Synthesis of allyloxycarbonyloxymethyl-5-fluorouracil and copolymerizations with N-vinylpyrrolidinone

Zuifang Liu,^a Nigel Fullwood^b and Steve Rimmer^{*a}

^aThe Polymer Centre, Lancaster University, Lancaster, UK LA1 4YA. E-mail: S.Rimmer@Lancaster.ac.uk ^bBiological Sciences, Lancaster University, Lancaster, UK

Received 15th March 2000, Accepted 17th May 2000 Published on the Web 3rd July 2000

Poly(N-vinylpyrrolidinone) (PNVP) bearing 5-fluorouracil (5-FU) moieties was synthesised via a three-step method. Firstly, 5-FU reacted with formaldehyde to form a mixture of mono- and disubstituted hydroxymethyl-5-FUs. The mixture was then treated with allyl chloroformate to afford

allyloxycarbonyloxymethyl-5-FUs. This reaction showed site-specificity: the hydroxymethyl goup at the N-1 position readily reacted with chloroformate whereas the N-3 hydroxymethyl group partially decomposed into formaldehyde and the amide group. 1-Allyloxycarbonyloxymethyl-5-FU (4) and NVP were copolymerized in dioxane using azobisisobutyronitrile as an initiator. The monomer reactivity ratios, $r_4 = 0.32$ and $r_{NVP} = 0.97$, were evaluated by both linear and non-linear methods.

Introduction

It has been reported that 5-fluorouracil (5-FU), a widely used antimetabolite, is effective in inhibiting the progression of disease such as proliferative vitreoretinopathy (PVR), which remains a leading cause of failure in retinal detachment surgery,¹ and various cancers.² Several polymeric routes to controlled administration of 5-FU have now been examined. These include encapsulation in synthetic or natural hydrogels,^{3–8} followed by diffusive release; encapsulation in degradable polymer devices from which release occurs as the polymer degrades;⁹⁻²⁰ conjugation to soluble peptides, which may then be attached to other polymers;²¹⁻²⁵ copolymerization of 5-FU monomers with other water soluble monomers^{26–35} or attachment of reactive 5-FU compounds to polymers.³⁶ Release from the three latter methodologies involves degradation of a group linking the polymer to 5-FU. In the case of the peptide linked species this degradation occurs through enzymatic attack on the peptide, which can also function as a targeting group, whereas in non-peptide degradable systems release relies on hydrolysis. Several 5-FU functional monomers have been reported. Thus, Cho et al. have reported the synthesis and polymerization of 1,2,3,6-tetrahydrophthaloyl, acrylic or itaconyl monomers with 5FU functionality.^{27–30,32–34} Berger et al. have shown that the action of 5-FU in PVR can be enhanced by coupling to dexamethasone using a degradable carbonate linking group.¹ The coupled molecule was not active but as the carbonate group hydrolysed, releasing only carbon dioxide as the by-product,¹ the two active drugs were released. Following on from this work we report here a synthesis of a polymerizable carbonate derivative of 5-FU and detail copolymerization behaviour with NVP. PNVP has been selected as the major component of the polymer backbone because of its excellent biocompatibility when used as vitreous substitute or when implanted in the vitreous body.^{1,37,38} The

synthetic route involves the preparation of allyloxycarbonyloxymethyl-5-fluorouracils.

Experimental

Materials

5-FU (Fluka) was used as supplied. N-Vinylpyrrolidinone (NVP) (Aldrich) was purified by distillation under reduced pressure prior to polymerization. Azobisisobutyronitrile (AIBN) (Merck) was recrystallised from methanol. Triethylamine (TEA) and solvents were purified and dried by standard procedures.³⁹ Distilled and deionised water was used throughout. Other chemicals were used as received from Aldrich.

Monomer synthesis

5-FU (1.3 g, 10 mmol) and 37 wt% formaldehyde in aqueous solution (1.14 g, 14 mmol) were added to water (10 g) in a 100 mL round-bottomed flask which was immersed in a 60 ± 1 °C oil bath. The reaction was conducted under magnetic agitation for 6 hours. The resultant solution was concentrated using a rotary evaporator and then dried in a vacuum oven at 60 °C for 48 hours. The obtained oily product was dissolved in dry acetonitrile (30 mL) in a twonecked round-bottomed flask containing TEA (1.37 g, 13.5 mmol). To this solution, allyl chloroformate (1.54 g, 12.4 mmol) was added dropwise during about 5 minutes under dry nitrogen at room temperature. A white precipitate formed as soon as the chloroformate was added. After stirring at room temperature for 3 hours, the mixture was filtered and acetonitrile was removed under reduced pressure. The resultant was then dissolved in dichloromethane (30 mL) and washed three times with HCl $(1.0 \text{ mol dm}^{-3})$, saturated NaHCO₃aq. (twice) and water (twice). The organic layer was dried over sodium sulfate,

J. Mater. Chem., 2000, 10, 1771-1775 1771

This journal is ① The Royal Society of Chemistry 2000



CHEMISTRY

filtered and finally concentrated by using a rotary evaporator. Column chromatography on silica gel (silica gel 60, Fluka) using a mixture of methanol and dichloromethane (1:20 v/v) afforded two fractions. Evaporation of the solvent gave 0.68 g (19% yield, based on charged 5-FU) of an oily compound and 0.83 g (34% yield) of a wax-like compound for the first and second fraction, respectively. ¹H NMR spectrometry revealed that the former contained mainly 1,3-bis(allyloxycarbonyloxy-

methyl)-5-fluorouracil (6), and the latter 1-allyloxycarbonyl-oxymethyl-5-fluorouracil (4).

Analytical data for 4: ¹H NMR (DMSO- d_6) δ = 4.54 (m, O-CH₂-CH=), 5.32 (m, =CH₂), 5.62 (s, N-CH₂-O), 5.95 (m, -CH=), 8.15 (d, CH, 5-FU), 12.03 (s, NH, 5-FU); ¹³C NMR (DMSO- d_6) δ = 68.4, 73.0, 118.7, 129.0, 129.7, 131.7, 137.2, 141.7, 149.2, 153.3, 157.1, 157.6; FT-IR/cm⁻¹ = 3200, 3050, 1700 (broad), 1490, 1400, 1350, 1250, 1150, 1100, 950, 800; *m*/z = 244 (ESI+); mp 82.2 °C (DSC).

Analytical data for 6: ¹H NMR (DMSO- d_6) δ = 4.64 (m, O-CH₂-CH=), 5.32 (m, =CH₂), 5.69 (s, N-CH₂-O, N-1 position), 5.85 (s, N-CH₂-O, N-3 position), 5.95 (m, -CH=), 8.31 (d, CH, 5-FU).

Polymerization

1 Preparation of linear copolymers. A series of polymerizations were performed with various monomer feed ratios of 4 and NVP. In a typical polymerization, AIBN (0.016 g, 0.98 mmol) and a mixture of NVP (as defined by the designated feed) and 4 (10 mmol, 2.16 g) in dioxane (20 mL) were charged to a two-necked round-bottomed flask equipped with a magnetic stirrer bar, nitrogen inlet and reflux condenser. The solution was deoxygenated by bubbling with nitrogen at room temperature for 1 hour. Polymerization was then started by immersing the flask in an oil bath at 60 ± 1 °C. Samples (0.2 mL) were taken as the polymerization progressed and these were added to sample tubes containing 1.5 mL propan-2-ol (HPLC grade). The sample tubes were shaken for 20 minutes and then centrifuged to separate the precipitated polymer from the solution. The supernatants were finally analysed by high pressure liquid chromatography (HPLC)

and the residual monomer levels were calculated using a standard calibration curve. Pure linear polymer samples, for NMR spectroscopy, were prepared by double reprecipitation from methanol into diethyl ether.

Analysis

200 MHz ¹H nuclear magnetic resonance (NMR) spectra were recorded in dimethyl sulfoxide- d_6 (DMSO- d_6) with tetramethylsilane (TMS) as an internal standard using a Varian NMR 200 spectrometer (Oxford, UK). Polymerization was followed by measuring the residual monomer concentrations using HPLC (column: Waters C8 3.9×150 mm, at 30 °C; eluent: 30 vol% methanol + 70 vol% 10 mM ammonium acetate aqueous solution; flow rate: 1.0 mL min^{-1} ; detector: UV_{234 nm} and UV_{262 nm} for NVP and monomer **4**, respectively). The molecular weights of copolymers of **4** and NVP with different monomer feed ratios were determined by size exclusion chromatography (SEC) (column: PSS HEMA 8 × 300 nm, at 70 °C; eluent 0.1% (w/v) ammonium acetate in dimethylacetamide; flow rate: 1.0 mL min^{-1} ; refractive index detector). The calibration curves were obtained using poly(ethylene oxide) standards.

Results and discussion

Preparation and characterization of monomers

5-FU can react with formaldehyde (in stoichiometric excess) to give 3 in quantitative yield.^{40–42} In the current work, reaction 1 in Scheme 1 was conducted in dilute aqueous solution using a ratio of 1.0 mole 5-FU to 1.4 mole formaldehyde and afforded a mixture of 1-hydroxymethyl-5-fluorouracil (1), 3-hydroxymethyl-5-fluorouracil (2) and 1,3-bis(hydroxymethyl)-5-fluorouracil (3). Fig. 1A shows the ¹H NMR spectrum and the assignments of the product of reaction 1 after 6 hours. It is evident that the product was a mixture of 1, 2 and 3. The product compositions were determined by ¹H NMR and the results after 3 and 6 hours are given in Table 1. As expected, the amount of 1 was higher than that of 2 throughout the reaction, whilst the amount of 3 increased as the reaction progressed. Finally, a mixture comprising 31.7 mol% 1, 21.1 mol% 2 and 32.7 mol% 3 was obtained.



Scheme 1

1772 J. Mater. Chem., 2000, 10, 1771–1775



Fig. 1 ¹H NMR spectra: A, products of reaction 1, 6 hours; B, product of reaction 2, after work-up.

After drying, this mixture was treated with allyl chloroformate (reaction 2) in the presence of TEA. ¹H NMR spectroscopy of the product revealed that the amount of N-1 substituted monomer, 4 (56.3 mol%), in the reaction mixture was significantly larger than that of the disubstituted monomer, 6 (31.5 mol%), and only a small amount of the N-3 substituted monomer, 5 (1.0 mol%), was detected (Fig. 1B). By examining the NMR spectra in Fig. 1, it is clear that the product distribution was significantly different from the product distribution predicted by reference to the starting mixture, containing 1, 2 and 3. This implies different behaviours of the hydroxymethyl groups at the N-1 and N-3 positions; that is, demethylolation was a function of the substitution pattern. After work-up and column separation, the two major components, 4 and 6, were isolated in 34% and 19% yield (based on charged 5-FU), respectively. It is noteworthy that the amount of isolated 6 (1.9 mmol) is significantly smaller than the amount predicted by the amount of 3 (ca. 3.3 mmol) present in the starting material. In contrast, the amount of 4 (3.4 mmol) is slightly larger than the amount expected based on the amount of 1 (3.2 mmol) present in the starting material. These results suggest that 1, 2 and 3 undergo demethylolation in competition with allyloxyformylation so that a fraction of 3 undergoes demethylolation at N-3 followed by allyloxyformylation of the remaining hydroxymethyl group at N-1. Thus reaction of 3 generated both 4 and 6. Similarly, 1 could react readily to form 4 but a fraction of 1 underwent demethylolation. Since 5 was produced only in low yield it is reasonable to assume the N-3-

methanol is more easily removed than the N-1 methanol group. On preparing 5-FU derivatives from 1.3-bis(hydroxymethyl)-5-FU and some chloroformates, Nagase et al.⁴³ reported that the reaction occurred exclusively on the N-1 position at the early stage and the remaining chloroformate then reacted with the N-3 hydroxymethyl group to give disubstituted compounds. In the current study, the amount of allyl chloroformate (12.4 mmol) was larger than the total amount of hydroxymethyl groups (11.8 mmol, calculated from the composition result). However, only partial conversion of 3 to 6 was observed. Furthermore, no signal arising from the proton on the hydroxymethyl group at the N-3 position was detectable (see Fig. 1B). These results suggest that, under the reaction conditions, the hydroxymethyl group at the N-1 position could be activated to react with allyl chloroformate. The hydroxymethyl group at the N-3 position, on the other hand, reacted with chloroformate by decomposition into formaldehyde and NH group. This is further supported by the fact that only a very small amount of 5 was formed from about 2.1 mmol of 2.

Copolymerization of monomer 4 with NVP

Isolated monomer **4** was copolymerized with NVP at various feed ratios (reaction 3 in Scheme 1). Fig. 2 shows the variations of the conversions of both **4** and NVP with time for a typical polymerization. It is evident that **4** polymerized at a slightly slower rate than NVP. A typical ¹H NMR spectrum for one of the copolymers is shown in Fig. 3. The characteristic signal corresponding to the proton on the 5-FU residue at around

Table 1 Product compositions for reaction 1 in Scheme 1

Reaction time/h	Product composition (mol%)		
	1	2	3
3	32.1	22.5	17.7
6	31.7	21.1	32.7



Fig. 2 Conversion of monomer *versus* polymerization time [**4**: NVP=44:56 (mol/mol)].

8.15 ppm is clearly seen. However, the initial attempt to determine the copolymer compositions by using the ¹H NMR spectroscopy was not successful because of the wide and incompletely resolved peaks arising from the protons on the NVP repeat unit. To evaluate the monomer reactivity ratios, the copolymer compositions were determined from the residual monomer levels measured by HPLC. The composition of one final copolymer prepared using about 44 mol% of 4 in the feed was also determined by elemental analysis (performed by C.H.N Analysis Ltd, Leicester, UK). The results obtained by the two methods are in good agreement (the fractions of monomer 4 in the copolymer are 0.35 and 0.33 determined by HPLC and by elemental analysis, respectively). In order to satisfy the well-known differential equation relating the copolymer composition to the monomer composition, data with monomer conversions lower than 10% were used for the evaluation of the monomer reactivity ratios. The compositions of the monomer feed and of the corresponding copolymers are given in Table 2. A pair of reactivity ratios, $r_4 = 0.31 \pm 0.06$ and $r_{\rm NVP} = 0.97 \pm 0.02$, was obtained by using the Kelen-Tüdõs linear regression method.⁴⁴ These data were then used as initial approximate values for the non-linear least-square Tidwell-Mortimer method,⁴⁵ which gave $r_4 = 0.32$ and $r_{\text{NVP}} = 0.97$. The 95% confidence ellipse is shown in Fig. 4. Molecular weights of the copolymers were determined by SEC and plotted against the fraction of monomer 4 in the monomer feed in Fig. 5. It can be seen that as expected an increase in the fraction of 4 resulted in a decrease in the molecular weight.

 Table 2 Monomer conversion and compositions of the feed and the copolymers

f^{a}	F^b	Conversion of 4 (%)	Conversion of NVP (%)
0.15	0.14	6.8	7.3
0.20	0.18	7.8	8.8
0.29	0.25	6.2	7.8
0.44	0.36	6.7	9.4
0.60	0.47	5.5	9.1
^{<i>a</i>} Molat lymer.	r fraction	of 4 in monomer feed. ${}^{b}N$	Iolar fraction of 4 in copo-



Fig. 3 1 H NMR spectrum of copolymer prepared under the conditions shown in Fig. 2.



Fig. 4 95% confidence ellipse for the reactivity ratios determined by the non-linear least-squares method.



Fig. 5 Variation of copolymer molecular weight with fraction of 4 in the monomer feed. The monomer conversions are presented in Table 2.

Conclusion

We report the synthesis of a new 5-FU functional monomer and its copolymerization with NVP. The allyl carbonate monomer can be successfully copolymerized with NVP over a wide composition range. The reactivity ratios imply that copolymerization would lead to polymers that are slightly rich in NVP at low conversion and rich in the new monomer towards the end of the polymerization. However, the values (r_4 =0.32, r_{NVP} =0.97) were found to be sufficiently close to minimize the production of homopolymer during the polymerization. Although a substantial decrease in molecu-

Acknowledgements

The authors wish to thank EPSRC, UK (GR/M36526) for the financial support to this project.

References

- A. S. Berger, C. K. Cheng, P. A. Pearson, P. A. Shton, P. A. Crooks, T. Cynkowski, G. Cynkowska and G. J. Jaffe, Invest. Ophthalmol. Visual Sci., 1996, 37, 2318.
- D. Putnam and J. Kopecek, Adv. Polym. Sci., 1995, 122, 55. 2
- 3 O. Garcia, M. D. Blanco, J. A. Martin and J. M. Teijon, Eur. Polym. J., 2000, 36, 111.
- 4 Y. Luo, Y. Aso and S. Yoshioka, Chem. Pharm. Bull., 1999, 47, 579
- O. Garcia, M. D. Blanco, C. Gomez and J. M. Teijon, Polym. 5 Bull., 1997, 38, 55.
- T. D. Blanco, O. Garcia, R. Olmo, J. M. Teijon and I. Katime, 6 I. Chromatogr. B: Biomed. Appl., 1996, 680, 243.
- 7 X. M. Li, W. M. Shen, C. J. Liu, S. I. Nishimoto and T. Kagiya, Radiat. Phys. Chem., 1991, 38, 377.
- 8 C. Zinutti, F. Kedzierewicz, M. Hoffman and P. Maincent, J. Microencapsulation, 1994, 11, 555.
- 9 Y. H. E. Lin and R. C. Vasavada, J. Microencapsulation, 2000, 17,
- S. Einmahl, M. Zignani, E. Varesio, J. Heller, J. L. Veuthey, 10
- C. Tabatabay and R. Gurny, *Int. J. Pharm.*, 1999, 185, 189.
 M. B. Sintzel, J. Heller, S. Y. Ng, C. Tabatabay, K. SchwachAbdellaoui and R. Gurny, *J. Controlled Release*, 11 1998, 55, 213.
- T. H. Zhou, H. Lewis, R. E. Foster and S. P. Schwendeman, 12 J. Controlled Release, 1998, 55, 281.
- G. A. Peyman, D. H. Yang, B. Khoobehi, M. H. Rahimy and 13 S. Y. Chin, *Ophthalmic Surgery and Lasers*, 1996, **27**, 384. L. K. Chiu, W. J. Chiu and Y. L. Cheng, *Int. J. Pharm.*, 1995, **126**, 14
- 169 15 G. E. Trope, Y. L. Cheng, H. Sheardown, G. S. Liu, I. A. Menon,
- J. G. Heathcote, D. S. Rootman, W. J. Chiu and L. Gould, Can. J. Ophthalmol., 1994, 29, 263.
- S. F. Bernatchez, A. Merkli, T. L. Minh, C. Tabatabay, J. M. Anderson and R. Gurny, *J. Biomed. Mater. Res.*, 1994, 16 **28**, 1037.
- L. W. Seymour, R. Duncan, J. Duffy, S. Y. Ng and J. Heller, 17 J. Controlled Release, 1994, 21, 201.

- 18 K. Ciftci, A. A. Hincal, H. S. Kas, M. T. Ercan and S. Ruacan, Eur. Pharm. Sci., 1994, 1, 249.
- 19 T. Moritera, Y. Ogura, Y. Honda, R. Wada, S. H. Hyon and Y. Ikada, Invest. Ophthalmol. Visual Sci., 1991, 32, 1785
- 20 J. Heller, Y. F. Maa, P. Wuthrich, S. Y. Ng and R. Duncan, J. Controlled Release, 1991, 16, 3.
- M. Nichifor, E. H. Schacht and L. W. Seymour, J. Controlled 21 Release, 1997, 48, 165.
- K. S. Kim, D. S. Lim and S. H. Cho, Korea Polym. J., 1996, 4, 16. 23
- M. Nichifor, E. H. Schacht and L. W. Seymour, J. Controlled Release, 1996, 39, 79. 24
- D. Putnam and J. Kopecek, *Bioconjugate Chem.*, 1995, **6**, 483. M. Nichifor, E. H. Schacht, L. W. Seymour, D. Anderson and 25
- M. Shoaibi, *J. Bioact. Compat. Polym.*, 1997, **12**, 265. S. M. Lee, W. M. Choi, C. S. Ha and W. J. Cho, *J. Polym. Sci.* 26
- Part A: Polym. Chem., 1999, 37, 2619. 27 J. G. Park, C. S. Ha and W. J. Cho, J. Polym. Sci. Part A: Polym.
- Chem., 1999, 37, 2113.
- 28 J. G. Park, C. S. Ha and W. J. Cho, J. Polym. Sci. Part A: Polym. Chem., 1999, 37, 1589.
- J. G. Park, S. H. Kim, C. S. Ha and W. J. Cho, J. Polym. Sci. Part 29 A: Polym. Chem., 1998, 36, 2985.
- 30 W. M. Choi, I. D. Chung, N. J. Lee, Y. W. Lee, C. S. Ha and W. J. Cho, J. Polym. Sci. Part A: Polym. Chem., 1998, 36, 2177.
- 31 A. Kishida, H. Goto, K. Murakami, K. Kakinoki, M. Akashi and T. Endo, J. Bioact. Compat. Polym., 1998, 13, 222.
- J. G. Park, W. M. Choi, N. J. Lee, C. S. Ha and W. J. Cho, 32 J. Polym. Sci. Part A: Polym. Chem., 1998, 36, 1625
- J. Polym. Sci. Part A: Polym. Chem., 1956, 50, 1625.
 W. M. Choi, I. D. Jung, N. J. Lee, S. H. Kim, C. S. Ha and W. J. Cho, Polym. Adv. Technol., 1997, 8, 701.
 N. J. Lee, C. S. Ha and W. J. Cho, J. Macromol. Sci., Pure Appl. 33
- 34 Chem., 1992, 29, 161.
- T. Akiyama, Y. Takesue, M. Kumegawa, H. Nishimoto and 35 S. Ozaki, Bull. Chem. Soc. Jpn., 1991, 64, 2266.
- M. Nichifor, V. Coessens and E. H. Schacht, J. Bioact. Compat. 36 Polym., 1995, 10, 199.
- М. J. Bruining, P. S. Edelbroek Hoogendoorn, 37 H. G. T. Blaauwgeers, C. M. Mooy, F. H. Hendrikse and L. H. Koole, J. Biomed. Mater. Res., 1999, 47, 189.
- Y. Hong, T. V. Chirila, S. Vijayasekaran, W. Shen, X. Lou and 38 P. D. Dalton, J. Biomed. Mater. Res., 1998, 41, 650.
- W. L. F. Armarego and D. D. Perrin, Purification of Laboratory 39 Chemicals, 4th edn., Butterworth-Heinemann, Bath, UK, 1996.
- S. Ahmad, S. Ozaki, T. Nagase, M. Iigo, R. Tokuzen and 40 A. Hoshi, *Chem. Pharm. Bull.*, 1987, 35, 4137.
 Y. Ohya, H. Kobayashi and T. Ouchi, *React. Polym.*, 1991, 15,
- 41 153
- 42 J. C. Maurizis and B. Pucci, Macromol. Chem. Phys., 1999, 200, 1351.
- T. Nagase, K. Shiraishi, Y. Yamada and S. Ozaki, Heterocycles, 43 1988, 27, 1155.
- T. Kelen and F. Tüdős, J. Macromol. Sci. Chem., 1975, A9, 1. 44
- P. W. Tidwell and G. A. Mortimer, J. Polym. Sci., 1965, A3, 45 369.