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Cross-Linked Dendrimer Hosts Containing Reporter Groups for Amine Guests

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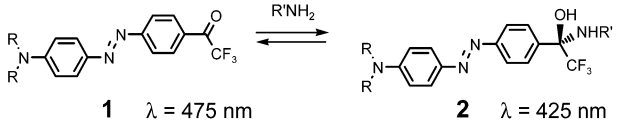
Considerable attention has been focused on improving the properties of molecularly imprinted polymers (MIPs).[1,2] Recently, we disclosed[3] a strategy for imprinting a single molecular template within a protein-sized dendritic polymer.[4] This proof of principle study showed that a porphyrin could template the formation of a macromolecular host whose eight carboxylic acid groups within a cross-linked dendrimer selectively bound porphyrin guests presenting four or eight hydrogen bond acceptor sites.

Although porphyrins serve as spectrophotometric indicators of binding, an ideal host would itself signal the complexation. Additionally, one would like to know whether the same approach would succeed with a smaller template and dendrimer presenting fewer binding contacts. Herein we report the integration of a chromogenic reporter group into our monomolecular imprinting approach. We also apply a rigorous test of imprinting by comparing the cross-reactivities of two dendrimers derived from different templates, a method occasionally applied to MIPs.[5] In the current work, the test reveals a guest-dependent kinetic binding effect masquerading as evidence of a highly selective two-point imprinting process.

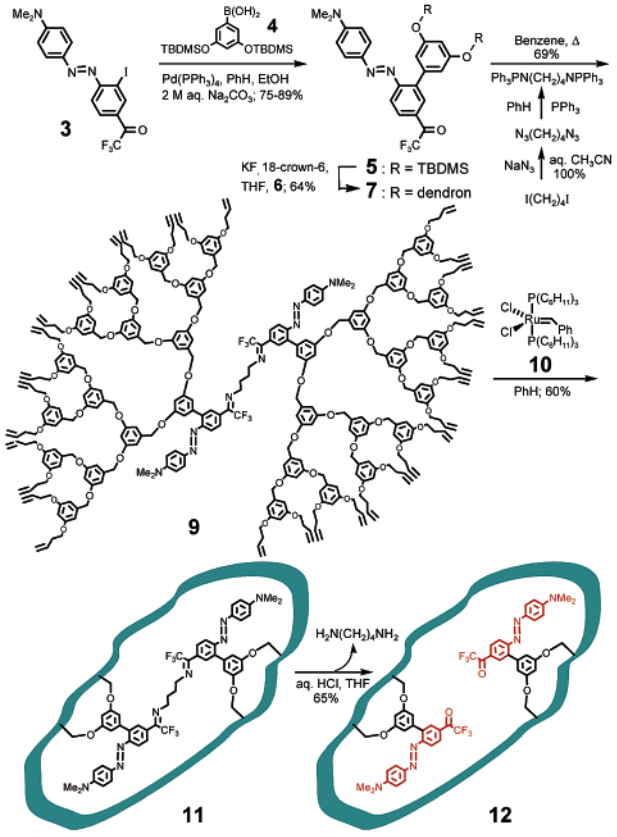
An appealing chromogenic reporter for amines is (trifluoroacetyl)azobenzene dye **1**,[6] studied by Mohr in the context of polymer-based amine sensors.[7] Dye **1** reversibly forms hemiaminal **2** resulting in a ~50 nm blue shift that is readily seen as a change in solution color from red-orange to yellow. In THF, the measured association constant (K_{assoc}) for the complexation of butylamine by **1** (R = Me) was ca. 150−300 M^{-1}.



The dendron used in this study was the same as that used for the porphyrin imprinting[3] and for the synthesis of cored dendrimers.[8] Compound **3**, synthesized in four steps from *N*,*N*-dimethylaniline and 4-fluorobromobenzene (Scheme 1), was used to link the dendron to the dye.[9] Thus, boronic acid **4**, made in two steps from 5-iodo resorcinol, underwent Suzuki coupling with **3** to give **5**, which, in turn, was deprotected and trapped in situ with third-generation dendritic bromide **6** (structure not shown) to afford the dendron-dye conjugate **7** in good yield.

Two-point covalent imprinting was examined by using 1,4-diaminobutane (putrescine, **8**) as a template. Although **8** formed a 2:1 complex with **7** (see **2**), it was not stable under the high-dilution conditions needed for the unimolecular cross-linking reaction. The two dendrons were instead linked to the diamine core through the bis-imine (i.e., **9**).[10] Thus, 1,4-diazidobutane was refluxed with triphenylphosphine to form the bis(iminophosphorane) intermediate which was treated with dye **7** to provide **9**.[11] RCM reaction of **9** using Grubbs' catalyst **10**[12] provided **11** as a mixture of isomeric

Scheme 1



cross-linked dendrimers.[3,8] The MALDI-MS spectrum of **11** showed formation of 13−16 out of a possible 16 cross-links.[9] Bis(imine) **11** was readily hydrolyzed to give **12**, whose MALDI-MS spectrum was consistent with loss of the core. Likewise, the λ_{max} shift from 436 to 480 nm indicated full conversion of the imine to the ketone.

Qualitative binding studies of **12** with **8** and butylamine in THF indicated much stronger binding of diamine **8** than butylamine. Thus, 8 equiv of **8** changed the color of a 30 μM solution of **12** in THF from red-orange (λ_{max} = 480 nm) to yellow (λ_{max} = 431 nm), whereas under the same conditions butylamine showed a negligible color change. A 10.7 ppm upfield shift in the ^{19}F NMR signal due to the trifluoromethyl group of **12** confirmed that the binding involved formation of a hemiaminal with **8**.[13] A Job plot indicated 1:1 stoichiometry (**12**·**8**), and the binding reversibility was shown by diluting a solution of complex whereupon the color reverted to orange-red and gave spectra of mostly unbound **12**.

Complexation studies in THF afforded an apparent association constant (K_{app} = 2.7 × 10^4 M^{-1}) for **12**·**8** that was ca. 200-fold higher than that for butylamine·**7** (K_{app} ≈ 140 M^{-1}). The advantage of two-point binding is less when compared to the stepwise K_{app} for formation of a 2:1 complex between butylamine and **12** ($K_{app(11)}$

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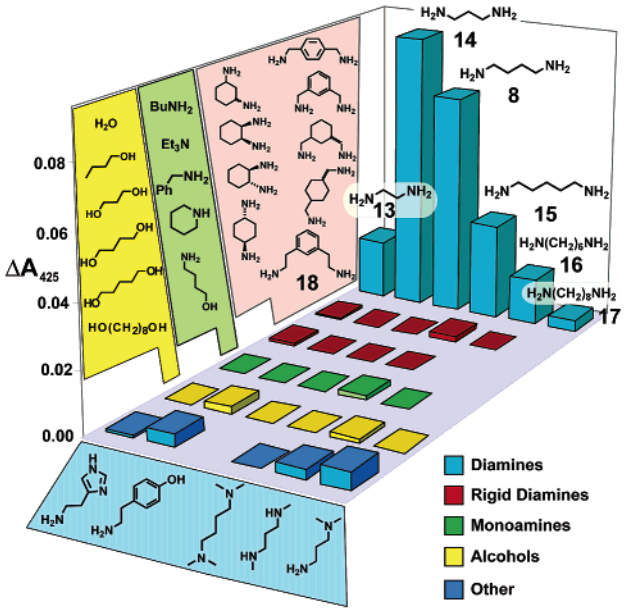

Figure 1. Binding selectivity profile for **12**.

= 500 M^{-1}, $K_{app(12)}$ = 1000 M^{-1}), but this comparison is complicated by the interaction between the dye sites.

The chromogenic response shown by **12** allowed its selectivity profile to be established readily using a small library of amino and alcoholic targets (Figure 1). A simple, qualitative screening assay involved adding 1 equiv of each guest to a 20 μM solution of **12** in THF and measuring the increase in absorbance at λ = 425 nm (ΔA_{425}) 30 s after mixing.

The remarkable level of selectivity for the straight-chain diamines (e.g., **13**−**17**, Figure 1), in particular for **8** and **14**, suggests a cross-linked dendrimer with an effectively imprinted binding site showing a high degree of shape and functional group discrimination. However, a key control experiment would be to show that the same chemistry outlined in Scheme 1, but using a different diamine template, ideally one weakly bound by **12**, gives an imprinted dendrimer with a different binding profile, in other words, to demonstrate *different cross-reactivities for two dendrimers imprinted with different templates*. To apply this stringent test, a second cross-linked dendrimer was synthesized from 1,3-bis(2-azidoethyl)benzene.[9] Rather than showing selective binding of its template (i.e., **18**), a diamine that was weakly bound by **12**, this new dendrimer showed a guest binding profile very similar to that seen for **12** (Figure 1).[9] In another control study, a third dendrimer was prepared by treating **7** with **10** at a concentration that favors formation of a cross-linked dimer. The dimer, purified by size exclusion chromatography (SEC) and appearing identical to **12** by ^{1}H NMR, MALDI-MS, and analytical SEC, also showed guest binding properties similar to those of **12**.

Molecular modeling studies[9] indicated a very open cross-linked framework for **12** with sufficient flexibility to allow considerable adjustment of the distance between azo dye units. Thus, the selectivity for straight-chain diamines exhibited might reflect a kinetic effect wherein diamines **8** and **14** bound rapidly because they are the least sterically hindered and best able to employ an intramolecular general base catalyzed addition mechanism. The latter is a type of bifunctional catalysis that has been documented extensively by Hine,[14] and specifically in the formation of carbinol amines by Page and Jencks.[15] Whatever the origin, the selectivity shown by **12** was clearly not maintained over time. In longer-term (12−24 h) screening assays, there were smaller differences between diamines **8** and **13**−**17** as each exhibited a higher degree of complex formation. Several other diamines, including the cyclohexyl diamines and *N*-methylated diamines, achieved 30−60% of the signal changes seen with **8** and **13**−**17**.

Described herein is an approach to macromolecular hosts that show rapid, selective, high affinity, two-point binding of straight-chain diamine guests. Over longer times, the hosts are more promiscuous, binding a broader range of diamines. However, the binding site, made by cross-linking the two template-linked dendrons, carrying 16 alkene groups each, does not arise from template-mediated imprinting. This is in contrast to our previous study wherein a porphyrin template holding 8 dendrons each with 8 alkenes produced an effective imprint. Current efforts are focused on further defining the design rules for this monomolecular imprinting approach and looking at new scaffolds that might be generally applicable. It is clear that the integration of reporter units as binding groups is a compelling advance because it allows rapid screening of guests and small guest libraries. Reporter group-cored dendrons for other functional groups are currently under development.

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Supporting Information Available: Synthetic details and additional binding and characterization data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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