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INTERNUCLEOTIDE CLEAVAGE DURING UNBLOCKING IN OLIGONUCLEOTIDE SYNTHESIS BY THE PHOSPHOTRIESTER APPROACH

J. H. van Boom, P. M. J. Burgers and P. H. van Deursen

Gorlaeus Laboratoria der Rijksuniversiteit, Postbus 75, Leiden, The Netherlands.

R. Arentzen and C. B. Reese

Department of Chemistry, King's College London, Strand, London WC2R 2LS, England.

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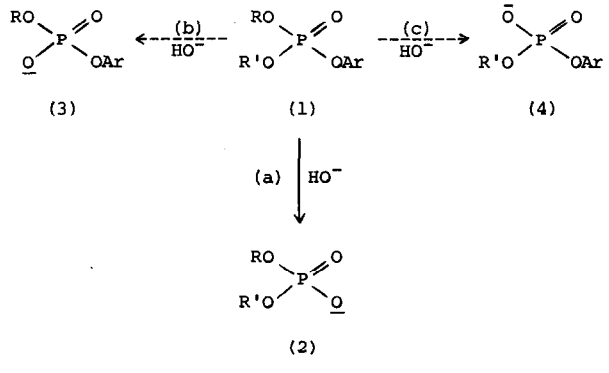
Our original choice[1] of phenyl as the protecting group for internucleotide linkages in the synthesis of oligonucleotides by the phosphotriester approach was based on the assumption that phenyl dialkyl phosphates (1; Ar=Ph) would undergo alkaline hydrolysis virtually exclusively by mode (a) (Scheme 1) to give dialkyl phosphates (2). We anticipated that the alternative modes of hydrolysis [(b) and (c)], involving alkoxide ion leaving groups, to give aryl alkyl phosphates [(3) and (4)] would be much less favourable.

Scheme 1



However, in a recent study[a] concerned with the synthesis of oligothymidylic acids by the phosphotriester approach, we found that unblocking[3] of fully-protected $(Tp)_7T$ (heptaphenyl ester) gave only 52% of the desired octamer. Chromatography of the hydrolysate on DEAE-cellulose and other chromatographic and electrophoretic evidence indicated clearly that the comparatively low yield was due to a significant amount of cleavage (corresponding to modes (b) and (c), Scheme 1), probably at each of the seven internucleotide linkages. The unblocking of fully-protected $(Tp)_3T$ (triphenyl ester) gave only 79% of the desired tetramer. Internucleotide cleavage occurs to at least the same extent in the unblocking of fully-protected oligoribonucleotides. Thus only 80% of UpUpU was obtained from the fully-protected trinucleoside diphosphate (diphenyl ester). Some of the possible products of hydrolysis of a fully-protected oligonucleotide are indicated in Scheme 2.

Scheme 2

$$\underline{Xp}Y\underline{p}Z\underline{p}\text{---} \longrightarrow XpYpZp\text{---} + [X + \underline{p}YpZp\text{---}] + [X\underline{p} + YpZp\text{---}] + [XpY + \underline{p}Zp\text{---}] +$$

$$\underline{p} = -O-\overset{\overset{O}{\|}}{\underset{\underset{OPh}{|}}{P}}-O-$$

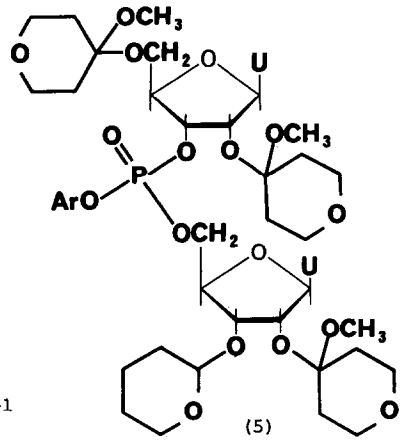
TABLE

Alkaline Hydrolysis of Fully-Protected Uridylyl-(3'→5')-Uridine Aryl Esters[a] (5) with 0.1 M-NaOH/Dioxan-Water (1:4, v/v) at 20°

Ar	pK_a of ArOH	$t_{\frac{1}{2}}$[b] (min)	Yield of Uridine[c] (%)
C_6H_5-	9.92	40	4.2
2-FC_6H_4-	8.72	7.5	2.4
2-ClC_6H_4-	8.40	6.5	1.8
3,5-$Cl_2C_6H_3$-	8.19	3.0	1.2
2,4-$Cl_2C_6H_3$-	7.85	2.5	0.8
2,5-$Cl_2C_6H_3$-	7.51	1.8	0.7

[a] The initial substrate concentrations were 0.001 M. [b] First-order kinetics were observed.

[c] Uridine, uridine 5'-phenyl phosphate and further hydrolysis products of uridine 3'-phenyl phosphate (uridine 2',3'-cyclic phosphate, uridine 2'- and 3'- phosphates) are formed by acidic hydrolysis (0.01 M-hydrochloric acid) of the alkaline hydrolysis products. The yield of uridine, which is estimated by liquid-liquid chromatography, is based on the possible formation of one molecule of uridine per molecule of (5).



U = uracil-1

(5)

An obvious method of decreasing the amount of internucleotide cleavage which accompanies unblocking is to modify the phenyl residue in such a way that the corresponding arylate ion becomes a better leaving group. As previously intimated[2], this may readily be accomplished by the introduction of halogen substituents in appropriate positions. The data in the Table clearly demonstrate that the extent of internucleotide cleavage, as indicated by the amount of uridine released, in the unblocking of fully-protected uridylyl-(3'→5')-uridine (5) can be decreased by, for example, a factor of six by the introduction of chloro-substituents in the 2- and 5- positions of the phenyl residue. Thus, by adopting this modification, the extent of cleavage can be kept below 1% per internucleotide linkage. It should then be possible to obtain quite acceptable yields in the unblocking even of comparatively large fully-protected oligonucleotides.

Although this approach is undoubtedly useful, it should be noted (Table) that a decrease in the amount of internucleotide cleavage is accompanied by an even larger increase in the rate of phosphotriester hydrolysis.[4] Thus the fully-protected 2,5-dichlorophenyl ester of UpU (5; Ar = 2,5-$Cl_2C_6H_3$-) undergoes alkaline hydrolysis *ca.* 22 times as fast as the phenyl ester (5; Ar=Ph). Such an increase in the rate of phosphotriester hydrolysis introduces a new problem if the aryl protecting groups are to be used in conjunction with other base-labile protecting groups (*e.g.* for alcoholic hydroxyl functions) and if it is desirable that the latter groups should be selectively removable.

However, it very fortunately appears that it will not be necessary to use 2,4- or 2,5- dichlorophenyl protecting groups to prevent the amount of cleavage per internucleotide linkage from exceeding 1%. We have recently observed that phosphotriester hydrolysis with 0.1 M-potassium hydroxide in water-dimethyl sulphoxide (1:9, v/v) leads to appreciably less internucleotide cleavage than has been observed previously[2] with sodium hydroxide in aqueous dioxan solution. Thus experiments with phenyl esters both in the ribose (with fully-protected UpUpU) and deoxyribose (with fully-protected TpTpTpT) series suggest that the extent of internucleotide cleavage is ca. 3 times less in 90% dimethyl sulphoxide than in aqueous dioxan solution, under the conditions described above. Preliminary experiments have suggested that hydrolysis with 7 M-ammonia in water-dimethyl sulphoxide (1:1, v/v) leads to even less internucleotide cleavage. We are therefore actively investigating the properties of other hydrolytic media in the hope that it will be possible to use the phenyl protecting group in the synthesis of comparatively large oligonucleotides and still confine the amount of internucleotide cleavage in the unblocking process within reasonable limits.

We thank the Nuffield Foundation for financial support.

Footnotes and References

[1]C. B. Reese and R. Saffhill, Chem. Comm., 767 (1968).

[2]N. J. Cusack, C. B. Reese and J. H. van Boom, Tetrahedron Letters, 2209 (1973).

[3]Unblocking was carried out at 20° first by treating the fully-protected oligomer with 0.2 M-NaOH in dioxan-water (1:1, v/v). The terminal 3'- and 5'- acid-labile protecting groups were then removed by hydrolysis with hydrochloric acid (pH 2).

[4]It can also be seen from the Table that an increase in the dissociation constant of the phenol has a proportionately smaller effect on the rate of phosphotriester hydrolysis.