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# Synthesis of Conformationally Restricted Dinucleotides by Ring-Closing Metathesis

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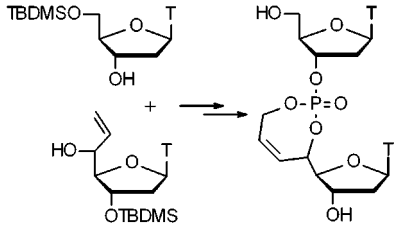
## ABSTRACT




**The ring-closing metathesis reaction has been used in the synthesis of conformationally restricted dinucleotides as well as a 3′-nucleotide analogue. From bis-allylic nucleoside phosphates obtained from simple nucleoside precursors, the synthesis of unsaturated cyclophosphates has been accomplished using either Grubbs' catalyst or an improved analogue. Hereby, the conformational freedom of the nucleic acid phosphordiester linkage has been efficiently constrained.**

Conformationally restricted oligonucleotides have been intensively investigated for their high-affinity nucleic acid recognition and potential therapeutic and biotechnological applications.[1] Thus, conformational restriction has been introduced with very promising results by, for example, the incorporation of nucleoside analogues with bi- and tricyclic carbohydrate moieties,[2] by 2′-modified ribonucleosides,[3,4] and by modifications in the natural phosphordiester linkage.[4,5] The introduction of constrained heterocycles as nonionic nucleotide linkages has been presented,[4,6] in some cases involving the furanose moiety in bicyclic ring systems.[4,7] However, the incorporation of conformational restriction of the intact phosphordiester linkage has not been explored. Hereby, we introduce a simple chemical methodology in the preparation of conformationally restricted oligonucleotides. Taking advantage of a ring-closing metathesis (RCM) reaction, a diastereomeric mixture of dinucleotides with the phosphordiester linkages constrained as uncharged phosphortriesters in a seven-membered unsaturated ring system has been synthesized.

The RCM synthetic method has recently been intensively investigated for its convenient and very general applications in the synthesis of medium and large ring systems.[8] Especially, the introduction of Grubbs' catalyst **1** (Table 1)

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**Table 1.** RCM Reactions on Mono- and Dinucleotides[a]


1 2

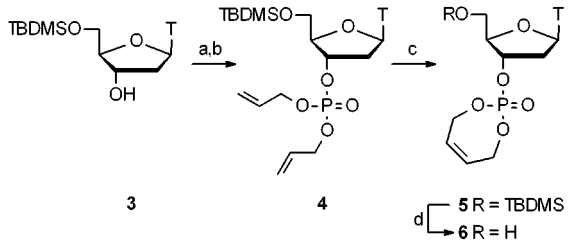
| | | | | |
|---|---|---|---|---|
| **4** → **5** | 52%[b] | 20 h | 97%[b] | 45 min |
| **10** → **11** | 65%[b,d] | 48 h | 91%[c] | 2 h |

[a] All RCM reactions (Schemes 1 and 2) performed in $CH_2Cl_2$ at 40 °C with substrate concentrations of 0.02 M; yields and reaction times given. [b] 10 mol % of catalyst. [c] 5 mol % of catalyst. [d] Yield estimated via $^{31}P$ NMR from a mixture of product and starting material isolated in approximately 80% yield. Cy = cyclohexyl. Mes = 2,4,6-trimethylphenyl (mesityl).

has enlarged the scope of this reaction and prompted us to introduce this method in the synthesis of nucleic acid derivatives. Thus, RCM reactions have been used in peptide chemistry, for example, for the synthesis of cyclic di- and oligopeptides[9] as well as for the construction of a short conformationally restricted α-helix model.[10] Very recently, the catalyst **2**[11] (Table 1) has shown improved efficiency and stability compared to **1** and comparable functional group tolerance.[8,11] The use of RCM reactions in the formation of phosphorus-containing heterocycles has also recently been presented including cyclic phosphonates.[12] However, this is to our best knowledge the first report on cyclic phosphortriesters produced by the RCM method.

To explore the general applicability of the RCM reaction on nucleotide substrates, we decided to synthesize a simple cyclic phosphortriester from a single nucleoside precursor. Thus, the protected thymidine derivative **3** was converted to the bis-allyl substituted 3′-nucleotide **4** (Scheme 1) using

**Scheme 1.** Synthesis of a 3′-Nucleotide Cyclophosphate[a]

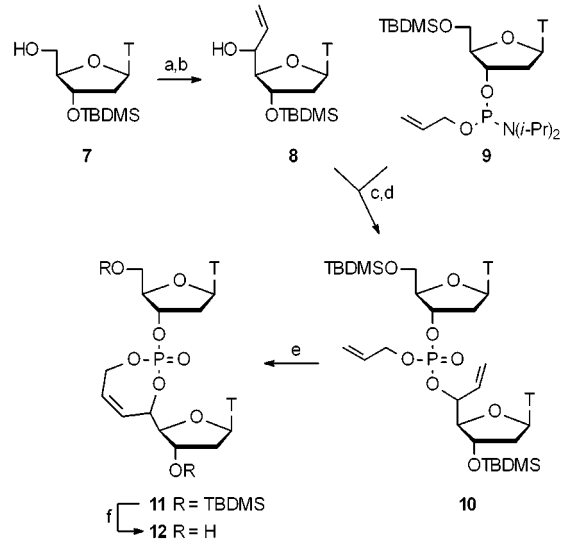



[a] Legend: (a) $(CH_2{=}CHCH_2O)_2PN(i\text{-}Pr)_2$, 1*H*-tetrazole, $(i\text{-}Pr)_2NH$, $CH_3CN$; (b) *t*-BuOOH, toluene, $CH_2Cl_2$, 80% (2 steps); (c) **1** or **2** (Table 1); (d) 90% TFA, 93%. T = thymin-1-yl.

a commercially available phosphoramidite reagent and well-known phosphoramidite technology[13] followed by a smooth oxidation of the phosphite triester intermediate. An RCM reaction on this substrate was approached using standard conditions with Grubbs' catalyst **1** in refluxing dichloromethane. This afforded the expected product **5** in a reasonable 52% yield after a relatively long reaction time (Table 1). The product was verified by MS and NMR.[14]

This result prompted us to investigate the further use of the RCM reaction in a nucleic acid context. To make a conformationally restricted dinucleotide with the smallest possible ring including an intact phosphortriester functionality, we chose a 5′-vinyl-substituted thymidine as a convenient nucleoside monomer. The protected thymidine derivative **7** (Scheme 2) was oxidized using the Dess−Martin periodinane[15] and used in a Grignard reaction affording, as expected, both diastereomers of **8** (in a ∼1:1 ratio) and in an acceptable 41% yield. All attempts to improve this by Cu(I) additives[16] failed. The use of $CeCl_3$ in connection with the Grignard reagent[17] improved the result marginally to a 43% yield but

**Scheme 2.** Synthesis of Cyclophosphate Dinucleotides[a]




[a] Legend: (a) Dess−Martin periodinane, $CH_2Cl_2$ 80%; (b) vinylMgBr, THF, 41%; (c) 1*H*-tetrazole, $(i\text{-}Pr)_2NH$, $CH_3CN$; (d) *t*-BuOOH, toluene, $CH_2Cl_2$, 87% (2 steps); (e) **1** or **2** (Table 1); (f) 90% TFA, 95%. T = thymin-1-yl.

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(14) Selected data for **4**−**6**. $^{31}P$ NMR $\delta_P$ ($CDCl_3$, 121.5 MHz with 85% $H_3PO_4$ as external standard): **4** −0.66; **5** 3.85; **6** ($CD_3OD$) 3.95. HR MALDI FT-MS *m*/*z* $[M - Na]^+$ (found/calcd): **4** (539.1940/539.1935); **5** (511.1633/511.1636).

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(16) Cu(I) salts have been used in combination with other Grignard reagents, e.g., allylMgBr and CuCN were used in the synthesis of 5′-allylthymidine derivatives from **7**: Wang, G.; Middleton, P. J. *Tetrahedron Lett.* **1996**, *37*, 2739.

(17) $CeCl_3$ has been successfully used in combination with several Grignard reagents including vinylMgBr, e.g.: Bender, S. L.; Moffett, K. K. *J. Org. Chem.* **1992**, *57*, 1646.

with a 1:1.3 diastereomeric ratio. Even though separation of the diastereomers should be possible, we chose to use the equimolar mixture of **8** in our first investigation. The known allyl phosphoramidite **9**[18] was synthesized from **3** and coupled with the mixture of alcohols **8** using standard phosphoramidite coupling conditions[13,18] to give a mixture of phosphite triesters. This was without purification oxidized to give the mixture of four diastereomeric dinucleotides **10** in a good yield and an equimolar ratio.

The RCM reaction with **10** as the substrate using **1** gave the expected product **11**, but in a relatively low yield and after a long reaction time (Table 1). However, the use of catalyst **2** improved this result significantly, giving product **11** in a very good (91%) yield and after a much shorter reaction time. The reaction could be conveniently followed by $^{31}$P NMR as the four diastereomers of **10** displayed four signals in the −0.8 to −2.0 ppm range whereas the corresponding four diasteromeric products **11** displayed four signals in the 1.7 to 2.8 ppm range.[19] This deshielding of the phosphorus atom was also observed in the synthesis of **5**[14] and could be deduced to the construction of a constrained ring. Furthermore, product **11** was confirmed by MS data[19] showing the expected loss of the mass of ethylene and $^1$H NMR showing the loss of terminal allyl groups. Acidic desilylation gave the unprotected dinucleotides **12**[19] in high yield, after a standard procedure using TBAF had failed. The efficiency of **2** as the best catalyst for these RCM reactions was further demonstrated with the first nucleotide substrate **4** giving the product **5** in a fast and very high yielding reaction (Table 1). A similar desilylation procedure afforded the unprotected 3′-nucleotide cyclophosphate **6** (Scheme 1).

The present results clearly demonstrate that the RCM reaction is perfectly compatible with the chemistry of nucleotide structures, and the technology has proved very useful in the construction of conformationally restricted nucleotide analogues. Thus, conformational restriction has been introduced into the phosphordiester linkage by a very simple and convenient method. The preparation of the four pure diasteromers of **12** and the introduction of these into oligonucleotides is in progress in our laboratory. We plan to incorporate these uncharged dimers at strategic positions in otherwise unmodified oligonucleotides using the conventional automatic solid-phase technology based on phosphoramidite chemistry.[13] However, we expect that alternative methods[20] have to be applied for the deprotection of these oligonucleotides in order to leave the unsaturated cyclophosphate moieties unaffected.

We expect the restriction in conformational freedom of the natural nucleic acid structure introduced by these constrained ring structures to allow the construction of oligonucleotide sequences with efficient recognition of complementary nucleic acid sequences. Thus, two relatively flexible bonds in the nucleic acid backbone are controlled by the ring structure. Furthermore, the olefinic moiety opens the possibility of simple and diverse functionalization. We also expect the present RCM methodology to apply for the synthesis of other conformationally restricted di- and oligonucleotides using solution as well as solid phase chemistry. Hence, this very simple strategy using easily available alkenic nucleoside building blocks (such as **8** and **9**) and subsequent metathesis reactions reveals general opportunities in reducing the flexibility of nucleic acids.

In conclusion, we have introduced the first ring-closing metathesis reaction in the construction of nucleotide derivatives. Hereby, the conformationally restricted dinucleotides **12** were synthesized by a short and convenient route. These are expected to reveal oligonucleotides with interesting applications in nucleic acid based technologies.

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**Supporting Information Available:** Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) The four diastereomers present in **10**, **11**, and **12** arise from the introduction of a (5′)-stereocenter and an asymmetric phosphorus atom. Selected data for **10**−**12**. $^{31}$P NMR $\delta_P$ ($CDCl_3$, 121.5 MHz with 85% $H_3PO_4$ as external standard): **10** −1.97, −1.46, −1.25, −0.85; **11** 1.80, 2.00, 2.42, 2.71; **12** ($CD_3OD$) 1.91, 1.91, 2.41, 2.80. HR MALDI FT-MS *m*/*z* $[M - Na]^+$ (found/calcd): **10** (863.3462/863.3454); **11** (835.3141/835.3141).

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