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An approach to heterobifunctional poly(ethyleneglycol) bioconjugates

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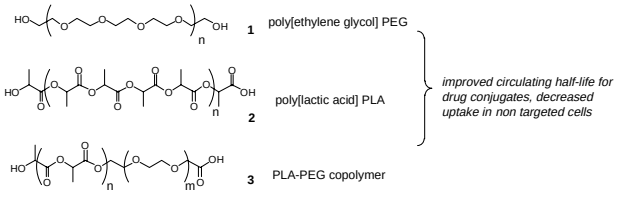
Abstract—A family of differentially substituted poly(ethyleneglycol) building blocks has been assembled from commercially available material. Their utility is demonstrated by formation of amino acid conjugates, image contrast agents, gold nanoparticles, and functional antibody conjugates. Application in the cellular trafficking of antitumoral agent conjugates is expected.

One of the goals of targeted drug delivery is to enhance uptake at the desired site of action using the minimum effective dose of agent.[1] In the case of many solid tumors, it has been shown that derivatizing or encapsulating drugs with polymers including poly(ethyleneglycols) **1** can greatly improve their circulating half-lives and in some cases enhance uptake in tumors by exploiting the leaky vasculature.[2] This strategy can also help overcome immunogenic reactions to protein based drugs, and is currently being employed in a clinical trial of an enediyne–chromoprotein complex, with encapsulation in a block-copolymer.[3] In the field of prostate cancer, liposome encapsulated drugs have been explored as have polymer coupled agents. One of these, a poly(ethyleneglycol) (PEG) linked doxorubicin, has shown enhanced efficacy.[4]While PEG derivatives



continue to be evaluated,[2] there is considerable interest in related polymers including lactic acids (**2**) and hybrids, e.g., the PEG—polylactic acids (PLA, **3**), a nanoparticle conjugate of which has been shown to adhere to prostate membrane specific antigen (PMSA) expressing cells when coupled to aptamers that bind PMSA.[5] Accordingly, there is considerable interest in derivatives of reagent-grade polymers, which can be readily tailored for specific purposes, e.g., attachment of aptamers, fluorescent imaging agents, antibodies, and motifs to enhance nuclear uptake.[6]

Our initial objective was to demonstrate a route to bifunctional building blocks commencing with commercially available starting materials. An uncapped 1.5K average molecular weight PEG diol **4** (Aldrich) was selected and subjected to desymmetrization (Scheme 1). Bis tosylation to **5** can be avoided by use of silver oxide/KI, and the resulting monotosylate was converted to thioacetate **6** via nucleophilic displacement with the potassium salt.[7] Compound **6** proved highly versatile for production of numerous derivatives, the thio group selected on the basis of potential adduction to surfaces using established thiol chemistry.[8] Conversion to the corresponding mesylate of **6** proved more efficient than the tosylate, allowing azido displacement to give **7**, which may have application as a photoaffinity tag. Interestingly, microwave displacement (CEM Navigator system) was highly efficient giving an 80% yield of product

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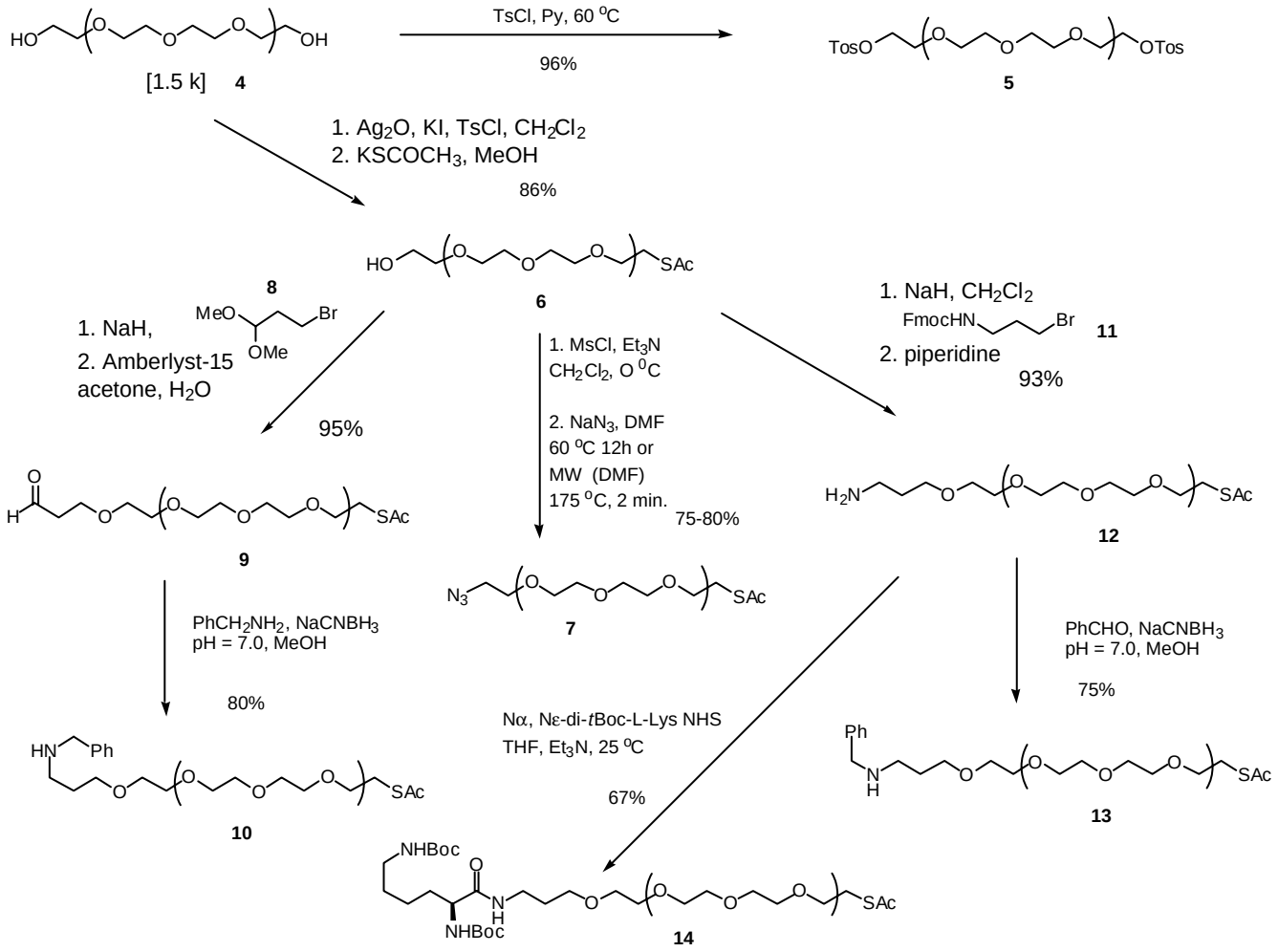
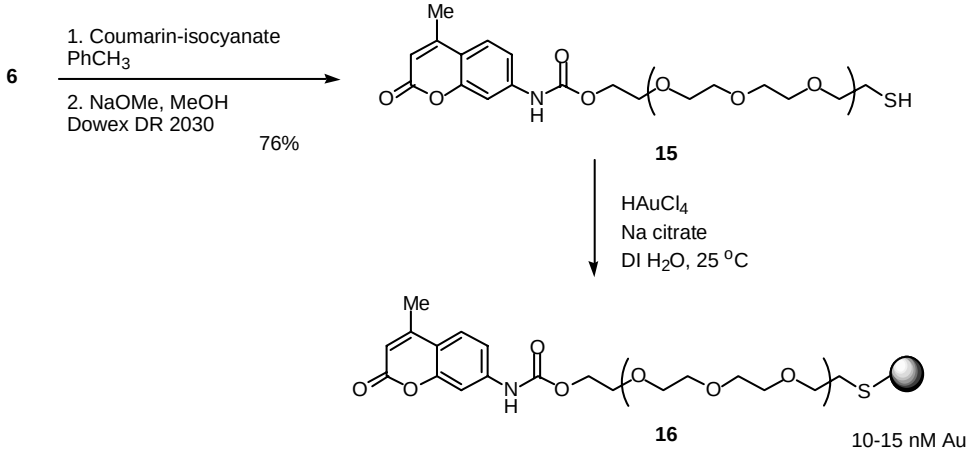


Scheme 1. Preparation of bifunctional PEG building blocks.



Scheme 2. Preparation of coumarin and (Au) nanoparticle conjugates.

within 2 min. To introduce carboxaldehyde functionality, addition of bromoacetal **8** to the alkoxide salt of **6** was conducted and then the aldehyde was unmasked using Amberlyst resin. Product **9** was subjected to specimen reductive amination, benzylamine giving amine **10** in good yield without compromise of the thioacetate group. Despite numerous efforts, attempted Staudinger reduction of azide **7** was unsuccessful,[9] thus we sought alternate means to introduce amine functionality. Remedy was found by alkylation with bromide **11**, which in

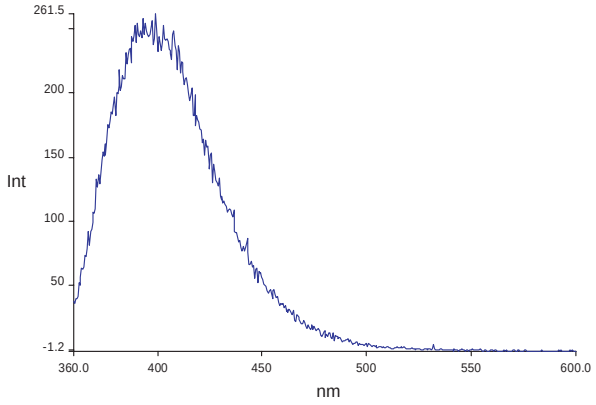


Figure 1. Fluorescence of 16.

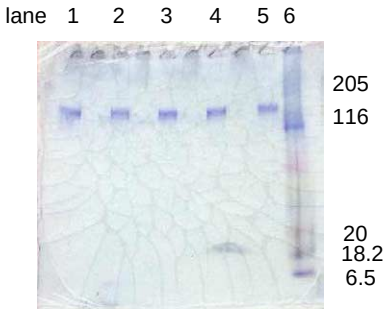


Figure 2. Coomassie stained gel of IgG adducts. Goat anti-mouse IgG (lanes 1–3), adducts **17** and **18** (lanes 4 and 5) and MW markers (lane 6).

unmasking gave reagent **12**. Complementary reductive amination using **12** was achieved, benzaldehdye giving product **13** in good yield. More significantly, peptide bond formation from **12** was also achievable, a protected lysine derivative giving adduct **14** in good yield via use of the activated NHS ester. With seven different derivatives accessible, we turned attention to the production of bioconjugates and sought to demonstrate the utility of the masked thiol terminus for conjugation to biocompatible nanocarriers. Substrate **6** was coupled with a coumarin derivative, and the product subjected to careful deprotection (Scheme 2). The resulting thiol (**15**) was coupled with freshly prepared gold nanoparticles, prepared from reduction of $HAuCl_4$ using de-ionized citrate solution.[10] The product conjugates **16** (approx. 10–15 nM based on Coulter analysis)[11] were re-suspended and subjected to fluorescence assays. The coumarin label showed characteristic properties (λ_{Ex} 326 nm, λ_{Em} 396 nm, Fig. 1), and permitted cellular visualization assays, which confirmed trafficking into cytosol using phase contrast microscopy (Keck 3D fusion microscope). Given the apparent ease with which gold nanoparticles can be functionalized using thiol chemistry, coupled with their bio-compatibility, we anticipate many applications of this strategy will be forthcoming.[10]

The next objective was to demonstrate attachment to antibodies.[12] Accordingly, derivative **9** was successfully subjected to reductive amination with a commercially available goat anti-mouse antibody (Scheme 3). The resulting adduct **17** was then converted to a labeled substrate by unmasking the terminal thiol group and coupling with an activated fluorescein derivative, both of which proceeded without incident. The adduct **18** displayed excellent fluorescence characteristics, permitting cellular affinity studies. Spectroscopic analysis revealed a ratio of 2 moles of labeled PEG per antibody (based on IgG_{abs} 280 nm and F5M E_{max} of 73,000 at 494 nm). Additionally, the competency of the antibody conjugates could be verified by conventional gel (Coomassie stain, Fig. 2) and by Western assay using an anti-FITC rabbit IgG-HRP.[13]

In summary, a series of functionalized PEG building blocks bearing reactive functionality have been assembled. Their utility has been demonstrated in the preparation of bioconjugates and image contrast agents. Application of this chemistry in drug carrier conjugates is an immediate and ongoing priority which will be reported in due course.[14]

Acknowledgment

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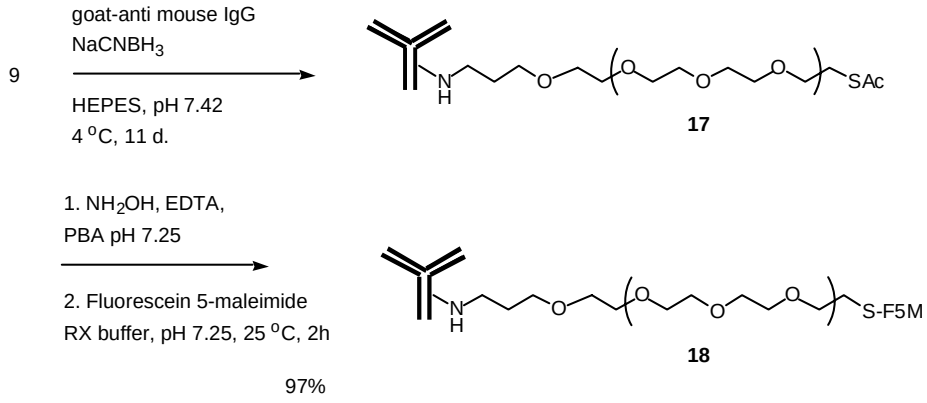

Scheme 3. Preparation of heterobifunctional Mab-PEG derivatives.

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