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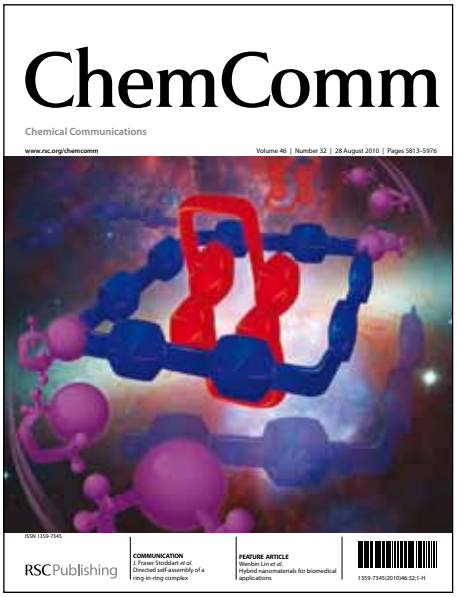

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Covalent Attachment and Release of Small Molecules from Functional Polyphenylene Dendrimers

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Abstract: Herein, we report the synthesis of 2nd generation PPDs functionalized with free thiol moieties within the scaffold, which were used as anchor points for the covalent attachment of guest species (*p*-nitrophenol derivatives) through the oxidative formation of disulfide linkages. The disulfide bonds were then cleaved under reductive conditions using dithiothreitol to discharge the molecules.

Nanostructures capable of encapsulating guest molecules and releasing them through a controlled mechanism have tremendous potential for a range of applications.[1-6] Polyphenylene dendrimers (PPDs) represent a unique class of macromolecules based on their monodisperse, shape-persistent structure, which can be functionalized at the core, scaffold, and surface.[7-14] The ability to functionalize PPDs in a site-specific nature allows for the synthesis of well-defined, highly functional dendrimers that could be used as hosts for encapsulation and release studies.[15-17]

Previously, our group has investigated PPDs as hosts for small molecules through thermodynamic interactions between the scaffold of the dendrimers and guest species. Former work described the synthesis of 2nd generation PPDs internally functionalized with eight carboxylic acid groups that were used to bind 3-4 proflavin hydrochloride dye molecules.[18] Upon this encapsulation the polar dye species became soluble in organic solvents (hexanes, $CHCl_3$, *etc*.). Recently, further studies were carried out to investigate the driving forces behind the encapsulation of small molecules within PPD scaffolds through isothermal titration calorimetry (ITC) analysis.[19] It was found that the uptake of non-polar guest molecules (benzene and toluene) in unfunctionalized PPDs was entropically driven through the release of solvent molecules and their molecular exchange with the guests. Additionally, when the PPDs were synthesized with polar scaffolds (*i.e.* carboxylic acids, pyridines, nitrobenzenes) and loaded with polar species (acetonitrile, acetone, benzaldehyde, *etc*.) the encapsulation process was influenced by enthalpic factors, such as hydrogen bonding and π-π interactions. Yet a significant area that is often overlooked in encapsulation and release studies is the stability of the assemblies, where thermodynamic stabilization may not provide the necessary strength to retain the guest molecules until the desired release site.

Our group enhanced the stability of PPD based guest/host structures by introducing azo-benzene functionalities into the dendrimer scaffold that underwent a reversible cis-trans isomerization upon irradiation at 450 nm (cis-trans) or 365 nm (trans-cis).[20] "Open" PPDs (trans- isomer) were loaded with *p*-nitrophenol units and upon isomerization to the cis- structure two guest molecules were sterically sealed per macromolecule. These loaded macromolecules proved stable despite multiple precipitations and washings, while the encapsulated molecules were only released upon isomerization back to the trans-structure.

In an effort to further improve the introduction of guest species into PPD nanostructures, we report the covalent attachment of small molecules within their scaffolds through the use of disulfide bonds. These linkages are known to cleave under reductive conditions[21-24] which allowed us to achieve stable macromolecular structures with a controlled release mechanism as illustrated in **Scheme 1**. 2nd Generation PPDs were synthesized with eight thiol moieties decorating the scaffold. A thiol-functionalized *p*-nitrophenol derivative was chosen as the guest molecule for two reasons: 1) its attachment and release to the PPDS can be clearly characterized by nuclear magnetic resonance (NMR), ultra-violet and visible (UV-Vis) spectroscopies, and matrix-assisted laser desorption ionization time of flight (MALDI-TOF) spectrometry; 2) it provides a comparison for the dendrimer functionalization efficiency of this system as compared to the encapsulation and release approach of the photo-switchable azo-benzene functionalized PPDs.[20]

Pyrene was chosen as the dendrimer core to act as a fluorescent tag, and four tetraethynyl-biphenyl spacers were introduced at the 1, 3, 6, and 8 positions to increase the free volume of the cavities (Supporting Information **Scheme 1**, **Compound 1**). A thioacetate functionalized cyclopentadienone was synthesized (S.I. **Scheme 1**, **Compound 2**) and used in a Diels-Alder cycloaddition with the pyrene core, followed by the deprotection of the triisopropylsilyl (TIPS) groups upon reaction with tetrabutyl ammonium fluoride to afford the 1st generation PPD (SI **Scheme 1**, **SI-PPD 1**). MALDI-TOF spectrometry confirmed the synthesis of a monodisperse dendrimer with a molecular weight $[M/Z]_{exp}$=3228.10 g/mol ($[M/Z]_{theor.}$=3230.95g/mol).

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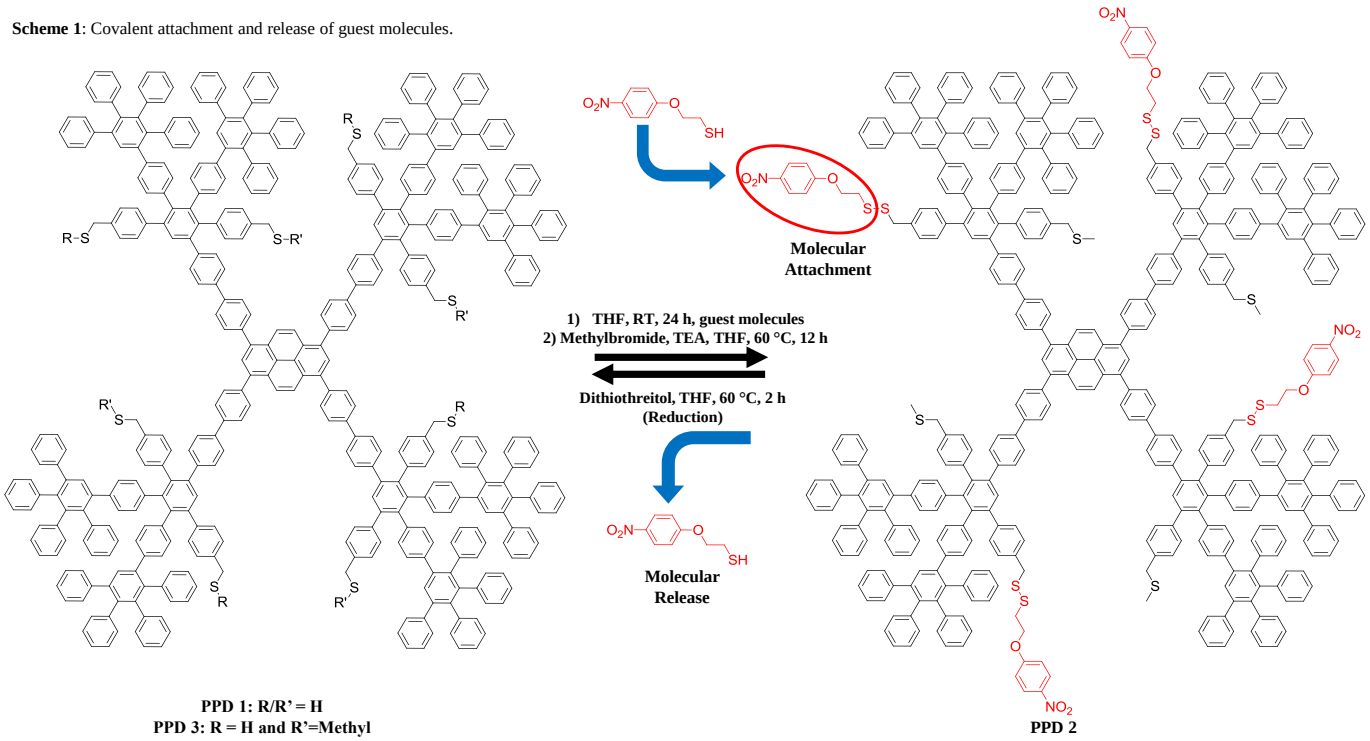
Scheme 1: Covalent attachment and release of guest molecules.



SI-**PPD 1** underwent an additional Diels-Alder reaction with a tetraphenylcyclopentadienone to achieve a monodisperse 2nd generation dendrimer (SI **Scheme 1**, **SI-PPD 2**) with a $[M/Z]_{exp}$=6082.54 g/mol ($[M/Z]_{theor.}$=6081.21 g/mol), as characterized by MALDI-TOF spectrometry. The thioacetate functionalities of **SI-PPD 2** were deprotected to the resulting thiols by reacting them with excess dimethyl amine to yield **PPD 1** (**Scheme 1**) in quantitative yield. MALDI-TOF spectrometry confirmed the molecular weight to be $[M/Z]_{exp}$=5745.66 ($[M/Z]_{theor.}$= 5745.24 g/mol; **Figure 2 A**), and ^{1}H NMR spectroscopy showed a signal shift for the benzylic protons of the scaffold from 4.0 ppm (adjacent to the thioacetate) to 3.8 ppm (adjacent to the thiol), while the signal for the methyl groups of the thioacetate moieties at 2.4 ppm completely disappeared (**SI- Figure 5**).

Thiol functionalized dendrimers (**PPD 1**) were reacted with 2-(4-nitrophenoxy)ethanethiol (50 equivalents, SI-**Compound 4**) in THF at room temperature open to atmospheric oxygen to covalently attach the small molecules within the scaffolds *via* oxidative disulfide bond formation, as seen in **Scheme 1**. The macromolecular structures were precipitated in methanol, which is a good solvent for 2-(4-nitrophenoxy)ethanethiol and a poor solvent for the PPDs, then further purified by dialysis in THF for 24 h to remove any non-reacted molecules. According to MALDI-TOF spectrometry ($[M/Z]_{exp}$=6535.10 g/mol, **Figure 2 B**), we were able to covalently bind four 2-(4-nitrophenoxy)ethanethiols (molecular weight ~ 198 g/mol) per dendrimer, where the molecular weight and NMR analysis confirmed defined structures without a distribution of the number of bound species. Shorter reaction periods (4-20 h) led to the attachment of fewer guest species (1-3 molecules), while extended reaction times (≥ 24 h) did not result in an increased degree of functionalization. This indicates that the loading capacity of the dendrimers was limited by the free volume of the dendritic cavities, and thus steric constraints led to an upper limit of 4 guest molecules per host. Additionally, we did not investigate the use of oxidizing agents to encourage the formation of the disulfide bonds because of the possibility of cyclodehydrogenation of the polyphenylene backbone, which has been extensively reported in literature.[17]

Furthermore, the residual thiols were end-capped with methylbromide in the presence of triethylamine (TEA) in THF to yield **PPD 2** (**Scheme 1**) to prevent additional oxidative reactions. MALDI-TOF spectrometry showed an increase in molecular weight of 55 g/mol (**Figure 2 C**) that equates to four methyl end-capping groups per dendrimer, again showing that there were four residual thiols after reaction with the 2-(4-nitrophenoxy)ethanethiol.

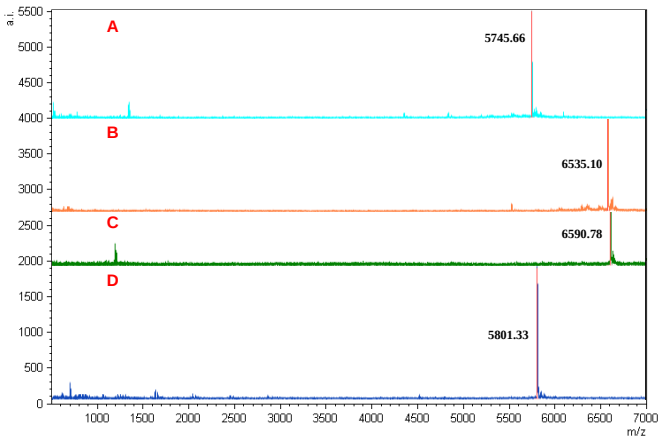


Figure 2: MALDI-ToF spectra of A) **PPD 1** B) **PPD 2** before end-capping residual thiols C) **PPD 2** D) **PPD 3**.

^{1}H NMR spectroscopy was used to calculate the number of 2-(4-nitrophenoxy)ethanethiol and end-capped methyl groups within the dendrimers. As seen in **SI-Figure 6 A**, integrating the proton signals from the nitrophenol derivatives (A) and methylthiols (F) against those of the PPDs (C and D), it was determined that there were four small molecules and four methyl end-capped thiols in each dendrimer.

A very important aspect of the molecular design of these dendrimers was their optical properties, specifically the photoluminescence of the pyrene, so it was necessary to characterize the influence of the nitrophenol derivatives, a known chromophore. **Figure 4** shows the UV-Vis absorption of the pyrene core, sequential dendrimer generations, and the PPDs loaded with the nitrophenol derivatives. The unfunctionalized 2nd generation PPDs had a maximum absorption at λ_{max}~290 nm with an absorption from the pyrene core at λ~380 nm, while 2-(4-nitrophenoxy)ethanethiol had a λ_{max}~310 nm. **PPD 2** still had a λ_{max}~290 nm, but displayed a shoulder ~310 nm related to the attached nitrophenol derivatives. There was a $\lambda_{emission}$~450 nm ($\lambda_{excitation}$=400 nm) for the dendrimers with and without the guest molecules, as characterized by photoluminescence spectroscopy, which was expected since there is no overlap between the photoluminescence of the pyrene core and absorption of the nitrophenol derivatives.

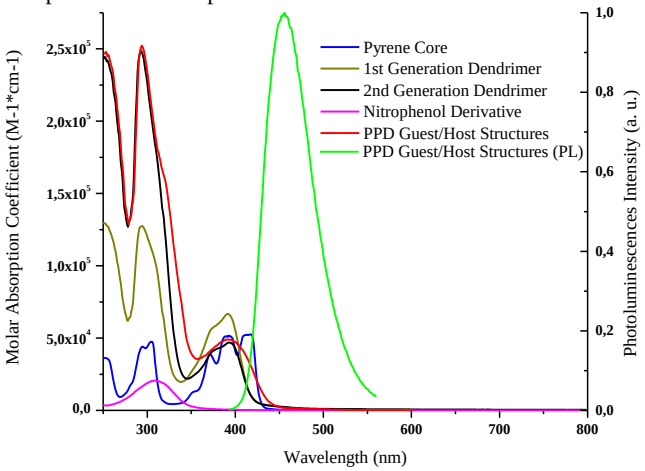


Figure 4: UV-Vis and photoluminescence spectra for the pyrene core, 1st and 2nd generation PPDs, 2-(4-nitrophenoxy)ethanethiol, and functionalized PPDs.

Next it was imperative to demonstrate the ability to cleave the bond between the covalently bound molecules and PPDs under reductive conditions. **PPD 2** was reacted with dithiothreitol (20 equivalents) in THF at 60 °C for 2 h to reduce the disulfide bonds, and thus release the guest species. The dendrimers were precipitated in methanol and purified by dialysis in THF for 24 h to remove the nitrophenol derivatives. **Figure 2 D** shows the MALDI-TOF spectrum of **PPD 3** post-reduction with a $[M/Z]_{exp}$=5801.33 g/mol (($[M/Z]_{theor.}$=5800.56 g/mol) that is 789 g/mol less than **PPD 2,** which correlates to the loss of the four nitrophenol derivatives. ^{1}H NMR spectroscopy reveals the disappearance of the signals associated with the 2-(4-nitrophenoxy)ethanethiols at 3.1, 4.0, and 8.2 ppm (**SI-Figure 6A**: protons A, B, and E), while the signal for the benzylic protons of the scaffold shifted from 3.7 ppm (adjacent to the disulfide linkage) to 3.6 when adjacent to the thiols (**SI-Figure 6B**). This demonstrated the quantitative cleavage of the disulfide bonds under reductive conditions that led to the release of the nitrophenol derivatives by a controlled mechanism. Additionally, this process highlighted the stability of the covalently attached molecules, since they remained within the scaffold through vigorous purification steps until the disulfide bonds were cleaved.

Conclusions

We report the synthesis of 2nd generation polyphenylene dendrimers that were functionalized with eight thiols throughout their scaffolds. These thiols were used as anchor points to covalently bind guest moieties (*i.e.* 2-(4-nitrophenoxy)ethanethiol) through oxidative disulfide bond formation, which yielded chemically stable macromolecular structures with four molecules per dendrimer. Furthermore, under reductive conditions the disulfide linkages could be quantitatively cleaved resulting in the discharge of all the nitrophenol derivatives. To our knowledge this represents the first report of the covalent attachment of small molecules to the scaffold of a dendrimer, which can be chemically cleaved, yielding stable multicomponent PPDs that possesses a trigger for the controlled release of the guest species.

Notes and references

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† Electronic Supplementary Information (ESI) available: Synthetic schemes, experimental procedures, and additional ^{1}H NMR, MALDI-ToF, and UV-Vis characterization. See DOI: 10.1039/c000000x/

1 K. Greish, T. Sawa, J. Fang, T. Akaike, H. Maeda, *J. Control Release*, 2004, **97**, 219.
2 T. Fojo, C. Grady, *N. Engl. J. Med.*, 2009, **360**, 563.
3 H. Tan, T. Zhan, W. Y. Fan, *J. Phys. Chem. B*, 2006, **110**, 21690.
4 S. J, Guillaudeu, M. E. Fox, Y. M. Haidar, E. E. Dy, F. C. Szoka, M. J. M. Fréchet, *Bioconjugate Chem.*, 2008, **19**, 461.
5 C. C. Lee, J. A. MacKay, M. J. M. Fréchet, F. C. Szoka, *Nat. Biotechnol.*, 2005, **23**, 1517.
6 M. J. Maciejewski, *Macromol. Sci. Chem.*, 1982, **17**, 689.
7 L. Liu, R. Breslow, *J. Am. Chem. Soc.*, 2003, **125**, 12110.
8 T. M. Miller, T. X. Neenan, R. Zaya, H. E. Bair, *J. Am. Chem. Soc.*, 1992, **114**, 1018.
9 F. Morgenroth, E. Reuther, K. Müllen, *Angew. Chem. Int. Ed. Engl.*, 1997, **36**, 631.
10 M. Müller, C. Kübel, K. Müllen, *Chem. Euro. J.*, 1998, **4**, 2099.
11 K. Harre, G. Wegner, *Polymer*, 2006, **47**, 7312.
12 F. Jaiser, D. Neher, A. Meisel, H. G. Nothofer, T. Miteva, A. Herrmann, K. Müllen, U. Scherf, *J. Chem. Phys.*, 2008, **129**, 114901.
13 N. Krasteva, Y. Fogel, R. E. Bauer, K. Müllen, Y. Joseph, N. Matsuzawa, A. Yasuda, T. Vossmeyer, *Adv. Funct. Mater.*, 2007, **17**, 881.
14 M. Stemmler, F. D. Stefani, S. Bernhardt, R. E. Bauer, M. Kreiter, K. Müllen, W. Knoll, *Langmuir*, 2009, **25**, 12425.
15 J. Sakamoto, K. Müllen, *Org. Letters*, 2004, **6**, 4277.
16 S. Kobayashi, H. Uyama, S. Kimura, *Chem. Rev.*, 2001, **101**, 3793.
17 D. Türp, T. T. T. Nguyen, M. Baumgarten, K. Müllen, *New J. Chem.* 2012, **36**, 282.
18 R. E. Bauer, J. C. G. Clark, K. Müllen, *New. J. Chem.*, 2007, **31**, 1275.
19 T. T. T. Nguyen, D. Türp, D. Wang, B. Nolacher, F. Laquai, K. Müllen, *J. Am. Chem. Soc.*, 2011, **133**, 11194.
20 K. Chiad, M. Grill, M. Baumgarten, M. Klapper, K. Müllen, *Macromolecules*, 2013, **46**, 3554.
21 W. Zhang, S. E. Tichy, L. M. Pérez, G. C. Maria, P. A. Lindahl, E. E. Simanek, *J. Am. Chem. Soc.*, 2003, **125**, 5086.
22 G. Saito, J. A. Swanson, K. D. Lee, *Adv. Drug. Del. Rev.*, 2003, **2**, 199.
23 A. R. Frand, J. W. Cuozzo, C. A. Kaiser, *Trends in Cell Biology*, 2000, **10**, 203.
24 H. F. Lodish, N. Kong, *J. Bio. Chem.*, 1993, **268**, 20598.