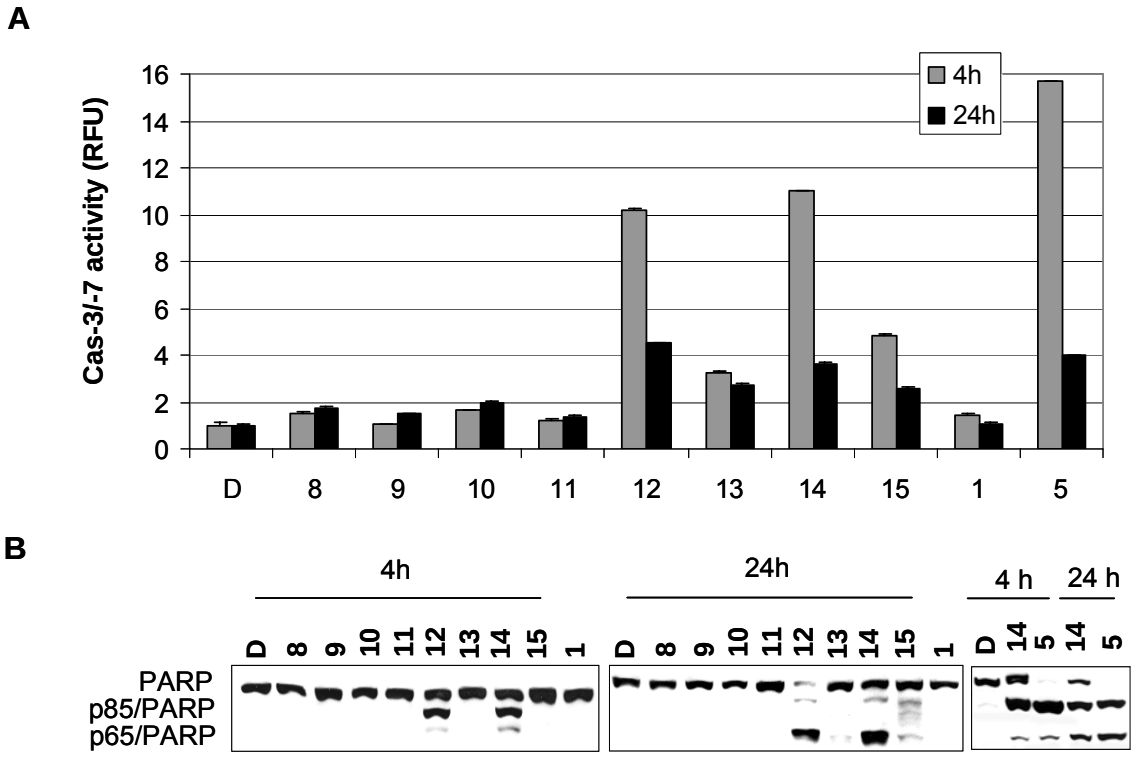


Figure 5. *O*-Acetylated EGCG analogs induce apoptotic cell death in cultured tumor cells. (A) A cell-free caspase-3/-7 activity assay was done as described in Experimental. (B) Western blot analysis using specific antibodies to PARP.

CONCLUSION

We have synthesized enantioselectively the A-ring analog compound **8** of (-)-EGCG, the active ingredient of green tea, and compound **9**, analog of the natural catechin **6** from *C. salvifolius*, as well as their enantiomers **10** and **11**. They show proteasomal inhibitory activities under cell free conditions, at a potency lower than that of (-)-EGCG. The results suggest that the hydroxyl groups in ring A of (-)-EGCG play a role in enhancing the activity. The acetylated derivatives of these compounds show cytotoxic activites and proteasomal inhibition after cellular intake, presumably acting as pro-drugs.

EXPERIMENTAL

General. The starting materials and reagents, purchased from commercial suppliers, were used without further purification. Anhydrous THF was distilled under nitrogen from sodium benzophenone ketyl.

Anhydrous methylene chloride was distilled under nitrogen from CaH_2. Anhydrous DMF was distilled under vacuum from CaH_2. Reaction flasks were flame-dried under a stream of N_2. All moisture-sensitive reactions were conducted under a nitrogen atmosphere. Flash chromatography was carried out using silica-gel 60 (70-230 mesh). The melting points were uncorrected. ^1H and ^{13}C NMR (400 MHz) spectra were measured with TMS as internal standard when $CDCl_3$ and acetone-d_6 were used as solvent. High-resolution electrospray ionization (ESI) mass spectra were recorded using a QTOF-2 Micromass spectrometer.

(*E*)-[3-(5-Benzyloxy)-(2-*tert*-butyldimethylsiloxy)phenyl]-[1-(3,4,5-*tris*(benzyloxy)phenyl)]-propene (20). To a solution of 4-benzyloxyphenol (0.601 g, 3.00 mmol), (*E*)-3,4,5-*tris*-(benzyloxy)cinnamyl alcohol (1.36 g, 3.00 mmol) and triphenylphosphine (1.18 g, 4.50 mmol) in toluene (20.0 mL) cooled to –15-20 °C was added di-*iso*propyl azodicarboxylate (DIAD, 886 μL, 4.50 mmol) dropwise. The reaction mixture was stirred for 30 min and the solvent was evaporated *in vacuo*. The obtained mixture was purified by flash SiO_2 column chromatography (hexane/EtOAc, 4:1) to give (*E*)-[3-(5-benzyloxy)-(2-hydroxy)phenyl]- [1-(3,4,5-tris(benzyloxy)phenyl)]propene (0.353 g, 19%) as a yellow oil and 4-benzyloxy phenyl-(*E*)-2-propen-3-(3,4,5-*tris*(benzyloxy)phenyl ether (1.02 g, 54%) as a white solid. To a solution of (*E*)-1-[3-(5-benzyloxy)-(2-hydroxy)phenyl]-[1-(3,4,5-*tris*(benzyloxy)-phenyl)]propene (353 mg, 556 mmol) in DMF (8.20 mL) were added imidazole (302 mg, 4.44 mmol) and *tert*-butyldimethylsilyl chloride (335 mg, 2.22 mmol). The reaction mixture was stirred for 18 h at rt. The mixture was diluted with H_2O at 0 °C and extracted with AcOEt (30.0 mL x 3). The organic layer was washed with brine (20.0 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The obtained oil was dried under a reduced pressure at 50 °C to remove DMF. Purification by flash SiO_2 column chromatography (hexane/EtOAc, 20:1) furnished the title compound (157 mg, 38%) as a white solid. 4-Benzyloxy phenyloxy-(*E*)-2-propen-3-(3,4,5-tris(benzyloxy)phenyl ether (1.02 g) was dissolved in toluene (32.2 mL) and cooled to 0 °C in an ice bath. Trimethylaluminum solution (2.0 M in toluene, 3.22 mL, 6.44 mmol) was added dropwise into the solution and the ice bath was removed to warm the reaction mixture to rt. After the reaction mixture was stirred for 30 min, the reaction was quenched with 10% aqueous hydrochloric acid solution at 0 °C. The mixture was extracted with AcOEt (3 x 50.0 mL) and the combined organic layers were washed with brine (50.0 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The obtained orange solid (1.03 g) was dissolved in DMF (24.0 mL) and imidazole (885 mg, 13.0 mmol) and *tert*-butyldimethylsilyl chloride (1.22 g, 8.10 mmol) were added to the solution and then the mixture was stirred at rt for 22 h. The reaction mixture was diluted with H_2O at 0 °C and extracted with AcOEt (50.0 mL x 3). The organic layer was washed with brine (50.0 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The obtained oil was dried up under a reduced pressure at 50 °C to remove DMF. Purification of

the obtained orange brown oil (1.29 g) by flash SiO_2 column chromatography (hexane/EtOAc, 20:1) gave the title compound (611 mg, 82%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.45-7.20 (m, 20 H), 6.82 (br s, 1 H), 6.73-6.69 (m, 2 H), 6.65 (s, 2 H), 6.29 (d, *J* = 15.4 Hz, 1 H), 6.19 (dt, *J* = 15.4, 7.3 Hz, 1 H), 5.09 (s, 4 H), 5.04 (s, 2 H), 4.97 (s, 2 H), 3.46 (d, *J* = 7.3 Hz, 2 H), 1.01 (s, 9 H), 0.21 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 154.03, 153.82, 148.36, 138.82, 138.72, 138.22, 138.12, 134.39, 132.58, 131.76, 129.54, 129.47, 129.41, 129.34, 129.14, 129.08, 128.82, 128.77, 128.71, 128.59, 128.52, 128.49, 128.37, 119.85, 117.93, 113.66, 106.81, 76.20, 72.14, 71.45, 34.65, 26.81, 19.21, -3.164; HRMS m/z calculated for $C_{49}H_{52}O_5Si$ (M + H) 749.3662, found 749.3655.

(+)-(*1R, 2R*)-3-[(4-Benzyloxy)-1-hydroxyphen-2-yl]-1-[3,4,5-*tris*(benzyloxy)]phenyl]-1,2-di-hydroxyl-propane (21). AD-mix-beta (2.20 g) and methanesulfonamide (147mg, 1.55 mmol) were dissolved in a mixture of *tert*-BuOH (9.00 mL) and H_2O (9.00 mL). The mixture was stirred at rt for 5 min, and then cooled to 0 °C. A solution of (*E*)-1-[3-(5-benzyloxy)-(2-*tert*-butyldimethyl-siloxy)phenyl]-[1-(3,4,5-*tris*(benzyloxy)phenyl)]propene (**20**, 579 mg, 773 μmol) in CH_2Cl_2 (9.00 mL) was added to the mixture. After the mixture was stirred at 0 °C for 24 h, total four times of additions of AD-mix-beta (1.10 g) and methanesulfonamide (74.2 mg, 780 μmol) were done in 24 h intervals. The reaction was quenched with a mixture of saturated aqueous $Na_2S_2O_3$ solution at 0 °C. After stirred for a while, the mixture was extracted with AcOEt (3 x 50.0 mL). The combined organic layers were washed with brine (50.0 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The obtained crude (756 mg) was dissolved in THF (18.0 mL) and then tetrabutylammonium fluoride solution (1.0 M in THF, 850 μL, 850 μmol) was added dropwise to the solution. The mixture was stirred for 50 min at rt. The reaction was diluted with water at 0 °C and the resulting mixture was extracted with AcOEt (3 x 50.0 mL). The combined organic layers were washed with brine (50.0 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The obtained orange brown amorphous solid (592 mg) was purified by flash SiO_2 column chromatography (hexane/EtOAc, 1:1) to give the title compound (383 mg, 74%) as a pale yellow solid: $[\alpha]_D$ + 11.3 (*c* 1.015, $CHCl_3$, 20 °C); ^{1}H NMR ($CDCl_3$) δ 7.32-7.12 (m, 20 H), 6.72 (d, *J* = 8.0 Hz, 1 H), 6.65 (dd, *J* = 8.0, 3.1 Hz, 1 H), 6.45 (s, 2 H), 6.33 (d, *J* = 3.1 Hz, 1 H), 4.98-4.88 (m, 6 H), 4.81 (s, 2 H), 4.15 (d, *J* = 7.0 Hz, 1 H), 3.79 (ddd, *J* = 14.3, 7.0, 3.5 Hz, 1 H), 2.49 (dd, *J* = 14.3, 3.5 Hz, 1 H), 2.41 (dd, *J* = 14.3, 7.0 Hz. 1 H); ^{13}C NMR ($CDCl_3$) δ 153.02, 152.68, 149.94, 138.16, 137.81, 137.47, 137.06, 136.24, 128.97, 128.82, 128.81, 128.50, 128.28, 128.17, 127.85, 127.81, 125.93, 119.33, 117.96, 114.17, 110.03, 106.83, 77.60, 76.93, 75.52, 71.39, 71.01, 35.08; HRMS m/z calculated for $C_{43}H_{40}O_7Na$ (M + Na) 691.2672, found 691.2656.

(-)-(*2R, 3R*)-6-Benzyloxy-2-[3,4,5-*tris*(benzyloxy)phenyl]-3-hydroxychromane (22). To a solution of (+)-(*1R, 2R*)-3-[(4-benzyloxy)-1-hydroxyphen-2-yl]-1-[3,4,5-*tris*(benzyloxy)]phenyl]-1,2-dihydroxypro-

pane (148 mg, 221 μmol) and triethyl orthoformate (54.7 μL, 332 μmol) in CH_2Cl_2 (4.50 mL) was added pyridinium *p*-toluenesulfonate (27.9 mg, 111 μmol). The reaction mixture was stirred at rt for 20 min, and then the solvent of the reaction mixture was evaporated *in vacuo*. The resulting pale residue was dissolved in CH_2Cl_2 (4.50 mL) and the solution was cooled to 0 °C. Acetyl bromide (500 μL) was added dropwise to the solution at 0 °C and the reaction mixture was stirred for 45 min. The solvent of the mixture was evaporated *in vacuo* and then the obtained orange red oil was dissolved in acetone (6.50 mL). K_2CO_3 (15.3 mg, 111 μmol) was added to the solution and the mixture was stirred at rt for 3.0 h. The reaction mixture was diluted with MeOH (6.50 mL) and K_2CO_3 (15.3 mg, 111 μmol) was added. The mixture was stirred for 14 h, and then the mixture was diluted with water at 0 °C. The mixture was extracted with AcOEt (3 × 30.0 mL) and the combined organic layers were washed with brine (30.0 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The obtained brown solid was purified by flash SiO_2 column chromatography (hexane/EtOAc, 5:1) to give the title compound (83.0 mg, 58%) as a pale yellow solid: $[\alpha]_D$ – 3.9 (*c* 0.390, $CHCl_3$, 20 °C); ^{1}H NMR ($CDCl_3$) δ 7.40-7.13 (m, 20 H), 6.76 (d, *J* = 8.4 Hz, 1 H), 6.71 (dd, *J* = 8.4, 3.0 Hz, 1 H), 6.67-6.60 (m, 1 H), 6.64 (s, 2 H), 5.07-4.90 (m, 8 H), 4.51 (d, *J* = 8.4 Hz, 1 H), 3.89 (ddd, *J* = 16.3, 8.4, 5.7 Hz, 1 H), 2.92 (dd, *J* = 16.3, 5.7 Hz, 1 H), 2.76 (dd, *J* = 16.3, 8.4 Hz. 1 H), 1.65 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 153.49, 153.33, 148.48, 139.00, 138.08, 137.54, 137.16, 133.81, 128.89, 128.88, 128.82, 128.50, 128.27, 128.24, 128.18, 127.87, 127.78, 121.26, 117.40, 115.88, 115.12, 107.02, 82.19, 75.53, 71.53, 70.97, 68.49, 33.44.; HRMS *m/z* calculated for $C_{43}H_{39}O_6$ (M + H) 651.2747, found 651.2760.

(-)-(2*R*, 3*R*)-6-Benzyloxy-2-[3,4,5-*tris*(benzyloxy)phenyl]chroman-3-yl 3,4,5-*tris*(benzyloxy)]benzoate (23). To a solution of 3,4,5-*tris*benzyloxybenzoic acid (30.1 mg, 68.3 μmol) in CH_2Cl_2 (1.00 mL) was added oxalyl chloride (1.00 mL, 11.6 mmol). The mixture was refluxed at 65 °C for 2 h. After the solvent of the reaction was evaporated *in vacuo*, the resulting oil was dried completely under a reduced pressure for 2 h. The obtained white solid was dissolved in CH_2Cl_2 (500 μL) and cooled to 0 °C. 4-Dimethylaminopyridine (10.2 mg, 83.3 μmol) was added to the solution and the mixture was stirred for 5 min. A solution of (-)-(2*R*, 3*R*)-6-benzyloxy-2-[3,4,5-*tris*(benzyloxy)phenyl]-3-hydroxychromane (21.7 mg, 33.3 μmol) in CH_2Cl_2 (500 μL) was added dropwise at 0 °C and the mixture was stirred at rt overnight. The solvent of the mixture was evaporated *in vacuo*. The resulting residue was purified by flash SiO_2 column chromatography (hexane/EtOAc, 10:1) to give the title compound (19.6 mg, 55%) as a colorless amorphous: $[\alpha]_D$ - 29 (*c* 0.980, $CHCl_3$, 20 °C); ^{1}H NMR ($CDCl_3$) δ 7.37-7.11 (m, 37 H), 6.85 (d, *J* = 8.7 Hz, 1 H), 6.79 (dd, *J* = 8.7, 3.2 Hz, 1 H), 6.623-6.59 (m, 1 H), 6.61 (s, 2 H), 5.35 (ddd, *J* = 6.4, 6.4, 5.1 Hz, 1 H), 4.99-4.88 (m, 14 H), 2.93 (dd, *J* = 17.0, 5.1 Hz, 1 H), 2.80 (dd, *J* = 17.0, 6.4 Hz, 1 H); ^{13}C

NMR ($CDCl_3$) δ 165.51, 153.37, 153.22, 152.75, 148.08, 142.98, 138.68, 138.04, 137.68, 137.46, 137.10, 136.81, 134.13, 128.89, 128.83, 128.81, 128.76, 128.74, 128.49, 128.44, 128.33, 128.27, 128.25, 128.15, 128.10, 127.93, 127.81, 127.78, 125.15, 120.14, 117.27, 115.87, 115.22, 109.41, 106.43, 78.60, 75.46, 75.40, 71.54, 71.46, 70.91, 70.37, 29.42.; HRMS *m/z* calculated for $C_{71}H_{60}O_{10}Na$ (M + Na) 1095.4084, found 1095.4149.

(-)-(2*R*, 3*R*)-6-Hydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl (3,4,5-trihydroxy)benzoate (8). To a solution of (-)-(2*R*, 3*R*)-6-benzyloxy-2-[3,4,5-*tris*(benzyloxy)phenyl]chroman-3-yl [3,4,5-*tris*(benzyloxy)]benzoate (43.9 mg, 40.9 μmol) in THF (4.00 mL) and MeOH (4.00 mL) was added palladium hydroxide on carbon powder (20% Pd, 45.0 mg). The mixture was stirred under a H_2 atmosphere at rt for 1 h, filtered and eluted with MeOH. The eluate was evaporated *in vacuo*. The obtained pale brown amorphous solid (23.4 mg) was purified by flash SiO_2 column chromatography (AcOEt/hexane, 2:1) to give the title compound (12.9 mg, 71%) as a colorless solid: $[\alpha]_D$ - 37 (*c* 0.645, MeOH, 20 °C); ^{1}H NMR ($CDCl_3$) δ 6.94 (s, 2 H), 6.76 (d, *J* = 8.8 Hz, 1 H), 6.61 (dd, *J* = 8.8, 3.1 Hz, 1 H), 6.51 (d, *J* = 3.1 Hz, 1 H), 6.39 (s, 2 H), 5.38 (ddd, *J* = 5.4, 5.4, 4.5 Hz, 1 H), 5.07 (d, *J* = 5.4 Hz, 1 H), 4.92 (br s, 7 H), 2.99 (dd, *J* = 16.8, 4.5 Hz, 1 H), 2.80 (dd, *J* = 16.8, 5.4 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 168.44, 152.90, 149.05, 147.83, 147.20, 140.74, 134.73, 132.03, 122.13, 121.86, 118.56, 117.38, 116.76, 111.00, 110.95, 107.08, 107.00, 80.04, 79.93, 71.99, 71.94, 30.07.; HRMS *m/z* calculated for $C_{22}H_{18}O_{10}Na$ (M + Na) 465.0798, found 465.0811.

(-)-(2*R*, 3*R*)-6-Acetoxy-2-(3,4,5-triacetoxyphenyl)chroman-3-yl (3,4,5-triacetoxy)-benzoate (12). To a solution of crude (-)-(2*R*, 3*R*)-6-hydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl (3,4,5-trihydroxy)-benzoate (20.1 mg, 45.4 μmol) in pyridine (50.0 μL) was added acetic anhydride (50.0 μL, 529 mmol) and the mixture was stirred at rt for 1.5 h. The solvent was evaporated *in vacuo* and the resulting residue was dried completely under a reduced pressure. The obtained colorless amorphous solid (33.4 mg) was purified by flash SiO_2 column chromatography (hexane/EtOAc, 2:1) to give the title compound (16.5 mg, 49%) as a colourless amorphous solid: $[\alpha]_D$ - 42 (*c* 0.760, $CHCl_3$, 20 °C); ^{1}H NMR ($CDCl_3$) δ 7.67 (s, 2 H), 7.16 (s, 2H), 6.97 (d, *J* = 9.1 Hz, 1 H), 6.92 (dd, *J* = 9.1, 2.7 Hz, 1 H), 6.81 (d, *J* = 2.7 Hz, 1 H), 5.47 (ddd, *J* = 5.8, 5.8, 5.2 Hz, 1 H), 5.33 (d, *J* = 5.8 Hz, 1 H), 3.17 (dd, *J* = 17.3, 5.2 Hz, 1 H), 2.95 (dd, *J* = 17.3, 5.8 Hz, 1 H), 2.32-2.23 (m, 21 H); ^{13}C NMR ($CDCl_3$) δ 170.19, 167.89, 167.84, 167.02, 166.59, 163.88, 150.84, 144.80, 143.89, 143.71, 139.31, 136.67, 134.74, 127.94, 122.66, 122.58, 121.62, 119.67, 118.79, 117.59, 77.40, 70.56, 28.59, 21.36, 20.90, 20.86, 20.44; HRMS *m/z* calculated for $C_{36}H_{32}O_{17}$ (M + H) 736.1639, found 736.1623.

(-)-(2*R*, 3*R*)-6-Benzyloxy-2-[3,4,5-*tris*(benzyloxy)phenyl]chroman-3-yl (4-benzyloxy)benzoate (24). To a solution of 4-benzyloxybenzoic acid (82.9 mg, 363 μmol) in CH_2Cl_2 (5.00 mL) was added oxalyl chloride (3.00 mL, 34.9 mmol). The mixture was refluxed at 65 °C for 3 h. After the solvent of the reaction was evaporated *in vacuo*, the resulting oil was dried completely under a reduced pressure for 2 h. The obtained white solid was dissolved in CH_2Cl_2 (1.00 mL) and cooled to 0 °C. 4-Dimethylaminopyridine (44.3 mg, 363 μmol) was added to the solution and the mixture was stirred for 5 min. A solution of (-)-(2*R*, 3*R*)-6-benzyloxy-2-[3,4,5-*tris*(benzyloxy)phenyl]-3-hydroxychromane (94.5 mg, 145 μmol) in CH_2Cl_2 (2.00 mL) was added dropwise at 0 °C and the mixture was stirred at rt overnight. The solvent of the mixture was evaporated *in vacuo* and the obtained residue was purified by flash SiO_2 column chromatography (hexane/EtOAc, 5:1) to give the title compound (62.2 mg, 50%) as a white solid: $[\alpha]_D$ - 33 (*c* 0.400, $CHCl_3$, 20 °C); ^{1}H NMR ($CDCl_3$) δ 7.79 (d, *J* = 8.8 Hz, 2 H), 7.38-7.10 (m, 25 H), 6.85 (d, *J* = 8.8 Hz, 2 H), 6.83 (d, *J* = 8.8 Hz, 1 H), 6.77 (dd, *J* = 8.8, 2.9 Hz, 1 H), 6.63 (s, 2 H), 6.60 (brs, 1 H), 5.41-5.34 (m, 1 H), 5.07 (d, *J* = 25.6 Hz, 1 H), 4.98 (s, 2 H), 4.95 (s, 2 H), 4.92 (s, 2 H), 4.91 (s, 4 H), 2.97 (dd, *J* = 16.4, 4.0 Hz, 1 H), 2.83 (dd, *J* = 16.4, 5.6 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 165.58, 163.00, 153.34, 153.14, 148.10, 138.10, 137.52, 137.23, 136.43, 134.19, 132.04, 128.97, 128.87, 128.83, 128.74, 128.52, 128.41, 128.24, 128.13, 128.06, 127.79, 127.73, 122.74, 120.21, 117.35, 115.75, 115.25, 114.82, 106.41, 78.69, 75.42, 71.46, 70.91, 70.38, 69.94, 29.69.; HRMS *m/z* calculated for $C_{57}H_{49}O_8$ (M + H) 861.3427, found 861.3469.

(-)-(2*R*, 3*R*)-6-Hydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl (4-hydroxy)benzoate (9). To a solution of (-)-(2*R*, 3*R*)-6-benzyloxy-2-[3,4,5-*tris*(benzyloxy)phenyl]chroman-3-yl 4-benzyloxybenzoate (39.3 mg, 45.6 μmol) in THF (3.50 mL) and MeOH (3.50 mL) was added palladium hydroxide on carbon powder (20% Pd, 40 mg). The mixture was stirred under a H_2 atmosphere at rt for 1 h, filtered and elutedg with MeOH. The eluate was evaporated *in vacuo*. The obtained pale yellow amorphous solid (23.3 mg) was purified by flash SiO_2 column chromatography (AcOEt/hexane, 2:1) to give the title compound (13.1 mg, 70%) as a colorless amorphous solid: $[\alpha]_D$ - 57 (*c* 0.230, MeOH, 20 °C); ^{1}H NMR ($CDCl_3$) δ 7.73 (d, *J* = 8.9 Hz, 2 H), 6.78-6.73 (m, 3 H), 6.62 (dd, *J* = 8.9, 2.3 Hz, 1 H), 6.51 (d, *J* = 2.3 Hz, 1 H), 6.42 (s, 2 H), 5.37 (ddd, *J* = 6.2, 5.8, 5.0 Hz, 1 H), 5.03 (d, *J* = 6.2 Hz, 1 H), 4.89 (br s, 5 H), 3.04 (dd, *J* = 17.4, 5.0 Hz, 1 H), 2.85 (dd, *J* = 17.4, 5.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 166.14, 162.39, 150.97, 147.18, 145.81, 132.79, 131.71, 131.66, 129.87, 120.92, 120.03, 116.60, 115.35 (115.31), 114.94, 114.75, 105.31, 105.24, 78.41 (78.32), 70.18 (70.13), 28.82 (diastereomer).; HRMS *m/z* calculated for $C_{22}H_{19}O_8$ (M + H) 411.1080, found 411.1092.

(-)-(2*R*, 3*R*)-6-Acetoxy-2-(3,4,5-triacetoxyphenyl)chroman-3-yl (4-acetoxy)benzoate (13). To a

solution of crude (-)-(2*R*, 3*R*)-6-hydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl (4-hydroxy)benzoate (20.4 mg, 49.7 μmol) in pyridine (50.0 μL) and THF (1.00 mL) was added acetic anhydride (93.4 μL, 994 μmol) and the mixture was stirred at rt for 3.5 h. The solvent was evaporated *in vacuo* and the resulting pale brown solid (28.5 mg) was purified by flash SiO_2 column chromatography (hexane/EtOAc, 2:1) to give the title compound (23.7 mg, 77%) as a colorless amorphous solid: $[\alpha]_D$ - 31 (*c* 1.00, $CHCl_3$, 20 °C); ^{1}H NMR ($CDCl_3$) δ 7.93 (d, *J* = 8.7 Hz, 2 H), 7.17 (s, 2H), 7.12 (d, *J* = 8.7 Hz, 2 H), 6.98 (d, *J* = 8.7 Hz, 1 H), 6.93 (dd, *J* = 8.7, 2.4 Hz, 1 H), 6.82 (d, *J* = 2.4 Hz, 1 H), 5.49 (ddd, *J* = 5.9, 5.5, 4.7 Hz, 1 H), 5.36 (d, *J* = 5.5 Hz, 1 H), 3.18 (dd, *J* = 16.9, 4.7 Hz, 1 H), 2.94 (dd, *J* = 16.9, 5.9 Hz, 1 H), 2.30 (s, 3 H), 2.27 (s, 3 H), 2.26 (s, 3 H), 2.25 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 170.12, 169.05, 167.91, 167.04, 165.28, 154.86, 150.91, 144.76, 143.91, 136.96, 134.76, 131.68, 127.36, 122.60, 121.98, 121.67, 119.83, 118.86, 117.49, 77.64, 69.97, 28.57, 21.42, 21.37, 20.91, 20.45; HRMS *m/z* calculated for $C_{32}H_{28}O_{13}Na$ (M + Na) 643.1428, found 643.1432.

(-)-(1*S*, 2*S*)-3-[(4-Benzyloxy)-1-hydroxyphen-2-yl]-1-[3,4,5-*tris*(benzyloxy)]phenyl]-1,2-di-hydroxy-propane (21). The synthesis of this compound was the same as the procedure described for the *1R, 2R*-isomer but using AD-mix-alpha (2.95 g). The obtained brown solid (827 mg) was purified by flash SiO_2 column chromatography (hexane/EtOAc, 1:1) to give the title compound (521 mg, 75%) as a pale yellow solid: $[\alpha]_D$ –10.4 (*c* 1.030, $CHCl_3$, 20 °C); ^{1}H NMR ($CDCl_3$) δ 7.92 (br s, 1 H), 7.44-7.22 (m, 20 H), 6.84 (d, *J* = 8.7 Hz, 1 H), 6.77 (dd, *J* = 8.7, 2.9 Hz, 1 H), 6.55 (s, 2 H), 6.43 (d, *J* = 2.9 Hz, 1 H), 5.11-5.01 (m, 6 H), 4.92 (s, 2 H), 4.28 (d, *J* = 7.7 Hz, 1 H), 3.91 (ddd, *J* = 7.7, 7.2, 2.4 Hz, 1 H), 3.65 (br s, 1 H), 2.72 (br s, 1 H), 2.62 (dd, *J* = 15.0, 2.4 Hz, 1 H), 2.51 (dd, *J* = 15.0, 7.2 Hz. 1 H); ^{13}C NMR ($CDCl_3$) δ 153.04, 152.64, 150.00, 138.25, 137.81, 137.46, 137.05, 136.13, 128.95, 128.81, 128.80, 128.49, 128.27, 128.26, 128.16, 127.81, 127.79, 125.84, 119.33, 117.98, 114.16, 106.82, 77.61, 76.92, 75.48, 71.39, 71.00, 35.02; HRMS *m/z* calculated for $C_{43}H_{40}O_7Na$ (M + Na) 691.2672, found 691.2687.

(+)-(2*S*, 3*S*)-6-Benzyloxy-2-[3,4,5-*tris*(benzyloxy)phenyl]-3-hydroxychromane (22). Cyclization of *1S, 2S*-**21** gave residue (175 mg) which was purified by flash SiO_2 column chromatography (hexane/EtOAc, 4:1) to give the title compound (161 mg, 94%) as a pale yellow solid: $[\alpha]_D$ + 3.8 (*c* 2.04, $CHCl_3$, 20 °C); ^{1}H NMR ($CDCl_3$) δ 7.39-7.17 (m, 20 H), 6.77 (d, *J* = 8.6 Hz, 1 H), 6.73 (dd, *J* = 8.6, 2.9 Hz, 1 H), 6.67-6.64 (m, 1 H), 6.66 (s, 2 H), 5.09-4.94 (m, 8 H), 4.53 (d, *J* = 8.3 Hz, 1 H), 3.91 (ddd, *J* = 9.2, 8.3, 5.5 Hz, 1 H), 2.95 (dd, *J* = 16.7, 5.5 Hz, 1 H), 2.79 (dd, *J* = 16.7, 9.2 Hz. 1 H), 1.55 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 153.46, 153.31, 148.45, 138.94, 138.05, 137.51, 137.13, 133.76, 128.89, 128.82, 128.50, 128.27, 128.25, 128.18, 127.86, 127.79, 121.26, 117.40, 115.83, 115.08, 106.95, 82.19, 75.52, 71.48, 70.93, 68.48, 33.44.; HRMS *m/z* calculated for $C_{43}H_{38}O_6Na$ (M + Na) 673.2566, found 673.2571.

(+)-(2*S*, 3*S*)-6-Benzyloxy-2-[3,4,5-*tris*(benzyloxy)phenyl]chroman-3-yl 3,4,5-*tris*(benzyloxy)benzoate (23). The compound was prepared from *2S, 3S*-**22**. The resulting pale yellow amorphous (198 mg) was purified by flash SiO_2 column chromatography (hexane/EtOAc, 6:1) to give 132 mg (90%) of the title compound as a pale yellow amorphous: $[\alpha]_D$ + 25 (*c* 0.482, $CHCl_3$, 20 °C); ^{1}H NMR ($CDCl_3$) δ 7.38-7.11 (m, 37 H), 6.86 (d, *J* = 8.4 Hz, 1 H), 6.80 (dd, *J* = 8.4, 2.2 Hz, 1 H), 6.62 (d, *J* = 2.2 Hz, 1 H), 6.61 (s, 2 H), 5.36 (ddd, *J* = 5.6, 5.6, 4.5 Hz, 1 H), 5.12-4.89 (m, 14 H), 5.08 (d, *J* = 5.6 Hz, 1 H), 2.94 (dd, *J* = 16.5, 4.5 Hz, 1 H), 2.81 (dd, *J* = 16.5, 5.6 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 165.53, 153.38, 153.23, 152.76, 148.09, 143.00, 138.70, 138.05, 137.69, 137.47, 137.12, 136.82, 134.14, 128.93, 128.91, 128.84, 128.82, 128.78, 128.76, 128.59, 128.50, 128.45, 128.34, 128.29, 128.26, 128.17, 128.11, 127.94, 127.84, 127.82, 127.80, 125.16, 120.15, 117.28, 115.88, 115.22, 109.42, 106.44, 78.61, 75.47, 75.42, 71.56, 71.47, 70.93, 70.38, 29.42.; HRMS *m/z* calculated for $C_{71}H_{61}O_{10}$ (M + H) 1073.4265, found 1073.4305.

(+)-(2*S*, 3*S*)-6-Hydroxy-2-[(3,4,5-trihydroxyphenyl)]chroman-3-yl 3,4,5-trihydroxybenzoate (10). This compound was prepared from *2S, 3S*-**23**. The obtained pale pink amorphous (66.6 mg) was purified by flash SiO_2 column chromatography (AcOEt/hexane, 2:1) to give 39.6 mg (73%) of the title compound as a pale yellow amorphous: $[\alpha]_D$ + 37 (*c* 0.482, MeOH, 20 °C); ^{1}H NMR ($CDCl_3$) δ 6.97-6.93 (m, 2 H), 6.76 (d, *J* = 8.9 Hz, 1 H), 6.61 (br d, *J* = 8.9 Hz, 1 H), 6.51 (br s, 1 H), 6.39 (s, 1 H), 5.38 (ddd, *J* = 5.7, 4.8, 4.5 Hz, 1 H), 5.07 (d, *J* = 4.8 Hz, 1 H), 4.96 (br s, 7 H), 2.98 (dd, *J* = 16.7, 4.5 Hz, 1 H), 2.81 (dd, *J* = 16.7, 5.7 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ168.46, 152.94, 149.06, 147.85, 147.23, 140.84, 134.75, 132.05, 122.09, 121.87, 118.58, 117.34, 116.76, 110.97, 107.07, 107.01, 80.03 (79.97), 71.99 (71.96), 30.08 (diastereomer).; HRMS *m/z* calculated for $C_{22}H_{19}O_{10}$ (M + H) 443.0978, found 443.0994.

(+)-(2*S*, 3*S*)-6-Acetoxy-2-(3,4,5-triacetoxyphenyl)chroman-3-yl (3,4,5-triacetoxy)benzoate (14). Acetylation of **10** gave a yellow amorphous solid (33.7 mg) which was purified by flash SiO_2 column chromatography (hexane/EtOAc, 1:1) to give the title compound (33.2 mg, 77%) as a colourless amorphous solid: $[\alpha]_D$ + 33 (*c* 0.250, $CHCl_3$, 20 °C); ^{1}H NMR ($CDCl_3$) δ 7.67 (s, 2 H), 7.16 (s, 2 H), 6.97 (d, *J* = 9.3 Hz, 1 H), 6.93 (dd, *J* = 9.3, 2.7 Hz, 1 H), 6.81 (d, *J* = 2.7 Hz, 1 H), 5.48 (ddd, *J* = 6.7, 6.0, 4.7 Hz, 1 H), 5.33 (d, *J* = 6.0 Hz, 1 H), 3.17 (dd, *J* = 17.0, 4.7 Hz, 1 H), 2.95 (dd, *J* = 17.0, 6.7 Hz, 1 H), 2.31-2.23 (m, 21 H); ^{13}C NMR ($CDCl_3$) δ170.12, 167.92, 167.87, 167.05, 166.61, 163.91, 150.86, 144.82, 143.91, 143.73, 139.33, 136.70, 134.76, 127.97, 122.69, 122.60, 121.64, 119.69, 118.81, 117.62, 77.43, 70.59, 28.60, 21.39, 20.93, 20.90, 20.47; HRMS *m/z* calculated for $C_{36}H_{32}O_{17}$ (M + H) 736.1639, found 736.1616.

(+)-(2*S*, 3*S*)-6-Benzyloxy-2-[3,4,5-*tris*(benzyloxy)phenyl]chroman-3-yl (4-benzyloxy)benzoate (24).

The compound was prepared from *2S, 3S*-**22** using the same procedures for *2R, 3R*-**24**. Flash SiO_2 column chromatography (hexane/EtOAc, 10:1) gave the title compound with impurity. Recrystallization (hexane-AcOEt) gave the title compound (153 mg, 60%) as a white solid: $[\alpha]_D$ + 34 (*c* 0.752, $CHCl_3$, 20 °C); ^{1}H NMR ($CDCl_3$) δ 7.87 (d, *J* = 8.7 Hz, 2 H), 7.47-7.20 (m, 25 H), 6.93 (d, *J* = 8.7 Hz, 2 H), 6.91 (d, *J* = 8.7 Hz, 1 H), 6.85 (dd, *J* = 8.7, 2.4 Hz, 1 H), 6.71 (s, 2 H), 6.68 (d, *J* = 2.4 Hz, 1 H), 5.45 (ddd, *J* = 7.1, 6.3, 5.1 Hz, 1 H), 5.15 (d, *J* = 6.3 Hz, 1 H), 5.08-4.97 (m, 10 h), 3.05 (dd, *J* = 16.5, 5.1 Hz, 1 H), 2.91 (dd, *J* = 16.5, 7.1 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 165.61, 163.01, 153.35, 153.15, 148.10, 138.52, 138.10, 137.52, 137.24, 136.44, 134.20, 132.05, 129.00, 128.90, 128.85, 128.76, 128.54, 128.43, 128.26, 128.15, 128.08, 127.81, 127.75, 122.74, 120.21, 117.35, 115.75, 115.26, 114.82, 106.39, 78.70, 75.44, 71.47, 70.93, 70.40, 69.95, 29.68.; HRMS *m/z* calculated for $C_{57}H_{49}O_8$ (M + H) 861.3427, found 861.3464.

(+)-(2*S*, 3*S*)-6-Hydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl (4-hydroxy)benzoate (11). The obtained colorless amorphous solid (84.0 mg) was purified by flash SiO_2 column chromatography (hexane/AcOEt, 1:1) to give the title compound (67.5 mg, 92%) as a colorless amorphous solid: $[\alpha]_D$ + 31 (*c* 0.534, MeOH, 20 °C); ^{1}H NMR ($CDCl_3$) δ 7.73 (d, *J* = 7.9 Hz, 2 H), 6.78-6.73 (m, 3 H), 6.62 (dd, *J* = 7.9, 2.9 Hz, 1 H), 6.52 (d, *J* = 2.9 Hz, 1 H), 6.42 (s, 2 H), 5.37 (ddd, *J* = 6.3, 5.4, 4.6 Hz, 1 H), 5.02 (d, *J* = 5.4 Hz, 1 H), 4.90 (br s, 5 H), 3.04 (dd, *J* = 16.7, 4.6 Hz, 1 H), 2.85 (dd, *J* = 16.7, 6.3 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 166.16, 162.40, 150.97, 147.19, 145.82, 132.80, 131.72, 131.69, 129.90, 120.93, 120.05, 116.62, 115.38 (115.33), 114.96, 114.77, 105.34, 105.28, 78.42 (78.35), 70.19 (70.16), 28.84 (diastereomer).; HRMS *m/z* calculated for $C_{22}H_{19}O_8$ (M + H) 411.1080, found 411.1100.

(+)-(2*S*, 3*S*)-6-Acetoxy-2-(3,4,5-triacetoxyphenyl)chroman-3-yl (4-acetoxy)benzoate (15). The title compound (37.5 mg, 73%) was obtained as a colourless amorphous solid: $[\alpha]_D$ + 33 (*c* 0.430, $CHCl_3$, 20 °C); ^{1}H NMR ($CDCl_3$) δ 7.93 (d, *J* = 8.8 Hz, 2 H), 7.17 (s, 2 H), 7.13 (d, *J* = 8.8 Hz, 2 H), 6.98 (d, *J* = 8.8 Hz, 1 H), 6.93 (dd, *J* = 8.8, 2.6 Hz, 1 H), 6.82 (d, *J* = 2.6 Hz, 1 H), 5.49 (ddd, *J* = 5.8, 5.5, 4.4 Hz, 1 H), 5.36 (d, *J* = 5.8 Hz, 1 H), 3.18 (dd, *J* = 17.2, 4.4 Hz, 1 H), 2.94 (dd, *J* = 17.2, 5.5 Hz, 1 H), 2.30 (s, 3 H), 2.28 (s, 3 H), 2.26 (s, 3 H), 2.26 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 170.13, 169.07, 167.93, 167.06, 165.30, 154.88, 150.93, 144.77, 143.93, 136.99, 134.78, 131.70, 127.38, 122.62, 121.99, 121.59, 119.85, 118.87, 117.52, 77.67, 69.99, 28.57, 21.44, 21.39, 20.93, 20.47; HRMS *m/z* calculated for $C_{32}H_{28}O_{13}$ (M + H) 620.1529, found 620.1527.

Cell culture. Human leukaemia HL60 cells were cultured in RPMI supplemented with 10% (v/v) fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cell cultures were maintained in a 5% CO_2 atmosphere at 37 ^{o}C.

Inhibition of purified 20S proteasome activity. Purified rabbit 20S proteasome (17.5 ng) was incubated with 10 μM of fluorogenic peptide substrate Suc-Leu-Leu-Val-Tyr-AMC in 100 μL of assay buffer (25 mM Tris–HCl, pH 7.5) in the presence of compounds **8**-**11** at various concentrations or equivalent volume of solvent DMSO (D) as control. After 2 h incubation at 37˚C, inhibition of proteasomal chymotrypsin-like activity was measured by the release of hydrolyzed AMC groups, as previously described.[12]

Cell proliferation assay

The MTS assay was used to determine the effects of *O*-acetyl protected and unprotected EGCG analogs on proliferation of HL60 leukemia cells by plating 100 μL of 200,000 cells/mL in a 96-well plate. Cells were allowed to recover for 24 h, followed by the addition of each compound at 50 μM and incubated at 37 ºC. At the end of the treatment, 20 μL of MTS solution was added to the wells and incubated for 2 to 4 h. The presence of the bioreduced colored formazan product was measured by absorbance at 490 nm using a Victor 3 Multilabel Counter (PerkinElmer, Boston, MA, USA). All samples were assayed in triplicate in three independent experiments, and the mean value for each experiment was calculated. The results are displayed as mean (± standard deviation) and are expressed as percentage of the control, which was considered to be 100%.

Cell extract preparation and Western blot analysis. Whole cell extracts were prepared as previously described.[31] For Western blot analysis, the cell extract was separated by SDS-PAGE and transferred to a nitrocellulose membrane, followed by visualization using the enhanced chemiluminescence (ECL) reagent (Amersham Biosciences, Piscataway, NJ), as previously described.[12]

Inhibition of the proteasomal chymotrypsin-like activity in intact tumor cells. HL60 cells were treated with each compound for 4 or 24 h, harvested, and lysed as described previously.[12] After the additional 2 h incubation with the fluorogenic peptide substrate specific for the proteasomal CT-like activity, production of hydrolyzed AMC groups was measured, as described above.

Caspase-3/-7 activity assay. Proteins extracted from cells were incubated in 100 μL of assay buffer (25 mM Tris-HCl, pH 7.5) with 20 μmol/L fluorogenic substrate specific for caspase-3/-7 activity. After 3 h incubation at 37˚C, release of hydrolyzed AMC groups was measured as described above.

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