Abstract

Functionalized PEG derivatives are finding ever-increasing applications in the areas of pharmaceuticals and targeted drug delivery by their judicious incorporation into nanoparticle-forming surfactants and delivery vehicles. As such, the development of bifunctional PEG azide derivatives that possess terminal hydroxyl-, carboxylate- methoxyl- and optionally protected-amine moieties and that can be utilized to quantify their level of azide substitution in our intermediates.

In this paper, we describe the synthesis of a range of novel mono- and bifunctional azido-PEGs (1), their uncatalyzed click reactions with four dipolarophiles, and identification of optimal reaction conditions to afford triazoles (2), whose 1H-NMR spectra can be conveniently utilized to quantify their level of azide substitution.

Discussion

Click reactions have found widespread utility in both chemistry and biology applications.1 The [3+2] cycloaddition reaction between azide and alkynyl activated alkyne moieties generally proceeds under mild thermal or metal-catalyzed conditions and produces stable triazole linkages that can be used to quantitate their level of azide substitution. Simple integrals for click adduct are low, and thus we envisioned use of a click reaction to form substituted triazole derivatives.

Monitoring of the PEG azide reaction and determination of final product purity is oftentimes problematic since TLC or HPLC analysis may fail to adequately resolve the reaction constituents and the 400 MHz 1H-NMR chemical shift of the newly formed PEG-CN, N3 to overlap with the 13C satellites (Table 1). This issue is especially pronounced with the higher molecular weight (i.e., 10-20 K) PEG analogues that have larger satellites. In order to overcome this limitation, we sought to identify a simple, rapid, and robust protocol that would afford products whose 1H-NMR signals were distinctly different and shifted far away from the massive PEG proton manifold. To this end, we envisioned use of a click reaction to form substituted triazole derivatives.

In our model studies, we surveyed a range of solvents and conditions for copper-free click reactions with mPEG-N3 3.9K (% type 3) with four dipolarophiles: dimethyl and diethyl acetylenedicarboxylate (DMAD, DEBD, respectively), and diphenyl and phenylethynylphosphonate (DPA, PA, respectively).2 The former clickers both worked well while the latter alkyynes did not react without catalysis (Table 1). We chose DEBD as the preferred dipolarophile due to its high reactivity, low cost, and elimination of the regiosubstitution issue associated with use of unsymmetrical alkyne in this reaction. Note that yields are not optimized as we removed aliquots for NMR reaction monitoring.

With suitable mild reaction conditions in hand, we continued our survey using DEBD as the dipolarophile. Several additional examples of PEG azides selected from classes (4) to (7) were used and the results are shown in Table 2. All azido reactants were precipitated from CH2Cl2/Et2O 1.15 to 2.0. The precipitated 2K from larger PEG azides (18-20K, 1630-1830 PEG-H) are best analyzed by 600 MHz 1H-NMR, which results in both greater SN ratio and quantitation accuracy.

With this general synthetic approach to our functionalized PEG azides, both mono- and di-functional PEG azides have been synthesized in good overall yield. The 400 MHz 1H-NMR spectra of the resultant click adducts show several distinct new signals clearly resolved from the PEG proton manifold, typically observed at δ 3.35-3.65 ppm. To this end, we optimized 1H-NMR procedure for PEG azide quantitation. (opt. THF, NaHCO3, ~1 atm, 18-36 hr)

For copper-free click reactions with mPEG-N3 3.5K (% type 3): 1. Toluene, 50-60°C, 4 h, (~100% convn); no rxn with DPA (uncatalyzed) in CH2Cl2/Et2O 1.15 to 2.0. The precipitated 2K azides were isolated/ppt’d. The precipitated 2K was purified by silica gel flash column chromatography (FCC) and in good overall yield.

Table 1: Synthesis of Poly(ethylene glycol) Azides

<table>
<thead>
<tr>
<th>Substitution</th>
<th>Reaction Conditions</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Toluene, 50-60°C, 4 h</td>
<td>(~100%)</td>
</tr>
<tr>
<td>2</td>
<td>CH2Cl2/Et2O 1.15 to 2.0</td>
<td>~1 atm, 18-36 h</td>
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Table 2: Further Examples of PEG-N3 Click Reaction

<table>
<thead>
<tr>
<th>Substitution</th>
<th>Reaction Conditions</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Toluene, 50-60°C, 4 h</td>
<td>(~100%)</td>
</tr>
<tr>
<td>4</td>
<td>CH2Cl2/Et2O 1.15 to 2.0</td>
<td>~1 atm, 18-36 h</td>
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Procedure for PEG Azide Quantitation

1. To a 10% w/w solution of PEG-N3 in ethanol was added DEBD (2-3 eq.); the solution was stirred under argon at ambient RT or heated as described in Table 1.

2. Monitor reaction progress in CD3CN (also D2O, CH3OH) by the appearance of new, distinct triazole-derived signals and the disappearance of the PEG-CN, N3 methylene proton signals, which may be distinct or overlap with the right-side PEG 13C satellites. Careful integration and peak subtraction essential!

3. Workup: Evaporate reaction solvent, pump residue in vacuo, zpt’ed from CH2Cl2/Et2O mixtures, EtOAc washing, vacuum drying.

4. Run NMR (CD3CN) and measure 1H integration for the new and/or distinct signal, take the sum, and divide by total integrals used, to afford average % substitution. Repeat twice for n = 3.

Conclusions

• Prepared a range of novel mono- and bifunctional PEG azides (1-7) and surveyed their uncatalyzed click reactions.

• Identified DEBD as the preferred dipolarophile and optimal reaction conditions to afford triazoles (2).

• 1H-NMR (400-600 MHz) spectra of the resultant click adducts (2) show several distinct new signals that can be used to quantify the level of azide substitution in our PEG derivatives.

• Scope and Limitations:

  • Preferred sodium acetate, chloroform (CDCl3), water (D2O).
  • Alkynyl azides (MoD, EDH) are generally satisfactory but slowly react with DMAD and DEBD, need to use excess dipolarophile with slower reacting PEG-N3 substrates.

  • DPA and PA do not participate in an uncatalyzed click rxn.

  • PEG substrates contain an unpurified amino group from complex mixtures of Click and Michael addition-derived byproducts, which are inseparable.

  • In accessible and THF, unreacted N3-PEG-G is afforded the desired triazole as the major product but also appear to produce a minor inseparable byproduct. Use of chloroform eliminates this issue.

References