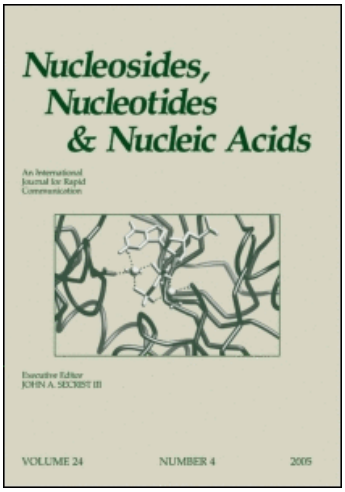


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Ring-Closing Metathesis Reactions in Nucleic Acid Chemistry—Cyclic Dinucleotides for Targeting Secondary Nucleic Acid Structures

Poul Nielsen[a]; Philip Børsting[a]
[a] Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, Odense M, Denmark

To cite this Article Nielsen, Poul and Børsting, Philip(2005) 'Ring-Closing Metathesis Reactions in Nucleic Acid Chemistry—Cyclic Dinucleotides for Targeting Secondary Nucleic Acid Structures', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 349 — 352
To link to this Article: DOI: 10.1081/NCN-200059776
URL: http://dx.doi.org/10.1081/NCN-200059776

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):349–352, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200059776

Taylor & Francis
Taylor & Francis Group

RING-CLOSING METATHESIS REACTIONS IN NUCLEIC ACID CHEMISTRY—CYCLIC DINUCLEOTIDES FOR TARGETING SECONDARY NUCLEIC ACID STRUCTURES

Poul Nielsen and Philip Børsting ▫ *Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, Odense M, Denmark*

▫ *Cyclic dinucleotides are synthesized using a ring-closing metathesis protocol and incorporated into oligonucleotides. A stabilization of a three-way junction is observed by an oligodeoxynucleotide containing a central 2′-C to 3′-phosphate connection.*

INTRODUCTION

The application of olefin metathesis technology is one the most important recent breakthroughs in synthetic organic chemistry and extremely useful for the construction of medium to large rings.[1–3] We have recently applied ring-closing metathesis (RCM) technology in nucleic acid chemistry for the construction of conformationally restricted cyclic di- and trinucleotides and hereby introduced a general strategy for constructing restricted nucleic acid fragments.[4–9] This strategy is summarized in Figure 1.

By the incorporation of the cyclic dinucleotides into oligonucleotides, an artificial bending of the standard nucleic acid structure and hereby a preorganization for the formation of other secondary nucleic acid structure than duplexes and triplexes is envisioned. A general tool for modeling and targeting different secondary structures is hereby introduced. Several nucleoside building blocks all containing terminal double bonds have been prepared and investigated, and from these a series of dinucleotides containing two terminal double bonds in various positions have been prepared and studied as substrates for RCM reactions.[4–9] Recently, we have focused on introducing dinucleotides with different linkages between the 2′-position and the subsequent 3′-phosphate.[10] In this line, the most

The Nucleic Acid Center is funded by the Danish National Research Foundation for the studies on nucleic acid chemical biology.

Address correspondence to Poul Nielsen, Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, Odense M DK-5230, Denmark.

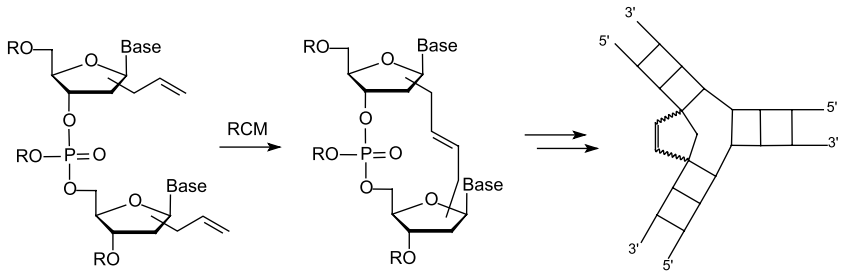
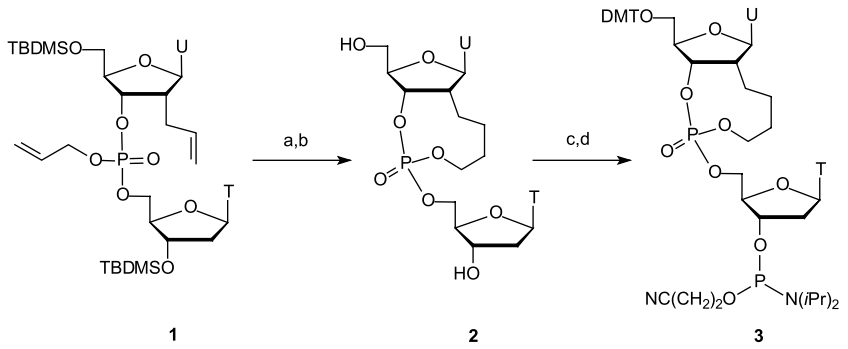


FIGURE 1 The general strategy: a) dinucleotides are made from nucleoside building blocks decorated with terminal double bonds; b) cyclizations by RCM reactions afford cyclic dinucleotides with all-carbon linkages; and c) incorporation of these dinucleotides into oligonucleotides might result in stabilised secondary structures like a three-way junction.

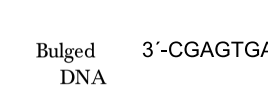
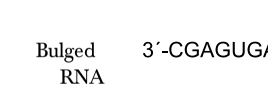
efficient RCM reaction was obtained on a dinucleotide substrate **1** prepared from a 2′-*C*-allyl-2′-deoxyuridine derivative (Scheme 1).[10] By the application of a tandem RCM-hydrogenation strategy,[7,11] followed by separation into the pure phosphorus epimers and deprotection, the cyclic dinucleotide **2** was obtained in a good overall isolated yield of the major epimer of 48%. The phosphorus configuration of the two epimers was not determined, but the major isomer was converted to the appropriate phosphoramidite **3** and incorporated successfully into oligonucleotides. No cleavage of the phosphortriester moiety during the included treatment with ammonia was observed. However, the minor phosphorus epimer of **2** was also converted to a phosphoramidite but the subsequent incorporation into oligonucleotides disclosed after MALDI-MS analysis a significant amount of an ammonia adduct.

The cyclic dinucleotide **2** (major isomer) was incorporated in the middle of a standard DNA sequence as well as in a corresponding DNA/LNA mixmer, and the hybridization of these sequences with different DNA and RNA complements was examined (Table 1). The DNA/LNA mixmer is expected to induce overall A-type duplex formation.[12] As expected, the incorporation of **2** results in a significant



SCHEME 1 a) see Steffansen et al.;[10] b) chromatographic separation *then* 90% aq. TFA, 100%; c) DMTCl, 2,6-lutidine, DMSO, 64%; d) $NC(CH_2)_2OP(N(iPr)_2)_2$, dicyanoimidazole, CH_3CN, 81%.

TABLE 1 Hybridization Data of Oligonucleotides Containing **2** (Major Isomer)

Target sequences		Investigated oligonucleotides			
		5′-GCTCACTTCTCCCA	5′-GCTCA**C2**CTCCCA	5′-GC^{L}TC^{L}AC^{L}TTC^{L}TC^{L}CC^{L}A	5′-GC^{L}TC^{L}AC^{L}**2**C^{L}TC^{L}CC^{L}A
DNA	3′-CGAGTGAAGAGGGT-5′	54.1	39.1 (−15.0)	76.3	61.7 (−14.6)
RNA	3′-CGAGUGAAGAGGGU-5′	60.2	47.9 (−12.3)	>90	79.3 (≤11)
Bulged DNA		42.1	36.1 (−6.0)	67.5	61.2 (−6.3)
Bulged RNA		49.7	44.8 (−4.9)	82.6	76.0 (−6.6)
Stem-loop DNA		26.4	27.1 (+0.7)	52.2	51.9 (−0.3)
Stem-loop RNA		36.8	39.0 (+2.2)	71.3	70.8 (−0.5)

All values are T_m values/°C measured in a medium salt buffer (Na_2HPO_4 [15 mM], NaCl [100 mM], EDTA [0.1 mM], pH 7.0) using 1.0 μM concentrations of each strand. ΔT_ms are given in brackets. C^{L} is the LNA-5-methylcytidine monomer.

destabilization of all standard duplexes (A- or B-type, DNA:DNA or DNA:RNA, ΔT_m −11 to −15°C). Toward bulged DNA and RNA complements (i.e., incorporation of a G in between the two As opposite the central dinucleotide), a less pronounced drop in affinity for the modified oligomers compared to the standard sequences was observed (ΔT_m −4.9 to −6.6°C). When the target was an RNA stem-loop sequence, an increase in affinity (ΔT_m +2.2°C) was observed for the standard ODN sequence containing the cyclic moiety. In the LNA-modified sequences and/or with a DNA stem-loop complement, no significant influence of the cyclic structure was observed (ΔT_m −0.5 to +0.7°C).

In summary, a small but clear thermal stabilization of a three-way junction has been observed. This stabilization was evident also with increased (Mg^{2+}), which leads to a general stabilization of the secondary structure (data not shown). This result demonstrates the potential of our general strategy toward stabilization and targeting of secondary nucleic acid structures by the RCM-based synthesis of conformationally restricted cyclic dinucleotides and corresponding oligonucleotides. We expect this strategy in combination with intense modeling to be a generally useful tool in nucleic acid–based chemical biology, structure- and function-relation studies, diagnostics, and therapeutics.

REFERENCES

1. Fürstner, A. Olefin metathesis and beyond. Angew. Chem., Int. Ed. **2000**, *39*, 3012–3043.
2. Trnka, T.M.; Grubbs, R.H. The development of L_2X_2Ru = CHR olefin metathesis catalysts: an organometallic success story. Acc. Chem. Res. **2001**, *34*, 18–29.
3. McReynolds, M.D.; Dougherty, J.M.; Hanson, P.R. Synthesis of phosphorus and sulfur heterocycles via ring-closing olefin metathesis. Chem. Rev. **2004**, *104*, 2239–2258.
4. Sørensen, A.M.; Nielsen, P. Synthesis of conformationally restricted dinucleotides by ring closing metathesis. Org. Lett. **2000**, *2*, 4217–4219.
5. Sørensen, A.M.; Nielsen, K.E.; Vogg, B.; Jacobsen, J.P.; Nielsen, P. Synthesis and NMR-studies of dinucleotides with conformationally restricted cyclic phosphotriester linkages. Tetrahedron **2001**, *57*, 10191–10201.
6. Børsting, P.; Sørensen, A.M.; Nielsen, P. A ring-closing metathesis strategy towards conformationally restricted di- and trinucleotides. Synthesis **2002**, *6*, 797–801.
7. Børsting, P.; Nielsen, P. Chem. Commun. **2002**, 2140–2141.
8. Kirchhoff, C.; Nielsen, P. Dinucleotides of 4′-*C*-vinyl- and 5′-*C*-allylthymidine as substrates for ring-closing metathesis reactions. Tetrahedron Lett. **2003**, *44*, 6475–6478.
9. Børsting, P.; Freitag, M.; Nielsen, P. Dinucleotides containing two allyl groups by combinations of allyl phosphortriesters, 5-allyl-, 2′-*O*-allyl- and 2′-arabino-*O*-allyl uridine derivatives as substrates for ring-closing metathesis. Tetrahedron **2004**, *60*, 10955–10966.
10. Steffansen, S.I.; Christensen, M.S.; Børsting, P.; Nielsen, P. Synthesis of conformationally restricted nucleic acid fragments using ring-closing alkene and enyne metathesis reactions. Adjacent proceeding in this issue of *Nucleosides Nucleotides and Nucleic Acids*.
11. Louie, J.; Bielawski, C.W.; Grubbs, R.H. Tandem catalysis: the sequential mediation of olefin metathesis, hydrogenation, and hydrogen transfer with single-component Ru complexes. J. Am. Chem. Soc. **2001**, *123*, 11312–11313.
12. Bondensgaard, K.; Petersen, M.; Singh, S.K.; Rajwanshi, V.K.; Kumar, R.; Wengel, J.; Jacobsen, J.P. Structural studies of LNA: RNA duplexes by NMR: conformations and implications for RNase H activity. Chem. Eur. J. **2000**, *6*, 2687–2695.