

## Dynamic Combinatorial Chemistry with Novel Dithiol Building Blocks: Towards New Structurally Diverse and Adaptive Screening Collections

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**Abstract:** We describe the synthesis of a range of novel dithiol-functionalized building blocks and demonstrate how they can be used to generate new structurally diverse dynamic combinatorial libraries. A proof-of-principle experiment using the catecholamine dopamine revealed that molecular recognition changed the library composition under biocompatible conditions and identified new promising candidate receptors of this biologically important neurotransmitter.

**Key words:** dynamic combinatorial chemistry, thiols, receptors, macrocycles, drugs

A high level of structural (shape) diversity is widely recognized as a valuable characteristic in synthetic compound libraries used for biological screening.<sup>1–5</sup> Indeed, it is generally regarded as a prerequisite for broad biological activity, which is particularly valuable in phenotypic screening experiments.<sup>1,2,6</sup> However, the synthesis (and indeed biological screening) of molecules is very expensive, in terms of time, money, and resources.<sup>1</sup> Thus there is a desire to achieve structural diversity in synthetic compound collections in the most efficient manner possible.<sup>1,7</sup>

The compounds in typical synthetic libraries are usually prepared as discrete, stable entities via several synthetic steps or manipulations. Once isolated such compounds are then typically tested individually for their biological effects.<sup>8</sup> Dynamic combinatorial chemistry (DCC) offers a conceptually different approach towards the efficient generation of structurally diverse libraries and the identification of hits against biological targets.<sup>8</sup> In DCC a relatively small collection of starting compounds (building blocks) are selected, which are capable of reacting together by the formation of reversible linkages to form a collection of larger-sized compounds, known as a dynamic combinatorial library (DCL).<sup>9,10</sup> Under thermodynamic conditions the concentration of each member is dictated by its relative stability.<sup>8–10</sup> If an external biological entity (e.g., a biomolecule, receptor, or enzyme) is added to the library, this will interact, via noncovalent interactions, with the equilibrating species, thus changing the equilibrium profile of the DCL.<sup>8,10</sup> The compound that best interacts with the biological entity will be stabilized most, leading to an

amplification of the best binder at the expense of other species in the mixture. This compound can then be detected and isolated.<sup>10,11</sup> The DCC approach conceivably allows for the generation of structurally diverse compound collections in an extremely time- and resource-efficient fashion; a wide range of substances can be assembled from relatively few building blocks, without need to synthesize each member individually (cf. standard ‘static’ synthetic compound collections).<sup>8</sup> In addition, due the fact that a DCL is dynamic and adaptive, the target biological substance itself can be used to select an active ligand/inhibitor directly from the library pool, thus greatly simplifying, and increasing the efficiency of the screening process.<sup>8</sup> Because of this selection process, DCC can lead to the discovery of unexpected and unpredictable ligands/receptors for biological substances.<sup>12</sup> Consequently, DCC has considerable potential as a tool for the discovery of new ligands for biomolecules in general and drug discovery in particular.<sup>9</sup>

Our group has a continuing interest in the efficient generation of structurally diverse compound collections for biological screening.<sup>13</sup> We sought to explore the potential of DCC in this context. Herein we describe the synthesis of a range of novel dithiol-functionalized building blocks that can potentially be used to generate structurally diverse DCL. In proof-of-principle work two such libraries were generated. Experiments using the catecholamine dopamine revealed that molecular-recognition changes in the library compositions where possible under biocompatible conditions and identified new promising candidate receptors of this biologically important neurotransmitter.

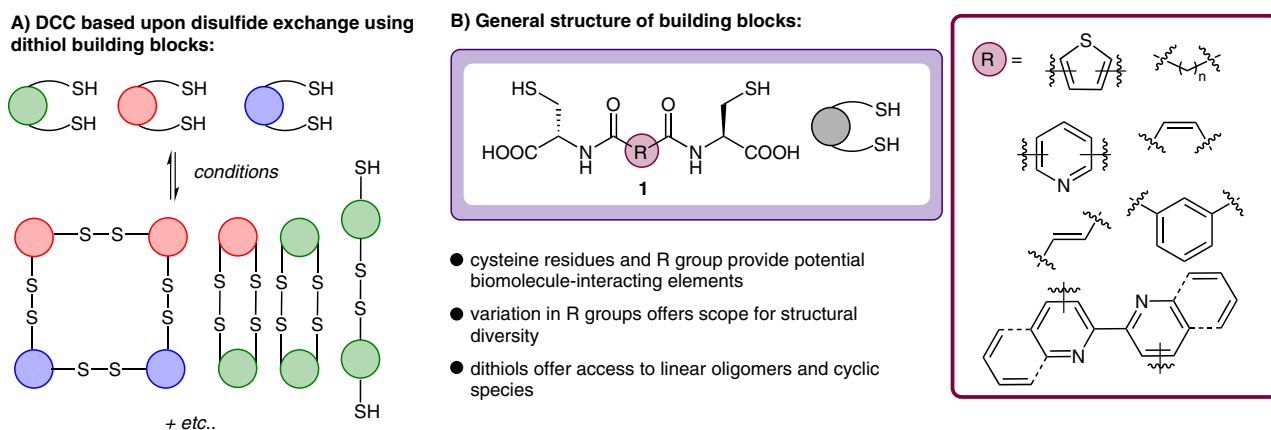
Our studies began with a consideration of the type of reversible reaction we wanted to use as the basis for DCL generation. Disulfide exchange was chosen; disulfide DCL can be generated simply by dissolution of thiol-containing building blocks at pH 8.0 under air, which allows for the screening of biologically relevant targets under near-physiological conditions.<sup>10</sup> Building blocks containing two thiol groups (dithiols) were targeted as these could potentially form not only linear disulfide-bonded species (e.g., dimers, trimers, etc.) but also larger, macrocyclic-type disulfides which could possibly explore more of the surface of a target biomolecule (Figure 1). Other functionality suitable for molecular recognition was also desired in the building blocks in order to increase the likelihood that the resulting DCL members could interact

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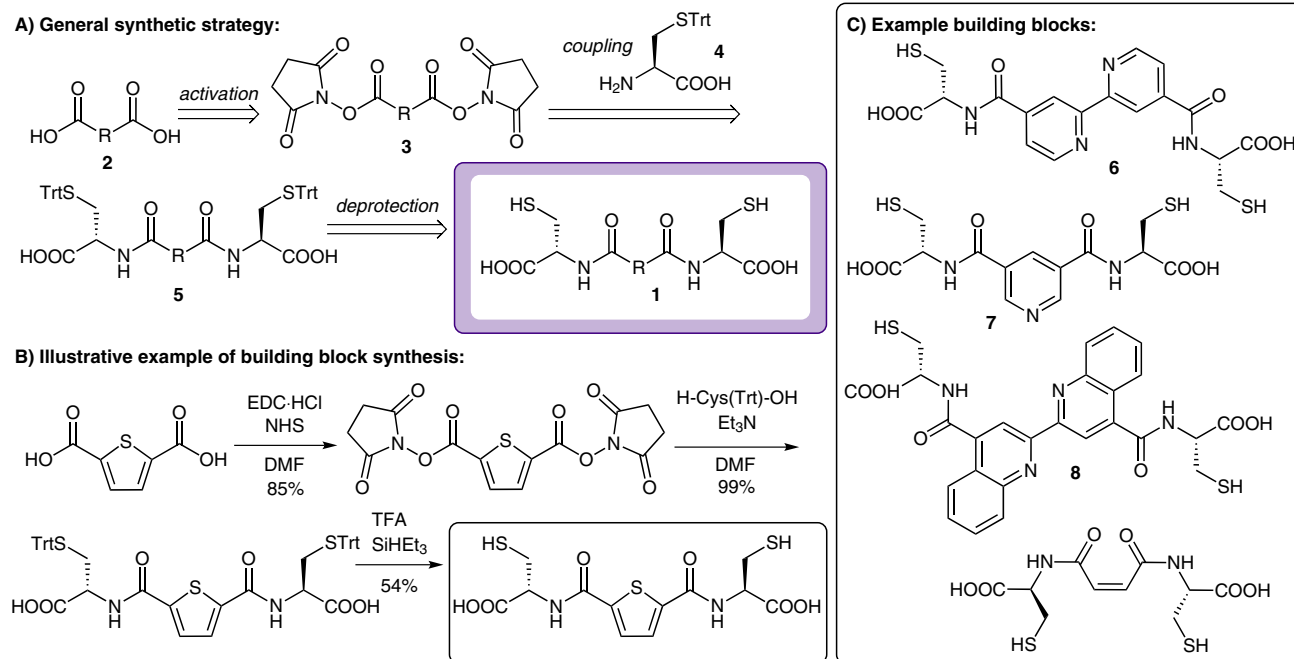
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**Figure 1** A) A general illustration of DCC based upon disulfide exchange using three dithiol building blocks. Under certain conditions the building blocks can interact to form higher-molecular-weight species; some examples of the types of structures that can result (linear and cyclic) are shown. B) The general structure of dithiol building blocks employed in this study.

strongly with biological target macromolecules. In addition, structural variation in the building blocks was deemed important in the context of enhancing the structural diversity of the resulting DCL. This was thought to be crucial both in terms of the biorelevant diversity of the library (vide supra) and also in the context of adaptive library behavior.<sup>14</sup> We thus targeted the synthesis of a number of structurally diverse dithiol derivatives of the general form **1**, based around two cysteine residues connected by different aromatic, heteroaromatic, alkene, or alkane units (Figure 1). These building blocks were expected to generate a structurally diverse collection of library compounds, each containing a variety of features suitable for biomolecule recognition (e.g., hydrogen bonds, ionic interactions,  $\pi$ - $\pi$  interactions, hydrophobic interactions, etc.).

A structurally diverse range of thiophene-, isophthalic acid-, pyridine-, bipyridine-, biquinoline-, alkane-, and alkene-based dithiol building blocks were generated from the appropriate commercially available or readily prepared diacids **2** by a three-step sequence: (1) carbonyl group activation to form compounds **3**; (2) coupling with trityl-protected cysteine (**4**) to furnish compounds **5**, and (3) acid-mediated deprotection (Scheme 1).<sup>15</sup> For thiophene- and isophthalic acid based building blocks carbonyl group activation was achieved using a combination of *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) or *N,N*-dicyclohexylcarbodiimide (DCC).<sup>13</sup> For the other types of building blocks, NHS and trifluoroacetic anhydride were used.<sup>16</sup> In total 14 building blocks were generated. Representative examples are shown in Scheme 1. For full details see the



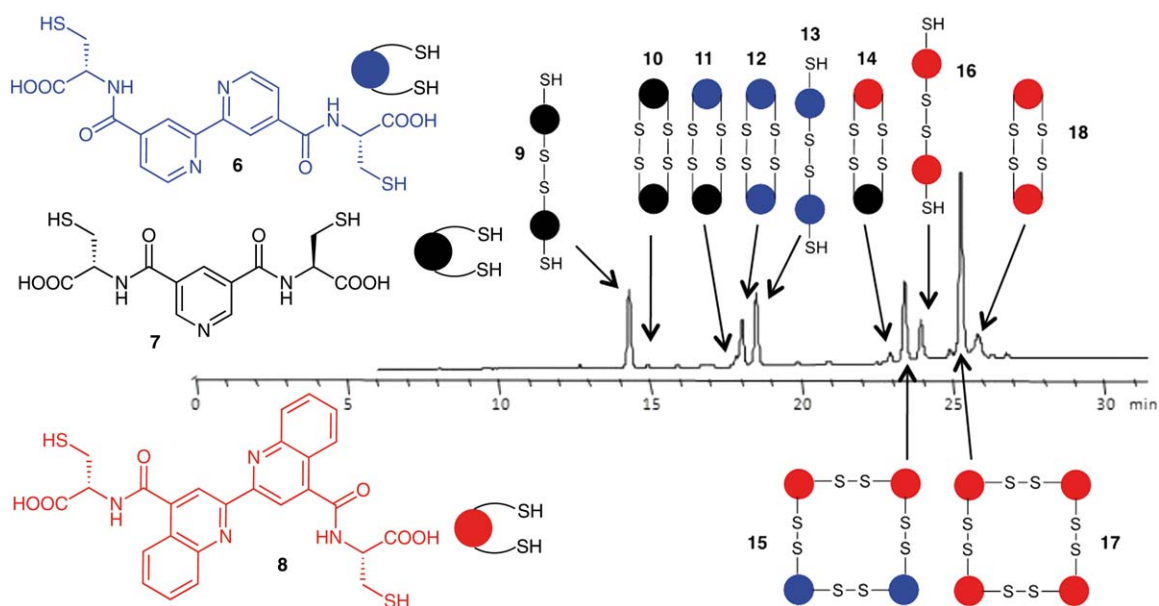
**Scheme 1** A) Overview of the synthetic strategy for the generation of the dithiol building blocks. B) A representative example of building block synthesis. C) Examples of the dithiol building blocks generated.

Supporting Information. With these building blocks in hand we were in a position to investigate whether they could be used to generate a DCL. As a proof-of-principle equimolar amounts of building blocks **6**, **7**, and **8** were mixed together (total building block concentration of 2 mM) in an aqueous solution (pH 8.0) under air and the solution stirred at room temperature in a capped vial for seven days. An aliquot of solution was then removed and analyzed by LC–MS (Figure 2). There were ten detectable species present (**9–18**), the mass data being consistent with linear homodimers **9**, **13**, and **16**, cyclic homodimers **10**, **12**, and **18**, cyclic heterodimers **11** and **14**, and two tetramers **15** and **17** (Figure 2). This experiment indicated that building blocks **6–8** were indeed capable of interacting to form a collection of structurally diverse higher-molecular-weight species. In addition, the presence of library members **11**, **14**, and **15** containing two different building blocks demonstrated that efficient mixing had occurred. A second proof-of-principle experiment involving the mixing of two building blocks under the same conditions led to a library of six different compounds (see Supporting Information for full details).

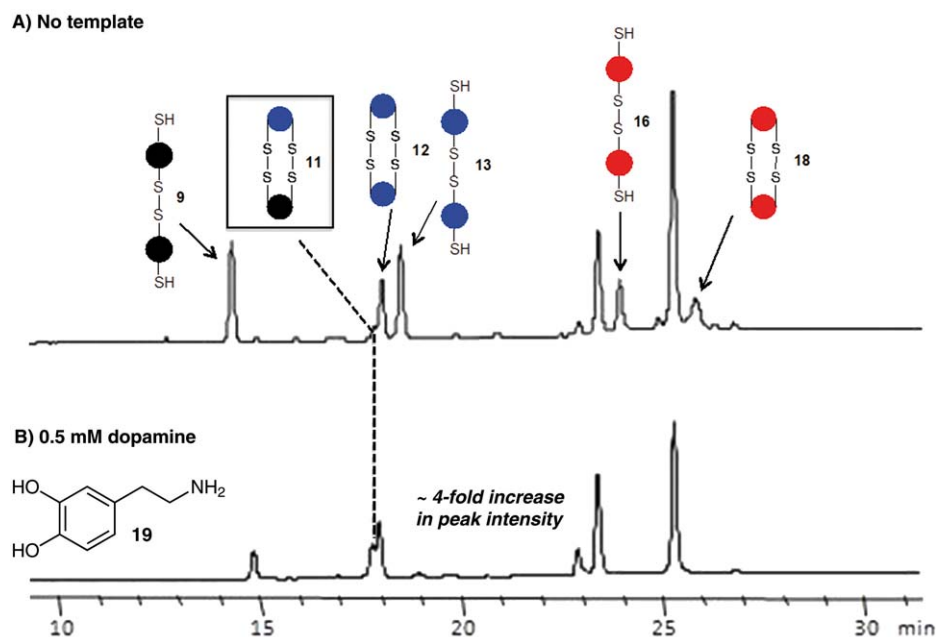
After acquiring evidence that combinations of our disulfide-based building blocks could interact to form collections of diverse higher-molecular-weight species, we next sought to examine whether the thermodynamic equilibrium of these collections could be altered. That is, could the compositions of such libraries be affected by the addition of an external agent, thereby allowing the amplification (and thus identification) of members capable of interacting with the agent? The catecholamine neurotransmitter dopamine (**19**) was chosen as a test biomolecule. Building blocks **6–8** (total building-block concentration of 2 mM) and dopamine (hydrochloride salt, 0.5 mM concentration) were mixed together in aqueous solution (pH 8.0) under air and the solution stirred at room temperature in a

capped vial for seven days. An aliquot of solution was then removed and analyzed by LC–MS. The chromatogram was then compared to that obtained previously when these three building blocks were combined in the absence of dopamine under the same reaction conditions. In the presence of dopamine there was an increase in relative concentration (assumed proportional to peak intensity) of six species **10–12**, **14**, **15**, and **17** at the expense of linear species **9**, **13**, **16**, and **18**. This implies that **10–15** and **17** interact more strongly with dopamine than **9**, **13**, **16**, and **18**. Compound **11** was observed to have the largest increase in peak intensity (and thus relative concentration) when library formation was carried out in the presence of dopamine. It can thus be inferred that **11** is the best receptor for this biologically important molecule present in the DCL that results from the combination of building blocks **6–8** (Figure 4). A similar experiment was carried out using two building blocks and dopamine. Again the data obtained indicated that library composition was affected by the presence of the biomolecule (the best binders, **20** and **21**, are shown in Figure 4, for full details see Supporting Information). These two proof-of-principle experiments suggest that molecular-recognition-induced changes in library composition are indeed possible, under biologically relevant conditions, with our disulfide-DCC approach. The precise binding mode of **11**, **20**, and **21** with dopamine is not known. However, it can be expected that dopamine will be protonated under the DCL conditions (pH 8.0) and that the carboxylic acid groups of **11**, **20**, and **21** will be deprotonated; therefore it is plausible that recognition of dopamine takes place, at least in part, through ionic interactions (though it is expected that interactions between the hydrophobic groups present in both species will also be important).<sup>17</sup>

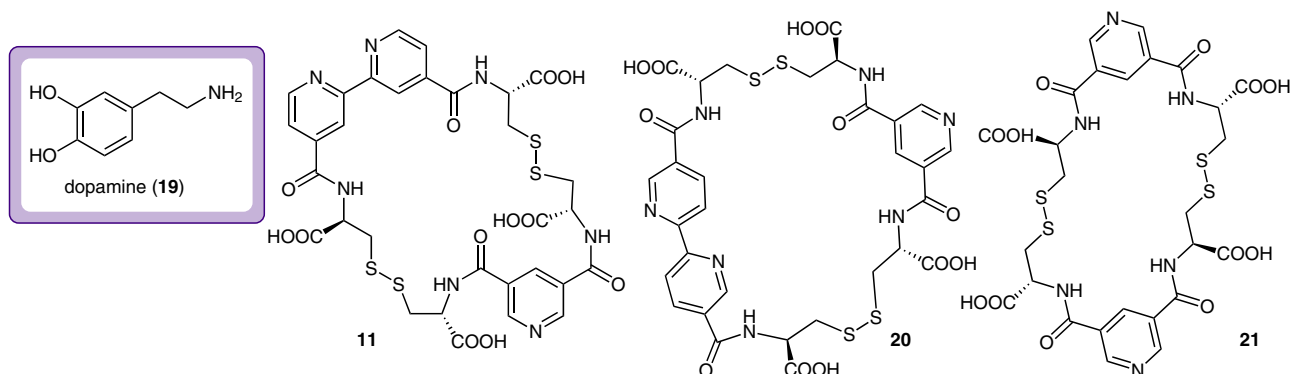
In conclusion, we have described the synthesis of a range of novel dithiol building blocks that can be used to form



**Figure 2** LC–MS trace of the library of compounds resulting from the combination of building blocks **6**, **7**, and **8** (total building block concentration of 2 mM in aq solution at pH 8.0, stirred at r.t. in a capped vial for 7 d).



**Figure 3** LC-MS trace of the library of compounds resulting from the combination of building blocks 6–8 (total building-block concentration of 2 mM in aqueous solution at pH 8.0), prepared A) in the absence of dopamine and B) in the presence of dopamine (**19**, 0.5 mM). In both cases mixtures were stirred at r.t. in a capped vial for 7 d prior to analysis. Note the loss of peaks attributed to compounds in the library generated in the presence of dopamine, and the fourfold increase in the intensity of the peak attributed to **11**.



**Figure 4** Structure of candidate receptors of dopamine (**19**) identified in this study. Compound **11** was identified by the experiment illustrated in Figure 3. Compounds **20** and **21** were identified by a second experiment, which is described in the Supporting Information.

structurally diverse DCL. Experiments with the catecholamine neurotransmitter dopamine indicate that such DCL are responsive to biological entities under physiologically relevant conditions. New promising candidate receptors of this biologically important neurotransmitter were identified. This is noteworthy as the design of synthetic receptors that bind biologically relevant guests in aqueous solution is a considerable challenge.<sup>18a</sup> These building blocks could conceivably be exploited in future DCC-based screening endeavors for the identification of biological useful molecules. Currently, we are exploring their use in the identification of inhibitors of protein–protein interactions, and the results of this work will be reported in due course.

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**Supporting Information** for this article is available online at <http://www.thieme-connect.com/ejournals/toc/synlett>.

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