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Target-induced selection of ligands from a dynamic combinatorial library of mono- and bi-conjugated oligonucleotides

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Abstract—A dynamic library of 15 mono- and bi-conjugated oligonucleotides was generated from a pool of three aldehydes and an oligonucleotide bearing two reactive amino groups. Addition of complementary target to the equilibrating mixture of imines resulted in selective amplification of one conjugate. UV-melting experiments confirmed that it was the best ligand among those that were tested. This study emphasizes that dynamic combinatorial chemistry can be used to simultaneously identify the type and the location of appended residues for stabilizing oligonucleotide complexes.

1. Introduction

Oligonucleotide ligands are becoming increasingly interesting for their use as nucleic acid-targeting drugs, probes in molecular diagnosis, molecular biology tools and in various biotech applications.[1–4] Whereas natural DNA or RNA oligonucleotides, either rationally designed or identified through in vitro selection, represent lead compounds, there is generally a need for the optimization of their properties (affinity, specificity, nuclease resistance, cellular permeability). In this goal, attachment of non-nucleic acid groups at the carbohydrate moiety of nucleotides represents an attractive way.[5–7] Design of such modifications is not trivial. It requires structural data and molecular modeling experiments that are difficult and time-consuming. So, the development of combinatorial methods allowing for the rapid synthesis and screening of large populations of conjugated oligonucleotides is a challenging task,[8,9] in which dynamic combinatorial chemistry (DCC) may offer a complementary route.

DCC has attracted increasing interest over recent years as an alternative approach to traditional combinatorial chemistry (CC) that combines in a single step the library generation and screening processes.[10,11] The principle difference between DCC and CC is that DCC involves the use of reversible reactions to generate an equilibrating mixture of molecules, that is, a dynamic combinatorial library (DCL). In contrast to the static character of a traditional combinatorial library based on irreversible connections between building blocks, a DCL is able to respond to a change in its environment. Molecular recognition events due to the addition of a target molecule would induce a shift in the equilibrium towards the preferential formation of the compounds that bind to the target. Thus, DCC offers in situ screening of the combinatorial library simply by comparing its composition in absence or in presence of the target. Since its formulation in the mid 1990s, DCC selection experiments have been performed by using various biological target molecules,[12] including nucleic acids.[13–16] We have recently established that DCC can be used to identify covalently appended small molecules that stabilize oligonucleotide complexes.[17]

Our initial study showed that reversible exchange between imines formed from a 2′-amino-nucleotide, incorporated into a oligonucleotide ligand, and small set of aldehydes is influenced by the presence of a nucleic acid target, which promotes increased formation of the strongest binding conjugated products. This study was performed both in the context of a DNA duplex and a RNA–RNA kissing complex. In each case, after reduction of the imino link, a chemically stable conjugated oligonucleotide ligand with an increased affinity for this

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target was identified. Here we report an extension of this work, which illustrates further the potency of DCC in the field of oligonucleotide ligands. We demonstrated that in addition to the selection of the best-fitted appended residue, this methodology simultaneously allows the selection of the best-fitted appending site within the oligonucleotide ligand.

2. Results and discussion

For this study, the oligonucleotide ligand was a 10-mer oligoribonucleotide (**2**, Fig. 1) incorporating two 2′-deoxy-2′-aminouridines (U*) in positions 3 and 9.[18] This reactive ligand was allowed to reversibly react with a set of three aldehydes (4-[3-(dimethylamino)propoxy]-benzaldehyde hydrochloride (**3**), nalidixic aldehyde (**4**), 3-hydroxy-4-methoxybenzaldehyde (**5**), Fig. 1) both in absence and in presence of the complementary oligoribonucleotide **1** (Fig. 1) as the target. In these conditions, a DCL of 15 different imines (six mono-conjugated and nine bi-conjugated products) was potentially generated and screened for affinity to the target. Reactions were carried out in the presence of sodium cyanoborohydride ($NaBH_3CN$) and selective imine reduction provided the corresponding mixtures of stable amine analogs, which were subsequently analyzed by RP-HPLC.[†] The results obtained from this experiment are presented in Figure 2, which shows the HPLC profiles of conjugated oligonucleotide mixtures generated in absence (Fig. 2A) or in presence (Fig. 2B) of the complementary target. In each case, 15 peaks were merely detected, which correspond to the expected 15 products.[‡] Comparison of the two chromatograms clearly indicated that one product (peak *a*, Fig. 2) was significantly overproduced (+105%) in the presence of the target.[§] In accordance with the DCC principle, and as we previously observed,[17] this amplification effect occurred at the expense of the other products.

MALDI-TOF mass spectrometry (MS) analysis of the amplified product (peak *a*, Fig. 2, m/z = 3358.7) revealed a mass increase of 199.4, as compared with **2** (m/z = 3159.3). Such an increase can be unambiguously assigned to the covalent attachment of one nalidixic residue (calculated mass increases for a mono-conjugation with **3**, **4** and **5** are 191.3, 200.3 and 136.2, respectively). This suggests that among the three aldehydes, only one, namely nalidixic aldehyde (**4**), was able to yield an imino conjugated oligonucleotide that stabilizes the duplex

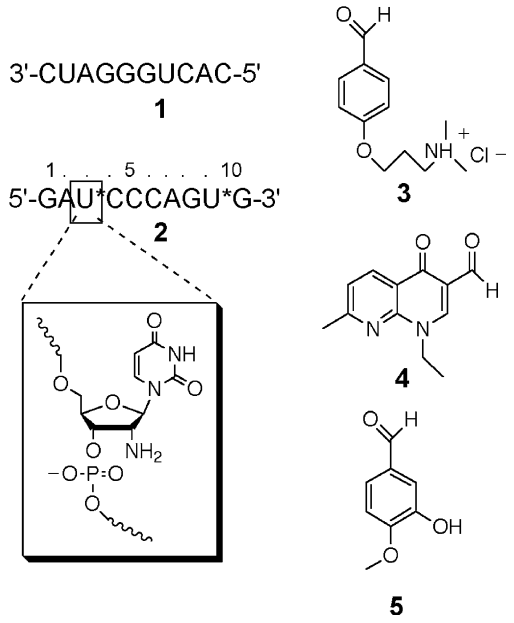

Figure 1. Structures of reacting aldehydes (**3**–**5**) that were screened by DCC for stabilizing a RNA duplex formed between target oligoribonucleotide **1** and complementary **2** incorporating 2′-amino-2′-deoxyuridines in positions 3 and 9.

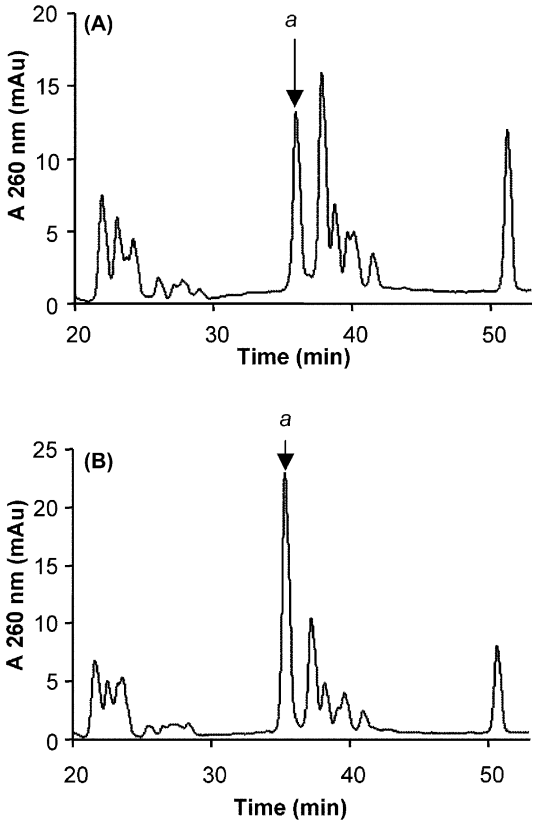

Figure 2. RP-HPLC profiles of the mixture of products formed between **2** and the set of aldehydes (**3**–**5**, Fig. 1) in absence (A) or in presence (B) of RNA target **1**. Peak *a* corresponds to target-amplified product. HPLC conditions are given in supporting information.

formed with complementary **1**. Indeed, when the previous experiment was repeated with only nalidixic alde-

[†] Compounds **3** (1 mM), **4** (0.2 mM) and **5** (1.2 mM) were incubated with **2** (0.05 mM) at room temperature (~22 °C) in a 20 mM phosphate buffer (pH 6.0) containing 20 mM NaCl, 140 mM KCl, 3 mM $MgCl_2$ and 2.5 mM $NaBH_3CN$. Reactions were carried out in absence or in presence of **1** (0.05 mM) for 20 h. Prior RP-HPLC analysis, reaction mixtures were dialyzed (Slide-A-Lyzer Mini Dialysis Units, 3500-MW cutoff, Pierce), in 3 L of water (MilliQ grade) for ~16 h, in order to remove free aldehydes that could overlap with peak products.

[‡] This was confirmed by HPLC analysis of products formed by reacting **2** with different mixtures of two out of the three aldehydes **3**–**5**.

[§] Amplification percentages were calculated from HPLC peak areas.

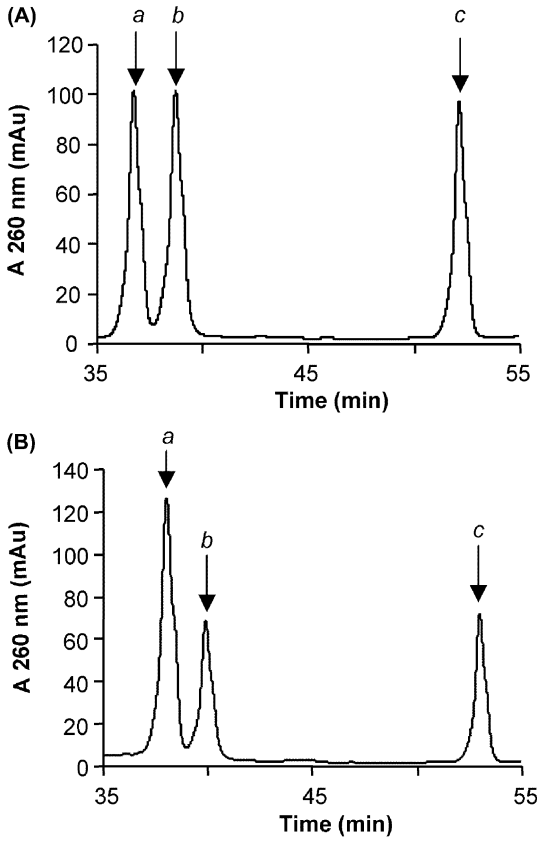

Figure 3. RP-HPLC profiles of the mixture of products formed between reactive oligonucleotide ligand **2** and nalidixic aldehyde (**4**) in absence (A) or in presence (B) of RNA target **1**. HPLC conditions were identical to those used in Figure 2. Peak *a* coelutes with peak *a* in Figure 2 and corresponds to product **6** (Fig. 4). Peaks *b* and *c* correspond to compounds **7** and **8**, respectively (Fig. 4).

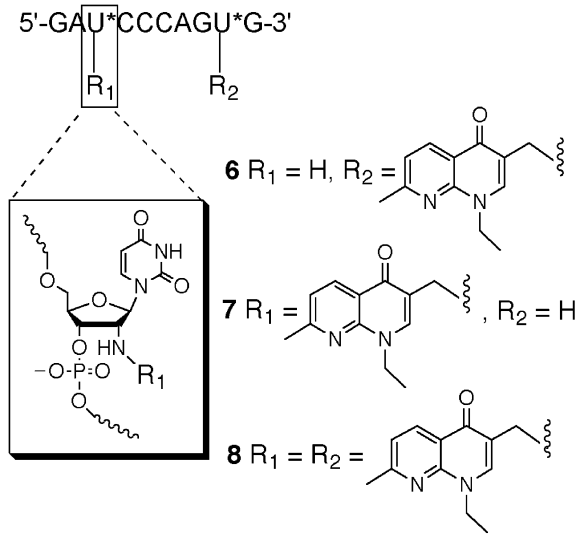

Figure 4. Structures of products formed during the reaction between oligonucleotide **1** and nalidixic aldehyde (**4**) in presence of $NaBH_3CN$.

Table 1. Melting temperatures (T_m) of RNA duplexes and amplification effects observed in the DCC experiment carried out with **4**

Duplex	T_m (°C)[a]	ΔT_m (°C)	Amplification (%)
1 + **2**	52.7		
1 + **6**	54.3	+1.6	+52
1 + **7**	51.4	−1.3	−30
1 + **8**	52.7	0	−22

[a] T_m values were assessed in 20 mM phosphate buffer (pH 6.0) containing 140 mM KCl, 20 mM NaCl and 3 mM $MgCl_2$, at 260 nm, and 1 μM duplex concentration. Standard deviation did not exceed ±0.5 °C.

hyde, the same mono-conjugated product (peak *a*, Fig. 3) was amplified (+52%) at the expense of the other mono-conjugated (peak *b*, Fig. 3, −30%, *m/z* = 3357.8) and the bi-conjugated (peak *c*, Fig. 3, −22%, *m/z* = 3557.9) products.

Then, the position of nalidixic residue within the sequence of amplified mono-conjugated product was determined after time dependent snake venom phosphodiesterase (SVP, 3′-exonuclease) digestion of a HPLC-purified sample and subsequent analysis of the resulting mixture of fragments by MALDI-TOF MS (see supporting information).[19] An increased resistance to SVP digestion of this product, as compared to the unconjugated oligonucleotide **2**, was observed. Whereas the latter compound was extensively digested after 5 min reaction, MS spectrum of the digestion mixture of amplified nalidixic mono-conjugate, obtained under the same conditions, still showed intense peaks corresponding to the intact product (*m/z* = 3358.7, $[M-H]^-$ and *m/z* = 1678.7, $[M-2H]^{2-}$), and to the fragment resulting from the removal of the first 3′-residue, (*m/z* = 3012.8, $[(M-G)-H]^-$ and *m/z* = 1506.1, $[(M-G)-2H]^{2-}$). These results suggested that the nalidixic residue is close to the 3′-terminus where it hinders the 3′-exonuclease activity of SVP. This was confirmed by a 505.5 mass unit difference between peak fragments $[(M-G)-H]^-$ and $[(M-UG)-H]^-$ (*m/z* = 2507.3), thus allowing to definitely characterize the amplified mono-conjugated product as **6** (Fig. 4).

Next, to verify that the selection occurring during the DCC process was related to the thermodynamic stabilities of duplexes formed, UV-monitored melting experiments were carried out. The melting temperature (T_m) values obtained (Table 1) shows that the binding properties of nalidixic-conjugated oligonucleotides **6**, **7** and **8** (Fig. 4) correlates well with the ranking order of amplification effects observed in the DCC experiment reported in Figure 3. In the light of T_m values, it turns out that only nalidixic group at position 3 has a favourable duplex stabilizing effect. Interestingly, the duplex formed between bi-conjugated product **8** and **1** presents the same stability as the duplex **1**+**2**. This may be interpreted as a consequence of additive effects of nalidixic residues at positions 3 and 9. These results suggest that the duplex stabilizing effect of nalidixic group is not unspecific, but rather occurs in a sequence-dependent way.

3. Conclusion

In conclusion, the results of this study emphasize that DCC is a powerful methodology allowing to

simultaneously identify the most efficient appended groups and the most appropriate appending sites for the formation of a stable oligonucleotide duplex. Thus, we also propose this methodology to identify the site where a reporter group of interest (a cleaving or a fluorescent agent) could be introduced in an oligonucleotide ligand without disrupting its binding properties. We are currently exploring the implementation of such a dynamic process in the selection of modifications that could optimize binding properties of in vitro selected aptamers.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2004.11.110.

References and notes

1. Crooke, S. T. *Annu. Rev. Med.* **2004**, *55*, 61–95.
2. Sun, J. S.; Helene, C. *Nucleos. Nucleot. Nucl.* **2003**, *22*, 489–505.
3. Toulmé, J. J.; Di Primo, C.; Moreau, S. *Prog. Nucleic Acid Res. Mol. Biol.* **2001**, *69*, 1–46.
4. Osborne, S. E.; Matsumura, I.; Ellington, A. D. *Curr. Opin. Chem. Biol.* **1997**, *1*, 5–9.
5. Gait, M. J. *Cell Mol. Life Sci.* **2003**, *60*, 844–853.
6. Manoharan, M. *Antisense Nucleic Acid Drug Dev.* **2002**, *12*, 103–128.
7. Gait, M. J. *Curr. Opin. Biotechnol.* **1991**, *2*, 61–68.
8. Altman, R. K.; Schwope, I.; Sarracino, D. A.; Tetzlaff, C. N.; Bleczinski, C. F.; Richert, C. *J. Comb. Chem.* **1999**, *1*, 493–508.
9. Mokhir, A. A.; Tetzlaff, C. N.; Herzberger, S.; Mosbacher, A.; Richert, C. *J. Comb. Chem.* **2001**, *3*, 374–386.
10. Lehn, J. M. *Chem. Eur. J.* **1999**, *9*, 2455–2463.
11. Rowan, S. J.; Cantrill, S. J.; Cousins, G. R.; Sanders, J. K.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 898–952.
12. Ramstrom, O.; Lehn, J. M. *Nat. Rev. Drug Discovery* **2002**, *1*, 26–36.
13. Klekota, B.; Hammond, M. H.; Miller, B. L. *Tetrahedron Lett.* **1997**, *38*, 8639–8642.
14. Klekota, B.; Miller, B. L. *Tetrahedron* **1999**, *55*, 11687–11697.
15. Karan, C.; Miller, B. L. *J. Am. Chem. Soc.* **2001**, *123*, 7455–7456.
16. Whitney, A. M.; Ladame, S.; Balasubramanian, S. *Angew. Chem., Int. Ed.* **2004**, *43*, 1143–1146.
17. Bugaut, A.; Toulmé, J. J.; Rayner, B. *Angew. Chem., Int. Ed.* **2004**, *43*, 3144–3147.
18. 5′-*O*-(4,4′-Dimethoxytrityl)-2′-trifluoroacetamido-2′-deoxyuridine was synthesized as previously reported: McGee, D. P.; Vaughn-Settle, A.; Vargeese, C.; Zhai, Y. *J. Org. Chem.* **1996**, *61*, 781–785, and converted into β-cyanoethylphosphoramidite according to: Mc Bride, L. J.; Caruthers, M. H. *Tetrahedron Lett.* **1983**, *24*, 245–248.
19. Pieles, U.; Zurcher, W.; Schar, M.; Moser, H. E. *Nucleic Acids Res.* **1993**, *21*, 3191–3196.