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Stereoselective synthesis of highly functionalised P-stereogenic nucleosides via palladium-catalysed P–C cross-coupling reactions

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ABSTRACT

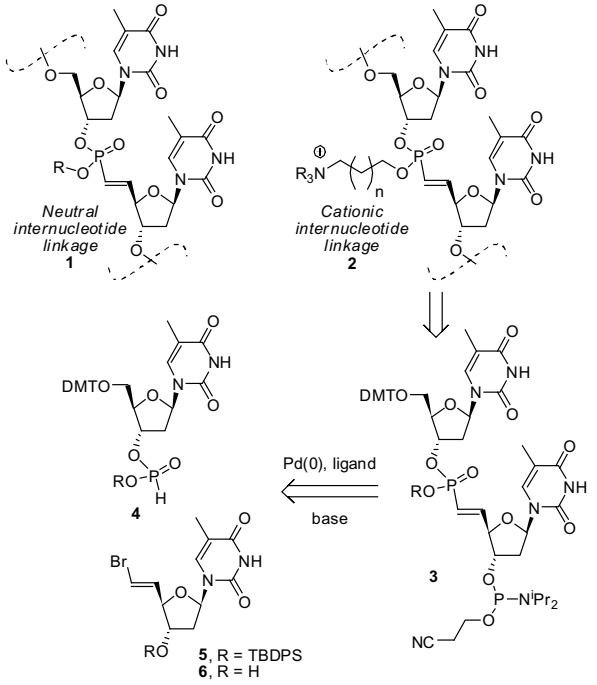
We have shown that the configuration at the P-stereogenic centre in a range of *H*-phosphonates can be assigned using a combination of chromatographic mobility, ^{1}H and ^{31}P NMR data. We have also shown that the individual P-stereoisomers can be cross-coupled with vinyl and aryl halides, in the presence of a palladium(0) catalyst, to afford the corresponding vinyl- and arylphosphonates in a stereocontrolled manner.

The ability to modulate gene function in a sequence-specific manner at either the RNA (antisense,[1] RNAi[2]) or the DNA (antigene)[3] level has stimulated much interest in the synthesis and biological evaluation of a wide range of nucleic acid-derived oligomers. Many modifications to the natural phosphodiester oligonucleic acid backbone have been examined in order to overcome problems associated with nuclease degradation, and significant progress has been made towards increasing their useful lifetime.[4]

Our own studies in this area have focussed upon replacing the internucleotide phosphodiester linkage with a nuclease-resistant vinylphosphonate moiety **1**,[5] and we have used these materials as probes with which to study helicase,[6] nuclease[7] and polymerase activity[7] (Scheme 1). The vinylphosphonate internucleotide modification was incorporated into oligomers by using suitably modified dinucleotide phosphoramidite reagents **3**, which in turn were synthesised from the corresponding *H*-phosphonate **4** and vinyl bromide **5** building blocks via palladium(0)-catalysed P–C cross-coupling[8] (Scheme 2).

As a prelude to examining the potential therapeutic applications of these modified oligonucleotides, we wished to explore the possibility of synthesising neutral **1**[9] and cationic **2**[10] versions of the vinylphosphonate internucleotide linkage as these may be better candidates for efficient transport across cellular membranes. In principle, we could easily prepare neutral versions by using base-stable alkyl groups on the *H*-phosphonate in place of the cyanoethyl-protecting group used previously, and cationic versions could be prepared in a similar way using pendant groups that could be protonated under physiological conditions (i.e., amines and guanidines).[10] As the target phosphonate diesters possess a P-stereogenic centre, which will be retained in the final oligomer, we needed to develop a method for accessing single P-stereoisomers of these compounds, as different biological activities had been observed previously for other modified oligomers that bear P-stereogenic centres.[11] We now wish to report our initial findings on the development of such a method, which allows stereocontrolled access to P-stereogenic vinylphosphonates using palladium-catalysed P–C cross-coupling as a key step.

It is well know that the stereogenic centre in P-chiral *H*-phosphonates is configurationally stable at room temperature,[12] and that the P–H bond can be oxidised with overall retention of



Scheme 1. Retrosynthesis of vinylphosphonate-linked oligonucleotides.

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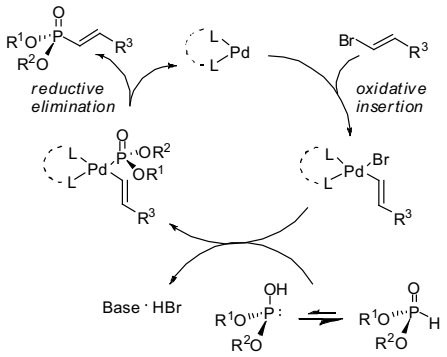
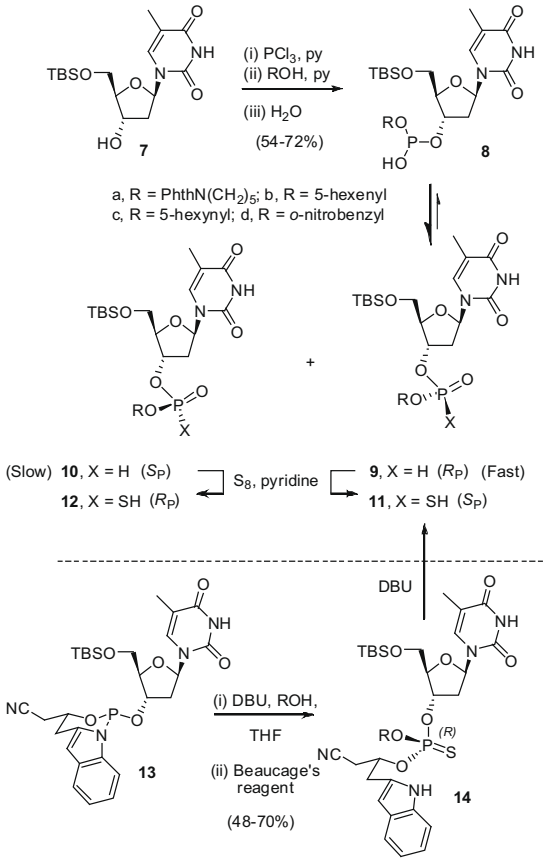


Scheme 2. Proposed catalytic cycle for P–C cross-coupling.

stereochemistry.[13] Furthermore, it has been shown that P-chiral phosphinates[14] and phosphine–borane complexes[15] undergo transition metal-mediated cross coupling reactions with overall retention of stereochemistry at phosphorus. Thus, as a starting point for our own work, we decided to explore the use of single *H*-phosphonate P-stereoisomers in the palladium-catalysed cross-coupling reaction, as this should afford single isomers of the desired vinylphosphonate products (Scheme 2).

A range of *H*-phosphonates were synthesised by either hydrolysis of the corresponding phosphoramidite,[16] or sequential substitution of PCl_3 with 5′-OTBS-thymidine **7**, an appropriate alcohol and then water (Scheme 3). A 50:50 mixture of (R_P)-**9** and (S_P)-**10** isomers was obtained during this reaction, and these were separated using flash column chromatography to afford the stereochemically pure *H*-phosphonates. Although we could separate the two isomers, we did not know at this stage which was the R_P and which was the S_P configured material, so our next task was to assign the absolute configuration at the P-stereogenic centre.



Scheme 3. Synthesis and stereochemical assignment of *H*-phosphonates.

As we knew of no direct method for assigning the stereochemistry at phosphorus, we decided to oxidise the *H*-phosphonates to the corresponding phosphorothioates, **11** and **12**, and then correlate these with the same materials produced via an independent, stereocontrolled route. For this purpose, we used the P-chiral indole-substituted phosphoramidite **13** of Just[17] as our starting material, as this had been used previously to prepare P-chiral phosphorothioates of known stereochemistry. Thus, Just's phosphoramidite **13** was prepared according to the known procedure[17] in good yield, and nucleophilic substitution with the chosen alcohol then gave the corresponding phosphites, which were immediately oxidised with Beaucage's reagent[18] to give the phosphorothioate diesters **14** with retention of stereochemistry. Elimination of the chiral auxiliary with DBU finally gave the phosphorothioates **11** as single S_P stereoisomers, whose ^1H and ^{31}P NMR data were then compared to those obtained for the equivalent compounds produced previously via oxidation of the *H*-phosphonates **9** and **10** (Scheme 3).

It was found that the data for phosphorothioates **11** (produced from the 'Fast' *H*-phosphonates **9**) were identical to those produced for the same compounds prepared from Just's phosphoramidite **13**, thus confirming them as the S_P stereoisomers. As it is known that oxidation proceeds with retention of stereochemistry, we could assign the precursor 'Fast' *H*-phosphonates **9** as possessing the R_P absolute configuration,[19] and also the remaining 'Slow' *H*-phosphonates **10** as having the S_P configuration. It is striking that in all cases the R_P configured *H*-phosphonates have higher chromatographic mobility (denoted 'Fast') than the S_P configured materials (denoted 'Slow').[20] Inspection of the ^1H and ^{31}P NMR data for the R_P and S_P *H*-phosphonates also revealed characteristic trends within the two stereochemical series. For example, the chemical shift of the P–H ^1H resonance was always at higher field for the R_P series than the equivalent proton in the S_P series. Also the ^{31}P resonance was always at higher field in the R_P series than it was in the S_P series. It thus appears that a combination of chromatographic mobility, ^1H and ^{31}P NMR data can be used to assign the absolute configuration of the P-stereogenic centre (Table 1).[21]

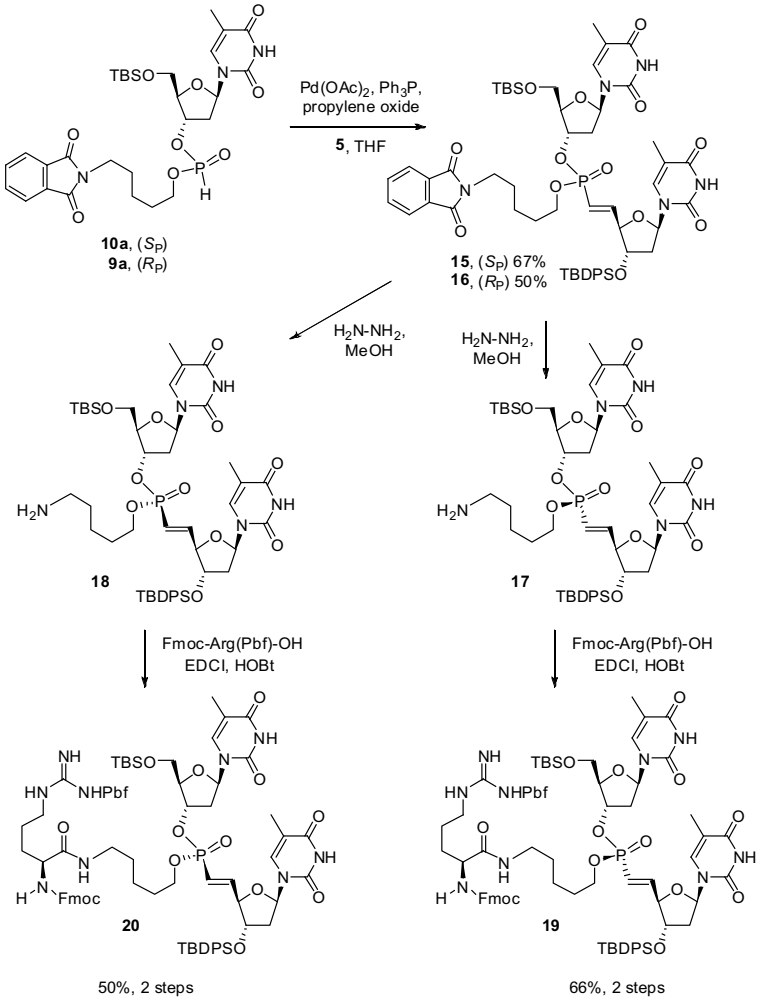
Having determined the stereochemistry at the P-stereogenic centre of some *H*-phosphonates, we were ready to examine their behaviour in palladium-catalysed P–C cross-coupling reactions. Thus, each diastereoisomer of the phthalimide-containing *H*-phosphonates (R_P)-**9a** and (S_P)-**10a** was reacted with the thymidine-derived vinyl bromide **5**[5] using a catalyst system derived from $Pd(OAc)_2$, Ph_3P and propylene oxide (Scheme 4).

Pleasingly, the desired vinylphosphonates (S_P)-**15** (67%) and (R_P)-**16** (50%) were isolated as single diastereoisomers (Scheme 4). In order to demonstrate the potential utility of the phthaloyl-protected amines, each was exposed to hydrazine hydrate in

Table 1

Entry	Compound	Mobility[a]	^1H δ ppm[b] (P–H)	^{31}P δ ppm[c] (P–H)	R_P/S_P
1	**9a**	Fast	6.86	8.2	R_P
2	**10a**	Slow	6.89	8.4	S_P
3	**9b**	Fast	6.83	7.9	R_P
4	**10b**	Slow	6.86	8.1	S_P
5	**9c**	Fast	6.83	7.9	R_P
6	**10c**	Slow	6.87	8.1	S_P
7	**9d**	Fast	7.06	8.5	R_P
8	**10d**	Slow	7.09	8.6	S_P

[a] Relative chromatographic mobility on SiO_2 gel.
[b] 500 MHz, $CDCl_3$.
[c] 121.5 MHz, $CDCl_3$.

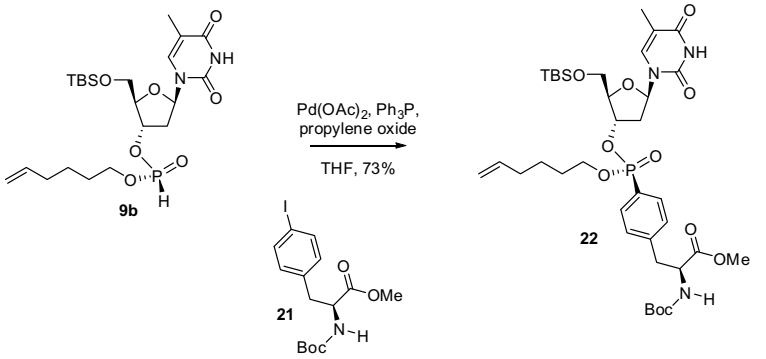


Scheme 4. Stereospecific palladium-catalysed cross-coupling reactions.

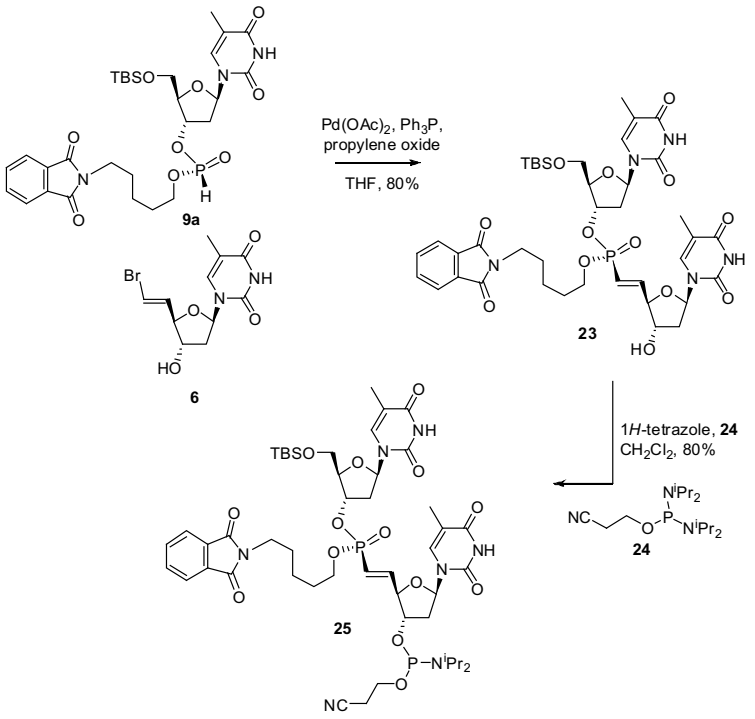
methanol to facilitate deprotection to afford the corresponding primary amines (S_P)-**17** and (R_P)-**18** (Scheme 4). As the primary amines were quite difficult to handle due to their polarity, we decided to show that they could be further derivatised via peptide-bond formation. Thus, conjugation of **17** and **18** with Fmoc-Arg(Pbf)-OH[22] gave the corresponding amides **19** and **20** in good yields for the two-step procedure.

Stimulated by this success, and with the obvious potential to use this chemistry to prepare nucleic acid-peptide conjugates, we next explored the direct cross-coupling of nucleotide and amino acid-derived coupling partners. Thus, the hexenyl-substituted *H*-phosphonate (R_P)-**9b** was cross-coupled with the protected *p*-iodophenylalanine derivative **21** to afford the nucleic acid-amino acid hybrid **22** (73%) as a single stereoisomer (Scheme 5).

We concluded this study by performing a cross-coupling reaction between the phthalimide-containing *H*-phosphonate (R_P)-**9a** and the thymidine-derived vinylbromide **6**, which contains an unprotected 3′-OH group. As expected, the desired vinylphosphonate **23** was isolated in good yield (80%) as a single isomer, and this material was then converted into its corresponding



Scheme 5. Synthesis of nucleic acid-amino acid hybrid.



Scheme 6. Preparation of a vinylphosphonate-containing phosphoramidite reagent for oligonucleotide synthesis.

phosphoramidite **25** using the standard procedure (Scheme 6). We are now studying the incorporation of these modified nucleotides into oligomers, and we will report our findings on this work in due course.

In conclusion, we have shown that the configuration at the P-stereogenic centre in a range of *H*-phosphonates can be assigned using a combination of chromatographic mobility, ^{1}H and ^{31}P NMR data. We have also shown that the individual P-stereoisomers can be cross-coupled with vinyl- and aryl halides, in the presence of a palladium(0) catalyst, to afford the corresponding vinyl- and arylphosphonates in a stereocontrolled manner.

Acknowledgements

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