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Tandem ring-closing metathesis and hydrogenation towards cyclic dinucleotides

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Cyclic dinucleotides containing a butylene nucleobase–phophotriester connection are synthesised by a tandem ring-closing metathesis and hydrogenation reaction.

Conformational restriction of nucleic acid fragments has attracted a lot of attention due to important potential applications in therapeutics and diagnostics.[1,2] Thus, oligonucleotides containing nucleoside monomers with bi- and tricyclic carbohydrate moieties have demonstrated high affinity for complementary nucleic acids.[2–4] Furthermore, conformationally restricted dinucleotides mimicking tertiary structure elements[5–7] *e.g.* the anticodon loop in bacterial tRNA[7] have been synthesised and investigated.[5–7] Recently, the ring-closing metathesis (RCM) methodology has shown to be a powerful tool for introducing conformationally restricting rings into biomolecules such as peptide structures.[8,9] Especially, the ruthenium based precatalysts such as **1** (Scheme 1) developed by Grubbs and co-workers[10] have broadened the scope of RCM.[11] We have recently applied RCM (and **1**) in the construction of bicyclic nucleosides[12] and of conformationally restricted dinucleotide[13–15] and trinucleotide structures[15] containing additional unsaturated rings involving phosphortriester internucleoside linkages.[13–15] We hereby present important synthetic improvements to this RCM based strategy. Thus, the general lability of the phosphortriesters is hereby explored and the subsequent synthetic demand for saturated phosphortriester analogues is solved by a tandem RCM-hydrogenation protocol.

The dinucleotide structures are synthesised from nucleoside building blocks containing allyl moieties followed by RCM-reactions. The synthesis of the dinucleotide **6a** has been described recently.[15] Thus, **3a** was coupled to the allylphosphoramidite **2**[16] followed by oxidation to give the dinucleotide **4a** which in an RCM-reaction afforded the cyclic dinucleotide **5a** and, after deprotection, **6a** as a mixture of stereoisomers‡ (Scheme 1).[15] Hereby, we present the similar construction of the bis-(2′-deoxy)dinucleotide. Thus, the known 5-allyl-2′-deoxyuridine **7**[17] was converted to **3b** in 64% yield over three steps using standard protection/deprotection procedures. Subsequently, the dinucleotide **4b** was synthesised as an epimeric mixture by coupling of **2** and **3b** using 1*H*-tetrazole as an activator followed by oxidation of the resulting phosphite in 64% overall yield. Finally, an RCM reaction applying the same conditions as before,[15] *i.e.* the precatalyst **1** in CH_2Cl_2 afforded in 58% yield **5b** as an epimeric mixture. Thus, MS-data confirmed the loss of the mass of ethylene, whereas the ^{1}H NMR signals verified the conversion of terminal to internal double bonds.§ Two major products were formed corresponding to the two phosphorus epimers with (*E*)-configuration of the 2-butene linker in combination with only trace amounts of the (*Z*)-configured isomers.§

Whereas the phosphortriester moieties were stable towards acidic treatment as exemplified by the deprotection of **5a** to give **6a**, the lability towards basic conditions was proven to be significant. Thus, when **6a** was dissolved in deuterated pyridine, the solvent acted as a nucleophile towards the phosphate bound methylene of the 2-butene linker opening the cyclic structure to give the zwitterionic dinucleotide **8a** as the only major product as judged from NMR. Also MS verified a covalently bound pyridinium moiety. When **6a** was treated with concentrated aqueous ammonia for 24 h at room temperature, a similar ring-opening reaction affording **9a** was complete. An analogueous ring-opening reaction as well as contemporary double desilyla-

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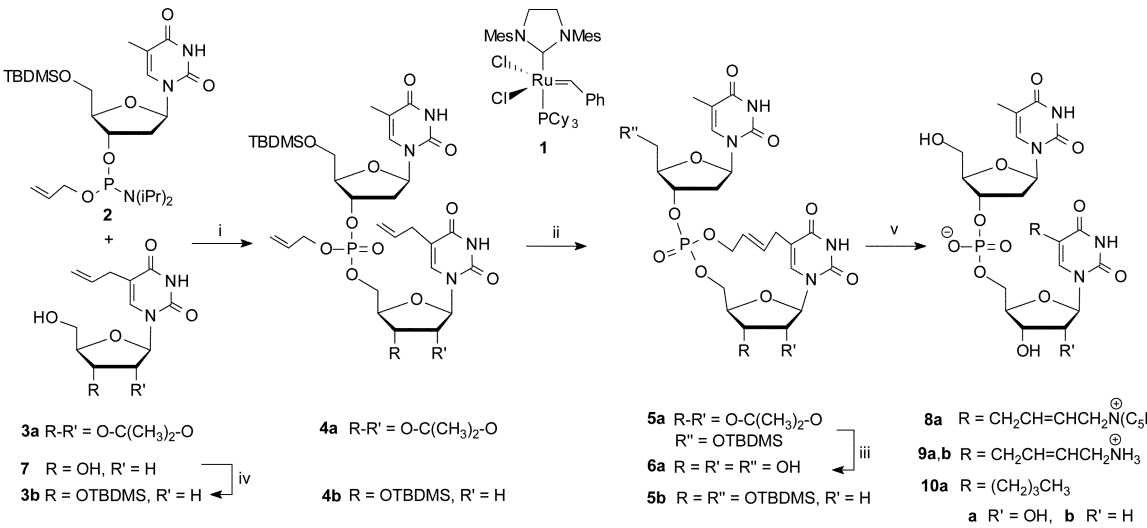

Scheme 1 *Reagents and conditions*: i, a. 1*H*-tetrazole, CH_3CN, b. *t*-BuOOH, CH_3CN, 96% (**4a**), 64% (**4b**); ii, 5 mol% **1**, CH_2Cl_2, 40 °C, 47% (**5a**), 58% (**5b**); iii, 90% aq. TFA, 96%; iv, a. DMT-Cl, $AgNO_3$, pyridine, b. TBDMSCl, $AgNO_3$, pyridine, c. *p*-toluenesulfonic acid, CH_2Cl_2, MeOH, 0 °C, 64%; v, pyridine-d_5, quant. (**8a**); 32% aq. NH_3, rt., quant. (**9a**); 20 mol% Pd/C, H_2, MeOH, quant. (**10a**); 32% aq. NH_3, 55 °C, quant. (**9b**).

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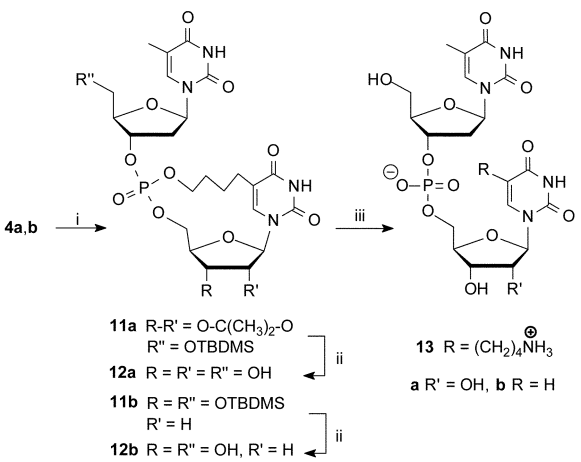
tion was seen when **5b** was subjected to hot aqueous ammomnia affording the zwitterion **9b**.

Since the present significant electrophilic character of the phosphortriester moiety must at least in part be deduced to its allylic nature, a hydrogenation of the 2-butene linker was approached. However, when **6a** was subjected to standard hydrogenation conditions, *i.e.* atmospheric pressure of hydrogen and a palladium catalyst, this led surprisingly not only to the hydrogenation of the double bond but also to reduction of the phosphortriester linkage to give the dinucleotide **10a** in a quantitative yield. Palladium hydroxide as a catalyst gave the same result. A solution to the problem of hydrogenation was given by a method of Grubbs and co-workers who recently presented a tandem ring-closing metathesis and hydrogenation procedure in which catalyst **1** was applied also as a hydrogenation catalyst.[18] Thus, a solution of **4a** in CH_2Cl_2 was refluxed with 5 mol% of **1** until completion of the RCM reaction as detected by TLC and then subjected to 1000 psi H_2 at 50 °C overnight to give **11a** as an epimeric mixture in 54% (Scheme 2). Thus, while the RCM reaction proceeded in moderate yield, the hydrogenation was quantitative as indicated by the yield, which is similar to the yield of the RCM reaction alone, and by MS and NMR as no presence of the unsaturated compound **5a** could be detected after hydrogenation. **11a** was deprotected using the usual acidic treatment[14] to give **12a** in 85% yield.¶ Applying the same conditions, **11b** was synthesised as an epimeric mixture in an even higher 63% yield from **4b**. Deprotection gave **12b** in quantitative yield.¶ The stability towards nucleophiles of these saturated phosphortriesters was examined and, as expected, found to be significantly improved compared to the unsaturated analogues. Thus, **12a** did not react at all with deuterated pyridine within 24 h, and treatment with concentrated aqueous ammonia for 24 h at room temperature resulted in only approximately 10% conversion to **13a** according to ^{31}P NMR. A harsher treatment of **12a** with ammonia at 55 °C for 5 days resulted, however, in complete conversion to **13a**. Similar properties were seen in the 2′-deoxy series as treatment of **12b** with 32% aq. NH_3 at 55 °C in 5 days gave complete conversion to **13b**, whereas less than 10% conversion was seen after 24 h at room temperature.

In summary, the RCM protocol has proven very efficient in preparing the conformationally restricted cyclic dinucleotides **5a**,**b**. The potential use of these compounds, however, is hampered by the high base lability, which is not compatible with standard solid phase oligonucleotide synthesis applying base-labile protecting groups and solid support connections. The saturated counterparts **12a**,**b** are much more stable, and the standard deprotection conditions usually applied (*i.e.* conc. ammonia at rt) afforded only a slow cleavage of the conformationally restricting rings. Thus, the incorporation of **12a**,**b** into oligonucleotides should be straightforward as it has been with analogous dinucleotides with shorter nucleobase–phosphortriester linkages.[5,6] The lability of the allylic phosphortriesters gives, on the other hand, the interesting opportunity of conjugation to the nucleobase applying other nucleophiles. Thus, transferring the RCM chemistry to solid phase oligonucleotide synthesis reveals the 2-butene linkage as a platform for postsynthetic functionalisation[19] *e.g.* towards zwitterionic DNA.[20]

In conclusion, the present RCM-based strategy has proven very convenient towards conformationally restricted cyclic dinucleotides. Importantly, the precatalyst **1** has proven very useful also as an efficient and selective hydrogenation catalyst even towards compounds unobtainable by conventional hydrogenation methods. We expect the conformational restriction introduced, and the present RCM methodology in general, to be a very important tool towards nucleic acid analogues mimicking tertiary nucleic acid structures.



Scheme 2 *Reagents and conditions*: i, 5 mol% **1**, CH_2Cl_2, 40 °C then 1000 psi H_2, 50 °C, 54% (**11a**), 63% (**11b**); ii, 90% aq. TFA, 85% (**12a**), quant. (**12b**); iii, 32% aq. NH_3, 55 °C, quant. (**13a**), quant. (**13b**).

Notes and references

‡ **6a** contains a 10 : 10 : 1 : 1 mixture of the (E,S_P), (E,R_P), (Z,S_P) and (Z,R_P) stereoisomers, respectively.[15]

§ *Selected data*: for **4b**: ^{1}H NMR ($CDCl_3$) δ 5.00–5.41 (4H, m, CH=CH_2), 5.84–5.95 (2H, m, CH=CH$_2$); ^{31}P NMR ($CDCl_3$) δ −0.61. For **5b**: ^{1}H NMR ($CDCl_3$) δ 5.84–5.98 (2H, m, CH=CH); ^{31}P NMR ($CDCl_3$) δ −0.65, 2.01; IR (KBr) 3431, 3065, 1694, 987, 780 cm^{-1}.

¶ *Selected data*: for compound **12a**: ^{1}H NMR (DMSO-d_6) δ 1.50–1.70 (4H, m, CH_2CH_2), 1.76–1.80 (3H, br s, CH_3), 2.28–2.43 (4H, m, CH_2-U, T-H2′,2″), 3.60–3.65 (2H, m, T-H5′,5″), 3.98–4.39 (8H, m, T-H4′, U-H5′,5″,4′,3′,2′, CH_2-OP), 4.93–4.99 (1H, m, T-H3′), 5.80 (0.5H, d, J 2.3 Hz, U-H1′), 5.87 (0.5H, d, J 3.9 Hz, U-H1′), 6.18–6.24 (1H, m, T-H1′), 7.28 (0.5H, s, U-H6), 7.41 (0.5H, s, U-H6), 7.67–7.71 (1H, br s, T-H6), 11.34–11.40 (2H, m, NH); ^{31}P NMR (DMSO-d_6) δ −0.58, 0.29; IR (KBr) 3419, 1695, 1024, 786 cm^{-1}. HiRes MALDI FT-MS: [MNa$^+$] found/calc. m/z 625.1507/625.1517. For compound **12b**: ^{1}H NMR: (DMSO-d_6) δ 1.50–1.70 (4H, m, CH_2CH_2), 1.76–1.79 (3H, br s, CH_3), 2.10–2.42 (6H, m, CH_2-dU, 2 × H2′,2″), 3.50–4.40 (9H, m, 2 × H5′,5″,4″, dU-H3′, CH_2-OP), 4.93–4.97 (1H, m, T-H3′), 6.18–6.29 (2H, m, 2 × H1′), 7.31 (0.5H, s, dU-H6), 7.43 (0.5H, s, dU-H6), 7.66–7.70 (1H, br s, T-H6), 11.31–11.35 (2H, m, NH); ^{31}P NMR (DMSO-d_6) δ −0.71, 0.00; IR (KBr) 3435, 1690, 1022, 784 cm^{-1}; HiRes MALDI FT-MS: [MNa$^+$] found/calc. m/z 609.1584/609,1568.

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