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Study of the green tea polyphenols catechin-3-gallate (CG) and *epi*catechin-3-gallate (ECG) as proteasome inhibitors

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Abstract—The green tea polyphenol catechin-3-gallate (CG) and *epi*catechin-3-gallate (ECG) were synthesized enantioselectively via a Sharpless hydroxylation reaction followed by a diastereoselective cyclization. Their potencies to inhibit the proteasome activity were measured. The unnatural enantiomers were found to be equally potent to the natural compounds.

1. Introduction

According to a number of epidemiological studies, regular drinking of green tea has been associated with reduced risk of several forms of cancer.[1] In 1996, a development plan with respect to tea as a possible agent for cancer prevention has been formulated by the National Cancer Institute of the United States.[2] The biological effects of green tea are often attributed to the polyphenols, in particular, the catechins.[3] Using liquid chromatography combined with atmospheric pressure chemical ionization-mass spectrometry, at least 12 catechins have been identified in green tea infusions.[4] They are (−)-*epi*afzelechin (EZ, **1**), (+)-catechin (C, **2**), (−)-*epi*catechin (EC, **3**), (−)-gallocatechin (GC, **4**), (−)-*epi*gallocatechin (EGC, **5**), their respective 3-gallate esters (−)-EZG (**6**), (+)-CG (**7**), (−)-ECG (**8**), (−)-GCG (**9**), and (−)-EGCG (**10**), as well as two 3-(3′-methyl)gallate esters (−)-ECMG (**11**) and (−)-EGCMG (**12**). Although a number of cancer-related proteins are affected by tea polyphenols, the exact mechanism of tea-mediated cancer prevention is not known.[5] Recently, it was reported that the naturally occurring ester bond-containing green tea polyphenols, such as EGCG, GCG, and ECG, possess the ability to inhibit proteasome activity in vitro and in vivo.[6] In contrast, the polyphenols without the gallate ester function, such as EGC, GC, and EC, are not inhibitors of proteasome.

The proteasome is a multicatalytic protease responsible for the degradation of most cellular proteins. The eukaryotic proteasome contains three known activities: chymotrypsin-like mediated by the β5-subunit, trypsin-like by the β2-subunit and the caspase-like by the β1-subunit.[7] Since the ubiquitin/proteasome-dependent degradation pathway plays an important role in the up-regulation of cell proliferation and down-regulation of cell death, proteasome inhibitors have been considered as potential anticancer drugs.[8] Indeed, MLN-341 (formerly PS-341), a dipeptidyl boronic compound and a potent and selective inhibitor of the chymotrypsin-like activity of the 20S proteasome, has recently been approved for the treatment of hematological malignant neoplasms.[9] In the case of natural green tea polyphenols, the selective inhibition of the chymotrypsin-like activity of proteasome by EGCG and other gallate esters of catechins may offer an approach to develop drug candidates for cancer prevention or therapy.[6] Through our synthetic efforts, we had recently prepared the natural (−)-EGCG and (−)-GCG as well as the enantiomers (+)-EGCG and (+)-GCG.[10] Surprisingly, it was found that the unnatural enantiomers were equally or more

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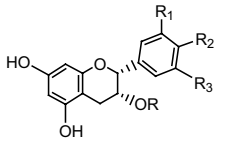
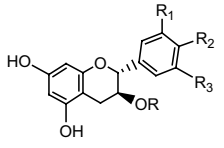
effective as the natural compounds in inhibiting the chymotrypsin-like activity of proteasome.[11] Based on these and similar in vitro results, a mechanistic model to account for their inhibition of proteasome has been proposed.[12] In this model, the green tea polyphenol gallate ester, for example, (−)-EGCG, binds to the active site of the β5-subunit of 20S proteasome. The A ring of (−)-EGCG acts as the phenyl ring of a phenylalanine mimic, binding to the hydrophobic S1 pocket of the β5-subunit. The ester bond of (−)-EGCG is then in reasonable proximity (about 3.18) from the N-terminal Thr (Thr 1) OH function, which is responsible for the protease catalytic activity. Inhibition is then due to the irreversible transfer of the gallate moiety from (−)-EGCG to the hydroxy oxygen of Thr 1. This model was evaluated using in silico docking calculations in conjunction with biological activities of a number of natural occurring or synthetic analogues. It was found that the calculated binding free energies between the enzyme and the inhibitors correlated quite well with the measured in vitro IC_{50} values (Fig. 1).[12]

In order to further validate this model, we have embarked on the synthesis of the natural (+)-CG (**7**) and (−)-ECG (**8**) as well as their enantiomers and examined their inhibition of proteasome. As far as we are aware, there has been no literature report on the enantioselective synthesis of these compounds.[13]



1: R=R_1=H, R_2=OH
3: R=R_1=H, R_2=R_3=OH
5: R=H, R_1=R_2=R_3=OH
6: R=gallate, R_1=R_3=H, R_2=OH
8: R=gallate, R_1=H, R_2=R_3=OH
10:R=gallate, R_1=R_2=R_3=OH
11:R=3'-methylgallate, R_1=H, R_2=R_3=OH
12:R=3'-methylgallate, R_1=R_2=R_3=OH



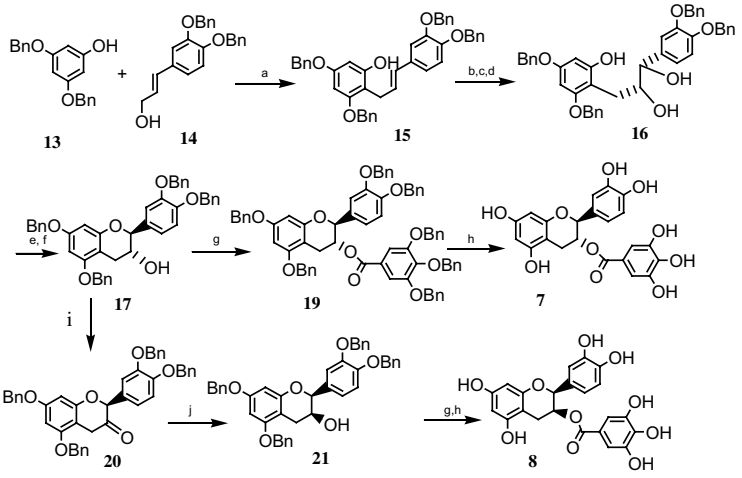
2: R=R_1=H, R_2=R_3=OH
4: R=H, R_1=R_2=R_3=OH
7: R=gallate, R_1=H, R_2=R_3=OH
9: R=gallate, R_1=R_2=R_3=OH

Figure 1. Structure of catechins and analogs.

2. Results and discussion

2.1. Enantioselective synthesis of CG (7) and ECG (8)

The syntheses and **7** and **8** followed the sequence of reactions outlined in Scheme 1. Friedel–Craft alkylation of 3,5-bis(benzyloxy)phenol (**13**) with 3′,4′-bis(benzyloxy)cinnamyl alcohol (**14**) under acidic conditions gave compound **15**. The double bond retained the *trans* stereochemistry as evident from the coupling constant of the vinyl hydrogens. Protection of the phenolic hydroxy group as the *t*-butyldimethylsilyl (TBS) ether was followed by Sharpless asymmetric dihydroxylation of **15** with AD-mix-α. Subsequent deprotection of the TBS group gave the optically active compound (+)-**16** in 48% yield. The configurations of the two newly created chiral centres were assigned to have the *1S*, *2S* stereochemistry on the basis of the model normally used for Sharpless asymmetric hydroxylation, and was later confirmed by identity of the final product CG (**7**). Cyclization of (+)-**16** with ethyl orthoformate under catalytic pyridinium *p*-toluenesulfonate conditions, followed by methanolysis of the intermediate formate ester, gave the cyclized compound (+)-**17** in 73% yield. Compound **17** had the *trans* stereochemistry as evident from its ^{1}H NMR spectrum. Furthermore, since there was an inversion at the benzylic position, the stereochemistry of (+)-**17** must be *2R*, *3S* (chromane numbering). Acylation of (+)-**17** with 3,4,5-tris(benzyloxy)galloyl chloride (**18**) gave the protected gallate ester (+)-**19**. Hydrogenolysis of (+)-**19** with $Pd(OH)_2$/C gave (+)-CG (**7**) with *2R*, *3S* configuration whose optical activity and NMR spectra were in agreement with values reported for the natural compound.[14] On the other hand, when compound **15** was converted to the dihydroxy compound (−)-**16** with AD-



Scheme 1. Synthesis of CG and ECG. (a): $H_2SO_4(SiO_2)/CH_2Cl_2$, rt; (b): TBSCl/imidazole/DMF, rt; (c): AD-mix/$CH_3SO_2NH_2/H_2O$/*t*-BuOH/CH_2Cl_2, 0 °C; (d): TBAF/THF, rt; (e): $CH(OEt)_3$/PPTS/$(CH_2Cl)_2$, rt; (f): K_2CO_3/MeOH/DME, rt; (g): 3,4,5-tris(benzyloxy)benzoyl chloride/DMAP/CH_2Cl_2, rt; (h): $H_2/Pd(OH)_2$ on charcoal/MeOH/THF, rt; (i): Dess–Martin periodinane/CH_2Cl_2, rt; (j): L-Selectride/THF, −78 °C–rt.

mix-β, and the same reaction sequence was followed, (−)-CG (**7**) was obtained. It had the same NMR spectra as the (+)-enantiomer but with opposite optical rotation.

Dess–Martin oxidation of compound (+)-**17** with the *2R*, *3S* configuration gave the ketone compound (+)-**20** with *2R* stereochemistry. Selective hydride reduction of (+)-20 gave the *cis* epimer (−)-**21**, now with the *2R*, *3R* stereochemistry. Acylation of (−)-**21** with **18** gave **22**, which was followed by hydrogenolysis to give (−)-ECG with the same optical rotation as the natural (−)-ECG (**8**), with *2R*, *3R* configuration. The ent-(+)-ECG was obtained by the same reaction sequence starting from (−)-**17**.

2.2. Inhibition of proteasome

We then tested whether these synthetic compounds, (−)-ECG, (+)-ECG, (−)-CG and (+)-CG, can inhibit the proteasomal chymotrypsin-like activity, using (−)-EGCG as a positive control.[6,11,12] We first used a purified 20S prokaryotic proteasome. The chymotrypsin-like activity of purified 20S prokaryotic proteasome was significantly inhibited by the synthetic (−)-ECG and (+)-CG with IC_{50} values of 0.58 and 0.73 μM, respectively (Table 1). Furthermore, the synthetic ent-(+)-ECG and ent-(−)-CG were also equally potent to inhibit the chymotrypsin-like activity of purified proteasome (Table 1). As expected, (−)-EGCG acts as a potent inhibitor of the proteasomal chymotrypsin activity ($IC_{50} = 0.30$ μM in Table 1), similar to what we reported previously.[6,12] In our previous study,[12] we have shown that (−)-ECG and (−)-CG were able to inhibit the chymotrypsin-like activity of a purified rabbit 20S proteasome with similar potencies (IC_{50} values of 0.71 and 0.51 μM, respectively). When both (+)-ECG and (+)-CG were tested using the purified rabbit 20S proteasome, we observed similar potencies to those reported in Table 1.

We next tested the potencies of these compounds in inhibiting the 26S proteasome activities in a leukemia Jurkat T cell extract. All the four compounds, (−)-ECG, (+)-ECG, (−)-CG, and (+)-CG, greatly inhibited the chymotrypsin-like activity of 26S proteasome, with IC_{50} values of 3.5, 3.0, 4.5, and 3.5 μM, respectively (Table 1). As a positive control, (−)-EGCG at 2.0 μM inhibited 50% of the 26S proteasomal chymotrypsin-like activity (Table 1). Furthermore, (−)-ECG, (+)-ECG, (−)-CG and (+)-CG also inhibited the caspase-like activity of the 26S proteasome in the cell extract, with IC_{50} values ranging from 3.7 to 5.3 μM. However, these four compounds had much less inhibitory activities to the trypsin-like activity: only 3–10% inhibition at 10 μM (Table 1). Therefore, these new synthetic polyphenol compounds are potent inhibitors of the proteasomal chymotrypsin-like (and caspase-like) activities.

The nearly equal potencies of the synthetic enantiomeric (−)-CG and (+)-ECG as their natural compounds in proteasome inhibition corroborate our previous observations on GCG and EGCG where similar potencies were observed for both the natural and the synthetic enantiomers.[11,12] Any mechanistic model to account for the proteasome inhibition activities of the green tea catechin gallate esters will have to take these observations into consideration. Our previous in silico docking calculation suggested that the natural (−)-EGCG binds to the β5-chymotrypsin active site of proteasome. In this model, the fairly hydrophobic A–C rings of (−)-EGCG are oriented in the S1 pocket of the β5-subunit, the B ring projected up into solvent, and the gallate (G) ring ester bond-carbon located 3.18 Å away from the hydroxyl of Thr 1.[12] Experimentally, the synthetic ent-(+)-EGCG was found to be slightly more potent than the natural (−)-EGCG with regard to purified 20S proteasome.[11] The docking studies revealed that (+)-EGCG was also oriented in the proteasome β5-subunit, with the A–C rings in the S1 pocket and the B ring in solvent. However, in order that the gallate (G) group of (+)-EGCG was to bind in the same relative position to the hydroxyl of Thr 1 as (−)-EGCG, the A–C rings of (+)-EGCG had to flip 180° (in relation to the A–C rings) to attain similar orientation/conformation. Due to the partial symmetry of the A–C rings, with O1 being isostere of C4 (in C ring) and B ring similar to G ring, the model successfully accounted for the similar activities of (+)-EGCG and the natural (−)-EGCG.[12] This mechanistic and binding model could also be used to interpret the proteasome inhibition properties of the natural occurring GCG, ECG, and CG, which are less potent than EGCG in proteasome inhibition.[12] Their binding energies to the proteasome's active site have all increased relative to that of EGCG due to either the change of relative stereochemistry of the B and G rings, or the absence of one hydroxyl group in the B ring. The calculated binding free energies between the enzyme and these compounds correlated reasonably well with the measured in vitro IC_{50} values.[12] Furthermore, since the partial symmetry of the A–C rings is preserved between the natural compounds and the synthetic enantiomers, the model can also be used to explain the nearly equal potencies of the unnatural enantiomers.

Table 1. Effects of tea polyphenols on various proteasome activities

Compound	IC_{50} for chymotrypsin-like activity of the purified 20S prokaryotic proteasome (μM)	IC_{50} for chymotrypsin-like activity of Jurkat T cell extract (μM)	IC_{50} for caspase-like activity of Jurkat T cell extract (μM)	Inhibition of trypsin-like activity of Jurkat T cell extract at 10 μM (%)
(−)-ECG	0.58	3.5	5.3	3
(+)-ECG	0.73	3.0	4.2	3
(−)-CG	0.75	4.5	3.9	10
(+)-CG	0.73	3.5	3.7	10
(−)-EGCG	0.30	2.0	3.1	2

3. Experimental

3.1. General

The starting materials and reagents, purchased from commercial suppliers, were used without further purification. Literature procedures were used for preparation of 3,5-bis(benzyloxy)phenol (**13**),[15] 3,4,5-tris(benzyloxy)-benzoic acid,[16] silica gel supported H_2SO_4,[10] and (*E*)-3,4-bis(benzyloxy)cinnamyl alcohol (**14**).[17] 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. ^{1}H NMR and ^{13}C NMR (400 MHz) spectra were measured with TMS as an internal standard when $CDCl_3$ and acetone-d_6 were used as a solvent. High-resolution (ESI) MS spectra were recorded using a QTOF-2 Micromass spectrometer.

3.2. (*E*)-3,4-Bis(benzyloxy)cinnamyl alcohol (14)

3,4-Bis(benzyloxy)benzaldehyde (9.0 g, 42.4 mmol) was dissolved in dry THF (150 mL) under a nitrogen atmosphere and cooled in ice bath. To this solution triethyl phosphonoacetate (9.5 g, 42.4 mmol) was added. Sodium hydride (1.7 g, 60% dispersion in mineral oil, 42.4 mmol) was then added in 10 batches. The mixture was allowed to be stirred at rt for 2 h. Saturated aqueous $NaHCO_3$ solution was added. The organic phase was separated, and the aqueous layer was extracted with EtOAc. The organic phases were combined, dried ($MgSO_4$) and evaporated to afford a solid. The solid was washed with hexane to remove the mineral oil to yield ethyl (*E*)-3,4-bis(benzyloxy)cinnamate (10.1 g, 92.0% yield): mp 80–81 °C.

To a solution of ethyl (*E*)-3,4-bis(benzyloxy)cinnamate (9.5 g, 24.4 mmol) in dry THF (120 mL) at −78 °C under a nitrogen atmosphere, DIBAL (36 mL, 1 M solution in hexane, 54 mmol) was added dropwise. The mixture was stirred at −78 °C for 1 h and then at rt for another 1 h. The mixture was cooled to 0 °C and poured into a stirred mixture of hexane (250 mL) and saturated aqueous Na_2SO_4 solution (5 mL). The resulting mixture was stirred until a large quantity of solid was formed. The mixture was filtered, and the solid was thoroughly washed with EtOAc. The organic solutions were combined and dried ($MgSO_4$). The residue after evaporation of the solvent was washed again with hexane, and the solid was collected and recrystallized in EtOAc and hexane to afford (*E*)-3, 4-bis(benzyloxy)cinnamyl alcohol (7.9 g, 71% overall yield based on 3,4-dihydroxybezaldehyde): mp 75–76 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.44–7.29 (m, 10H), 6.98 (s, 1H), 6.84 (bs, 2H), 6.43 (d, $J = 15$ Hz, 1H), 6.12 (dt, $J = 15$, 5.8 Hz, 1H), 5.12 (s, 2H), 5.11 (s, 2H), 4.20 (dd, $J = 5.8$, 1.3 Hz, 2H), 1.90 (br s, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 148.8, 148.6, 137.0, 130.0, 128.4, 127.7, 127.2, 127.1, 126.7, 120.1, 114.7, 112.7, 71.2, 71.1, 63.5.

3.3. (*E*)-3-[2,4-Bis(benzyloxy)-6-hydroxyphenyl]-1-[3,4-bis(benzyloxy)phenyl]propene (15)

At rt under an N_2 atmosphere, 25% H_2SO_4/SiO_2 (1.6 g, 4 mmol) was added in one batch to the stirred mixture of 3,5-bis(benzyloxy)phenol (3.06 g, 10 mmol) and (*E*)-3,4-bis (benzyloxy)cinnamyl alcohol (3.46 g, 10 mmol) in dry CH_2Cl_2 (50 mL). The resulting mixture was stirred at rt overnight. After filtration and evaporation, the residue was purified by column chromatography on silica gel (benzene) to afford the desired compound as white amorphous mass (3.0 g, 47.3% yield): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.43–7.32 (m, 20H), 6.95 (d, $J = 1.2$ Hz, 1H), 6.84–6.81 (m, 2H), 6.37 (A of AB, $J = 15.5$ Hz, 1H), 6.27 (d, $J = 1.7$ Hz, 1H), 6.16 (d, $J = 1.7$ Hz, 1H), 6.14–6.10 (B of ABt, $J = 15.5$, 5.8 Hz, 1H), 5.12 (s, 2H), 5.11 (s, 2H), 5.01 (s, 2H), 4.98 (s, 2H), 3.55 (d, $J = 5.8$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 158.5, 157.6, 155.5, 148.7, 148.1, 137.0, 136.8, 136.6, 130.9, 129.8, 128.3, 128.2, 128.1, 127.7, 127.5, 127.4, 127.2, 127.1, 127.0, 126.4, 119.6, 114.8, 112.3, 106.7, 94.8, 93.4, 76.9, 71.1, 70.1, 69.8, 26.1; HRMS (ESI) calcd for $C_{43}H_{38}O_5$ (M+Na) 657.2617, found 657.2631.

3.4. (−)-(*1R,2R*)-3-[2,4-Bis(benzyloxy)-6-hydroxyphenyl]-1-[3,4-bis(benzyloxy)phenyl]propane-1,2-diol ((−)-16)

The propene **15** (3.0 g, 4.7 mmol) was dissolved in dry DMF (50 mL), and to this solution imidazole (1.4 g) and TBSCl (1.7 g) were added successively. The resulting mixture was stirred at rt overnight, and then saturated Na_2CO_3 solution was added to quench the reaction. The mixture was extracted with EtOAc. The organic layers were combined, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatograph on silica gel (*n*-hexane and EtOAc 6/1 v/v) to afford [3,5-bis(benzyloxy)-2-[3-[3,4-bis(benzyloxy)phenyl]allyl]-phenoxy]-*tert*-butyldimethyl-silane (3.1 g). This material was used in the next step without further purification.

AD-mixβ (12.8 g) and methanesulfonamide (0.85 g) were dissolved in a solvent mixture of *t*-BuOH (60 mL) and H_2O (60 mL). The resulting mixture was stirred at rt for 5 min, then the mixture was cooled to 0 °C and a solution of [3,5-bis(benzyloxy)-2-[3-[3,4-bis(benzyloxy)-phenyl]-allyl]phenoxy]-*tert*-butyldimethylsilane (3.1 g) in dichloromethane (60 mL) was added. After the mixture had been stirred overnight, a total of four batches of AD-mixβ (6.4 g each) and methanesulfonamide (0.43 g each) were added in 24 h intervals. After another 24 h of stirring at 0 °C, TLC showed that the reaction was completed. Then a 10% $Na_2S_2O_3$ aqueous solution was added to quench the reaction. The mixture was extracted with EtOAc. The organic phases were combined, dried ($MgSO_4$) and evaporated. The residue was redissolved in THF (30 mL), and TBAF (10 mL, 1 M in THF) was added. The resulting mixture was stirred at rt for 4 h, and saturated $NaHCO_3$ solution was added. The

mixture was extracted with EtOAc and the organic layers were combined, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography on silica gel (5% EtOAc/CH_3Cl) and then recrystallized in EtOAc to give a white solid (2.1 g, 32% yield based on compound **13**): mp 160–162 °C; $[\alpha]_D$ −12.8 (*c* 0.5, CH_3Cl/MeOH 3/1 v/v); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.41–7.25 (m, 18H), 7.10 (d, $J = 6.9$ Hz, 2H), 6.92 (d, $J = 1.5$ Hz, 1H), 6.80 (AB, $J = 8$ Hz, 2H), 6.27 (d, $J = 2.2$ Hz, 1H), 6.19 (d, $J = 2.2$ Hz, 1H), 5.10 (AB, $J = 12.2$ Hz, 2H), 5.05 (s, 2H), 5.02 (s, 2H), 4.88 (AB, $J = 11.8$ Hz, 2H), 4.44 (d, $J = 6.8$ Hz, 1H), 3.97–3.93 (m, 1H), 2.92 (A of AB, $J = 14.5$, 3.6 Hz, 1H), 2.74 (B of AB, $J = 14.5$, 8.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz); δ 158.8, 157.5, 157.2, 148.6, 148.5, 136.8, 136.7, 136.6, 136.5, 133.1, 128.3, 128.2, 128.1, 127.7, 127.5, 127.4, 127.3, 127.1, 126.9, 126.4, 119.7, 114.2, 113.3, 105.8, 95.6, 93.1, 76.9, 70.9, 70.7, 69.8, 69.7, 26.1; HRMS (ESI) calcd for $C_{43}H_{40}O_7$ (M+Na) 691.2672, found 691.2677.

Using the same procedure with AD-mixα instead of AD-mixβ, (+)-(*1S,2S*)-3-[2,4-bis(benzyloxy)-6-hydroxyphenyl]-1-[3,4-bis(benzyloxy)phenyl]propane-1,2-diol ((+)-**16**) ($[\alpha]_D$ +12.8 (*c* 0.5, CH_3Cl/MeOH 3/1 v/v)) was prepared and it had identical NMR spectra as the (−)-isomer.

3.5. (−)-(2*S*,3*R*)-5,7-Bis(benzyloxy)-2-[3,4-bis(benzyloxy)-phenyl]chroman-3-ol ((−)-17)

To a suspension of (*1R,2R*)-3-[2,4-bis(benzyloxy)-6-hydroxyphenyl]-1-[3,4-bis(benzyloxy)phenyl]propane-1,2-diol (2.1 g, 3.1 mmol) in 1,2-dichloroethane (50 mL) was added triethyl orthoformate (2 mL), followed by PPTS (500 mg, 2.0 mmol). The mixture was stirred at rt for 20 min and the solid dissolved. The mixture was heated to 60 °C for 5 h until TLC showed the reaction had been completed. After evaporation of the solvent, the residue was redissolved in DME (30 mL) and MeOH (30 mL), K_2CO_3 (500 mg) was added, and the mixture was stirred at rt overnight. The solvent was evaporated, and the residue was purified by flash chromatography on silica gel (EtOAc/hexane, 1/3 v/v) to afford the desired product as white solid (1.5 g, 73% yield): mp 136–138 °C; $[\alpha]_D$ −3 (*c* 1.0, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.43–7.31 (m, 20H), 7.02 (s, 1H), 6.94 (d, $J = 0.9$ Hz, 2H), 6.27 (d, $J = 2.2$ Hz, 1H), 6.20 (d, $J = 2.2$ Hz, 1H), 5.19 (m, 4H), 5.02 (s, 2H), 4.98 (s, 2H), 4.62 (d, $J = 8.2$ Hz, 1H), 3.99–3.97 (m, 1H), 3.13 (A of AB, $J = 16.4$, 5.6 Hz, 1H), 2.67 (B of AB, $J = 16.4$, 8.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 158.5, 157.5, 155.0, 149.0, 136.8, 136.7, 136.6, 128.3, 128.2, 127.7, 127.6, 127.5, 127.3, 127.2, 126.9, 126.8, 120.3, 114.7, 113.6, 102.0, 94.1, 93.5, 81.3, 71.0, 70.9, 69.8, 69.6, 67.9, 27.4; HRMS (ESI) calcd for $C_{43}H_{39}O_6$ (M+H) 651.2747, found 651.2712.

Using the same procedure with (+)-**16** as the starting material, (+)-(*2R,3S*)-5,7-bis(benzyloxy)-2-[3,4-bis(benzyloxy)phenyl]chroman-3-ol ((+)-**17**), $[\alpha]_D$ +3 (*c* 1.0, CH_2Cl_2), was prepared with identical NMR spectra as the (−)-isomer.

3.6. (−)-(2*S*,3*R*)-5,7-Bis(benzyloxy)-2-[3,4-bis(benzyloxy)-phenyl]chroman-3-yl 3,4,5-tris(benzyloxy)benzoate ((−)-19)

Under an N_2 atmosphere, a solution of 3,4,5-tris(benzyloxy)benzoic acid (270 mg, 0.61 mmol) was refluxed with oxally chloride (1 mL) in dry CH_2Cl_2 (10 mL) and one drop of DMF for 3 h. The excess oxally chloride and solvent were removed by distillation and the residue was dried under vacuum for 3 h and dissolved in CH_2Cl_2 (2 mL). This solution was added dropwise to a solution of (−)-**17** (200 mg, 0.3 mmol) and DMAP (75 mg, 0.62 mmol) in CH_2Cl_2 (15 mL) at 0 °C. The mixture was stirred at rt overnight, then saturated $NaHCO_3$ aqueous solution was added. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The organic phases were combined, dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography on silica gel (5% EtOAc/benzene) to afford the desired compound (260 mg, 81.0%). Recrystallization in $CHCl_3$ and ether gave a white powder: mp 149–151 °C; $[\alpha]_D$ −36.8 (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.38–7.30 (m, 35H), 7.19 (s, 2H), 6.99 (d, $J = 1.8$ Hz, 1 H), 6.90 (Ab, $J = 8.3$, 1.8 Hz, 2H), 6.29 (AB, $J = 2.1$ Hz, 2H), 5.47 (q, $J = 6.6$ Hz, 1H), 5.10 (s, 1H), 5.09 (s, 2H), 5.06 (s, 2H), 5.03–5.02 (m, 4H), 5.00 (d, $J = 2.7$ Hz, 2H), 3.08 (AB, $J = 16.5$, 5.4 Hz, 1H), 2.86 (AB, $J = 16.5$, 6.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 158.7, 158.2, 155.2, 148.9, 148.8, 137.2, 137.1, 136.9, 136.8, 131.4, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.5, 127.4, 127.2, 127.1, 119.4, 115.0, 113.4, 100.9, 94.6, 78.3, 71.3, 70.0, 69.8, 66.2, 28.1; HRMS (ESI) calcd for $C_{71}H_{61}O_{10}$ (M+Na) 1095.4084, found 1095.4080.

Using the same procedure with (+)-**17** as the starting material, (+)-(*2R,3S*)-5,7-bis(benzyloxy)-2-[3,4-bis(benzyloxy)phenyl]chroman-3-yl 3,4,5-tris(benzyloxy)-benzoate ((+)-**19**), $[\alpha]_D$ +36.8 (*c* 2.0, $CHCl_3$) was prepared with identical NMR spectra as the (−)-isomer.

3.7. (−)-3-*O*-Galloylcatechin ((−)-7)

Under an H_2 atmosphere, $Pd(OH)_2/C$ (20%, 400 mg) was added to a solution of (−)-**19** (260 mg, 0.24 mmol) in a solvent mixture of THF/MeOH (1:1 v/v, 50 mL). The resulting reaction mixture was stirred at rt under H_2 for 6 h, TLC showed that the reaction was completed. The reaction mixture was filtered to remove the catalyst. The filtrate was evaporated, and the residue was rapidly purified by flash chromatograph on silica gel (2%AcOH/10% MeOH/CH_2Cl_2, then 2%AcOH/20% MeOH/CH_2Cl_2) to afford (−)-CG (80 mg, 74% yield): mp 248–251 °C (decompose); $[\alpha]_D$ −54 (*c* 0.9, EtOH), lit. $[\alpha]_D$ −51.5 (*c* 0.2, EtOH);[18] ^1H NMR (acetone-d_6/D_2O, 3/1, v/v, 400 MHz): δ 7.11 (s, 2H), 7.03 (s, 1H), 6.87 (AB, $J = 8.1$ Hz, 2H), 6.16 (d, $J = 2$ Hz, 1H), 6.07 (d, $J = 2$ Hz, 1H), 5.44 (q, $J = 6.6$ Hz, 1H), 5.15 (d, $J = 6.6$ Hz, 1H), 3.03 (A of AB, $J = 16$, 5.3 Hz, 1H), 2.82 (B of AB, $J = 16$, 6.6 Hz, 1H); ^{13}C NMR (acetone-d_6/D_2O, 3/1, v/v, 400 MHz): δ 165.7, 156.3, 155.9, 154.8, 144.7, 144.4, 138.1, 129.6, 119.8, 118.1, 115.0, 113.5,

108.9, 98.2, 95.3, 94.3, 77.7, 69.6, 23.7; HRMS (ESI) calcd for $C_{22}H_{19}O_{10}$ (M+H) 443.0978, found 443.1016.

Using the same procedure with (+)-**19** as the starting material. the naturally occurring compound (+)-3-*O*-galloylcatechin ($[\alpha]_D$ +54 (*c* 0.9, EtOH); Lit +56 (*c* 0.9, EtOH))[18] was prepared with identical NMR spectra as the (−)-isomer.

3.8. (+)-(2*R*)-5,7-Bis(benzyloxy)-2-[3,4-bis(benzyloxy)-phenyl]chroman-3-one ((+)-20))

Dess–Martin periodinane (6.3 mL, 15% g/mL in CH_2Cl_2, 2.2 mmol) was added in one batch to a stirred solution of (+)-**17** (650 mg, 1.0 mmol) in CH_2Cl_2 (30 mL) under an N_2 atmosphere. The mixture was stirred at rt for about 2 h till TLC showed the absence of starting material. Subsequently, saturated $NaHCO_3$ solution (15 mL) and 10% $Na_2S_2O_3$ aqueous solution (15 mL) were added to quench the reaction. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography on silica gel (benzene) and then recrystallized in $CHCl_3$ and ether to afford the desired compound (500 mg, 76.0%): mp 141–143 °C, lit 142–143.5 °C;[18] $[\alpha]_D$ +38 (*c* 2.1, $CHCl_3$); lit. $[\alpha]_D$ +38.5 (*c* 2.08, $CHCl_3$);[18] ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.42–7.25 (m, 20H), 6.95 (d, $J = 1.4$ Hz, 1H), 6.89 (AB, $J = 8.2$ Hz, 2H), 6.35 (AB, $J = 1.9$ Hz, 2H), 5.23 (s, 1H), 5.13 (s, 2H), 5.10 (d, $J = 2.9$ Hz, 2H), 5.01 (s, 2H), 5.00 (s, 2H), 3.64 (AB, $J = 21.5$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 205.0, 159.4, 157.0, 154.4, 149.1, 148.9, 137.0, 136.9, 136.4, 128.6, 128.5, 128.4, 128.1, 128.0, 127.8, 127.7, 127.5, 127.3, 127.2, 127.1, 119.9, 114.6, 113.2, 101.9, 95.7, 95.0, 83.0, 71.1, 71.0, 70.2, 70.0, 33.6; HRMS (ESI) calcd for $C_{43}H_{37}O_6$ (M+H) 649.2590, found 649.2580.

Using the same procedure with (−)-**17** as the starting material, (−)-(2*S*)-5,7-bis(benzyloxy)-2-[3,4-bis(benzyloxy)phenyl]chroman-3-one ((−)-**20**), $[\alpha]_D$ −38 (*c* 2.1, $CHCl_3$), was prepared with identical NMR spectra as the (+)-isomer.

3.9. (−)-(2*R*,3*R*)-5,7-Bis(benzyloxy)-2-[3,4-bis(benzyloxy)-phenyl]chroman-3-ol ((−)-21)

Under an N_2 atmosphere, the ketone (+)-**20** (500 mg, 0.77 mmol) was dissolved in dry THF (10 mL), and the solution was cooled to −78 °C. Then L-Selectride (1.5 mL, 1 M solution in THF, 1.5 mmol) was added dropwise. The resulting solution was stirred at −78 °C overnight. When TLC showed the reaction was completed, saturated $NaHCO_3$ aqueous solution (10 mL) was added to quench the reaction. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic phases were dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatograph on silica gel (5% EtOAC/benzene) and then recrystallized with ethanol and EtOAC to afford the desired product (450 mg, 90%) as a white solid: mp 129–130 °C, lit 129.5–130;[18] $[\alpha]_D$ −27 (*c* 2.2, EtOAc), lit. $[\alpha]_D$ −27.7 (*c* 2.16, EtOAc);[18] ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.43–7.30 (m, 20H), 7.13 (d, $J = 1.6$ Hz, 1H), 6.95 (AB, $J = 8$, 1.6 Hz, 2H), 6.26 (s, 2H), 5.17 (s, 2H), 5.15 (s, 2H), 5.02 (s, 4H), 4.88 (s, 1H), 4.17 (b, 1H), 3.00 (A of AB, $J = 17.2$, 1.3 Hz, 1H), 2.92 (B of AB, $J = 17.2$, 4.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 165.0, 158.8, 157.6, 154.9, 152.2, 148.9, 148.8, 142.3, 137.3, 136.9, 136.8, 136.6, 136.4, 130.9, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 127.5, 127.4, 127.3, 127.1, 124.8, 119.9, 114.7, 113.2, 108.9, 101.3, 94.2, 93.6, 78.2, 75.0, 71.2, 71.0, 70.0, 69.8, 24.2; HRMS (ESI) calcd for $C_{43}H_{39}O_6$ (M+H) 651.2747, found 651.2730.

Using the same procedure with (−)-**20** as the starting material, (+)-(2*S*,3*S*)-5,7-bis(benzyloxy)-2-[3,4-bis(benzyloxy)phenyl]chroman-3-ol ((+)-**21**), $[\alpha]_D$ +27 (*c* 2.2, EtOAc), was prepared with identical NMR spectra as the (−)-isomer.

3.10. (−)-(2*R*,3*R*)-5,7-Bis(benzyloxy)-2-[3,4-bis(benzyloxy)-phenyl]chroman-3-yl 3,4,5-tris(benzyloxy)benzoate ((−)-22)

Following the procedure used for the preparation of (−)-**19** but with (−)-**21** as starting material, (−)-(2*R*,3*R*)-5,7-bis(benzyloxy)-2-[3,4-bis(benzyloxy)phenyl]-chroman-3-yl 3,4,5-tris(benzyloxy)benzoate was obtained (80% yield) as a white solid: mp 122–124 °C, lit 122–123.5 °C;[18] $[\alpha]_D$ −71 (*c* 2.2, CH_2Cl_2), lit. $[\alpha]_D$ −70.7 (*c* 1.94, CH_2Cl_2);[2] ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.37–7.15 (m, 37H), 7.03 (s, 1H), 6.89 (AB, $J = 8$ Hz, 2H), 6.37 (s, 1H), 6.31 (s, 1H), 5.60 (bs, 1H), 5.04 (bs, 1H), 5.00 (m, 12H), 4.74 (AB, $J = 11$ Hz, 2H), 3.07 (bs, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 164.8, 158.7, 157.9, 155.6, 152.2, 148.8, 148.7, 142.3, 137.3, 137.0, 136.9, 136.7, 136.3, 130.9, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.6, 127.3, 127.2, 127.1, 124.8, 119.9, 114.5, 113.4, 108.9, 100.8, 94.5, 93.8, 77.5, 74.9, 71.0, 70.8, 70.0, 69.8, 68.4, 26.0; HRMS (ESI) calcd for $C_{71}H_{61}O_{10}$ (M+H) 1073.4265, found 1073.4230.

Using the same procedure with (+)-**21** as starting material, (+)-(2*S*,3*S*)-5,7-bis(benzyloxy)-2-[3,4-bis(benzyloxy)-phenyl]-chroman-3-yl 3,4,5-tris(benzyloxy)benzoate ((+)-**22**), $[\alpha]_D$ +71 (*c* 2.2, CH_2Cl_2), was prepared with identical NMR spectra as the (−)-isomer.

3.11. (−)-3-*O*-Galloylepicatechin ((−)-8)

Following the procedure for the preparation (−)-7, but with (−)-**21** as starting material, (−)-3-*O*-gallo*yl*epi catechin was obtained (80% yield): mp 253–255°C (decompose), lit 257–259 °C;[18] $[\alpha]_D$ −157 (*c* 0.3, acetone), lit. 160.6 (*c* 0.2, acetone);[18] ^{1}H NMR (acetone-d_6/D_2O, 3/1, v/v, 400 MHz): δ 7.12 (d, $J = 1.9$ Hz, 1H), 7.06 (s, 2H), 6.94 (A of AB, $J = 8.2$, 1.9 Hz, 1 H), 6.83 (B of AB, $J = 8.2$ Hz, 1H), 6.09 (AB, $J = 2.2$ Hz, 2H), 5.49 (bs, 1 H), 5.13 (s, 1H), 3.09 (A of AB, $J = 17.3$, 4.5 Hz, 1H),

2.97 (B of AB, $J = 17.3$ Hz, 1H); ^{13}C NMR (acetone-d_6/D_2O, 3/1, v/v, 400 MHz): δ 166.9, 157.1, 157.0, 156.6, 145.7, 145.1, 139.1, 130.8, 120.9, 118.9, 115.8, 114.8, 109.9, 98.7, 96.3, 95.5, 77.8, 69.9, 26.3; HRMS (ESI) calcd for $C_{22}H_{19}O_{10}$ (M+H) 443.0978, found 443.1016.

Using the same procedure with (+)-**21** as starting material, (+)-3-*O*-galloyl*epi* catechin, $[\alpha]_D$ +155 (*c* 0.3, acetone), was prepared with identical NMR spectra as the (−)-isomer.

Inhibition of purified 20S proteasome activity—The chymotrypsin-like activity of a purified 20S prokaryotic proteasome (*Methanosarcina thermophile*, recombinant, *E. coli*; from Calbiochem) or a purified rabbit 20S proteasome (Boston Biochem) was measured as follows. A quantity of 0.5 g of purified 20S proteasome was pre-incubated with each synthetic tea polyphenol at 0.01, 0.1, 0.5, 1.0, 10, and 25 μM for 30 min at 4 °C in a 96-well plate. This was followed by an additional incubation with 20 μM fluorogenic peptide substrate, Suc-Leu-Leu-Val-Tyr-AMC (for the proteasomal chymotrypsin-like activity), for 1 h at 37 °C in 100 μL of assay buffer (20 mM Tris–HCl, pH 8.0). After that, the plate was directly used for measurement of the hydrolyzed 7-amido-4-methyl-coumarin (AMC) groups by a Victor^3 1420 Multilabel Counter equipped with computer.

Inhibition of the proteasome activity in whole cell extracts—A whole cell extract (10 μg) of Jurkat T cells was pre-incubated with each synthetic tea polyphenol for 30 min at 4 °C in a 96-well plate, as described above, followed by an additional incubation with 20 μM of fluorogenic peptide substrates Suc-Leu-Leu-Val-Tyr-AMC (for the proteasomal chymotrypsin-like activity), benzyloxycarbonyl (Z)-Leu-Leu-Glu-AMC (for the proteasomal caspase-like activity), and Z-Gly-Gly-ArgGR-AMC (for the proteasomal trypsin-like activity) for 1 h at 37 °C. The hydrolyzed AMCs were then determined as described above.

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