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Photoswitchable Dynamic Combinatorial Libraries: Coupling Azobenzene Photoisomerization with Hydrazone Exchange

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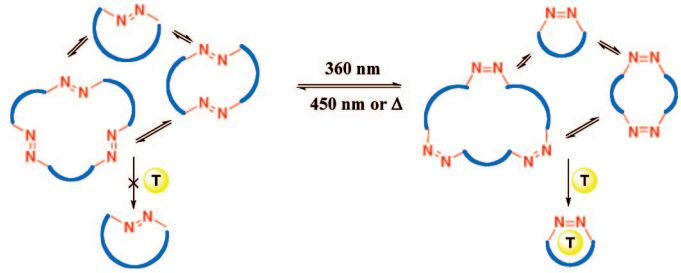

The novel azobenzene-based monomer **1** was prepared, equipped with the necessary functionality to undergo simultaneous dynamic exchange processes: hydrazone exchange and photoisomerization. Acid-promoted hydrolysis of the azobenzene building block produced a dynamic combinatorial library of cyclic oligomers, while multibuilding block libraries were also generated upon addition of proline-based monomers. Libraries equilibrated under thermal conditions were dominated by trans isomers of the azobenzene macrocycles, whereas light-induced isomerization resulted in a conformational change of the library members to their corresponding *cis*-azo form. In the presence of a pentaproline template, a slower rate of thermal relaxation of the *cis*-azobenzene species $\mathbf{1}_c$ was observed, resulting in stabilization and amplification of this receptor due to favorable binding interactions. The facile identification and application of such photoswitchable receptors have the potential to allow for greater control over molecular recognition events.

Introduction

Dynamic combinatorial chemistry (DCC) is an attractive alternative to rational design in the development of novel receptors in which molecular recognition guides the synthesis of complex host systems from simple building blocks using reversible linkages under thermodynamic control (Figure 1).[1] It takes advantage of the ability of a molecular target to template the preferential bond formation of the strongest target binders, upon which desired receptors are amplified at the expense of other oligomers.[2] DCC not only allows for the discovery of nonintuitive receptors, but it also provides access to structures that may otherwise be unobtainable by traditional synthesis.[3]

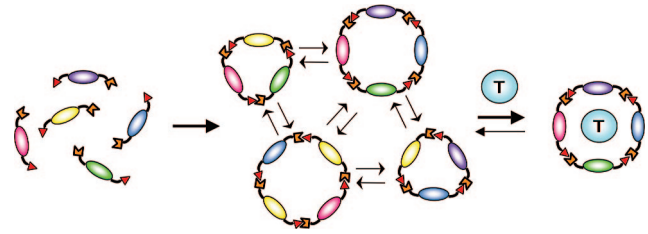

FIGURE 1. Generation and templation of a dynamic combinatorial library (DCL).

In recent years, DCC has found application among a variety of disciplines, ranging from drug discovery to material science.[4]

Incorporating building blocks with new features and potentially interesting recognition elements is desirable in order to

(1) For reviews, see: (a) 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. (b) Lehn, J.-M. *Chem.—Eur. J.* **1999**, *5*, 2455–2463. (c) Ladame, S. *Org. Biomol. Chem.* **2008**, *6*, 219–226. (d) Ludlow, R. F.; Otto, S. *Chem. Soc. Rev.* **2008**, *37*, 101–108. (e) Otto, S. *Curr. Opin. Drug Disc. Devel.* **2003**, *6*, 509–520.

(2) For example, see: (a) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *Science* **2002**, *297*, 590–593. (b) Baolu, S.; Stevenson, R.; Campopiano, D. J.; Greaney, M. F. *J. Am. Chem. Soc.* **2006**, *128*, 8459–8467. (c) Voshell, S. M.; Lee, S. J.; Gagne, M. R. *J. Am. Chem. Soc.* **2006**, *128*, 12422–12423.

(3) (a) Lam, R. T. S.; Belenguer, A.; Roberts, S. L.; Naumann, C.; Jarrosson, T.; Otto, S.; Sanders, J. K. M. *Science* **2005**, *308*, 667–669. (b) Hamilton, D. G.; Feeder, N.; Teat, S. J.; Sanders, J. K. M. *New J. Chem.* **1998**, *101*, 9–1021.

(4) (a) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *Drug Discov. Today* **2002**, *7*, 117–125. (b) Karan, C.; Miller, B. L. *Drug Discov. Today* **2000**, *5*, 67–75. (c) Lehn, J.-M. *Prog. Polym. Sci.* **2005**, *30*, 814–831.

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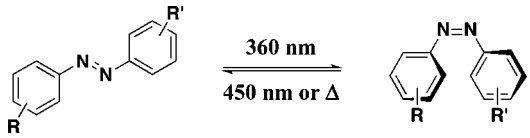


FIGURE 2. Cis−trans isomerization of azobenzene derivatives.

expand the present applications of DCC. In particular, several groups have developed "doubly dynamic" DCLs through the incorporation of two different reversible reactions which can be triggered independently, demonstrating the benefit of incorporating multiple equilibria in a single system.[5] For example, Otto and co-workers have prepared DCLs which feature two simultaneous covalent exchange reactions, disulfide and thioester exchange.[6] In this system, the two reversible reactions are addressed sequentially. First, library equilibration occurs based on thioester exchange only, and then in the presence of atmospheric oxygen both reactions occur simultaneously. Eliseev and co-workers have explored doubly dynamic DCLs as well by combining noncovalent metal coordination with imine exchange, which can be used as independent equilibrium processes controlled by different types of external intervention, oxidation/reduction of the metal template, and change in the pH and temperature of the medium.[7] Furthermore, a system has been reported in which three dynamic linkages, disulfide, imine, and coordinative bonds, were shown to be capable of simultaneous reversible exchange.[8] Although the three types of dynamic linkages were demonstrated to be mutually compatible, both transmetalation and covalent imine exchange were used to alter the equilibrium between disulfides, allowing for greater control over the degree of self-sorting.

Herein, we report a further expansion in the area of multilevel dynamic libraries by combining two reversible processes, hydrazone exchange and photoinduced isomerization. The two exchange processes involved in our double-level DCLs is advantageous in that it offers a higher degree of control over the library composition in the investigation of potential targets. While hydrazone exchange facilitates the traditional formation and interconversion of an assembly of macrocycles under acidic conditions, photoinduced isomerization can be applied for the development of switchable receptors. We aimed to develop a DCL from which we could identify switchable receptors with which one could photomodulate molecular recognition processes as a direct result of the distinct conformational changes of azobenzene.

Azobenzene has been widely used as an optical trigger for various photoresponsive systems due to its pronounced changes in geometry upon light-induced isomerization. Azobenzene is an attractive photoswitch due to its high phototstability, facile isomerization resulting in good quantum yields, and extremely fast and reversible isomerization processes (picosecond time scale).[9] At thermal equilibrium, the trans isomer is dominant, but irradiation to the photostationary state converts the trans isomer to its corresponding cis form (Figure 2). The reverse process is also feasible photochemically (at 450 nm) or thermally, although thermal relaxation to the trans state is a slow process (hour-to-day time scale).[10]

The conformational change that is induced upon isomerization of azobenezene derivatives has been successfully exploited to control the biological properties of various systems, such as folded peptides[11] and helical polymers,[12] by either disrupting, changing, or enhancing secondary structure. Recently, progress has been made in the development of photoswitches that covalently modify target proteins and reversibly present and withdraw a ligand from its binding site as a result of photoisomerization of an azobenzene linker, allowing for rapid and selective manipulation of protein function.[13] The photoswitchable properties of azobenzenes have also been utilized to manipulate the properties of host−guest systems involving crown ethers[14] and cyclodextrins,[15] while also finding applications as small molecule inhibitors.[16]

Despite its extensive use in other applications, particularly in the field of molecular recognition, photochemistry has yet to be widely employed in the design of dynamic combinatorial libraries. Eliseev and co-workers reported an early example which integrated photoisomerization into DCC, making use of an unsaturated dicarboxylate monomer in the development of anionic receptors for arginine.[17]

Hydrazone exchange is well suited for DCC, as much success has been met with this reversible reaction in the preparation of DCLs,[18] and the required functionality can be incorporated into an azobenzene derivative in a straightforward manner. The hydrazone linkage is formed from a hydrazide and an aldehyde under acidic conditions. While acid catalyzes both the initial formation and the interconversion of an assembly of macrocycles, neutralization yields stable, isolable products.[19]

These two types of exchange were merged in the design of a novel azobenzene-containing building block appended with the appropriate functionality for hydrazone exchange. We have investigated the generation of various macrocycles incorporating our designed azobenzene building block **1** via DCC (Figure 3), examining both single building block libraries and libraries with more than one building block, adding to the structural diversity of the library. The additional building blocks include the proline-based monomers **2** and **3**.[20] We report the composition of DCLs derived from the azobenzene building block **1** as a single

(5) (a) Miller, B. L.; Klekota, B. *Tetrahedron* **1999**, *55*, 11687–11697. (b) ten Cate, A. T.; Dankers, P. Y. W.; Sijbesma, R. P.; Meijer, E. W. *J. Org. Chem.* **2005**, *70*, 5799–5803.

(6) Leclaire, J.; Vial, L.; Otto, S.; Sanders, J. K. M. *Chem. Commun.* **2005**, 1959–1961.

(7) Goral, V.; Nelen, M. I.; Eliseev, A. V.; Lehn, J.-M. *Proc. Nat. Acad. Sci. U.S.A.* **2001**, *98*, 1347–1352.

(8) Sarma, R. J.; Otto, S.; Nitschke, J. R. *Chem.—Eur. J.* **2007**, *13*, 9542–9546.

(9) Renner, C.; Moroder, L. *ChemBioChem* **2006**, *7*, 868–878.

(10) Kumar, G. S.; Neckers, D. C. *Chem. Rev.* **1989**, *89*, 1915–1925.

(11) For example, see: (a) Renner, C.; Kusebauch, U.; Loweneck, M.; Milbradt, A. G.; Moroder, L. *J. Peptide Res.* **2005**, *65*, 4–14. (b) Pieroni, O.; Fissi, A.; Angelini, N.; Lenci, F. *Acc. Chem. Res.* **2001**, *34*, 9–17. (c) Jurt, S.; Aemissegger, A.; Guntert, P.; Zerbe, O.; Hilvert, D. *Angew. Chem., Int. Ed.* **2006**, *45*, 6297–6300.

(12) For a review, see: (a) Natansohn, A.; Rochon, P. *Chem. Rev.* **2002**, *102*, 4139–4175. (b) Iftime, G.; Labarthet, F. L.; Natansohn, A.; Rochon, P. *J. Am. Chem. Soc.* **2000**, *122*, 12646–12650. (c) Tie, C.; Gallucci, J. C.; Parquette, J. R. *J. Am. Chem. Soc.* **2006**, *128*, 1162–1171.

(13) Harvey, J. H.; Trauner, D. *ChemBioChem* **2008**, *9*, 191–193.

(14) Shinkai, S.; Manabe, O. *Top. Curr. Chem.* **1984**, *121*, 67–104.

(15) Ueno, A.; Tomita, Y.; Osa, T. *Tetrahedron Lett.* **1983**, *24*, 5245–5248.

(16) Abell, A. D.; Jones, M. A.; Neffe, A. T.; Aitken, S. G.; Cain, T. P.; Payne, R. J.; McNabb, S. B.; Coxon, J. M.; Stuart, B. G.; Pearson, D.; Lee, H. Y.-Y.; Morton, J. D. *J. Med. Chem.* **2007**, *50*, 2916–2920.

(17) Eliseev, A. V.; Nelen, M. I. *J. Am. Chem. Soc.* **1997**, *119*, 1147–1148.

(18) For example, see: (a) Matsumoto, M.; Nicholas, K. M. *J. Org. Chem.* **2007**, *72*, 9308–9313. (b) Liu, J.; West, K. R.; Bondy, C. R.; Sanders, J. K. M. *Org. Biomol. Chem.* **2007**, *5*, 778–786. (c) Simpson, M. G.; Watson, S. P.; Feeder, N.; Davies, J. E.; Sanders, J. K. M. *Org. Lett.* **2000**, *2*, 1435–1438. (d) Roberts, S. L.; Furlan, R. L. E.; Otto, S.; Sanders, J. K. M. *Org. Biomol. Chem.* **2003**, *1*, 1625–1633.

(19) Cousins, G. R. L.; Poulsen, S.-A.; Sanders, J. K. M. *Chem. Commun.* **1999**, 1575–1576.

(20) Furlan, R. L. E.; Ng, Y.-F.; Cousins, G. R. L.; Redman, J. E.; Sanders, J. K. M. *Tetrahedron* **2002**, *58*, 771–778.

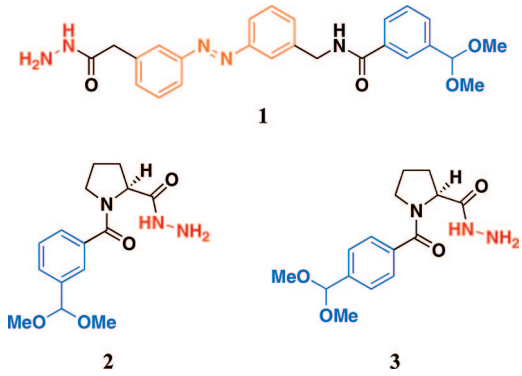


FIGURE 3. Azobenzene building block **1** and proline building blocks **2** and **3** functionalized with a hydrazide (red) and protected aldehyde (blue) to facilitate hydrazone exchange.

component and in mixtures, along with the effect of photoisomerization on the composition of such libraries.

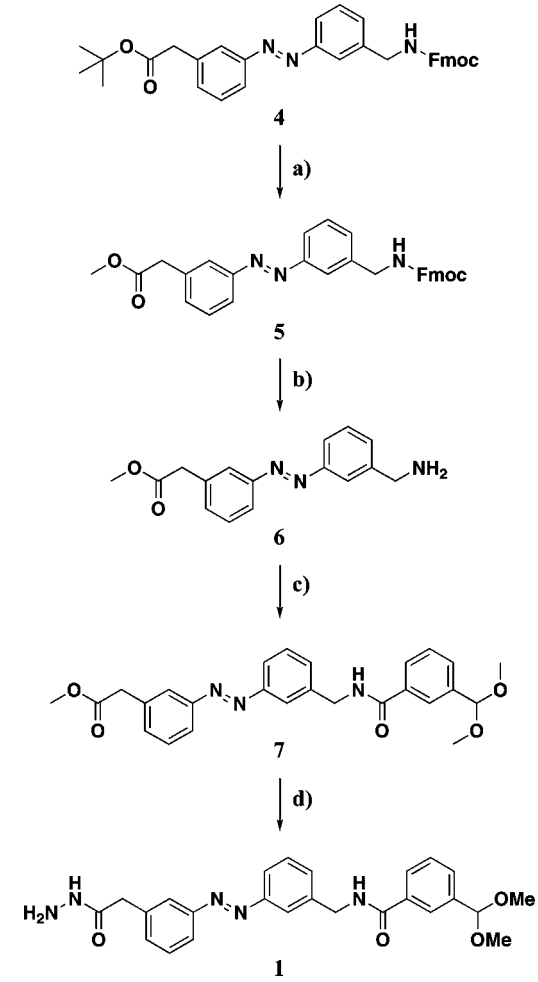
Results and Discussion

Synthesis of Building Blocks and Preparation of DCLs. The azobenzene building block **1** was synthesized from the corresponding fully protected azobenzene amino acid **4** (Scheme 1). The preparation of **4** relies on the reaction of a nitrosobenzene with an aniline as described by Hilvert and co-workers.[21] The nitrosobenzene was prepared in two steps from commercially available *m*-nitrophenylacetic acid, whereas the aniline was prepared in two steps from commercially available 3-nitrobenzylamine hydrochloride. The protected amino acid **4** was transesterified to the corresponding methyl ester **5**, followed by Fmoc deprotection under basic conditions, to yield the free amine **6**.[22] The coupling of **6** with 3-carboxybenzaldehyde dimethoxy acetal via standard HOBt/HBTU coupling to yield **7** followed by hydrazinolysis of the methyl ester afforded the desired building block **1** as a mixture of cis and trans isomers, containing the necessary functionality to facilitate hydrazone exchange.

Deprotection and subsequent cyclization of **1** (4 mM) was accomplished using an excess of TFA (100 mM). Because we were met with solubility problems upon cyclization in most solvents, libraries prepared with building block **1** alone were generated in DMSO. Reactions were monitored daily by LC−MS for 4−5 days, although thermal equilibrium was reached after 4 days. Most cyclization occurred within 24 h, and the initial library distribution did not change drastically after the first day. Although linear species were observed within hours of monomer deprotection, the libraries were composed of entirely cyclic macrocycles upon reaching equilibrium. This DCL gave relatively simple distributions dominated by macrocyclic monomers and dimers, along with smaller trimer peaks (Figure 4a).

Each library member was observed in both the cis and trans conformations, and the isomers were easily identified due to different retention times and a large difference in absorbance of trans and *cis*-azobenzene at 320 nm (see Figure S9. Supporting Information).[23] The cyclic hydrazones were found in a cis to trans ratio of about 20:80 at thermal equilibrium. To ascertain the reversible nature of the library and confirm that the reaction mixture had reached equilibrium, the library was also generated from pure cyclic trans monomer. As expected, the final product distribution was the same (not shown).

SCHEME 1. Synthesis of Building Block 1[a]



[a] Conditions: (a) H_2SO_4, MeOH (94%); (b) TAEA, CH_2Cl_2 (68%); (c) 3-carboxybenzaldehyde dimethoxy acetal, HOBt, HBTU, TEA, DMF (60%); (d) $NH_2NH_2 \cdot H_2O$, MeOH (65%).

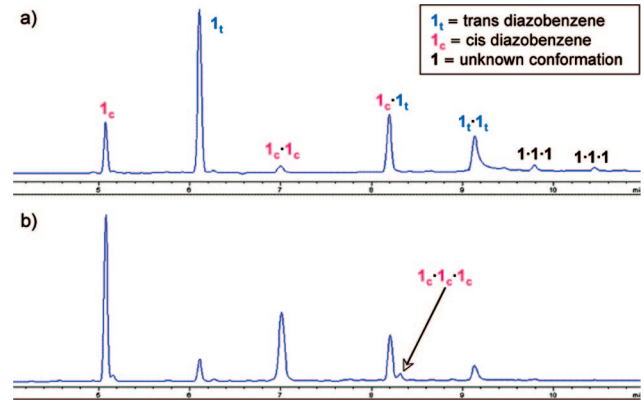


FIGURE 4. HPLC traces at 280 nm of a DCL of **1** (4 mM) (a) at thermal equilibrium and (b) after isomerization. The chromatogram *y*-axes are on the same scale.

(21) (a) Aemissegger, A.; Krautler, V.; van Gunsteren, W. F.; Hilvert, D. *J. Am. Chem. Soc.* **2005**, *127*, 2929–2936. (b) A similar synthesis of the corresponding free acid of **4** was reported by Renner and co-workers: Dong, S.-L.; Loweneck, M.; Schrader, T. E.; Schreier, W. J.; Zinth, W.; Moroder, L.; Renner, C. *Chem.—Eur. J.* **2006**, *12*, 1114–1120.

(22) (a) Unable to prepare methyl ester **5** directly due to the instability of the methyl ester of 4-nitrosobenzoic acid. (b) Ulysse, L.; Chmielewski, J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2145–2146.

(23) Griffiths, J. *Chem. Soc. Rev.* **1972**, *1*, 481–493.

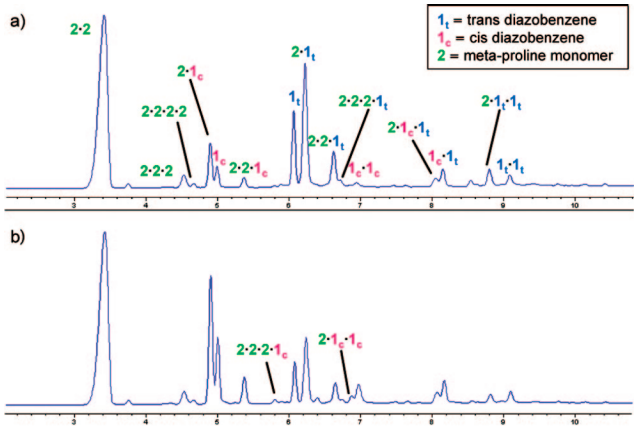


FIGURE 5. HPLC traces at 280 nm of a DCL of **1** (4 mM) and **2** (4 mM) (a) at equilibrium and (b) immediately after photoisomerization. The chromatogram *y*-axes are on the same scale.

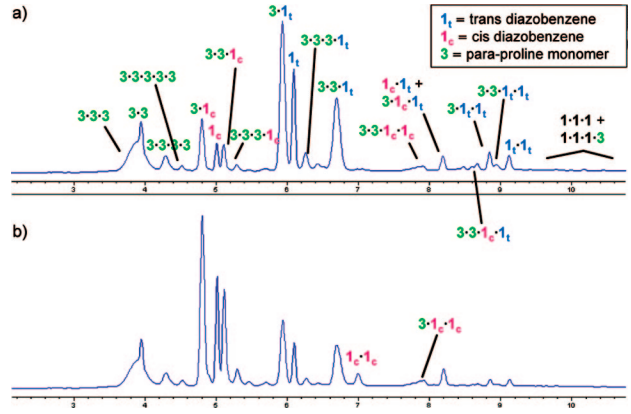


FIGURE 6. HPLC traces at 280 nm of a DCL of **1** (4 mM) and **3** (4 mM) (a) at equilibrium and (b) immediately after photoisomerization. The chromatogram *y*-axes are on the same scale.

Library Diversification. To further diversify the library and expand upon the number of host macrocycles generated, the flexible azobenzene building block **1** was reacted with the more rigid proline-based building blocks **2** and **3**, differing only in the position of the dimethoxyacetal substituent (meta and para, respectively). The necessary functional groups for hydrazone exchange were similarly installed in these monomers, following modified literature procedures.[19,20]

The DCLs were prepared (4 mM in each monomer) in a $CHCl_3$−DMSO (85:15 v/v) solution, as the proline monomer has been found to assist with solubility and $CHCl_3$ is a more amenable solvent for templation studies. After equilibration, these multibuilding block DCLs generated complex library distributions with upward of 20 identifiable species and up to pentameric cyclic oligomers in some cases (Figures 5a and 6a).

The distinct differences between the distribution of species in the libraries generated with **2** versus **3** portray the sensitivity of DCLs to slight structural changes in the building blocks. Proline monomer **2** is found to self-sort to a much larger extent than **3**, giving rise to the proline homodimer (**2**•**2**) as the dominant library member in DCLs generated from **1** and **2**, whereas the heterodimer (**1**•**3**) is found to be the major species in DCLs generated from **1** and **3**.

Effect of Photoisomerization on Library Distribution. To investigate the dual nature of our libraries and their application in the development of photoswitchable hosts, we examined the effect of light-induced isomerization on the composition of our

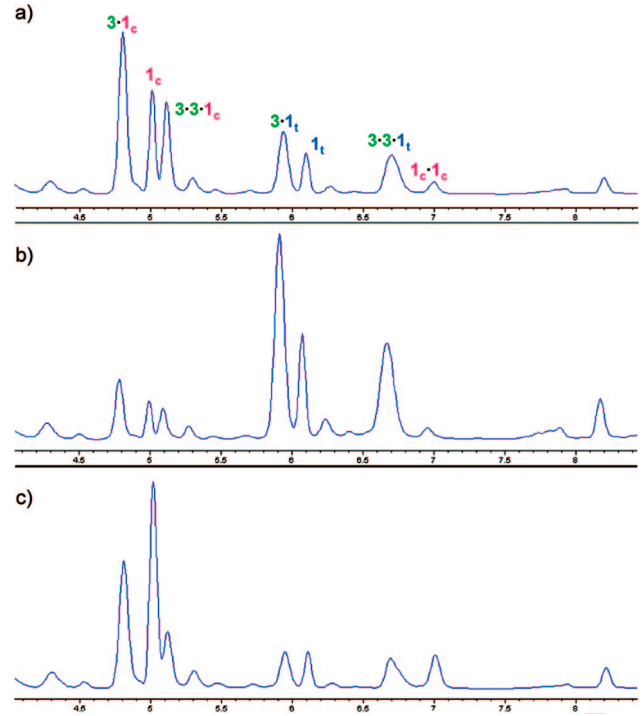


FIGURE 7. Part of the HPLC traces at 280 nm of a DCL of **1** (4 mM) and **3** (4 mM) (a) immediately after irradiation of an equilibrated library, (b) 6 days after irradiation where there has been adequate time for thermal relaxation, and (c) equilibrated in the photostationary state with repetitive isomerization. The chromatogram *y*-axes are on the same scale.

libraries. Upon irradiation of equilibrated DCLs at 365 nm for 5 min, each library distribution is shifted significantly in favor of the cis isomers. This assisted in confirming our assignments of the different isomers. Further isomerization does not occur with longer light exposure. After irradiation of the single building block library, the azobenzene cyclic monomer species is present in a cis:trans ratio of 87:13 as observed by LC−MS (Figure 4b). In this library, a similar large increase of the cis/cis azobenzene homodimer ($\mathbf{1_c \cdot 1_c}$) is observed, accompanied by a significant decrease of the trans/trans azobenzene homodimer ($\mathbf{1_t \cdot 1_t}$) and a minor decrease in the cis/trans heterodimer ($\mathbf{1_c \cdot 1_t}$). Comparable changes are also observed with the trimer macrocycles (**1**•**1**•**1**), as well as with oligomers containing one or more proline monomer units in the multibuilding block libraries (Figures 5b and 6b).

Immediately after photoisomerization, the libraries are stored in the dark, although with time they return to thermal equilibrium. The rate of conversion back to thermal equilibrium seems to be solvent dependent, taking on the order of a week or less for the libraries generated in mostly $CHCl_3$ but longer for those in DMSO only. Hydrazone exchange within the irradiated DCL (Figure 7a) and relaxation back to thermal equilibrium are competitive processes, occurring simultaneously, and resulting in a DCL dominated by trans macrocycles (Figure 7b). In contrast, libraries which are equilibrated under photochemical conditions with repetitive irradiation allow for hydrazone exchange to occur in the absence of thermal relaxation (Figure 7c). By comparing parts a and c of Figure 7, both in the photostationary state, it is evident that the order of equilibration and photoisomerization along with the conditions under which the DCLs are equilibrated considerably influence the distribution of macrocycles. The integration of these two dynamic reversible

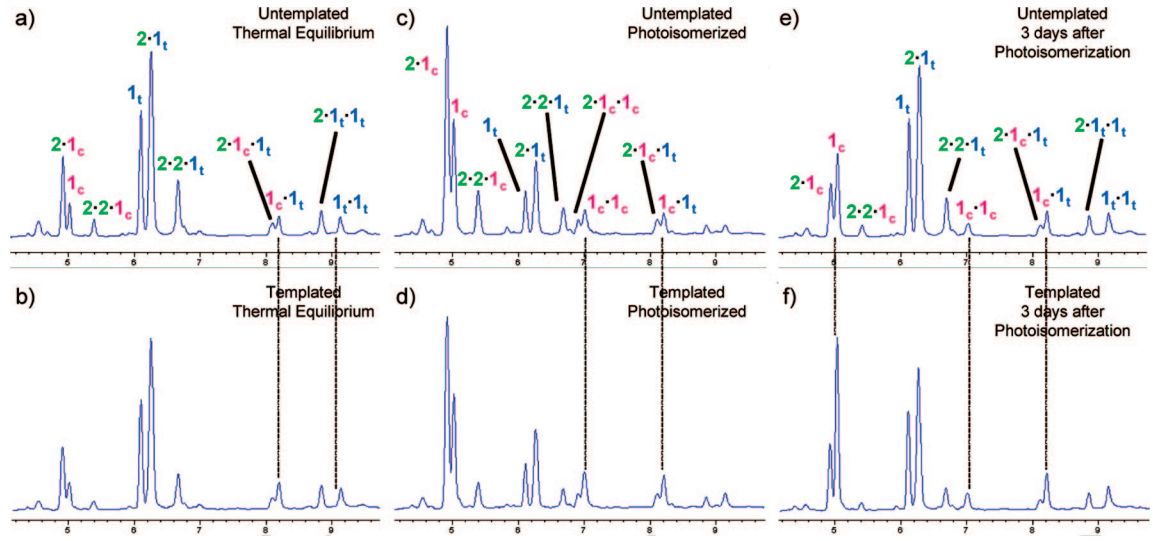


FIGURE 8. Part of the HPLC trace at 280 nm of a DCL made from **1** (4 mM) and **2** (4 mM) (a) at thermal equilibrium without an added template (b) at thermal equilibrium in the presence of a pentaproline peptide (25 mM) (c) immediately after irradiation, untemplated, (d) immediately after irradiation with a pentaproline peptide, (e) 3 days after irradiation, untemplated, and (f) 3 days after irradiation with a pentaproline peptide. Note that (e) and (f) are not meant to represent DCLs which have returned to thermal equilibrium. Amplified species are indicated with the dashed lines between untemplated and templated libraries. The chromatogram *y*-axes are on the same scale.

processes permits various distributions of species to be generated under different conditions within a single DCL. Such versatility within a library may prove to be valuable in expanding the applications of DCC, as well as in the identification of photoswitchable receptors in this context.

Azobenzene monomeric building blocks which were subjected to photoisomerization before cyclization were also investigated. As expected, upon acid catalysis the irradiated monomers underwent simultaneous cyclization and thermal relaxation, which over time resulted in DCLs identical to those generated with the thermodynamically favored trans monomers.

Templation Studies. Because photoisomerization converts the library to a photostationary state rather than a thermally equilibrated state, if a guest binds a specific cis macrocycle it would be expected to inhibit conversion of that receptor back to its trans conformation due to favorable binding interactions. Although this is not amplification in the traditional sense, it would nonetheless result in an increased amount of the cis receptor in the templated library versus the corresponding untemplated DCL. This is indeed what was observed when the libraries were equilibrated in the presence of a pentaproline guest.

We chose to investigate an oligoproline peptide guest due to the role of polyproline helices in many important protein−protein interactions.[24] When a photoswitchable DCL consisting of monomers **1** and **2** was thermally equilibrated in the presence of a five-residue oligoproline peptide, the $\mathbf{1_c}\cdot\mathbf{1_t}$ heterodimer is amplified by about 48% relative to the untemplated library (Figure 8a,b and Figure 9). Immediately following photoisomerization, amplification of both $\mathbf{1_c}\cdot\mathbf{1_t}$ and $\mathbf{1_c}\cdot\mathbf{1_c}$ is observed relative to the untemplated library, yet there has been little time for requilibration of the newly isomerized macrocycles at this point (Figure 8c,d).

In returning to a state of thermal equilibrium, a slower rate of thermal relaxation of some library members was observed in comparison to the untemplated library, indicative of favorable

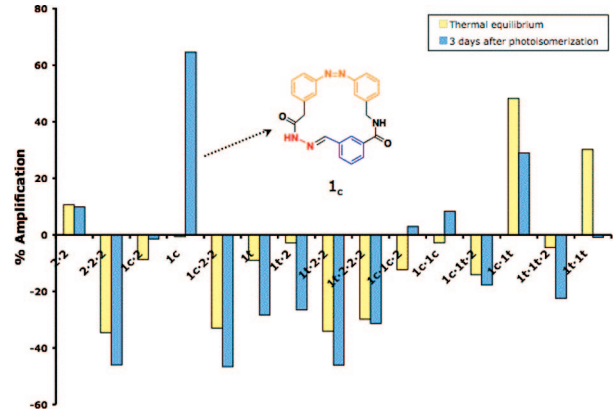


FIGURE 9. Comparison between the extent of amplification of each library member in the polyproline templated library (made from **1** and **2**) at thermal equilibrium (yellow) and 3 days after irradiation (blue). (Percent amplification = [(% area of library member in templated DCL − % area of library member in untemplated DCL)/% area of library member in untemplated DCL] × 100).

host−guest binding interactions. This rate difference is most apparent with the cyclic cis monomer $\mathbf{1_c}$. As seen in parts e and f of Figure 8 along with Figure 9, 3 days after the DCLs were irradiated at 365 nm, host $\mathbf{1_c}$ is significantly amplified in the polyproline templated DCL. As expected, this amplification is observed in DCLs containing monomer **1** and either proline monomer (**2** or **3**). In contrast, $\mathbf{1_t}$ is not amplified in the thermally equilibrated library, suggesting that the conformation of the azobenzene controls binding. The fact that $\mathbf{1_c}$ is only amplified after photoisomerization is likely a result of not only the low concentration of *cis*-azobenzene macrocycles at thermal equilibrium but also the competitive equilibria at play. Presumably at higher concentrations of the cis monomer, the favorable interactions which result in templation of $\mathbf{1_c}$ out-compete other equilibria. This type of phenomenon is not unprecedented in dynamic combinatorial libraries[25] and reinforces the fact that

(24) Ball, L. J.; Kuhne, R.; Schneider-Mergener, J.; Oschkinat, H. *Angew. Chem., Int. Ed.* **2005**, *44*, 2852–2869.

(25) (a) Corbett, P. T.; Sanders, J. K. M.; Otto, S. *Angew. Chem., Int. Ed.* **2007**, *46*, 8858–8861. (b) Severin, K. *Chem.—Eur. J.* **2004**, *10*, 2565–2580.

the thermally equilibrated and photoisomerized libraries represent different libraries with different behaviors which indeed should be considered independently.

Unfortunately, as is often the case with hydrazone libraries, the binding constants of the isolated host−guest complex could not be measured because the host is not soluble under non-equilibrating (i.e., nonacidic) conditions, except in DMSO, which disrupts binding.[2c,26]

The observed stabilization of a *cis*-azobenzene via binding is not unprecedented. Such stabilization has been previously reported in the context of binding tethered, photoswitchable maleimide−azobenzene−glutamate ligands.[27] Polyproline peptides and other such guests which form more favorable binding interactions with one isomer of a specific host are especially attractive due to the potential to photomodulate the binding to these receptors, therefore achieving a larger degree of control over such molecular recognition processes.

Limitations of the Azobenzene Hydrazide DCLs. Despite the desirable properties of azobenzenes, based on our initial studies there are also some limitations with azobenzene building blocks that must be noted. First, in the presence of nucleophiles such as halide counterions, the *cis*-azobenzene isomers are rapidly converted to their trans counterparts under the acidic conditions necessary for hydrazone exchange. This clearly perturbs the equilibrium, yet is unrelated to favorable binding interactions, and limits the range of guests that can be studied. Second, the azobenzene moiety can undergo surprisingly facile reduction to hydrazobenzene in the presence of reducing agents including thiols such that azobenzene isomerization is not compatible with disulfide or thioester exchange.[28]

Conclusion

This work reports the design of an azobenzene-based hydazide monomer that can undergo thermodynamically controlled oligomerization and cyclization under acidic conditions, either in single or multibuilding block libraries. Although the thermally equilibrated DCLs favor the trans isomers, we have shown that we can photochemically control the library distribution by irradiating the systems at appropriate wavelengths. The incorporation of these two reversible processes in a single library is advantageous in that it offers a higher degree of control over the library composition and its organization in the investigation of potential targets. In exploring the templation of a polyproline peptide, we have demonstrated that we can stabilize and amplify an inherently less stable *cis*-azobenzene macrocycle through templation with an appropriate guest. The development of hosts for such proline rich sequences via DCC may help to elucidate their role as potential protein interaction domains.

Photoresponsive libraries of this type are useful in that, despite the nature of the guest, binding can be controlled by irradiation at appropriate wavelengths. This shows the potential to, in one system, both identify and synthesize novel host receptors and take advantage of the optical properties of azobenzene to control binding.

(26) (a) Bulos, F.; Roberts, S. L.; Furlan, R. L. E.; Sanders, J. K. M. *Chem. Commun.* **2007**, 3092–3093. (b) Ludlow, R. F.; Liu, J.; Li, H.; Roberts, S. L.; Sanders, J. K. M.; Otto, S. *Angew. Chem., Int. Ed.* **2007**, *46*, 5762–5764.

(27) Gorostiza, P.; Volgraf, M.; Numano, R.; Szobota, S.; Trauner, D.; Isacoff, E. Y. *Proc. Nat. Acad. Sci. U.S.A.* **2007**, *104*, 10865–10870.

(28) Boulegue, C.; Loweneck, M.; Renner, C.; Moroder, L. *ChemBioChem* **2007**, *8*, 591–594.

Experimental Section

Synthesis. (*E*)-Methyl 2-(3-((3-((((9*H*-Fluoren-9-yl)methoxy)-carbonylamino)methyl)phenyl)diazenyl)phenyl)acetate (5). In 27 mL of methanol, 0.1 M in H_2SO_4, 0.29 g (0.53 mmol) of **4** was dissolved, and the solution was refluxed for 6 h. After removal of the solvent, water was added to precipitate the product, and it was extracted three times with EtOAc. The organic extracts were dried over $MgSO_4$, and the solvent was evaporated to obtain an orange solid (94%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.83−7.81 (m, 4H), 7.75 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 7.5 Hz, 2H), 7.50−7.44 (m, 2H), 7.41−7.36 (m, 4H), 7.31−7.26 (m, 2H), 5.26 (t, J = 6.0 Hz, 1H), 4.47−4.45 (m, 4H), 4.23 (t, J = 6.8 Hz, 1H), 3.72 (s, 2H), 3.70 (s, 3H). HRMS-ESI(+): m/z $[M + H]^+$ calcd for $C_{31}H_{27}N_3O_4$ 506.2080, found 506.2062; m/z $[M + Na]^+$ calcd for $C_{31}H_{27}N_3NaO_4$ = 528.1899, found = 528.1907.

(*E*)-Methyl 2-(3-((3-(Aminomethyl)phenyl)diazenyl)phenyl)acetate (6). To a solution of 0.26 g (0.52 mmol) of **5** in 5.2 mL of dichloromethane was added 3.9 mL (26 mol) of tris(2-aminoethyl)amine. The mixture was stirred for 1 h and then washed with saturated NaCl followed by phosphate buffer pH 5.5. The organic extracts were dried over $MgSO_4$. After the solvent was evaporated, the resulting oil was purified by flash chromatography (MeOH with 10% NH_4OH/CH_2Cl_2 1:9) to obtain 0.18 g (68%) of a bright orange oil. The product was isolated as a mixture cis and trans isomers, with the cis isomer ranging from 10 to 20% as determined by NMR integrations. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.85−7.77 (m, 4H), 7.49−7.37 (m, 4H), 3.96 (s, 2H), 3.72 (s, 2H), 3.70 (s, 3H). HRMS-ESI(+): m/z $[M + H]^+$ calculated for $C_{16}H_{17}N_3O_2$ = 284.1399, found = 284.1402.

(*E*)-Methyl 2-(3-((3-((3-(Dimethoxymethyl)benzamido)methyl)phenyl)diazenyl)phenyl)acetate (7). A solution of 0.022 g (0.112 mmol) of 3-carboxybenzaldehyde dimethoxy acetal, 0.015 g (0.112 mmol) of HOBt, and 0.042 g (0.112 mmol) of HBTU in 700 μL of DMF was added to 0.032 g (0.112 mmol) of **6**. To the mixture was added 47 μL (0.336 mmol) of triethylamine, and the solution was stirred for 4 h. After the addition of brine, the product was extracted with EtOAc. The organic phase was washed with sodium bicarbonate and dried over $MgSO_4$, and the solvent was evaporated. The resulting orange oil was purified by flash chromatography (MeOH with 10% NH_4OH/CH_2Cl_2 1:19) to obtain 0.031 g (60%) of a bright orange oil. The product was again isolated as a similar mixture of isomers, with a small amount of the corresponding deprotected aldehyde (5−10%) which was carried through without a problem. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.86−7.80 (m, 6H), 7.57 (d, J = 7.5 Hz, 1H), 7.48−7.37 (m, 5H), 5.39 (s, 1H), 4.72 (d, J = 5.7 Hz, 2H), 3.71 (s, 2H), 3.69 (s, 3H), 3.29 (s, 6H). HRMS-ESI(+): m/z $[M + Na]^+$ calcd for $C_{26}H_{27}N_3NaO_5$ = 484.1849, found = 484.1861.

(*E*)-3-(Dimethoxymethyl)-*N*-(3-((3-(2-hydrazinyl-2-oxoethyl)phenyl)diazenyl)benzyl)benzamide ((*E*)-1). A solution of **7** (0.032 g, 0.069 mmol) in anhydrous methanol (0.8 mL) was treated with hydrazine monohydrate (33.5 μL, 0.69 mmol). The reaction was left overnight under nitrogen before removal of the solvent under vacuum to give an orange oil which was purified by flash chromatography (MeOH with 10% NH_4OH/CH_2Cl_2 gradient, 1:39 to 1:19 to 1:9) to afford the monomer **1** (65%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.88−7.77 (m, 6H), 7.58 (d, J = 7.5 Hz, 1H), 7.50−7.36 (m, 5H), 5.39 (s, 1H), 4.74 (d, J = 5.7 Hz, 2H), 3.63 (s, 2H), 3.30 (s, 6H). HRMS-ESI(+)$^+$: m/z [M + Na] calcd for $C_{25}H_{27}N_5NaO_4$ = 484.1961, found = 484.1958.

(*Z*)-3-(Dimethoxymethyl)-*N*-(3-((3-(2-hydrazinyl-2-oxoethyl)phenyl)diazenyl)benzyl)benzamide ((*Z*)-1). (*E*)-1 was photolyzed at 365 nm. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.81 (s, 1H), 7.73 (d, J = 7.8 Hz, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.38 (t, J = 7.8 Hz, 1H), 7.14−7.01 (m, 3H), 6.75−6.58 (m, 5H), 5.71 (d, J = 12 Hz, 2H, NH_2), 5.37 (s, 1H), 4.46 (d, J = 5.7 Hz, 2H), 3.34 (s, 2H), 3.28 (s, 6H).

(*S*)-*N*-(3-Dimethoxymethylbenzoyl)prolinecarboxylic Acid Hydrazide (2). Monomer **2** was prepared according to literature procedures,[20] except the amide bond formation was accomplished with HBTU/HOBt as the coupling reagent rather than EDC, as described below. A solution of 0.059 g (0.302 mmol) of 3-carboxybenzaldehyde dimethoxy acetal, 0.041 g (0.302 mmol) of HOBt, and 0.115 g (0.302 mmol) of HBTU in 1.5 mL of DMF was added to 0.050 g (0.302 mmol) of L-proline methyl ester hydrochloride. To the mixture was added 126 μL (0.906 mmol) of triethylamine, and the solution was stirred for 1.5 h. After the addition of brine, the product was extracted with EtOAc. The organic phase was washed with sodium bicarbonate and dried over $MgSO_4$, and the solvent was evaporated to give 0.088 g (94%) of a colorless oil. The hydrazinolysis reaction of the methyl ester was carried out as previously reported to afford **2**.

(*S*)-*N*-(4-Dimethoxymethylbenzoyl)prolinecarboxylic Acid Hydrazide (3). The synthesis of (*S*)-*N*-(4-dimethoxymethylbenzoyl)-prolinecarboxylic acid hydrazide (**3**) was carried out using 4-carboxybenzaldehyde dimethoxy acetal and the same conditions as those reported above for monomer **2**.

Polyproline Peptide (Ac-Pro-Pro-Pro-Pro-Pro-NH_2). The peptide synthesis was performed on a Tetras Peptide Synthesizer using Applied Biosystems PAL-PEG amide resin. The peptide was synthesized on a 0.0215 mmol scale (50 mg resin). Coupling reagents were HOBt/HBTU in DMF. The N-terminus was acylated with a solution of 5% acetic anhydride and 6% 2,6-lutidine in DMF. Cleavage was performed by hand with a cocktail of 95% TFA/2.5% triisopropylsilane/2.5% H_2O. The peptide was purified by semipreparative reversed-phase HPLC on a C18 column at a flow rate of 4 mL/min. The purification was achieved using a linear gradient of A and B (A: 95% H_2O/5% CH_3CN with 0.1% TFA; B: 95% CH_3CN/5% H_2O with 0.1% TFA) and elution was monitored at 214 nm. Once purified, the peptide was lyophilized and characterized by ESI-MS. ESI-MS (+): m/z $[M+H]^+$ calculated for $C_{27}H_{40}N_6O_6$ = 545.30, found = 545.3.

Dynamic Combinatorial Chemistry. (a) DCL with Monomer 1. The single-component library was prepared by making a 4 mM building block solution of **1** in DMSO containing 100 mM of TFA. The resulting solution equilibrated at room temperature for at least 4 days. The reaction was monitored daily by LC-MS (3 μL injections). Separations were performed using H_2O−acetonitrile gradients with 0.2% formic acid (t = 0 min: 100% water, flow rate 1.0 mL/min; t = 2 min: 75% water, flow rate 1.0 mL/min; t = 3.5 min: 66% water, flow rate 1.0 mL/min; t = 6.5 min: 57% water, flow rate 1.5 mL/min; t = 11 min: 30% water, flow rate 1.5 mL/min), with the left column temperature set to 40 °C and the right column temperature set to 50 °C to optimize separation. Multiple wavelengths were used for analysis (220 nm, 254 nm, 280 nm, and 320 nm). The cis and trans isomers were assigned based on the strong absorbance of trans azobenzene at 320 nm, along with the shift in the distribution of isomers upon isomerization.

(b) DCL with Monomers 1 and 2 or 1 and 3. The mixed library was prepared by making a 1:1 solution of the building blocks (4 mM each) in $CHCl_3$−DMSO (85:15 v/v) containing 100 mM of TFA. The resulting solution was stirred at room temperature for at least 4 days for equilibration. The reactions were monitored daily by LC−MS as described above for the single component library. In the case of templation studies, a second library was equilibrated in the presence of 25 mM of a five-residue polyproline peptide.

Photoisomerization. Libraries were irradiated at 365 nm for 5 min in the dark with a Spectroline long wave UV pencil lamp (1,000 $\mu W/cm^2$ of 365 nm radiation at 1 in.). Once irradiated, solutions were continually stored in the dark at room temperature. LC−MS analysis was continued on a daily basis, and light exposure was minimized during analysis.

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Supporting Information Available: General experimental details; ^1H NMR spectra for (*E*)-**1**, (*Z*)-**1**, and compounds **2**−**7**; UV−vis spectra of (*E*)-**1** and (*Z*)-**1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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