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# A simple method for the preparation of PEG-6-mercaptopurine for oral administration

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**Abstract**—A new and efficient method for the synthesis of PEG-6-mercaptopurine is described. The key feature of the proposed approach is the protection of the thiol group against metabolic inactivation. Preliminary in vivo and in vitro evaluations of the macromolecular prodrug have been carried out.


6-Mercaptopurine [6-MP] is a water insoluble purine analogue, well known for its antitumor and immunosuppressive properties. The drug is used, at the dose of 1.5 mg/kg/*die*, in the treatment of a variety of conditions, including rheumathologic disorders, prevention of rejection following organ transplantation, and inflammatory bowel disease.

However, oral absorption of 6-MP is erratic with only 10–50% of the administered dose reaching the systemic circulation. The limiting factors in the use of 6-MP are its very short half-life in plasma (0.5–1.5 h) and its very variable bioavailability (about 16%). Strategies to circumvent this problem have included the use of azatioprine [AZA, Imuran®, dose of 2.5 mg/kg/*die*], a low molecular prodrug of 6-MP containing an imidazole group attached to the sulfur at the 6-position of the purine ring. This substitution serves to decrease the rate of enzymatic and non-enzymatic inactivation. In the blood AZA is converted to parent drug by non-enzymatic attack of sulfhydryl-containing compound, such as glutathione, on the sulfide bond between the purine and the imidazole ring of AZA. 6-MP is further metabolized via three pathways. Two pathways metabolize 6-MP to presumed inactive metabolites: 6-thiouric acid via xanthine oxidase and 6-methyl mercaptopurine via thiopurine methyl transferase. 6-MP is also metabolized to the presumed active metabolites, the 6-thioguanine nucleotides via hypoxanthine phosphoribosyl transferase and other enzymes.[1] The 6-thioguanine nucleotides are also responsible for the cytotoxic side effects associated with this drug. Common problems encountered with the use of 6-MP, like low solubility and rapid inactivation, could be overcome by PEGylation. PEGylation defines the modification of a molecule by the covalent linking of one or more poly(ethylene glycol) [PEG] chains. PEG is non-toxic, non-immunogenic, non-antigenic, highly water soluble, and FDA approved. The PEG–drug conjugates have several advantages: a prolonged residence in body, a decreased degradation by metabolic enzymes and a reduction or elimination of protein immunogenicity.[2]

Prodrugs are often biologically inert or substantially inactive forms of the parent or active compound. The release rate of active drug, typically by hydrolysis, is influenced by several factors, but especially by the type of bond joining the parent drug to the modifier. Incorporating a polymer, like PEG, as part of a prodrug system has been suggested to increase the circulating life of drug. Studies on PEG-based double prodrug of 6-MP using a benzyl elimination [BE] system for controllably delivering of parent drug in vivo were reported.[3]

Protected sulfur-linked poly(ethylene glycols) are also known, although these form polymer–drug conjugates via covalent disulfide bonds, not via covalent thiol bonds. By utilizing the sulfhydryl bond as the basis for linkage, it is possible to take advantage of the reactivity of such linkages to plasma enzymes in vivo.

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An alternative of the double prodrug strategy is the use of a simple chemical approach, herein described, that enables the protection of the thiol group of 6-MP by the formation of a thioether bond. $PEG_{2000}$ was used for the synthesis of the 6-MP prodrug. The hydroxylic groups of the polymer were activated to chloro derivative by an excess of thionyl chloride (Fig. 1).[4] The attachment of 6-MP to chlorinate polymer was performed by means of cesium fluoride and Celite (521) [CsF–Celite] in ACN with a good yield. The CsF–Celite assisted coupling of aliphatic and aromatic thiols with various alkyl, acyl, benzyl, and benzoyl halides resulted in thioethers and thioesters.[5]

In a typical reaction to a mixture of 6-MP and CsF–Celite in ACN, PEG-$Cl_2$ was added (Fig. 1). Then the mixture was stirred at reflux up to completion of the reaction, indicated by TLC monitoring. The product was purified by extraction with $CH_2Cl_2$/$NaHCO_3$ and recrystallized from EtOH.[6]

In order to gain some preliminary information about the potential use of PEG-mercaptopurine as a drug delivery system, the conjugate was subjected to hydrolysis at 37 ± 0.1 °C in buffer solutions at physiological pHs, simulated gastric juice, pH 1.2, endosomial compartments, pH 5.5, and extracellular fluids, pH 7.4, and in plasma. Samples were regularly taken out of the incubation mixture and the quantity of released mercaptopurine was quantified with the HPLC method.[7]

The PEG-6-MP conjugate was freely water soluble at room temperature, stable in aqueous solutions at various pHs (Fig. 2) and in vitro plasma dissociation assay (Fig. 3), indicating that 6-MP is very little released from conjugate by non-enzymatic hydrolysis.

To evaluate the biological performance of the adduct, a physiological solution of PEG-6-MP was orally

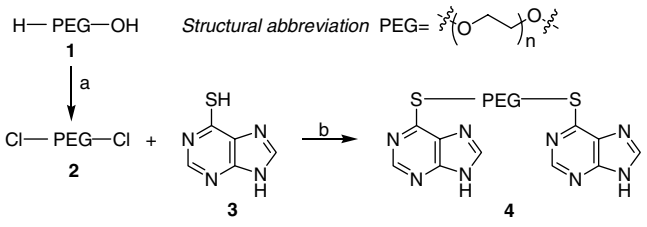


**Figure 1.** Synthesis of PEG-6-MP prodrug. (a) $PEG_{2000}$ (1 equiv), $SOCl_2$, (3 equiv), $CH_2Cl_2$, reflux under nitrogen, 5 h, 96%; (b) 6-MP (1.1 equiv), $PEG_{2000}$-$Cl_2$ (1 equiv), CsF–Celite (1.5 equiv), ACN, reflux, 48 h, 85%. TLC: isopropanol–MeOH–$H_2O$–$NH_4OH$ (60/22/20/1 v/v), $R_f$ = 0.80.

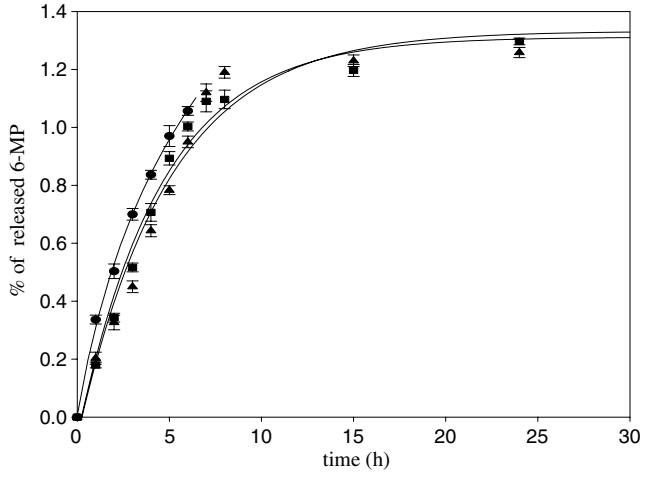


**Figure 2.** 6-MP release profile from PEG-6-MP at pH 1.2 ●, simulated gastric juice; pH 5.5 ■, endosomial compartments; and pH 7.4 ▲, extracellular fluids. Each value is mean ± SD for three experiments. Ten milligrams of PEG-6-MP (1.34 mg of 6-MP) was dissolved in 1 ml of each buffer solution and samples were taken at suitable intervals. Buffer solutions: pH 1.2: HCl/NaCl 0.1 M; pH 5.5: $CH_3COOH$/$CH_3COONa$ 0.1 M; pH 7.4: $KH_2PO_4$/$K_2HPO_4$ 0.1 M.

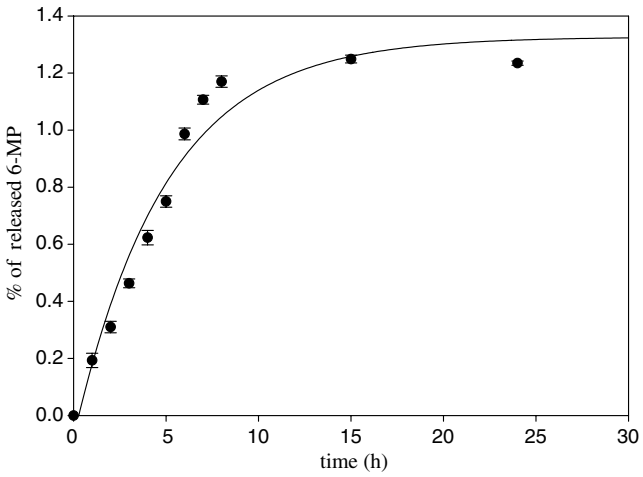


**Figure 3.** Stability of the PEG-6-MP in plasma. Each value is mean ± SD for three experiments. Ten milligrams of PEG-6-MP (1.34 mg of 6-MP) was dissolved in 1 ml of plasma and samples were taken at suitable intervals.

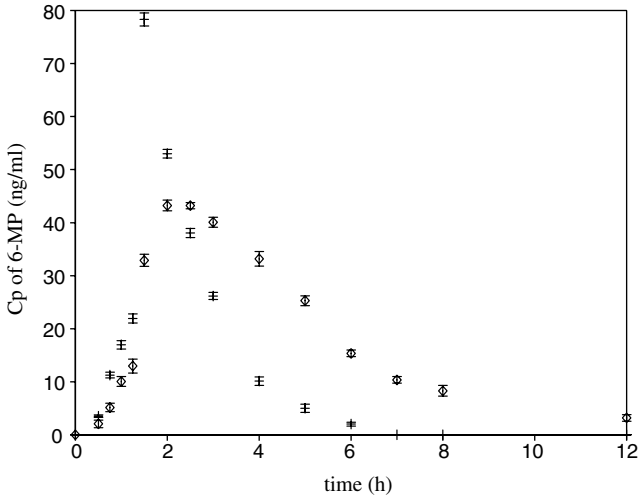


**Figure 4.** Concentration–time curve for 6-MP following 100 mg of AZA [+] (55 mg of 6-MP) or 410 mg of PEG-6-MP [◊] (55 mg of 6-MP) administered orally to a medication-free, non-smoking, healthy European subjects. Each value is mean ± SD for three administrations. AUC for AZA: 128 ng/min/ml; for PEG-6-MP: 206 ng/min/ml.

administered to three human subjects. The same voluntaries were treated in the same way with a table of azathioprine as control drug. The blood samples were taken at regular time intervals and centrifuged. The plasma was stored at −80 °C until analysis. The 6-MP was extracted from plasma by SPE method and quantified by HPLC.[7,8]

Figure 4 is a plasmatic concentration–time curve for 6-MP following oral administration of 100 mg AZA and 410 mg PEG-6-MP (each 55 mg 6-MP) to three healthy subjects. It is interesting to notice that 6-MP administration in the shape of PEG prodrug allows for therapeutic levels for larger period than in the case of AZA administration. In fact, AZA gave a plasma concentration of 6-MP that immediately increased after administration, but decreased with time. On the contrary, the plasma concentration of drug administration of its polymeric derivative was initially lower, when compared with the administration of AZA, but decreased more slowly with time. The 6-MP plasma concentration after administration of the polymeric prodrug is initially lower when compared with the administration of AZA, but decreases very slowly with time. Two different processes contribute to the different blood levels of 6-MP. The first is the passage of the AZA and PEG-6-MP from the intravascular space into the tissue and cells. The second is the faster degradation of the AZA by reductive cleavage of the thioether bond than that of PEG-6-MP. The area under the curve [AUC], calculated by trapezoidal rule, of 6-MP for PEG prodrug was over 1.5 times in comparison with the AUC for AZA, suggesting a better bioavailability of drug from the PEG-6-MP.

In this paper, the synthesis of a polymeric mercaptopurine conjugate was described. Mercaptopurine was covalently linked to the activated macromolecular carrier, poly(ethylene glycol), via thioether formation. The simplicity, sensitivity, and rapidity of this synthesis allow it to be easily adapted for prodrug use.

The PEG-mercaptopurine prodrug showed some interesting peculiarities which make it attractive in the drug delivery for immunosuppressive treatment. It was very soluble in water and stable in physiological buffer, but, in vivo, it was able to release drug in a constant and effective manner.

## References and notes


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6. $^1$H NMR ($CDCl_3$): $\delta$ = 3.46–3.70 ppm (br s; $OCH_2CH_3$), 3.80 (t; 4H, $S–CH_2$), 8.22 (s; 1H, C*H* aromatic), 8.73 (s; 1H, C*H* aromatic), 13.6 (br s, 1H N*H*).
7. Chromatographic conditions. Column: 250 × 4.4 mm ID, packed with RP C18, 5 μm. Flow rate: 1 ml/min. Detection: 332 nm. (a) Mobile phase for analysis in buffer: water–methanol–acetonitrile (73/23/4 v/v) pH 2.5.; (b) Mobile phase for analysis in plasma: water–methanol–acetonitrile (84/14/2 v/v) pH 2.5.
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