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Organic Arsenicals As Efficient and Highly Specific Linkers for Protein/Peptide–Polymer Conjugation

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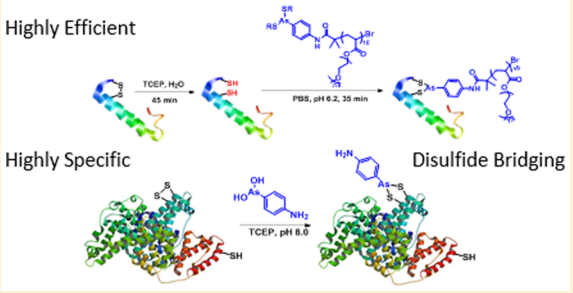
Supporting Information

ABSTRACT: The entropy-driven affinity of trivalent (in)organic arsenicals for closely spaced dithiols has been exploited to develop a novel route to peptide/protein–polymer conjugation. A trivalent arsenous acid (As(III)) derivative (**1**) obtained from *p*-arsanilic acid (As(V)) was shown to readily undergo conjugation to the therapeutic peptide salmon calcitonin (sCT) via bridging of the Cys[1]-Cys[7] disulfide, which was verified by RP-HPLC and MALDI-ToF-MS. Conjugation was shown to proceed rapidly (t < 2 min) *in situ* and stoichiometrically through sequential reduction–conjugation protocols, therefore exhibiting conjugation efficiencies equivalent to those reported for the current leading disulfide-bond targeting strategies. Furthermore, using bovine serum albumin as a model protein, the trivalent organic arsenical **1** was found to demonstrate enhanced specificity for disulfide-bond bridging in the presence of free cysteine residues relative to established maleimide functional reagents. This specificity represents a shift toward potential orthogonality, by clearly distinguishing between the reactivity of mono- and disulfide-derived (vicinal or neighbors-through-space) dithiols. Finally, *p*-arsanilic acid was transformed into an initiator for aqueous single electron-transfer living radical polymerization, allowing the synthesis of hydrophilic arsenic-functional polymers which were shown to exhibit negligible cytotoxicity relative to a small molecule organic arsenical, and an unfunctionalized polymer control. Poly(poly[ethylene glycol] methyl ether acrylate) ($PPEGA_{480}$, DP_n = 10, $M_{n,NMR}$ = 4900 g·mol^{-1}, $Đ$ = 1.07) possessing a pentavalent arsenic acid (As(V)) α-chain end was transformed into trivalent As(III) post-polymerization via initial reduction by biological reducing agent glutathione (GSH), followed by binding of GSH. Conjugation of the resulting As(III)-functional polymer to sCT was realized within 35 min as indicated by RP-HPLC and verified later by thermodynamically driven release of sCT, from the conjugate, in the presence of strong chelating reagent ethanedithiol.



INTRODUCTION

Nature can achieve sophisticated modification of peptides/proteins via a variety of post-translational transformations, altering the complexity, function, and diversity of biomacromolecules.[1] These modifications are efficient and site specific, with the desired structure, function, and activity of the biomolecules controlled throughout. In the laboratory, a number of chemical transformations exist through which peptides/proteins can be modified with functional small and/or macromolecules, commonly referred to as bioconjugation.[2−8] In brief, chemical ligation can be achieved intrinsically by exploiting the functionalities inherent in peptides/proteins.[9] Alternatively, extraneous (bio)chemical modification (e.g., mutation, peptide synthesis, ligation)[10] can be employed to introduce unnatural functionality into the peptide/protein structure to facilitate and direct conjugation.

The intrinsic approach harnesses the reactivity of amino acid side chains, for example, nucleophilic primary amines present in lysine residues and at the *N*-terminus of the peptide.[11,12] Though site specificity can be conferred by careful control of the pH, the relatively high natural abundance of lysine often results in multiple conjugation sites which can significantly reduce the activity of the target biomolecule. Enhanced site specificity can be attained by targeting less abundant nucleophilic cysteine, the side chain of which exists as a free thiol or as a disulfide with a second, proximal cysteine residue depending upon to redox environment.[13,14] Aromatic side chains present in tyrosine and tryptophan have also been targeted as conjugation sites. For example, tyrosine can be

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modified via three component Mannich reaction,[15] cyclic diazodicarboxamides[16] or diazonium coupling,[17] while examples of Pd-catalyzed, *O*-alkylation have also been reported.[18]

The soft nucleophilic character of the pendant thiol group presented by cysteine residues, combined with the low relative natural abundance make it an ideal target for highly specific ligation. Free thiol groups are readily modified by disulfide-bond metathesis upon reaction with functionalized pyridyl disulfide reagents.[19] Alternatively, their reactivity toward electrophiles can be exploited via substitution (α-halocarbonyls)[20] or conjugate (Michael) addition (α,β-unsaturated esters,[21] maleimides,[22] vinyl sulfones[23]) mechanisms resulting in stable thio-ether-bond formation. However, formation of the stable thio-ether linkages is generally considered to be irreversible which can limit the practicality of such transformations, particularly where reversibility is required for a resumption of bioactivity. Though maleimide conjugates can undergo hydrolytic decomposition to release peptides/proteins,[24,25] the discovery of (di)bromomaleimides, has introduced the potential for a specific, stoichiometric and reversible thiol conjugation strategy.[26,27]

Upon folding, proximal cysteine residues can combine to form intra- and/or interchain disulfide bonds.[28,29] These bonds contribute to the maintenance of the tertiary and quaternary structure present in peptides/proteins and are therefore crucial for structure, function, and activity. Naturally, their stability is controlled by changes in the local redox environment, which can be efficiently manipulated chemically using appropriate reducing and oxidizing agents.[30,31] Upon reduction of the disulfide bond, the resulting cysteine residues can be targeted as independent free thiols. However, where minimal disruption of the native structure is desirable, bridging dibromo-/dithiomaleimides[32,33] and bisulfones[34,35] have been shown to be efficient and stoichiometric reagents for pegylation and conjugation of functional polymers prepared by controlled radical polymerization (CRP).

(In)organic arsenic represents a (bio)chemical dichotomy between toxicity and therapy. On one hand arsenic is a well-established poison and is a known contaminant of base water supplies, particularly in South-East Asia.[36] On the other hand, the use of arsenic as a therapeutic dates back millenia as a common ingredient in Chinese medicine.[37] In the early 20th century Paul Ehrlich developed a number of organic arsenicals, including Salvarsan,[38] for the treatment of diseases including psoriasis, syphilis, and rheumatosis, and perhaps more notably, as chemotherapeutics.[39] However, due to concerns regarding toxicity, the use of arsenic in conventional medical practice was curtailed before a clinical renaissance in the treatment of acute promyelocytic leukemia (APL).[40] It was found the arsenic trioxide[41] (As_2O_3) induced complete remission in a high percentage of APL sufferers, particularly in combination with all-*trans* retinoic acid (ATRA) and other chemotherapeutics.[42,43]

Arsenic can exist in two biologically relevant oxidation states, trivalent arsenous acid (As(III)), and pentavalent arsenic acid (As(V)). In As_2O_3 it exists as trivalent As(III), in which the soft metalloid center has a high affinity for soft nucleophiles, such as thiols. This affinity is enhanced for chelating vicinal/proximal dithiols able to form cyclic dithiaarsanes which are entropically much more stable than monothiol adducts.[44,45] Such is the avidity of (in)organic arsenic for dithiols that it is implicated in the inactivation of a number redox enzymes, particular those of the thioredoxin family of enzymes.[46,47] A common feature of these enzymes is thiol-rich domains which can be cross-linked by As(III) significantly altering structure, function, and activity.[48] Though inorganic arsenic is associated with high cytotoxicity, local and systemic effects have been related to metabolic fate.[49−52] For example, inorganic arsenic metabolism is thought to proceed via cell uptake followed by two-electron reduction and methylation to form organic (di)methylarsenous acid (As(III)) derivatives which can be sequestered and then excreted in urine, evoking detoxification.[53] Thus, organic arsenicals are perceived to be more stable and less toxic owing to enhanced rates of excretion. Arsenobetaine[54,55] exhibits no toxic effects upon oral administration to mice up to 10 g/kg, and dimethylarsenic acid has been shown to be significantly less toxic toward normal progenitor cells than As_2O_3.[56] Consequently, the therapeutic potential of arsenic in combination with the diminished toxicity of its organic derivatives has paved the way for the emergence of a number of arsenic containing chemotherapeutic drug candidates.[57−59]

However, there are few reports on the incorporation of organic arsenicals into synthetic polymers, and no data regarding their toxicity.[60] Furthermore, despite the high affinity for proximal dithiols, there are no reports on the use of As(III) as a linker for peptide/protein−polymer conjugation. Consequently, we were inspired to investigate whether the affinity of As(III) for dithiols could be translated into a site specific strategy for peptide/protein bioconjugation through bridging disulfide bonds.

RESULTS AND DISCUSSION

Conjugation of an Organic Arsenical to Salmon Calcitonin (sCT). Calcitonin is a hormonal peptide secreted by neuroendocrine cells (*C*-cells)[61] in the thyroid of mammals and assists in controlling calcium and phosphorus levels through regulating their deposition into bones.[62] Also produced by submammalian species, including birds, reptiles and fish, calcitonin exists as a 32-amino acid peptide, the sequence of which differs depending on the species. Structurally, an important disulfide bridge between Cys^1-Cys^7 is conserved throughout the calcitonin family of peptides, which, upon reduction contains a pair of closely spaced thiols. It has been demonstrated previously that the disulfide present in sCT can be efficiently bridged by functional and polymeric disubstituted maleimides.[32,33] As a proof of concept, sCT was used as a model peptide for conjugation of As(III) containing organic arsenicals to closely spaced thiols.

Initially, the arsenic acid (As(V)) group of commercially available organic arsenical *p*-arsanilic acid was reduced, as described previously, to yield the arsenous acid (As(III)) **1**.[63] Following reduction to As(III), the affinity of the soft metalloid center for dithiols was exemplified by rapid chelation to ethanedithiol to form the cyclic dithiaarsane, which was also prepared in a one-pot process (Scheme 1).[64] For thermodynamic reasons, the dithiaarsanes were not considered for conjugation with attention focused on the arsenous acid (As(III)) functionality.

Two possible methods of conjugation were conceived at the outset. An *in situ* approach whereby reduction and conjugation proceed concurrently upon addition of reducing agent *tris*-(2-carboxyethyl)phosphine (TCEP) to a mixture of sCT and **1**. Alternatively, sequential reduction and conjugation proceeding via complete reduction of the disulfide bond prior to addition of **1**. Conjugation via the *in situ* method was realized within 2 min upon addition of TCEP to a solution of sCT and **1** at pH

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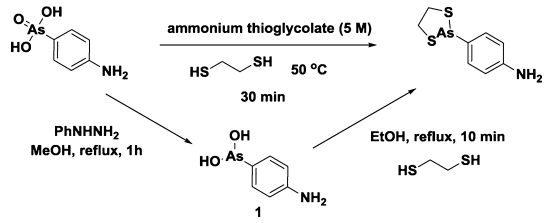
Scheme 1. Synthesis of Trivalent Organic Arsenicals by Chemical Modification of *p*-Arsanilic Acid via an *in Situ* and Sequential Reduction/Conjugation Approach, Respectively



6.2. Prior to addition of TCEP, HPLC analysis showed two peaks, one assigned to the small molecule arsenical (**1**, t = 2.7 min), the other to native sCT (t = 14.0 min). Conjugation of **1** was confirmed by a reduction in the peak intensity of **1** and shift in the retention time for the sCT conjugate relative to native sCT (t = 13.5 min, Figure 1A). The sequential reduction−conjugation approach was initiated by reduction of the disulfide bond by TCEP in water. Complete reduction of sCT was confirmed after 45 min by the characteristic shift to shorter retention time for the reduced sCT (t = 12.8 min) relative to native sCT (t = 14.0 min). The reduced peptide solution was then buffered to pH 6.2 in line with the *in situ* method, and a solution of **1** was added. Conjugation was achieved within 2 min as indicated by complete consumption of the reduced sCT peak and appearance of a new peak at a retention independent of, and in the absence of, any reformed native sCT peaks (t = 13.5 min, Figure 1B). Moreover, the peptide was shown to be thermodynamically released from the As(III) linker upon addition of an excess of ethanedithiol (EDT) yielding a mixture of reduced and native sCT. In addition to confirming conjugation, this observation also implies potential for a new approach to the controlled release of biomolecules from their polymer conjugates, the virtues of which have been the subject of a recent review.[65]

The HPLC data from both methods pointed to a single product being formed which was assigned as sCT with the disulfide bridged by organic arsenical **1**. This was supported by MALDI-ToF-MS which showed a mass increase of 166.74 Da consistent with bridging of the disulfide by **1** (Figure 2). In agreement with the HPLC data, no evidence of native or reduced sCT was detected. Furthermore, potential side products arising from less favored monothiol addition or reaction with His[17] as a competitive soft nucleophile for As(III) were not detected.

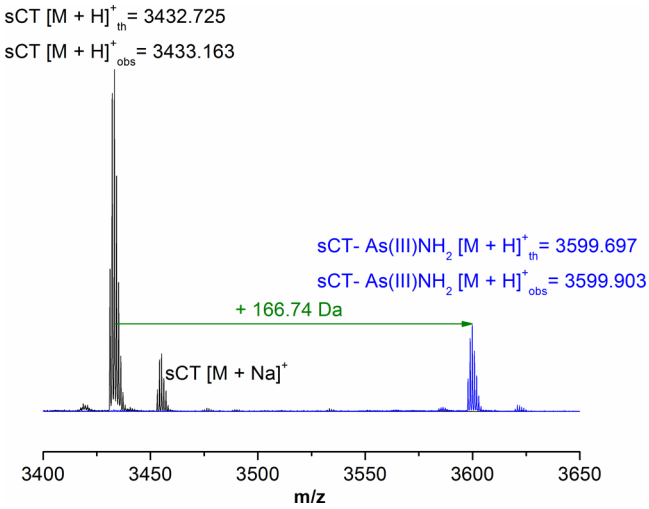


Figure 2. MALDI-ToF-MS showing complete conjugation (blue) of arsenical **1** to native sCT (black). Mass increase of +166.74 Da (green) is indicative of disulfide bridging.

Insight into Disulfide-Bond Specificity. The performance of small molecule arsenical **1** is equally as efficient as dibromomaleimide, the current gold standard in disulfide-bond targeted conjugation.[27,32] However, little is known regarding the relative specificities of these reagents for disulfides in the presence of single, reduced cysteine residues. An enhancement in the specificity for closely spaced, reduced disulfide thiols, would represent a significant advance in conjugation technology, particularly when targeting larger, more complex peptides/proteins (e.g., antibodies).

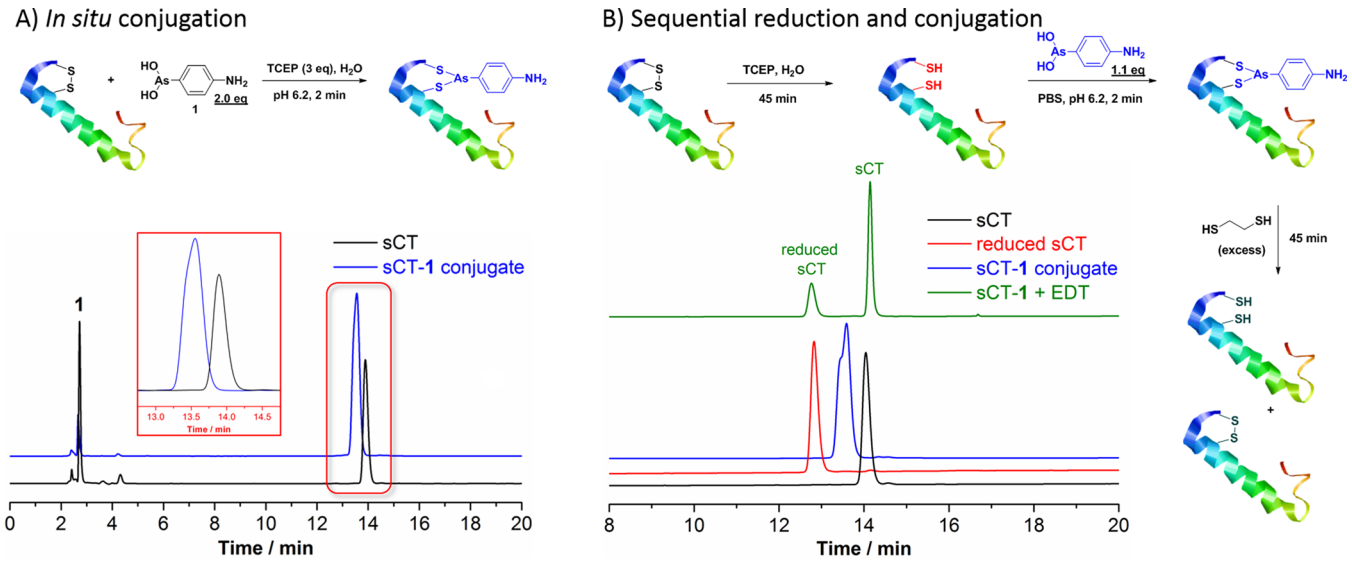


Figure 1. (A) RP-HPLC for the *in situ* conjugation (blue) of organic arsenical to sCT (black). (B) RP-HPLC for the sequential reduction (red) and conjugation (blue) of organic arsenical **1** to native sCT (black) followed by release of sCT (green) upon addition of ethanedithiol (EDT).

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Bovine serum albumin (BSA) contains 17 disulfide bonds and a single cysteine residue (BSA-Cys)[34] which is often partially, intermolecularly, oxidized.[66] Accordingly, quantification of the thiol content of native BSA by Ellman's assay,[2] relative to a thiol concentration calibration plot derived from cysteine standards (Figure S1), resulted in detection of <1 thiol per BSA molecule (0.61 thiols per BSA, Table 1, entry 1). The

Table 1. Thiol Concentrations and the Subsequent Number of Thiols Present in BSA Samples Determined From Ellman's Assay

entry	reagent (10 μM)	A_{412}	[SH] μM	no. SH
1	BSA	0.111	6.1	0.61
2	+NEM	0.015	−1.3	−
3	+DBM	0.035	0.3	0.03
4	+**1**	0.094	4.8	0.48
5	rBSA[i]	0.292	20.0	2.00
6	+NEM	0.061	2.2	0.22
7	+DBM	0.064	2.5	0.25
8	+**1**	0.154	9.4	0.94

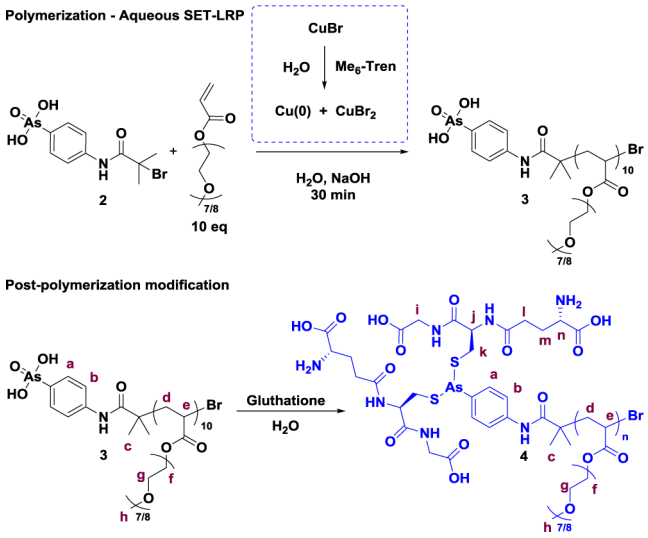
[i]BSA treated with TCEP (1 equiv).

extent to which arsenical **1** could react with the 0.61 available thiols of BSA-Cys[34] was then determined and compared to thiol reactive compounds *N*-ethylmaleimide (NEM), specific for monothiols, and dibromomaleimide (DBM), a disulfide-bond targeting reagent. Addition of these reagents to independent aliquots of native BSA, in the absence of any prior reduction revealed that both maleimide reagents reacted near quantitatively with the available thiol groups within 30 min (Table 1, entries 2−3). However, arsenical **1** exhibited limited reactivity indicating that, with respect to native BSA, only ≈20% of the available thiols had reacted over the same period of time (Table 1, entry 4). To ensure that the performance of arsenical **1** was not the result of a difference in reactivity relative to the maleimide reagents, an additional experiment in which the reaction of **1** with BSA-Cys[34] was monitored as a function of time was performed. This revealed that the maximum amount of binding of **1** to BSA-Cys[34] had occurred within 30−60 min after which the number of thiols detected was found to increase (i.e., binding decreased) as a function time (Table S1).

Treatment of native BSA with a stoichiometric amount of the reducing agent *tris*(carboxyethyl)phosphine (TCEP), at 25 °C for 2 h, resulted in an increase in the thiol content. A substoichiometric amount of TCEP (≈ 0.39 equiv) is sufficient to fully reduce BSA-Cys.[34] The remaining TCEP (≈ 0.61 equiv) can then act to reduce an equivalent amount of an accessible disulfide bond present in BSA. When averaged over all the molecules present, this resulted in the detection of 2 thiols per BSA molecule by Ellman's assay (Table 1, entry 5). With this in mind, incubation of reduced BSA (rBSA) with NEM, DBM, and arsenical **1** can therefore offer an insight into relative specificity of these reagents for the thiols originating from the intramolecular disulfide bonds in the presence of the free thiol from BSA-Cys.[34] Maleimide reagents (NEM, DBM) exhibited very little selectivity reacting with ≈90% of all available thiols (Table 1, entries 6−7). Conversely, under identical conditions, arsenical **1** reacted with only ≈54% of the available thiols of rBSA (Table 1, entry 8). The reaction of monothiol reagents with maleimides is well studied. Arsenicals can also react with monothiols, and this has been exploited below. However, the adducts formed are appreciably less stable, particular in the presence of chelating dithiols, which give entropically favored products.[67−71] Thus, we postulate, that upon reaction of rBSA with arsenical **1**, the detection of 1 thiol is predominantly attributed to the existence of nonreacted reduced BSA-Cys,[34] indicating that arsenical **1** specifically reacts via bridging any available reduced disulfides present in rBSA.

Arsenic Functional Polymers by Aqueous SET-LRP. In order to probe the viability of arsenic as a linker for peptide/protein conjugation, *p*-arsanilic acid was amidated using 2-bromoisobutyryl bromide furnishing a novel initiator (**2**) for Cu-mediated CRP (Scheme 2). Water-soluble acrylamide

Scheme 2. Synthesis of As(V)-Functional PPEGA$_{480}$ 3 by Aqueous SET-LRP Followed by Post-Polymerization Modification by Reduction and Complexation with Glutathione (GSH) To form the Bis(GSH)As(III)PPEGA Adduct 4



monomers *N*-isopropylacylamide (NIPAm) and *N*-acryloylmorpoline (NAM), and acrylate poly(ethylene glycol) methyl ether acrylate (PEGA$_{480}$) were polymerized via aqueous single electron-transfer living radical polymerization (SET-LRP) as described by Haddleton and co-workers (Table S2).[72−74] Using this technique, polymerizations were complete within 30 min, as determined by ^{1}H NMR, with good agreement between $M_{n,th}$ and $M_{n,exp}$ and narrow dispersities ($Đ$) attained from SEC (Table S2, Figures S2−S4). At the molecular weights targeted (∼10000 g·mol^{-1}), the existence of the α-arsenic end-group could be substantiated by the presence of aryl protons (7.4−7.8 ppm, Figures S3−S4) in the ^{1}H NMR (the signal is obscured by the N−H signal in the PNIPAm, Figure S2).

The reduction of As(V) to As(III) at the α-chain end proceeded via post-polymerization modification. For the purpose of conjugation, reduction of arsenic (As(V)) to arsenous (As(III)) acid was achieved with phenylhydrazine in refluxing methanol, as shown in Scheme 1, followed by purification by dialysis against pure water (3.5 kDa MWCO). Reduction was confirmed by a downfield shift of the aryl proton signals in ^{1}H NMR and SEC analysis revealed that the integrity of the polymers was retained (Figure S5).

Organic arsenicals are generally considered to be more stable and less cytotoxic than inorganic arsenicals. In the most recent United States Agency for Toxic Substances and Disease

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Registry (ATSDR) toxicology profile for arsenic,[75] LD_{50} values (rats and mice) of inorganic As(III)/As(V) and organic monomethylarsonic acid (MMA) were quoted as 15−175 and 102−3184 mg·kg^{-1}, respectively. With respect to the current investigation little is known regarding the toxicity of arsenicals when incorporated into polymer compositions, *i.e.*, polymeric arsenicals. As such, the toxicity of arsenic (As(V)) and dithiaarsane (As(III)) polymers were compared in a series of mouse cell lines: macrophages (RAW264.7), fibroblasts (3T3MEF), and dendritic like cells (DC2.4). The As(III) compounds showed minimal toxicity in all three cell lines, up to a concentration of 0.1 mg·mL^{-1}. The As(V) polymers also showed minimal toxicity in 3T3 and DC2.4 cells up to a concentration of 0.1 mg·mL^{-1}, and minimal toxicity in RAW264.7 cells up to 10 μg·mL^{-1} (Figure S8). The concentration of As(III/V) per chain was then varied by copolymerization of an As-functional acrylamide monomer with $PEGA_{480}$ (Table S3). Small molecule arsenical **2** was used as a control and found to be an order of magnitude more toxic than all the polymers investigated. Furthermore, the toxicity of $PPEGA_{480}$ copolymerized with increasing concentrations of arsenical was comparable to nonfunctional PPEGA up to a concentration of 0.1 μg·mL^{-1} (Figure S9).

Conjugation of a Polymeric Arsenical to Salmon Calcitonin (sCT). Arsenic acid (As(V)) end-functional $PPEGA_{480}$ was initially treated with phenylhydrazine to form the arsenous acid (As(III)) analogue (Figure S5). However, attempts to conjugate this polymer, by both *in situ* and sequential protocols were unsuccessful, even at stoichiometric excess (>20 equiv), resulting only in reformation of native sCT (Figure S10). This was attributed to loss of the arsenous acid As(III) end group as a result of oxidation, occurring during the extensive purification process via dialysis against water.

In an alternative approach, reduction of the end group from As(V) to As(III) was attempted using an excess of reduced glutathione (GSH).[76] At the outset, sparingly soluble *p*-arsanilic acid was added to 4 equiv of GSH in Milli-Q water. In an initial redox reaction, 2 equiv of GSH are required to reduce As(V) to As(III), forming oxidized glutathione (GSSG) as a byproduct. The remaining 2 equiv of GSH then bind As(III) forming a water-soluble *bis*(GSH) adduct. Isolation of the crude mixture by lyophilization and subsequent characterization by ^{1}H NMR revealed the formation of a 1:1 mixture of $(GSH)_2As(III)$ and GSSG (Figure S11). When this mixture was added to reduced sCT, conjugation efficiency was found to be on par with arsenical **2**, yielding the desired conjugate within 2 min (Figure S12). Encouraged by this result a low molecular weight arsenic acid (As(V)) $PPEGA_{480}$ was prepared (DP_n = 10, $M_{n,NMR}$ = 4900 g·mol^{-1}, $Đ$ = 1.07) and treated with an excess of GSH. Removal of the reaction byproduct (GSSG) and unreacted GSH, which could interfere in the conjugation reaction, was achieved by dialysis against Milli-Q water. Formation of the *bis*(GSH) adduct **4** was confirmed by ^{1}H NMR (Figure 3A) and supported by an anticipated reduction in retention time by RP-HPLC (Figure 3B) of the $PPEGA_{480}$ following modification. End group analysis by ^{1}H NMR via integration of the aryl protons of the arsanilic group (7.76−7.58 ppm) revealed 75% retention of the α-end group functionality.

Conjugation of the $(GSH)_2As(III)PPEGA_{480}$ **4** was initially attempted via sequential reduction of sCT and addition of the polymer in phosphate buffer (pH 6.2). At the stoichiometries employed for the small molecule arsenicals (1.2 equiv polymer), a sCT-**4** conjugate peak was detected by RP-HPLC

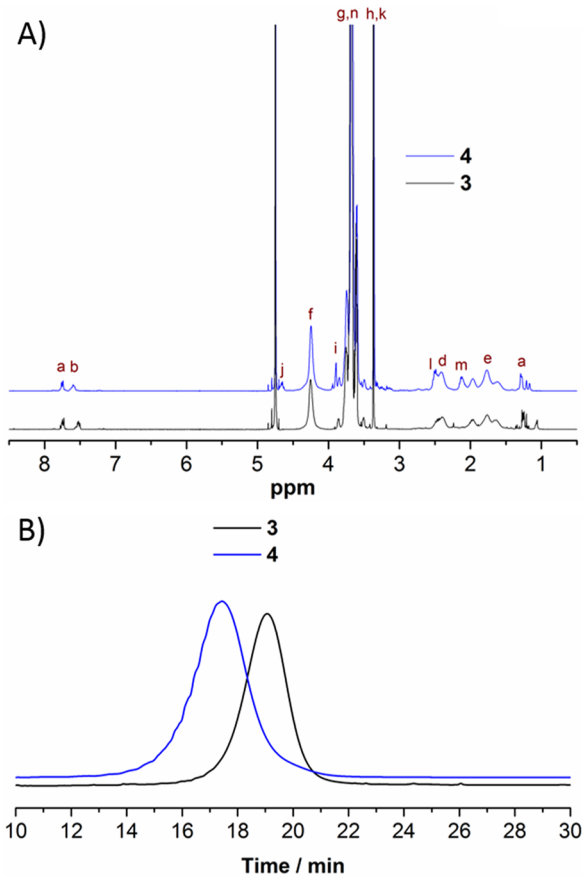

Figure 3. ^{1}H NMR (A) and RP-HPLC (B) data for the polymerization of $PEGA_{480}$ by aqueous SET-LRP **3** (black) followed by post-polymerization modification of the arsenic acid (As(V)) end group using GSH furnishing the bis(GSH)As(III)PPEGA adduct **4** (blue).

at a retention time of 16.9 min. However, the presence of a reduced sCT peak (t = 12.5 min) suggested the conjugation was not quantitative (Figure S13). Increasing the polymer stoichiometry to 2.5 equiv, relative to sCT, resulted in quantitative conjugation as confirmed by the presence of the same conjugate peak (t = 16.9 min), in the absence of any peaks corresponding to native and/or reduced sCT (Figure 4A).

sCT Release from the Peptide−Polymer Conjugate. The release of sCT from its conjugate with arsenical **1** was demonstrated above in the presence of an excess of EDT (Figure 1B). A similar outcome for the sCT-**4** conjugate would provide strong evidence of conjugate formation. Pure conjugate was obtained following extensive dialysis (MWCO 3.5 kDa) against Milli-Q water. The lyophilized product was then dissolved in Milli-Q water and EDT was added. A sample taken after 107 min (Figure 4B) revealed the absence of a conjugate peak. This coincided with the reappearance of a peak corresponding to reduced sCT (t = 12.5 min), and the appearance of new peaks assigned to the thermodynamically favored ethanedithiol functional polymer, (EDT)As(III)-$PPEGA_{480}$ (**5**, t = 23.5 min), and excess EDT (t = 30.5 min). To confirm the identity of polymer **5**, EDT was also added to an aqueous solution of polymer **4**. Characterization by RP-HPLC revealed that the resulting polymer possessed the same retention time as polymer **5** (Figure 4B).

The peptide was also released from the polymer conjugate under more biologically relevant conditions. Reduced lipoic

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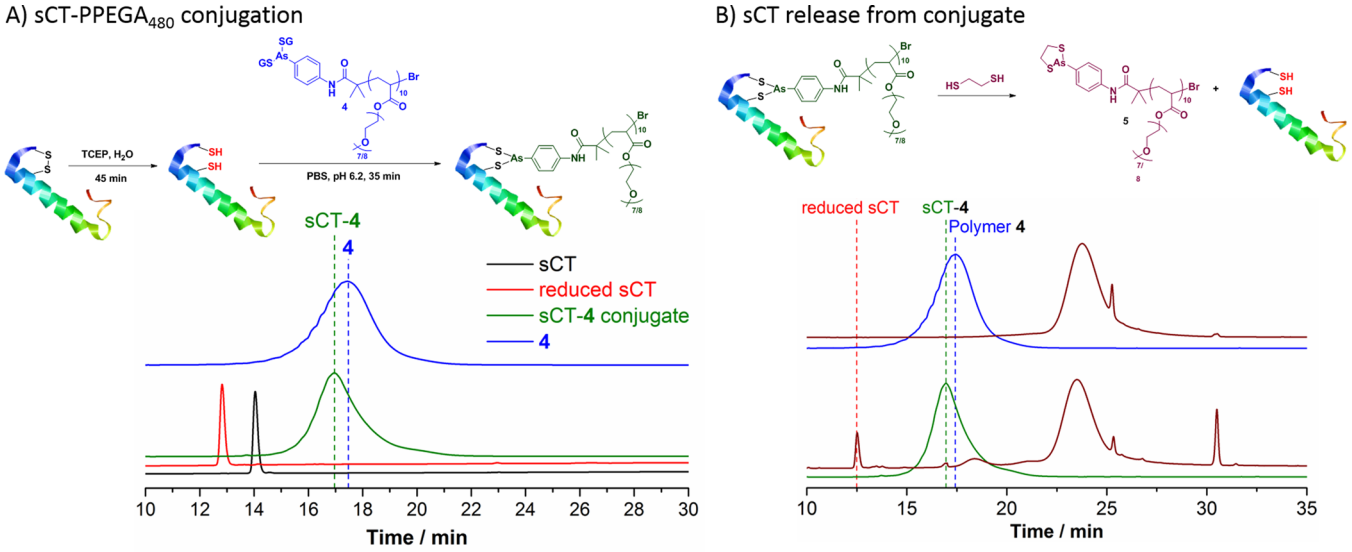

Figure 4. (A) Conjugation of sCT to $(GSH)_2$-As(III)$PPEGA_{480}$ **4** (2.5 equiv) via sequential reduction−conjugation. (B) Release of sCT from sCT-**4** using EDT (bottom spectra) and control reaction of **4** and EDT (top spectra).

acid was synthesized and added to a freshly prepared conjugation mixture. A sample taken after 30 min revealed the formation of a new lipoic acid-polymer conjugate peak which coincided with the reappearance of signals corresponding to native and reduced sCT (Figure S14). The thermodynamically driven release of sCT from its conjugates evokes the potential for targeted and controlled release of the peptide in the presence of biological chelating dithiols, as exemplified using reduced lipoic acid.

CONCLUSIONS

The first example of using organic arsenicals to couple polymers to biomolecules has been described. A trivalent arsenical (**1**) derived from *p*-arsanilic acid was shown to undergo efficient conjugation to the therapeutic peptide sCT. Conjugation proceeds through bridging of the Cys^1-Cys^7 disulfide, according to RP-HPLC and MALDI-ToF-MS, with an efficiency comparable to that of dibromomaleimides, the current gold standard for disulfide bridging. Owing to the development of sophisticated conjugation strategies, site-specificity in protein/peptide polymer conjugation has significantly improved in recent years. Here, we have extended this specificity to include selectivity for bridging disulfide bonds in the presence of free cysteine residues. Thus, organic arsenical **1** demonstrates a significant enhancement in specificity for bridging disulfides relative to other thiol reactive agents, including NEM and DBM). Arsenic functional polymers have been prepared by aqueous SET-LRP, which showed negligible cytotoxicity relative to small molecule, and unfunctionalized polymer controls. Arsenic end-functional $PPEGA_{480}$ has been conjugated to sCT, and it was demonstrated that the peptide can be chemically released from the polymer upon addition of strong chelating agents, such as ethanedithiol. It is believed that this highly specific, reversible approach to bioconjugation can elicit the potential for controlled release applications. Furthermore, the enhanced specificity engenders orthogonality between mono- and disulfide-derived dithiols which can be of benefit to the drug delivery community in the future design of biomaterials.

ASSOCIATED CONTENT

Supporting Information

Experimental details and supplementary schemes, tables, and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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