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SOLID PHASE SYNTHESIS OF THE 3'-TERMINAL NONADECARIBONUCLEOSIDE OCTADECAPHOSPHATE SEQUENCE OF YEAST ALANINE TRANSFER RIBONUCLEIC ACID†

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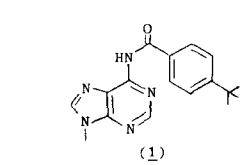
Summary: The rapid synthesis of the 3'-terminal decaribonucleoside nonaphosphate and nonadecaribonucleoside octadecaphosphate sequences of yeast $tRNA^{Ala}$ by the phosphoramidite approach on controlled pore glass is described; the synthetic products were found to be identical to the authentic oligoribonucleotides, prepared by the phosphotriester approach in solution.

Considerable progress has been made in recent years in the rapid chemical synthesis of oligo- and poly-deoxyribonucleotides (i.e. DNA sequences) on solid supports[1]. Progress has also been made in the chemical synthesis of oligo- and poly-ribonucleotides (i.e. RNA sequences) in solution[2,3], but this is still a time-consuming and experimentally demanding process. It is clearly a matter of great importance in connection with the development of certain areas of biological research that synthetic RNA should become more readily accessible.

Several reports concerned with the rapid solid phase synthesis of RNA sequences have already appeared[4-8] in the literature. The choice of the protecting group for the 2'-hydroxy functions is a matter of paramount importance in polyribonucleotide synthesis, and one of the main considerations to be taken into account in its selection is that it should be stable under the conditions required for 5'-unblocking. While all of the procedures so far reported involve the use of acid-labile modified trityl protecting groups for the 5'-hydroxy functions, the *tert*-butyldimethylsilyl[4,8], 2-nitrobenzyl[7] and tetrahydropyranyl[5,6] groups have been used for 2'-hydroxyl protection in solid phase polyribonucleotide synthesis. Although no satisfactory alternatives to modified trityl groups appear so far to be available for the protection of the 5'-hydroxy functions, we believe that it is desirable also to protect the 2'-hydroxy functions with an acid-labile protecting group[9]. As it seemed clear from recent studies[10,11] that the tetrahydropyranyl and methoxytetrahydropyranyl groups were insufficiently stable under the acidic conditions required for the removal of the 4,4'-dimethoxytrityl (DMTr) or 9-phenylxanthen-9-yl (Px) protecting groups, we have developed the 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl [Ctmp, as in (11)] group[12] for the protection of the 2'-hydroxy functions in rapid RNA synthesis. We now report the solid phase synthesis of the 3'-terminal decaribonucleoside nonaphosphate and the nonadecaribonucleoside octadecaphosphate sequences of yeast alanine transfer RNA ($tRNA^{Ala}$), using the phosphoramidite approach[1b] and the Ctmp and Px groups for the protection of the 2'- and 5'-hydroxy functions, respectively.



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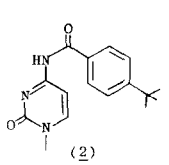

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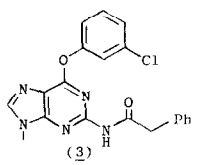

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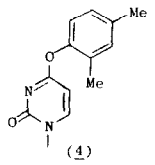

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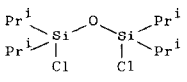

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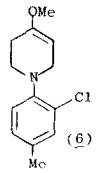

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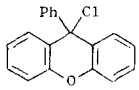

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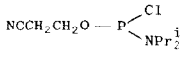

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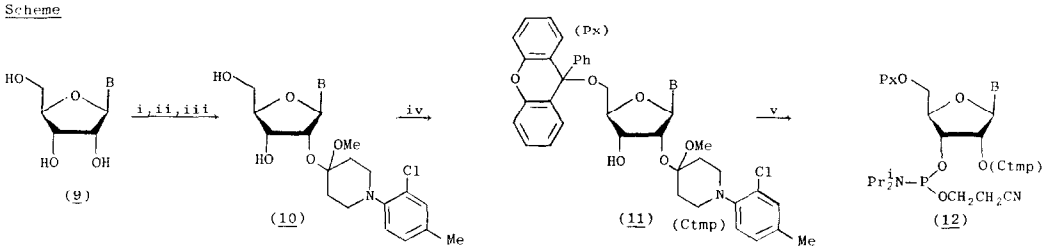
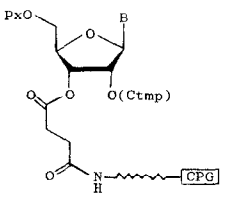
Scheme

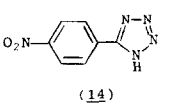


Reagents: (i) (5), imidazole/MeCN; (ii) (6), CF_3CO_2H/CH_2Cl_2; (iii) Et_4NF/MeCN; (iv) (7)/C_5H_5N; (v) (8), Pr^i_2NEt/tetrahydrofuran

The procedure for the preparation of the phosphoramidite building blocks is outlined in the Scheme. The base-protected ribonucleosides [(9; B = 1), (9; B = 2), (9; B = 3) and (9; B = 4)][2,13] were each converted in three steps into the corresponding 2'-O-(Ctmp) derivatives [(10; B = 1), (10; B = 2), (10; B = 3) and (10; B = 4), respectively] in overall yields of 39, 52, 57 and 38%. The latter compounds were then converted into the corresponding 9-phenylxanthen-9-yl derivatives (11; B = 1 - 4) in good yields (78-88%), and these products were allowed to react with 2-cyanoethyl di-isopropylphosphoramidochloridite[14] (8), under nitrogen, in the presence of di-isopropylethylamine in tetrahydrofuran solution at room temperature to give the required phosphoramidite building blocks (12; B = 1 - 4) also in good yields[15].



(13) a; B = (1)



(14)

The half-succinate ester[16] of the 2',5'-protected adenosine derivative (11; B = 1) was attached to functionalized controlled-pore glass (CPG)[17] in the usual way[16] to give the loaded support (13a). The nucleoside loading of (13a) was estimated to be 31 μmol/g. The synthesis of the 3'-terminal decaribonucleoside nonaphosphate sequence of yeast $tRNA^{Ala}$ was undertaken first. An Omnifit apparatus was used. At the end of 9 synthetic cycles[18], the solid

support was subjected to a three step procedure[20] that led to the release of the synthetic oligoribonucleotide from the solid support and to its total unblocking. The aqueous products were fractionated by ion-exchange liquid chromatography (LC) (Fig. 1a): the main peak (R_t = 11.5 min) was found to be homogeneous by LC on a reverse phase column and by gel electrophoresis (Fig. 2a). Furthermore, the electrophoretic mobility of this putative UpCpGpUpCpCpApCpCpA, synthesized on a solid support, was found to be identical [reverse phase LC and gel electrophoresis (Fig. 2a)] to authentic material synthesized[2,21] by the phosphotriester approach in solution. LC Analysis (reverse phase column) of the four ribonucleosides that resulted from the total digestion of UpCpGpUpCpCpApCpCpA in the presence of snake venom phosphodiesterase and alkaline phosphatase, revealed that, within the margin of experimental error, they had been obtained in the expected proportions.

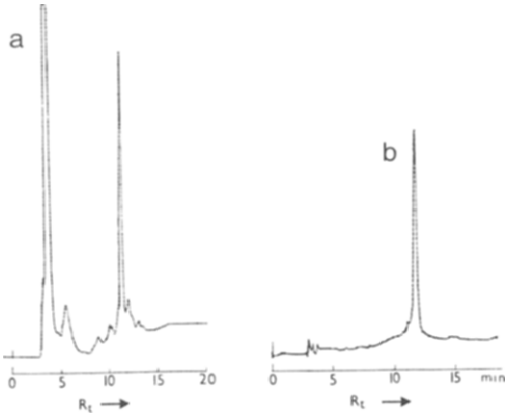


FIGURE 1: (a) LC profile (Hypersil APS column, eluted with pH 6.8 potassium phosphate buffer) of the crude products obtained in the solid phase synthesis of UpCpGpUpCpCpApCpCpA; (b) LC profile (APEX ODS column, eluted with 0.1 M triethylammonium acetate (pH 7.0)/acetonitrile) of the purified products obtained in the solid phase synthesis of ApUpUpCpCpGpGpApCpUpCpGpUpCpCpApCpCpA.

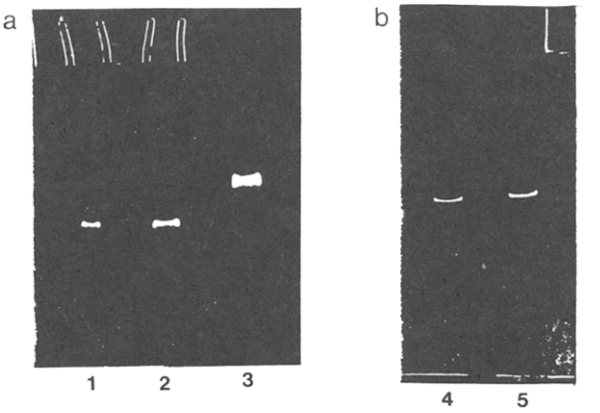


FIGURE 2: (a) Gel electrophoretogram (visualized by ultraviolet light) of UpCpGpUpCpCpApCpCpA, synthesized by the phosphotriester approach in solution (track 1), UpCpGpUpCpCpApCpCpA, synthesized on a solid support and purified by LC (track 2), ApUpUpCpCpCpGpApCpUpCpGpUpCpCpApCpCpA, synthesized on a solid support and not further purified (track 3); (b) Gel Electrophoretogram of purified ApUpUpCpCpGpGpApCpUpCpGpUpCpCpApCpCpA, synthesized on a solid support (track 4) and by the phosphotriester approach in solution (track 5).

The 3'-terminal nonadecanucleoside octadecaphosphate sequence of yeast $tRNA^{Ala}$ was synthesized on the loaded support (13a) in the same way. After the 18 appropriate cycles[18], the same three step releasing and unblocking procedure[20] was carried out to give putative ApUpUpCpCpGpGpApCpUpCpGpUpCpCpApCpCpA. The aqueous products were analyzed by gel electrophoresis and found to migrate as a single ultraviolet-absorbing band that was appreciably less mobile (Fig. 2a) than UpCpGpUpCpCpApCpCpA; after fractionation by LC on a reverse phase column, this material was homogeneous (Fig. 1b). When the latter purified material was analyzed by gel electrophoresis (Fig. 2b), it migrated as a sharp band with a mobility identical to that of authentic ApUpUpCpCpGpGpApCpUpCpGpUpCpCpApCpCpA, synthesized[2,21] by the phosphotriester approach in solution. The proportions of the four nucleosides obtained after enzymatic digestion (see above) were again as expected.

We believe that these preliminary studies clearly demonstrate the compatibility of the 2'-O-Ctmp and 5'-O-Px protecting groups in rapid RNA synthesis on a solid support. We further believe that the results obtained suggest that the automated synthesis of RNA should now be as easy and straightforward as that of DNA sequences.

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REFERENCES AND FOOTNOTES

† This article is dedicated to Professor Wolfgang Pfleiderer on the occasion of his sixtieth birthday.

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15 The yields [and ^{31}P n.m.r. spectra ($CDCl_3$, 36.4 MHz)] of (12; B = 1), (12; B = 2), (12; B = 3) and (12; B = 4) were 82% [δ 149.3, 151.5], 91% [δ 149.0, 151.3], 81% [δ 149.0, 151.5] and 86% [δ 149.1, 150.8], respectively.

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18 The synthetic cycle, which was closely similar to that used in the synthesis of DNA sequences by the phosphoramidite approach[16], consisted of the following ten steps: (1) 5'-unblocking [3% $CF_3CO_2H/ClCH_2CH_2Cl$, 1 min]; (2) washing [$ClCH_2CH_2Cl$, 4 min]; (3) washing [MeCN, 6 min]; (4) coupling [(12) (20 mol. equiv.), (14) (40 mol. equiv.)/MeCN, 15 min]; (5) washing [MeCN, 4 min]; (6) capping [Ac_2O, DMAP, 2,6-lutidine/tetrahydrofuran, 2 min]; (7) washing [MeCN, 3 min]; (8) oxidation [I_2 (50 mol. equiv.), H_2O, pyridine/tetrahydrofuran, 2 min]; (9) washing [MeCN, 5 min]; (10) washing [$ClCH_2CH_2Cl$, 4 min]. Solid phase synthesis was carried out on 0.03g (*ca.* 0.9 μmol) of functionalized controlled pore glass. 5-(4-Nitrophenyl)tetrazole[19] (14) was used for phosphoramidite activation and coupling [step (4)] was allowed to proceed for 15 min to ensure, as far as possible, that it went to completion. The extent of coupling in each cycle was monitored by the spectrophotometric assay of Px^+ ions released during 5'-unblocking [step (1)]; it was estimated to average *ca.* 93%.

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20 The three step procedure[2] involves treatment with (i) 0.3M-(N^1,N^1,N^3,N^3-tetramethylguanidinium) *E*-2-nitrobenzaldoximate in acetonitrile, 35°C, 17 hr; (ii) concentrated aqueous ammonia (*d* 0.88), 60°C, 17 hr; (iii) 0.01M-hydrochloric acid (pH 2), room temperature, 24 hr. Step (i) is required[2b] in order to unblock O-4 and O-6 of the uracil and guanine residues, respectively.

21 J.M. Brown, C. Christodoulou, A.S. Modak, C.B. Reese, and H.T. Serafinowska, unpublished observations.

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