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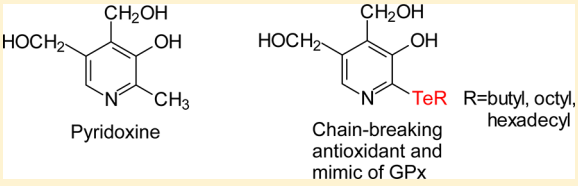
Turning Pyridoxine into a Catalytic Chain-Breaking and Hydroperoxide-Decomposing Antioxidant

Vijay P. Singh, Jia-fei Poon, and Lars Engman*

Department of Chemistry−BMC, Uppsala University, Box 576, SE-751 23 Uppsala, Sweden

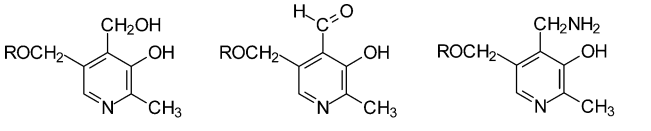
Supporting Information

ABSTRACT: Vitamin B6 is involved in a variety of enzymatic transformations. Some recent findings also indicate an antioxidant role of the vitamin in biological systems. We set out to turn pyridoxine (**1a**) into a catalytic chain-breaking and hydroperoxide-decomposing antioxidant by replacing the 2-methyl substituent with an alkyltelluro group. Target molecules **12** and derivatives **14**, **17**, **18**, and **20** thereof were accessed by subjecting suitably substituted 2-halopyridin-3-ols to aromatic substitution using sodium alkanetellurolates as nucleophiles and then LAH-reduction of ester groups. The novel pyridoxine compounds were found to inhibit azo-initiated peroxidation of linoleic acid an order of magnitude more efficiently than α-tocopherol in a water/chlorobenzene two-phase system containing *N*-acetylcysteine as a reducing agent in the aqueous phase. The most lipid-soluble pyridoxine derivative **20c** was regenerable and could inhibit peroxidation for substantially longer time (>410 min) than α-tocopherol (87 min). The chalcogen-containing pyridoxines could also mimic the action of the glutathione peroxidase enzymes. Thus, compound **20a** catalyzed reduction of hydrogen peroxide three times more efficiently than Ebselen in the presence of glutathione as a stoichiometric reducing agent.



INTRODUCTION

The water-soluble vitamin B6 has an important role in living systems. As a cofactor, it is involved in more than one hundred enzymatic transformations.[1] Vitamin B6 is the generic name for six interconvertible forms of the vitamin (vitamers) with similar structure. They include pyridoxine (**1a**), pyridoxal (**2a**), pyridoxamine (**3a**), and their respective 5′-phosphate esters **1b**, **2b**, and **3b**. Phosphate esters of pyridoxine and pyridoxamine are both oxidized in tissue to the biologically active pyridoxal-5′-phosphate (**2b**, PLP). Essentially all enzymes that rely on PLP as a cofactor act upon amino acids. Whereas the aldehyde condenses with the amine to form an imine, the pyridine moiety serves as an electron sink in subsequent transformations.[2−4] Various transamination-, deamination-, racemization-, decarboxylation-, and β-elimination reactions are brought about by these enzymes.[5]



The vitamin B6 family of 3-pyridinol compounds has been the subject of numerous structural modifications. Some of them were made with the purpose to produce artificial enzymes,[6,7] others with the intention to modify/change the activity of the cofactor.[8] Since the turn of the millennium it has been speculated that vitamin B6 compounds could also have a role as antioxidants and scavengers of reactive oxygen species in biological systems.[9−11] Evidence for their quenching of superoxide[12] and hydroxyl radicals[13] has been presented. However, the most convincing results when it comes to antioxidative capacity have been obtained in studies of their reactions with singlet oxygen.[14] Thus, compounds **1a**, **1b**, **2a**, and **3a** quench $^1O_2$ with bimolecular rate constants ranging from 5.5 to 7.5 × 10^7 M^{-1} s^{-1}.[15]

3-Pyridinols carrying strongly electron-donating substituents have been known for some time to be excellent chain-breaking antioxidants with reactivities toward peroxyl radicals matching or exceeding those recorded for α-tocopherol.[16] However, neither pyridoxine itself (*vide infra*) nor any of the other vitamin B6 vitamers are expected to possess any appreciable chain-breaking antioxidant capacity. In an effort to improve the radical trapping activity, Culbertson[17] some time ago introduced a strongly electron-donating dimethylamino group into the 3-pyridinol scaffolds of pyridoxine and pyridoxamine. Although the chain-breaking antioxidant capacity of these materials (**4a** and **4b**, respectively) was not well documented,[18] it is clear that **4b** reduced the formation of advanced glycation end products (AGE) more efficiently than the parent **3a** and nearly as efficiently as Trolox in model systems.

We have recently described another strategy for improving chain-breaking antioxidative capacity. Introduction of an alkyltelluro group into the *ortho*-position of phenols (for example **5a** and **5b**[19]) and 3-pyridinols (for example **6a** and **6b**[20]) was found to dramatically increase reactivity toward peroxyl radicals. In contrast to the parent phenols and pyridinols, the alkyltelluro-modified antioxidants were readily regenerable by *N*-acetylcysteine when assayed for their capacity to inhibit azo-initiated peroxidation of linoleic acid in a water/chlorbenzene two-phase system. In view of the antioxidant role of pyridoxine in biological systems we thought it would be interesting to modify the structure in such a way that the compound could also act in a

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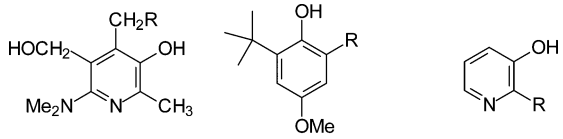
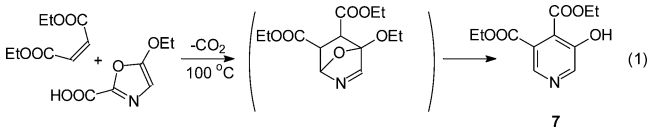


4a R=OH **4b** R=NH_2 **5a** R=H **5b** R=Te-*n*-Octyl **6a** R=H **6b** R=Te-*n*-Octyl

catalytic chain-breaking as well as hydroperoxide-decomposing fashion. It is our hope that the modified compounds would still be recognized in biological systems and that they would be suitable for treatment of disorders caused by or involving oxidative stress (such as atherosclerosis, stroke, rheumatoid arthritis, and Parkinsońs and Alzheimer's diseases). In the following we describe our attempts to install an alkyltelluro group in place of the 2-methyl group of pyridoxine (**1a**). The ability of newly prepared pyridoxine analogues to act as catalytic chain-breaking and hydroperoxide-decomposing antioxidants in model systems is also reported.

RESULTS

Synthesis. Introduction of alkyltelluro groups was envisioned taking advantage of an old approach to pyridinols[21] involving the decarboxylative Diels–Alder reaction of 5-ethoxyoxazole-2-carboxylic acid with diethyl maleate[22] (eq 1) under solvent-free

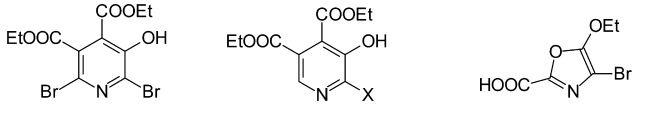


(1)

7

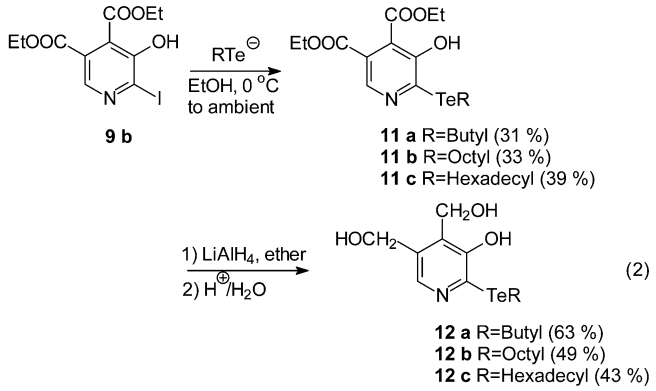
conditions. It was hoped that regioselective halogenation in position 2 of pyridinol **7**, followed by substitution with highly nucleophilic alkanetellurolate reagents and reduction of ester groups, would provide the desired target molecules.

Halogenation of pyridinol **7** turned out to be more troublesome than expected. Bromination with an equivalent amount of tetra-*n*-butylammonium tribromide in methylene chloride at ambient temperature afforded only the dibrominated pyridinol **8** along with unreacted starting material and none of the desired monobromination product **9a**. With a 2-fold excess of tetra-*n*-butylammonium tribromide, compound **8** was isolated in 90% yield. At lower temperature (−80 °C) we observed selective monobromination (**9a**:**8** ratio = 10:1), but the overall conversion of starting material was less than 20%. Attempts were also made to introduce bromine prior to the cycloaddition step. Thus, 4-bromo-5-ethoxyoxazole-2-carboxylic acid (**10b**) was prepared by bromination of 2-carboethoxy-5-ethoxyoxazole and alkaline hydrolysis of the ester. Unfortunately, attempted reaction of compound **10a** with diethyl maleate produced a complex product mixture from which the target molecule could not be isolated.



8 **9 a** X=Br **b** X=I **10 a** R=Et **b** R=H

We next turned the attention to electrophilic iodination. Iodine in the presence of sodium bicarbonate has previously been used for the introduction of iodine into pyridinols.[23] We were pleased to find that pyridinol **7** was selectively monoiodinated in position 2 when heated with this reagent at reflux in a 1:1 mixture of water and tetrahydrofuran for 4 h. (48% isolated yield of compound **9b**). Increasing the reaction time in order to improve the conversion of starting material resulted in formation of diiodinated product.



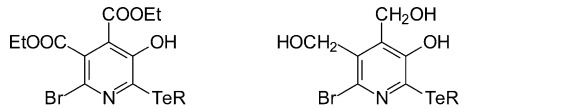
9 b

11 a R=Butyl (31 %)
11 b R=Octyl (33 %)
11 c R=Hexadecyl (39 %)

(2)

12 a R=Butyl (63 %)
12 b R=Octyl (49 %)
12 c R=Hexadecyl (43 %)

Iodopyridinol **9b** was found to undergo aromatic nucleophilic substitution at ambient temperature when treated with sodium alkanetellurolates generated by sodium borohydride reduction of the corresponding dialkyl ditellurides in ethanol. As shown in eq 2, the substitution products **11a–c** were isolated in only modest yields. The following reduction of ester groups to give target molecules **12a–c** was performed using lithium aluminum hydride in dry ether following literature methods.[24] Obviously, reductive cleavage of the weak carbon–tellurium bond was not a big problem in these reactions. When subjected to the same reaction conditions as **9b**, dibromo compound **8** afforded tellurides **13a–c**, and after reduction, brominated pyridoxine analogues **14a–c**. Surprisingly, the bromine in position 6 of the dibromopyridinol resisted substitution even at forcing conditions (refluxing ethanol and excess of tellurolate).



13 a R=Butyl (56 %)
13 b R=Octyl (48 %)
13 c R=Hexadecyl (44 %)

14 a R=Butyl (48 %)
14 b R=Octyl (59 %)
14 c R=Hexadecyl (45 %)

If the cycloaddition shown in eq 1 is carried out using the ethyl ester of 5-ethoxyoxazole-2-carboxylic acid, decarboxylation is no longer possible and the carboethoxy group will appear in position 6 of the pyridinol product **15a**. This compound was also subjected to bromination (**15b**; 72% yield using Bu_4NBr_3), substitution with sodium alkanetellurolates (**16a–c**), and ester reduction to afford pyridoxine derivatives **17a–c**. The poor isolated yields in the reduction this time is probably a consequence of the higher water solubility of these materials.

In an effort to reduce the hydrophilicity of some of the newly prepared pyridoxine derivatives, we decided to protect hydroxymethyl groups as acetonides. Stirring of compounds **12a–c** at room temperature for 20 h with 2,2-dimethoxypropane in acetone containing an equimolar amount of *p*-toluenesulphonic acid afforded compounds **18a–c** where the pyridinolic moiety was left untouched. Interestingly, when compounds **14a–c** were subjected to the similar reaction conditions, products **19a–c**, resulting from ketalization between the pyridinolic and 4-hydroxymethyl groups, were isolated. Workup of the reaction mixture after 2.5 h provided ketals **20a–c** as major products in addition to **19a**, **19b**, and **19c** (18%, 9%, and 15% isolated yields, respectively). Probably,

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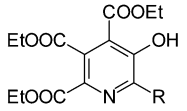
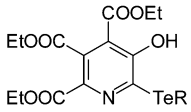
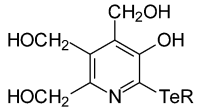


15 a R=H
15 b R=Br

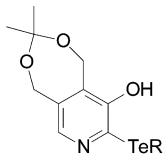


16 a R=Butyl (35 %)
16 b R=Octyl (29 %)
16 c R=Hexadecyl (25 %)

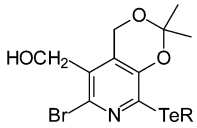


17 a R=Butyl (18 %)
17 b R=Octyl (22 %)
17 c R=Hexadecyl (14 %)

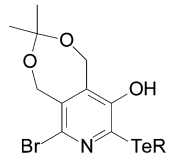
compounds **20a–c** are kinetically favored, whereas **19a–c** are thermodynamically more stable.



18 a R=Butyl (64 %)
18 b R=Octyl (63 %)
18 c R=Hexadecyl (54 %)

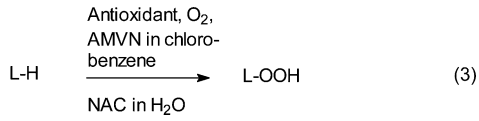


19 a R=Butyl (52 %)
19 b R=Octyl (58 %)
19 c R=Hexadecyl (15 %)



20 a R=Butyl (48 %)
20 b R=Octyl (65 %)
20 c R=Hexadecyl (47 %)

Inhibition Studies in a Two-Phase Lipid Peroxidation Model System. Azo-initiated peroxidation of linoleic acid or derivatives thereof has commonly been used for studying the chain-breaking capacity of synthetic and natural antioxidants. Some time ago, we modified this system to allow for regeneration of the lipid-soluble antioxidant by co-antioxidants contained in an aqueous phase.[25] In the experimental setup for this two-phase system (eq 3),



(3)

linoleic acid (L-H) and a trace amount of the antioxidant in chlorobenzenewere stirred with an aqueous phase containing *N*-acetylcysteine (NAC) as a stoichiometric co-antioxidant. 2,2′-Azobis(2,4-dimethylvaleronitrile AMVN) was added as an initiator, and the progress of peroxidation was monitored by HPLC analysis of the chlorbenzene layer (formation of conjugated diene hydroperoxide, L-OOH at 234 nm). When evaluated in this model, good chain-breaking antioxidants show an inhibited phase of peroxidation that can be clearly distinguished from the uninhibited phase that follows when the antioxidant is all consumed (*vide infra*). The slower the rate of LOOH formation during the inhibited phase (R_{inh}), the more efficiently does the antioxidant quench peroxyl radicals. The duration of the inhibited phase, T_{inh}, is another interesting parameter. If the co-antioxidant contained in the aqueous phase can continuously regenerate the antioxidant in the chlorobenzene layer, the inhibition time can be significantly extended.[19,20,26] α-Tocopherol (α-TOC) was used as a reference compound (see Table 1). Regardless of whether NAC (1 mM) was present in the aqueous phase, inhibition times and inhibited rates of peroxidation were essentially the same. Thus, *N*-acetylcysteine in the aqueous phase cannot regenerate the α-tocopheroxyl radical in the system before quenching of a second peroxyl radical and conversion to other, nonradical products.

All new pyridoxine analogues containing an alkyltelluro group in position 2 were evaluated for their chain-breaking capacity and regenerability in the model lipid peroxidation system (Table 1). In the absence of NAC in the aqueous phase, none of the compounds showed any appreciable chain-breaking activity (R_{inh} = 449–592 μM h^{-1}). This is due to oxidation at tellurium by residual amounts of linoleic acid hydroperoxide always contained in commercial samples of linoleic acid. The resulting telluroxides are known to act as poor retarders of lipid peroxidation rather than as inhibitors. *N*-Acetylcysteine will reduce telluroxides formed and keep catalyst tellurium in the active, divalent state. With few exceptions (diesters **13** and triesters **16** with R_{inh} values in the range of 84–140 and 86–124 μM h^{-1}, respectively), newly prepared pyridinolic antioxidants were as efficient (diesters **11** with R_{inh} values in the range of 24–25 μM h^{-1}) or significantly better quenchers of peroxyl radicals than α-tocopherol. Reduction of ester groups to the corresponding benzylic alcohols improved antioxidant capacity significantly. Thus, pyridoxine-like compounds **12** (R_{inh} values in the range of 0.8–2.8 μM h^{-1}) were approximately an order of magnitude more reactive than the corresponding esters **11**. Similarly, compounds **14** and **17** were more potent antioxidants than their respective ester precursors **13** and **16**.

We anticipated already at the outset of this project that the high water solubility of our target molecules could be a problem when compounds were to be evaluated in the lipid peroxidation model. If too much of the catalyst escapes from the chlorobenzene into the aqueous layer, the duration of the antioxidant protection will be reduced. Inspection of inhibition times (Table 1; T_{inh}) shows that many catalysts tested inhibit peroxidation for only marginally longer times than recorded for α-tocopherol (compounds **11–14**, **16**, **17**). However, there is a trend in each series of compounds that increasing lipophilicity in the alkyltelluro moiety causes an increase in the inhibition time (hexadecyltelluro > octyltelluro > butyltelluro). The effect is never dramatic, though. In contrast, an almost 2-fold increase in inhibition time was recorded for catalysts **12** and **14** when hydroxymethyl groups were protected as acetonides (compounds **18** and **20**). The most long-lasting antioxidant protection was offered by catalyst **20c**, which showed an inhibition time of more than 410 min. This result lends further support to the idea that a certain lipophilicity of the antioxidant is required in order to maintain a high enough concentration in the chlorobenzene layer to allow regeneration by the co-antioxidant contained in the aqueous phase. As we have seen before,[26] the limitation for the duration of the catalytic antioxidant protection seems to be the amount of thiol present in the aqueous phase. When all thiol is oxidized to the corresponding disulfide, peroxidation is no longer inhibited.

A free pyridinolic group in the organotellurium compound seems to be essential for good antioxidant performance. When acetonide formation with compounds **14** occurred between phenolic and neighboring hydroxymethyl groups (compounds **19**), both quenching capacity (R_{inh} = 46–79 μM h^{-1}) and regenerability (T_{inh} = 88–99 min) were drastically reduced.

For comparison, pyridoxine (**1a**) was also evaluated in the two-phase model. As anticipated, the compound slightly retarded peroxidation (R_{inh} = 457 μM h^{-1}) but did not quench peroxyl radicals. Peroxidation traces recorded with pyridoxine (**1a**), α-tocopherol, and the most regenerable pyridoxine analogue **20c** are shown in Figure 1. It should be clear from this figure that

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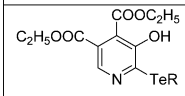
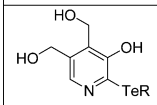
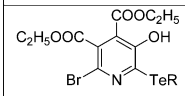
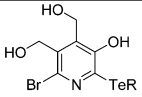
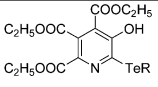
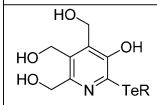
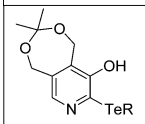
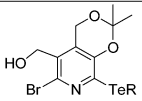
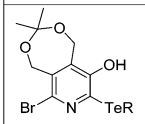
Table 1. Inhibited Rates of Linoleic Acid Peroxidation (R_{inh}) and Inhibition Times (T_{inh}) in the Presence and Absence of NAC (1 mM) for Antioxidants Tested in the Two-Phase Model System[d]

Antioxidant (40 μM)		With NAC		Without NAC	GPx activities
		R_{inh}[a] (μM h^{-1})	T_{inh}[b] (min)	R_{inh}[a] (μM h^{-1})	v_0 (μM min^{-1})[c]
	**11a** R = Butyl	24 ± 2	115 ± 10	483	
	11b R = Octyl	25 ± 3	132 ± 5	467	
	11c R = Hexadecyl	25 ± 2	149 ± 2	563	
	**12a** R = Butyl	2.8 ± 0.4	75 ± 6	479	148 ± 5
	12b R = Octyl	1.7 ± 0.6	115 ± 5	450	114 ± 2
	12c R = Hexadecyl	0.8 ± 0.3	159 ± 8	474	12 ± 2
	**13a** R = Butyl	140 ± 3	103 ± 6	481	
	13b R = Octyl	84 ± 7	106 ± 5	500	
	13c R = Hexadecyl	91 ± 6	120 ± 8	454	
	**14a** R = Butyl	6.4 ± 0.2	192 ± 1	472	169 ± 4
	14b R = Octyl	4.6 ± 1.2	195 ± 7	464	117 ± 1
	14c R = Hexadecyl	2.5 ± 0.1	204 ± 7	449	7 ± 2
	**16a** R = Butyl	124 ± 4	106 ± 6	482	
	16b R = Octyl	86 ± 3	90 ± 6	576	
	16c R = Hexadecyl	110 ± 5	106 ± 8	592	
	**17a** R = Butyl	11.2 ± 1.9	73 ± 3	484	195 ± 2
	17b R = Octyl	7.1 ± 1.6	143 ± 9	488	139 ± 1
	17c R = Hexadecyl	1.7 ± 0.6	172 ± 3	463	3 ± 1
	**18a** R = Butyl	4.3 ± 0.3	173 ± 4	466	173 ± 1
	18b R = Octyl	2.3 ± 0.3	259 ± 12	469	58 ± 2
	18c R = Hexadecyl	1.4 ± 0.4	276 ± 5	468	inactive
	**19a** R = Butyl	78 ± 5	90 ± 8	491	9 ± 2
	19b R = Octyl	79 ± 8	88 ± 9	499	inactive
	19c R = Hexadecyl	46 ± 5	99 ± 6	435	inactive
	**20a** R = Butyl	4.1 ± 0.9	308 ± 9	471	182 ± 2
	20b R = Octyl	4.2 ± 1.3	340 ± 9	458	11 ± 2
	20c R = Hexadecyl	2.6 ± 0.2	>410	482	3 ± 1
Pyridoxine (**1a**)		457 ± 12	-	533	inactive
α-TOC		25 ± 1	87 ± 6	24	
Ebselen		-	-	-	60 ± 1

[a]Rate of peroxidation during the inhibited phase (uninhibited rate ca. 650 μM h^{-1}). Errors correspond to ± SD for triplicates. [b]Duration of the inhibited phase of peroxidation. Reactions were monitored for 410 min. Errors correspond to ± SD for triplicates. [c]NADPH consumption for the initial 10 s of reaction. Values were corrected for the spontaneous oxidation of GSH (22 ± 3 μM min^{-1}) and the maximal initial consumption of NADPH that could be attributed to glutathione reductase reduction of telluroxide (Table S5 in the Supporting Information). Errors correspond to ± SD for triplicates. [d]Glutathione peroxidase-like activities of antioxidants as determined by initial rates of NADPH consumption (v_0) in the presence of H_2O_2, glutathione, and glutathione reductase.

introduction of tellurium in place of the methyl group in the 2-position of pyridoxine dramatically improves antioxidant quenching capacity as well as regenerability.

Glutathione Peroxidase-Like Antioxidant Activity. The glutathione peroxidases (GPx) are a small family of selenoenzymes that catalyze reduction of hydrogen peroxide and organic hydroperoxides to water and alcohols, respectively, using glutathione, GSH, as the stoichiometric reducing agent. The reason Nature selected selenium for this important task is probably that this element is easily redox-cycled. In the proposed

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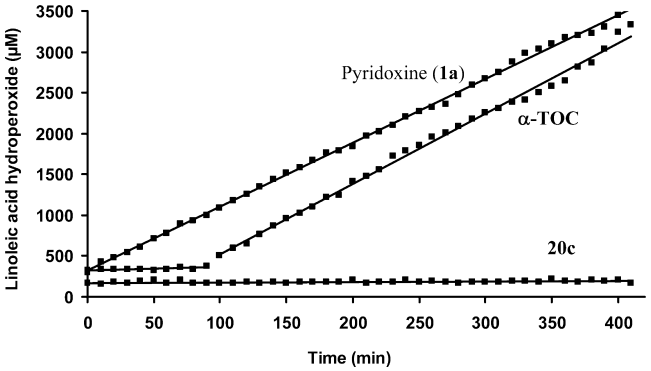
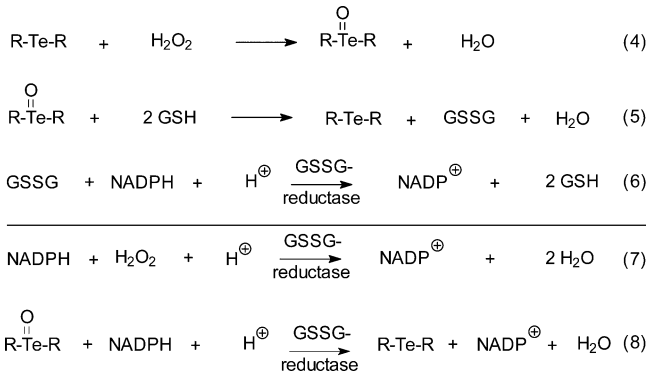


Figure 1. Peroxidation traces (linoleic acid hydroperoxide concentration vs time) recorded using compounds **20c**, ***α*-TOC**, and pyridoxine (**1a**) (40 μM) as antioxidants in the chlorobenzene layer in the presence of NAC (1 mM) in the aqueous phase.

mechanism for the action of active site selenocysteine, selenium traverses the selenol, selenenic acid, and selenosulfide oxidation states while hydrogen peroxide is reduced to water and two molecules of glutathione are oxidized to the corresponding disulfide, GSSG. Other types of chalcogen compounds also undergo facile redox cycling and can be useful as mimics of the glutathione peroxidase enzymes. This is especially true for divalent organotellurium compounds, R_2Te, which could be oxidized to the corresponding telluroxides R_2TeO.

The glutathione peroxidase-like activities of all new pyridoxine analogues were assessed by using the coupled reductase assay.[27] Telluroxide produced in the reaction of hydrogen peroxide with catalyst (eq 4) is reduced by GSH to the divalent state with



disulfide formation (eq 5). We have previously shown for other telluride/thiol/hydrogen peroxide systems that oxidation of telluride is much slower than reduction of telluroxide and that the resting state of the catalyst is telluride.[28] This is likely to be the case also in this system. Oxidized glutathione (GSSG) is then continuously reduced by GSSG-reductase with consumption of NADPH (eq 6). GPx-activities are shown in Table 1 as initial rates (v_0) of NADPH-consumption (eq 7) recorded by UV spectroscopy at 340 nm for the first 10 s of reaction. Values in Table 1 are corrected for the uncatalyzed background reaction of GSH with H_2O_2 and the slow initial consumption of NADPH that was recorded for each catalyst (4−17 μM/min) in separate experiments (normal assay conditions minus GSH). Obviously, telluroxides can act as substrates for GSSG-reductase as shown in eq 8. Ebselen (2-phenyl-1,2-benzisoselenazol-3-(2*H*)-one, a well-known GPx mimic,[29] was included as a reference compound and bench-mark (v_0 was 60 ± 1 μM/min in the presence of 2.5 mol % of this catalyst). Pyridoxine analogues **12a**, **14a**, **17a**, **18a**, and **20a**, carrying a butyltelluro group in place of a methyl in position 2, showed a GPx activity that was 2−3 times higher than that recorded for Ebselen. However, in all series of compounds the activity was reduced as the size of the alkyl group was increased (butyl > octyl > hexadecyl) and the catalytic activity of the hexadecyl derivatives could never match that recorded for Ebselen. The reaction of hydrogen peroxide with the organotellurium catalyst (eq 4) is likely to involve a nucleophilic attack of tellurium on oxygen. The rate of this reaction would be affected by a combination of electronic and steric effects. Electronically as well as sterically, the pyridinol moieties of compounds **12**, **14**, **17**, **18**, and **20** would seem to have a rather similar influence. Thus, we conclude that the varying GPx activities recorded largely reflect the steric hindrance of the alkyl group attached to tellurium. The poor performance of catalysts **19a−c** in the GPx assay can probably also be ascribed to steric hindrance around tellurium. Also, this is the only organotellurium compound studied that does not have a phenolic moiety in the *ortho*-position. The hydroxyl substituent may in fact assist by hydrogen bonding in the reaction of tellurium with hydrogen peroxide.

Not unexpectedly, pyridoxine (**1a**) did not show any GPx-like activity.

Catalytic Parameters and Consumption of Hydrogen Peroxide. The two most active GPx mimics, compounds **17a** and **20a**, were subjected to further kinetic studies. Maximum velocities (V_{max}) were obtained by recording initial rates of NADPH consumption (v_0) in the presence of increasing amounts of GSH. As shown in Figure 2 for compound **17a**, saturation kinetics was

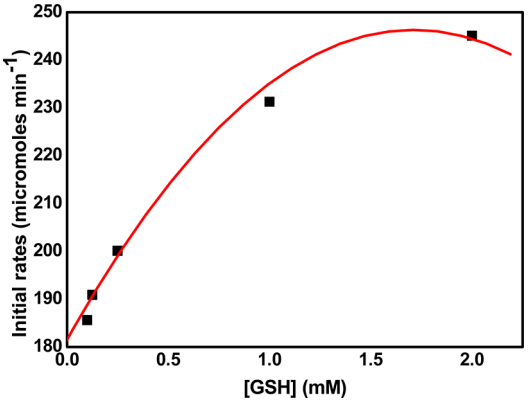


Figure 2. Effect of thiol concentration on initial rates of NADPH consumption for reduction of H_2O_2 (0.80 mM) by GSH (0.1−2.0 mM) in the presence of catalyst **17a**.

observed. From Lineweaver−Burk plots (Figures S1−S8 in Supporting Information) where the concentration of hydrogen peroxide was also varied, kinetic parameters such as the maximum velocity (V_{max}), Michaelis constant (K_M), catalytic constant (k_{cat}), and catalytic efficiency (η) were determined as shown in Table 2. In contrast to experiments where GSH was varied, saturation was not observed with increasing amounts of H_2O_2.

The performance of the GPx mimics was in a few cases followed for a much longer time (for the first 200 min of reaction) than during the initial phase of conversion of starting materials. As shown in Figure 3, essentially all hydrogen peroxide was reduced within 200 min in the presence of 5 mol % of catalysts **17a** and **20a**. The consumption of NADPH is mainly due to the catalyzed reaction during the first 50% of conversion,

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Table 2. Catalytic Parameters (V_{max}, K_M, k_{cat}, η) for Compounds 17a and 20a

catalyst	V_{max} (μM min^{-1})	K_M (mM)	k_{cat} (min^{-1})	η (mM^{-1} min^{-1})
		17a		
GSH (variable)	238.66	0.03	11.93	384.93
H_2O_2 (variable)	1121.70	2.52	56.08	22.25
		20a		
GSH (variable)	242.13	0.03	12.12	403.55
H_2O_2 (variable)	4179.02	15.92	208.95	13.12

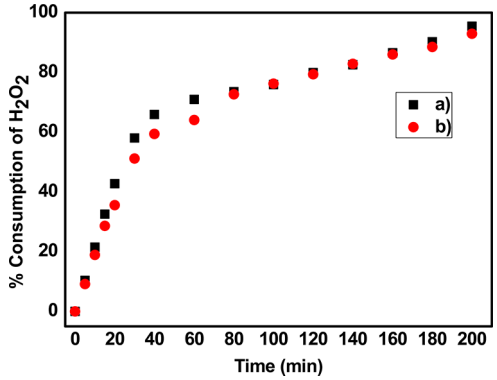

Figure 3. Consumption of H_2O_2 (in percent as determined by NADPH consumption) with time using (a) catalyst **17a** and (b) catalyst **20a**. Assay conditions: phosphate buffer (100 mM), pH 7.5, with EDTA (1 mM), GSH (0.25 mM), NADPH (0.2 mM), GR (1 unit/mL), organotellurium catalyst (10 μM), and H_2O_2 (0.20 mM).

but mostly due to the spontaneous background reaction of hydrogen peroxide with glutathione toward the end (for control experiments see Figures S9 and S10 in the Supporting Information).

DISCUSSION AND CONCLUDING REMARKS

Replacement of the 2-methyl group with alkyltelluro in the structure of pyridoxine has a rather dramatic effect on the antioxidant profile of the compound. Not only does the *ortho*-arrangement of 3-pyridinol and alkyltelluro groups add value in the form of a chain-breaking capacity exceeding that of α-tocopherol. The very presence of the heavy chalcogen tellurium in the molecule also implies that it could act as a preventive antioxidant and catalyze decomposition of hydroperoxides using thiols as stoichiometric reducing agents. The reasons for the latter, glutathione peroxidase-like activity of our antioxidants, has been discussed above in some detail. However, the mechanisms for quenching of peroxyl radicals and regeneration of the antioxidant in the two-phase peroxidation model, accompanied by thiol oxidation in the aqueous phase, are as yet not fully understood. Evans–Polanij plots of log $k_{inhibition}$ versus BDE(O–H) for phenols with similar steric crowding showed that introduction of an *ortho*-alkyltelluro group increased the reactivity toward peroxyl radicals with 2 orders of magnitude more than expected from substituent effects considerations.[19] This dramatic effect suggests that another mechanism than direct H-atom transfer from O–H could be operative with the chalcogen-containing antioxidants. We hypothesize that peroxyl radicals could add to tellurium with O-atom transfer[30] and the resulting alkoxyl radical abstract a hydrogen atom in a solvent cage from neighboring phenolic or pyridinolic groups. The formation of a telluroxide would provide a large thermodynamic driving force for the reaction. Catalyst regeneration by thiol contained in the aqueous phase then has to involve the usual reduction of telluroxide to telluride as well as a one-electron reduction/protonation of a phenoxyl radical. We are presently trying to find experimental evidence for this new antioxidant mechanism.

The novel pyridoxine compounds are likely to serve as excellent scavengers of reactive oxygen species. Since thiols can serve as stoichiometric reducing agents both for scavenging peroxyl radicals and hydroperoxides, the cellular environment with glutathione present in millimolar concentrations may provide the reducing capacity needed for catalysis. Although little is known about the toxicity of organotellurium compounds in biological systems,[31] it is clear that the small amounts of the element that we consume via the normal diet can be metabolized and excreted.

EXPERIMENTAL SECTION

^{1}H NMR spectra were recorded on 300 and 400 MHz spectrometers. ^{13}C spectra were recorded at 100 and 75 MHz. NMR chemical shifts are reported in ppm referenced to the solvent peak of $CDCl_3$ (7.26 ppm for ^{1}H and 77.20 ppm for ^{13}C, respectively), CD_3OD (3.31 ppm for ^{1}H and 49.00 ppm for ^{13}C, respectively), or DMSO-d_6 (2.50 ppm for ^{1}H and 39.52 ppm for ^{13}C, respectively). Melting points are uncorrected. High resolution mass spectra (HRMS) were obtained using a time-of-flight instrument equipped with electrospray ionization. Diethyl ether for LAH-reductions was dried over sodium/benzophenone. 5-Ethoxy-2-carboethoxyoxazole was obtained as a yellow, viscous, liquid cyclodehydration product by heating of ethyl *N*-ethoxalylglycinate (prepared by heating ethyl glycinate hydrochloride with diethyl oxalate and triethylamine in C_2H_5OH at 50–55 °C for 24 h[22a]) in refluxing chloroform containing phosphorus oxychloride ($POCl_3$) and triethylamine.[22b] Hydrolysis of the ester occurred in a 20% aqueous sodium hydroxide solution to provide the corresponding carboxylic acid.[22b] Heating of 5-ethoxyoxazole-2-carboxylic acid and 5-ethoxy-2-carboethoxyoxazole, respectively, with neat diethyl maleate at 100 °C afforded pyridinol **7** (as a yellow solid) and **15a** (as an oil).[22b] The compounds were purified by column chromatography on silica gel using pentane/ethyl acetate (80:20) as an eluent. Di-*n*-butyl ditelluride,[32] di-*n*-octyl ditelluride,[33] and di-*n*-hexadecyl ditelluride[34] were prepared according to literature methods.

2,6-Dibromo-4,5-bis(carboethoxy)-3-pyridinol (8). To a stirred solution of compound **7** (2.0 g, 8.36 mmol) in CH_2Cl_2 (10 mL) at room temperature was added dropwise tetrabutylammonium tribromide (8.06 g, 16.7 mmol) in CH_2Cl_2 (10 mL). After 1 h of stirring, water (2 mL) was added, and stirring continued overnight. The organic layer was separated and extracted with $CHCl_3$. The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated in vacuo. Purification by column chromatography using pentane/ethyl acetate (90:10) as eluent afforded the title compound as a white solid. Yield: 3.00 g (90%). Mp 72–74 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38–1.45 (several peaks, 6H), 4.39–4.50 (several peaks, 4H), 11.39 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.9, 14.1, 62.8, 64.4, 117.7, 125.6, 130.9, 133.1, 153.5, 164.8, 166.7. HRMS (TOF MS ES$^+$) m/z calcd for $C_{11}H_{11}Br_2NO_5$ $[M + H]^+$: 395.9082; found 395.9076.

2-Bromo-4,5-bis(carboethoxy)-3-pyridinol (9a). If the above reaction was carried out at −80 °C for 5–10 min only, compound **8** (1.3% yield) and the title compound **9a** (11% yield) were isolated as a light yellow oil along with unreacted starting material (82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.36–1.41 (several peaks, 6H), 4.37 (q, J = 7.2 Hz, 2H), 4.46 (q, J = 7.2 Hz, 2H), 8.13 (s, 1H), 10.12 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.9, 14.2, 62.5, 63.6, 119.2, 127.5, 135.6, 139.4, 151.8, 165.5, 166.9. HRMS (TOF MS ES$^+$) m/z calcd for $C_{11}H_{12}BrNO_5$ $[M + Na]^+$: 339.9797; found 339.9778.

2-Iodo-4,5-bis(carboethoxy)-3-pyridinol (9b). To a solution of compound **7** (1.20 g, 5.0 mmol) in THF (4.5 mL) and H_2O (4.5 mL) were added iodine (1.53 g, 5.5 mmol) and $NaHCO_3$ (0.506 g, 5.5 mmol) in one portion at room temperature. The reaction mixture was then stirred under reflux for 4 h. Residual iodine was quenched by the addition of a 10% aqueous solution of $Na_2S_2O_3$. The aqueous solution

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was extracted with $CHCl_3$ (3 × 10 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and filtered, and the solvent was evaporated under vacuum. The crude product was purified by column chromatography using pentane/ethylacetate (85:15) as eluent to afford the title compound as a yellow oil. Yield: 0.88 g (48%). Unreacted starting material could also be recovered from the column. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.36−1.40 (several peaks, 6H), 4.37 (q, J = 7.1 Hz, 2H), 4.44 (q, J = 7.1 Hz, 2H), 8.11 (s, 1H), 10.57 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.9, 14.2, 62.4, 63.7, 114.8, 116.3, 127.8, 140.4, 154.4, 165.7, 167.2. HRMS (TOF MS ES$^+$) m/z calcd for $C_{11}H_{12}NIO_5$ [M + Na]$^+$: 387.9658; found 387.9646.

4-Bromo-5-ethoxyoxazole-2-carboxylic Acid (10b). To a solution of 5-ethoxy-2-carboethoxyoxazole (0.54 g, 2.90 mmol) in CH_2Cl_2 (10 mL) was added tetrabutylammonium tribromide (1.40 g, 2.90 mmol) as described for preparation of compound **8**. After the usual workup and purification by column chromaography using pentane/ethyl acetate (96:4) as eluent a white crystalline bromination product **10a** was obtained as a solid (0.25 g, 32%). Mp 70−72 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39−1.55 (several peaks, 6H), 4.39−4.50 (several peaks, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 15.1, 62.8, 70.9, 93.4, 142.5, 154.7, 156.5. HRMS (TOF MS ES$^+$) m/z calcd for $C_8H_{10}BrNO_4$ [M + Na]$^+$: 285.9691; found 285.9690. Hydrolysis of the material (0.18 g, 0.68 mmol) with 20% aqueous NaOH solution and acidic workup afforded the title compound, 0.095 g (59%). Mp 92−94 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.35 (t, J = 7.0 Hz, 3H), 4.42 (q, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.8, 70.8, 92.3, 143.2, 155.4, 155.6. HRMS (TOF MS ES$^+$) m/z calcd for $C_6H_6BrNO_4$ [M − H + 2Na]$^+$: 279.9197; found 279.9182.

2-Bromo-4,5,6-tris(carboethoxy)-3-pyridinol (15b). Synthesized from compound **15a** (1.0 g, 3.21 mmol) in CH_2Cl_2 (3 mL) and tetrabutylammonium tribromide (2.0 g, 4.17 mmol) according to the procedure for preparation of compound **8**. The title compound was obtained as a white solid after purification by column chromatography using pentane/ethyl acetate (90:10) as an eluent. Yield: 0.90 g (72%). Mp 73−74 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37−1.45 (several peaks, 9H), 4.39−4.53 (several peaks, 6H), 12.07 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.8, 13.9, 14.3, 62.4, 62.6, 64.4, 116.2, 131.2, 133.9, 136.0, 156.5, 162.9, 165.3, 167.7. HRMS (TOF MS ES$^+$) m/z calcd for $C_{14}H_{16}BrNO_7$ [M + Na]$^+$: 412.0008; found 411.9994.

Typical Procedure for Introduction of Alkyltelluro Groups by Nucleophilic Aromatic Substitution. Butyl 4,5-Bis(carboethoxy)-3-hydroxy-2-pyridyl Telluride (11a). To a solution of di-*n*-butyl ditelluride (0.325 g, 0.88 mmol) in deoxygenated ethanol (5 mL) was added $NaBH_4$ (0.67 g, 1.76 mmol) under an inert atmosphere at 0 °C, and the mixture was stirred at room temperature until colorless. Then, compound **9b** (0.320 g, 0.88 mmol) in ethanol (2 mL) was added dropwise to the *in situ* prepared solution of *n*-BuTeNa at 0 °C. Additionally, the mixture was stirred at room temperature for 4−5 h and then heated at 60 °C for 2 h. The solvent was removed under reduced pressure, and the residue was dissolved in $CHCl_3$ and washed with water. The organic layers and $CHCl_3$ extracts from the aqueous layer were combined and dried over anhydrous Na_2SO_4. After evaporation and column chromatography using pentane/ethyl acetate (90:10) as eluent, the pure title compound was isolated as an yellow liquid. Yield: 0.115 g (31%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (t, J = 7.3 Hz, 3H), 1.34−1.48 (several peaks, 8H), 1.86 (quintet, J = 7.5 Hz, 2H), 3.15 (t, J = 7.5 Hz, 2H), 4.35 (q, J = 7.1 Hz, 2H), 4.40 (q, J = 7.1 Hz, 2H), 8.24 (s, 1H), 10.59 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 8.7, 13.7, 13.9, 14.3, 25.4, 33.7, 61.9, 63.2, 112.4, 124.4, 141.1, 141.2, 155.2, 166.9, 168.3. HRMS (TOF MS ES$^+$) m/z calcd for $C_{15}H_{21}NO_5Te$ [M + H]$^+$: 426.0560; found 426.0568.

The following compounds were analogously prepared starting from 2-halo-3-pyridinols **8**, **9b**, and **15** and the appropriate, *in situ* prepared, sodium alkanetellurolates (*n*-butylTeNa, *n*-octylTeNa, or *n*-hexadecylTeNa).

4,5-Bis(carboethoxy)-3-hydroxy-2-pyridyl Octyl Telluride (11b). Yellow liquid. Yield: 33%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, J = 6.7 Hz, 3H), 1.26−1.41 (several peaks, 16H), 1.87 (quintet, J = 7.6 Hz, 2H), 3.14 (t, J = 7.5 Hz, 2H), 4.30−4.44 (several peaks, 4H), 8.23 (s, 1H), 10.59 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 9.1, 13.9, 14.2, 14.3, 22.8, 29.1, 29.3, 31.6, 31.9, 32.4, 61.9, 63.1, 112.3, 124.4, 141.2, 155.2, 166.9, 168.3. HRMS (TOF MS ES$^+$) m/z calcd for $C_{19}H_{29}NO_5Te$ [M + H]$^+$: 482.1186; found 482.1177.

4,5-Bis(carboethoxy)-3-hydroxy-2-pyridyl Hexadecyl Telluride (11c). Yellow solid. Yield: 39%. Mp 49−51 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, J = 7.0 Hz, 3H), 1.25−1.38 (several peaks, 32H), 1.87 (quintet, J = 7.4 Hz, 2H), 3.14 (t, J = 7.5 Hz, 2H), 4.35 (q, J = 7.2 Hz, 2H), 4.40 (q, J = 7.2 Hz, 2H), 8.23 (s, 1H), 10.58 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.1, 13.9, 14.2, 14.3, 22.9, 29.2, 29.5, 29.7, 29.8, 29.9, 31.6, 32.1, 32.4, 61.9, 63.2, 112.4, 124.4, 141.2, 155.2, 166.9, 168.3. HRMS (TOF MS ES$^+$) m/z calcd for $C_{27}H_{45}NO_5Te$ [M + H]$^+$: 594.2438; found 594.2446.

4,5-Bis(carboethoxy)-6-bromo-3-hydroxy-2-pyridyl Butyl Telluride (13a). Yellow solid. Yield: 56%. Mp 39−41 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (t, J = 7.2 Hz, 3H), 1.36−1.47 (several peaks, 8H), 1.87 (quintet, J = 7.5 Hz, 2H), 3.13 (t, J = 7.5 Hz, 2H), 4.37−4.44 (several peaks, 4H), 11.19 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.6, 13.7, 13.9, 14.1, 25.4, 33.6, 62.4, 63.7, 112.7, 126.4, 128.9, 140.2, 155.9, 165.8, 167.4. HRMS (TOF MS ES$^+$) m/z calcd for $C_{15}H_{20}BrNO_5Te$ [M + H]$^+$: 503.9665; found 503.9651.

4,5-Bis(carboethoxy)-6-bromo-3-hydroxy-2-pyridyl Octyl Telluride (13b). Yellow solid. Yield: 48%. Mp 45−47 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, J = 6.8 Hz, 3H), 1.26−1.43 (several peaks, 16H), 1.88 (quintet, J = 7.5 Hz, 2H), 3.12 (t, J = 7.5 Hz, 2H), 4.37−4.43 (several peaks, 4H), 11.19 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.9, 13.9, 14.1, 14.3, 22.8, 29.1, 29.4, 31.5, 32.0, 32.3, 62.4, 63.7, 112.7, 126.4, 128.9, 140.2, 155.9, 165.8, 167.4. HRMS (TOF MS ES$^+$) m/z calcd for $C_{19}H_{28}BrNO_5Te$ [M + H]$^+$: 560.0291; found 560.0285.

4,5-Bis(carboethoxy)-6-bromo-3-hydroxy-2-pyridyl Hexadecyl Telluride (13c). Yellow solid. Yield: 44%. Mp 68−70 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, J = 6.9 Hz, 3H), 1.26−1.43 (several peaks, 32H), 1.88 (quintet, J = 7.5 Hz, 2H), 3.12 (t, J = 7.5 Hz, 2H), 4.37−4.45 (several peaks, 4H), 11.19 (br s, OH, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.9, 13.9, 14.1, 14.3, 22.9, 29.2, 29.5, 29.6, 29.7, 29.8, 29.9, 31.5, 31.6, 32.1, 32.3, 62.4, 63.7, 112.7, 126.4, 128.9, 140.3, 155.9, 165.8, 167.4. HRMS (TOF MS ES$^+$) m/z calcd for $C_{27}H_{44}BrNO_5Te$ [M + H]$^+$: 672.1543; found 672.1524.

Butyl 3-Hydroxy-4,5,6-tris(carboethoxy)-2-pyridyl Telluride (16a). Yellow solid. Yield: 35%. Mp 57−59 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, J = 7.5 Hz, 3H), 1.25−1.51 (several peaks, 11H), 1.90 (quintet, J = 7.4 Hz, 2H), 3.20 (t, J = 7.5 Hz, 2H), 4.36−4.48 (several peaks, 6H), 11.85 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 9.3, 13.7, 13.9, 14.0, 14.3, 25.6, 33.9, 61.9, 62.1, 63.8, 111.2, 127.9, 137.9, 139.4, 158.6, 164.2, 166.4, 168.4. HRMS (TOF MS ES$^+$) m/z calcd for $C_{18}H_{25}NO_7Te$ [M + H]$^+$: 498.0772; found 498.0779.

3-Hydroxy-4,5,6-tris(carboethoxy)-2-pyridyl Octyl Telluride (16b). Yellow solid. Yield: 29%. Mp 43−45 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, J = 7.0 Hz, 3H), 1.26−1.42 (several peaks, 19H), 1.91 (quintet, J = 7.7 Hz, 2H), 3.20 (t, J = 7.5 Hz, 2H), 4.34−4.48 (several peaks, 6H), 11.86 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.8, 13.9, 14.0, 14.2, 14.3, 22.8, 29.2, 29.5, 31.8, 32.0, 32.6, 61.9, 62.1, 63.8, 111.1, 127.9, 137.9, 139.4, 158.6, 164.1, 166.4, 168.4. HRMS (TOF MS ES$^+$) m/z calcd for $C_{22}H_{33}NO_7Te$ [M + H]$^+$: 554.1398; found 554.1413.

Hexadecyl 3-Hydroxy-4,5,6-tris(carboethoxy)-2-pyridyl Telluride (16c). Yellow solid. Yield: 25%. Mp 56−57 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, J = 7.7 Hz, 3H), 1.25−1.41 (several peaks, 35H), 1.90 (quintet, J = 7.5 Hz, 2H), 3.18 (t, J = 7.5 Hz, 2H), 4.34−4.46 (several peaks, 6H), 11.85 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.8, 13.9, 14.0, 14.2, 14.3, 22.9, 29.3, 29.5, 29.7, 29.8, 29.9, 31.8, 32.1, 32.6, 61.8, 62.1, 63.7, 111.1, 127.9, 137.9, 139.4, 158.6, 164.1, 166.3, 168.4. HRMS (TOF MS ES$^+$) m/z calcd for $C_{30}H_{49}NO_7Te$ [M + H]$^+$: 666.2649; found 666.2652.

Typical Procedure for LAH-Reduction of Carboethoxy Groups. 4,5-Bis(hydroxymethyl)-3-hydroxy-2-pyridyl Butyl Telluride (12a). To a stirred suspension of $LiAlH_4$ (0.094 g, 2.48 mmol) in dry ether (6 mL) was added dropwise compound **11a** (0.35 g, 0.83 mmol) in ether (2 mL) at 0 °C under an inert atmosphere. The reaction mixture was then stirred at room temperature for 1 h, and H_2SO_4 (10% aq) was added to quench the excess $LiAlH_4$. After 5 min the mixture was neutralized with NaOH (5% aq), and the separated aqueous layer was extracted several times with ethyl acetate. The combined organic

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extracts were dried over anhydrous Na_2SO_4 and evaporated. Purification of the residue by column chromatography on silica gel using pentane/ethyl acetate (50:50) as eluent afforded the title compound as a white solid. Yield: 0.175 g (63%). Mp 88−90 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 0.93 (t, J = 7.3 Hz, 3H), 1.44 (sextet, J = 7.9 Hz, 2H), 1.82 (quintet, J = 7.6 Hz, 2H), 3.05 (t, J = 7.5 Hz, 2H), 4.58 (s, 2H), 4.92 (s, 2H), 7.99 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 6.9, 13.9, 26.3, 35.3, 59.7, 60.5, 130.1, 132.7, 134.2, 143.2, 154.9. Anal. Calcd for $C_{11}H_{17}NO_3Te$: C, 38.9; H, 5.1; N, 4.1. Found: C, 39.0; H, 5.0; N, 4.0.

The following compounds were analogously prepared starting from appropriately substituted alkyl pyridyl tellurides.

4,5-Bis(hydroxymethyl)-3-hydroxy-2-pyridyl Octyl Telluride (12b). White solid. Yield: 49%. Mp 92−94 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 0.90 (t, J = 7.0 Hz, 3H), 1.29−1.41 (several peaks, 10H), 1.83 (quintet, J = 7.3 Hz, 2H), 3.04 (t, J = 7.5 Hz, 2H), 4.57 (s, 2H), 4.92 (s, 2H), 7.99 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 7.2, 14.5, 23.7, 30.1, 30.4, 33.0, 33.1, 33.3, 59.7, 60.5, 130.0, 132.7, 134.2, 143.2, 154.9. Anal. Calcd for $C_{15}H_{25}NO_3Te$: C, 45.6; H, 6.4; N, 3.5. Found: C, 45.5; H, 6.4; N, 3.5.

4,5-Bis(hydroxymethyl)-3-hydroxy-2-pyridyl Hexadecylyl Telluride (12c). White solid. Yield: 43%. Mp 99−102 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 0.90 (t, J = 7.0 Hz, 3H), 1.24−1.43 (several peaks, 26H), 1.83 (quintet, J = 7.6 Hz, 2H), 3.04 (t, J = 7.5 Hz, 2H), 4.58 (s, 2H), 4.92 (s, 2H), 7.99 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 7.2, 14.5, 23.8, 30.2, 30.5, 30.6, 30.7, 30.8, 30.9, 33.0, 33.1, 33.3, 59.7, 60.5, 130.0, 132.6, 134.3, 143.3, 154.9. Anal. Calcd for $C_{23}H_{41}NO_3Te$: C, 54.5; H, 8.1; N, 2.8. Found: C, 54.6; H, 8.2; N, 2.7.

4,5-Bis(hydroxymethyl)-3-hydroxy-6-bromo-2-pyridyl Butyl Telluride (14a). White solid. Yield: 48%. Mp 137−140 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 0.96 (t, J = 7.5 Hz, 3H), 1.44 (sextet, J = 7.1 Hz, 2H), 1.85 (quintet, J = 7.5 Hz, 2H), 3.05 (t, J = 7.3 Hz, 2H), 4.73 (s, 2H), 4.96 (s, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 7.9, 13.9, 26.3, 35.2, 60.3, 61.5, 131.1, 133.2, 134.8, 136.0, 154.9. Anal. Calcd for $C_{11}H_{16}BrNO_3Te$: C, 31.6; H, 3.9; N, 3.3. Found: C, 31.8; H, 3.9; N, 3.4.

4,5-Bis(hydroxymethyl)-3-hydroxy-6-bromo-2-pyridyl Octyl Telluride (14b). White solid. Yield: 59%. Mp 128−131 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 0.90 (t, J = 6.4 Hz, 3H), 1.30−1.42 (several peaks, 10H), 1.86 (quintet, J = 7.2 Hz, 2H), 3.04 (t, J = 7.4 Hz, 2H), 4.73 (s, 2H), 4.96 (s, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 8.3, 14.5, 23.7, 30.1, 30.4, 33.0, 33.3, 60.3, 60.5, 131.1, 133.2, 134.9, 136.0, 154.9. Anal. Calcd for $C_{15}H_{24}BrNO_3Te$: C, 38.0; H, 5.1; N, 2.9. Found: C, 38.1; H, 5.1; N, 3.0.

4,5-Bis(hydroxymethyl)-3-hydroxy-6-bromo-2-pyridyl Hexadecyl Telluride (14c). White solid. Yield: 45%. Mp 115−118 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 0.90 (t, J = 6.8 Hz, 3H), 1.24−1.42 (several peaks, 26H), 1.86 (quintet, J = 7.6 Hz, 2H), 3.04 (t, J = 7.5 Hz, 2H), 4.73 (s, 2H), 4.96 (s, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 8.3, 14.5, 23.8, 30.2, 30.5, 30.7, 30.78, 30.82, 30.85 33.0, 33.1, 33.3, 60.3, 60.6, 131.1, 133.2, 134.9, 136.9, 154.9. Anal. Calcd for $C_{23}H_{40}BrNO_3Te$: C, 47.1; H, 6.9; N, 2.4. Found: C, 47.2; H, 6.9; N, 2.4.

Butyl 3-Hydroxy-4,5,6-tris(hydroxymethyl)-2-pyridyl Telluride (17a). The title compound was obtained as a yellow solid by column chromatography on silica gel eluted with pentane/ethyl acetate (20:80). Yield: 18%. Mp 106−109 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 0.95 (t, J = 7.3 Hz, 3H), 1.45 (sextet, J = 7.2 Hz, 2H), 1.86 (quintet, J = 7.4 Hz, 2H), 3.08 (t, J = 7.6 Hz, 2H), 4.66 (s, 2H), 4.75 (s, 2H), 4.96 (s, 2H). ^{13}C NMR (75 MHz, CD_3OD): δ 7.1, 13.9, 26.4, 35.4, 57.3, 59.7, 64.2, 129.9, 131.4, 132.4, 151.6, 154.4. Anal. Calcd for $C_{12}H_{19}NO_4Te$: C, 39.1; H, 5.2; N, 3.8. Found: C, 39.5; H, 5.2; N, 3.7.

Octyl 3-Hydroxy-4,5,6-tris(hydroxymethyl)-2-pyridyl Telluride (17b). The title compound was obtained as a yellow solid by column chromatography on silica gel eluted with pentane/ethyl acetate (20:80). Yield: 22%. Mp 84−86 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 0.90 (t, J = 7.0 Hz, 3H), 1.30−1.42 (several peaks, 10H), 1.87 (quintet, J = 7.2 Hz, 2 H), 3.08 (t, J = 7.3 Hz, 2H), 4.66 (s, 2H), 4.76 (s, 2H), 4.96 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 6.4, 13.5, 21.6, 27.9, 28.2, 30.8, 30.9, 31.3, 55.3, 56.3, 62.7, 129.8, 129.9, 130.7, 151.2, 151.3. Anal. Calcd for $C_{16}H_{27}NO_4Te$: C, 45.2; H, 6.4; N, 3.3. Found: C, 45.4; H, 6.4; N, 3.3.

Hexadecyl 3-Hydroxy-4,5,6-tris(hydroxymethyl)-2-pyridyl Telluride (17c). The title compound was obtained as a white solid by column chromatography on silica gel eluted with pentane/ethyl acetate (20:80). Yield: 14%. Mp 96−98 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 0.90 (t, J = 7.0 Hz, 3H), 1.24−1.43 (several peaks, 26H), 1.87 (quintet, J = 7.6 Hz, 2H), 3.07 (t, J = 7.5 Hz, 2H), 4.65 (s 2H), 4.76 (s, 2H), 4.96 (s, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 7.4, 14.5, 23.8, 30.2, 30.5, 30.4, 30.80, 30.82, 33.1, 33.3, 33.5, 57.3, 59.8, 64.3, 129.9, 131.3, 132.5, 151.6, 154.4. Anal. Calcd for $C_{24}H_{43}NO_4Te$: C, 53.7; H, 8.1; N, 2.6. Found: C, 53.8; H, 8.1; N, 2.5.

Typical Procedure for Acetonide Formation. 8-Butyltelluro-3,3-dimethyl[1,3]dioxepino[5,6-*c*]pyridin-9-ol (18a). To a stirred solution of compound **12a** (100 mg, 0.295 mmol) in acetone (4 mL) were added 2,2-dimethoxypropane (0.5 mL) and *p*-toluenesulfonic acid monohydrate (56 mg, 0.295 mmol), and stirring was continued for 20 h at room temperature. During this period the solution turned yellowish. After pouring into a saturated $NaHCO_3$ solution, the aqueous layer was extracted with $CHCl_3$ and dried over anhydrous Na_2SO_4. Removal of the solvent under vacuo and purification of the residue by silica gel column chromatography using pentane/ethyl acetate (90:10) as eluent afforded the title compound as a yellow solid. Yield: 70 mg (64%). Mp 84−87 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, J = 7.3 Hz, 3H), 1.37 (sextet, J = 7.1 Hz, 2H), 1.51 (s, 6H), 1.75 (quintet, J = 7.5 Hz, 2 H), 2.90 (t, J = 7.7 Hz, 2H), 4.83 (s, 2H), 4.93 (s, 2H), 6.21 (br s, 1H), 7.93 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.7, 13.5, 23.9, 25.2, 34.1, 60.3, 61.5, 102.8, 128.1, 131.6, 135.9, 141.4, 151.3. HRMS (TOF MS ES$^+$) m/z calcd for $C_{14}H_{21}NO_3Te$ $[M + H]^+$: 382.0662; found 382.0654.

The following compounds were analogously prepared starting from the appropriately substituted alkyl pyridyl telluride.

3,3-Dimethyl-8-octyltelluro[1,3]dioxepino[5,6-*c*]pyridin-9-ol (18b). Yellow solid. Yield: 63%. Mp 63−67 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, J = 7.0 Hz, 3H), 1.23−1.34 (several peaks, 10H), 1.51 (s, 6H), 1.75 (quintet, J = 7.6 Hz, 2H), 2.88 (t, J = 7.4 Hz, 2H), 4.82 (s, 2H), 4.92 (s, 2H), 6.15 (br s, 1H), 7.92 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.0, 14.3, 22.8, 23.9, 28.9, 29.3, 31.9, 32.0, 32.2, 60.3,61.5, 102.8, 128.2, 131.6, 135.9, 141.4, 151.3. Anal. Calcd for $C_{18}H_{29}NO_3Te$: C, 49.7; H, 6.7; N, 3.2. Found: C, 49.4; H, 6.7; N, 3.3.

3,3-Dimethyl-8-hexadecyltelluro[1,3]dioxepino[5,6-*c*]-pyridin-9-ol (18c). White solid. Yield: 54%. Mp 65−67 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, J = 7.0 Hz, 3H), 1.23−1.25 (several peaks, 26H), 1.51 (s, 6H), 1.75 (quintet, J = 7.6 Hz, 2H), 2.88 (t, J = 7.4 Hz, 2H), 4.82 (s, 2H), 4.92 (s, 2H), 6.08 (br s, 1H), 7.93 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.2, 14.3, 22.9, 23.9, 29.0, 29.6, 29.7, 29.8, 29.9, 32.0, 32.1, 60.4, 61.6, 102.8, 127.8, 131.4, 136.1, 141.4, 151.3. Anal. Calcd for $C_{26}H_{45}NO_3Te$: C, 57.1; H, 8.3; N, 2.6. Found: C, 57.1; H, 8.3; N, 2.5.

6-Bromo-8-butyltelluro-3,3-dimethyl[1,3]dioxepino[5,6-*c*]-pyridin-9-ol (20a). Yellow solid. Yield: 48%. Mp 62−64 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, J = 7.3 Hz, 3H), 1.37 (sextet, J = 7.2 Hz, 2H), 1.51 (s, H), 1.76 (quintet, J = 7.5 Hz, 2H), 2.93 (t, J = 7.5 Hz, 2H), 4.89 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.7, 13.5, 23.8, 25.1, 33.9, 59.8, 63.8, 102.7, 126.3, 131.7, 134.7, 135.5, 151.5. Anal. Calcd for $C_{14}H_{20}BrNO_3Te$: C, 36.7; H, 4.4; N, 3.1. Found: C, 37.0; H, 4.5; N, 3.2.

6-Bromo-3,3-dimethyl-8-octyltelluro[1,3]dioxepino[5,6-*c*]-pyridin-9-ol (20b). Orange semisolid. Yield: 65%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, J = 7.0 Hz, 3H), 1.24−1.40 (several peaks, 10H), 1.50 (s, 6H), 1.78 (quintet, J = 7.6 Hz, 2H), 2.93 (t, J = 7.6 Hz, 2 H), 4.90 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.0, 14.3, 22.8, 23.8, 28.9, 29.3, 31.90, 31.96, 31.99, 59.8, 63.8, 102.6, 126.4, 131.7, 134.7, 135.5, 151.6. Anal. Calcd for $C_{18}H_{28}BrNO_3Te$: C, 42.1; H, 5.5; N, 2.7. Found: 42.3; H, 5.6; N, 2.8.

6-Bromo-3,3-dimethyl-8-hexadecyltelluro[1,3]dioxepino-[5,6-*c*]pyridin-9-ol (20c). White solid. Yield: 47%. Mp 68−70 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (t, J = 7.0 Hz, 3H), 1.25−1.30 (several peaks, 26H), 1.50 (s, 6H), 1.78 (quintet, J = 7.7 Hz, 2H), 2.93 (t, J = 7.5 Hz, 2H), 4.89 (s, 4H), 5.98 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 14.3, 22.9, 23.8, 29.0, 29.5, 29.6, 29.7, 29.8, 29.9, 31.9, 32.0, 32.1, 59.8, 63.8, 102.6, 126.3, 131.7, 134.6, 135.6, 151.5. Anal. Calcd for $C_{26}H_{44}BrNO_3Te$: C, 49.9; H, 7.1; N, 2.2. Found: C, 50.1; H, 7.2; N, 2.3.

6-Bromo-8-butyltelluro-5-hydroxymethyl-4*H*-1,3-dioxino-[4,5-*c*]pyridine (19a). Yellow semisolid. Yield: 52%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (t, J = 7.4 Hz, 3H), 1.44 (sextet, J = 7.1 Hz, 2H), 1.54 (s, 6H), 1.87 (quintet, J = 7.3 Hz, 2H), 3.12 (t, J = 7.5 Hz, 2H), 4.65

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(d, J = 3.1 Hz, 2H), 4.92 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 8.5, 13.7, 24.7, 25.4, 33.9, 59.1, 61.2, 101.3, 126.4, 127.5, 133.8, 134.7, 149.3. Anal. Calcd for $C_{14}H_{20}BrNO_3Te$: C, 36.7; H, 4.4; N, 3.1. Found: C, 37.0; H, 4.5; N, 3.1.

6-Bromo-5-hydroxymethyl-8-octyltelluro-4*H*-1,3-dioxino-[4,5-*c*]pyridine (19b). Yellow solid. Yield: 58%. Mp 58−60 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, J = 6.9 Hz, 3H), 1.27−1.43 (several peaks, 10H), 1.54 (s, 6H), 1.88 (quintet, J = 7.5 Hz, 2H), 3.10 (t, J = 7.6 Hz, 2H), 4.64 (s, 2H), 4.91 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 8.9, 14.3, 22.8, 24.7, 29.1, 29.4, 31.7, 32.0, 32.4, 59.1, 61.1, 101.2, 126.4, 127.5, 133.8, 134.7, 149.3. Anal. Calcd for $C_{18}H_{28}BrNO_3Te$: C, 42.1; H, 5.5; N, 2.7. Found: 42.3; H, 5.5; N, 2.7.

6-Bromo-8-hexadecyltelluro-5-hydroxymethyl-4*H*-1,3-dioxino[4,5-*c*]pyridine (19c). Light yellow solid. Yield: 15%. Mp 68−70 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, J = 7.0 Hz, 3H), 1.26−1.43 (several peaks, 26H), 1.55 (s, 6H), 1.88 (quintet, J = 7.5 Hz, 2H), 3.11 (t, J = 7.5 Hz, 2H), 4.65 (d, J = 3.1 Hz, 2H), 4.92 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 8.9, 14.3, 22.9, 24.8, 29.2, 29.6, 29.7, 29.8, 29.9, 31.8, 32.1, 32.4, 59.1, 61.2, 101.3, 126.4, 127.5, 133.8, 134.7, 149.3. HRMS (TOF MS ES$^+$) m/z calcd for $C_{26}H_{44}BrNO_3Te$ $[M + H]^+$: 628.1645; found 628.1647.

HPLC Peroxidation Assay. The experimental setup for recording inhibition times (T_{inh}) and inhibited rates of peroxidation (R_{inh}) during azo-initiated peroxidation of linoleic acid in a two-phase system was recently described.[35] Values reported in Table 1 for reactions performed in the presence of NAC are means ± SD based on triplicates.

Coupled Reductase Assay. The GPx-like activities of novel organotellurium compounds prepared were determined by UV spectroscopy following the protocol by Wilson[27] with slight modifications. The test mixture contained GSH (1 mM), ethylene diamine tetra acetate (EDTA, 1 mM), glutathione reductase (GR) (1 unit/mL), and β-nicotinamide adenine dinucleotide phosphate (NADPH, 0.2 mM) in potassium phosphate buffer (100 mM), pH 7.5. Catalysts (20 μM) were added to the test mixture at 21 °C, and the reaction was initiated by addition of H_2O_2 (0.8 mM). Initial reaction rates were based on the consumption of NADPH as assessed by UV spectroscopy at 340 nm. The initial reduction rate was determined at least 3−4 times and calculated from the first 10 s of reaction by using 6.22 mM^{-1} cm^{-1} as the extinction coefficient for NADPH. GPx data reported in Table 1 are means ± SD.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR data for all new compounds prepared. Results from kinetic studies of compounds prepared (maximum velocities, Lineweaver −Burk plots, control experiments, consumption of hydrogen peroxide in long-lime experiments). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: Lars.Engman@kemi.uu.se.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) NIH, Office of dietary supplements. Dietary Supplement Fact Sheet: Vitamin B6 (2013-01-10). http://ods.od.nih.gov/factsheets/VitaminB6-HealthProfessional/.

(2) John, R. A. *Biochim. Biophys. Acta* **1995**, *1248*, 81−96.

(3) Sharif, S.; Schagen, D.; Toney, M. D.; Limbach, H.-H. *J. Am. Chem. Soc.* **2007**, *129*, 4440−4455.

(4) Griswold, W. R.; Toney, M. D. *J. Am. Chem. Soc.* **2011**, *133*, 14823−14830.

(5) Combs, G. F., Jr. *The Vitamins: Fundamental Aspects in Nutrition and Health*, 4th ed.; Elsevier: Amsterdam, 2012.

(6) Murakami, Y.; Kikuchi, J.; Hisaeda, Y.; Hayashida, O. *Chem. Rev.* **1996**, *96*, 721−758.

(7) Liu, L.; Breslow, R. *Bioorg. Med. Chem.* **2004**, *12*, 3277−3287.

(8) See for example: (a) Kim, Y.-C.; Brown, S. G.; Harden, T. K.; Boyer, J. L.; Dubyak, G.; King, B. F.; Burnstock, G.; Jacobson, K. A. *J. Med. Chem.* **2001**, *44*, 340−349. (b) Pham, V.; Zhang, W.; Chen, V.; Whitney, T.; Yao, J.; Froese, D.; Friesen, A. D.; Diakur, J. M.; Haque, W. *J. Med. Chem.* **2003**, *46*, 3680−3687. (c) Kim, Y.-C.; Lee, J.-S.; Sak, K.; Marteau, F.; Mamedova, L.; Boeynaems, J.-M.; Jacobson, K. A. *Biochem. Pharmacol.* **2005**, *70*, 266−274. (d) Zhang, D.-H.; Bai, S.; Dong, X.-Y.; Sun, Y. *J. Agric. Food Chem.* **2007**, *55*, 4526−4531. (e) Shtyrlin, N. V.; Pavelyev, R. S.; Pugachev, M. V.; Sysoeva, L. P.; Musin, R. Z.; Shtyrlin, Y. G. *Tetrahedron Lett.* **2012**, *53*, 3967−3970. (f) Yu, J.-X.; Cui, W.; Bourke, V. A.; Mason, R. P. *J. Med. Chem.* **2012**, *55*, 6814−6821.

(9) Stocker, P.; Lesgards, J.-F.; Vidal, N.; Chalier, F.; Prost, M. *Biochim. Biophys. Acta* **2003**, *1621*, 1−8.

(10) Matxain, J. M.; Ristilä, M.; Strid, Å.; Eriksson, L. A. *J. Phys. Chem. A* **2006**, *110*, 13068−13072.

(11) Natera, J.; Massad, W.; Garcia, N. A. *Photochem. Photobiol. Sci.* **2012**, *11*, 938−945.

(12) Jain, S. K.; Lim, G. *Free Radical Biol. Med.* **2001**, *30*, 232−237.

(13) Matxain, J. M.; Padro, D.; Ristilä, M.; Strid, Å.; Eriksson, L. A. *J. Phys. Chem. B* **2009**, *113*, 9629−9632.

(14) (a) Osmani, A. H.; May, G. S.; Osmani, S. A. *J. Biol. Chem.* **1999**, *274*, 23565−23569. (b) Ehrenshaft, M.; Bilski, P.; Li, M. Y.; Chignell, C. F.; Daub, M. E. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9374−9378. (c) Matxain, J. M.; Ristilä, M.; Strid, Å.; Eriksson, L. A. *Chem.—Eur. J.* **2007**, *13*, 4636−4642.

(15) (a) Bilski, P.; Li, M. Y.; Ehrenshaft, M.; Daub, M. E.; Chignell, C. F. *Photochem. Photobiol.* **2000**, *71*, 129−134. (b) See also: Ohta, B. K.; Foote, C. S. *J. Am. Chem. Soc.* **2002**, *124*, 12064−12065.

(16) (a) Wijtmans, M.; Pratt, D. A.; Valgimigli, L.; DiLabio, G. A.; Pedulli, G. F.; Porter, N. A. *Angew. Chem., Int. Ed.* **2003**, *42*, 4370−4373. (b) Wijtmans, M.; Pratt, D. A.; Brinkhorst, J.; Serwa, R.; Valgimigli, L.; Pedulli, G. F.; Porter, N. A. *J. Org. Chem.* **2004**, *69*, 9215−9223. (c) Nam, T.; Rector, C. L.; Kim, H.; Sonnen, A. F.-P.; Meyer, R.; Nau, W. M.; Atkinson, J.; Rintoul, J.; Pratt, D. A.; Porter, N. A. *J. Am. Chem. Soc.* **2007**, *129*, 10211−10219. (d) Serwa, R.; Nam, T.; Valgimigli, L.; Culbertson, S.; Rector, C. L.; Jeong, B.-S.; Pratt, D. A.; Porter, N. A. *Chem.—Eur. J.* **2010**, *16*, 14106−14114. (e) Lu, J.; Cai, X.; Hecht, S. M. *Org. Lett.* **2010**, *12*, 5189−5191.

(17) (a) Culbertson, S. M.; Enright, G. D.; Ingold, K. I. *Org. Lett.* **2003**, *5*, 2659−2662. (b) Culbertson, S. M.; Vassilenko, E. I.; Morrison, L. D.; Ingold, K. I. *J. Biol. Chem.* **2003**, *278*, 38384−38394.

(18) Compounds **4a** and **4b** were found to retard allophycocyanin (APC) fluorescence as efficiently as Trolox in the presence of peroxyl radicals.

(19) (a) Amorati, R.; Pedulli, G. F.; Valgimigli, L.; Johansson, H.; Engman, L. *Org. Lett.* **2010**, *12*, 2326−2329. (b) Johansson, H.; Shanks, D.; Engman, L.; Amorati, R.; Pedulli, G. F.; Valgimigli, L. *J. Org. Chem.* **2010**, *75*, 7535−7541.

(20) (a) Kumar, S.; Johansson, H.; Kanda, T.; Engman, L.; Müller, T.; Jonsson, M.; Pedulli, G. F.; Petrucci, S.; Valgimigli, L. *Org. Lett.* **2008**, *10*, 4895−4898. (b) Kumar, S.; Johansson, H.; Kanda, T.; Engman, L.; Müller, T.; Bergenudd, H.; Jonsson, M.; Pedulli, G. F.; Amorat, R.; Valgimigli, L. *J. Org. Chem.* **2010**, *75*, 716−725.

(21) (a) Kondratjeva, G. Ya. *Khim. Nauka Prom-st.* **1957**, *2*, 666. (b) Harris, E. E.; Firestone, R. A.; Pfister, K., 3rd; Boettcher, R. R.; Cross, F. J.; Currie, R. B.; Monaco, M.; Peterson, E. R.; Reuter, W. *J. Am. Chem. Soc.* **1962**, *27*, 2705−2706.

(22) (a) Maeda, I.; Takehara, M.; Togo, K.; Asai, S.; Yoshida, R. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 1435−1437. (b) Kozikowski, A. P.; Isobe, K. *Heterocycles* **1978**, *9*, 1271−1275.

(23) (a) Thomsen, I.; Torsell, K. B. G. *Acta Chem. Scand.* **1991**, *45*, 539−542. (b) Massa, M. A.; Patt, W. C.; Ahn, K.; Sisneros, A. M.; Herman, S. B.; Doherty, A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2117−2122.
(24) (a) Korytnyk, W.; Angelino, N. *J. Med. Chem.* **1977**, *20*, 745−749. (b) Doktorova, N. D.; Ionova, L. V.; Karpeisky, M. Ya.; Padyukova, N. Sh.; Turchin, K. F.;V.; Florentiev, V. L. *Tetrahedron* **1969**, *25*, 3527−3553.
(25) Vessman, K.; Ekström, M.; Berglund, M.; Andersson, C.-M.; Engman, L. *J. Org. Chem.* **1995**, *60*, 4461−4467.
(26) (a) Kumar, S.; Johansson, H.; Engman, L.; Valgimigli, L.; Amorati, R.; Fumo, M. G.; Pedulli, G. F. *J. Org. Chem.* **2007**, *72*, 2583−2595. (b) Kumar, S.; Engman, L.; Valgimigli, L.; Amorati, R.; Fumo, M. G.; Pedulli, G. F. *J. Org. Chem.* **2007**, *72*, 6046−6055.
(27) (a) Wilson, S. R.; Zucker, P. A.; Huang, R.-R. C.; Spector, A. *J. Am. Chem. Soc.* **1989**, *11*, 5936−5939. (b) The method is based on a procedure described by Wendel: Wendel, A. *Methods Enzymol.* **1981**, *77*, 325−333.
(28) Engman, L.; Stern, D.; Pelcman, M. *J. Org. Chem.* **1994**, *59*, 1973−1979.
(29) Bhabak, K. P.; Mugesh, G. *Acc. Chem. Res.* **2010**, *43*, 1408−1419.
(30) Engman, L.; Persson, J.; Merényi, G.; Lind, J. *Organometallics* **1995**, *14*, 3641−3648.
(31) (a) Nogueira, C. W.; Zeni, G.; Rocha, J. B. T. *Chem. Rev.* **2004**, *104*, 6255−6285. (b) Ba, L. A.; Döring, M.; Jamier, V.; Jacob, C. *Org. Biomol. Chem.* **2010**, *8*, 4203−4216.
(32) Engman, L.; Cava, M. P. *Synth. Commun.* **1982**, *12*, 163−165.
(33) Li, Y.; Silverton, L. C.; Haasch, R.; Tong, Y. Y. *Langmuir* **2008**, *24*, 7048−7053.
(34) Nakamura, T.; Miyamae, T.; Nakai, I.; Kondoh, H.; Kawamoto, T.; Kobayashi, N.; Yasuda, S.; Yoshimura, D.; Ohta, T.; Nozoye, H.; Matsumoto, M. *Langmuir* **2005**, *21*, 3344−3353.
(35) (a) Shanks, D.; Amorati, R.; Fumo, M. G.; Pedulli, G. F.; Valgimigli, L.; Engman, L. *J. Org. Chem.* **2006**, *71*, 1033−1038. (b) Kumar, S.; Johansson, H.; Engman, L.; Valgimigli, L.; Amorati, R.; Fumo, M. G.; Pedulli, G. F. *J. Org. Chem.* **2007**, *72*, 2583−2595.