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AN IMPROVEMENT IN THE STEREOSPECIFIC SYNTHESIS OF DINUCLEOSIDE METHYLPHOSPHONATES

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The use of 1,1,1,3,3,3-hexafluoro-2-propanoxy as the leaving group for the title synthesis improves the yield and ease of resolution of the required monomers.

The synthesis of P-modified oligonucleotide analogues for use in antisense control of gene expression has received a great deal of attention in recent years. The antisense technique has potential in the development of antitumor and antiviral compounds, because of its inherent selectivity. P-modifications are employed to enhance binding, cell penetration, and lifetime of the agent in the target cell. The naturally occurring phosphodiester has been replaced with a number of other functionalities, such as carbonate, diisopropylsilyl, phosphorothioate, alkyl phosphotriester and alkylphosphonate.[1] The most successful modifications appear to be the last three. In each case, however, a new chiral center is formed at P. For a sequence containing n modified linkages, 2^n diastereoisomers are formed. There is considerable evidence to support the notion that the stereochemistry about the P atom affects the ability of the sequence to bind to its target. In order to optimise this binding, it is necessary to have a procedure which allows the introduction of the modified linkage in a stereospecific manner. To date, no such method is available for alkyl phosphotriesters. Engels and Jaeger[2] have described a route to predominantly Rp-configured thymidine dimers. Stec[3] has introduced a route to oligonucleoside methylphosphonates which allows for the introduction of either Rp- or Sp-configured linkages. In this procedure (see Scheme, shown for only one diasteroisomer), the nucleoside-3'-phosphonate **1** is produced as the racemic mixture and resolved chromatographically. The resolved monomer is then coupled with the 5'-hydroxyl of the second nucleoside **3**, which is activated by t-butyl magnesium chloride. In an early publication,[4] they reported some epimerisation, but this has been resolved by switching the solvent from THF to pyridine.

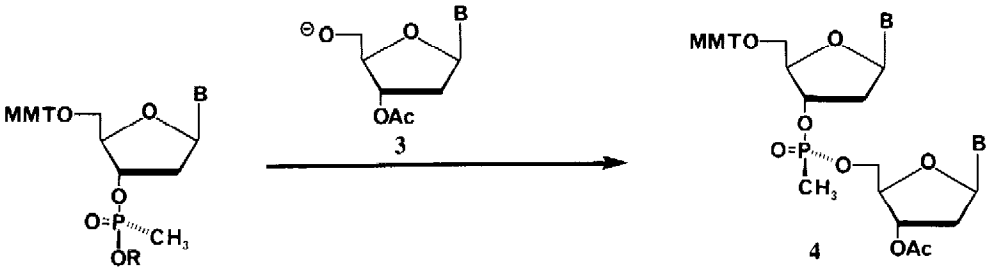
We became interested in this method as a route to stereopure antisense sequences. The resolution, as described, of the monomers **1** was difficult. We obtained a total yield of about 35%, with only about 10% each of the Rp and Sp isomer completely resolved. The remaining 15% was recovered as a mixture of the two epimers. Stec reports a 40% yield overall, but does not indicate the resolved yield. We decided to try to modify the monomer somewhat, in order to improve the efficiency of the resolution.

We needed a replacement for the p-nitrophenoxy group that would be stable to chromatography, but still be a sufficiently good leaving group to allow the coupling reaction to proceed. In addition, the anion

of this group should not cause the epimerisation described in ref 4. We chose the 1,1,1,3,3,3-hexafluoro-2-propanoxy group.

Monomer **2** was produced, essentially as described by Stec *et al.*, as follows. A solution of 5'-monomethoxytrityl thymidine (0.51 g, 1 mmol) in anhydrous pyridine (2.5 ml) was added dropwise, with stirring, to a solution of methylphosphonic dichloride (0.40 g, 3 mmol) in anhydrous pyridine (3.0 ml), over an hour. After a half an hour, a solution of 1,1,1,3,3,3-hexafluoro-2-propanol in anhydrous pyridine (4.5 ml) was added. After another hour, the reaction mixture was poured into saturated sodium bicarbonate solution and worked up by extraction with chloroform. After drying (sodium sulphate) and concentration, the monomers Rp- and Sp-**2** were resolved by silica gel column chromatography. The eluting solvent was chloroform:acetone (10:0 --> 10:4). In this system, the two diastereoisomers are essentially completely resolved. The overall yield of the synthesis is typically 65-70%, and the Rp and Sp diastereoisomers are recovered in approximately equal amounts. This represents a significant improvement in yield for the monomer. The chromatography is also complete in less than half the time. The two monomers were characterised by proton and phosphorus NMR.[5] In accordance with observations in the literature,[6] the faster eluting monomer displayed the more downfield shift in the ^{31}P NMR spectrum, and was tentatively assigned the Rp configuration, while the slower eluting monomer displayed the more upfield shift, and was assigned the Sp configuration.

SCHEME



1, R = p-nitrophenyl

2, R = 1,1,1,3,3,3-hexafluoro-2-propyl

MMT = monomethoxytrityl; Ac = acetyl; Th = thymidyl

We then proceeded to use these monomers in coupling reactions. The second nucleoside used for the coupling was 3'-O-acetyl thymidine. t-Butyl magnesium chloride (2.15 M, in THF) was added to a

solution of 3'-O-acetylthymidine (0.14 g, 0.40 mmol) in anhydrous THF (2.5 ml). To the resulting suspension was then added either Rp- or Sp-**2** (0.30 mmol), also in anhydrous THF (2.0 ml). Anhydrous DMF (0.1 ml) was added after an hour, and the reaction mixture was left to stir 20 hours. After aqueous workup and silica gel column chromatography, fully protected dimer **4** was isolated in 65-70% yield. No epimerisation was noted (^{31}P NMR). The use of the Grignard reagent as base appears to be necessary. Use of other bases such as diisopropyethylamine leads to no discernible reaction.

We then repeated the reaction using anhydrous pyridine as the solvent. Stec reports that use of this solvent reduced the coupling time, and eliminated a small amount of epimerisation. In our case, the coupling reaction in pyridine gave a higher yield (80%) but also resulted in a considerable amount of the wrong epimer (25-30% as judged by ^{31}P NMR). We decided to monitor the coupling reaction with Rp-**2** monomer by NMR to see why this was so. Along with signals for the starting material and the desired (Sp) dimer, we observed a new peak at about 34.66 ppm. This peak began to appear after 1-2 hours. At this time, a signal for the other (Rp) dimer, also appeared. The peak at 34.66 ppm does not appear when the reaction is run in THF. We speculated that this new peak might be due to displacement of the leaving group by pyridine. Subsequent displacement by **3** would result in formation of the product with overall retention of configuration at P. On the other hand, successive displacements by pyridine, followed ultimately by attack by **3** would result in epimerisation. We therefore repeated the reaction, in the absence of nucleoside **3**. The peak at 34.66 ppm appeared as before. After 4 hours, all the starting material had been consumed, nucleoside **3** was added, and the reaction left overnight. The NMR spectrum of the crude reaction mixture showed signals for the two diastereomeric dimers in nearly equal amounts. We therefore conclude that the peak at 34.66 ppm is probably due to monomer **2** in which the hexafluoro-2-propanoxy group has been displaced by pyridine. In the original coupling, complete epimerisation does not occur, and the desired Rp diastereoisomer is the main product. Thus it would appear that some of the starting monomer proceeds directly to the product, and the rest goes through the postulated pyridinium intermediate.

In a related series of experiments, we replaced the p-nitrophenoxy group with 2,2,2-trifluoroethoxy. The synthesis of the monomers proceeded as described above, with resolved yield similar to those for **1**, but the coupling reaction did not occur. Apparently, the trifluoroethoxy group is not a sufficiently good leaving group to undergo this reaction.

In conclusion, replacement of p-nitrophenoxy with 1,1,1,3,3,3-hexafluoro-2-propanoxy increases considerably the efficiency of monomer resolution in the stereospecific synthesis of oligonucleoside methylphosphonates. An interesting, though not synthetically useful, side reaction occurs when pyridine is used as the solvent. Yields for the coupling reaction are as good as those reported, and the coupling times in THF are comparable to those reported previously.

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3. Z. J. Lesnikowski, M. Jaworska, W. J. Stec, *Nucl. Acids Res.*, **18**, 2109-2115 (1990). Recently, this work was extended to incorporate homodimers of all four common deoxynuclotide methylphosphonates, with isolated yields of monomers ranging from 43-74% Resolved yields are not reported: Z. J. Leznikowski, M. M. Jaworska-Maslanka, W. J. Stec, *Nucleosides & Nucleotides*, **10**, 733-736 (1991). Methylphosphonylation of Ade, Cyt and Gua nucleosides was done with methylphosphonyltriazolyl derivatives.

4. Z. J. Lesnikowski, P. J. Wolkanin, W. J. Stec, *Tetrahedron Lett.*, **28**, 5535-5538 (1987).

5. Rp-**2**: Rf ($CHCl_3$:acetone, 10:4), 0.74; ^{31}P NMR 34.38 ($CHCl_3$), 34.55 (pyridine).
Sp-**2**: Rf ($CHCl_3$:acetone, 10:4), 0.55; ^{31}P NMR 33.99 ($CHCl_3$), 34.28 (pyridine).

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