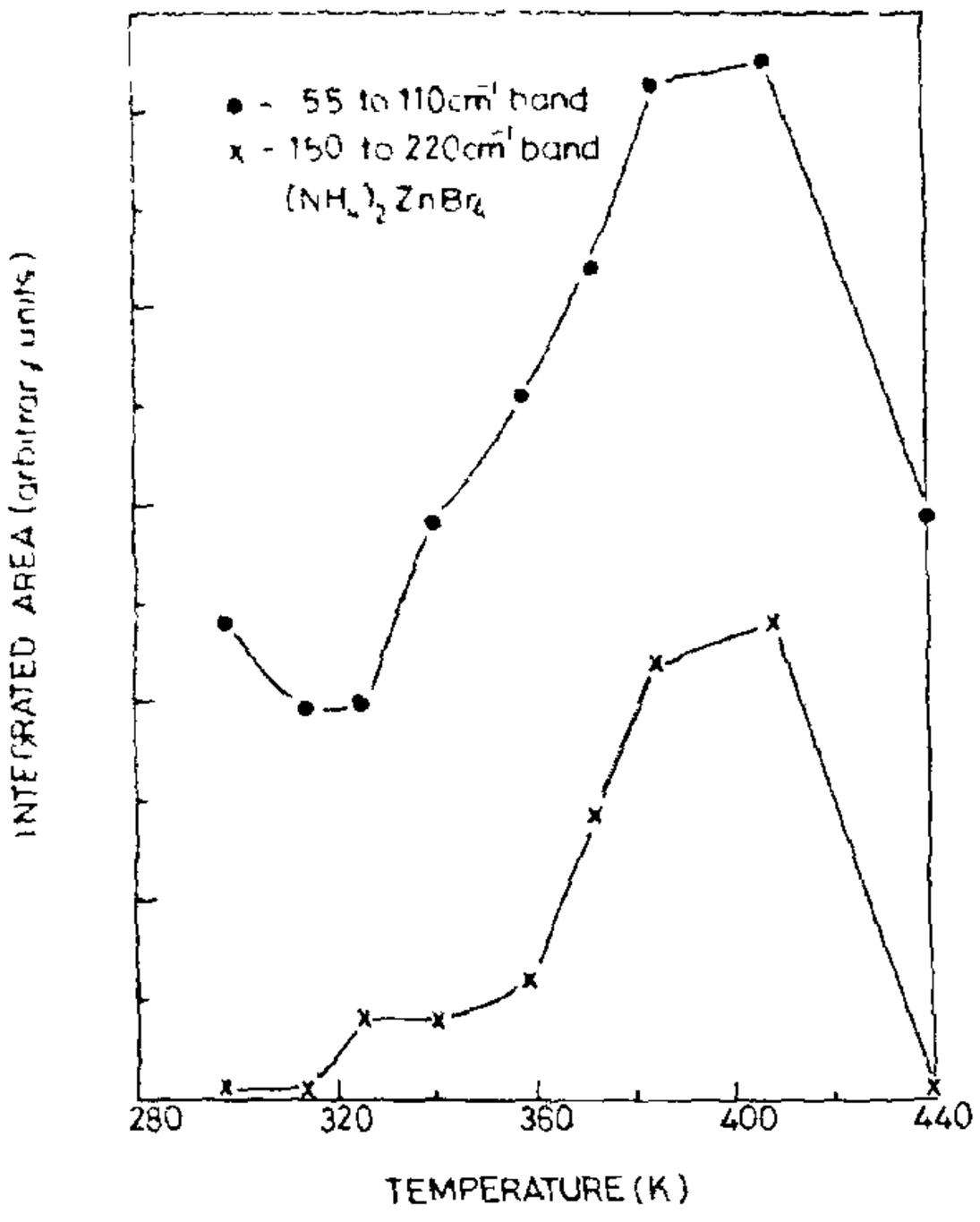


**Figure 3.** *Integrated areas of two bands vs temperature*

$(NH_4)_2ZnCl_4$[8]. No soft mode has been observed in $(NH_4)_2ZnBr_4$ but the present studies indicate a coupling of the internal vibrations of the $ZnBr_4^{2-}$ ion to the amplitude fluctuations in the incommensurate phase. The phase fluctuations probably give rise to the large increase in intensity of the total scattered light.

1 September 1984

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# A SIMPLIFIED PROTOCOL FOR THE SYNTHESIS OF OLIGODEOXY NUCLEOTIDES BY CONTINUOUS-FLOW PHOSPHOTRIESTER APPROACH ON SOLID SUPPORTS

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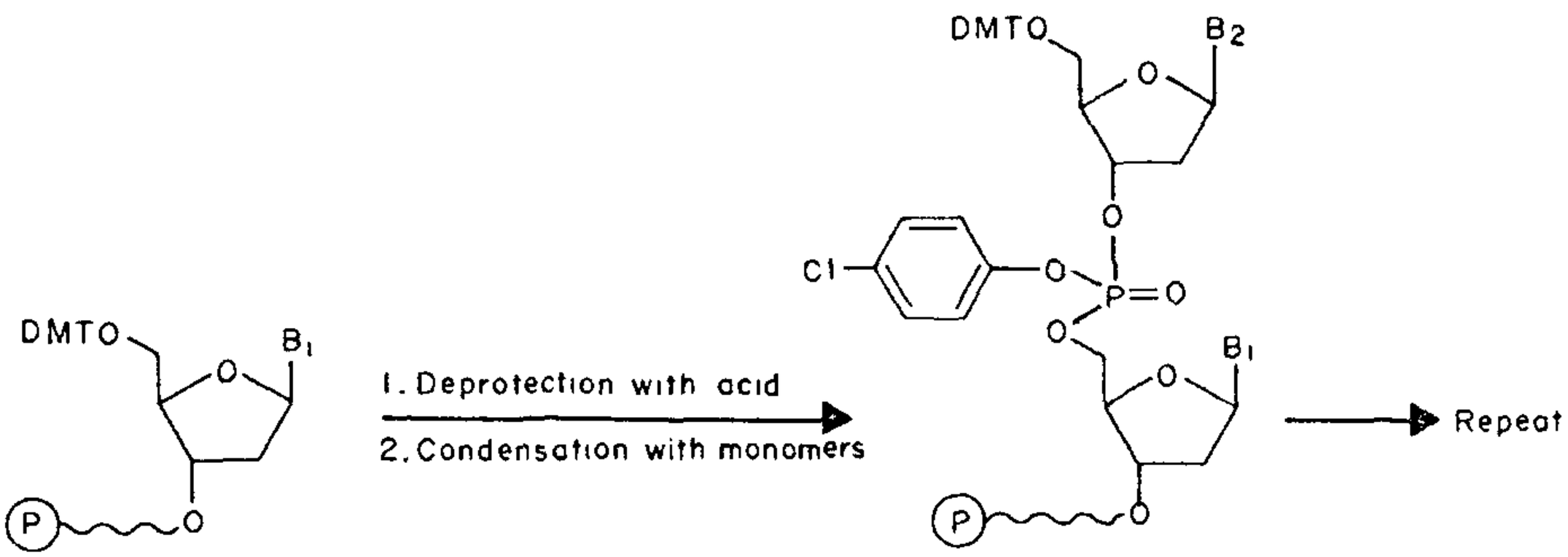
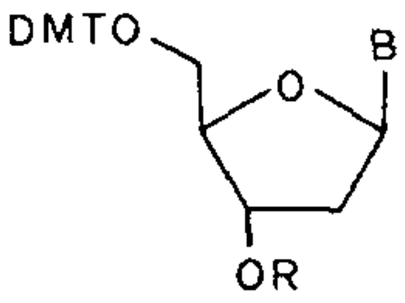
THE success of solid phase synthesis[1] of oligonucleotides (scheme 1) depends mainly on the choice of the support (chemical and mechanical stability), the condensing agent, the purities of the monomer building blocks and the solvents. There have been constant efforts in several laboratories to optimise each of these factors and extend the capability of the technique to routinely synthesise chains longer than 20 base pairs. The conditions become more stringent when continuous flow methods are adapted for solid phase synthesis where the resin is treated with reagents and solvents of volumes equivalent to 10 times the bed volume at each step. Typically the concentrations of the monomers used are about 4 equivalents over the resin loading value and these factors amplify the effect of minor reactive impurities, resulting in a gradual decrease of yield as the chain grows. Another important consideration is the minimum exposure to the acid washings to avoid undesirable depurination. Taking these facts into consideration and based on the idea mooted by Effimov *et al*[2] we present here a simplified protocol for routine synthesis of oligodeoxynucleotides on controlled pore glass resins by the phosphotriester approach. We have synthesised $d(G\text{-}C)_4$, $d(G\text{-}C)_5$ and d(GGTGGACCTC) by this method.

*Preparation of the monomers and functionalisation of the resin*

The protected nucleosides (I to IV) were prepared from the corresponding nucleosides (Sigma USA) by one-pot transient protection and tritylation[3]. They were then phosphorylated[4] with *p*-chlorophenyl phos-




**SCHEME 1**




I. B = $A^{Bz}$, R = H

II. B = $C^{Bz}$, R = H

III. B = T, R = H

IV. B = $G^{iBu}$, R = H

V. to VIII Same as I to IV with R = O‖P(O⁻ $Et_3NH^+$) - (4-chlorophenyl)

IX to XII Same as I to IV with R = CO $CH_2$ $CH_2$ COOH

phorodichloridate (prepared from $POCl_3$ and *p*-chlorophenol[5]) and the protected nucleotides (V to VIII) were purified as triethylammonium salts on short column chromatography over silica gel H (BDH, India). The nucleoside succinates (IX to XII) required for resin functionalisation were prepared by reaction of protected nucleosides (I to IV) with succinic anhydride and dimethylamino pyridine[4]. The controlled pore glass resin (Pierce) was functionalised by DCCI-mediated active anhydride procedure and the resin loadings were estimated by trityl analysis[4].

*Assembly of the oligonucleotide chains*

The assembly was done on an OMNIFIT (Cambridge, U.K.) manual DNA synthesiser equipped with a 6-way rotary valve and employing continuous flow method (driven by $N_2$ gas at 1.5 p.s.i) with a single system of solvents, acetonitrile:dichloromethane (8:2 v/v). Acetonitrile (E. Merck, India) was purified by refluxing over $P_2O_5$ for 5 hr, distillation, followed by refluxing and distillation over $CaH_2$. Dichloromethane (E. Merck, India) was passed over neutral alumina (Brockmann grade I) and distilled over $P_2O_5$. The resin (25 mg, 0.9 $\mu$m) was capped with 200 $\mu$l of acetonitrile:acetic anhydride: N-methyl imidazole (8:1:1, v/v) for 10 min before the synthesis and capping at subsequent steps was found unnecessary. The coupling mixture (100 $\mu$l) consisted of the monomer (12 mg, 13 $\mu$m) tri-isopropyl benzenesulphonyl chloride (20 mg, 65 $\mu$m), 10 $\mu$l of N-methyl imidazole in acetonitrile:dichloromethane (8:2, v/v). The following cycle was used with a flow rate of 1.5 ml/min.

| | |
|---|---|
| 1. Acetonitrile:dichloromethane (8:2, v/v) | 2 min. |
| 2. 3% Dichloroacetic acid in acetonitrile: dichloromethane (8:2, v/v) | 6 min. |
| 3. Acetonitrile:dichloromethane (8:2, v/v) | 2 min. |
| 4. Coupling (stop-flow) | 15 min. |

The yield at every step of condensation was 90–95% as monitored by a spectrophotometric estimation of the trityl washings. The trityl group was retained after the last coupling step.

At the end of the assembly, the resin was washed with dichloromethane and the oligonucleotide was recovered after deprotection in three stages: (i) The resin was treated with 4-nitrobenzaldoxime (35 mg) and 1,1,3,3-tetramethyl guanidine (25 $\mu$l) in 0.5 ml dioxane:water (1:1, v/v) for 20 hr with shaking at room temperature. It was then filtered off, washed with dioxane:water (1:1, 5 ml) and the filtrate was evaporated to a gum. (ii) The gum was treated with 15 ml of conc. ammonia (sp. gr. 0.91, BDH, India)

sealed in a flask and heated at 60 C for 20 hr. The excess ammonia was evaporated off. (iii) The product was then treated with 5 ml of 80 % acetic acid : water for 30 min at room temperature. It was extracted with ether and the aqueous layer was concentrated to get the crude product.

The products were purified by ion-exchange FPLC on Pharmacia Polyanion SI columns and the detailed methods will be reported elsewhere.

The key features of the present method are that (i) a single system of solvents has been used in the assembly procedure, reducing the number of mechanical manipulations, (ii) dichloroacetic acid has been used instead of trifluoro or trichloroacetic acids, as a good compromise between the efficiency of deprotection and minimal depurination, (iii) it avoids the use of troublesome pyridine unlike in most other phosphotriester methods, simultaneously retaining the efficiency of condensation, (iv) it competes effectively with the phosphite method in terms of speed, efficiency and ease, (v) all the protected nucleotides and the functionalised resins were made here at our laboratory using indegenious chemicals and reagents.

The author thanks Mr G V Rajendrakumar and Ms Sivakama Sundari for assistance in performing the experiments.

18 October 1984

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## SENSITISED PHOTOOXYGENATION OF ISORHAMNETIN-4′,5-DIMETHYLETHER

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DIOXYGENASE catalysed reactions are similar to sensitised photooxygenation involving singlet oxygen. A variety of reactions have been reported in the literature involving singlet oxygen with heterocyclic compounds[1–4]. The present study reports the reaction of singlet oxygen with isorhamnetin-4′,5-dimethylether.

Isorhamnetin-3,7-diglucoside isolated[5] from the fresh flowers of *Argemona mexicana* Linn. (Papaveraceae) was methylated and hydrolysed. The resulting product, isorhamnetin-4′,5-dimethylether (100 mg) was dissolved in pyridine containing a catalytic amount of the sensitiser (rose bengal, 5 mg). The solution was irradiated with 300 W tungsten lamp and air free from $CO_2$ was bubbled through it for 14 hr. The solvent was removed under reduced pressure and the residue extracted with ether and ethylacetate. The product was purified and the colourless solid that separated had $\lambda_{max}$(MeOH) 233, 264 nm and IR (Nujol mull) peaks at 3550(s), 2990(s), 1700, 1500, 1450(s), 1370, 1320, 1240, 1100 and 990 $cm^{-1}$.

The starting material (I) was recovered unchanged when the reaction was carried out without the sensitiser. It can thus be concluded that singlet oxygen produced from the interaction of triplet excited sensitiser with the ground state triplet oxygen reacts with isorhamnetin-4′,5-dimethylether to form the depsidë (II). The depside on hydrolysis gave 2-methoxy-4,6-dihydroxybenzoic acid and 3,4-dimethoxybenzoic acid both of which were appropriately characterised.

26 July 1984

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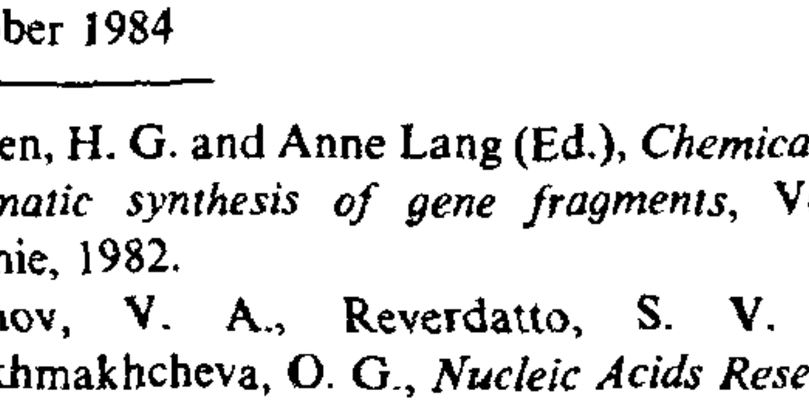
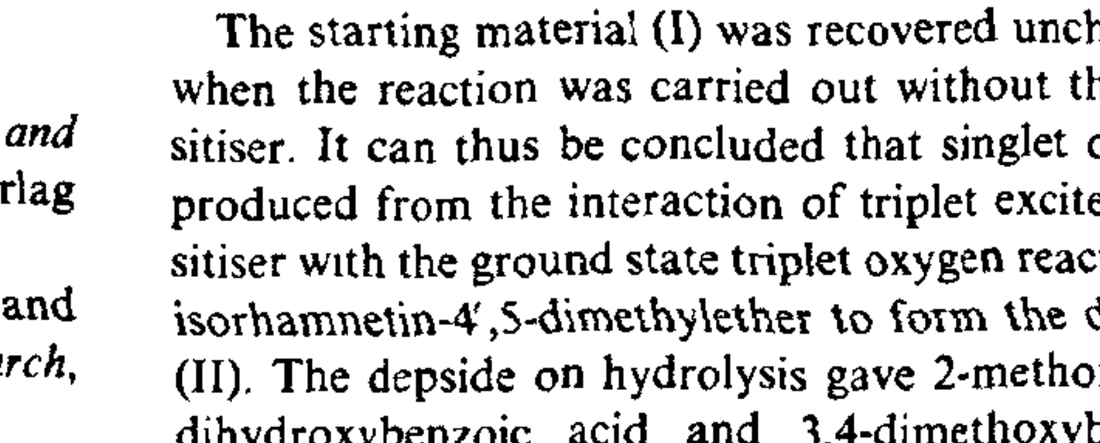



I




II

$R_1$ = Me, $R_2$ = H