DOI: 10.1002/chem.200801109

Selective Extraction of G-Quadruplex Ligands from a Rationally Designed Scaffold-Based Dynamic Combinatorial Library

Mads Corvinius Nielsen and Trond Ulven*[a]

Dynamic combinatorial chemistry (DCC) is a concept for library synthesis where a mixture of building blocks capable of reversible bond formation gives rise to a set of library members existing in equilibrium. Upon addition of a molecular template or target to a dynamic combinatorial library (DCL), the library members forming the strongest interaction with the target will be stabilized, and the equilibrium will shift to replenish the selected compounds, resulting in their overall enrichment.[1] In principle, this concept enables convenient one-pot synthesis and screening of large libraries. However, despite its attractive features, DCC has not yet become a standard item in the drug discovery toolbox. Among the reasons for this are the lack of methods for efficient analysis of larger libraries,[2] the difficulties associated with designing libraries of structures that make target interaction likely and with a size that makes them suitable starting points for optimization, and the limited availability of chemistry providing reliable dynamic equilibrium between library components under biological conditions.[3] The most satisfactory available solution to the last problem is probably the disulfide group. Its ability to undergo thiolate-catalyzed scrambling with high chemoselectivity in the presence of biomolecules in close to neutral aqueous buffers has made this functional group a preferred dynamic unit in DCLs aimed at biological targets. Most disulfide-based DCLs described hitherto may be divided into one of two groups: those formed from building blocks with one thiol attachment point, leading to dimeric libraries,[2,4–5] and those including building blocks with two or three attachment points, resulting in libraries of oligomeric or macrocyclic compounds.[6] Here we describe a third library design, taking advantage of disulfide scrambling, where the members are constructed from a central scaffold that may carry and equilibrate with several side chains.

It is well established that many nucleic acid sequences rich in guanine are capable of folding into secondary structures know as G-quadruplexes.[7] The ability of the chromosomal telomeres to form G-quadruplexes and the potential of developing new anticancer therapeutics that act by stabilizing these structures has lately received much attention.[8] Recent results furthermore indicate that >40% of human gene promoters contain motifs capable of G-quadruplex formation, and it has been proposed that this dynamic structure may directly regulate gene expression.[9] If this hypothesis is substantiated, compounds selectively modulating specific G-quadruplexes will surely be in high demand.

A substantial number of G-quadruplex stabilizing small molecules are currently known, although confirmed sequence-specific compounds are still scarce. The majority of efficient G-quadruplex ligands fit a general pharmacophoric pattern consisting of a central aromatic scaffold that is substituted with one or several positively charged side chains.[10] We have designed a DCL over this pattern consisting of three central aromatic scaffolds carrying one (**A**), two (**B**) or three (**C**) thiol handles in equilibration with three different side chains (**a**, **b** and **c**), each carrying a positively charged group (Figure 1). The scaffolds were mixed in a 3:6:10 ratio to provide equal amounts of each library member in the statistical distribution. The acridine scaffold (**A**) and the pyridine-2,5-dicarboxamide scaffold (**B**) are both elements from known potent G-quadruplex ligands like BRACO-19[11] and 831A,[12] however, it is notable that none of the three scaffolds are capable of G-quadruplex stabilization in the absence of side chains in the relevant concentration range. The positively charged side chains are present in large excess in order to i) counteract oligomerization of the central scaffolds, ii) function as a thiol/disulfide exchange buffer with tunable exchange rate (which is proportional to the thiolate concentration), and iii) provide a uniform concentration of each side chain throughout the library equilibration, so that it will maintain invariable availability in solution independent of its presence in amplified

[a] M. C. Nielsen, Prof. Dr. T. Ulven
Department of Physics and Chemistry
University of Southern Denmark
Campusvej 55, 5320 Odense M (Denmark)
Fax: (+45) 6615-8780
E-mail: ulven@ifk.sdu.dk

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.200801109.

 © 2008 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim
WILEY InterScience DISCOVER SOMETHING GREAT

compounds, thus facilitating amplification of library members with the highest target affinity. This design eliminates the risk of compromising the amplification of the strongest interacting compound due to competition for chains between scaffolds, despite the presence of members composed of up to four dynamic elements.[13]

All components were added as disulfide dimers, and equilibration was initiated by adding a fraction of one of the side chains as the free thiol to the pH 7.4 buffer solution. The libraries were analyzed by HPLC and ESI-MS, and we found the analysis much facilitated by taking advantage of the specific UV/Vis spectra of the scaffolds outside the absorbance range of the side chains. The ratios between the absorbance at different frequencies reveal the scaffold identity of any given peak in the HPLC chromatogram and indicate if it reflects a pure library member (Figure 2a). Eighteen of the 19 theoretical library members were identified and, with one exception, resolved with baseline separation in the chromatogram (Figure 2b). Peak 8 in the chromatogram illustrates the usefulness of monitoring multiple wavelengths, as the absorbance ratios did not fit any pure scaffold, but suggested that the peak was composed of an acridine (**A**) and a pyridine (**B**) scaffold, which was confirmed by HRMS. All other peaks fitted one of the three expected absorbance patterns, each corresponding to the compound identified by HRMS.[14]

The most active compounds were identified by an extraction protocol inspired from an elegant procedure by Balasubramanian and co-workers, in which the biotinylated DNA target is pulled out from the quenched library solution by streptavidin coated magnetic beads in order to liberate the bound compounds before recombination and determination of the overall amplification.[4] Rather than recombining the solutions, we separately analyzed the supernatant after denaturing the DNA target. Three libraries were constructed in parallel; one was incubated with telomeric DNA on G-quadruplex form, one with a related random coil DNA string, and one without DNA.[14] After equilibration, disulfide scrambling was stopped by acidification, and the DNA was extracted and denatured. In the case of the G-quadruplex DNA, the acridine **Ac** was extracted with high selectivity, constituting approximately 54% of scaffold-based components in the superna-

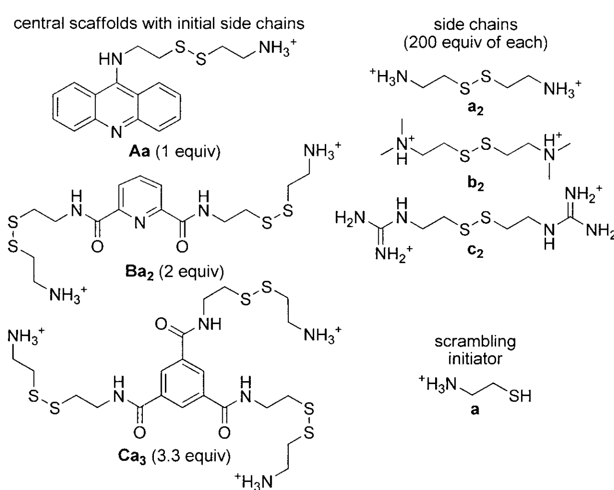

Figure 1. Initial components of the DCL.

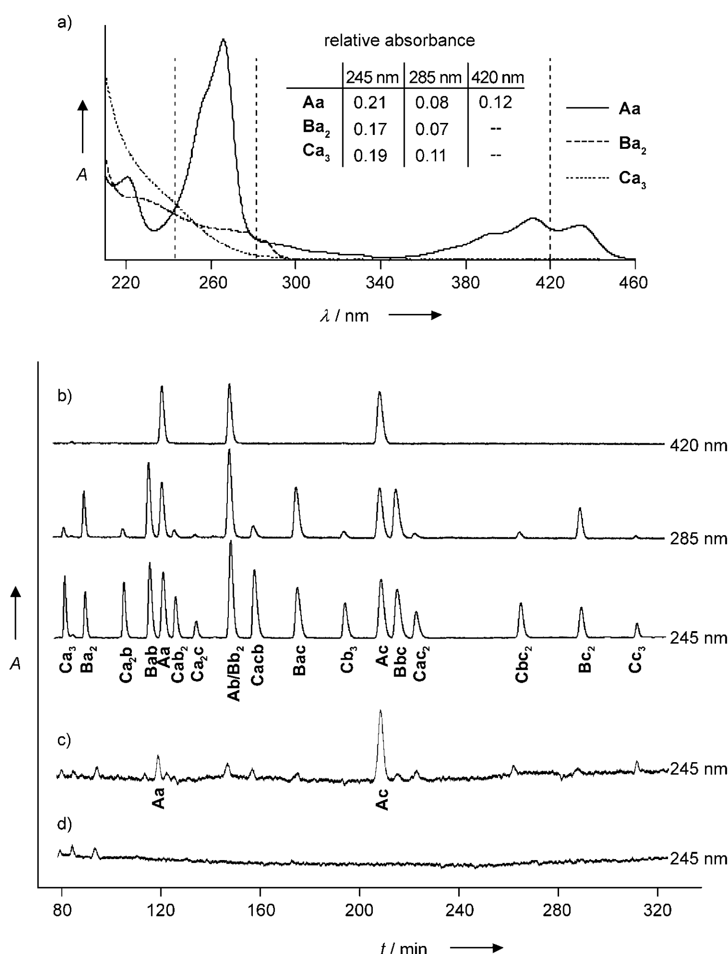

Figure 2. a) UV/Vis spectra of the initial library members **Aa**, $\mathbf{Ba_2}$ and $\mathbf{Ca_3}$, and relative absorbance at 245, 285 and 420 nm. b) HPLC chromatogram of library mixture without DNA. Central scaffolds are identifiable by the relative absorbance. c) HPLC chromatogram of composition extracted by G-quadruplex folded 5′-(biotin-d[$(GT_2AG_2)_5$])-3′.[14] d) HPLC chromatogram of composition extracted by 5′-(biotin-d[$(AGT_2AG)_5$])-3′.

 © 2008 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

tant (Figure 2c), compared to 8.3% in the reference library. Acridine **Aa**, constituting ≈10%, was the second most abundant extracted compound. The analogous procedure with a related random coil DNA oligomer did not result in identifiable amounts of any library member (Figure 2d).

The G-quadruplex stabilizing properties of the extracted compound **Ac** and of the three initial library components were evaluated by CD on a telomeric sequence.[15] The melting temperature of the **Ac** complex was determined 12.8°C higher than the G-quadruplex alone, compared to a ΔT_m of 8.6°C for the **Aa** complex and 6.1°C for the $\mathbf{Ca_3}$ complex (Figure 3). The CD curve and melting temperature of the $\mathbf{Ba_2}$ complex was found to be identical to the G-quadruplex without ligand. Dissociation constants (K_d) for the acridine compounds were determined by surface plasmon resonance (SPR) to 0.78±0.21 μM for **Ac** and 1.57±0.31 μM for **Aa**, corresponding agreeably to the increases in CD melting temperatures.[14]

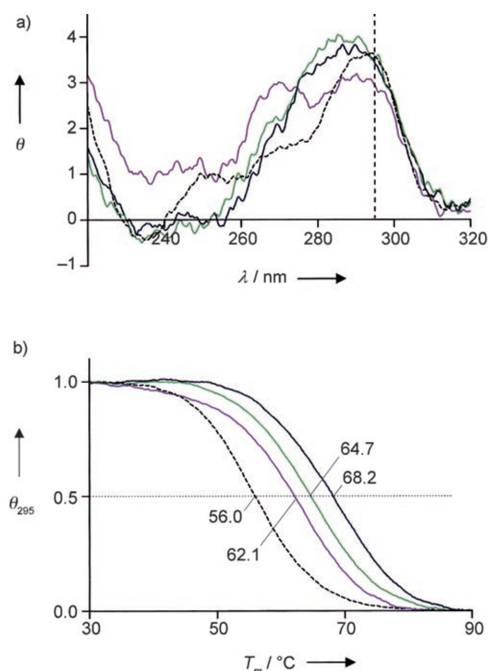

Figure 3. a) CD spectra of 5′-d[$G_3(T_2AG_3)_3$]-3′ (2 μM) at pH 7.4 in 10 mM Tris-HCl buffer containing 10 mM KCl alone (dashed line) and with 10 μM **Ac** (black line), **Aa** (green line), or $\mathbf{Ca_3}$ (purple line). b) Normalized CD melting curves of G-quadruplex alone (dashed line) and with **Ac** (black line), **Aa** (green line), or $\mathbf{Ca_3}$ (purple line) recorded at 295 nm.

A crucial requirement of DCLs in drug discovery is the ability to discriminate between several active compounds in the mixture. The data above indicate that the DCL described here contains at least three and possibly more compounds that interact with and stabilize telomeric G-quadruplexes, and that the most active of these is extracted with five-fold selectivity over a less but comparably active member, thus supporting the notion that DCC has the potential to play an important role in drug discovery. As the main objective of this work was to test the new design principle in a fully analyzable system, relatively few components were included. However, in the general application it is only required to identify the preferentially extracted compounds, which significantly simplify the analysis task. The DCL design principle described here can be easily extended to construction of larger libraries which may be aimed at various targets and consisting of compact and diverse members around scaffolds of special interest, and we expect to find the simple technique of recording chromatograms at multiple wavelengths to be increasingly valuable with such larger libraries by reducing the need for deconvolution.

Acknowledgements

We thank Dr. Jonas Borch and Dr. Kim F. Haselmann for advices and assistance with SPR and ESI-MS analysis. This research was supported by the Danish Natural Science Research Council (grant 272-05-0357) and the Danish Medical Research Council (grant 271-05-0409).

Keywords: combinatorial chemistry · disulfide exchange · DNA · drug design · G-quadruplex

[1] For reviews, see: a) P. T. Corbett, J. Leclaire, L. Vial, K. R. West, J. L. Wietor, J. K. M. Sanders, S. Otto, *Chem. Rev.* **2006**, *106*, 3652–3711; b) J. D. Cheeseman, A. D. Corbett, J. L. Gleason, R. J. Kazlauskas, *Chem. Eur. J.* **2005**, *11*, 1708–1716; c) S. J. Rowan, S. J. Cantrill, G. R. L. Cousins, J. K. M. Sanders, J. F. Stoddart, *Angew. Chem.* **2002**, *114*, 938–993; *Angew. Chem. Int. Ed.* **2002**, *41*, 898–952; d) J.-M. Lehn, *Chem. Eur. J.* **1999**, *5*, 2455–2463.

[2] An approach to overcome this problem using resin bound library components was recently described: a) B. R. McNaughton, B. L. Miller, *Org. Lett.* **2006**, *8*, 1803–1806; b) B. R. McNaughton, P. C. Gareiss, B. L. Miller, *J. Am. Chem. Soc.* **2007**, *129*, 11306–11307.

[3] a) O. Ramström, J. M. Lehn, *Nat. Rev. Drug Discovery* **2002**, *1*, 26–36; b) S. Ladame, *Org. Biomol. Chem.* **2008**, *6*, 219–226.

[4] a) A. M. Whitney, S. Ladame, S. Balasubramanian, *Angew. Chem.* **2004**, *116*, 1163–1166; *Angew. Chem. Int. Ed.* **2004**, *43*, 1143–1146; b) S. Ladame, A. M. Whitney, S. Balasubramanian, *Angew. Chem.* **2005**, *117*, 5882–5885; *Angew. Chem. Int. Ed.* **2005**, *44*, 5736–5739; c) A. Bugaut, K. Jantos, J. L. Wietor, R. Rodriguez, J. K. M. Sanders, S. Balasubramanian, *Angew. Chem.* **2008**, *120*, 2717–2720; *Angew. Chem. Int. Ed.* **2008**, *47*, 2677–2680.

[5] Representative dimeric disulfide DCLs: a) H. Hioki, W. C. Still, *J. Org. Chem.* **1998**, *63*, 904–905; b) O. Ramström, J. M. Lehn, *ChemBioChem* **2000**, *1*, 41–48; c) K. C. Nicolaou, R. Hughes, S. Y. Cho, N. Winssinger, H. Labischinski, R. Endermann, *Chem. Eur. J.* **2001**, *7*, 3824–3843; d) K. C. Nicolaou, R. Hughes, J. A. Pfefferkorn, S. Barluenga, A. J. Roecker, *Chem. Eur. J.* **2001**, *7*, 4280–4295; e) D. A. Erlanson, J. W. Lam, C. Wiesmann, T. N. Luong, R. L. Simmons, W. L. DeLano, I. C. Choong, M. T. Burdett, W. M. Flanagan, D. Lee, E. M. Gordon, T. O'Brien, *Nat. Biotechnol.* **2003**, *21*, 308–314; f) A. L. Kieran, A. D. Bond, A. M. Belenguer, J. K. M. Sanders, *Chem. Commun.* **2003**, 2674–2675; g) Y. Krishnan-Ghosh, S. Balasubramanian, *Angew. Chem.* **2003**, *115*, 2221–2223; *Angew. Chem. Int. Ed. Engl.* **2003**, *42*, 2171–2173; h) T. Hotchkiss, H. B. Kramer, K. J. Doores, D. P. Gamblin, N. J. Oldham, B. G. Davis, *Chem. Commun.* **2005**, 4264–4266; i) Z. Pei, R. Larsson, T. Aastrup, H. Anderson, J. M. Lehn, O. Ramström, *Biosens. Bioelectron.* **2006**, *22*, 42–48; j) S. Andre, Z. Pei, H. C. Siebert, O. Ramström, H. J. Gabius, *Bioorg. Med. Chem.* **2006**, *14*, 6314–6326; k) L. Milanesi, C. A. Hunter, S. E. Sedelnikova, J. P. Waltho, *Chem. Eur. J.* **2006**, *12*,

 © 2008 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

1081–1087; l) B. Danieli, A. Giardini, G. Lesma, D. Passarella, B. Peretto, A. Sacchetti, A. Silvani, G. Pratesi, F. Zunino, *J. Org. Chem.* **2006**, *71*, 2848–2853; m) B. M. Lienard, N. Selevsek, N. J. Oldham, C. J. Schofield, *ChemMedChem* **2007**, *2*, 175–179; n) B. M. Lienard, R. Huting, P. Lassaux, M. Galleni, J. M. Frere, C. J. Schofield, *J. Med. Chem.* **2008**, *51*, 684–688.

[6] Representative oligomeric/macrocyclic disulfide DCLs: a) S. Otto, R. L. E. Furlan, J. K. M. Sanders, *J. Am. Chem. Soc.* **2000**, *122*, 12063–12064; b) B. Brisig, J. K. M. Sanders, S. Otto, *Angew. Chem.* **2003**, *115*, 1308–1311; *Angew. Chem. Int. Ed.* **2003**, *42*, 1270–1273; c) A. L. Kieran, S. I. Pascu, T. Jarrosson, J. K. M. Sanders, *Chem. Commun.* **2005**, 1276–1278; d) S. Sando, A. Narita, Y. Aoyama, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2835–2838; e) J. Leclaire, L. Vial, S. Otto, J. K. M. Sanders, *Chem. Commun.* **2005**, 1959–1961; f) P. T. Corbett, L. H. Tong, J. K. M. Sanders, S. Otto, *J. Am. Chem. Soc.* **2005**, *127*, 8902–8903; g) P. T. Corbett, J. K. M. Sanders, S. Otto, *J. Am. Chem. Soc.* **2005**, *127*, 9390–9392; h) L. Vial, J. K. M. Sanders, S. Otto, *New J. Chem.* **2005**, *29*, 1001–1003; i) K. R. West, K. D. Bake, S. Otto, *Org. Lett.* **2005**, *7*, 2615–2618; j) R. Perez-Fernandez, M. Pittelkow, A. M. Belenguer, J. K. M. Sanders, *Chem. Commun.* **2008**, 1738–1740.

[7] J. T. Davis, *Angew. Chem.* **2004**, *116*, 684–716; *Angew. Chem. Int. Ed.* **2004**, *43*, 668–698.

[8] a) A. M. Burger, *Cancer Lett.* **2007**, *245*, 11–21; b) D. J. Patel, A. T. Phan, V. Kuryavyi, *Nucleic Acid Res.* **2007**, *35*, 7429–7455.

[9] J. L. Huppert, S. Balasubramanian, *Nucleic Acids Res.* **2007**, *35*, 406–413.

[10] D. Monchaud, M. P. Teulade-Fichou, *Org. Biomol. Chem.* **2008**, *6*, 627–636.

[11] M. Read, R. J. Harrison, B. Romagnoli, F. A. Tanious, S. H. Gowan, A. P. Reszka, W. D. Wilson, L. R. Kelland, S. Neidle, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 4844–4849.

[12] G. Pennarun, C. Granotier, L. R. Gauthier, D. Gomez, F. Hoffschir, E. Mandine, J. F. Riou, J. L. Mergny, P. Mailliet, F. D. Boussin, *Oncogene* **2005**, *24*, 2917–2928.

[13] B. de Bruin, P. Hauwert, J. N. H. Reek, *Angew. Chem.* **2006**, *118*, 2726–2729; *Angew. Chem. Int. Ed.* **2006**, *45*, 2660–2663.

[14] See Supporting Information.

[15] The CD curves of all complexes retain G-quadruplex characteristics, however, curves for **Ac, Aa**, and in particular $\mathbf{Ca_3}$ suggest partial conversion from the K^+ induced hybrid to parallel folding. See: A. Ambrus, D. Chen, J. Dai, T. Bialis, R. A. Jones, D. Yang, *Nucleic Acids Res.* **2006**, *34*, 2723–2735.

Received: June 7, 2008
Published online: September 12, 2008

 © 2008 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim