Tetrahedron Letters Vol. 21, pp 2841 - 2842
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0040-4039/80/0715-2841$02.00/0

COMPARISON OF RATES OF PHOSPHOTRIESTER FORMATION AND SULPHONATION BY COUPLING AGENTS IN OLIGODEOXYRIBONUCLEOTIDE SYNTHESIS BY THE PHOSPHOTRIESTER METHOD.

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ABSTRACT Rates of phosphotriester bond formation and amounts of sulphonation are compared for three popular coupling agents used in oligodeoxyribonucleotide synthesis by the phosphotriester approach.

A key issue in the chemical synthesis of oligodeoxyribonucleotides by the phosphotriester method[1] is the optimal coupling agent for reaction between a 3'-phosphodiester of one deoxynucleoside unit (I) and a 5'-hydroxyl group of another (II) to give a dinucleoside-3',5'-phosphotriester (III) (Fig. 1). Aromatic sulphonyl azolides are the reagents of choice, in particular 2,4,6-triisopropylbenzenesulphonyl tetrazole (TPStet, IVa)[2], mesitylenesulphonyl 3-nitro-1,2,4-triazole (MSNT, IVb)[3] or 2,4,6-triisopropylbenzenesulphonyl 3-nitro-1,2,4-triazole (TPSNT,IVc)[4]. A competing side reaction resultant from use of such coupling agents is sulphonation of the hydroxyl group in II to give the sulphonyl derivative V. For TPStet it has recently been shown that when the phosphate protecting group R_2 is alkyl rather than the more electron withdrawing aryl, greater sulphonation takes place, because the rate of phosphotriester formation is reduced but the rate of sulphonation is supposedly unaltered[5]. Large amounts of sulphonation have also been observed in oligoribonucleotide synthesis when the concentration of reactants was high[6]. To our knowledge no direct comparison of rates of phosphotriester formation to sulphonation in the deoxy series for these coupling agents at moderate concentrations (0.1M) has been published[7].

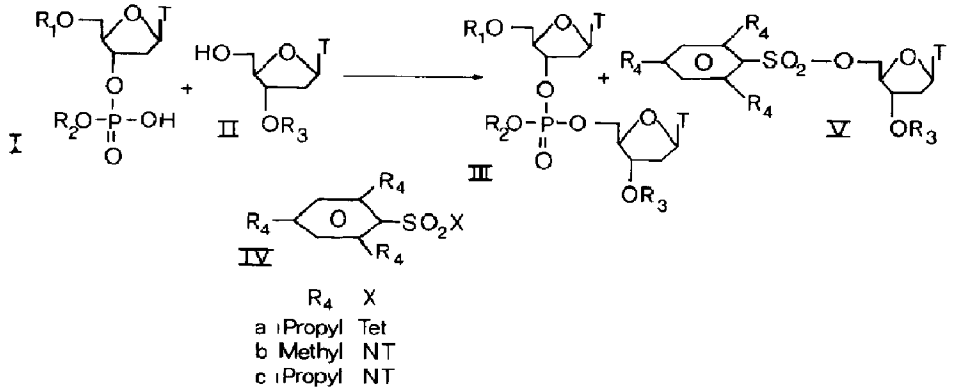

Figure 1

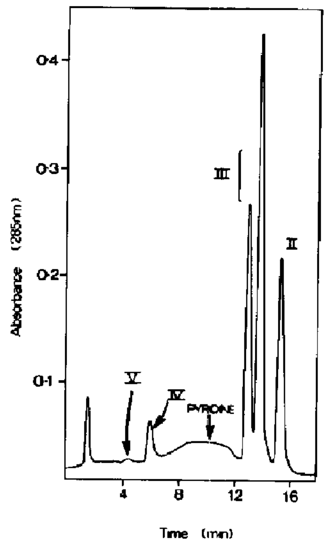

Figure 2

Figure 3

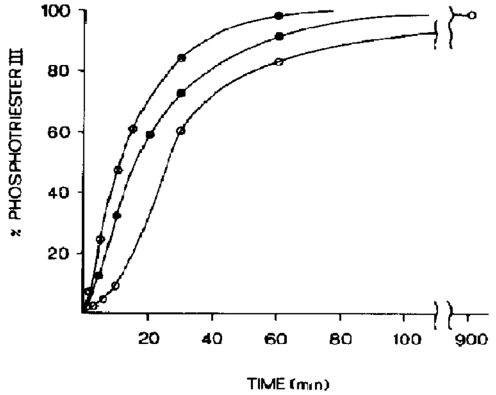

Fig. 2 H.p.l.c. profile, TPSNT, 30 min. Elution 2 mi 0% B, 20 min 0–20% B (A is $CHCl_3$/0.02% pyridine; B is $CHCl_3$/0.02% pyridine/20% methanol). (Compound I not eluted on this gradient). Flow 2 ml min^{-1}

Fig. 3 Plot of % of phosphotriester III against time of reaction: –⊗– MSNT; –●– TPStet; –○– TPSNT

Hence to 2'-deoxythymidine derivatives I (R_1=4,4'-dimethoxytrityl, R_2=p-chlorophenyl) (0.05 mmol) and II (R_3=acetyl)(0.04 mmol) in anhydrous pyridine (1 ml) at 20° in a dry box was added coupling agent (IVa,b or c) (0.1 mmol). At various times 100 µl aliquots were added to water (20 µl), the mixture evaporated, dissolved in chloroform and analysed on Lichrosorb Si60 (Merck 9387, 10 µ, 25 x 0.46 cm). Fig. 2 shows a typical separation. The molar percentage of phosphotriester III is plotted against reaction time (Fig. 3). The percentages of sulphonated product V at $t_{complete}$ for MSNT, TPSNT and TPStet were 0.8, 0.9 and 1.2% (± 0.3%) respectively. Final yields of III were 97–99% in each case. The results show that under the above conditions the rate of phosphotriester formation decreased in the order MSNT>TPStet>TPSNT but that sulphonation was equally small in each case. Thus sulphonation is not a serious problem if moderate reactant concentrations are used and that choice of coupling agent may be based on desired speed of coupling and lack of other side reactions (e.g. with nucleotide bases).

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7. In reference 4 the authors state that 3% and 7% sulphonylation was obtained at tcomplete in a model study using TPSNT and MSNT respectively. No volume of reaction is given but the description "a slightly viscous solution" suggests high concentrations.

We thank Drs. R.C. Sheppard and R.C. Titmas for helpful discussions and M. Singh for valuable assistance.

(Received in UK 7 May 1980)