Macromolecules

Volume 34, Number 11 May 22, 2001

# Communications to the Editor

## Toward Polymeric Anticancer Drug Cocktails from Ring-Opening Metathesis Polymerization

**Keith J. Watson, Donde R. Anderson, and SonBinh T. Nguyen***

*Department of Chemistry, Northwestern University, Evanston, Illinois 60208-3113*

*Received November 7, 2000*
*Revised Manuscript Received February 28, 2001*

**Introduction.** The use of functionalized polymers as scaffolds for the delivery of drugs is an area of research that has seen considerable growth during the past decade.[1−3] Drugs in the polymeric form offer several advantages over their monomeric precursors, including longer retention time in the body, lower toxicity, and a greater specificity of action. The treatment of cancer lends itself particularly well to this form of therapy, due in large part to the enhanced permeability and retention of macromolecules by cancerous tumors.[4] The basis of this phenomenon, known as the EPR effect, can be traced to enhanced tumor vascular permeability combined with a substantial decrease in the tumor lymphatic drainage system.[4]

Currently, many polymer-based drug delivery systems rely on grafting pharmaceutically active molecules to backbones that are comprised of either poly-*N*-(2-hydroxypropyl)methacrylamide (poly-HPMA)[5−7] or poly(ethylene glycol) (PEG).[8,9] Although these systems are inherently biocompatible, they often suffer from limited functionalizability. For example, a typical PEG unit has only two modifiable groups (the end groups) that can be used as drug attachment sites, which results in a low degree of drug incorporation into the polymer chain. Further, since most treatment strategies rely on a combination of multiple drugs to achieve optimum benefit,[10,11] well-defined block copolymers which are simultaneously functionalized with a high density of two or more drugs may be an extremely useful means of drug delivery. Herein, we communicate our initial efforts to utilize living ring-opening metathesis polymerization (ROMP) for the preparation of such materials.

The recent development and commercialization of well-defined, single-site cyclic olefin metathesis catalysts such as $Cl_2(PCy_3)_2Ru{=}CHPh$ (**1**) has resulted in a substantial increase in the number of monodisperse polymers and block copolymers that can be made using ROMP.[12−15] The exceptional functional group tolerance and selectivity of **1** are such that virtually any moiety that can be attached to a norbornene group can subsequently be included in the corresponding ROMP polymer, allowing for the isolation of heretofore unattainable macromolecules. To utilize ROMP in the synthesis of polymeric drugs, especially those that are relevant for cancer chemotherapy, several issues need to be addressed: (1) Are there known anticancer drugs that can be readily modified with a norbornene group? (2) Once obtained, will such functionalized molecules be polymerizable using catalyst **1**? (3) Will these new compounds be amenable to the synthesis of well-defined block copolymers? (4) What steps are necessary to maximize the biocompatibility and overall effectiveness of these macromolecules? We specifically address these questions in this report.

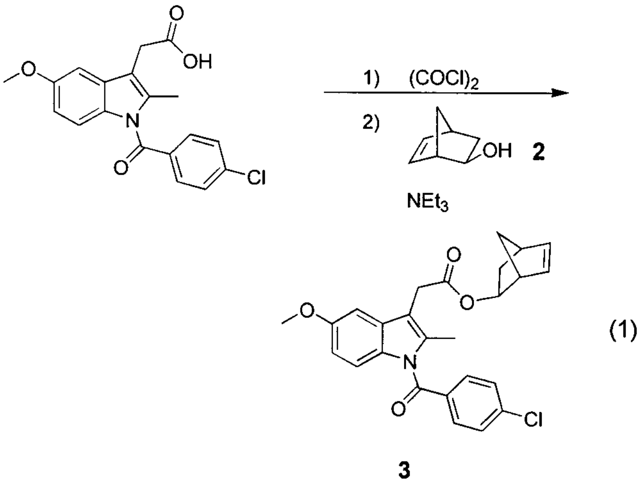
**Results and Discussion. a. Preparation of Norbornenyl-Modified Anticancer Agents.** In this investigation, three commercially available compounds with demonstrated anticancer activity were targeted for modification with *exo*-5-norbornen-2-ol (**2**), a useful norbornene intermediate that we have used for the synthesis of several complex ROMP monomers.[16−19] Indomethacin is a nonsteroidal antiinflammatory drug which has potent cancer-preventive and tumor-regressive activity.[10,20] For example, it has recently been shown that indomethacin is involved in the induction of apoptosis of colon cancer cells.[20] The most reactive group of indomethacin is a carboxylic acid functionality. We exploited this reactivity by first converting the acid to an acid chloride and then allowing it to react with **2**

---

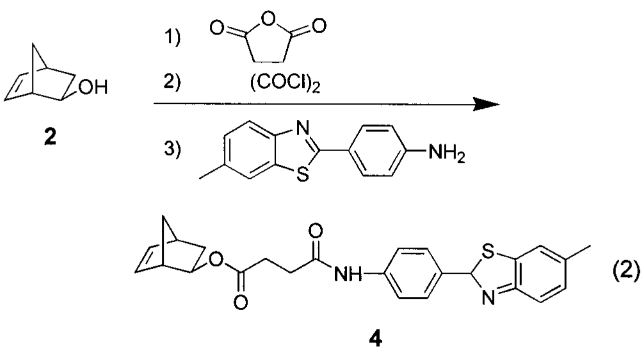
* To whom correspondence should be addressed. E-mail: stn@chem.northwestern.edu.

10.1021/ma001916t CCC: $20.00

**Scheme 1**



**Scheme 2**



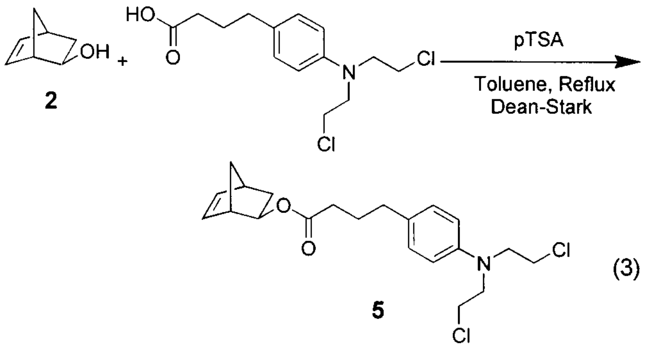
in the presence of triethylamine to yield the desired ROMP monomer **3** in greater than 90% yield (Scheme 1).

The second anticancer ROMP monomer targeted in this study was based on 2-(4-aminophenyl)-6-methylbenzothiazole, one of a series of (aminophenyl)benzothiazoles shown to exhibit significant activity against certain types of colon, lung, melanoma, renal, breast, and ovarian cancer,[21−23] although the mechanism of action of these drugs remains unknown. The free amino group is the most reactive functionality of these compounds and hence was utilized for the attachment of the norbornene. When **2** is treated with succinic anhydride in the presence of triethylamine, a ring-opening condensation reaction occurs, resulting in a carboxylic acid substituted norbornene. Conversion of the acid group to an acid chloride, followed by condensation with 2-(4-aminophenyl)-6-methylbenzothiazole, yielded the desired compound **4** in greater than 80% yield (Scheme 2). This product was found to be insoluble in most organic solvents (such as $CH_2Cl_2$), limiting the extent to which its polymerization activity could be probed (vide infra).

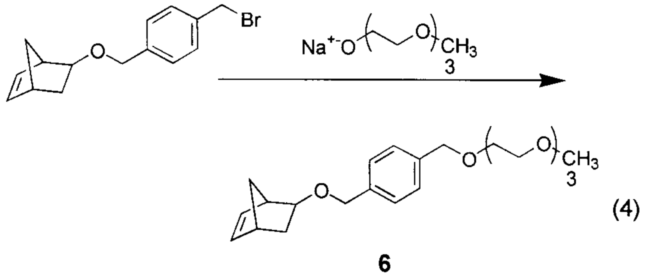
Chlorambucil is a bifunctional alkylating agent that has been utilized for the treatment of chronic lymphatic leukemia as well as malignant lymphomas such as Hodgkin's disease.[24−26] The free carboxylic acid group of chlorambucil was coupled to **2** under Dean−Stark conditions, yielding **5** in moderate yields (45%) as a clear oil (Scheme 3). This compound was found to be freely soluble in most organic solvents.

**b. Preparation of Norbornenyl-Modified Triethylene Glycol.** As described earlier, PEG-based drug delivery systems are advantageous because of their biocompatibility, which arises from their water solubility and lack of ionic character. A triethylene glycol-derivatized norbornene **6** was constructed as a model solublizing comonomer. Reaction of the sodium salt of triethylene glycol monomethyl ether with α-bromo-α′-(*exo*-5-norbornene-2-ol)-*p*-xylene[19] afforded **6** in nearly quantitative yield (Scheme 4). Although **6** is only slightly soluble in water, a true water-solubilizing comonomer can be targeted using a longer PEG chain segment (vide infra).

**Scheme 3**



**Scheme 4**



**c. Initial Survey of the ROMP Activity of 3−6 with 1.** To test the ROMP activity of **3**−**6** with **1**, four NMR tube polymerization experiments were set up and monitored by $^1H$ NMR spectroscopy. In these experiments, each monomer (20 equiv) was mixed with a catalytic amount of **1** (1 equiv) in the appropriate solvent ($CDCl_3$ for **3**, **5**, and **6**; DMF-$d_7$ for **4**). The conversion of monomer to polymer was signaled by the loss of resonances associated with the olefinic protons of the norbornene monomer (at 5.9 and 6.2 ppm). The simultaneous growth of resonances associated with the olefinic protons of the ring-opened polymer (from 5 to 5.4 ppm) was also observed, indicating that **1** was tolerant to each of the functionally diverse monomers **3**−**6**.

**d. Synthesis of Polymers and Block Copolymers.** After verifying the polymerization reactivity of monomers **3**−**6**, several larger scale (∼100 mg) polymerization experiments were performed. A ROMP homopolymer of **3** was isolated by stirring an excess of **3** with **1** in $CH_2Cl_2$ for 30 min at room temperature. Termination of the polymerization reaction with ethyl vinyl ether[27] and subsequent purification by precipitation yielded a polymer with a $M_n$ of 19 400 (vs polystyrene standards) and a PDI of 1.25. Similarly, a homopolymer of **5** was prepared and found to have a $M_n$ of 16 800 and a PDI of 1.26. Since these experiments and molecular weight determinations were carried out in $CH_2Cl_2$, larger scale ROMP experiments of the $CH_2Cl_2$ insoluble complex **4** were not carried out. A well-defined block copolymer of **3** and **5** was isolated by allowing an excess of **3** to fully react with **1**, followed by the injection of a solution of

**5** into the reaction vessel. Characterization of this polymer revealed a $M_n$ of 27 600 and a narrow PDI of 1.20. Finally, a random copolymer of **3** and **6** was synthesized by reacting a mixture of the two monomers with **1**. In this case, a larger $M_n$ of 78 300 was observed, as well as a broadening of the PDI to 2.03. In all cases, quantitative yields of polymer were obtained.

**Conclusions.** Herein, we have demonstrated that at least three anticancer drugs can be readily modified with a norbornene group and that the resulting multifunctional molecules can be polymerized using the ruthenium carbene initiator **1**. Further, these compounds are amenable to the synthesis of either block copolymers in combination with each other or random copolymers in combination with the triethylene glycol monomer **6**. To our knowledge, this report represents the simplest route to multifunctional pharmaceutical-containing block copolymers that contain a high density of drugs and a narrow polydispersity. The unique ability of ROMP initiators such as **1** to polymerize such diverse monomers under mild conditions should allow chemists to further capitalize upon these initial results. In addition, the narrow polydispersity obtained in living ROMP chemistry can be advantageous in the control of dosage in pharmacotherapy and pharmacokinetics. Future work in this area by our group will concentrate on improving the overall biological compatibility of these systems by increasing the length of the ethylene glycol chain of **6**, by using amide connections in place of ester linkages, and by incorporating the tetrapeptide linker Gly-Phe-Leu-Gly into **3**−**5**. This linker is enzymatically hydrolyzed in the cellular environment and has been extensively used in the field of drug delivery.[8] These studies, as well as an examination of the biological activity of these systems, will be the topic of future reports.

**Acknowledgment.** Financial support by the AFOSR, NSF (DMR-CAREER Grant 0094347), the Beckman Foundation, the Packard Foundation, the Dreyfus Foundation, and the Dupont Company is appreciated. K.J.W. acknowledges the Natural Sciences and Engineering Research Council of Canada (NSERC) for a predoctoral fellowship.

**Supporting Information Available:** Details of the synthesis and characterization of **3**−**6** as well as polymerization procedures and appropriate $^1$H NMR data. This information is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

(1) Chasin, M. *Biomed. Appl. Synth. Biodegrad. Polym.* **1995**, 1−15.

(2) Domb, A.; Amselem, S.; Shah, J.; Maniar, M. *Polym. Adv. Technol.* **1992**, *3*, 279−292.

(3) Duncan, R. *Anti-Cancer Drugs* **1992**, *3*, 175−210.

(4) Duncan, R. *Pharm. Sci. Technol. Today* **1999**, *2*, 441−449.

(5) Kopecek, J.; Kopeckova, P.; Minko, T.; Lu, Z. R. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 61−81.

(6) Minko, T.; Kopeckova, P.; Kopecek, J. *Int. J. Cancer* **2000**, *86*, 108−117.

(7) Tijerina, M.; Fowers, K. D.; Kopeckova, P.; Kopecek, J. *Biomaterials* **2000**, *21*, 2203−2210.

(8) Pechar, M.; Ulbrich, K.; Subr, V.; Seymour, L. W.; Schacht, E. H. *Bioconjugate Chem.* **2000**, *11*, 131−139.

(9) La, S. B.; Nagasaki, Y.; Kataoka, K. In *ACS Symposium Series: Poly(ethylene glycol): chemistry and biological applications*; Hilton, J. M., Zalipsky, S., Eds.; American Chemical Society: Washington, DC, 1997; Vol. 680, pp 99−118.

(10) Huang, Y.; Horvath, C. M.; Waxman, S. *Cancer Res.* **2000**, *60*, 3200−3206.

(11) Henderson, E. S.; Samaha, R. J. *Cancer Res.* **1969**, *29*, 2272−2280.

(12) Ivin, K. J.; Mol, I. C. *Olefin Metathesis and Metathesis Polymerization*, 2nd ed.; Academic Press: San Diego, 1997.

(13) Schuster, M.; Blechert, S. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2037−2056.

(14) Schwab, P.; France, M. B.; Ziller, J. W.; Grubbs, R. H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2039−2041.

(15) Schwab, P.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 100−110.

(16) Watson, K. J.; Zhu, J.; Nguyen, S. T.; Mirkin, C. A. *J. Am. Chem. Soc.* **1999**, *121*, 462−463.

(17) Watson, K. J.; Wolfe, P. S.; Nguyen, S. T.; Zhu, J.; Mirkin, C. A. *Macromolecules* **2000**, *33*, 4628−4633.

(18) Watson, K. J.; Zhu, J.; Nguyen, S. T.; Mirkin, C. A. *Pure Appl. Chem.* **2000**, *72*, 67−72.

(19) Watson, K. J.; Nguyen, S. T.; Mirkin, C. A. *J. Organomet. Chem.* **2000**, *606*, 79−83.

(20) Kang, H.-J.; Song, M.-J.; Choung, S. Y.; Kim, S. J.; Lee, M.-O. *Biol. Pharm. Bull.* **2000**, *23*, 815−819.

(21) Hutchinson, I.; Stevens, M. F. G.; Westwell, A. D. *Tetrahedron Lett.* **2000**, *41*, 425−428.

(22) Kashiyama, E.; Hutchinson, I.; Chua, M.-S.; Stinson, S. F.; Phillips, L. R.; Kaur, G.; Sausville, E. A.; Bradshaw, T. D.; Westwell, A. D.; Stevens, M. F. G. *J. Med. Chem.* **1999**, *42*, 4172−4184.

(23) Wells, G.; Bradshaw, T. D.; Diana, P.; Seaton, A.; Shi, D.-F.; Westwell, A. D.; Stevens, M. F. G. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 513−515.

(24) Beyer, U.; Roth, T.; Schumacher, P.; Maier, G.; Unold, A.; Frahm, A. W.; Fiebig, H. H.; Unger, C.; Kratz, F. *J. Med. Chem.* **1998**, *41*, 2701−2708.

(25) Dighiero, G.; Maloum, K.; Desablens, B.; Cazin, B.; Navarro, M.; Leblay, R.; Leporrier, M.; Jaubert, J.; Lepeu, G.; Dreyfus, B.; Binet, J.-L.; Travade, P. *N. Engl. J. Med.* **1998**, *338*, 1506−1514.

(26) McElwain, T. J.; Toy, J.; Smith, E.; Peckham, M. J.; Austin, D. E. *Br. J. Cancer* **1977**, *36*, 276−280.

(27) Wu, Z.; Nguyen, S. T.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 5503−5511.

MA001916T