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SCIENCE DIRECT
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 2875–2878

Dual side-reactions limit the utility of a key polymer therapeutic precursor

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Received 29 November 2005; revised 12 February 2006; accepted 23 February 2006
Available online 10 March 2006

Abstract—In contrast to literature reports, the activated polyacid poly(methacryloxysuccinimide) reacts with nucleophiles to give, initially, a high proportion of ring-opened residues. This copolymer then reacts intramolecularly to form a polymer with a high fraction of glutarimide residues. These side reactions occur to such an extent as to preclude the use of poly(methacryloxysuccinimide) as a precursor to polymethacrylamides.

The use of polymeric delivery systems has been shown to improve the pharmaceutical characteristics of many cancer chemotherapeutics,[1] with a number of liposome- and poly(ethylene glycol)-based drugs having been approved for clinical use in the past decade.[2] Another class of polymer therapeutic, of type **1** (Fig. 1), is based on poly[*N*-(2-hydroxypropyl)methacrylamide] (pHPMA), a biocompatible polymer originally developed as a plasma expander.[3] The prototypical doxorubicin-carrying copolymer PK1 (**1**, Biolinker = glycylphenylalanylleucylglycine, Drug = doxorubicin), shows improved anticancer activity and greatly reduced cardiotoxicity compared to free doxorubicin,[4] and is currently in Phase II clinical trials.[5] Several other pHPMA-based conjugates are currently at varying stages of development.[6]

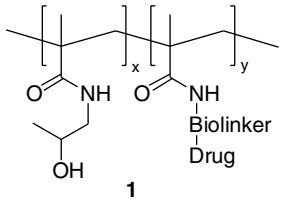

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Figure 1. General form of pHPMA polymer therapeutics.

The preparation of these conjugates has typically been carried out by chemical modification of a pHPMA-based copolymer formed by free radical polymerization;[7] however, this was improved with the publication of a report utilizing a poly(methacryloxysuccinimide) (pMAOS, **2**) precursor (Scheme 1).[8] In this approach, the precursor is reacted with amine-containing drug components and, if desired, targeting residues. The remaining active ester sites are then quenched by reaction with excess 1-amino-2-propanol (1A2P); the reaction progress is followed using FTIR to monitor the disappearance of the active ester imide band at 1735 cm^{-1}. By allowing a single precursor to be used in the preparation of conjugates with variable levels of drug and/or targeting moiety incorporation, this approach has the potential to greatly simplify the preparation of families of polymer therapeutics. Furthermore, a more chemically homogeneous polymer, with low polydispersity, is ensured through the use of controlled radical polymerization.[8,9]

As part of a program dealing with the incorporation of marine natural products into polymer therapeutics, attempts were made to utilize this chemistry; however, initial efforts to introduce suitably functionalized drugs to **2** gave considerably less than satisfactory results. As a consequence, the conversion of **2** to pHPMA has been

Keywords: pMAOS; pHPMA; NHS ester; NMR spectroscopy; Structure elucidation; Chemoselectivity.
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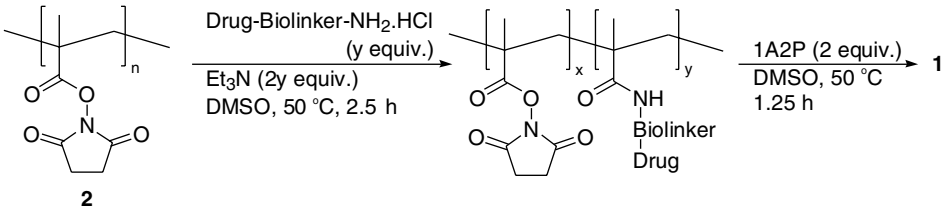
doi:10.1016/j.tetlet.2006.02.131



Scheme 1. Reported synthesis of polymer therapeutics, **1**, from **2**.

examined in more detail, and two dominating side reactions that severely limit the utility of **2** have been uncovered. Specifically, facile aminolytic ring opening of the polymer-bound succinimide moieties, as well as slower glutarimide formation through attack of an amide on a neighbouring activated ester have been observed.

When **2** ($M_n = 30$ kDa, polydisperity = 1.36) was reacted with 1A2P for 3 h at 50 °C, the polymeric product gave an unexpected signal, belonging to neither **2** nor pHPMA, at 2.5 ppm in the ^{1}H NMR spectrum. Analysis of this product by 2D NMR techniques (HSQC-DEPT and CIGAR) established that a significant degree of ring opening of the *N*-hydroxysuccinimide (NHS) moieties through attack by 1A2P at an imide carbonyl had occurred, to give copolymer **3** (Scheme 2), rather than the anticipated complete displacement of NHS by attack at the ester carbonyl. Signal integrals indicated that ~60% ring opening had occurred, and subsequent experiments with differing reaction conditions invariably gave ring-opened copolymers, with 50–65% ring opening. There are existing reports, albeit few, of NHS-activated esters undergoing ring-opening reactions in cases of high steric congestion of the ester carbonyl group or the incoming nucleophile.[10–12]

When significantly higher reaction temperatures or longer reaction times were employed, for example, 70 °C for 3 h or 50 °C for 24 h, water-insoluble polymer products were isolated. The formation of water-insoluble polymers from the reaction of pMAOS with ethanolamine has previously been reported, and was attributed at the time to ester cross-links formed by polymer-bound ethanolamine hydroxyl groups displacing a second NHS group.[13] Examination of the polymeric product by ^{1}H NMR spectroscopy, however, indicated this was not the case. Again, 2D NMR experiments (COSY and HSQC-DEPT) were employed to elucidate the structure of this polymer as **4** (Fig. 2), free of any hydroxamate ester moieties. The formation of **4** is proposed to occur through ring-closing attack of amides on (presumably) neighbouring active esters, to form *N*-substituted glutarimides. Such formation of imides from the aminolysis of pMAOS has been previously suggested,[14] although no characterization data were provided.

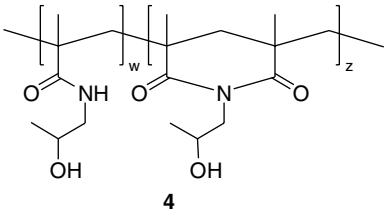
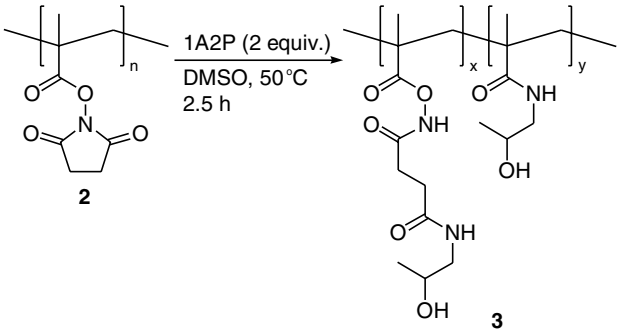


Figure 2. Structures of the water-insoluble polymer isolated after prolonged reaction of **2** in the presence or absence 1A2P.

To confirm the proposed chemistry, a pure sample of copolymer **3** was heated in DMSO-d_6 at 70 °C and the reaction followed by ^{1}H NMR spectroscopy. The liberation of hydroxamic acids **5** and **6** (Fig. 3) was clearly observed during the course of the experiment,[15] and upon completion of the reaction, copolymer **4** was isolated by size-exclusion chromatography (SEC) and characterized by ^{1}H NMR spectroscopy.

Attempts at synthesizing pHPMA from the reaction of **3** with 1A2P were invariably hindered by the formation of **4**, such that in an aminolysis of **3** in 1:1 1A2P/DMSO, approximately half of the hydroxamate moieties were displaced by the intramolecular glutarimide-forming reaction, with the remainder consisting of the desired amide functionality.

To confirm that the observed side reactions do indeed seriously limit the utility of **2**, two independently published protocols for aminolysis of pMAOS were replicated.[8,9] Analysis of the polymeric products by ^{1}H NMR revealed both to be copolymers comprised of



Scheme 2. Observed ring opening of **2**.

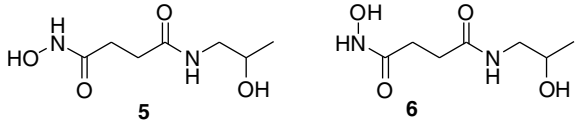


Figure 3. Structures of the two isomeric hydroxamic acids isolated from attempted aminolyses of polymer precursor **2**.