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Dinucleotides of 4′-*C*-vinyl- and 5′-*C*-allylthymidine as substrates for ring-closing metathesis reactions

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Abstract—Thymidine derivatives containing a 4′-*C*-vinyl group or a 5′-*C*-allyl group are synthesized and used as building blocks for three different dinucleotides. These are evaluated as substrates for ring-closing metathesis cyclisations, and a protected 5′-*C*-allylthymidine homo-dimer is found to be the most reactive. A protected precursor for a conformationally restricted cyclic dinucleotide with a four carbon 5′-*C* to 5′-*C* connection is hereby efficiently obtained, whereas a corresponding three carbon 4′-*C* to 5′-*C* connection is obtained in a lower yield.

The construction of conformationally restricted nucleic acid fragments is a powerful tool towards potential therapeutics and diagnostics.[1,2] Thus, oligonucleotides containing nucleoside monomers with restricted carbohydrate moieties have demonstrated high affinity for complementary nucleic acids.[2,3] On the other hand, conformationally restricted models of the secondary or tertiary structural elements found especially in RNA[4] has gained much less attention, though a few restricted dinucleotides have been synthesised and investigated,[5,6] e.g. as a model of the anticodon loop in a bacterial tRNA.[7]

We have recently applied the ring-closing metathesis (RCM) methodology[8] using the ruthenium based pre-catalyst **A** (Scheme 1) developed by Grubbs and co-workers[9] in the construction of conformationally restricted dinucleotide[10–12] and trinucleotide structures.[11] Thus, nucleoside monomers with an allyl substituent either at the 5-position of a uracil or at a 3′-*O*-phosphoramidite moiety,[11,12] or with a 5′-*C*-vinyl substituent[10] have been coupled to afford di- or trinucleotides. Subsequently, medium to large unsaturated rings have been formed by RCM in medium or high yields.[10–12] Nevertheless, the cyclic di- or trinucleotides formed involves phosphotriester internucleoside linkages[10–12] revealing two problems; (1) the chirality of the phosphorus leads to several isomeric products and (2) the allylic phosphotriester moiety demonstrates a high basic lability.[12] The latter problem, however, has been significantly reduced by saturation of the allylic moiety using a tandem RCM-hydrogenation protocol.[12]

In order to obtain even more stable and convenient molecules, cyclic dinucleotides with an intact, charged and achiral, phosphate internucleoside linkage were approached. Thus, we decided to perform dinucleotide couplings of nucleoside monomers on which terminal double bonds are placed on the 4′-*C* and/or on the 5′-*C* positions, and subsequently use these dinucleotides as substrates for RCM-reactions. Thus, a 4′-*C*-vinylthymidine derivative should be conveniently obtained,[13–15] and in contrary to 5′-*C*-vinylthymidine which we applied in a former study,[10] the 5′(*S*)-epimer of a 5′-*C*-allylthymidine derivative can be obtained by a completely stereoselective method.[16] Herein we present the preparation of the appropriately protected nucleoside monomers, their coupling into dinucleotides as well as the properties of these as substrates for RCM cyclisation reactions.

The 4′-*C*-hydroxymethyl thymidine derivative **1** was obtained from thymidine in five steps as published before.[17] Among the two primary hydroxyl groups the pro-(*S*) position has been shown to be the more reactive[17,18] and after tritylation of this, the other hydroxyl group was silylated to give after detritylation either **2**[19] or **3**[20] as the major products in reasonable yields (Scheme 1). Oxidation of the alcohol in **2** or **3** and subsequent Wittig methylenation[21] afforded the two 4′-*C*-vinyl thymidine derivatives **4** and **5**[14] in 51

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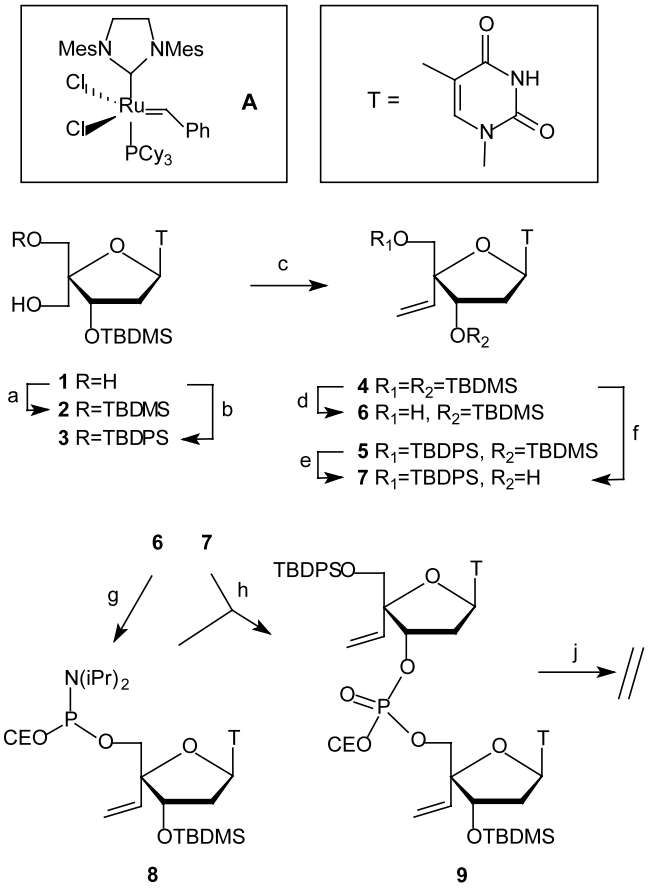
and 85% yield, respectively. The high yield of **5** compared to **4** might be ascribed to the higher basic stability of a primary TBDPS-ether.

In order to obtain the two appropriately mono-protected 4′-*C*-vinyl nucleosides, selective desilylations of **4** and **5** were approached. Thus, the primary silyl ether of the bis-TBDMS protected compound **4** was selectively hydrolysed under acidic conditions to give, conveniently, **6** in high yield. Also, the secondary silyl ether of **5** could be cleaved selectively applying TMS-triflate[22] to afford **7**. However, as the Wittig reaction leading to **5** was the more efficient, we attempted also to obtain the 3′-*O*-protected building block **6** from **5** using methods published to cleave selectively TBDPS-ethers over TBDMS-ethers.[23] Nevertheless, this conversion was not possible in our hands, and in conclusion, **6** was most conveniently produced via **4**. Finally, **7** was also obtained from **4** using full deprotection[24] and subsequent selective TBDPS-protection of the primary 5′-hydroxyl functionality.

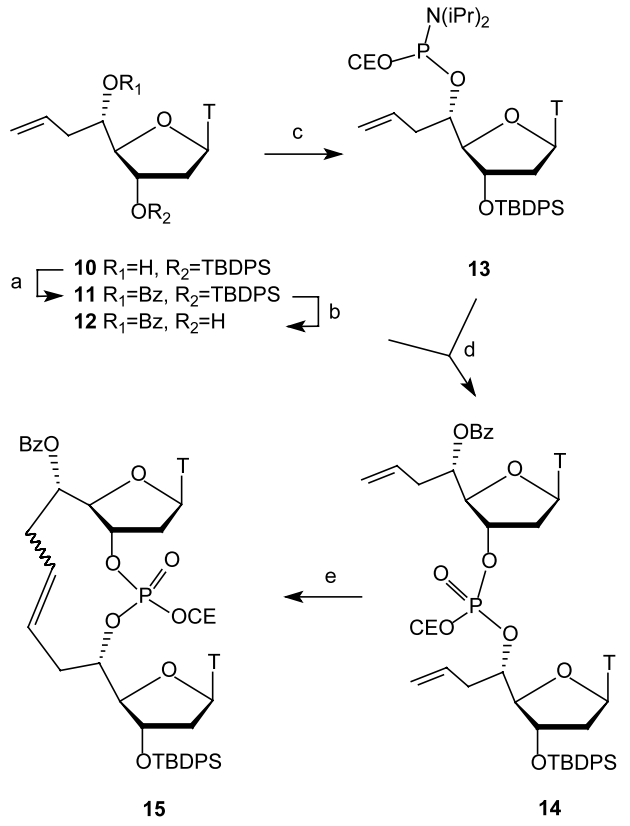
Phosphitylation of **6** using dicyanoimidazole as the activating reagent[25] gave in high yield the phosphoramidite **8** as a mixture of two epimers as judged from ^{31}P NMR. A standard nucleotide coupling reaction[26] of the alcohol **7** and the phosphoramidite **8** afforded the dinucleotide **9** after oxidation.[27] Due to the chiral phosphotriester moiety, the dinucleotide contains two epimers in almost equimolar yields as judged from ^{31}P NMR. Finally, **9** was investigated as a substrate for an RCM cyclisation. Thus, **9** was treated with 5 mol% of the precatalyst **A** in dichloromethane at 40°C. However, even though additional amounts of the precatalyst were added, no reaction was detected and after 48 h, the starting material **9**, completely unchanged as shown by MS and NMR, was almost quantitatively recovered. Similar attempts using solutions in dichloroethane at 80°C or in toluene at 100°C gave the same disappointing result. Steric hindrance of the vinyl moieties due to their 'neopentylic' positions might explain the low reactivity with the catalyst, and therefore, we turned our attention towards a 5′-*C*-substituted nucleoside analogue.

The introduction of a 5′-*C*-allyl moiety on thymidine derivatives has been accomplished from a 3′-*O*-protected 5′-aldehyde derivative by a conventional Grignard reaction affording both possible epimers[28] as well as by a completely stereoselective allylation using allyltrimethylsilane and $BF_3{\cdot}OEt_2$ as a Lewis acid catalyst.[16] We decided to follow the latter strategy and obtained the 5′(*S*)-isomer **10** in five steps from thymidine.[16] In order to obtain an appropriately 5′-*O*-protected derivative, **10** was esterified to give **11** which after conventional desilylation afforded the 5′-*O*-benzoyl protected compound **12** (Scheme 2).

Phosphitylation of **10** applying the same conditions as before with **6** afforded the phosphoramidite **13**, and subsequently, a standard nucleotide coupling of **12** and **13** followed by oxidation afforded the dinucleotide **14**.[27] This coupling reaction, however, was hampered by the low solubility of **12** in acetonitrile. In an appropriate mixture of acetonitrile and dichloromethane, the coupling reaction succeeded though never completely and **14** was isolated in 25% yield as an approximate 2:1 ratio of epimers as indicated from ^{31}P NMR. The dinucleotide **14** was finally subjected to 5 mol% of the precatalyst **A** in dichloromethane at 40°C, and this time a product was observed. After treatment with an additional amount of **A** the RCM cyclisation was completed and the product **15** isolated in good yield as a mixture of four isomeric products as clearly demonstrated from the ^{31}P NMR spectrum.[27] Thus, both *E*- and *Z*-isomers of both epimeric phosphotriesters were found, but the precise elucidation of the observed approx. 10:5:4:2 ratio was not performed. The RCM reaction did eventually succeed also with a lower 2×2 mol% loading of **A**.

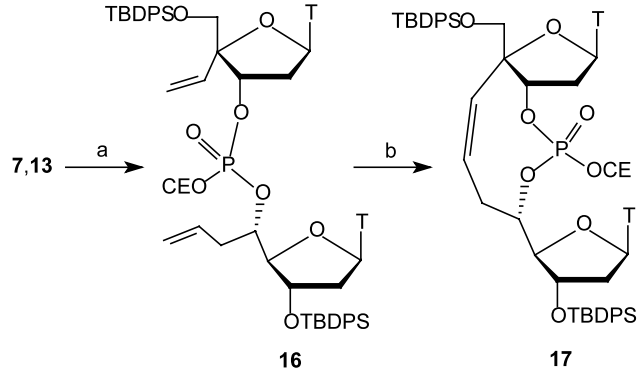


Scheme 1. *Reagents and conditions*: (a) (i) DMT-Cl, DMAP, pyr, rt, (ii) TBDMS-Cl, imidazole, DMF, rt, (iii) TFA, CH_2Cl_2, $CHCl_3$, Et_3SiH, rt, 55% (three steps); (b) (Ref. 20) (i) DMT-Cl, DMAP, pyr, rt, (ii) TBDPS-Cl, imidazole, DMF, rt, (iii) 80% aq. AcOH, THF, rt, 63% (three steps); (c) (i) Dess–Martin periodinane, CH_2Cl_2, rt, (ii) Ph_3PCH_3Br, *n*-BuLi, hexane, THF, 0°C, 51% **4** or 85% **5** (two steps); (d) 80% aq. AcOH, rt, 90%; (e) TMS-OTf, CH_2Cl_2, –30°C, 73%; (f) (i) 90% aq. TFA, rt, (ii) TBDPS-Cl, pyr, rt, 82% (two steps); (g) $(iPr_2N)_2PO(CH_2)_2CN$, 4,5-dicyanoimidazole, CH_2Cl_2, rt, 98%; (h) 1*H*-tetrazole, CH_3CN, rt, *then* *t*-BuOOH, toluene, 0°C, 68%; (j) A, *see text*, 0%. DMT = dimethoxytrityl, TBDMS = *tert*-butyldimethylsilyl, TBDPS = *tert*-butyldiphenylsilyl, CE = cyanoethyl, T = thymin-1-yl.



Scheme 2. *Reagents and conditions*: (a) BzCl, pyr, rt, 71%; (b) TBAF, THF, rt, 63%; (c) $(i\mathrm{Pr}_2\mathrm{N})_2\mathrm{PO}(\mathrm{CH}_2)_2\mathrm{CN}$, 4,5-dicyanoimidazole, $\mathrm{CH}_2\mathrm{Cl}_2$, rt, 77%; (d) 1*H*-tetrazole, $\mathrm{CH}_3\mathrm{CN}$, $\mathrm{CH}_2\mathrm{Cl}_2$, rt, *then* *t*-BuOOH, toluene, 0°C, 25%; (e) 4 mol% **A**, $\mathrm{CH}_2\mathrm{Cl}_2$, 40°C, 79%. TBDPS = *tert*-butyldiphenylsilyl, CE = cyanoethyl, T = thymin-1-yl.

In order to investigate the possibility of an RCM cyclisation of a mixed dinucleotide containing the 4′-*C*-vinyl nucleoside monomer, the alcohol **7** was coupled to the phosphoramidite **13** using the same reaction conditions as before (Scheme 3). The following dinucleotide **16** was easily obtained in high yield and an approximate 3:1 mixture of epimers.[27] The dinucleotide was



Scheme 3. *Reagents and conditions*: (a) 1*H*-tetrazole, $\mathrm{CH}_3\mathrm{CN}$, $\mathrm{CH}_2\mathrm{Cl}_2$, rt, *then* *t*-BuOOH, toluene, 0°C, 89%; (b) 15 mol% **A**, $\mathrm{ClCH}_2\mathrm{CH}_2\mathrm{Cl}$, 80°C, 38%. TBDPS = *tert*-butyldiphenylsilyl, CE = cyanoethyl, T = thymin-1-yl.

treated with 5 mol% of the precatalyst **A** in dichloromethane at 40°C but no reaction was detected. When the reaction was run in dichloroethane at 80°C with several consecutive additions of **A**, up to a total loading of 15 mol%, an RCM cyclisation did proceed to give after 5 days, and a chromatographic purification, 38% of the product **17** and 50% of recovered starting material **16**. The product contains, as indicated from NMR, one major product as well as smaller amounts of the other three possible isomers. This might be due to one of the two phosphotriester epimers being more favourably cyclised than the other, in combination with the fact that the *E*-configuration of the double bond might be much less favourable than the *Z*-configuration in the nine-membered ring. The latter suggestion seems probable from simple modelling.

In summary, the efficiency of the three dinucleotides as substrates for RCM cyclisation falls in the order **14**> **16**>**9**. Thus, the 5′-*C*-allyl group is much more reactive towards the catalyst than the more sterically hindered 4′-*C*-vinyl group, which in **9** does not react at all. On the other hand, the 4′-*C*-vinyl group can, in fact, react with the catalyst in RCM-reactions as reported recently for a nucleoside substrate giving efficiently a six-membered ring[15] and now with **16** affording a larger ring. Apparently, the catalyst reacts first with the 5′-*C*-allyl group in **16** (or alternatively a 3′-*O*-allylgroup)[15] and next in an intramolecular reaction with the hindered 4′-*C*-vinyl group.

Following these observations, we conclude that the dinucleotide of 5′-*C*-allylnucleosides is the best choice for the future construction of conformationally restricted cyclic dinucleotides and subsequent oligonucleotides. However, the dinucleotide **14** was, unfortunately, synthesised from the less efficient coupling reaction. Nevertheless, we believe that these problems are due to the choice of a benzoyl ester as the 5′-*O*-protecting group, and for future examinations, we plan to use alternatively protected analogues of **14**. The efficient RCM reaction should not be compromised by the use of other protecting groups. Also, the tandem RCM-hydrogenation protocol, to give in situ, a saturated ring is expected from our former investigations[12] to be straightforward.

In conclusion, we have proved that nucleoside monomers containing 4′-*C*-vinyl and 5′-*C*-allyl groups can be readily obtained and used in the preparation of dinucleotides. Furthermore, we have found that a dinucleotide containing two 5′-*C*-allyl groups is an efficient substrate for RCM reactions, whereas dinucleotides containing one or two 4′-*C*-vinyl groups are less efficient. Further investigations towards cyclic dinucleotides with a four-carbon 5′-*C* to 5′-*C* connection and oligonucleotides containing this conformationally restricting nucleic acid fragment is in progress in our laboratory. We expect this and other cyclic dinucleotide structures to be attractive building blocks towards nucleic acid secondary and tertiary structural mimics, and we expect the present RCM strategy to be an important tool in nucleic acid chemical biology and drug development.

Acknowledgements

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