Catalysis and communication in dynamic molecular networks

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Chapter 3

Cyclen Derivatives in Dynamic Combinatorial Chemistry. Disulfide Libraries
3.1 Introduction

Reversible disulfide exchange is probably the most popular chemical reaction to form DCLs\textsuperscript{1-3} and has been also the most used for the recognition of biological targets.\textsuperscript{4-9} It is easy to perform and, importantly, disulfides are chemically compatible with a large number of other functional groups and therefore rarely interfere with molecular recognition. A difference with respect to reversible hydrazone chemistry is that the building blocks used for disulfide exchange have symmetrical linkages (thiols). This means that any two building blocks are able to react with each other, unlike hydrazones that require the union between two different functional groups (hydrazide and aldehyde). As a result, disulfide libraries are often simpler to design than those made from hydrazones. An effective pH suitable for biological applications is an additional reason why disulfide DCLs are widely investigated.

As explained in Chapter 2, 1,4,7,10-tetraazaacyclododecane (cyclen) is a cyclic polyamine useful for the binding of metal ions. In this regard, cyclen was chosen to be the core of a dithiol building block with the aim of making disulfide libraries containing cyclen-bound metal ions. The presence of the metal ions in the library members would provide a potential recognition and a catalytic centre. In the literature, no cyclen containing DCLs are known, although other structures containing nitrogen atoms have been used for recognition purposes.\textsuperscript{10,11}

In this chapter, the synthesis and application of a cyclen-dithiol building block in disulfide DCLs are described. The addition of metal ions to these libraries is also discussed.

3.2 Study of cyclen-disulfide libraries

3.2.1 Introduction

Disulfide libraries containing cyclen were prepared by mixing building blocks equipped with thiol groups. Since thiolates in solution are required for these libraries to be dynamic, a pH between 7 and 9 is normally used. Multiple dithiol building blocks had been tested in our group for the formation of dynamic combinatorial libraries of disulfides,\textsuperscript{11-20} so we already accumulated a considerable amount experience in this chemistry. In view of the
promising results previously achieved, a cyclen-bearing building block similar to the one used for hydrazone chemistry (Chapter 2) was synthesized.

3.2.2 Synthesis of a cyclen-thiol building block

A cyclen containing two thiol groups able to form disulfides was designed to be used in disulfide DCLs. Thiol building block 10 was decorated with two phenyl groups that could provide π-π stacking interactions. The aromatic groups increase the possibilities of achieving molecular recognition by offering an apolar environment for organic molecules. The synthesis of 10 started from bare cyclen 1. The two more electrophilic nitrogen atoms were protected with t-BOC groups. The protected cyclen was reacted with t-BOC-protected p-(bromomethyl)-thiophenol which had been previously synthesized. Only the unprotected nitrogen atoms were then available to perform the nucleophilic substitution on the brominated carbon atom of reagent 8. In the last step, an acidic hydrolysis yielded the final product with an overall yield of 35% (Scheme 3.1).

![Scheme 3.1 Synthetic route of dithiol building block 10.](image)

3.2.3 Cyclen containing disulfide libraries

Thiol libraries of 1.00 mM building block concentration were prepared by dissolving 10 in borate buffer at pH 8.0. After 3 days, 80% of 10 had been oxidized into disulfides. The disulfide composition at this time consisted of 95% of cyclic dimer and a 5% of cyclic monomer 11 (Figure 3.2). The presence of the latter species was unexpected. Due to the high degree of strain in its structure, it was considered to be a high-energy species.
Indeed, this disulfide was not a thermodynamically stable species but the result of a kinetically controlled reaction. This fact was evident from an analysis of a similarly prepared library in presence of Cu(II) as an oxidation catalyst which revealed an increased proportion of cyclic monomer compared to the library in absence of the metal (Figure 2.2).

As a strategy to increase the diversity of the dynamic disulfide combinatorial network, other dithiol molecules were also included to participate in the formation of mixed disulfide species. Building blocks 13 and 14 were mixed together with 10 to form a ternary library of disulfides. An assortment of cyclic dimeric, trimeric and tetrameric structures was identified in the mixture (Figure 3.3). This diversity of products is a desirable feature in a library and prompted an initial screening of binding to potential guest molecules.

![Figure 3.2](image)

**Figure 3.2** HPLC analysis of a disulfide library made from building block 10 in presence (above) and absence (below) of Cu(II) as a catalyst of thiol oxidation. A broad peak probably corresponding to the presence of larger oligomers was observed during washing of the column after analysis of the Cu(II) containing DCL. Conditions: 1.00 mM of 10 in borate buffer 50 mM, pH = 8.0.
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Figure 3.3 HPLC analysis of a disulfide library made from building blocks 10, 13 and 14 after 8 days. Conditions: 1.00 mM of total building block concentration in borate buffer 50 mM, pH 8.0.

With the aim of shifting the library composition, some molecules were tested as templates (Figure 3.4). While 15 was known to amplify the cyclic trimer made from 13, 16 had been reported to bind to other carboxylic-containing disulfide library members. Molecules 1, 15-18 are positively charged in aqueous media and / or contain aromatic residues that are able to provide electrostatic and π-π stacking interactions with the library members. However, none of those molecules was able to produce any template effect in the library made from 10, 13 and 14.

Although no response from the library was detected in presence of any of the added molecules, the ability of 10 to combine with other thiol molecules and provide disulfide structures, encouraged us to continue with the study of these libraries for the formation of catalysts. For this purpose, metal ions were tested in combination with disulfide libraries.
Figure 3.4 The HPLC analysis of libraries made from 10, 13 and 14 in presence of 1, 15-18 did not reveal any templating effect. The main peak corresponds to the cyclic dimer made from 10 and 13. Conditions: 0.33 mM concentration of each building block and 1.00 mM concentration of template in borate buffer 50 mM, pH 8.0. The difference in the appearance of the top trace and that shown in Figure 2.3 is due to the fact that they were obtained using different LC instruments.

3.3 Metal salts and disulfides

The presence of the cyclohex moiety within 10 gives the possibility to bind metal ions to the dithiol building block. Such metals could act as recognition centers for guest molecules and, in some cases, also as catalysts. Up to now, no examples of aqueous disulfide libraries in the presence of transition metals have been reported, although some examples are found in organic solvents.\textsuperscript{22-24} Interactions between sulfur and metal ions\textsuperscript{25-27} are known to occur and could lead to the inactivation of the metal\textsuperscript{28} and interference with the disulfide exchange process. Our aim was to explore the compatibility of metal salts to be used within the disulfide libraries without disturbing their dynamic nature.

Initial tests combining metal ions with 10 were performed to obtain some information about the kinetics of the ligand coordination to the metals. Solutions of 10 in borate buffer were prepared and Co(III), Ni(II), Fe(III), Cu(II) or Zn(II) salts were added to them. The UV-vis absorption spectra of the solutions were recorded over time revealing that the coordination was slow, similarly to the coordination of metals to the hydrazide building block.
block bearing cyclen (Figure 3.5). However, the HPLC traces of disulfide libraries made from 10 and metal ions, showed some effect of the metals on the product distribution of the libraries (Figure 3.6). Cu(II) increased the amount of cyclic monomer as previously stated, as a result of a catalytic thiol oxidation process, but the total peak area observed in the chromatogram consistently decreased as compared to the sample in absence of metal. Presumably, the fast oxidation of thiols led to the formation of larger oligomers which eluted during the column washing step. Ni(II) and Co(II) behaved similarly, slightly accelerating the oxidation process. Only Ga(III) seemed to delay the thiol oxidation process. In any case, the metal ions by themselves did not produce any templating effect in the library.

![Figure 3.5](image)

**Figure 3.5** The binding of Cu(II) to 10 was monitored over time by UV-vis spectrometry. The shown spectra correspond to time measurements of 5, 10, 20, 35, 60 and 150 minutes. Conditions: 0.10 mM 10 and 0.02 mM Cu(NO₃)₂ in borate buffer 50 mM pH 8.0.

Attempts to achieve metal-mediated templating effects were carried out. The successful thymidine recognition by the hydrazide building block prompted us to test this molecule in disulfide DCLs. For that purpose, 1 eq of Zn(NO₃)₂ was added to a solution of 10 in borate buffer (in D₂O) 50 mM, pD 9.0. At this stage, a slight turbidity appeared probably coming from the formation of insoluble Zn(OH)₂. The addition of thymidine to that suspension produced more precipitation. This phenomenon was impossible to revert by addition of organic solvents, and therefore the experiment was abandoned at that point.
The presence of metal ions (1 eq) in the disulfide libraries made of 10 (1.00 mM) in borate buffer 50 mM pH 8.0 did not affect the distribution of the library members. Cu(II) accelerated the oxidation process as well as Ni(II) and Co(II) although the latter to a lesser extent. Ga(III) slowed down the formation of disulfides. Libraries were analyzed after 48h.

These negative results forced us to change our strategy once again and make use of a pre-synthesized metal complex to prepare disulfide libraries. After the successful complexation of Zn(II) to a hydrazide building block, the formation of the analogous dithiol-Zn(II) complex was attempted. Following a similar procedure to the synthesis of the Zn-hydrazide complex from Chapter 2, building block 10 and an excess of Zn(NO$_3$)$_2$ were mixed in boiling deoxygenated MeOH to obtain building block 19 (Figure 3.7).

The preparation of disulfide libraries from the metal complex 19 consistently resulted in precipitation upon oxidation of the thiol groups. The addition of isopropanol, EtOH or MeOH to the solution failed to re-dissolve the precipitate. Other libraries containing 19 in
combination with different and more soluble thiol building blocks were prepared with the hope of forming soluble dithiols. Cysteine, dithiols 13 and 14 or ethanethiol were tested with no positive results. Libraries with a lower concentration (0.2 mM) of 19 were also tested but precipitation was still observed. Unfortunately, these persistent solubility problems led us to abandon the development of disulfide libraries using Zn-dithiol building block 19. A possible solution for this precipitation issue might involve the use of charged building blocks with better solubilization properties. Alternatively, the modification of building block 19 by addition of polar functional groups could also solve the problem. On the other hand, the variation of the metal without further modification of the building blocks is not expected to change the solubility characteristics of the library, unless additional coordination by soluble ligands is occurring.

3.4 Conclusions

A study of the behavior of disulfide dynamic combinatorial libraries containing cyclen has been presented in this chapter. Dithiol molecules containing cyclen were mixed with other thiol building blocks which, upon oxidation, formed disulfide dynamic combinatorial libraries containing cyclic dimers, trimers and tetramers. The use of metal salts in these libraries was restricted to previously synthesized metal-dithiol complexes in view of the slow kinetics for the formation of the complex. The use of Zn-dithiol building block 19 for the formation of metal containing disulfide combinatorial libraries was frustrated by precipitation problems.

3.5 Experimental

Reagents and solvents

All reagents and solvents were obtained from commercial sources and used without further purification unless otherwise specified.
NMR analysis
NMR spectra were obtained on a Varian AS 400 MHz instrument. $^1$H chemical shifts are reported as $\delta$ in ppm relative to residual protonated solvent resonances. $^{13}$C chemical shifts are reported as $\delta$ in ppm and measured relative to solvent references. Coupling constants are reported in Hertz.

HPLC analysis
Analytic HPLC was carried out on Hewlett Packard 1050 or 1100 systems coupled to UV detectors and the data were processed using HP Chemstation software. Separations were performed on a reversed phase Waters Symmetry C8 column (4.6 x 150 mm, 3.5 μm particle size). Aliquots of 3 μL of library solution were injected. Doubly distilled water, HPLC-S-grade acetonitrile from Biosolve and formic acid were used to prepare the eluents:
eluent A = water + 5.0 % acetonitrile + 0.10 % formic acid
eluent B = acetonitrile + 5.0 % water + 0.10 % formic acid
Chromatography was performed at 45 °C using UV detection at 260 nm and a constant flow rate of 1.00 mL / min. The HPLC analysis method was as follows:

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<th>Time (min)</th>
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LC-MS analysis
For the LC-MS measurements an Accela High Speed LC system (ThermoFisher Scientific, Courtaboeuf, France) was coupled to a LTQ-Fleet Ion Trap Mass Spectrometer. Mass spectra (positive ion mode) were obtained using the following conditions: sheath gas flow rate 30, aux. gas flow rate 10, sweep gas flow rate 5, ionization spray voltage 3.50 kV, capillary temperature 330 °C, capillary voltage 12 V, tube lens 40 V.
The LC method employed was the same as that one used for HPLC. The flow was split after the LC to allow 0.30 mL / min to enter the mass spectrometer.

Synthetic procedures

4,4’-((1,4,7,10-tetraazacyclododecane-1,7-diyl)bis(methylene))dibenzenethiol (10)

1. Boc protection of cyclen (1): 21
To a solution of 1,4,7,10-tetraazacyclododecane (cyclen) (1.43 g, 8.00 mmol) in 60 mL of chloroform, N-(tert-butoxycarbonyloxy) succinimide (3.44 g, 16.0 mmol) was added. The reaction mixture was stirred for 2 days at r.t. The solvent was then removed by under vacuum and 60 mL NaOH (3.0 M) was added to the remaining residue. An extraction with 3 x 30 mL chloroform was performed, the organic extracts were combined, dried over MgSO₄ and the solvent was removed under vacuum. 1,7-di-Boc protected cyclen was obtained as a white solid (2.80 g, 94%). ¹H-NMR (CDCl₃, 200 MHz): 3.30 (m, 10H), 2.69 (m, 8H), 1.37 (s, 18H).

2. Boc protection of methylthiophenol (4): 29
A solution of Boc-anhydride (2.16 g, 14.4 mmol) in 50 mL of acetonitrile was cooled in an ice bath. A solution containing DMAP (0.59 g, 4.80 mmol) and TEA (2.94 g, 30.0 mmol) in 40 mL of acetonitrile was added to the solution of Boc-anhydride. p-Methylthiophenol (1.20 g, 9.60 mmol) was added gradually and the reaction mixture was stirred overnight at r.t. The solvents were then removed under vacuum. The remaining yellow solid was dissolved in 20 mL of chloroform and washed with 3 x 30 mL water and the organic phase dried over MgSO₄. The solvent was removed under vacuum and the residue was purified by column chromatography using
Hex:AcOEt (40:1) as eluent to obtain 0.80 g (50% yield) of O-tert-butyl S-p-tolyl carbonothioate. \(^1\)H-NMR (CDCl\(_3\), 400 MHz): 7.40 (d, J = 8.0Hz, 2H), 7.19 (d, J = 8.0Hz, 2H), 2.36 (s, 3H), 1.50 (s, 9H). \(^13\)C-NMR(CDCl\(_3\), 100 MHz): 168.2 (C\(_{\text{quat}}\)), 139.6 (C\(_{\text{quat}}\)), 134.9 (2xCH), 129.9 (C\(_{\text{quat}}\)), 125.0 (2xCH\(_2\)), 85.4 (C\(_{\text{quat}}\)), 28.2 (3xCH\(_3\)), 21.3 (CH\(_3\)).

3. Bromination of O-tert-butyl S-p-tolyl carbonothioate (6): N-bromosuccinimide (1.27 g, 7.13 mmol) was added to 20 mL of carbon tetrachloride. O-tert-butyl S-p-tolyl carbonothioate (1.50 g, 6.70 mmol) and benzyol peroxide (58 mg, 0.24 mmol) were then added and the reaction mixture was refluxed for 2 hours. After the solution cooled down, the remaining solid was filtered and the solvents were evaporated in vacuo. The crude product was purified by column chromatography using Hex:AcOEt (20:1) as eluent to obtain S-(4-(bromomethyl)phenyl) O-tert-butyl carbonothioate (0.76 g, 59%) as a white solid. M.p. 85.3 – 86.2 °C. \(^1\)H-NMR (CDCl\(_3\), 400 MHz): 7.50 (d, J = 8.0Hz, 2H), 7.41 (d, J = 8.0Hz, 2H), 4.47 (s, 2H), 1.52 (s, 9H). \(^13\)C-NMR(CDCl\(_3\), 100 MHz): 167.4 (C\(_{\text{quat}}\)), 138.9 (C\(_{\text{quat}}\)), 135.0 (2xCH), 129.7 (2xCH), 127.1 (C\(_{\text{quat}}\)), 85.8 (C\(_{\text{quat}}\)), 32.5 (CH\(_2\)), 28.2 (3xCH\(_3\)). HR-MS calcd (M-Na\(^+\)) 324.9868, found 324.9855.

4. Synthesis of di-tert-butyl 4,10-bis(4-((tert-butoxycarbonyl)thio)benzyl)-1,4,7,10-tetraazacyclododecane-1,7-dicarboxylate (9): In a round bottomed flask, S-(4-(bromomethyl)phenyl) O-tert-butyl carbonothioate (0.82 g, 2.71 mmol), 1,7-bis((tert-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane (0.31 g, 0.83 mmol) and Na\(_2\)CO\(_3\) (0.90 g, 8.50 mmol) were added to 15 mL of DMF. The mixture was stirred overnight at r.t. It was then filtered, diluted with 100 mL brine and extracted with 3 x 100 mL dichloromethane. The organic phase was washed with 2 x 100 mL brine, dried over MgSO\(_4\) and the solvents were removed in vacuo. The final residue was purified by column chromatography on alumina with Hex:AcOEt (20:1) and (1:1) to obtain 0.33 g (49%) of product. \(^1\)H-NMR (CDCl\(_3\), 300 MHz): 7.44 (d, J = 7.5Hz, 4H), 7.31 (d, J = 7.5Hz, 4H), 3.62 (s, 4H), 3.40 (m, 8H), 2.67 (m, 8H), 1.50 (s, 18H), 1.30 (s, 18H). \(^13\)C-NMR (CDCl\(_3\), 75 MHz): 176.7 (2xC\(_{\text{quat}}\)), 167.5
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(2xC quat), 143.0 (2xC quat), 134.9 (4xCH), 129.6 (4xCH), 127.2 (2xC quat), 85.6 (2xC quat), 85.3 (2xC quat), 64.1 (2xC H 2), 41.8 (2xC H 2), 28.5 (6xC H 3), 28.1 (6xC H 3). HR-MS calcd (M-H+) 817.4238, found 817.4235.

5. Deprotection of di-tert-butyl 4,10-bis(4-(((tert-butoxycarbonyl) thio)benzyl)-1,4,7,10-tetraazacyclododecane-1,7-dicarboxylate (9): (80 mg, 0.10 mmol) of 9 were dissolved in 10 mL of degassed ethanol and the solution was cooled in an ice bath under a N 2 atmosphere. HCl conc. (10 mL) was added dropwise through a septum and the mixture was stirred overnight. Solvents were removed in vacuo and the white solid residue was washed with degassed ethanol yielding 41 mg, (99%) of 4,4′-((1,4,7,10-tetraazacyclododecane-1,7-diyl)bis(methylene))dibenzenethiol. M.p. 227.0 -230.2 °C. 1H-NMR (D 2 O, 400 MHz): 7.42 (d, J = 8.1 Hz, 4H), 7.25 (d, J = 8.1 Hz, 4H), 3.72 (s, 4H), 3.13 (m, 8H), 2.88 (m, 8H). 13C-NMR((CD 3 ) 2 NC(O)D/D 2 O, 50 MHz): 133.6 (2xC quat), 133.4 (2xC quat), 132.9 (4xC H), 130.3 (4xC H), 57.6 (2xC H 2), 48.9 (4xC H 2), 44.4 (4xC H 2). HR-MS calcd (M-H+) 415.1985, found 415.1978.

4,4′-((1,4,7,10-tetraazacyclododecane-1,7-diyl)bis(methylene))dibenzenethiol, Zn(II) complex (19)

For the synthesis of this compound the same procedure as described for molecule 21 in Chapter 2 was employed. M.p. 293.3 - 294.7 °C. 1H-NMR (200 MHz, D 2 O): δ 7.30 (d, J = 8.0 Hz, 4H), 7.09 (d, J = 8.0 Hz, 4H), 3.59 (s, 4H), 3.07 (m, 10H), 2.79 (m, 8H). 13C-NMR (100 MHz, DMSO): δ 166.0 (2xC=O(C(NH-)), 142.9 (2xC quat), 132.5 (2xC quat), 129.2 (4xC H), 127.4 (4xC H), 60.9 (4xC H), 51.7 (4xC H), 47.0 (4xC H). HR-MS calcd (M-H+) 479.1266, found 479.1276.

Dithiol building blocks 13 12 and 14 15 were prepared as described previously.
Chapter 3

Preparation of libraries

For a typical disulfide library, building block 1 (1.00 mM) was dissolved in borate buffer 50 mM pH 8.0. The final sample was prepared by mixing 800 µL of the disulfide solution and 200 µL of IPA. The solutions containing the libraries were stirred at about 800 r.p.m. at room temperature.
3.6 References


Chapter 3


