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

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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]

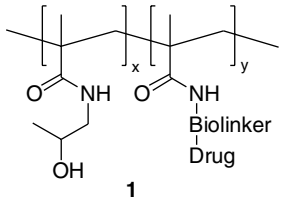

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Figure 1. General form of pHPMA polymer therapeutics.

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

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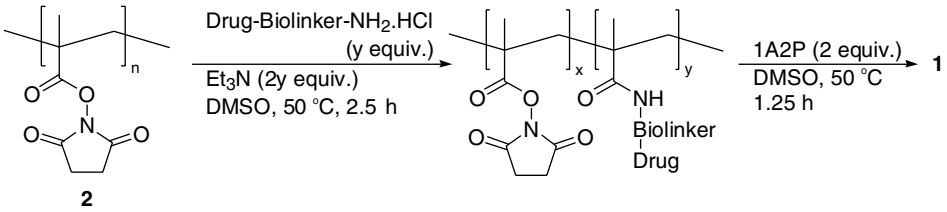


Scheme 1. Reported synthesis of polymer therapeutics, **1**, from **2**.

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.

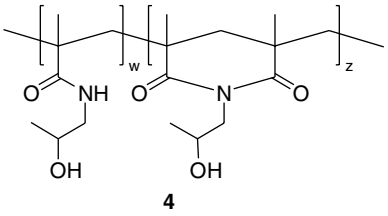
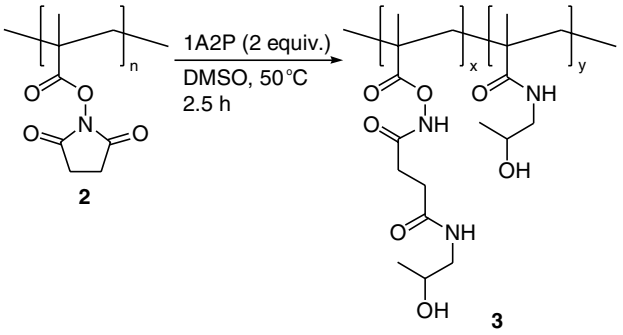


Figure 2. Structures of the water-insoluble polymer isolated after prolonged reaction of **2** in the presence or absence 1A2P.

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**.

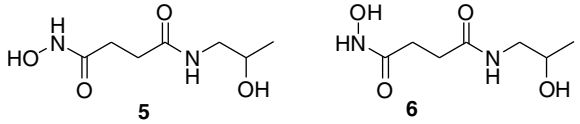


Figure 3. Structures of the two isomeric hydroxamic acids isolated from attempted aminolyses of polymer precursor **2**.

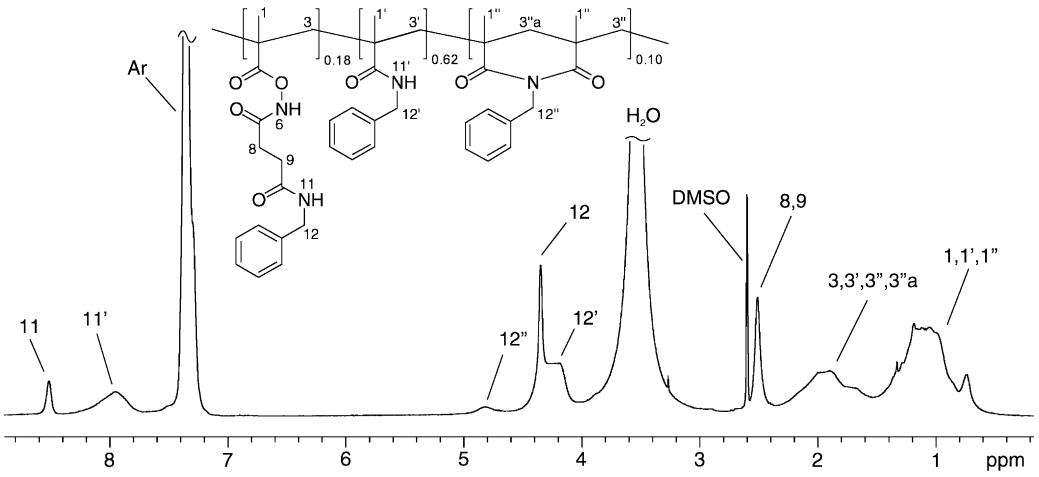


Figure 4. ^{1}H NMR spectrum of the product from reaction of **2** with benzylamine, showing both ring-opened and ring-closed side products.

the desired amide and the undesired ring-opened succinimide and ring-closed glutarimide units, which stands in contrast to the published accounts. It is presumably due to their use of only IR and GPC for polymer analysis that Godwin et al. did not observe the formation of side products **3** and **4**.[8]

Monge and Haddleton did use NMR analysis,[9] and close examination of the published ^{1}H spectrum reveals a small unexplained signal at 4.8 ppm. After following their procedures this same resonance was observed in our polymeric product. The application of 2D NMR techniques (COSY and HSQC-DEPT) allowed assignment of the signal at 4.8 ppm as the benzyl methylene of an *N*-benzylglutarimide entity. Monge and Haddleton did not report the formation of any ring-opened material. This is most probably a consequence of the work-up protocol used—in vacuo concentration of the reaction mixture followed by precipitation of the polymer resulting in prolonged exposure of the polymeric product to the benzylamine nucleophile. When repeating their procedure, two alternative work-up procedures were used to explore this possibility. The polymeric product was examined following direct purification of the reaction mixture on LH-20 immediately after completing the reaction (^{1}H NMR spectrum shown in Fig. 4). The second approach was in vacuo concentration of the reaction mixture (0.03 mmHg; 7 days at rt) followed by LH-20 chromatography. It was found that concentration in vacuo led to a significant decrease in the ring-opened material and explains why Monge and Haddleton observed no ring-opened material. Interestingly, this occurs with no significant increase in glutarimide formation, confirming that this cyclization reaction is less pronounced at lower temperatures.

In conclusion, the use of **2** as a precursor to polymethacrylamides is compromised by extensive formation of side products. In particular, the sterically hindered NHS esters are prone to ring opening, and intramolecular attack by amides on neighbouring activated esters leads to glutarimide formation.

Acknowledgements

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References and notes

1. Duncan, R. *Nat. Rev. Drug Disc.* **2003**, *2*, 347–360.
2. Allen, T. M.; Cullis, P. R. *Science* **2003**, *303*, 1818–1822.
3. Šprincl, L.; Exner, J.; Šterba, O.; Kopeček, J. *J. Biomed. Mater. Res.* **1976**, *10*, 953–963.
4. Yeung, T. K.; Hopewell, J. W.; Simmonds, R. H.; Seymour, L. W.; Duncan, R.; Bellini, O.; Grandi, M.; Spreafico, F.; Strohalm, J.; Ulbrich, K. *Cancer Chemother. Pharmacol.* **1991**, *29*, 105–111.
5. Bilim, V. *Curr. Opin. Mol. Ther.* **2003**, *5*, 326–330.
6. Rihova, B.; Kubackova, K. *Curr. Pharm. Biotechnol.* **2003**, *4*, 311–322.
7. Rihova, B.; Bilej, M.; Vetvicka, V.; Ulbrich, K.; Strohalm, J.; Kopeček, J.; Duncan, R. *Biomaterials* **1989**, *10*, 335–342.
8. Godwin, A.; Hartenstein, M.; Müller, A. H. E.; Brocchini, S. *Angew. Chem.* **2001**, *113*, 614–617; *Angew. Chem., Int. Ed.* **2001**, *40*, 594–597.
9. Monge, S.; Haddleton, D. M. *Eur. Polym. J.* **2004**, *40*, 37–45.
10. Degrand, C.; Limoges, B.; Blankespoor, R. L. *J. Org. Chem.* **1993**, *58*, 2573–2577.
11. Paquet, A.; Bergeron, M. *Can. J. Chem.* **1982**, *60*, 1806–1808.
12. Šavrda, J. *J. Org. Chem.* **1977**, *42*, 3199–3201.
13. Ringsdorf, H. *J. Polymer Sci.* **1975**, *51*, 135–153.
14. Pedone, E.; Li, X.; Koseva, N.; Alpar, O.; Brocchini, S. *J. Mater. Chem.* **2003**, *13*, 2825–2837.
15. These leaving groups were isolated and characterized by 2D NMR and HRMS. Compound **5**: colourless oil; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.39 (s, 1H), 8.70 (br s, 1H), 7.82 (t, 1H, $J = 5.5$ Hz), 4.65 (d, 1H, $J = 4.4$ Hz), 3.62 (m, 1H), 2.98 (m, 2H), 2.33 (t, 2H, $J = 7.5$ Hz), 2.18 (t, 2H, $J = 7.5$ Hz), 1.01 (d, 3H, $J = 6.2$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 171.3, 168.6, 65.3, 46.5, 30.7, 28.0, 21.2; HRMS (ESI): calcd for $C_7H_{15}N_2O_3$ [MH^+-O] 175.1082, found 175.1083. Compound **6**: ^{1}H NMR

(500 MHz, DMSO-d_6) δ: 7.86 (t, 1H, $J = 5.6$ Hz), 7.37 (br s, 1H), 6.82 (br s, 1H), 4.73 (br s, 1H), 3.70 (m, 1H), 3.06 (td, 2H, $J = 5.9$, 2.0 Hz), 2.38 (m, 4H), 1.09 (d, 3H, $J = 6.1$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.7, 171.7, 65.3, 46.5, 30.74, 30.66, 21.2; HRMS (ESI): calcd for $C_7H_{15}N_2O_4$ [MH^+] 191.1032, found 191.1031.