ORGANIC LETTERS

2008
Vol. 10, No. 15
3291−3294

Dynamic Combinatorial Libraries Constructed on Polymer Scaffolds

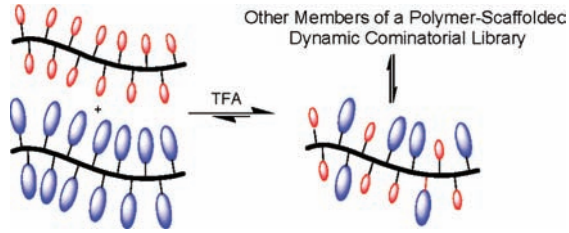
David A. Fulton*

School of Chemistry, Newcastle University, Newcastle Upon Tyne NE1 7RU, U.K.

d.a.fulton@ncl.ac.uk

Received May 20, 2008

ABSTRACT



Functionalized polymers were prepared by grafting acylhydrazides onto a polyvinylbenzaldehyde scaffold through reversible hydrazone linkages. The dynamic nature of these linkages allows the functionalized polymers to exchange and reshuffle their appendages, and the resultant mixture of polymers can be considered as a dynamic combinatorial library constructed upon a polymer scaffold. The dynamic nature of these functionalized polymers was demonstrated.

The preparation of synthetic macromolecules which can mimic natural proteins in terms of function such as catalysis or molecular recognition presents a formidable challenge to chemists. In the 1990s, Menger and co-workers utilized[1] a "combinatorial" approach to developing polymers possessing catalytic activity, and more recently, Schrader and co-workers have used[2] a small set of functionalized monomers to prepare libraries of copolymers and successfully screened these libraries to find selective protein binders. Both of these approaches to the design of functional synthetic macromolecules are fundamentally different from that of Nature, where through processes of selection and replication effective functional macromolecules have evolved.

Recently, dynamic combinatorial chemistry[3] (DCC) has emerged as a tool for the discovery of host−guest systems with the potential for catalysis and molecular recognition. DCC exploits collections of dynamic combinatorial libraries (DCLs), where all library members are in equilibrium and can interconvert with one another through a reversible chemical process. Addition of a template can lead to further stabilization (e.g., through noncovalent interactions) of those library members which can most effectively bind to the template. Consequently, the entire DCL can re-equilibrate, amplifying the concentration of effective binders of the template and consuming library members which are ineffective binders.

The application of concepts from DCC to the discovery of synthetic macromolecules which can mimic proteins is intriguing. Natural proteins consist of amino acid residues within very specific sequences, and it is the nature of the amino acid side chains and their precise location upon the peptide backbones which are largely responsible for protein function.[4] Importantly, peptides cannot exchange or reshuffle their amino acid constituents without access to the complex cellular machinery found in biology.[5] We envisaged a wholly synthetic polymer-based system where functionalized residues are grafted onto a preformed polymer scaffold through

(1) (a) Menger, F. M.; Eliseev, A. V.; Migulin, V. A. *J. Org. Chem.* **1995**, *60*, 6666–6667. (b) Menger, F. M.; West, C. A.; Ding, J. *Chem. Commun.* **1997**, 633–634. (c) Menger, F. M.; Ding, J.; Barragan, V. *J. Org. Chem.* **1998**, *63*, 7578–7579.

(2) Koch, S. J.; Renner, C.; Xie, X.; Schrader, T. *Angew. Chem., Int. Ed.* **2006**, *45*, 6352–6355.

(3) Corbett, P. T.; Leclaire, J.; Vial, L.; West, K. R.; Wietor, J.-L.; Sanders, J. K. M.; Otto, S. *Chem. Rev.* **2006**, *106*, 3652–3711.

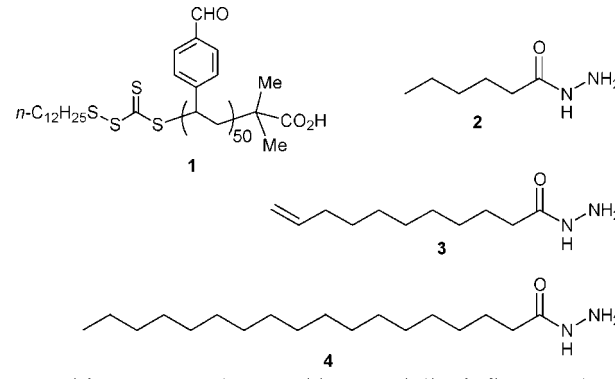
(4) Berg, J. M.; Tymoczko, J. L.; Stryer, L., M. *Biochemistry*, 5th ed.; W. H. Freeman and Company: New York, 2002; Chapter 3, pp 41−73.

10.1021/ol8011309 CCC: $40.75
Published on Web 06/28/2008

dynamic covalent linkages.[6,7] The reversibility of these linkages allows residues to exchange and reshuffle their positions upon the polymer scaffold, and the resulting mixture of constitutionally dynamic[8] polymers can be considered to possess the attributes of a DCL constructed upon a polymer scaffold. We hypothesize that such DCLs should be able to respond to the addition of templates in the same manner as any other DCL. A further advantage of this system is that polymer scaffolds of very precise lengths can be prepared using controlled/"living" radical polymerization methods[9–11] offering a degree of flexibility over the size and complexity of the resultant DCLs.

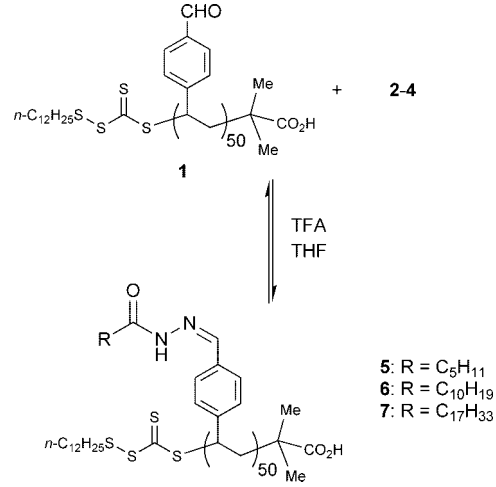
We report here the preparation of a "simple" polymer-scaffolded DCL constructed from a preformed polymer scaffold adorned through dynamic covalent hydrazone bonds with simple acylhydrazide residues, and demonstrate the dynamic nature of this system.

Acylhydrazone exchange was chosen as the dynamic reaction to append residues to suitably functionalized polymer scaffolds as it is a well-studied and successful reaction in dynamic covalent chemistry.[3,12] Acylhydrazone linkages are formed from the acid-catalyzed condensation of acylhydrazides with aldehydes, and the resulting acylhydrazone bonds are readily broken and formed under acidic conditions,[13] while neutralization yields kinetically stable products. These reaction conditions are compatible with many molecular recognition events that could potentially influence the equilibrium distribution of the resulting DCL.



Recent reports describing the preparation of well-defined polymers bearing aldehyde functionalities,[14] coupled with the apparent lack of reports of well-defined polymers bearing acylhydrazide functionalities, led us to base our DCL upon a polyaldehyde scaffold. Polyvinylbenzaldehyde (**1**) (DP = 50, PDI = 1.11) was prepared by reversible addition fragmentation chain-transfer polymerization[9] of vinylbenzaldehyde[15] according to the method described by Wooley and co-workers.[14c] Acylhydrazides (**2**−**4**) containing C_5, C_{10},or C_{17} alkyl chains, respectively, were chosen as simple model residues of differing molecular weights to graft onto the polyvinylbenzaldehyde scaffold. These acylhydrazides were prepared in high yields by simple hydrazinolysis of their corresponding methyl esters.

Scheme 1. Functionalization of Polyvinylbenzaldehyde with Acylhydrazides through Hydrazone Bond Formation



The functionalization of **1** (Scheme 1) was performed using an excess of acylhydrazides **2**−**4** in THF (concentration

(5) For a report describing the generation of a DCL of peptides using nonspecific proteases under conditions in which both hydrolysis and synthesis occur, effectively allowing amino acid residues to exchange and reshuffle their positions within a peptide framework without access to complex celluar machinery, see: Swann, P. G.; Casanova, R. A.; Desai, A.; Frauenhoff, M. M.; Urbancic, M.; Slomczynska, U.; Hopfinger, A. J.; Breton, G. C.; Venton, D. L. *Biopolymers* **1996**, *40*, 617–625.

(6) To the best of our knowledge, the only reported example of the functionalization of pre-formed polymer chains through dynamic covalent bonds where dynamic nature has been demonstrated is: Higaki, Y.; Otsuka, H.; Takahara, A. *Macromolecules* **2004**, *37*, 1696–1701.

(7) (a) The functionalization of pre-formed polymers through dynamic covalent bonds is conceptually different to dynamic covalent polymers prepared by linking monomers together through dynamic covalent bonds. For some examples of such dynamic covalent polymers, see: Otsuka, H.; Aotani, K.; Higaki, Y.; Takahara, A. *J. Am. Chem. Soc.* **2003**, *125*, 4064–4065. (b) Niu, W.; O'Sullivan, C.; Rambo, B. M.; Smith, M. D.; Lavigne, J. J. *Chem. Commun.* **2005**, 4342–4344. (c) Kamplain, J. W.; Bielawski, C. W. *Chem. Commun.* **2006**, 1727–1729. (d) Ono, T.; Fujii, S.; Nobori, T.; Lehn, J.-M. *Chem. Commun.* **2007**, 4360–4362. (e) Ruff, Y.; Lehn, J.-M. *Angew. Chem., Int. Ed.* **2008**, *47*, 3556–3559.

(8) (a) Lehn, J.-L. *Prog. Polym. Sci.* **2005**, *30*, 814–831. (b) Lehn, J.-M. *Chem. Soc. Rev.* **2007**, *36*, 151–160.

(9) (a) Moad, G.; Rizzardo, E.; Thang, S. H. *Aust. J. Chem.* **2005**, *58*, 379–410. (b) Perrier, S.; Takolpuckdee, P. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 5347–5393. (c) Moad, G.; Rizzardo, E.; Thang, S. H. *Aust. J. Chem.* **2005**, *59*, 669–692.

(10) (a) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921–2990. (b) Kamigaito, M.; Ando, T.; Sawamoto, M. *Chem. Rev.* **2001**, *101*, 3689–3746.

(11) Hawker, C. J.; Bosman, A. W.; Harth, E. *Chem. Rev.* **2001**, *101*, 3661–3688.

(12) Rowan, S. J.; Cantrill, S. J.; Cousins, G. R. L.; Sanders, J. K. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 898–952.

(13) (a) Recent work as shown that the well known dynamic covalent reactions—hydrazone and oxime formation—can be catalyzed in water using aniline as a catalyst. These findings open up the possibility of developing water-soluble polymer-scaffolded DCLs. See: Dirksen, A.; Hackeng, T. M.; Dawson, P. E. *J. Am. Chem. Soc.* **2006**, *128*, 15602–15603. (b) Dirksen, A.; Dirksen, S.; Hackeng, T. M.; Dawson, P. E. *Angew. Chem., Int. Ed.* **2006**, *45*, 7581–7584.

(14) (a) Hwang, J. Y.; Li, R. C.; Maynard, H. D. *J. Controlled Release* **2007**, *122*, 279–286. (b) Li, R. C.; Hwang, J.; Maynard, H. D. *Chem. Commun.* **2007**, 3631–3633. (c) Sun, G.; Cheng, C.; Wooley, K. L. *Macromolecules* **2007**, *40*, 793–795. (d) Yang, S.-K.; Weck, M. *Macromolecules* **2008**, *41*, 346–351.

(15) (a) Rodgers, C. J.; Dickerson, T. J.; Wentworth, P.; Janda, K. D. *Tetrahedron* **2005**, *61*, 12160–1216. (b) Manzotti, R.; Reger, T. S.; Janda, K. D. *Tetrahedron Lett.* **2000**, *41*, 8417–8420.

of **1** approximately 0.2 mmol) with TFA catalyst, and each reaction was monitored by gel permeation chromatography (GPC) until no further changes in retention times were observed, indicating equilibrium was reached within 2 h in all three cases.

Analysis of each reaction at equilibrium by GPC (Table 1) revealed the formation of higher molecular weight

Table 1. Comparison of M_n (Measured by GPC), M_n (Calculated), and PDIs for Polymers **1** and **5**−**7**

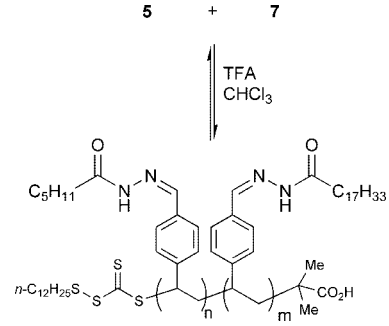
polymer	M_n (GPC)	M_n (calc)[a]	PDI[b]
1	6660	6964	1.11
5	7650	12570	1.13
6	11600	15980	1.13
7	14600	20890	1.14

[a] For polymer **1**, calculated from ^{1}H NMR. For polymers **5**−**7**, the calculated M_n was based on all aldehyde functions of polymer **1** reacting with acylhydrazides to form hydrazones. [b] By GPC.

polymers **5**−**7**, respectively, indicating the successful ligation of the acylhydrazides onto the polymer scaffolds. Under these conditions, the polydispersity index (PDI) of the functionalized polymers was essentially identical to that of the bare polymer **1**, indicating that all aldehyde functions of the polymer have reacted and that the functionalization does not change basic polymer properties. The measured M_n of each functionalized polymer, however, was lower than expected if all aldehyde functions on the polymer had reacted.[16] Each reaction was neutralized by treatment with anion-exchange resin (Amberlyst 21) and polymers **5** and **7** were purified through Sephadex LH-20.[17] The ^{1}H NMR spectra of **5** and **7** were exceptionally broad,[18] but IR spectroscopy of both polymers displayed the absence of the carbonyl stretch at 1702 cm^{-1} present in **1** and the presence of an C═N stretch at 1662 cm^{-1}, suggesting that hydrazone formation is an effective reaction for the functionalization of polymers.[19]

To demonstrate the dynamic nature of these functionalized polymers, **5** and **7** were mixed (Scheme 2) in THF (concentration of each approximately 0.2 mM). A catalytic quantity of TFA (concentration of TFA approximately 5 mM) was

Scheme 2. Hydrazone Exchange of Polymers **5** and **7**



then added and the mixture allowed to equilibrate at room temperature. GPC analysis showed the disappearance of the peaks at 14.61 and 15.34 min and the appearance of a new peak at 15.02 min. The mixture reached equilibrium within 2 h, and the GPC profiles of the reaction before the addition of TFA and at equilibrium are shown in Figure 1. The same

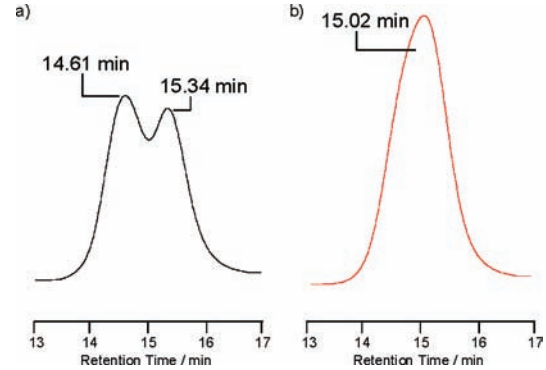


Figure 1. GPC profiles of (a) a THF solution of polymers **5** and **7** before the addtion of TFA catalyst and (b) the mixture at equilibrium after the addition of TFA catalyst.

mixture can also be reached by reaction of 50 equiv of **2** and 50 equiv of **4** with **1** under similar conditions, affording polymer with identical elution volume and PDI. These findings suggest strongly that both the polymer functionalized with C_5 residues and that functionalized with C_{17} residues have undergone hydrazone exchange to form a mixture of polymers functionalized with *both* the C_5 and C_{17} residues.

Such a polymer-based DCL possesses the potential to display vast molecular diversity. The theoretical number of unique members of such a DCL will depend upon the number of aldehydes (or other functional groups) (x) upon the polymer, the number of different types of residue available (y) and the percentage of functional groups on the polymer which have reacted. For the "simple" DCL described based on a polymer with 50 aldehydes ($x = 50$) and two different residue types ($y = 2$), assuming 100% ligation of the polymer leads to a DCL theoretically possessing around 2^{50} unique

(16) Polymers **5**−**7** are structurally different from the polystyrene standards used for GPC calibration, so differences between measured and theoretical M_n are not unexpected.

(17) Comparison of the GPC profiles of the polymers before and after purification indicated identical retention times and PDIs.

(18) The ligation of acylhydrazide residues onto **1** was investigated by ^{1}H NMR titration experiments. These revealed that as hydrazone bonds form, the aldehyde signal ($\delta = 9.81$ ppm) of the polymer deceases in intensity as the CONH signal ($\delta = 10.80$ ppm) of the forming hydrazone bonds increases in intensity. As the stoichiometry of the ligating residues approaches that of the aldehydes of the polymer the ^{1}H NMR spectra became increasingly broad, preventing meaningful interpretation of the aldehyde and hydrazone signals.

(19) (a) The laboratory of Weck (ref 14d) have described recently the functionalization of ketone-functionalized polymers with acylhydrazides through hydrazone bonds, although the dynamic nature of these bonds has not been investigated: Van Horn, B. A.; Iha, R. K.; Wooley, K. L. *Macromolecules* **2008**, *41*, 1618–1626. (b) Van Horn, B. A.; Wooley, K. L. *Soft Matter* **2007**, *3*, 1032–1040. (c) Taniguchi, I.; Mayes, A. M.; Chan, E. W. L.; Griffith, L. G. *Macromolecules* **2005**, *38*, 216–219. See refs 14a and 14b.

members; it is likely that under normal experimental conditions much of this diversity will be "virtual".[20]

Theoretical work[21] on DCLs by the groups of Moore, Severin, Sanders, and Otto make it possible to comment on how such polymer-scaffolded DCLs will respond in the presence of template. Amplification factors for library members which are able to bind successfully a template are predicted[21b] to be higher if the equilibrium distribution is dominated by monomeric building blocks, and aggregates are present in only small amounts. Matching this criteria with polymer-scaffolded DCLs is straightforward, as the library can simply be designed to contain a large excesses of residues relative to polymer scaffold. Furthermore, the complexity of polymer-scaffolded DCLs can by tailored simply be controlling the length of the scaffold and the number of residue types available. Work by Otto and Sanders suggests[21c] that libraries containing $10-10^6$ compounds still allows useful amplification of the single best binder, and oligomeric scaffolds would allow access to libraries possessing this order of diversity. For very large libraries, theoretical work by Moore predicts[21a] that after reequilibration 5% of a library population can become up to 10^4 times greater than the original library at binding a template. If this 5% of the library population can be isolated by, e.g., binding to a resin-supported template followed by washing, theoretically one can evolve rapidly effective functional molecules, although detailed information about structure will remain elusive.

(20) Lehn, J.-M. *Chem. Eur. J.* **1999**, *5*, 2455–2463.

(21) (a) Moore, J. S.; Zimmerman, N. W. *Org. Lett.* **2000**, *2*, 915–918. (b) Grote, Z.; Scopelliti, R.; Severin, K. *Angew. Chem., Int. Ed.* **2003**, *42*, 3821–3825. (c) Corbett, P. T.; Otto, S.; Sanders, J. K. M. *Org. Lett.* **2004**, *6*, 1825–1827. (d) Corbett, P. T.; Otto, S.; Sanders, J. K. M. *Chem. Eur. J.* **2004**, *10*, 3139–3143.

One drawback of utilizing polymer scaffolds such as polystyrene which cannot be subdived is the inability to establish sequence information, although the residual composition of a library member or group of members should be starightforward to determine. Polymer scaffolds based on peptides would present the possibility of sequencing with Edman degradation methods after a library has been kinetically fixed.

In summary, we have prepared mixtures of functionalized polymers possessing the attributes of a DCL and demonstrated their dynamic nature. We are currently expanding the complexity of polymer-scaffolded DCLs by preparing residues incorporating functional groups found in amino acid side chains and investigating the preparation and characterization of DCLs upon short oligomers. We then aim to demonstrate how such polymer-scaffolded DCLs can respond to transition-state analogue templates. We believe polymer-based DCLs have great potential in (1) the discovery of enzyme-like catalysts, (2) the discovery of polymer-based receptors for small molecules, (3) the creation of polymer-based protein-specific receptors, and (4) combinatorial materials research.

Acknowledgment. Research Councils UK (fellowship to D.A.F.), the Royal Society (for provision of a University Research Grant), and Newcastle University are gratefully thanked for generous support.

Supporting Information Available: Experimental procedures, spectroscopic data for new compounds, and GPC profiles of polymers. This material is available free of charge via the Internet at http://pubs.acs.org.

OL8011309