ORGANIC LETTERS

2006
Vol. 8, No. 22
4993−4996

A Retro-Diels–Alder Reaction to Uncover Maleimide-Modified Surfaces on Monolayer-Protected Nanoparticles for Reversible Covalent Assembly

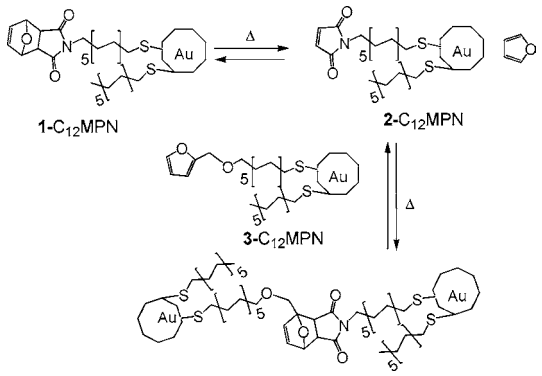
Jun Zhu, Arnold J. Kell, and Mark S. Workentin*

Department of Chemistry, The University of Western Ontario, London, Ontario, Canada N6A 5B7

mworkent@uwo.ca

Received June 28, 2006

ABSTRACT



Maleimide-modified monolayer-protected gold nanoparticles (MPGN) are prepared from the protected furan–maleimide via the thermally reversible Diels–Adler reaction when required. These maleimide-MPGNs serve as a general platform allowing for a Diels–Alder reaction with furan-modified MPN to prepare larger 3D networks reversibly.

Developing methodologies capable of efficiently and reproducibly functionalizing self-assembled monolayers (SAMs) and monolayer protected nanoparticles (MPNs) is an important challenge in nanotechnology. An area of emerging importance is that of biochip technology, where it is critical that the chemistry employed in anchoring biomolecules to surfaces (SAM or MPN) be applicable to numerous biomolecules without having to synthesize or alter the biomolecule prior to surface functionalization.[1−4] Mrksich and co-workers highlighted the elegant simplicity of a maleimide-modified SAM and how it can be employed in the generation of a series of protein and carbohydrate biochips.[5−7] They showed the importance of the maleimide moiety for these combinatorial reactions, where the reaction is ideal as it is very fast and selective and will readily react with any substrate with a thiol or an amine moiety, circumventing the tedious derivatization of substrates prior to surface attachment. Maleimide-functionalized moieties have been exploited to anchor proteins or DNA to surfaces through reaction with

(1) (a) Love, J. C.; Estroff, L. A.; Kriebel, J. K.; Nuzzo, R. G.; Whitesides, G. M. *Chem. Rev.* **2005**, *105*, 1103. (b) Katz, E.; Willner, I. *Angew. Chem. Int. Ed.* **2004**, *43*, 6042.

(2) Xiao, S.; Brunner, S.; Wieland, M. *J. Phys. Chem. B* **2004**, *108*, 16508.

(3) (a) You, C.-C.; Verma, A.; Rotello, V. M. *Soft Mater.* **2006**, 190. (b) Dreschler, U.; Erdogan, B.; Rotello, V. M. *Chem.-Eur. J.* **2004**, *10*, 5570.

(4) Daniel, M.; Astruc, D. *Chem. Rev.* **2004**, *104*, 293.

(5) Yonzon, C. R.; Jeoung, E.; Zou, S.; Schatz, G. C.; Mrksich, M.; Van Duyne, R. P. *J. Am. Chem. Soc.* **2004**, *126*, 12669.

(6) Houseman, B. T.; Gawalt, E. S.; Mrksich, M. *Langmuir* **2003**, *19*, 1522.

(7) Smith, J. C.; Lee, K.; Wang, Q.; Finn, M. G.; Johnson, J. E.; Mrksich, M.; Mirkin, C. A. *Nano Lett.* **2003**, *3*, 883.

10.1021/ol0615937 CCC: $33.50
Published on Web 10/05/2006

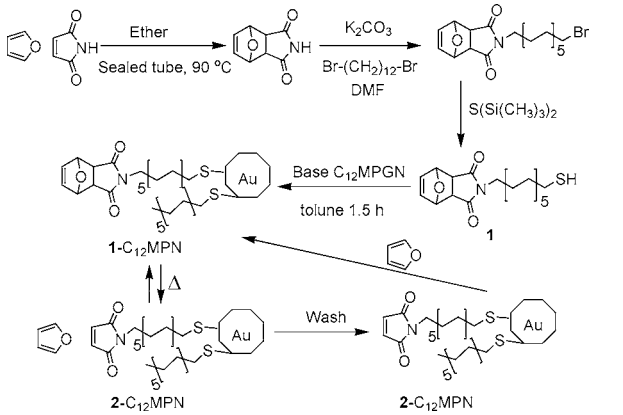
the maleimide moiety,[2,8] and generating complex features on surfaces.[9] Generally these modifications rely on the Michael addition reaction between a thiol or amine group of the biomolecule to the conjugated double bond in the maleimide moiety. The Diels−Alder reaction has also found use as a means to modify SAMs.[10]

Diels−Alder reactivity between furan and maleimides has been frequently used for the preparation of thermally responsive polymers and dendrimers.[11,12] A similar methodology was recently reported as a way to modifiy silicon surfaces.[13] However, because maleimide is such a potent scavenger of thiols, SAM and MPN surfaces on noble metals cannot be synthesized directly from the parent thiol. Instead they must either be functionalized directly with maleimide derivatives from disulfide moieties, which act to remove the reactive thiols with respect to Michael addition reactions, or by reacting a separately prepared surface modified such that it will react with a substituted maleimide moiety in solution, for example: reacting an alcohol- or amine-modified SAM or MPN surface with maleimidopropionic acid esters in a separate reaction. With respect to MPNs, the reducing conditions employed in nanoparticle synthesis will reduce the double bond of the maleimide functionality, rendering it unreactive to Michael addition reactions.[14]

This paper presents an approach to the derivatization of MPN surfaces with a reactive maleimide moiety using the thermally reversible Diels−Alder reaction of furan with a maleimide. We show that by preparing a surface with a furan−maleimide adduct one can efficiently generate, reversibly, a maleimide-modified MPN via the thermally activated retro-Diels−Alder reaction. The approach we present is beneficial as it allows us to better control the amount of the protected maleimide (and thus maleimide) that is incorporated onto the MPN surface as it does not depend on the efficiency of reaction of maleimide moieties in a separate reaction. More importantly, our approach allows the uncovering of the reactive maleimide moiety when it is required. Of course, the maleimide-modified MPN, once unprotected, can be used as a reactive platform for surface modification by Michael addition reactions with thiol or amine nucleophiles, such as those described on SAMs. In this report we illustrate its versatility in Diels−Alder chemistry with other modified MPNs for reversible formation of MPN networks.

The protected maleimide (**1**) required was prepared from the S_N2 reaction of a 3, 6-Endoxo-Δ^4-tetrahydrophthalhide (furan-masked maleimide) with an excess of 1,12-dibromododecane in DMF in the presence of potassium carbonate; the 3, 6-Endoxo-Δ^4-tetrahydrophthalimide was syntheized by a Diels−Alder reaction between maleimide and furan. Following purification, the resulting bromide was reacted with either hexmethyldisilathiane and TBAF in THF to generate the thiol, **1** or by converting the bromide to the thioacetate followed by hydrolysis to the thiol (Scheme 1).

Scheme 1. Reaction Sequence Leading to the Reversible Formation of the Maleimidio-Modified MPGN (**2**-C_{12}MPN)



Compound **1**, was then incorporated into a dodecanethiolate-modified MPN (C_{12}MPN) using a place exchange reaction to form a mixed ligand **1**-C_{12}MPN. The C_{12}MPN was prepared via the Brust−Schiffrin methodology by mixing an organic solution of a 1:1 ratio of dodecanethiol/hydrogen tetrachloroaurate under reducing conditions.[15] The **1**-C_{12}MPN was purified, and the incorporation of **1** was confirmed by ^{1}H NMR spectroscopy. A portion of the ^{1}H NMR spectrum highlighting the key resonances (indicated by arrows) from the adduct on the MPN are illustrated in Figure 1a, namely the broad resonances at 5.65 ppm due to the alkene protons, those at 4.95 ppm due to the two bridgehead protons, and those at 3.5 ppm due to the methylene protons α to the nitrogen. The ring fused protons appear at 2.1 ppm (full spectral details are available in the Supporting Information). **1**-C_{12}MPN was further characterized by TGA (Figure 2), IR spectroscopy and was shown by high-resolution transmission electron microscopy (HR-TEM) to have an average core size of 2.1 ± 0.2 nm (Figure 3a).

Following the preparation and purification of the masked maleimide-modified MPN (**1**-C_{12}MPN), the maleimide moiety can then be uncovered essentially quantitatively through the retro-Diels−Alder reaction to **2**-C_{12}MPN at elevated temperature. Figure 1b is a representative ^{1}H NMR spectrum corresponding to **2**-C_{12}MPN after heating **1**-C_{12}MPN to temperatures of 100−110 °C for 12 h and washing with 95% ethanol to remove the liberated furan following the retro-

(8) Jin, L.; Horgan, A.; Levicky, R. *Langmuir* **2003**, *19*, 6968.
(9) (a) Wang, X.; Zhou, D.; Rayment, T.; Abell, C. *Chem. Commun.* **2003**, 474. (b) Zhou, D.; Sinniah, K; Abell, C.; Rayment, T. *Langmuir* **2002**, *18*, 5055.
(10) (a) Gawalt, E. S.; Mrksich, M. *J. Am. Chem. Soc.* **2004**, *126*, 15613. (b) Chan, E. W. L.; Yousaf, M. N.; Mrksich, M. *J. Phys. Chem. A* **2000**, *104*, 9315. (c) Su, J.; Mrksich, M. *Langmuir* **2003**, *19*, 4867. (d) Kwon, Y.; Mrksich, M. *J. Am. Chem. Soc.* **2002**, *124*, 806. (e) Dillmore, W. S.; Yousaf, M. N.; Mrksich, M. *Langmuir* **2004**, *20*, 7223.
(11) (a) Chen, X.; Dam, M. A.; Ono, K.; Mal, A.; Shen, H.; Nutt, S. R.; Sheran, K.; Wudl, F. *Science* **2002**, *295*, 1698 and references therein. (b) Adachi, K.; Achimuthu, A. K.; Chujo, Y. *Macromolecules* **2004**, *37*, 9793.
(12) McElhanon, J. R.; Wheeler, D. R. *Org. Lett.* **2001**, *3*, 2681.
(13) Wang, Y.; Cai, J.; Rauscher, H.; Behm, R. J.; Goedel, W. A. *Chem.—Eur. J.* **2005**, *11*, 3968.
(14) Example of reduction of α,β-unsaturated ketones during MPN synthesis: Keonig, S.; Chechik, V. *Langmuir* **2003**, *19*, 9511. While the authors show that this can be minimized by control of the reaction conditions, in our hands the maleimide double bond was reduced significantly.
(15) Brust, M.; Walker, M.; Bethell, D.; Schiffrin, D. J.; Whyman, R. *J. Chem. Soc., Chem. Commun.* **1994**, 801.

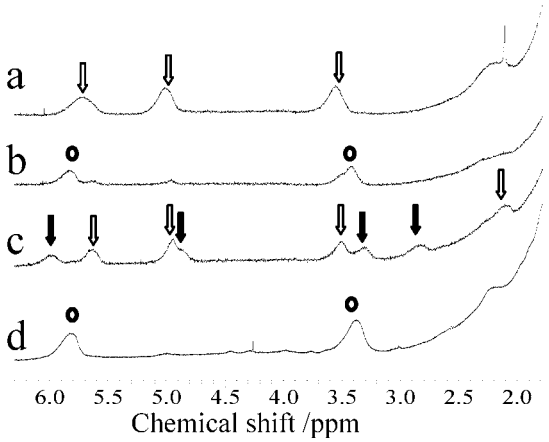


Figure 1. ^{1}H NMR of the Diels−Alder reaction and retro-Diels−Alder reaction between **2**-C_{12}MPN and furan: (a) **1**-C_{12}MPN; (b) **2**-C_{12}MPN, after retro-Diels−Alder reaction and washing to remove liberated furan; (c) Diels−Alder reaction of the liberated **2**-C_{12}-MPN with added furan at room temperature for 12 h showing formation of both exo- and endo-**1**-C_{12}MPN; (d) retro-Diels−Alder reaction to recover **2**-C_{12}MPN.

Diels−Alder reaction. The broad resonances (indicated by open circles) at 5.80 and 3.40 ppm are due to the alkene protons on the maleimide and the methylene protons α to the nitrogen, respectively. Note that there is very little indication of any remaining protected maleimide in this spectrum. This process to form the maleimide MPN avoids the problem of competitive Michael addition reactions with

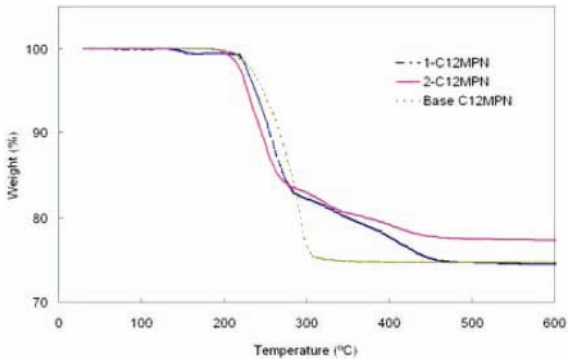


Figure 2. The thermal gravimetric analysis of (a) **1**-C_{12}MPN (blue); (b) **2**-C_{12}MPN (pink); (c) base C_{12}MPN (green). Temperature increase rate: 10 °C/min.

the thiol that would occur if one were to try to make **2**-C_{12}-MPN directly from the corresponding maleimide thiol. A maleimide MPN can be prepared via the direct synthesis of the MPN with the appropriate disulfides, but the functionalization of MPNs with maleimide exclusively leads to insoluble products and significant reduction of the double bond of the maleimide. The present methodology is fast and provides a means of masking the maleimide and uncovering it when it is desired.

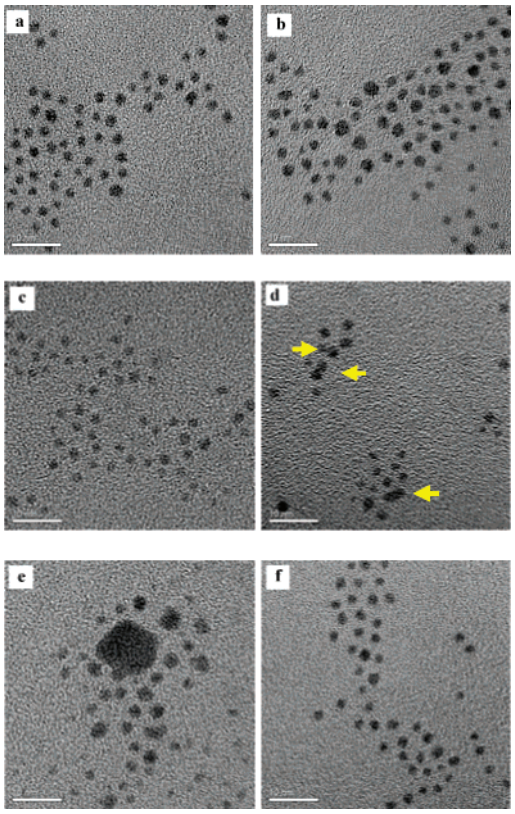


Figure 3. High-resolution TEM of the forward and retro Diels−Alder reactions between **2**-C_{12}MPN and **3**-C_{12}MPN: (a) **1**-C_{12}MPN; (b) **2**-C_{12}MPN; (c) **3**-C_{12}MPN; (d) **2**-C_{12}MPN and **3**-C_{12}MPN mixed for 24 h; (e) **2**-C_{12}MPN and **3**-C_{12}MPN mixed for 48 h; (f) heating the precipitate formed in (e) for 1 h. (Scale shown in the HR-TEM images is 10 nm.)

The resulting **2**-C_{12}MPN can then be mixed with furan to reform **1**-C_{12}MPN. It is noteworthy that the Diels−Alder reaction between furan and **2**-C_{12}MPN results in the generation of both endo and exo adducts of the product, in a ca. 2:1 ratio (Figure 1c). In this figure the open arrows indicate those resonances associated with the exo product (as in Figure 1a), and the solid arrows indicate resonances associated with the endo product, observed during the synthesis of **1**. Heating the sample **1**(endo/exo)-C_{12}MPN shown in Figure 1c at 50 °C converts the product exclusively to the exo product, analogous to that which occurs in solution. Once **1**-C_{12}MPN is reformed, it is possible to again uncover the maleimide to **2**-C_{12}MPN at elevated temperatures. The MPNs remains readily soluble after several cycles of the Diels−Alder/retro-Diels−Alder reaction. Both the forward and retro Diels−Alder reactions were monitored by ^{1}H NMR spectroscopy; the retro reaction is accompanied by some liberation of the thiolate ligand from the MPN surface as disulfide. TEM of the MPNs taken after three cycles shows only a small increase in the average core size (2.5 ± 0.5 nm) and polydispersity (Figure 2b). This slight increase in core size is consistent with the small amount of ligand liberated as disulfide in each heating cycle, the majority of which occurs in the first heating cycle as has been observed with other

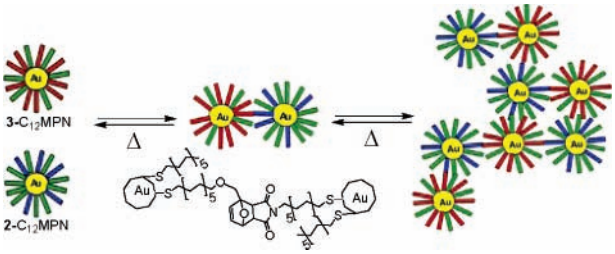
MPNs previously.[16] The resulting MPN can be purified easily with a wash with 95% ethanol and is readily redissolved in benzene. The series of reactions from **1**- C_{12}MPN to **2**- C_{12}MPN to **1**- C_{12}MPN and back to **2**- C_{12}MPN is shown in Figure 1 and nicely illustrates that the reaction can readily occur and does not affect the solubility of the MPNs following heating. It is noteworthy to point out that the maleimide moiety is stable when anchored to the MPN surface as illustrated by the lack of evidence for the Michael addition reaction between thiolate and maleimide in the ^{1}H NMR spectrum of **2**-MPN even months after its initial preparation.

Figure 2 shows the TGA of C_{12}MPN, **1**-C_{12}MPN, and **2**-C_{12}MPN, respectively. For the base C_{12}MPN, the dodecanthiolate was decomposed from the MPN surface from 220 to 300 °C with a total mass loss of 25%, consistent with that expected for the 2.2 ± 0.2 nm MPN. The mass loss of the maleimide ligands from the **2**-C_{12}MPN is around 270−450 °C. The TGA of **1**-C_{12}MPN shows evidence for the retro-Diels−Alder reaction to **2**-C_{12}MPN, even as a solid sample. The mass loss for **1**-C_{12}MPN occurs in three steps: the first step starting from 100 to 130 °C, and accounting for only 0.6% is likely due to the release of furan during the thermally induced retro-Diels−Alder reaction, followed by a mass loss of 16.9% from 240 to 270 °C, which is due to the loss of dodecanthiolate ligands and then followed by the loss of the maleimide ligands, formed during the TGA experiment, from 270 to 450 °C (mass loss 8.0%). The mole ratio of furan/maleimide moieties/dodecanthiolate on the **1**-C_{12}MPN is about 1:1:3, which is confirmed by the ^{1}H NMR integration results.

As illustrated simply above, modification of **2**-C_{12}MPN by Diels−Adler reactions with appropriate dienes in solution can occur, but here we show the utility of the reversible Diels−Alder reaction between **2**-C_{12}MPN with MPNs protected by a furan moiety (**3**-C_{12}MPN) for the preparation of larger assemblies. To this end 2-{[(12-mecaptododecyl)oxy]-methyl}furan (**3**) was synthesized, and then **3**-C_{12}MPN was prepared by the place-exchange method, purified, and characterized by ^{1}H NMR and IR spectroscopy, TGA, and TEM; the latter shows a particle with an average core size of 2.2 ± 0.2 nm. Full characterization details are provided in the Supporting Information. Unfortunately, the forward and retro-Diels−Alder reaction between **2**-C_{12}MPN and **3**-C_{12}MPN cannot be directly characterized by ^{1}H NMR spectroscopy because only a small fraction of the maleimide and furan end groups on the MPNs need to undergo a Diels−Alder reaction before the resulting dimers/trimers, and other aggregates begin to precipitate out of the solution. However,

(16) Hostetler, M. J.; Templeton, A. C.; Murray, R. W. *Langmuir* **1999**, *15*, 3782.

Scheme 2. Forward and Retro Diels−Alder Reactions between Maleimide-Modified MPGNs (**2**-C_{12}MPN) and Furan-Modified MPGN (**3**-C_{12}MPN)



HR-TEM provides more direct evidence for the Diels−Alder induced covalent assembly and retro-Diels−Alder dissociation between **2**-C_{12}MPN and **3**-C_{12}MPN (d, e, and f of Figure 3), illustrated in Scheme 2. After mixing **2**-C_{12}MPN with **3**-C_{12}MPN for 24 h, dimers and other larger aggregates are found (Figure 3d). Further reaction results in larger covalent aggregates (Figure 3e), which eventually precipitate and can be visibly observed. It is clear that there is a covalent reaction between these two MPNs because if **3**-C_{12}MPN is mixed with an MPN modified with a succinimide (and not maleimide) then no aggregation occurs. Heating the resulting precipitate at 100 °C for 1 h allows for the retro-Diels−Alder reaction, and the precipitated particles readily redissolve into the solution (Figure 3f). This reversibility also supports large, insoluble aggregates made up of discreet MPNs and not precipitated gold. Moreover, the forward and backward Diels−Alder reaction between these two kinds of MPNs has very good reversibility even after 30 cycles.

In this study we have provided a simple and efficient route to prepare, reversibly, MPNs modified with a reactive maleimide moiety. Further, we have demonstrated the reversible formation of 3D MPN assemblies utilizing the reversible Diels−Alder reaction between maleimide- and furan-modified MPNs, respectively. These maleimide-modified MPNs provide routes to expanding the scope of Diels−Alder reactions of **2**-C_{12}MPN with other dienes for controlled surface modifications. The maleimide moiety is also a reactive platform for further modifications via Michael additions with solution-based thiols and amines. We will report on the scope of reactions in the future.

Acknowledgment. NSERC, Western, PREA and CFI are thanked for financial support.

Supporting Information Available: General experimental details, details of synthesis and characterization of **1**, **3**, **1**-, **2**-, and **3**-C_{12}MPN. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0615937