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An improved procedure for the synthesis of vinylphosphonate-linked nucleic acids

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Abstract

The synthesis of a range of 5′-deoxy-5′-methylidene phosphonate-containing thymidine dimers has been achieved using a palladium-catalysed cross coupling reaction as a key step. The optimised reaction conditions comprising of palladium acetate, 1,1′-bis(diphenylphosphino)ferrocene and propylene oxide in THF were found to couple a range of *H*-phosphonates and vinyl bromide **3** in fair to excellent yield.

Keywords: antisense; palladium coupling; vinyl bromide; oligonucleotide; modified backbone.

The potential to inhibit protein synthesis in vivo by binding a short 'antisense oligonucleotide' to an appropriate 'sense' messenger RNA (mRNA) has stimulated considerable interest in the potential use of these materials as therapeutic agents.[1] Due to a number of well-documented limitations, naturally occurring phosphodiester-linked nucleic acids (DNA and RNA **1**, Fig. 1) are not suitable drug candidate molecules. One of their main limitations is their instability towards a number of nuclease enzymes. Many attempts have been made to increase nuclease resistance by chemical modification of the oligonucleotide backbone,[1,2] and we have recently reported our own work in this area.[3] In particular we have been interested in the synthesis of nucleotides possessing a vinylphosphonate internucleotide linkage[4] and we were able to show that a thymidine dimer possessing this backbone modification could be synthesised using a palladium catalysed carbon–phosphorous coupling as a key step (Fig. 1).[3,5] Using tetrakis(triphenylphosphine)-palladium(0) as catalyst, triethylamine as base and DMF as the solvent, we were able to obtain the modified dimer **4** in a pleasing 42% yield. In this letter we now wish to describe further studies in this area which have led to the development of a new coupling protocol and the synthesis of a new range of interesting modified nucleotide dimers.

Although we were pleased at the initial 42% yield for this coupling, we realised that it needed to be improved upon if we were going to have a feasible route to modified oligonucleic acids.

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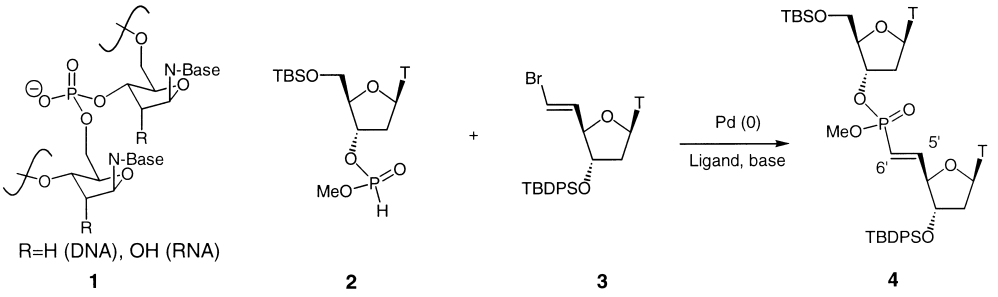
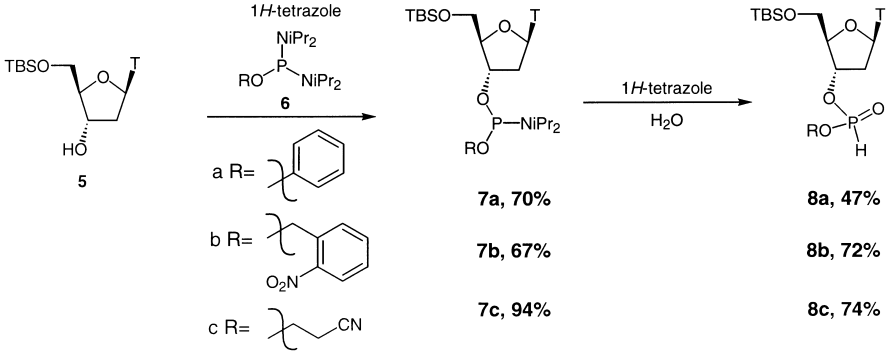


Figure 1.

Upon examining the composition of the mass balance of the reaction it was obvious that the major by-product formed was the secondary alcohol **5**. The formation of **5** can easily be accounted for by the hydrolysis of the *H*-phosphonate coupling partner **2**. Control reactions quickly confirmed this hypothesis, as **2** was hydolysed to **5** in quantitative yield by treatment with triethylamine in DMF at room temperature in less than one hour. This result was slightly concerning as it appeared that decomposition of the *H*-phosphonate coupling partner was a rather facile process under the coupling reaction conditions. In order to address this potentially serious problem we needed to either stabilise the *H*-phosphonate towards hydrolysis or improve the rate of the coupling reaction. Ideally we hoped to find conditions to achieve both of these goals, but we first decided to address the problem of increasing the rate of coupling step. Over the last few years there have been massive improvements in the area of transition metal-catalysed amine arylation reactions.[6] It has been found that sterically demanding and/or chelating ligands are especially good in this type of transformation. Due to the obvious similarities between this and our carbon–phosphorous coupling reaction, we wondered whether analogous changes to our catalyst would result in a useful rate enhancement.

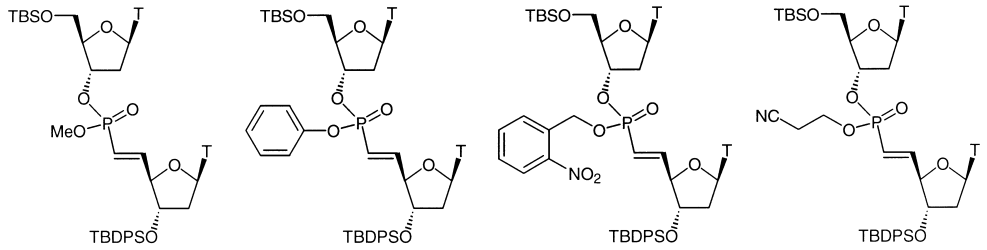
We therefore decided to examine the use of the catalyst generated in situ from commercially available 1,1′-bis(diphenylphosphino)ferrocene (dppf) and palladium acetate in refluxing THF. Rather gratifyingly, we found that this combination of catalyst and solvent resulted in the formation of the coupled product **4** in a much improved 78% yield. Changing the ligand to dppf and solvent to THF also resulted in an unexpected simplification of the isolation and purification procedure. After the reaction was judged complete by TLC analysis, the volatiles were removed in vacuo and the crude residue was subjected to purification by flash column chromatography. When PPh_3 was used as ligand, the purification of **4** was rather laborious, as it was often contaminated with the almost co-polar triphenylphosphine oxide. The use of dppf as ligand completely removed this inconvenience, and **4** could be isolated in pure form from the initial isolation and purification procedure. With this success in hand we now wanted to test the scope and limitations of this catalyst system. In order to do this we first needed to synthesise a range of additional *H*-phosphonate coupling partners. The *H*-phosphonates **8a–c** were synthesised from the alcohol **5**, via the phosphoramidites **7a–c**, using standard procedures (Scheme 1).[7]

We were now in a position to examine the palladium-catalysed couplings of **8a–c** with the vinyl bromide **3**. Firstly, **8a** and **8b** were coupled using the newly developed dppf/THF catalyst system (the cyanoethanol-protected *H*-phosphonate **11** was not subjected to these reaction conditions due to its sensitivity to amine bases). In both cases we were able to isolate the desired coupled products **9** and **10**, albeit in rather modest yields (21% and 24%, respectively, Fig. 2). We were



Scheme 1.

somewhat disappointed with these results, especially since we were using the 'improved' catalyst system. Once again we isolated significant amounts of the hydrolysis product **5** from the reaction mixtures, and it seemed that the rate of coupling was slower than the rate of decomposition for the more hindered *H*-phosphonates **8a** and **8b**. To overcome this problem we next examined the use of an alternative HBr scavenger to replace the triethylamine. The use of propylene oxide for this purpose has been reported previously in the synthesis of arylphosphonates and phosphinates,[8] and this looked ideal for our purposes. We therefore repeated the coupling reactions of **8a** and **8b** with **3**, using 1.2 equivalents of propylene oxide in place of the 4.5 equivalents of triethylamine. To our pleasure this resulted in increased yields of the desired coupled products **9** and **10** (53% and 65%, respectively). Having eliminated the need for amine base in the reaction conditions, we could now examine the coupling of **8c** without fearing removal of the cyanoethyl protecting group. Thus, **8c** was coupled with **3** using the new coupling conditions ($Pd(OAc)_2$, dppf, THF, propylene oxide (1.2 equiv.), reflux) to yield the desired dimer **11** in excellent yield (72%).

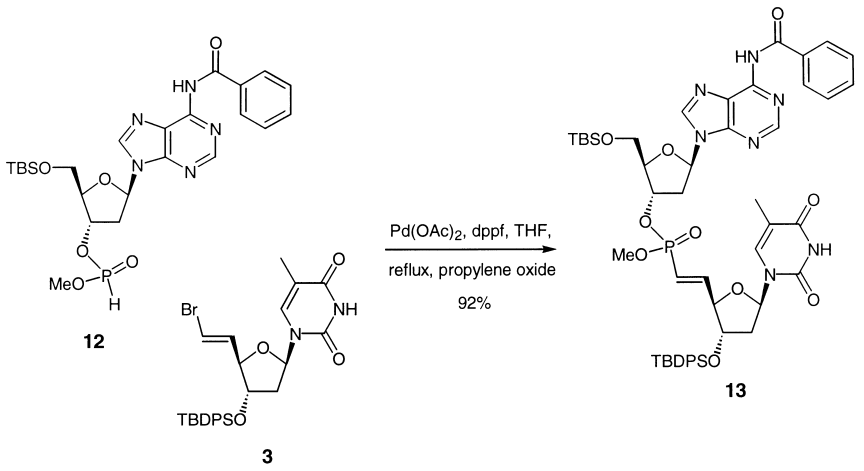


Et_3N[a]	78%	21%	24%	—
O [b]	60%	53%	65%	72%

Figure 2. Conditions: (a) $Pd(OAc)_2$, dppf, THF, Δ, 20 h, Et_3N (4.5 equiv.); (b) $Pd(OAc)_2$, dppf, THF, Δ, 20 h, propylene oxide (1.2 equiv.)

These results indicate that the dppf/THF/propylene oxide coupling conditions are well suited to a fairly wide range of *H*-phosphonate coupling partners. Although we were very pleased with these findings, we were acutely aware that if this methodology was to find any use in synthesising

biologically important modified nucleic acid sequences we would have to be able to access materials containing purine as well as pyrimidine bases. As a preliminary study in this area we synthesised the *N*-benzoyl-protected *H*-phosphonate **12** to act as a purine-containing coupling partner.[9] We were delighted to find that coupling of **12** with **3**, using the latest evolution of our coupling conditions (vide supra), gave the desired *N*-protected AT dimer **13** in an excellent 92% yield (Scheme 2).



Scheme 2.

In summary, we have developed a convenient coupling strategy to allow rapid access to a range of backbone modified nucleic acids. The conditions outlined above seem to have some degree of generality and are tolerant of a number of potentially sensitive functional groups. We have also demonstrated for the first time that a mixed purine–pyrimidine sequence can be synthesised in high yield. We are currently developing this methodology further, and examining the chemistry necessary to incorporate these modified dimers into oligonucleotide sequences. The results of these studies will be reported in due course.

Acknowledgements

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3. Abbas, S.; Hayes, C. J. *Synlett* **1999**, 1124.
4. See also: Zhao, Z.; Caruthers, M. H. *Tetrahedron Lett*. **1996**, *37*, 6239.
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9. The *H*-phosphonate **12** was synthesised in a similar manner to the *H*-phosphonate **2**, which we have reported previously. The synthesis involves selective TBS-5′-protection of commercially available *N*-benzoyl-2′-deoxyadenosine, coupling with *N*,*N*-diisopropylmethylphosphonamidic chloride and then tetrazole-mediated hydrolysis in methylene chloride.