

## Novel Condensing Reagents for Polynucleotide Synthesis

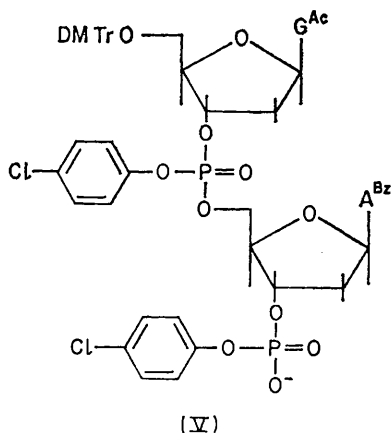
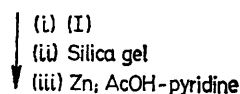
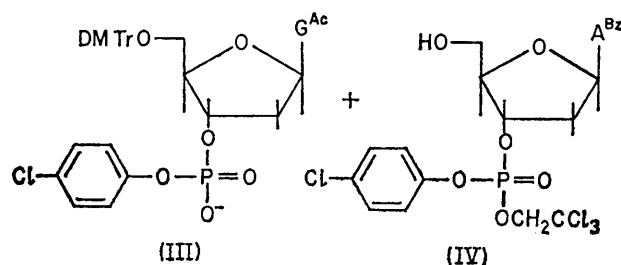
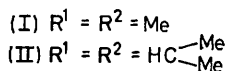
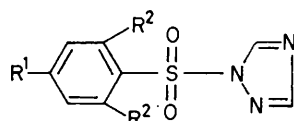
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*Summary* Arylsulphonyl 1,2,4-triazolides have been developed as new condensing reagents in the synthesis of polynucleotides of defined sequence.

RECENTLY, we reported the synthesis of penta-decanucleotides containing thymidine base by an improved triester approach.<sup>1</sup> During work on defined sequence synthesis,

we observed that the yields of the oligonucleotides containing guanine base are quite low (ca. 25%). Cramer *et al.*<sup>2</sup>



have also reported a very low yield (10%) for the synthesis of oligonucleotides containing the guanine group which may

be attributed to the liberation of hydrogen chloride, during the condensation reaction, from tri-isopropylbenzenesulphonyl chloride.

We now report the use of arylsulphonyl triazolides as novel condensing reagents for the synthesis of polynucleotides, to give improved yields. The triazolides (I) and (II) are easily prepared in almost quantitative yields by condensing the corresponding arylsulphonyl chlorides with 1H-1,2,4-triazole in the presence of triethylamine (equimolar ratio) in tetrahydrofuran or chloroform solution. The triazolides,† 1-(mesitylenesulphonyl)-1H-1,2,4-triazole (I), m.p. 135° and 1-(2,4,6-tri-isopropylbenzenesulphonyl)-1H-1,2,4-triazole (II), m.p. 112° activate effectively not only the phosphomonoester but also phosphodiester bond to form the diester and triester in good yields.

Using (I) we have conveniently achieved the synthesis of two hexanucleotides containing a hetero-sequence in fairly high yields. Protected dinucleotide (V) was prepared by condensing 5'-O-dimethoxytrityl-N<sup>2</sup>-acetylguanosine 3'-O-(p-chlorophenyl) phosphate (III) (1 mmol) with N<sup>6</sup>-benzoyl adenosine 3'-O-[(p-chlorophenyl) βββ-trichloroethyl phosphate] (IV) (1.2 mmol) in the presence of (I) (3 mmol) for 24 h at room temperature in anhydrous pyridine. After work up, chromatographic purification on silica-gel, and treatment with zinc in acetic acid-pyridine solution, the dinucleotide (V) was obtained in 54% overall yield. Using similar conditions various fully protected trinucleotides containing base sequences such as d-GAG, d-AAT, d-TGT, and d-CGG were also synthesized in 48, 74, 62, and 50% yields respectively. Finally the fully protected hexanucleotides (d-AATTGT and d-GAGCGG) were obtained using (I) in 45% and 38% yields. All these compounds were checked for purity on silica-gel t.l.c. plates<sup>3</sup> and final characterization was achieved by spleen phosphodiesterase degradation of the hexanucleotides after treatment of the fully protected compounds with dilute alkali, zinc in acetic acid and pyridine solution, and 80% acetic acid.

Compared to the corresponding arylsulphonyl chlorides, the rate of reaction with (I) is slightly slower but the yield is considerably higher. Tri-isopropylbenzenesulphonyl triazolide (II) is found to be less reactive than (I). Recently, Russian workers<sup>4</sup> have reported arylsulphonyl imidazolides as useful condensing reagents for the chemical synthesis of polynucleotides, but we found that the rate of reaction using arylsulphonyl triazolide is three times as fast as using arylsulphonyl imidazolide. Work is in progress to achieve the synthesis of a longer chain using mesitylenesulphonyl triazolide (I) as a condensing reagent.

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† Characterized satisfactorily by elemental and spectral analyses.

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<sup>2</sup> J. C. Catlin and F. Cramer, *J. Org. Chem.*, 1973, **38**, 245.

<sup>3</sup> J. J. Michniewicz, C. P. Bahl, K. Itakura, N. Katagiri, and S. A. Narang, *J. Chromatography*, 1973, **85**, 159.

<sup>4</sup> Yu. A. Berlin, O. G. Chakhmakheva, V. A. Efimov, M. N. Kolosov, and V. G. Korobko, *Tetrahedron Letters*, 1973, **16**, 1353.