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Reduction-Sensitive Tioguanine Prodrug Micelles

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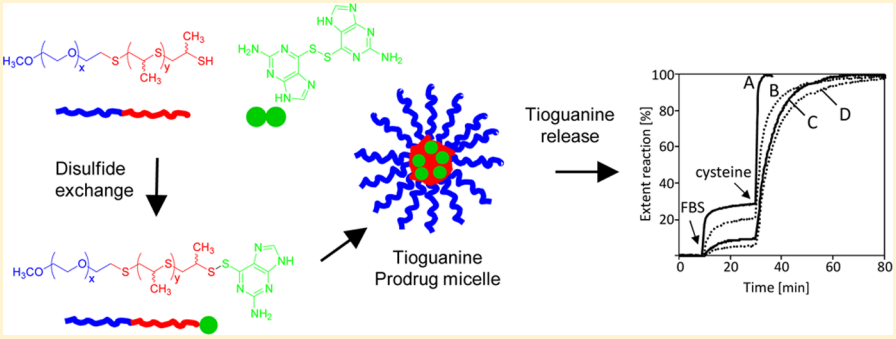
Supporting Information



ABSTRACT: Colloidal drug and prodrug conjugates have unique targeting characteristics for tumor vasculature from the blood and for the lymphatics draining a tissue injection site. Tioguanine and tioguanine-generating prodrugs have been investigated as anticancer and immunosuppressive agents, including use in cancer immunotherapy. Recently we developed block copolymers of poly(ethylene glycol)-*bl*-poly(propylene sulfide) that self-assemble in aqueous solutions to form micellar structures. Since the polymers carry a free terminal thiol group resulting from the ring-opening polymerization of the propylene sulfide monomer, we sought to prepare prodrug block copolymers with tioguanine linked by a reduction-sensitive disulfide bond. The synthesis involved a disulfide exchange between the oxidized form of tioguanine and the polymer. Spectroscopic data is presented to support the proposed reaction. The polymers self-assembled when dispersed in water to form tioguanine prodrug micelles with a size range between 18 and 40 nm that released tioguanine in response to cysteine and serum as shown spectroscopically. In comparison with a poly(ethylene glycol) prodrug polymer, we show that the rate of tioguanine release can be controlled by changing the poly(propylene sulfide) block length and that the tioguanine remains bioactive with cultured cells.

KEYWORDS: *polymeric prodrug, block copolymer, micelle, tioguanine, reduction-sensitive disulfide bond*

INTRODUCTION

Tioguanine (TG) is a drug that has been used in the treatment of acute myelocytic leukemia and has been shown to be active in both animal and human neoplasms.[1] The mechanism of cytotoxicity of TG is believed to be primarily due to its incorporation into DNA and subsequent repair-resistant DNA damage.[2] Although most research has focused on characterizing its biological effects on cancer eradication, TG and its derivatives, as well as the closely related drug mercaptopurine (MP), have also been implicated as an immunosuppressant.[3] For example, TG has also been shown to be useful as an antiarthritic.[4] Interestingly, it has been reported that TG and MP can potentiate active immunotherapy in L-1210 leukemia tumor-bearing mice treated with a concanavalin A-bound leukemia vaccine, related to the abrogation of the effects of suppressor cells.[5] As such, the drug's cytotoxicity is interesting both in direct cancer therapy and in cancer immunotherapy.

One of the problems of using TG as the parent drug is its metabolic inactivation by methylation of the sulfur atom by thiopurine *S*-methyltransferase.[6] A popular way of minimizing this effect has been the design of prodrugs, i.e., compounds that need to be chemically or enzymatically transformed in the body before exhibiting their pharmacological action.[7] Prodrugs of TG mainly involve functionalization of the metabolically unstable sulfur atom. One of the problems still remaining, especially with the use of small molecule prodrugs, is their unfavorable pharmacokinetics due to the rapid diffusion throughout the body, potentially leading to adverse effects. Here, colloidal forms, such as nanoparticle or micelle prodrugs, may offer more promise. Colloidal forms in the blood accumulate in tumors via the inherent leakiness of the tumor vasculature relative to healthy vasculature.[8] Moreover, we have shown that, after intradermal injection, colloidal forms, less than ca. 50 nm in size, rapidly accumulate in the lymph nodes that drain the injection site,[9] offering the ability to target immune cells in a lymph node specific manner.

Recently we developed block copolymers (PEG-*bl*-PPS) composed of hydrophilic block, poly(ethylene glycol) (PEG), and hydrophobic block, poly(propylene sulfide) (PPS). These

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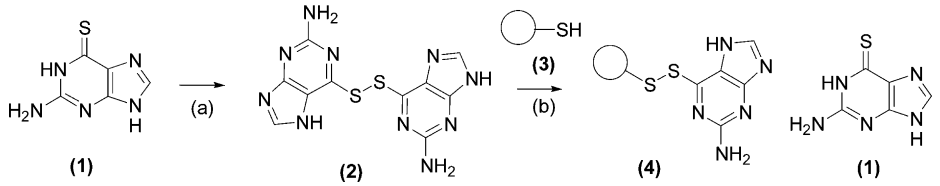
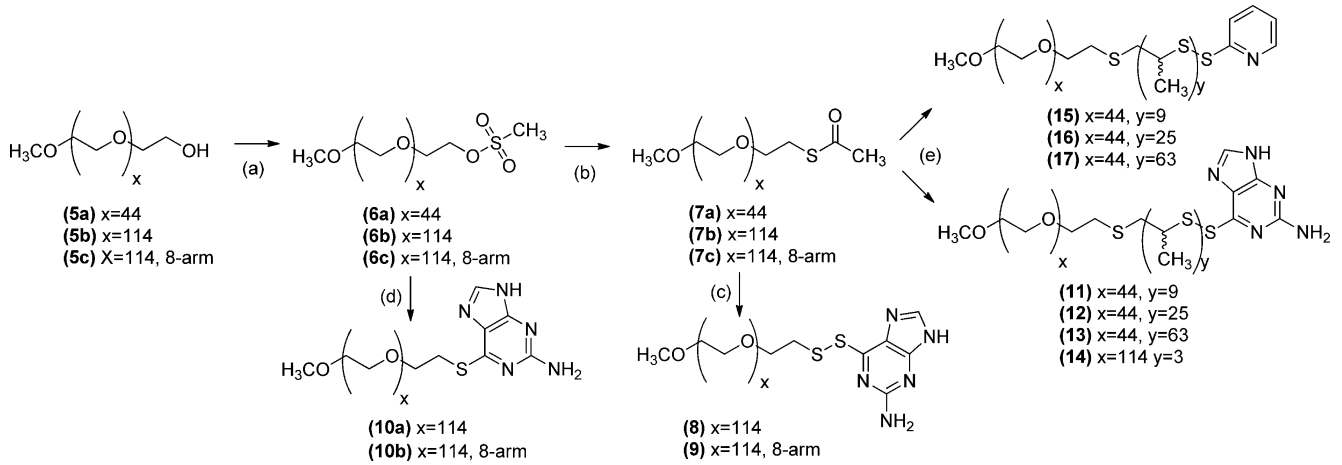
Scheme 1. Approach To Make Reduction-Sensitive Tioguanine (TG) Polymer Conjugates[a]



[a](a) Oxidation of TG (**1**) with I_2 in $NaHCO_3$(aq) leads to tioguanine disulfide (TG-SS-TG) (**2**), which (b) undergoes disulfide exchange with a thiol-containing polymer (**3**) to yield reduction-sensitive polymer-SS-TG polymer conjugates (**4**) with release of TG.

Scheme 2. Synthetic Scheme for Making the Different Polymer−TG Conjugates[a]



[a](a) CH_3SO_2Cl, Et_3N, toluene; (b) $KSCOCH_3$, DMF; (c) AcOH, TG-SS-TG, DMSO for (**8**), (1) $NaOCH_3$, DMF, (2) AcOH, TG-SS-TG, DMSO for (**9**); (d) TG, K_2CO_3, DMF, 80 °C; (e) (1) $NaOCH_3$, propylene sulfide, DMF, (2) AcOH, TG-SS-TG, DMSO for **11**−**14**, or (1) $NaOCH_3$, propylene sulfide, DMF, (2) AcOH, PY-SS-PY, DMSO for **15**−**17**.

block copolymers are made by ring-opening polymerization of propylene sulfide monomers (PS) using PEG-thiolate as a macroinitiator.[10] This ring-opening polymerization yields a terminal thiolate, i.e., PEG-*bl*-PPS-S^-. Oxidation of the terminal thiol group after polymerization or proper end-capping can lead to either ABA (with a central disulfide) or AB block copolymers, respectively. These polymers self-assemble in water to form spherical or wormlike micelles or vesicles, depending on the relative block lengths.[11] With the thiol group present at the end of the polymer immediately after synthesis, we envisioned to covalently attach TG by a reduction-sensitive disulfide bond at this site. This disulfide bond would allow for TG-release upon endocytosis via the reductive cysteine environment in the endo/lysosome.[12]

■ EXPERIMENTAL SECTION

The experimental details can be found in the Supporting Information.

■ RESULTS

Scheme 1 shows our approach in introducing TG (**1**) via a reduction-sensitive disulfide bond by reaction of a thiol-containing polymer (**3**) with the disulfide of TG (TG-SS-TG) (**2**).

Oxidation of TG with I_2 in saturated $NaHCO_3$ (aq) afforded the disulfide (TG-SS-TG), which was characterized by ^1H and ^{13}C NMR and HRMS. TG-SS-TG (**2**) had been reported before,[13] and synthesis using this procedure gave the same analytical results. Both solids showed the same mass by HRMS, and the ^1H NMR spectrum in deuterated DMSO displayed 3 signals at 12.6, 8.0, and 6.4 ppm in a ratio 1:1:2 assigned to the NH, CH, and NH_2 protons.

Reaction of the TG-SS-TG (**2**) with poly(ethylene glycol)-thiol ($mPEG_{114}$-SH), either starting from the thiol directly or made *in situ* from the thioacetate by transesterification with sodium methoxide followed by acidification with acetic acid (Scheme 2), afforded the mixed disulfide $mPEG_{114}$-SS-TG (**8**).

The ^1H NMR spectrum after workup showed the presence of the three characteristic signals of TG, and the triplet at 2.9 ppm, due to CH_2 next to the SH group, had disappeared and a new triplet appeared at 3.1 ppm suggesting the formation of the disulfide linkage. To further support the proposed disulfide exchange reaction, we performed a NMR measurement in which we reacted $mPEG_{114}$-SH with excess TG-SS-TG in acidified deuterated DMSO. As can be seen (Figure S1B in the Supporting Information), the region between 6.0 and 7.0 ppm shows two additional signals at slightly higher values than that of TG-SS-TG at 6.4 ppm (Figure S1D in the Supporting Information). We assign these signals at 6.48 and 6.54 ppm to $mPEG_{114}$-SS-TG and TG, respectively, for which the spectra run under the same conditions are shown in Figures S1A and S1C in the Supporting Information. Further support for the formation of the $mPEG_{114}$-SS-TG (**8**) conjugate comes from UV−vis measurements upon addition of cysteine in aqueous solution.

Figure S2 in the Supporting Information shows the UV spectra in water before and after addition of cysteine. As can be seen, the absorbance maximum at 316 nm shifts to 340 nm with increase of extinction. The measured spectrum is super-

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imposable on that of an authentic sample of TG (data not shown).

^{1}H NMR of the deuterated water solution reveals that the triplet at 3.1 ppm (Figure S3B in the Supporting Information), previously assigned to the CH_2 group next to the disulfide, disappears and a triplet occurs at 2.7 ppm after adding cysteine (Figure S3C in the Supporting Information). This signal is also present for mPEG$_{114}$-SH (Figure S3A in the Supporting Information) and can be assigned to the CH_2 group next to the SH group. At the same time a fraction of cysteine is oxidized to cystine as can be seen by comparing Figures S3C and S3D in the Supporting Information. The integral ratio of the signals at 4.1 ppm, the two CH protons of cystine, and 2.7 ppm assigned to C*H*$_2$SH of about 1:1 shows that mPEG$_{114}$-SS-TG (**8**) has been completely reduced. In the aromatic region, where only the CH proton of TG can be observed due to deuterium exchange of NH and NH_2 with D_2O, the release of TG can be seen by the shift of the signal from 8.16 to 8.08 ppm (Figure S4 in the Supporting Information).

TG release is better visible when the experiment is repeated in undeuterated water and the sample after lyophilization measured in deuterated DMSO (Figure S5 in the Supporting Information). In the region between 5.5 and 8.5 ppm, the tautomeric forms of TG clearly can be seen.[14] The broad signal in Figure S5B (Supporting Information) is due to the $-NH_3^+$ groups of cysteine and cystine. It is to be noted that the tautomeric forms are not observed in Figure 1C (Supporting Information) due to the presence of acetic acid.

It having been shown that the disulfide linkage can be cleaved, cell experiments were done to confirm that TG retained its biological activity. HeLa cells were cultured in the presence of 8-arm-PEG$_{114}$-SS-TG (**9**) of which the synthetic details are shown in Scheme 2. We also prepared the nonreductive sensitive 8-arm-PEG$_{114}$-S-TG (**10b**) having a thioether bond between the polymer and TG as a negative control. As can be seen from Figure 1, only the 8-arm-PEG$_{114}$-SS-TG is able to reduce metabolic activity of HeLa cells as measured by the MTT assay. The loss is due to dying cells that were visible under a light microscope (data not shown). The same cell-toxicity curve was obtained when mPEG$_{114}$ instead of the 8-arm-PEG$_{114}$ was used (Figure S6 in the Supporting Information).

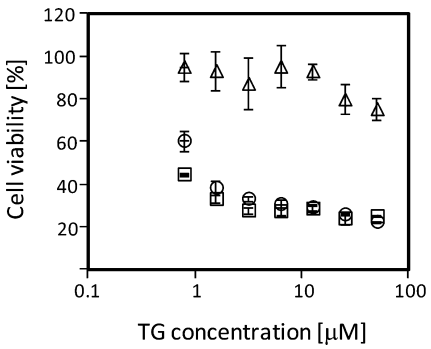

Figure 1. Cell viability data (N = 3) as measured by the MTT assay of reduction-sensitive 8-arm-PEG$_{114}$-SS-TG (squares), nonreductive sensitive 8-arm-PEG$_{114}$-S-TG (triangles), and TG (circles) after culturing with HeLa cells for 1 day show that 8-arm-PEG$_{114}$-SS-TG is bioactive.

Having established the TG conjugation protocol, we extended this approach to block copolymers of mPEG and poly(propylene sulfide) (PPS) polymers. Transesterification of mPEG-thioacetate with sodium methoxide followed by anionic ring-opening polymerization of propylene sulfide (PS), acidification with AcOH, and reaction with TG-SS-TG yielded the mPEG$_{44}$PPS$_x$-SS-TG block copolymers (**11**−**13**) (Scheme 2). These block copolymers self-assembled when dispersed in water from *N*-methylpyrrolidone (NMP) solutions (Table 1).

Table 1. Dynamic Light Scattering Results for the PEG-PPS Micelles and PEG Conjugates in Water

polymer	*Z*-av[a]	PDI[b]
mPEG$_{44}$PPS$_9$-SS-TG	16	0.17
mPEG$_{44}$PPS$_{25}$-SS-TG	17	0.08
mPEG$_{44}$PPS$_{63}$-SS-TG	35	0.12
mPEG$_{44}$PPS$_9$-SS-PY	17	0.16
mPEG$_{44}$PPS$_{25}$-SS-PY	16	0.09
mPEG$_{44}$PPS$_{63}$-SS-PY	34	0.12
mPEG$_{114}$-SS-TG	nd[c]	nd[c]
mPEG$_{114}$PPS$_3$-SS-TG	nd[c]	nd[c]
8-arm-PEG$_{114}$	9.8[d]	0.16[d]

[a]Diameter in nm as measured by the Z-average obtained by fitting the correlation function using the cumulant method. [b]Polydispersity index (PDI) calculated from fitting the correlation function using the cumulant method. [c]Not detected. [d]Was measured in PBS.

For comparison, the pyridyl (PY) terminated polymers (**15**−**17**) were prepared.[15] The TG and PY terminated polymers were characterized by NMR and GPC (Figure S7 in the Supporting Information). From Table 1 and the CMC determination (Figure S8 in the Supporting Information), it can be seen that replacing PY with TG does not affect the micelle formation significantly. As was observed for the 8-arm-PEG$_{114}$, micelle solutions were bioactive as can be seen in Figure 2 where PEG$_{44}$PPS$_{63}$-SS-TG micelles were incubated with the OVA-B16-F10 melanoma cell line. As a negative control, the PY-terminated polymer was included. Clearly, it can be seen that the micelles are able to reduce cell survival in a dose dependent matter. At higher concentration the PY polymer also reduces cell viability.

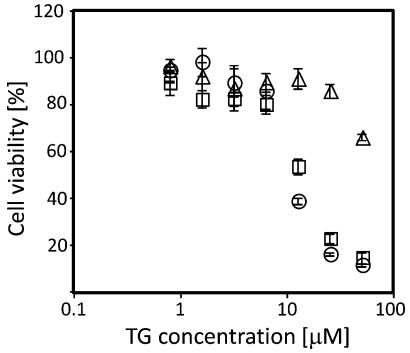

Figure 2. Cell viability data (N = 3) as measured by the MTT assay of reduction-sensitive mPEG$_{44}$PPS$_{63}$-SS-TG (squares), mPEG$_{44}$PPS$_{63}$-SS-PY (triangles), and TG (circles) after culturing with OVA-B16-F10 melanoma cells for 3 days.

To assess the qualitative kinetics of TG release from the polymers and micelles, we measured the increase of absorbance at 340 nm (Figure S2 in the Supporting Information) with time in the presence of cysteine at pH 5 and pH 7.4. As can be seen from Figure 3, TG is released under both near neutral and

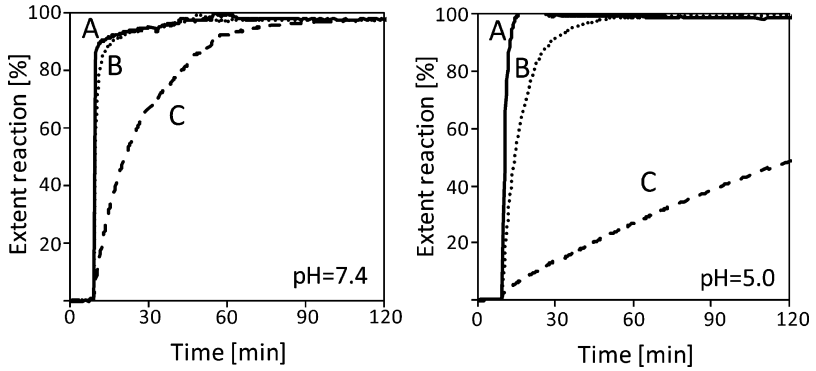

Figure 3. Release of TG as function of time in response to the addition cysteine at pH 7.4 and 5.0: (A) $mPEG_{114}$-SS-TG, (B) $mPEG_{114}PPS_3$-SS-TG, (C) $mPEG_{44}PPS_{63}$-SS-TG.

acidic conditions, and the release is clearly slower for the $PEG_{44}PPS_{63}$-SS-TG micelles at both pH values.

Also for the micelles, the pH has a significant effect on the release of TG, which is not the case for $mPEG_{114}$-SS-TG. This slower release is likely to be due to pH, since the micelles have similar sizes in the different buffer solutions (Supporting Information Table S1). TG release in the presence of 75% fetal bovine serum, which contains proteins with free thiol groups and small amounts of glutathione/cysteine, was also tested, and the same trend was observed (Figure 4). The TG release

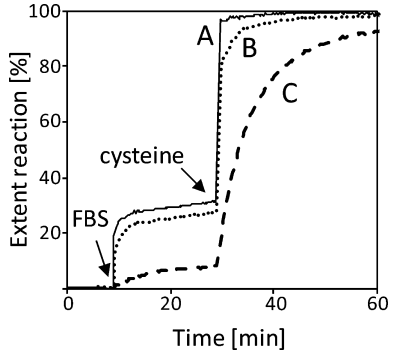

Figure 4. Release of TG as function of time in response to the addition of fetal bovine serum (FBS) and cysteine as indicated by the arrows at pH 7.4: (A) $mPEG_{114}$-SS-TG, (B) $mPEG_{114}PPS_3$-SS-TG, (C) $mPEG_{44}PPS_{63}$-SS-TG.

experiments in serum were done to have an indication about stability of the TG conjugates in the circulation. After the absorbance became constant, cysteine was added to make sure that the different formulations all released the same amount of TG.

From an initial screening using $mPEG_{114}$-SS-TG (**8**) and $mPEG_{44}PPS_{63}$-SS-TG (**13**), we observed a slower release for the micelles than for the PEG (Figure 3). To see whether this difference in release profile was due to the micellar structure, we prepared a $mPEG_{114}PPS_3$-SS-TG conjugate (**14**), which did not form micellar structures as confirmed by DLS, and measured its TG release. From Figures 3 and 4 it can be seen that release is slightly slower than for PEG alone but still faster than for the micelles. We prepared a series of mPEG-PPS block copolymers with different block composition, and as can be seen from Figure 5, TG release can be controlled by changing the block length.

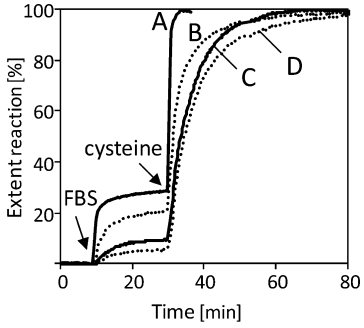

Figure 5. Release of TG as function of time in response to the addition of fetal bovine serum (FBS) and cysteine as indicated by the arrows at pH 7.4: (A) $mPEG_{114}$-SS-TG, (B) $mPEG_{44}PPS_9$-SS-TG, (C) $mPEG_{44}PPS_{63}$-SS-TG, (D) $mPEG_{44}PPS_{25}$-SS-TG.

DISCUSSION

One of the major disadvantages of the use of small drug molecules is their rapid diffusion throughout the body, leading to side effects in tissue that should not be affected. A very powerful approach addressing this issue is the conjugation of the small molecule to a polymeric carrier that can drastically change the pharmacokinetics of the drug.[7b,16] However, for the drug to become biologically active, it needs to be cleaved from the polymeric carrier, either chemically or enzymatically. This so-called prodrug approach has been widely used to improve drug efficacy.[7] For TG, several prodrugs have been synthesized, with some examples given in Scheme 3.

Most of the TG prodrugs exploit the increased levels of cytoplasmic glutathione in cancer cell tissue for regeneration of the parent TG.[3a,4,17] TG-disulfides where R is an alkyl chain (**18**) were among the first compounds to be screened. Thiamiprin (**19**) and AVTG (**20**) are both activated by glutathione, although enzymatic catalysis has been implicated in case of **20**.[4,18] Two reports have appeared on enzyme-activated prodrugs using either β-lyase present in the liver (**21**)[3b] or esterase in plasma (**22**).[19] The latter is the only report that has made polymeric prodrugs by conjugating TG to PEG polymers.

As a part of our own cancer vaccine research, we were interested in using tioguanine as a means to improve vaccine efficacy. There has been a report showing that both TG and the closely related MP can potentiate active immunotherapy in L-1210 leukemia tumor-bearing mice treated with a concanavalin A-bound leukemia vaccine.[5] Using our micelle platform based on poly(ethylene glycol)-poly(propylene sulfide) block copolymers (PEG-PPS), we were interested in whether we could

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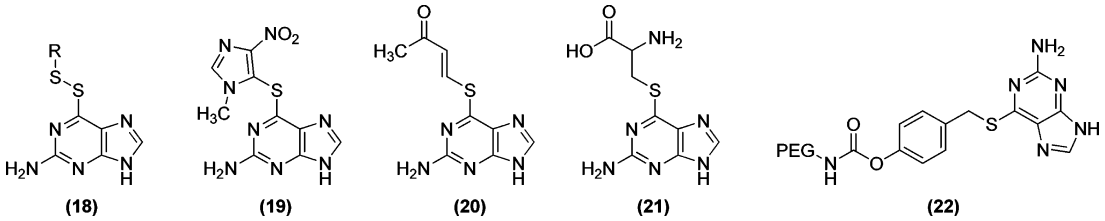
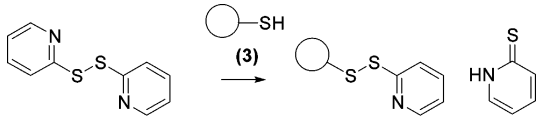
Scheme 3. Prodrugs of Tioguanine That Have Been Reported



deliver TG to a subset of immune cells in the lymph node. Since these polymers carry a thiol group, after anionic ring-opening polymerization of the PS monomer, we investigated whether we could use this group to link TG by a reduction-sensitive disulfide bond (-SS-) to the polymer (Scheme 1). Cleavage of this bond could occur in the reductive environment inside the endo/lysosome after endocytosis.[12] In this way, self-assembly of the PEG-PPS-SS-TG polymers in aqueous solutions gives the TG prodrug micelles. Advantage of this micelle approach is a possible targeting of a defined subset of immune cells that cannot be obtained with the free drug due to its rapid diffusion throughout the body. In addition, small drugs are able to cross cellular membranes, which is different from micelles that can only enter the cell by endocytosis. Since endocytosis is cell-type dependent and cell-type specific, a difference in cellular uptake can be expected.

The key point in preparing TG prodrug micelles was the disulfide exchange reaction of thiol-containing polymers with TG-SS-TG. The compound 2,2′-pyridyl disulfide (**23**) is known to undergo disulfide exchange with thiols with release of pyridinethione (**25**) as shown in Scheme 4. This well

Scheme 4. The Well-Known Disulfide Exchange Reaction of 2,2′-Pyridyl Disulfide with Thiol-Containing Polymers To Give the Mixed Disulfide with Release of Pyridinethione



established chemistry links molecules by a reduction-sensitive disulfide bond.[20] Because of the similar chemical structures of TG-SS-TG and 2,2′-pyridyl disulfide, we reasoned that TG-SS-TG may be able to undergo a disulfide exchange reaction as well.

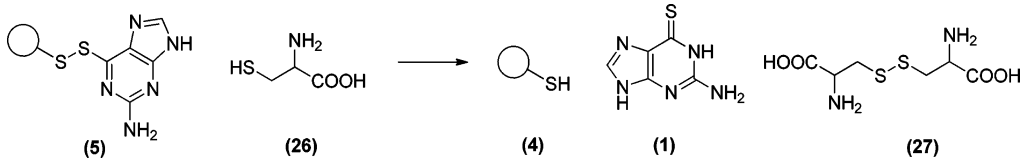
To test our hypothesis, we synthesized TG-SS-TG by oxidation of TG with I_2 in aqueous $NaHCO_3$(aq) and characterized it by ^1H and ^{13}C NMR and HRMS. For the TG-SS-TG reported, these data had been lacking.[13]

As a model reaction for the proposed disulfide exchange reaction, we employed $mPEG_{114}$-SH as the reaction partner. From ^1H NMR spectroscopy we could show convincing data for the proposed exchange reaction, i.e., the formation of $mPEG_{114}$-SS-TG and release of TG. Furthermore using cysteine as a model we showed the regeneration of TG. Of the two main thiols present in the body, glutathione and cysteine, we chose the latter, as it is the only reducing agent present in the endo- and lysosome.[12a] The release experiment at pH 5 shows that in principle TG can be cleaved under acidic conditions; a drop in pH from pH 7.4 to 5 occurs during maturation of the endosome. Diffusion of released TG through the endosomal membrane allows TG to enter the cytoplasm and exerts its biological effect. Based on the NMR and UV data (Figures S2 and S3 in the Supporting Information), the sequence depicted in Scheme 5 for cysteine-induced TG release can be proposed. This reaction is pH dependent as it involves a nucleophilic attack of the thiolate of cysteine onto the disulfide bond. That released TG was still bioactive was shown in Figure 1 by the decrease in cell viability of HeLa cultured in the presence of 8-arm-PEG_{114}-SS-TG. TG release can be induced by biomolecular components in serum and/or cells. We have not attempted to separate intracellular from extracellular release, and the cell toxicity data merely show that TG remains bioactive.

Having established the chemistry, we focused on making PEG-PPS-SS-TG prodrug micelles using the same disulfide exchange chemistry. In order to characterize the TG-containing micelles, we prepared the PY-end-capped conjugates as previously reported.[15] By splitting the polymerization mixture in two and end-capping with either TG-SS-TG or PY-SS-PY, we ensured that both polymers had the same block composition and that any different behavior would only be due to TG. The only marked difference between the TG and PY conjugate was the reduced solubility in tetrahydrofuran (THF): whereas the PY conjugates easily dissolved in THF, the TG conjugates proved to be insoluble. This difference in solubility is caused by the presence of the TG group, and in line with the fact that TG itself is only soluble in highly polar solvents like DMSO. However, employing this solvent for making micelles, gel-like solutions formed when added to water. Therefore we used NMP. As can be seen from Table 1, TG did not affect the sizes of the micelles and all samples were monodisperse.

When measuring TG release in the presence of cysteine, we observed a marked difference between $mPEG_{114}$-SS-TG and $mPEG_{44}PPS_{63}$-SS-TG micelles as shown in Figure 3. This

Scheme 5. Proposed Reaction for Cysteine-Induced Polymer-SS-TG Cleavage



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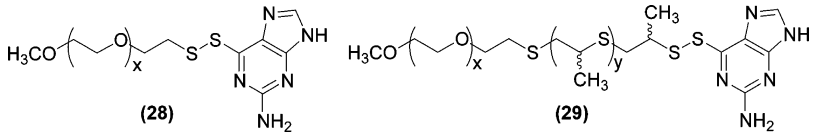
Scheme 6. Chemical Structures of the mPEG-SS-TG Conjugates and mPEG-PPS-SS-TG Block Copolymers Showing the Difference in Disulfide Structure



difference was even more pronounced in serum, as can be seen from Figure 4. Initially, we hypothesized this difference might arise from a difference in disulfide structure as was recently reported for disulfide-linked antibody–maytansinoid conjugates.[21] In the case of $mPEG_{44}PPS_{63}$-SS-TG, the disulfide is attached to a secondary carbon atom. The extra CH_3 methyl group increases the electron density on the disulfide and would explain a lower reactivity due an electronic effect (Scheme 6).

In order to test this hypothesis, we prepared $mPEG_{114}PPS_3$-SS-TG, having a PPS block sufficiently short not to form micelles, yet having the same disulfide structure as present in $mPEG_{44}PPS_{63}$-SS-TG. By dynamic light scattering, we confirmed that $mPEG_{114}PPS_3$-SS-TG did not form micelles (Table 1). Measuring TG release of the polymer in response to cysteine only showed a small decrease in release rate relative to $mPEG_{114}$-SS-TG, but still much faster than the micelles from $mPEG_{44}PPS_{63}$-SS-TG (Figures 3 and 4). This clearly demonstrates that the micellar structure is the main factor responsible for the decrease in release rate of TG.

An explanation for the above-observed behavior is that the hydrophobic core of PPS creates a microenvironment around TG, which excludes water and therefore the highly hydrophilic cysteine. However this explanation can only partially be true because the release data show that, finally, all TG is released. This release can better be explained by the dynamic nature of the micelle, with the TG moiety being sometimes intra- and other times extramicellar. One could interpret the TG release profile as a measure of this dynamic behavior. To investigate whether we could further slow down TG release, we also tested block copolymers with 9 and 25 PS units. Surprisingly the 25 unit polymer showed similar release kinetics as the 63 unit polymer. Since the 25 and 63 unit polymers also show a difference in micelle size by dynamic light scattering, we believe this to be due to a structural difference. However, from a size point of view, micelles of about 20 nm in diameter are preferred over 40 nm in terms of lymph node targeting,[9] and furthermore the amount of drug that can be delivered on a mass basis is higher.

As can be seen from Table 1, the micelles having 9 and 25 PS units form structures with a size of about 18 nm. We included the 8-arm-PEG in our study due to its size of about 10 nm, which gives it some particle-like character and possibly makes its pharmacokinetics different from a simple methoxy-PEG.

From the TG release data at pH 7.4 (Figure 3), it can be concluded that TG release is very fast in the case of $mPEG_{114}$-SS-TG and slower for $mPEG_{44}PPS_{63}$-SS-TG, with a half-life of 12 min. Though a direct comparison is not possible between our TG prodrug micelles and thiamiprin (**19**), it is worth mentioning that the half-life of this drug in the presence of glutathione under pseudo-first-order conditions at pH 6.8 is 18 min.[4] In serum, where TG release is mainly induced by thiol-containing proteins, the prodrug micelles have the advantage over a small molecule like thiamiprin, in that it is more difficult for both partners (the thiol and the disulfide) to interact due to steric hindrance imposed by the PEG layer, as well as slower diffusion of the micelle as compared to thiamiprin. This effect is clearly illustrated by comparing the serum-induced TG release curves of the $mPEG_{114}$-SS-TG and micelles in Figures 4 and 5.

Fast TG release as discussed above is not suited in delivering TG to tumor tissue. In order to accumulate at the tumor site by the enhanced-permeation and retention (EPR) effect,[8] the micelles would have to circulate several hours. We however believe that in our application, where lymph node-residing immune cells will be targeted, the micelles may be useful for two reasons. First, it only requires less than 10 min after intradermal injection for nanoparticles to arrive in the lymph node, a time span that might be short enough to avoid TG release and observe a difference between free drug and TG prodrug micelles. Second, the level of thiol-containing molecules like cysteine, glutathione, and thiol-containing proteins encountered in the complex environment *in vivo* might be completely different from the simple bovine serum-induced release profiles presented in this paper. We are therefore currently exploring the TG prodrug micelles as well as the 8-arm-PEG *in vivo* in mouse models.

■ CONCLUSIONS

We prepared PEG polymers that carry TG bound via a reduction-sensitive disulfide linkage. These polymers were prepared by a previously unknown disulfide exchange reaction between PEG-thiol and tioguanine disulfide. We characterized and presented spectroscopic data to support the proposed disulfide exchange reaction and showed that TG can be recovered by incubating the polymer in the presence of cysteine or serum. Furthermore the polymers were shown to be bioactive in a cytotoxicity assay using HeLa cells. Using the same disulfide exchange chemistry, we prepared block copolymers of poly(ethylene glycol) and poly(propylene sulfide). The block copolymers self-assembled in water to form tioguanine prodrug micelles and, in this form, released TG at a much slower rate than the corresponding PEG polymers. The micelles were able to reduce cell survival using OVA-B16-F10 melanoma cells.

■ ASSOCIATED CONTENT

Supporting Information

Additional experimental details, ^1H NMR and UV spectra of the disulfide exchange reaction and cysteine-induced TG cleavage, GPC charts of block copolymers, DLS data of micelles in different buffer solutions, and CMC determination. 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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