Cyclodextrin-Derived Diorganyl Tellurides as Glutathione Peroxidase Mimics and Inhibitors of Thioredoxin Reductase and Cancer Cell Growth

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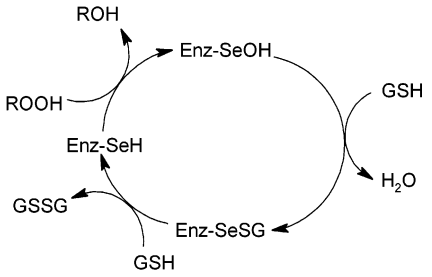
Water-soluble diorganyl tellurides of the alkyl aryl or dialkyl type were prepared by treatment of mono-6-tosyl-β-cyclodextrin with sodium alkanetellurolates or arenetellurolates or sodium telluride. The novel cyclodextrin-derived organotelluriums were evaluated for their capacity to catalyze the reduction of hydrogen peroxide, *tert*-butyl hydroperoxide, and cumene hydroperoxide in the presence of glutathione, NADPH, and GSSG-reductase (coupled reductase assay). Cyclodextrins **4d** and **4e**, carrying 4-(*N*,*N*-dimethylamino)phenyltelluro and *n*-butyltelluro groups, respectively, were the most efficient glutathione peroxidase mimics. Reduction of lipophilic cumene hydroperoxide often proceeded 10−20 times faster than reduction of the more hydrophilic hydroperoxides, which cannot bind into the hydrophobic interior of the cyclodextrin. Thus, it seems that the carbohydrate moiety acts as a binding site for the hydroperoxide substrate. The cyclodextrin derivatives were also evaluated for their capacity to inhibit thioredoxin reductase/thioredoxin and cancer cell growth in culture. IC_{50} values for inhibition of thioredoxin or thioredoxin/thioredoxin reductase were in the submicromolar range for the best inhibitors (compounds **4d** and **5**). Two of the compounds (**4c** and **5**) were found to inhibit the growth of MCF-7 cells in culture with IC_{50} values in the low micromolar range.

Introduction

Oxidants, many of which are free radicals, are present in biological systems under normal physiological conditions. To prevent undesired radical-induced damage, the organism is equipped with several lines of antioxidant defense. These act either by interception of free radicals (superoxide dismutases, vitamin E, ascorbate, glutathione, and uric acid) or by destruction of precursors to free radicals (catalase and glutathione peroxidases). The selenium-containing glutathione peroxidases[1] are selenoproteins, a class of enzymes of which about a dozen are known with respect to structure and function in mammals.[2] These enzymes catalyze the reduction of hydrogen peroxide, fatty acid hydroperoxides, and phospholipid and cholesterol hydroperoxides using glutathione (GSH) and other thiols as stoichiometric reducing agents. The enzyme catalytic site contains a selenocysteine residue where selenium undergoes redox cycling (Scheme 1). Enzyme-bound selenol (Enz-SeH) reduces hydrogen peroxide and other hydroperoxides to produce the corresponding selenenic acid (Enz-SeOH). This reacts with 2 equiv of reduced glutathione (GSH), first to produce a selenosulfide (Enz-SeSG) and finally to regenerate the active selenol form of the enzyme. The five varieties of the enzyme presently known serve their protective function in cells (GSH-Px-1),[3] plasma (GSH-Px-P),[4] membranes (PHGPX),[5] the gastrointestinal tract (GSHPx-GI),[6] and in the sperm nuclei.[7] Considerable

Scheme 1[a]



[a] GSH = γ-Glutamyl cysteinyl glycine.

efforts have been made recently to find compounds that could mimic the properties of the glutathione peroxidase enzymes. Ebselen (2-phenyl-1,2-benzisoselenazol-3(2*H*)-one), ebselen homologues, selenenamides, diselenides, α-phenylselenoketones, and selenium-containing enzymes, antibodies, and cyclodextrins have all been demonstrated to catalyze the reduction of hydroperoxides in the presence of thiols.[8−10]

These catalysts serve as precursors of selenols, selenenic acids, or selenosulfides, and the catalytic mechanism probably resembles that proposed for the glutathione peroxidase enzymes (Scheme 1).[11] Some time ago, we[12−15] and others[16] found that diorganyl tellurides could also mimic the glutathione peroxidases. However, the catalytic mechanism is distinctly different from the three-step cycle proposed for the enzymatic reaction. Thus, catalysis relies on redox cycling of the heteroatom between the two oxidation states II and IV (Scheme 2; oxidation with a hydroperoxide is shown). In principle, all mild oxidants (such as hydrogen peroxide, organic

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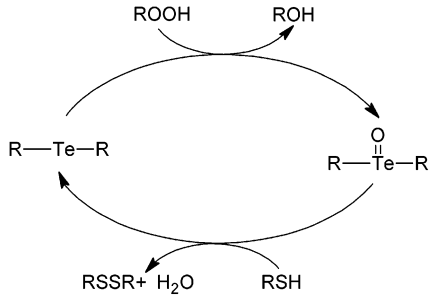
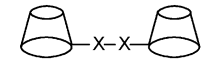
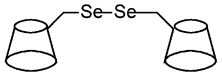
Scheme 2



hydroperoxides, hypochlorite, halogens) will convert diorganyl tellurides to the corresponding tellurium(IV) species. Reduction is then brought about by mild reducing agents such as thiols or ascorbate. For studies and applications of glutathione peroxidase mimics in hydrophilic environments, water-soluble compounds[17] would be required. In this respect, cyclodextrins are an attractive class of materials. Carbohydrate-based compounds of this type are readily available, bind hydrophobic substrates to their cavities in water solution, and have two rims of hydroxyl groups that can be used for anchoring them.[18] In the most common pharmaceutical applications, their role is to enhance the solubility, stability, and bioavailability of various drug molecules. However, they may also bind substrates and then either catalyze their reactions or mimic a step in an enzymatic catalytic sequence.[19]



1a X = Se
b X = Te



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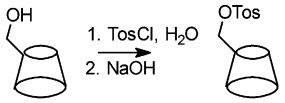
Among chalcogen-containing cyclodextrins, 2,2′-diselenobis(2-deoxy-β-cyclodextrin) (**1a**),[20] its tellurium analogue **1b**,[21] and 6,6′-diselenobis(6-deoxy-β-cyclodextrin) (**2**)[22] were recently shown to act as glutathione peroxidase mimics. Since these materials are diselenides[9] or ditellurides,[23] they will enter the catalytic cycle that has been proposed for the enzyme (Scheme 1). We thought it would be interesting to prepare some tellurium-based cyclodextrins that could mimic the glutathione peroxidases according to the alternative mechanism (Scheme 2) and evaluate their antioxidative properties and some of their biological effects.

Synthesis. For introducing functional diversity into β-cyclodextrins, mono-6-tosyl-β-cyclodextrin (**3**) is one of the commonly used starting materials.[24] Several methods for its preparation have been published with everything from poor to moderate reported yields.[25−28] In one recent procedure, *p*-toluenesulfonic anhydride was used to introduce a tosyl group with a reported yield of 61%.[25] However, in our hands this method was not very useful for obtaining the desired product. Instead, the procedure by Petter,[26] involving treatment of β-cyclodextrin with *p*-toluenesulfonyl chloride, was slightly modified (eq 1). Thus, tosyl chloride and β-cyclodextrin

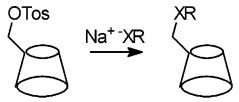


(1)

3

were stirred in water for 2 h at ambient temperature, after which aqueous NaOH was added to complete the reaction. Ammonium chloride was then added to adjust the pH to ca. 8. The white precipitate of compound **3** that formed by cooling of the solution (32% isolated yield) could be used in the following steps without any further purification.

The desired cyclodextrin-derived diorganyl tellurides were obtained by addition of preformed (from sodium borohydride and the appropriate diorganyl ditelluride) ethanolic solutions of sodium organyltellurolates to aqueous suspensions of 6-tosyl-β-cyclodextrin (eq 2).



(2)

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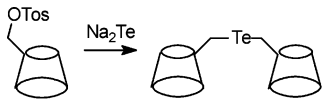
4a X = Te; R = C_6H_5
b X = Te; R = C_6H_4-4-OMe
c X = Te; R = C_6H_4-4-OH
d X = Te; R = C_6H_4-4-NMe_2
e X = Te; R = *n*-Bu
f X = Se; R = C_6H_5

After heating at 60 °C for 24 h under an inert atmosphere, addition of water caused precipitation of the diorganyl tellurides **4a**−**e** (23−62% isolated yields). Traces of diorganyl ditelluride present in the products due to air oxidation of the sensitive sodium organyltellurolates was easily removed by washing the product with dichloromethane. Phenylseleno derivative **4f** was similarly prepared in 40% yield from sodium benzeneselenolate. This and other β-cyclodextrin-derived organoseleniums have previously been prepared in DMF using a similar protocol.[29]

We also thought it would be interesting to incorporate more than one β-cyclodextrin moiety into the diorganyl telluride scaffold. Sodium telluride was therefore prepared by sodium borohydride reduction of elemental tellurium.[30] When added to a suspension of mono-6-tosyl-β-cyclodextrin, the water-soluble diorganyl telluride **5** was formed (eq 3). After filtration through



(3)

3 **5**

Celite to remove some elemental tellurium, addition of ethanol caused precipitation of the crude product. The isolated yield of recrystallized compound **5** (from water/ethanol) was 54%.

Results and Discussion

Glutathione Peroxidase-like Properties. The glutathione peroxidase-like activity of the cyclodextrin derived diorganyl tellurides **4** and **5** was assessed by their ability to catalyze the reaction between hydro-

Scheme 3

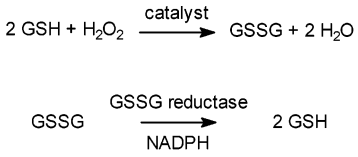
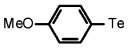
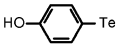
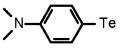


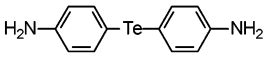
Table 1. Hydroperoxide Decomposing Capacity of Cyclodextrin-Derived Organotellurium Compounds As Determined by the Coupled Reductase Assay

Compound	% Catalysis[a]		
	Hydroperoxide		
XR =	H_2O_2	*t*-BuOOH	Cumene hydroperoxide
 **4a**	170	560	2075
 **4b**	230	640	2200
 **4c**	225	940	1850
 **4d**	430	1240	2370
 **4e**	520	3680	6240
 **4f**	100	120	105
 **5**	290	4280	5520

[a] The catalyst's percentage increase of the basal reaction rate between GSH and hydroperoxide was calculated as rate of NADPH consumption + 5 mol % catalyst (μM/min)/rate of NADPH consumption + vehicle (μM/min) × 100.

peroxides and glutathione in an aqueous buffer at physiological pH. The oxidation of glutathione (γ-glutamyl cysteinyl glycine; GSH) to the corresponding disulfide (GSSG) was measured indirectly by spectrophotometrically assessing the oxidation of NADPH in the presence of glutathione reductase at 340 nm (coupled reductase assay;[31] Scheme 3).

Data (Table 1) for hydrogen peroxide, *tert*-butyl hydroperoxide. and cumene hydroperoxide are expressed as a catalytic efficiency (percent catalysis), determined by reference to the respective control rates in the absence of catalyst, for each hydroperoxide. As expected, the phenylseleno derivative **4f** of β-cyclodextrin did not show any catalytic activity with the three hydroperoxides tested. The problem is that the oxidation of selenium is too slow. Among organotelluriums **4**, the cyclodextrin **4e** carrying a butyltelluro group was the best catalyst with all three hydroperoxides tried (520, 3680, and 6240% catalysis, respectively, with hydrogen peroxide, *tert*-butyl hydroperoxide and cumene hydroperoxide). This result is in line with previous findings that compounds carrying alkyltelluro moieties are better catalyst than those carrying aryltelluro groups.[10] Among cyclodextrins **4** carrying aryltelluro moieties, there is a trend that catalytic efficiency decreases with decreasing electron density at tellurium. Thus, the following order of catalytic efficiency was found with hydrogen peroxide and *tert*-butyl hydroperoxide as substrates: **4d** > **4c** ≈ **4b** > **4a**. With cumene hydroperoxide, the phenyltelluro derivative **4a** was slightly more active than the 4-hydroxyphenyl derivative **4c**. Apparently, nucleophilic attack of tellurium on the hydroperoxide is facilitated as the electron density at tellurium increases. The most notable result from the coupled reductase assay is the specificity for reduction of cumene hydroperoxide. With many of the catalysts (**4a**,**b**,**e**), cumene hydroperoxide is reduced 10−12 times faster than hydrogen peroxide. *tert*-Butyl hydroperoxide is also reduced more rapidly than hydrogen peroxide, but often only by a factor of 3−4 (catalysts **4a**−**d**). Some previously studied organotellurium glutathione peroxidase mimics not anchored to cyclodextrins, bis(4-aminophenyl)telluride (**6**)[12] and



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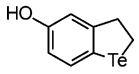

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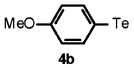
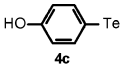
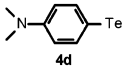
5-hydroxy-2,3-dihydrobenzo[*b*]tellurophene (**7**),[15] were found to reduce cumene hydroperoxide as well as *tert*-butyl hydroperoxide only 3−4 times faster than hydrogen peroxide. A similarly modest selectivity for cumene hydroperoxide was observed for the cyclodextrin-derived diselenide[20] and ditelluride[21] glutathione peroxidase mimics prepared by Luo. Thus, it seems that the cyclodextrin part of compounds **4** acts as a binding site for the lipophilic hydroperoxide. The effect was even more pronounced with catalyst **5**, which carries two cyclodextrin moieties. In the presence of this compound, cumene hydroperoxide was reduced almost 20 times faster than hydrogen peroxide (Table 1). It is expected that cumene hydroperoxide interacts with the cyclodextrin in such a way as to bring the HOO group into close proximity to tellurium at the primary face of the cyclodextrin.

Inhibition of Thioredoxin Reductase and Cancer Cell Growth. The thioredoxin system—a collective name for thioredoxin (TrX), thioredoxin reductase (TrxR), and NADPH—is a powerful cellular protein disulfide reductase system that is present in all living organisms. TrxR is a selenocysteine-containing dimeric FAD-containing enzyme that catalyzes the NADPH-dependent reduction of the active site disulfide in oxidized Trx. The reduced Trx is a hydrogen donor for ribonucleotide reductase—the essential enzyme for DNA synthesis—and a powerful protein reductase with many functions in cell growth and redox regulation. There are two mammalian thioredoxin reductases, Trx-1, found in the cytoplasm and nucleus of cancer cells, and Trx-2, found in the mitochondria.[32,33] Trx-1 protein levels have been

Table 2. Thioredoxin Reductase and Cancer Cell Growth Inhibiting Capacity of Cyclodextrin-Derived Organotellurium Compounds

Compound XR =	Inhibition TrxR/Trx[a] IC_{50} (μM)	Inhibition TrxR[b] IC_{50} (μM)	Inhibition MCF-7[c] IC_{50} (μM)
 **4a**	7.6	1.6	10.1
 **4b**	7.6	1.6	4.6
 **4c**	4.0	18.0	1.6
 **4d**	3.4	0.26	10.4
 **4e**	1.2	18.0	6.0
 **4f**	18.0	17.8	10.2
 **5**	0.5	2.4	1.4

[a] Inhibition of thioredoxin reductase in the presence of thioredoxin and insulin. [b] Inhibition of thioredoxin reductase. [c] Inhibition of growth of MCF-7 breast cancer cells in culture.

observed to be significantly elevated in several human primary cancers. Therefore, the TrxR/Trx-1 redox system provides an attractive target for cancer drug development. Studies of organotellurium inhibitors[34] of TrxR indicated that compounds with good antioxidative capacity, especially hydroperoxide-decomposing ability, were very efficient.[35] The organotellurium compounds investigated also inhibited the growth of human cancer cells in culture, but the poor water solubility of these materials was a restriction during administration to animals. We therefore thought it would be worthwhile to test the cyclodextrin-derived diorganyl tellurides **4** and **5** for their capacity to inhibit TrxR and cancer cell growth in culture.

TrxR activity was measured as the increase in absorbance at 405 nm that occurs as added dithionitrobenzoic acid (DTNB) is reduced by thiols produced in an incubation containing Trx, TrxR, NADPH, insulin, and the inhibitor (Table 2). This assay reflects the combined effects of the inhibition of Trx and TrxR. TrxR activity was also assessed in incubations of DTNB, TrxR, NADPH, and inhibitor and measured as the initial increase in absorbance at 405 nm (Table 2). This assay reflects the inhibition of TrxR only. With some exceptions (vide infra; compounds **4c** and **4e**), the IC_{50} values (concentrations required to inhibit thionitrobenzoate formation by 50%) obtained using the two methods were rather similar. The effect of the compounds on growth of MCF-7 breast cancer cells in culture was also studied. This cell line was chosen for its relatively high level of thioredoxin gene expression. Inhibition data expressed as IC_{50} values (concentration required to inhibit cell growth by 50% of that observed in a control incubation) are shown in Table 2.

All compounds except for selenium derivative **4f** inhibited TrxR/Trx with IC_{50} values in the interval 0.5−10 μM. These results are in line with those from a previous, comparative study of diaryl chalcogenides inhibitors that showed that organotellurium compounds are much better inhibitors than their organoselenium counterparts.[34] Compounds shown to possess good peroxide decomposing activity (Table 1) also turned out to be among the best inhibitors of TrxR/Trx [cyclodextrins carrying butyltelluro (**4e**), 4-(dimethylamino)phenyltelluro (**4d**) and 4-hydroxyphenyltelluro (**4c**) groups; biscyclodextrin compound **5**]. However, some of these materials (compounds **4c** and **4e**) were significantly poorer inhibitors in the TrxR assay. This could indicate that they are inhibitors of Trx rather than TrxR in the combined assay.

In the MCF-7 cell culture assay, most of the hydrophilic cyclodextrin compounds showed only moderate activity with IC_{50} values in the range of 5−10 μM. The failure of several other good, water-soluble (containing sulfopropyl groups) organotellurium inhibitors of TrxR to kill MCF-7 cells in culture was also recently observed. However, two of the compounds—4-hydroxyphenyltelluro-derived cyclodextrin **4c** and biscyclodextrin **5**—were more potent (IC_{50} values were 1.6 and 1.4 μM, respectively). Except for compound **5** (which is also a good inhibitor in the TrxR/Trx and TrxR assays) there is only a weak correlation between inhibition of the thioredoxin system and inhibition of cancer cell growth in the MCF-7 assay. This could be due to differences in the cellular uptake of the compounds.

In this and previous work we have found that a variety of organotellurium compounds inhibit the thioredoxin system at low micromolar concentrations. However, the mechanisms of inhibition are, as yet, unclear. According to a tentative hypothesis previously forwarded by us, the oxidized organotellurium compound (presumably a tellurium(IV) oxide) could serve to cross-link (oxidize) active site selenocysteine residues or thiols crucial for enzyme activity.[35] However, it turned out that an in situ prepared telluroxide was a poorer inhibitor of the thioredoxin system than the corresponding telluride. This suggests that tellurium(IV) compounds are not crucial for the events responsible for enzyme inhibition by organotelluriums. In addition to two-electron oxidation, organotelluriums are also known to undergo one-electron oxidation by various oxidizing agents. Maybe it is this capacity that makes them interfere with the thioredoxin system.

Conclusions and Outlook

Novel cyclodextrin-derived diorganyl tellurides of the alkyl aryl or dialkyl type were found to catalyze the reduction of hydrogen peroxide, *tert*-butyl hydroperox-

ide, and cumene hydroperoxide in the presence of glutathione. Thus, these compounds act as mimics of the glutathione peroxidase antioxidant enzymes. Reduction of the most lipophilic hydroperoxide (cumene hydroperoxide) proceeded 10−20 times faster than reduction of the two more hydrophilic ones. It therefore seems that the hydrophilic cavity provided by the carbohydrate moiety of the catalyst acts as a binding site for the hydroperoxide substrate. We feel that compounds of this type could find use as various types of "antioxidant pharmacotherapy", i.e., remedies for pathological conditions characterized by an elevation in the cellular steady-state concentration of reactive oxygen derived species ("oxidative stress"). For example, oxidative stress has been implicated in chronic inflammatory disorders, adult respiratory distress syndrome, atherosclerosis, ischemia/reperfusion injury, and cataract.

Like other organotelluriums, the novel cyclodextrin compounds were also found to interfere with the thioredoxin system and to inhibit cancer cell growth. IC_{50} values for inhibition of thioredoxin or thioredoxin/thioredoxin reductase were in the submicromolar range for the most active compounds, and two of them were found to inhibit the growth of MCF-7 cells in culture with IC_{50} values in the low micromolar range. We plan to test these compounds in other cell lines and study their effects in vivo.

Experimental Section

Melting points were recorded on a Stuart Scientific heated block and are uncorrected. ^1H and ^{13}C spectra were recorded at 400 and 100 MHz, respectively, on a Varian 400 MHz Unity instrument. For proton spectra, the residual peak of $CHCl_3$ was used as the internal reference (7.26 ppm), while the central peak of $CDCl_3$ (77.0 ppm) was used as the reference for carbon spectra. Mass spectra were recorded using a Bruker Reflex III MALDI-TOF. Molecular peaks are given for ^{130}Te. Elemental analyses were carried out by Analytical Laboratories, Lindlar, Germany. Since the cyclodextrin derivatives were extensively hydrated (compound **4b** was analyzed as a heptahydrate), elemental analysis was not a satisfactory proof of purity. β-Cyclodextrin hydrate, tosyl chloride, and diphenyl diselenide were purchased from Aldrich and used as supplied. Diphenylditelluride,[37] bis(4-methoxyphenyl)ditelluride,[37] bis-[4-(dimethylamino)telluride,[37] and di-*n*-butylditelluride[38] were prepared according to the literature methods. Spectral data for 6-(phenylseleno)-6-deoxy-β-cyclodextrin matched those reported in the literature.[29]

Bis(4-Hydroxyphenyl)ditelluride. Under an inert atmosphere at −78 °C, ^{t}BuLi (9.5 mL, 1.5 M) was added dropwise to 4-bromophenol (1.02 g, 5.90 mmol) in THF (25 mL). The solution was stirred at −78 °C for 30 min. The solution was allowed to warm to room temperature over 20 min and then added to a separate flask containing tellurium (737 mg, 5.78 mmol). The solution was stirred for a further 2 h, after which all the tellurium had reacted, and the mixture was opened to air and diethyl ether (100 mL) was added. Saturated aqueous NH_4Cl (80 mL) was added, the organic layer was separated, and the aqueous layer was extracted with DCM (2 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered through Celite, and evaporated in vacuo. The product was isolated as a 1/1.2 mixture of bis(4-hydroxyphenyl)telluride and the title ditelluride (925 mg), and due to the unstable nature of the matrial (detelluration), it was used without further purification: ^1H NMR ($CDCl_3$) δ 6.84 (d, 2H, J = 10.8 Hz), 7.24 (d, 2H, J = 10.8 Hz).

6-*O*-Monotosyl-β-cyclodextrin (3). A mixture of *p*-toluenesulfonyl chloride (2.50 g, 13.1 mmol) and β-cyclodextrin hydrate (10.0 g, 8.68 mmol) in water (220 mL) was stirred at ambient temperature for 2 h under an inert atmosphere. Aqueous NaOH (2.5 M, 40 mL) was added and the solution was stirred for 10 min before unreacted *p*-toluenesulfonyl chloride was filtered off. Ammonium chloride (11.6 g) was added to lower the pH to approximately 8. The solution was cooled and the resultant white precipitate was collected by filtration. The white powder was washed with acetone and dried under vacuum to afford 3.55 g (32%) of the title compound. Spectral data matched that reported in the literature.[26]

Typical Procedure. 6-(Phenyltelluro)-6-deoxy-β-cyclodextrin (4a). To a suspension of mono-6-tosyl-β-cyclodextrin (505 mg, 0.392 mmol) in H_2O (3 mL) under an inert atmosphere was added a solution of sodium benzenetellurolate [generated from diphenylditelluride (193 mg, 0.471 mmol) and sodium borohydride (40 mg, 1.06 mmol) in EtOH (5 mL)]. The reaction mixture was heated at 60 °C for 16 h. The mixture was cooled to room temperature, and H_2O (20 mL) and DCM (40 mL) were added. The aqueous layer, which contained the product as a suspension, was separated and washed with DCM (2 × 40 mL). Filtration afforded a white solid which was made into a slurry with H_2O (10 mL). The slurry was filtered, washed with DCM (40 mL), and dried to afford the title compound (270 mg, 52%) as a white powder: mp 263−264 °C (dec); ^1H ($(CD_3)_2SO$) δ 3.12−3.70 (m, 41H), 4.50 (m, 7H), 4.82 (m, 7H), 5.73 (m, 14H),7.16 (m, 3H), 7.69 (d, 2H, J = 6.8 Hz); ^{13}C ($(CD_3)_2SO$) δ 59.9, 72.0, 72.5, 73.0, 81.5, 102.0, 112.1, 127.3, 129.1, 137.9; MS calcd for $(C_{48}H_{73}O_{34}Te)^+$ m/z 1323.3061, found m/z 1323.3077.

The following compounds were similarly prepared.

6-(4-Methoxyphenyltelluro)-6-deoxy-β-cyclodextrin (4b): white powder (37%); mp 268−269 °C (dec); ^1H ($(CD_3)_2SO$) δ 2.98−3.83 (m, 44H), 4.36−4.55 (m, 7H), 4.80−4.86 (m, 7H), 5.59−5.82 (m, 14H), 6.76 (d, 2H, J = 8.3 Hz), 7.61 (d, 2H, J = 8.3 Hz); ^{13}C ($(CD_3)_2SO$) δ 55.1, 59.9, 71.9, 72.4, 73.0, 81.5, 101.5, 101.9, 115.1, 140.1, 159.2; MS calcd for $(C_{49}H_{75}O_{35}Te)^+$ m/z 1353.3167, found m/z 1353.3210. Anal. Calcd for $C_{49}H_{76}O_{35}Te{\cdot}7H_2O$: C, 39.79; H, 6.15. Found: C, 39.53; H, 5.89.

6-(4-Hydroxyphenyltelluro)-6-deoxy-β-cyclodextrin (4c): white powder (23%); mp 249−250 °C (dec); ^1H (CD_3OD) δ 3.47−3.55 (m, 7H), 3.71−3.94 (m, 14H), 4.64 (m, 28 H), 6.66 (d, 2H, J = 8.4 Hz), 7.61 (d, 2H, J = 8.4 Hz); ^{13}C (CD_3OD) δ 61.9, 73.7, 74.2, 74.9, 82.9, 99.8, 103.8, 117.7, 142.8, 159.2; MS calcd for $(C_{48}H_{73}O_{35}Te)^+$ m/z 1339.3010, found m/z 1339.3015.

6-(4-*N*,*N*-Dimethylaminophenyltelluro)-6-deoxy-β-cyclodextrin (4d): white powder (39%); mp 267−268 °C (dec); ^1H ($(CD_3)_2SO$) δ 2.84 (s, 6H), 3.16−3.62 (m, 41H), 4.31−4.51 (m, 7H), 4.75−4.83 (m, 7H), 5.59−5.82 (m, 14H), 6.53 (d, 2H, J = 8.4 Hz), 7.49 (d, 2H, J = 8.4 Hz); ^{13}C ($(CD_3)_2SO$) δ 59.9, 72.0, 72.4, 73.0, 81.5, 101.9, 113.3, 128.8, 140.1, 150.0; MS calcd for $(C_{50}H_{78}O_{34}NTe)^+$ m/z 1367.3562, found m/z 1367.3591.

6-(Butyltelluro)-6-deoxy-β-cyclodextrin (4e): white powder (62%); mp 248−249 °C (dec); ^1H ($(CD_3)_2SO$) δ 0.84 (t, 3H, J = 8.0 Hz), 1.30 (m, 2H), 1.62 (m, 2H), 2.58 (t, 2H, J = 7.6 Hz), 3.21−3.61 (m, 41H), 4.40−4.48 (m, 7H), 4.76−4.87 (m, 7H), 5.51−5.79 (m, 14H); ^{13}C ($(CD_3)_2SO$) δ 2.1, 12.8, 24.0, 33.4, 59.4, 71.5, 71.9, 72.5, 81.0, 101.4; MS calcd for $(C_{46}H_{77}O_{34}Te)^+$ m/z 1303.3373, found m/z 1303.3359.

6,6′-Telluro-bis(6-deoxy-β-cyclodextrin) (5). To a suspension of mono-6-tosyl-β-cyclodextrin (997 mg, 0.774 mmol) in H_2O (6 mL) was added sodium telluride [generated from 49 mg (0.384 mmol) of tellurium in H_2O (1 mL) and 70 mg (1.85 mmol) of sodium borohydride in EtOH (5 mL)].[30] The reaction mixture was stirred at 60 °C for 16 h. The solution was filtered through Celite prior to cooling. Water (20 mL) was added, followed by the addition of ethanol to precipitate the product. Further recrystallization from water and ethanol furnished the title compound (486 mg, 54%) as a white powder: mp 265−266 °C (dec); ^1H ($(CD_3)_2SO$) δ 3.28−3.70 (m, 82H), 4.31−4.52 (m, 14H), 4.81−4.87 (m, 14H), 5.60−5.81 (m, 28H); ^{13}C ($(CD_3)_2SO$) δ 59.9, 72.0, 72.4, 73.0, 81.6, 101.9; MS calcd for $(C_{84}H_{137}O_{68}Te)^+$ m/z 2363.6352, found m/z 2363.6392.

Coupled Reductase Assay. The glutathione peroxidase-like activity of the compounds under study was assessed by their ability to catalyze the reaction between hydroperoxides

and glutathione in an aqueous buffer at physiological pH. The oxidation of GSH to GSSG was measured indirectly by spectrophotometrically assessing the stimulated oxidation of NADPH in the presence of glutathione reductase. Incubations were conducted at room temperature in a Shimadzu Model 160 spectrophotometer recording at 340 nm with air as a reference. They were constructed in the following manner: Incubations in quartz cuvettes were with 50 mM potassium phosphate buffer, pH 7.4. Additions and recordings were made in the following order (all final concentrations): NADPH (250 μM), GSH (1 mM), test substance (50 μM), record baseline, GSSG reductase (1 unit), record, hydroperoxide (1 mM), record the decline in absorbance. Rate assessments were performed when the decline in absorbance was constant for at least 20 s. Control experiments revealed that the observed catalytic action of the compounds was not influenced by increasing amounts of GSSG reductase (0.5, 1.0, and 2.0 units) in the incubation. Controls also showed that none of the compounds directly interacted with the reduction of GSSG (250 and 500 μM) by the reductase. Shown below are initial rates of NADPH consumption ±SEM (μM/min) for the various combinations of catalysts and hydroperoxides (including controls). Shown in Table 1 are the catalytic efficiencies, determined by reference to the respective control rates in the absence of catalyst, for each hydroperoxide. Three independent experiments were performed with each combination of catalyst and hydroperoxide.

	consumption (μM/min)		
catalyst	H_2O_2 NADPH	*t*-BuOOH NADPH	cumene hydroperoxide NADPH
control	8 ± 0.4	2.0 ± 0.1	2.7 ± 0.2
4a	13.6 ± 0.8	11.2 ± 0.3	56.0 ± 1.6
4b	18.6 ± 0.8	12.8 ± 0.5	60.0 ± 4.0
4c	17.8 ± 1.6	12.8 ± 0.8	52.0 ± 4.0
4d	26.5 ± 0.7	24.8 ± 0.8	64.0 ± 3.2
4e	41.6 ± 3.6	73.6 ± 6.2	168.5 ± 21.3
4f	8.1 ± 0.4	2.4 ± 0.4	2.8 ± 0.2
5	23.2 ± 0.8	85.6 ± 4.0	150.4 ± 8.2

TrxR/Trx Assay. Thioredoxin reductase/thioredoxin-dependent insulin reducing activity was measured in an incubation with a final volume of 60 μL containing 100 mM HEPES buffer, pH 7.2, 5 mM EDTA (HE buffer), 1 mM NADPH, 1.0 μM thioredoxin reductase, 0.8 μM thioredoxin, 2.5 mg/mL bovine insulin, and inhibitor. Incubations were for 30 min at 37°C in flat-bottom 96-well microtiter plates. The reaction was stopped by the addition of 50 μL of 6 M guanidine HCl, 50 mM Tris, pH 8.0, and 10 mM DTNB, and the absorbance measured at 412 nM.

TrxR Assay. Assays of TrxR were carried out in flat-bottom 96-well microtiter plates. TrxR activity was measured in a final incubation volume of 60 μL containing HE buffer, 10 mM DTNB, 1.0 μM TrxR, and 1 mM NADPH and inhibitor. Compounds were diluted in HE buffer and added to the wells as 20 μL aliquots, and TrxR was then added, also as 20 μL aliquots in HE buffer. To ensure uniform coverage of the bottom of the well the plate was spun briefly at 3000g. To start the reaction, NADPH and DTNB were added as a 20 μL aliquot in HE buffer and the plate was moved to the plate reader which had been preheated to 37 °C. The optical density at 412 nm was measured every 10 s and initial linear reaction rates measured.

Growth Inhibition Assay. Compound cytotoxicity was measured using modifications of the MTT assay as described by Mosmann[39] and Carmichael.[40] Human MCF-7 breast cancer cells were seeded at 3000 cells/well into 96-well plates in Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS). After 16 h at 37 °C and 5.5% CO_2, drugs were added to the wells at concentrations ranging from 0.1 to 20 μM. The cells were further incubated for 72 h, after which 40 μL per well of a 2.5 μg/μL solution of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) was added and an additional 3 h, 37 °C, 5.5% CO_2 incubation was performed. At the end of the incubation time, the untransformed MTT was removed from each well by aspiration and 150 μL per well of DMSO (dimethyl sulfoxide) was added. The plate was shaken to ensure full solubilization of the formazan dye followed by dual optical density readings of 595 and 655 nm using a multiwell microplate spectrophotometer (Molecular Device Corp., Menlo Park, CA). Cytoviability of control cells was considered to be 100%. For the treated cells viability was expressed as a percentage of control cells. All determinations were carried out in triplicate.

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