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Synthesis and Antioxidant Capacity of Novel Stable 5-Tellurofuranose Derivatives

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Novel stable tellurium-containing carbohydrate derivatives are prepared from hexitols and pentitols through a double nucleophilic substitution with NaHTe in PEG-400. These tellurosugars react at very high rates with two-electron oxidants, including hypochlorous and peroxynitrous acid, formed at sites of inflammation, and show considerable promise as protective agents against oxidative damage.

Previous studies have shown that selenocysteine plays a fundamental role as a component in the the active site of a number of important protective enzymes in mammalian cells, including glutathione peroxidases (GPx), thioredoxin reductases (TrxR) and some methionine sulfoxide reductases (MSRs).[1, 2] GPx, together with its co-factor glutathione (GSH), catalyzes the reduction of potentially damaging H_2O_2 and hydroperoxides.[3] TxrR and its protein partner, thioredoxin, are key components of cellular reducing systems that maintain protein thiols in a reduced active form.[2, 4] The family of MSRs, together with thioredoxin as a source of reducing equivalents, reduce methionine sulfoxide residues formed on peptides and proteins, back to native methionine (Met) residues, thereby protecting proteins against irreversible damage and loss of activity.[5] In light of these data, a considerable number of low-molecular-mass selenium species have been synthesized and tested as GPx mimetics and direct antioxidants, with Ebselen being the most widely studied.[6] Many of the selenium species examined, including mono- and di-selenides, have low aqueous solubilities that hinder their biological use, although we (and others) have recently established synthetic routes that allow the incorporation of selenium in to carbohydrates and related structures, affording highly-water soluble species.[7-10] These materials are scavengers of a wide range of biological oxidants including those generated by the inflammatory enzyme myeloperoxidase (hypochlorous acid, HOCl; hypobromous acid, HOBr; hypothiocyanous acid, HOSCN), peroxynitrous acid (ONOOH), peroxides (H_2O_2, ROOH) and singlet oxygen ($^1O_2$), with second order rate constants, k_2, 10–100 fold higher than their sulfur analogues.[7,8,11] Furthermore, these materials protect human plasma proteins against oxidative damage,[11] and strongly promote healing of gastric ulcers and skin wounds in animal models.[12, 13]

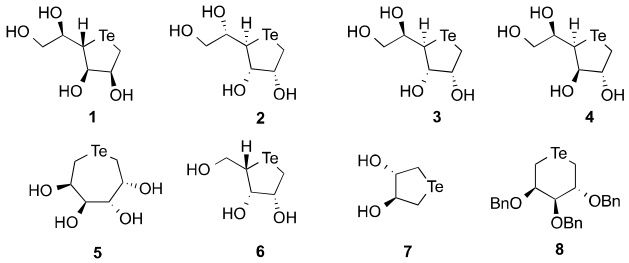

Figure 1. Te-carbohydrates.

Organotellurides have been shown to react two-to-three orders of magnitude more rapidly with free radicals than their selenium counterparts,[14, 15] and water-soluble organotellurides exhibit glutathione and thiol peroxidase activities,[16] however their reactivities with oxidants such as HOCl and ONOOH are unknown. In the light of the potential potent biological activities of tellurium-containing carbohydrates, such as **1** (Figure 1), we expected these tellurium species to exhibit superior oxidant scavenging activities than their selenium counterparts. Indeed, isolated reports on the reactivities of diaryl tellurides toward oxidants lends support to this hypothesis, with some previously synthesised materials exhibiting potent GPx activity,[17-19] effects on mitochondria,[20] redox sensitivity,[20, 21] rapid reaction with ONOOH and other oxidants (though absolute kinetic data were not established),[23-26] and are potent inhibitory agents against lipid peroxidation.[27, 28] These compounds are generally poorly water

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Electronic Supplementary Information (ESI) available: Experimental procedures for new compounds; ^{1}H and ^{13}C NMR spectra of compounds **1** to **7**. See DOI: 10.1039/x0xx00000x

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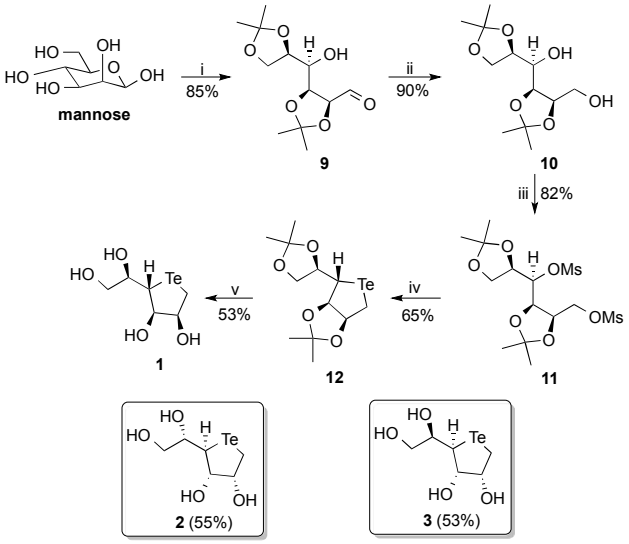
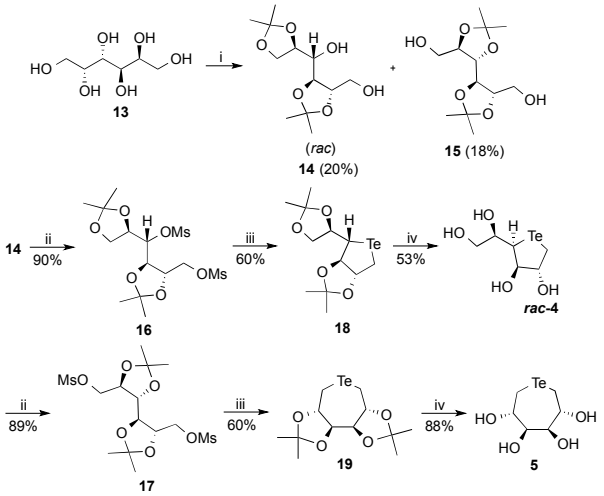
soluble, limiting their use in aqueous systems. Water-soluble cyclodextrin species bearing tellurium substituents have been reported, with these showing potent GPx activities, as well as a capacity to inhibit thioredoxin reductase activity at nanomolar concentrations, and cancer cell growth, with IC_{50} values in the low micromolar range.[29] Despite these positive data, and the potent biological activity of the corresponding selenium-species, there are no reports to date on the synthesis of stable (ring) tellurium-modified carbohydrates, or their oxidant scavenging activities; however, benzyl-protected telluropyranose derivatives (e.g., **8**) have been reported, although these could not be deprotected.[30]

We report here the synthesis and oxidant scavenging activity of carbohydrate derivatives **1**–**7** that are also cyclic tellurides (Figure 1). These novel and stable[§] compounds exhibit very high oxidant scavenging capacities, with some of the highest known rate constants for reaction with HOCl and ONOOH.



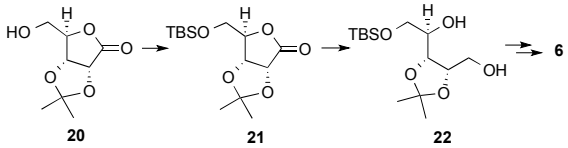
Scheme 1. Reagents and conditions: (i) *p*-TSA, 2,2-dimethoxypropane, Me_2CO; (ii) $NaBH_4$, MeOH; (iii) Et_3N, MsCl, CH_2Cl_2; (iv) Te, $NaBH_4$, PEG-400; (v) TFA, H_2O, CH_2Cl_2.

Starting from commercially available carbohydrates, a number of tellurosugar analogues with varying stereochemistries and functionalities were prepared. Accordingly, D-mannose underwent reaction with 2,2-dimethoxypropane in the presence of catalytic *p*-toluenesulfonic acid and acetone under anhydrous conditions to afford the protected D-mannose **9** (85% yield) (Scheme 1). The extracted product **9** was recrystallized and reacted with sodium borohydride ($NaBH_4$) in methanol to give diol **10** (90% yield), which was converted into the corresponding dimesylate **11** (82% yield), by reaction with methanesulfonyl chloride (MsCl) in dichloromethane (CH_2Cl_2) and triethylamine. Separately, the nucleophilic tellurium species (NaHTe) was generated *in situ* by the reaction of elemental tellurium with $NaBH_4$ in EtOH, after which it was added to dimesylate **11** with stirring for 1 h at 70 °C, however the desired product **12** was not obtained. Decreasing the reaction temperature to 55 °C, did however give **12** in 10% yield after 2h. Repetition of this reaction in polyethylene glycol (PEG-400) instead of EtOH under the same conditions, gave **12** in 65% yield after 1 h, suggesting that PEG-400 is important for this transformation.[‡] The cyclic telluride **12** was deprotected by treatment with catalytic trifluoroacetic acid (TFA) and H_2O in CH_2Cl_2 at room temperature for 1 h, followed by purification by silica-gel flash chromatography to afford 1,4-dideoxy-D-tellurotalitol (**1**) as a yellow solid in 53% isolated yield (Scheme 1). The synthetic pathway described above was extended to other carbohydrates. 1,4-Dideoxy-L-tellurotalitol (**2**), the enantiomer of **1**, was obtained in 55% yield, from L-mannose (Scheme 1) however **3**, which was obtained in 53% crude yield from D-gulonic acid-1,4-lactone, proved to be unstable and unsuitable for further evaluation.



Scheme 2. Reagents and conditions: (i) H_2SO_4, $CuSO_4$, Me_2CO; (ii) Et_3N, MsCl, CH_2Cl_2; (iii) Te, $NaBH_4$, PEG-400; (iv) TFA, H_2O, CH_2Cl_2.

In a complementary procedure, galactitol (**13**) underwent reaction with acetone in the presence of anhydrous $CuSO_4$ under acidic conditions to afford a mixture of racemic and meso diols, **14** and **15**, respectively, following a literature procedure (Scheme 2);[31] **14** and **15** were separated by fractional recrystallization and converted to the corresponding dimesylates **16** and **17** (90% and 89% yields respectively). Dimesylates **16** and **17** were reacted with NaHTe (as above), to afford the cyclic tellurides **18** and **19** in 60% yield. Deprotection with TFA and H_2O in CH_2Cl_2 and purification by flash chromatography led to decomposition of *rac*-**4**, however **5** was obtained as a crystalline, hygroscopic orange solid.



Scheme 3. Preparation of 1,4-dideoxy-L-tellurolyxitol (**6**).

We also explored the effect of the length of the side-chain attached to C-4 of the cyclic telluride by preparing 1,4-dideoxy-L-tellurolyxitol (**6**), which was prepared from 2,3-isopropylidene-D-ribonic acid 1,4-lactone (**20**) as the starting carbohydrate (Scheme 3). The free alcohol was first protected with *tert*-butyldimethylsilyl chloride (TBSCl) to afford the silane

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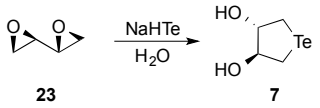
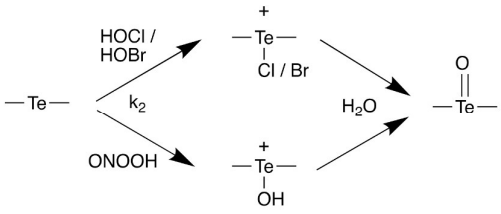
21 in almost quantitative yield. The protected lactone **21** was reduced to diol **22** with $NaBH_4$ (82% yield), after which it was converted into tellurolyxitol **6** using similar procedures to those described above; **6** was isolated as a yellow solid in 45% yield; further details are found in the ESI.



23 **7**

Scheme 4. Preparation of D,L-*trans*-3,4-dihydroxy-1-tellurolane.

For comparison purposes, racemic D,L-*trans*-3,4-dihydroxy-1-tellurolane (**7**) was synthesized by the addition of an aqueous solution of NaHTe to 1,3-butadiene bisepoxide (**23**), as a yellow solid in 98% yield after 1 h (Scheme 4). To the best of our knowledge, *trans*-dihydroxytellurolane **7** appears to be novel, and there are no literature reports on the use of this protocol. Indeed, Klayman reported that NaHTe reacts very slowly in water and affords no product.[32] To overcome this potential problem, we directly added an excess of $NaBH_4$ to the reaction mixture, which resulted in an increase in reactivity and product yield. It should be noted that **7** is the tellurium analogue of DHS_{red}, a known biological antioxidant.[12, 29] It is interesting to note that 1,4-dideoxy-L-tellurolyxitol (**6**) and the 1,4-dideoxytellurotalitol isomers (**1**, **2**) were inert, isolable solids, while **3** and **4** were more labile. We are unable to provide an explanation for these observations. The telluropane **5**, on the other hand, is stabilised through the larger ring that can better accommodate the longer C-Te bond distances.



Scheme 5. Proposed mechanism of reaction of oxidants with Te-sugars. Initial fast reaction with the oxidant (with second order rate constant, k_2) is followed by reaction with H_2O to give the corresponding telluroxide.

Scavenging of the biologically important oxidants HOCl and HOBr by the Te-compounds (Scheme 5), was investigated using a competition kinetics approach in which oxidation of Fmoc-methionine (FmocMet) to Fmoc-methionine sulfoxide (FmocMetSO) was used as a reference reaction, with the second order rate constant for this reference reaction, k_2 taken as 1.5×10^8 M^{-1} s^{-1}.[33] Representative scavenger plots (for further details see the ESI) are shown in Figure 2A, and the resulting k_2 values are collected in Table 1. Rate constants for reaction of ONOOH with the Te-compounds were determined by stopped-flow spectroscopy, by monitoring the decay of ONOOH at 302 nm in the absence and presence of added Te compound as described previously,[11] with the resulting pseudo-first-order rate constants (k_{obs}) subsequently plotted against the Te-sugar concentration, to give k_2 (Figure 2B, Table 1).

The k_2 values obtained indicate that these novel Te-sugars are potent oxidant scavengers. The simplest structures **7** and **6** exhibit the fastest rate constants for reaction with HOCl, with values comparable to those determined previously for the free amino acid Cys (k_2 ~ 3.2×10^8 $M^{-1}s^{-1}$) and other thiols,[33] and of the same magnitude as the analogous selenosugars,[8] but faster, in general, than those of thioethers (e.g. Met; k_2 ~ 3.4×10^7 $M^{-1}s^{-1}$ [33]). As expected the two stereoisomeric Te-sugars gave values that were not statistically different from each other. The rate constants determined for HOBr are faster than those determined for HOCl which is in contrast to the data reported for the selenium analogues,[8] but in line with the enhanced reactivity of some amino acid side chains with HOBr compared to HOCl (cf. previously reported data[34-36]).

Table 1. Second order rate constants, k_2, at 22° C and pH 7.4 (in 10 mM phosphate buffer for HOCl and HOBr, 100 mM phosphate buffer for ONOOH) for reaction of the oxidants HOCl, HOBr, and ONOOH with the Te sugars. For further details, see the ESI.

Compound	$k_{2\ HOCl}$, $M^{-1}s^{-1}$	$k_{2\ HOBr}$, $M^{-1}s^{-1}$	$k_{2\ ONOOH}$, $M^{-1}s^{-1}$
7	$(2.0\pm0.4) \times 10^8$	$(1.1\pm0.3) \times 10^8$	$(1.6\pm0.3) \times 10^5$
1	$(5.2\pm0.6) \times 10^7$	$(4.7\pm0.7) \times 10^8$	-
2	$(6.0\pm0.7) \times 10^7$	$(2.5\pm0.7) \times 10^8$	$(1.5\pm0.3) \times 10^4$
5	$(2.6\pm0.1) \times 10^7$	$(5.4\pm1.3) \times 10^8$	$(5.3\pm0.4) \times 10^3$
6	$(1.5\pm0.1) \times 10^8$	$(2.6\pm0.2) \times 10^9$	$(4.1\pm0.2) \times 10^4$

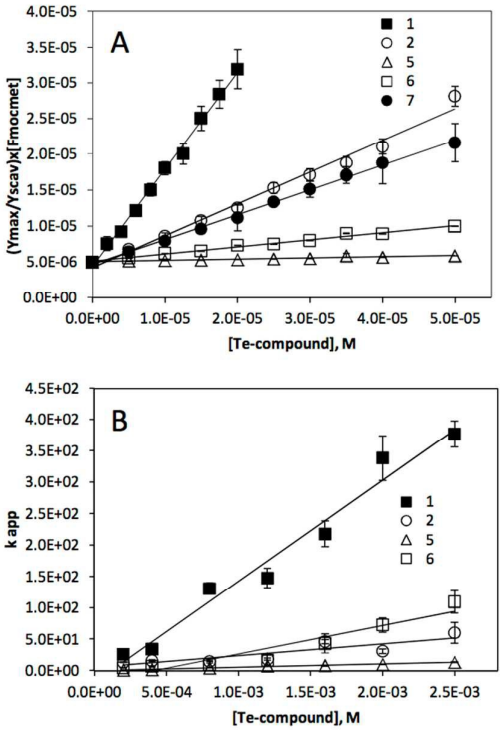


Figure 2. Kinetic analysis of: (A) the reaction of HOCl with Te-compounds determined by competition kinetics with FmocMet with UPLC analysis, and (B) for reaction with ONOOH as determined from direct kinetic determination of k_{obs} by fitting of exponential decay curves to the experimental data for ONOOH decay at 302 nm in the absence and presence of the added Te compounds.

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In the case of peroxynitrous acid (ONOOH), **7** was the most efficient scavenger, with the seven-membered ring **5** exhibiting the slowest rate constant among the Te-sugars examined. The variation between the highest and lowest values for these Te-sugars is approximately 30-fold. The reasons for this much larger variation compared to the data for HOCl and HOBr, is unclear at present, and warrants further investigation. These rate constants for reactions of the Te sugars with ONOOH are significantly higher than those for reaction with the sulfur-containing amino acids Cys and Met, which have k_2 values in the 10^2–10^3 $M^{-1}s^{-1}$ range, and other protein side-chains (e.g., Trp[11] and references therein), and also significantly greater than for the corresponding seleno sugars which have k_2 values of ~2.5 x 10^3 $M^{-1}s^{-1}$.[11] These data therefore indicate that some of these Te sugars (e.g., **7**) are efficient scavengers of ONOOH, and should be effective protective agents against damage induced by this oxidant.

In summary, we herein report the synthesis and potent oxidant scavenging activity of a family of novel, stable, water soluble tellurium-containing sugars. These materials show very high reactivities toward biologically important two-electron oxidants, with the overall order of oxidant reactivity being HOBr > HOCl > ONOOH.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

‡ In our experience, NaHTe is very sensitive to trace amounts of O_2, much more so than NaHSe. PEG-400 generally affords higher yields than EtOH/THF, which we ascribe to the lower solubility of adventitious O_2.

§ Melting points for **1**–**7** could not be determined as they decompose above 80 °C. However, **1**, **2**, **5**–**7** are stable solids that were shipped from Melbourne to Copenhagen without special precautions. The compounds were stored at -20 °C with minimal exposure to light, and were unchanged over one year.

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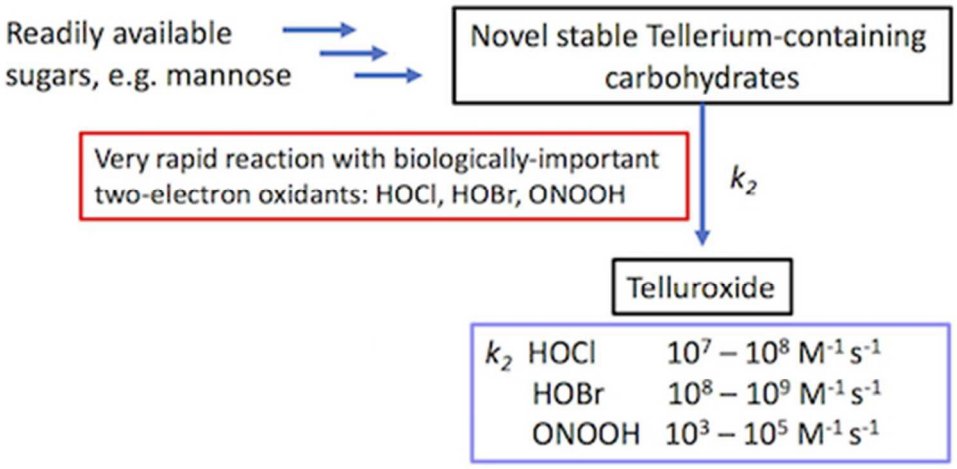

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TOC entry

Novel stable tellurium-containing carbohydrates are prepared; these react very rapidly with two-electron oxidants and show promise as protective agents.