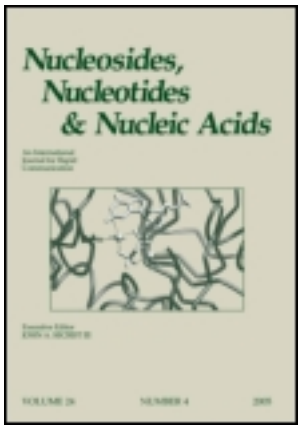

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▫ *In the aim of constructing conformationally restricted nucleic acid fragments for the recognition of secondary RNA structures, we have synthesized different mono- and dinucleotides containing extra rings. These rings were prepared by ring-closing alkene or enyne metathesis reactions from nucleotide substrates in which double or triple bonds have been introduced.*

INTRODUCTION

Long single stranded RNAs tend to fold into secondary structures. This makes the primary structure of RNA less accessible.[1] Secondary structures in RNA such as hairpins and loops are therefore targets for modified oligonucleotides. If an oligonucleotide has to bind, e.g., across a hairpin loop, a preorganization of the oligonuclotide to form the right bend would be preferable.[2] Recently, we have introduced the idea of using the ring-closing metathesis (RCM) reaction for the synthesis of conformationally restricted nucleic acid fragments targeting secondary nucleic acid structures.[3–8] In this study, we present our efforts toward cyclic dinucleotides with linkages between the 2′-position and the phosphate. Furthermore, we expand the scope of this strategy towards the first enyne metathesis reactions with nucleoside or nucleotide substrates. In general, we have applied Grubbs 2nd generation precatalyst **A**[9] (Scheme 1) in this study.

First, four different nucleoside building blocks were synthesized using uridine **1** as the starting material. Thus, a selective protection of the 3′-*O*- and 5′-*O*-positions by the TIPDS-group to give **2** was followed by oxidation of the 2′-position giving **3**. After a Grignard reaction leading to **4**, the appropriate deprotection and

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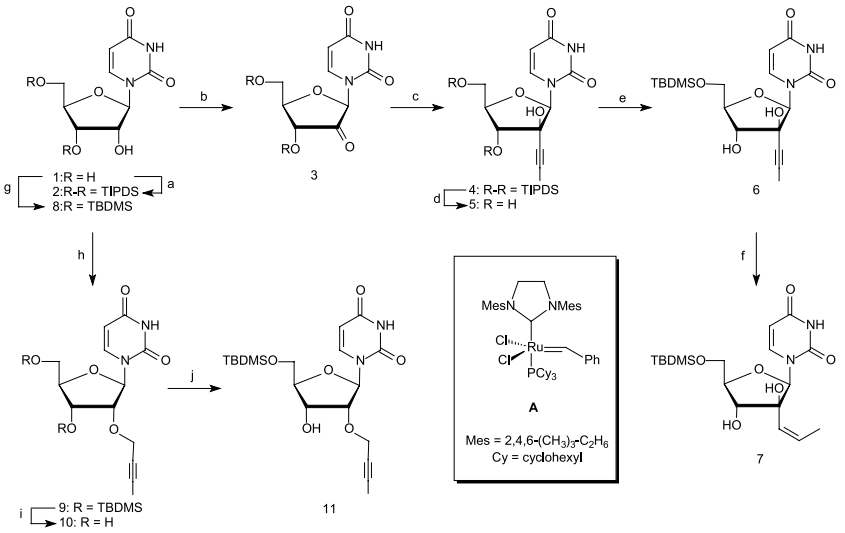
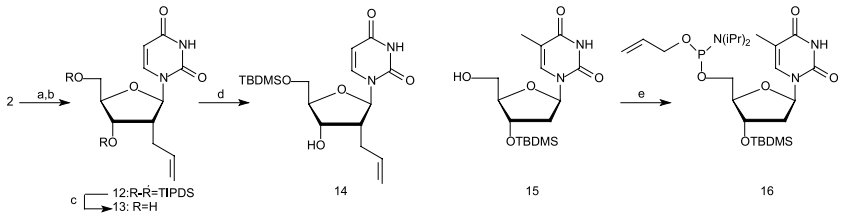


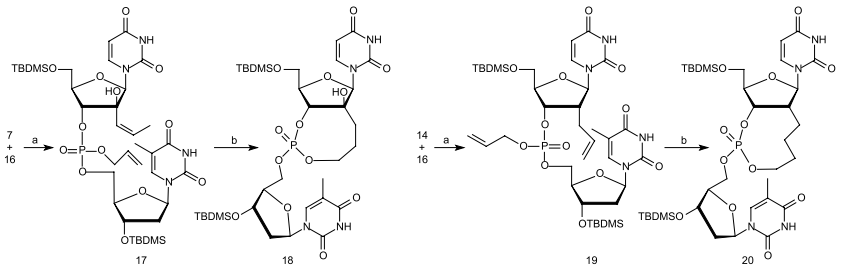
SCHEME 1 a) $TIPDSCl_2$, pyridine, 85%; b) CrO_3, Ac_2O, pyridine, CH_2Cl_2, 93%; c) propynyl-MgBr, $CeCl_3$, THF, 69%; d) TBAF, THF, 86%; e) TBDMSCl, imidazole, DMF, 70%; f) H_2, Lindlar Cat., quinoline, MeOH, 95%; g) TBDMSCl, DABCO, $AgNO_3$, THF; 79%; h) 1-bromo-2-butyne, NaH, THF, 75%; i) TBAF, THF, 84%; j) TBDMSCl, imidazole, DMF, 55%.

reprotection reactions gave the first monomer **6**, which, after controlled reduction of the triple bond, gave the second monomer **7** (Scheme 1). The TIPDS-protection proved to be problematic when used in an alkylation reaction leading to partial hydrolysis of the TIPDS-group. Therefore, the TBDMS-group was introduced for selective 3′-*O*- and 5′-*O*-protection,[10] and a subsequent 2′-*O*-alkylation gave the intermediate **9**. After deprotection and reprotection, the third monomer **11** was obtained.

A well-known radical-reaction[11] was used for synthesizing the last monomer (Scheme 2). Again, **2** was used as the starting material and converted in two steps to the 2′-*C*-allyl-2′-deoxy derivative **12**. Deprotection and reprotecton afforded the monomer **14**. The phosphoramidite **16** was prepared from 3′-*O*-TBDMS-thymidine **15** as a building block for incorporating the allyl group via the internucleotide phosphate.



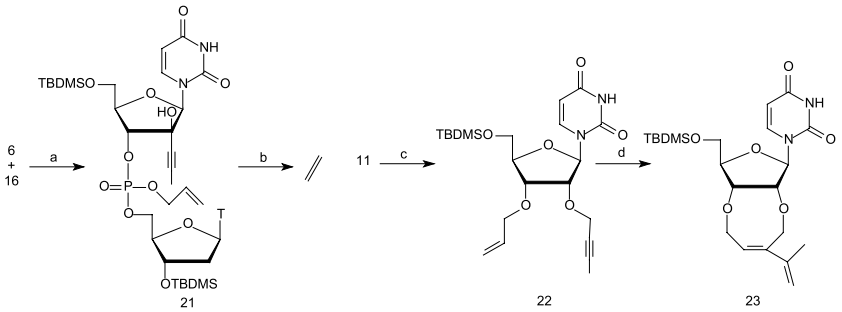
SCHEME 2 a) PhOCSCl, pyridine, CH_2Cl_2, 74%; b) allylSnBu$_3$, AIBN, toluene, 72%; c) TBAF, THF *then* Amberlite IR-120, pyridine/MeOH/H_2O, 90%; d) TBDMSCl, $AgNO_3$, pyridine, 85%; e) CH_2 = $CHCH_2OP(N(iPr))_2$, dicyanoimidazole, CH_2Cl_2, 63%.



SCHEME 3 a) 1H-tetrazole, CH_3CN *then* ^tBuOOH, **17** 65%, **19** 85%; b) **A**, CH_2Cl_2 *then* 70 atm H_2, **18** 11%, **20** 65%.

Monomer **7** and **14** were both coupled to the phosphoramidite **16** and the resulting dimers **17** and **19** were subjected to a tandem RCM/hydrogenation procedure leading to the saturated products **18** and **20** in a low and good yield, respectively (Scheme 3). The latter was subsequently separated to give the two pure phosphorus epimers, which were deprotected and incorporated into oligonucleotides.[12] Finally, the monomer **6** was coupled to phosphoramidite **16** giving a dimer **21** as a substrate for enyne metathesis. However, under varying metathesis conditions the dimer did not react at all. Instead, an enyne-nucleoside model was synthesis by alkylating **11** with allylbromide giving the enyne **22**, which successfully was ring-closed to give the diene **23**. This nucleoside model possesses some of the qualities that have been experienced to positively contribute to ring formation, i.e., heteroatoms in the 1- and 4-positions of the formed ring.[13]

In summary, nucleosides with allyl or propenyl moieties in the 2′-positions have been successfully incorporated into dinucleotides being substrates in a tandem RCM/hydrogenation procedure. On the other hand a dinucleotide containing a 2′-*C*-propynyl moiety was not a substrate for an enyne metathesis reaction. In contrast, a 3′-*O*-allyl-2′-*O*-(2-butynyl)-nucleoside was successfully ring-closed, leaving hope for the use of enyne metathesis for the synthesis of conformationally restricted dinucleotides with a potential for further functionalization. We expect the present metathesis-based strategy for the preparation of conformationally restricted nucleic



SCHEME 4 a) Dicyanoimidazole, CH_2Cl_2, 52%; b) **A**, CH_2Cl_2; c) CH_2 = $CHCH_2Br$, NaH, THF, 38%; d) **A**, CH_2Cl_2, 71%.

acid fragments to be a general future tool for modeling and targeting secondary nucleic acid structures (Scheme 4).

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