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Organotellurium scaffolds for mass cytometry reagent development†

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Mass cytometry (MC) is a powerful tool for studying heterogeneous cell populations. In previous work, our laboratory has developed an MC probe for hypoxia bearing a methyl telluride mass tag. The methyl telluride was unoptimized, displaying stability and toxicity limitations. Here, we investigate three classes of organotelluriums as MC mass tags: methyl tellurides, trifluoromethyl tellurides and 2-alkyl-tellurophenes. NMR was used to compare the stability of these compounds in aqueous and organic solutions and the compounds were analysed for toxicity in Jurkat cells. The methyl tellurides were moderately stable to aerobic oxidation in organic solution under dry ambient conditions. The trifluoromethyl tellurides were stable to aerobic oxidation in organic solution but decomposed in aqueous solution. The 2-alkyl-tellurophenes proved to be stable in both organic and aqueous solutions under ambient conditions and showed limited toxicity (IC_{50} > 200 μM) in cell based assays. The synthetic feasibility, chemically stability, and limited toxicity of tellurophenes suggests these groups will be good choices for MC reagent development.

Introduction

Characterization of single cells in tissue samples requires a highly parameterized assay.[1] Fluorescence-based flow cytometry (FC) has been the method of choice to study heterogeneous cell populations as it allows for 5–10 parameters to be routinely analyzed.[2] However, FC cannot be used for highly parameterized assays (>20 parameters) due to the spectral overlap of the fluorophores used for analyte detection.[3] A solution to this problem is to substitute the optical detection and fluorescently tagged antibodies in FC for mass detection with an inductively-coupled plasma mass spectrometer (ICP-MS) and isotope-tagged antibodies. This technology, known as mass cytometry (MC), is capable of detecting numerous bioorthogonal isotopes (theoretically > 100) with single mass unit resolution over multiple orders of magnitude.[1] MC allows experiments analogous to flow cytometry but with significantly greater parameterization. MC has been used to detect and quantify 34 cellular parameters simultaneously to reveal the drug response across a human hematopoietic continuum.[4]

MC experiments can be done by using commercially available reagents which are composed of antibodies labeled with metal chelating polymers that bind a range of high molecular weight metal isotopes, usually lanthanides. We are interested in developing novel MC probes that can be used to assay cellular biochemistry. Fig. 1 depicts the general requirements of an MC probe. Ideally, the mass tag should be accessible in a high yielding synthesis amenable to isotope incorporation, be stable under biologically-relevant conditions and have low toxicity. Recently we reported an MC-probe (Telox) for measuring cellular hypoxia.[5] This probe used a 2-nitroimidazole as the activity group for hypoxic-specific labelling and a methyl telluroether functionality as mass tag for MC detection.[5] Tellurium was chosen to be the element for detection as it is known to form stable bonds with carbon and it has eight naturally occurring isotopes that can be accessed to generate a series of uniquely identifiable, biologically indistinguishable MC probes using the same chemistry.

Although Telox demonstrated an important concept, the telluroether functionality was unoptimized, having moderate

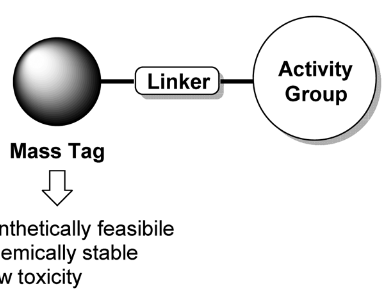

Fig. 1 The general requirements and design of a mass cytometry probe.

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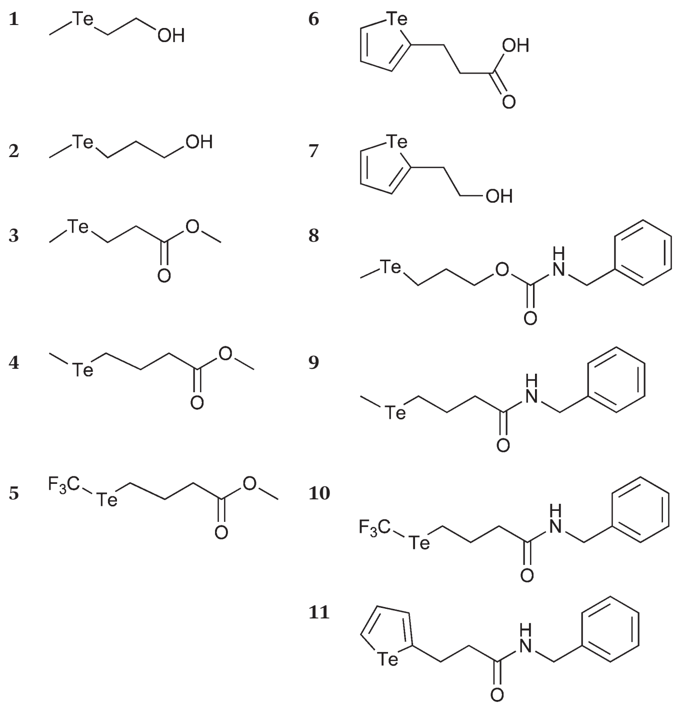
stability and a metabolic LD_{50} value close to the required assay concentration. These issues have motivated our laboratory to investigate alternative organotellurium moieties as mass tags for future activity-based probes.

The first organotellurium compound was synthesized by Wöhler in 1840.[6] Increasingly organotellurium compounds are being investigated in living systems, although this area of research remains underdeveloped.[7,8] Tellurium has no known biological role in prokaryotic or eukaryotic cells. In biological systems, tellurium metabolism is poorly understood, however it is presumed to follow the metabolic pathway of its analogue, selenium. Microorganisms have been found to methylate inorganic tellurium to volatile or ionic species for excretion. Experimental evidence of this process is scarce due to the instability of the metabolites.[7] However the number reports of cellular studies involving aryl, vinylic, alkynyl and alkyl telluroethers in biological systems are increasing.[9–14] The majority of this research has been based upon the ability of aryl telluroethers to mimic glutathione peroxidase activity providing, in some cases, resistance to oxidative stress, and, in other cases, disregulating redox homeostasis leading to apoptosis.[15,16] Recent murine studies have shown diverse effects from the expected toxicity of an amino acid-based aryl telluroether to increased memory in mice treated with an alkyl telluroether.[17,18]

Here we describe the synthesis, aqueous/aerobic stability, and *in vitro* toxicity of a series of alkyl telluroethers and tellurophene functional groups. We hypothesized that the organotellurium exhibiting the most favourable properties with respect to these three criteria would be an improved mass tag component for the next generation of MC probes.

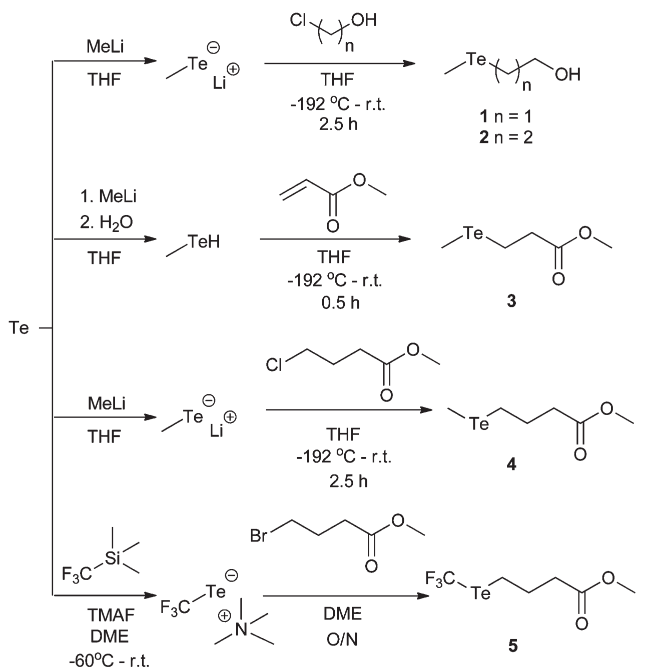
Table 1 Organotellurium compounds investigated



Results and discussion

Methyl telluroethers: synthesis and stability

The organotellurium functionality that was initially investigated was the methyl telluroether due to its small size and ease of synthesis (Table 1, compounds **1–4**, **8–9**). Aryl telluroethers were not investigated due to numerous reports of their redox-activity in living systems and their reported instability under ambient light.[19] The methyl telluroethers were synthesized from nucleophilic lithium methyl tellurolate, using a modified procedure first established by N. Khun, followed by reaction with the desired electrophile (Scheme 1).[20] The synthesis of compound **3** required quenching of the methyl telluroate with water to generate the tellurol prior to the Michael-style addition to methyl acrylate. The yields of these additions ranged from 66 to 91%.



Scheme 1 The synthesis of compounds **1–5**. The yields of the following reactions were: **1** = 66%, **2** = 74%, **3** = 85%, **4** = 91%, and **5** = 50%.

The relative chemical stability of the methyl telluroethers (**1–4**) were quantified using ^{1}H NMR by integration of the CH_3-Te signals with respect to a residual DMSO-d_5 internal standard. Samples were prepared as solutions of DMSO-d_6 and placed under a slow, continuous stream of dry ambient atmosphere in a clear glass desiccator. This setup allowed the stability of the compounds to be monitored without interference from atmospheric water (Fig. 2).

Compounds **1–4** all degraded significantly over the course of the 24 h incubation. Compound **4** was the most stable alkyl telluride investigated, degrading approximately 15% over

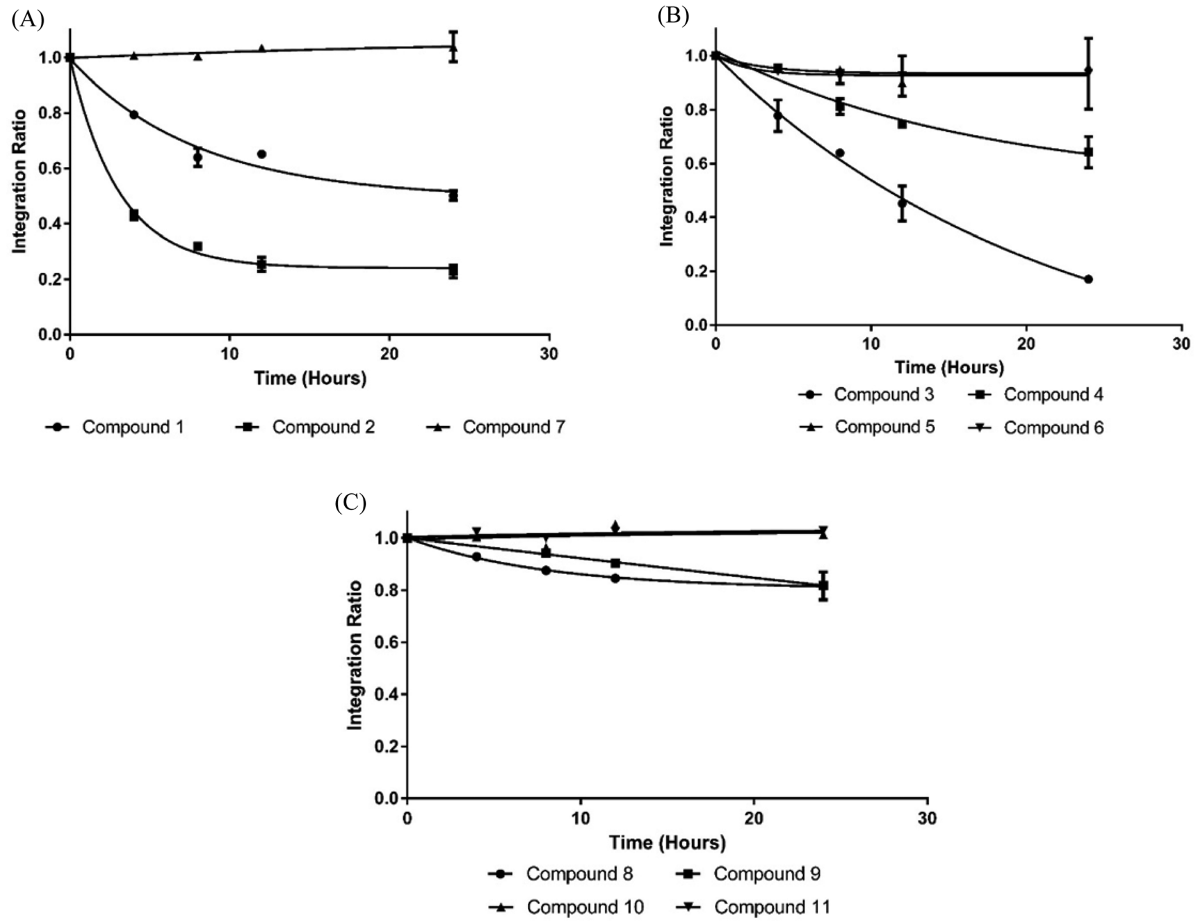


Fig. 2 ^{1}H NMR stability experiment of compounds **1–11**. (A) Compounds **1**, **2** and **7**. (B) Compounds **3**, **4**, **5** and **6**. (C) Compounds **8**, **9**, **10** and **11**. The organotellurium compounds (~150 μM) in DMSO-d_6 with 1,3,5 – trioxane, the secondary internal standard. The compounds were kept in clear glass 20 mL vials. The vials were kept in a moisture free environment for 24 hours with continuous supply of ambient atmosphere dried using a series of bubblers containing phosphoric acid, potassium hydroxide and calcium sulfate. Aliquots were analyzed by ^{1}H NMR and the ratio between the DMSO-d_5 internal standard and the protons β to the tellurium nucleus at time 0 were taken and normalized to generate a degradation plot. Experimental error was calculated by generating triplicate integration data from individual NMR spectra. This error takes into consideration integration bias and instrument fluctuations.

24 hours (Fig. 2B). Compounds **2** (Fig. 2A) and **3** (Fig. 2B) showed the greatest degradation, approximately 75% and 85%, respectively (Fig. 2A and 2B).

The alkyl tellurides are presumed to undergo oxidation under the experimental conditions. During incubation the initially yellow solutions became colourless with the formation of white precipitate, at varying rates. This phenomena has been previously observed and is presumed to be the telluroxide species forming polymeric structures or the formation of TeO_2.[21,22] In addition, it has been observed previously that solutions of Telox exhibited ^{1}H NMR absorptions consistent with chalcogen oxidation.[5,23] Alkyl telluride compounds are also known to undergo hemolytic bond cleavage and this may result in the observed small quantities of dimethyl ditelluride and dimethyl telluride.[24–26] These species are volatile and, in compounds **1** and **2**, are observed only in the early time points. The same species are observed in the 8 hour sample from compound **3**. In addition, methyl acrylamide was produced during the degradation of compound **3**. The remaining organic components resulting from these degradations could not be identified and may be lost due to their low molecular weight and volatility. To reduce the propensity for oxidative degradation of the telluroethers the trifluoromethyl telluroethers, **5** and **10**, and the tellurophenes **6**, **7** and **11** were investigated.

Trifluoromethyl telluroethers: synthesis and stability

Compound **5** bearing a trifluoromethyl group is expected have reduced electron density at the tellurium center and thus should oxidize more slowly. Compound **5** was synthesized by the generation of tetramethylammonium trifluoromethyl tellurolate *in situ* by treating tellurium metal with trimethyl-(trifluoromethyl)silane and tetramethylammonium fluoride.[27] Methyl-4-bromobutyrate was added to the solution to give the product **5** in 50% yield (Scheme 1).

The stability of the trifluoromethyl telluride **5** was evaluated under the same conditions as compounds **1–4** (Fig. 2B).

Comparison of the structurally related compounds **4** and **5** supported our hypothesis that reducing electron density at the Te centre would stabilize the compound, as no degradation was observed over the 24 hour incubation.

Tellurophenes: synthesis and stability

Tellurophenes have not been evaluated in biological systems, and only recently has the first water soluble tellurophene been reported.[28] Tellurophenes possess interesting photophysical properties and have been investigated as light harvesting agents for solar cell applications and in materials chemistry.[29–31] The chalcogen analogue, selenophenes, have been investigated in biological systems with promise as antioxidant molecules. Through computational analysis, ground state aromaticity of tellurophenes suggest these compounds to be more stabilized than selenophenes.[32] We hypothesized that the aromatic nature of the tellurophene would provide greater chemical stability over the telluroether derivatives under the desired biological conditions.

The tellurophenes were synthesized *via* the addition of Te^{2-} to a mono-functionalized diacetylene in a synthesis modified from Stephens and Sweat's initial report.[33,34] In synthesizing these tellurophenes, the generation of Te^{2-} was a key step. Commonly, an aqueous suspension of Te^0 is treated with $NaBH_4$. However, we found the use of a basic Rongalite ($NaHOCH_2SO_2$) solution reproducibly generated Te^{2-} and gave higher yields of the desired tellurophene.[35]

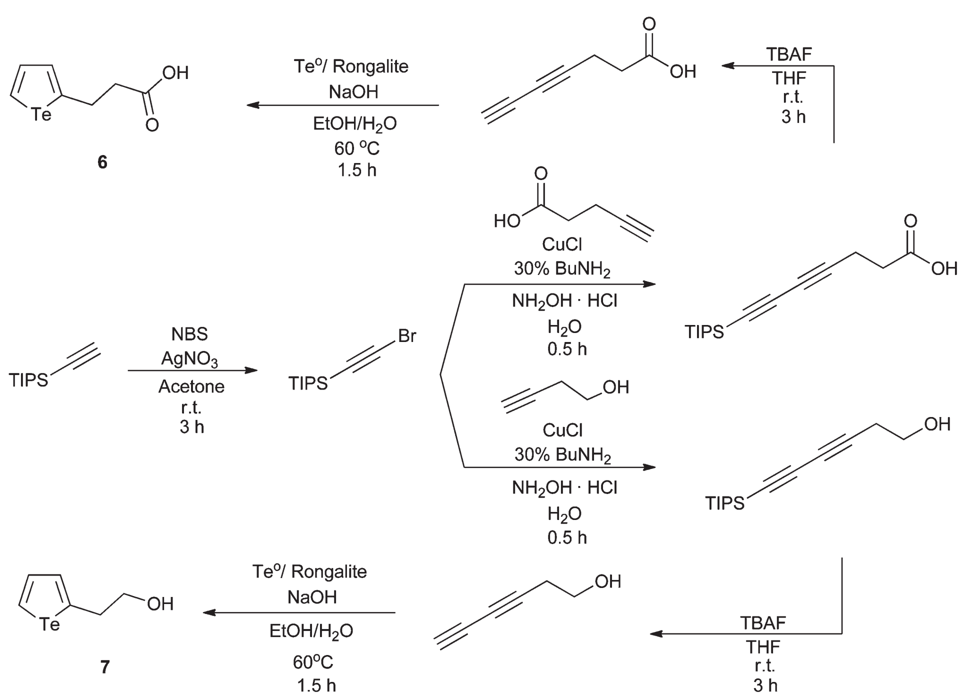
The required trialkylsilyl diacetylenes can be generated in excellent yield using the Cadiot-Chodkiewicz cross-coupling reaction (Scheme 2).[36] Bromination of triisopropylsilyl acetylene using *N*-bromosuccinimide and silver nitrate gave the known coupling intermediate using the conditions of Wulff *et al.*[37] This compound was then coupled using CuCl in 30% $BuNH_2$ with the desired acetylene component. Compound **6** required the addition of 4-pentynoic acid and compound **7** required the addition of 3-butyn-1-ol. The trialkylsilyl protected diacetylene compounds were deprotected using tetrabutylammonium fluoride and cyclized into tellurophenes **6** and **7** using a basic solution of Rongalite and tellurium metal in good yield (Scheme 2).

The stabilities of the two tellurophenes were studied using the same protocol as the alkyl telluride species (Fig. 2A and B). Compounds **6** and **7** had improved stability in comparison to the methyl alkyl telluroethers. Both tellurophene compounds degraded insignificantly over the 24 hour incubation having similar stability to the trifluoromethyl telluroether **5**.

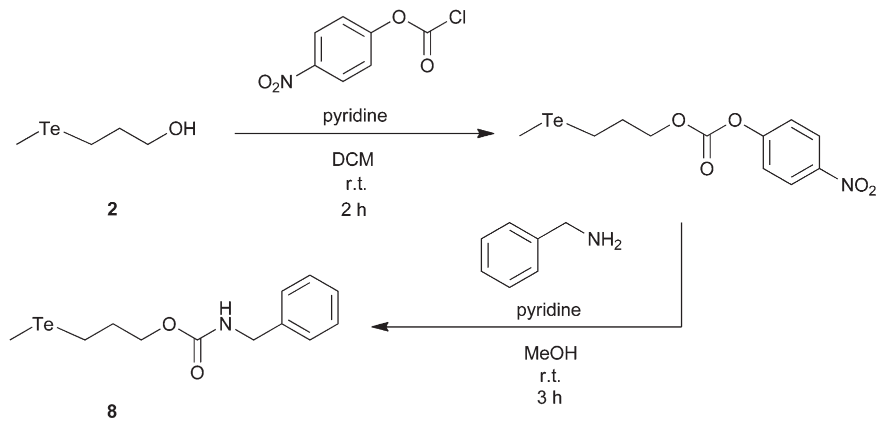
Benzylamine-conjugated organotellurium derivatives: synthesis and stability

Future MC probes will require the conjugation of the organotelluriums to biologically relevant functional groups. Here, two conjugation reactions are considered that would provide a means to label primary amines; a carbamylation and an amidation reaction. Furthermore, forming benzylamine derivatives of the organotellurium compounds **2**, **4**, **5** and **7**, lead to compounds (**8–11**) with more comparable partition coefficients for cell toxicity studies (Schemes 3 and 4).

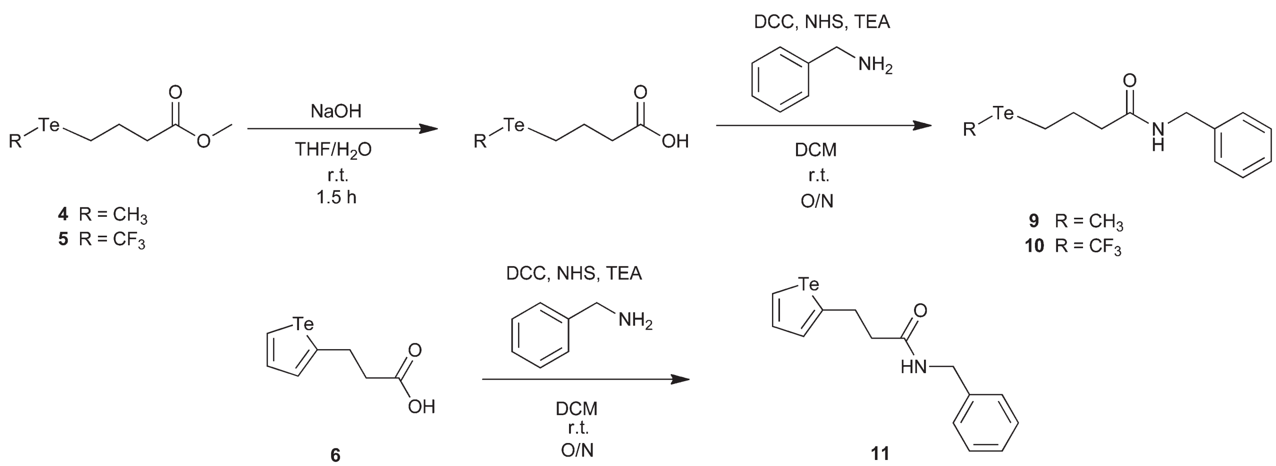
Compound **8** was synthesized *via* a *p*-nitrophenyl carbonate ester generated by the treatment of compound **2** with *p*-nitrophenyl chloroformate. The reactive carbonate could be



Scheme 2 The synthesis of compounds **6** & **7**. The yields of the reactions were: **6** = 70% & **7** = 66%.



Scheme 3 Synthesis of compound **8**. The yeild over two steps was 77%.



Scheme 4 Amidation of compounds **4–6** with benzylamine. The yields of the reactions were: **9** = 81%, **10** = 30%, & **11** = 81%.

purified and stored. Compounds **9** and **10** were synthesized by hydrolysis of the methyl ester and, after isolation of the carboxylic acid, coupling proceeded with DCC in the presence of NHS and benzylamine. Compound **11** was synthesized analogously to **9** and **10** but required the use of column chromatography for purification.

The stabilities of these benzylamine derivatives were assessed using the aforementioned ^{1}H NMR assay (Fig. 2C). The rate of degradation of these compounds mirrored those of the underivatized compounds with the methyl telluride species, compounds **8** and **9**, degrading 20–25% over the incubation and compounds **10** and **11** being stable over the incubation. Interestingly the carbamate **8** was considerably more stable than the parent alcohol **2** suggesting the alcohol may directly contribute to the degradation mechanism.

Stability in PBS buffer

Of the compounds evaluated, the trifluoromethyl telluroether-amide (**10**) and the tellurophene-amide (**11**) exhibited the best stabilities under aerobic conditions. To further validate these compounds as potential MC mass tags, the stability was also studied in a buffered aqueous solution by dissolving the compounds in a 50/50 solution of DMSO-d_6/PBS-d buffer. The compounds were kept in an environment exposed to air and ambient light at room temperature. ^{19}F NMR was used to study compound **10** using a trifluoroacetic acid internal standard while ^{1}H NMR was used to study the degradation of compound **11** using the DMSO-d_5 internal standard. Disappointingly, as shown in Fig. 3, the trifluoromethyl telluroether **10** showed 60% degradation after 24 hours. However, under the same conditions the tellurophene **11** was stable.

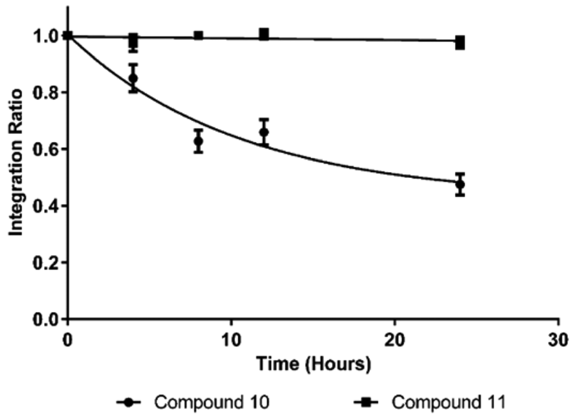


Fig. 3 Stability studies of compounds **10** and **11** in 50% DMSO-d_6, PBS-d buffer solutions. The ^{19}F NMR spectra of compound **10** and ^{1}H NMR spectra of compound **11** were taken at shown time points. Compound **10** used trifluoroacetic acid as the internal standard and compound **11** used DMSO-d_5.

Cellular toxicity

To finally investigate these organotellurium compounds as potential probe moieties for MC, the metabolic toxicity of the compounds was evaluated. Organotellurium compounds are often described as toxic, with aryl telluroethers showing cellular toxicity below 100 μM across a range of cell lines under different assay conditions.[9–11,38,39] Here, we investigated the toxicity of compounds **8–11** in Jurkat cells after a 24 hour incubation using the metabolic probe WST-1 as per manufacturer's instructions. Compounds **8**, **9** and **10** are expected to show degradation over the time frame of the toxicity assay, based on our NMR stability studies, and, as such these experiments show the relative toxicities of the compounds and their resulting degradation products (Table 2). Compound **8** had an apparent IC_{50} value of 610 μM, but with a large experimental error due to the lack of solubility of compound **8** at higher concentrations. Compounds **9** and **10** were more toxic with an IC_{50} < 200 μM, however, the tellurophene **11** was less toxic with an IC_{50} of 280 μM. These data suggest that, in general, the alkyl telluroethers and the tellurophenes are less toxic than previously investigated aryl telluroethers.[9–14] The IC_{50} values of the reported organotellurium compounds provide promise as mass tags for activity based MC probes since the probe concentrations required in these experiments will typically be below ~100 μM.[5]

Table 2 IC_{50} values of the organotellurium compounds **8–11**

Compound	IC_{50} (μM)
8	610 ± 290[a]
9	180 ± 60
10	130 ± 20
11	280 ± 30

[a] Compound **8** has a large experimental error due to the lack of solubility. All cells could not be killed at the maximal concentration acceptable for the experiment (2 mM).

Conclusion

MC is a powerful analytical tool. Tellurium has valuable characteristics as a mass tag for MC; including eight stable isotopes, small functional group size and low polarity. We have synthesized and characterized various organotellurium compounds functionalized for MC probe development. The alkyl telluride species, although synthetically tractable, have limitations, mainly due to poor compound stability. For future work, alkyl telluride species lacking β-protons could be investigated to improve stability. However, since the tellurophene moiety is available in good yield, is chemically stable and is sufficiently non-toxic it warrants further investigation as an MC mass tag. The applications of this functionality may extend beyond MC; for example, due to the similarity of tellurophenes and thiophenes, this moiety introduces a new building block for medicinal chemists.

Acknowledgements

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