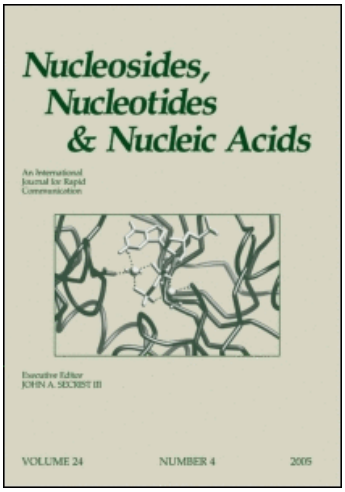

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A NEW APPROACH TO THE SYNTHESIS OF LINEAR AND CYCLIC OLIGORIBONUCLEOTIDES

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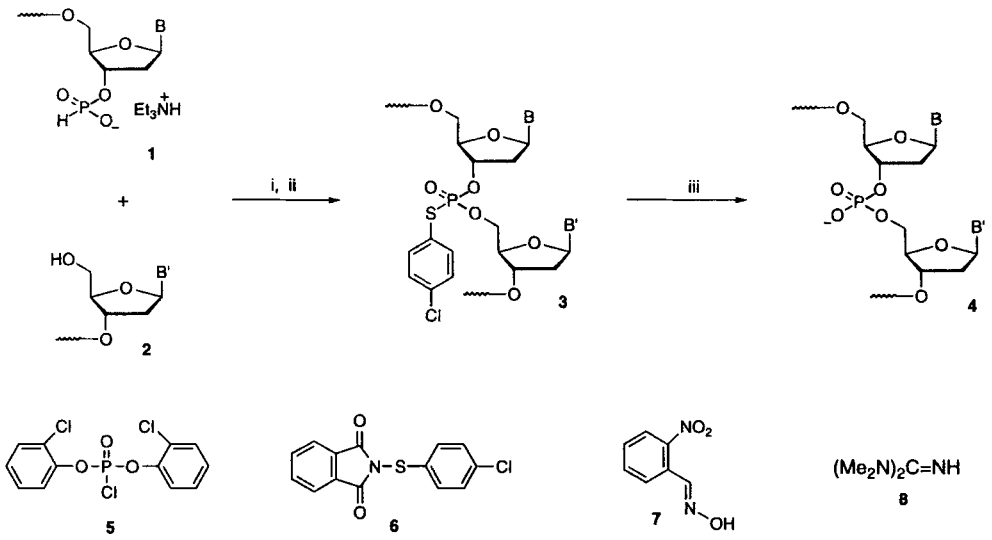
Abstract: The tetraribonucleoside triphosphate **15** and the cyclic tetraribonucleotide **16** have been prepared by a recently reported triester approach in solution, involving *H*-phosphonate coupling.

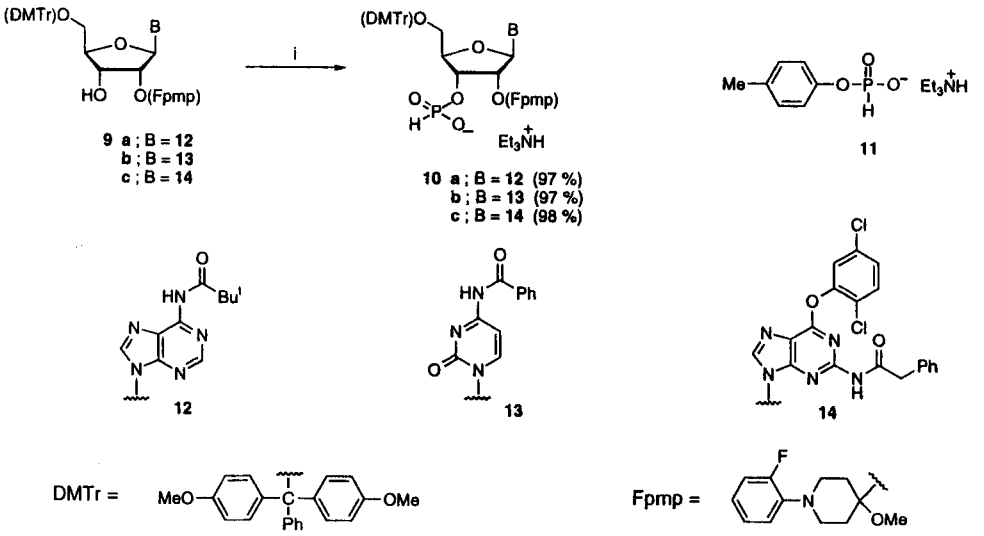
We recently reported[1] a new approach, based on *H*-phosphonate coupling[2], to the synthesis of oligodeoxyribonucleotides in solution. This approach is indicated in outline in Scheme 1. *H*-Phosphonate coupling between a protected nucleoside or oligonucleotide 3'-*H*-phosphonate **1** and a protected nucleoside or oligonucleotide with a free 5'-hydroxy function **2** may be effected rapidly by treatment with di-(2-chlorophenyl) phosphorochloridate **5** at -40°C. The intermediate *H*-phosphonate diester is not isolated but is allowed to react *in situ* with *N*-[(4-chlorophenyl)-sulfanyl]phthalimide[3] **6**, also at -40°C, to give the fully-protected dinucleoside phosphoro-



Scheme 1 *Reagents and conditions*: i, **5**,C_5H_5N, CH_2Cl_2, -40 °C, 5-10 min; ii, a, **6**, C_5H_5N, CH_2Cl_2, -40 °C, 15 min; b, C_5H_5N - H_2O, -40 °C to room temp.; iii, **7**, **8**, MeCN, room temp., 12 h

thioate **3**. Side-reactions are not observed. In the first unblocking step (step iii), the *S*-(4-chlorophenyl) phosphorothioate **3** is converted smoothly and quantitatively into the corresponding phosphodiester **4** by treatment with *E*-2-nitrobenzaldoxime[4] **7** and N^1, N^1, N^3, N^3-tetramethylguanidine (TMG) **8** in acetonitrile solution. The subsequent unblocking steps required will depend on the other protecting groups that have been used.

We have since further developed this methodology, and now report its application to the synthesis both of linear and cyclic oligoribonucleotides. In order to prepare the target sequences, triethylammonium 5'-*O*-(DMTr)-2'-*O*-(Fpmp)-ribonucleoside 3'-*H*-phosphonates **10 a-c**, derived from adenosine, cytidine and guanosine, were required. The latter building blocks **10 a-c**, were prepared (Scheme 2) in 97, 97 and 98% yields, respectively, by a previously reported procedure[5], and were isolated as stable colourless solids.



Scheme 2 *Reagents and conditions*: i, a, **11**, Me_3C·COCl, C_5H_5N, -30 °C, 30 min, b, H_2O, C_5H_5N, room temp., 1h

r(ApCpGpU)

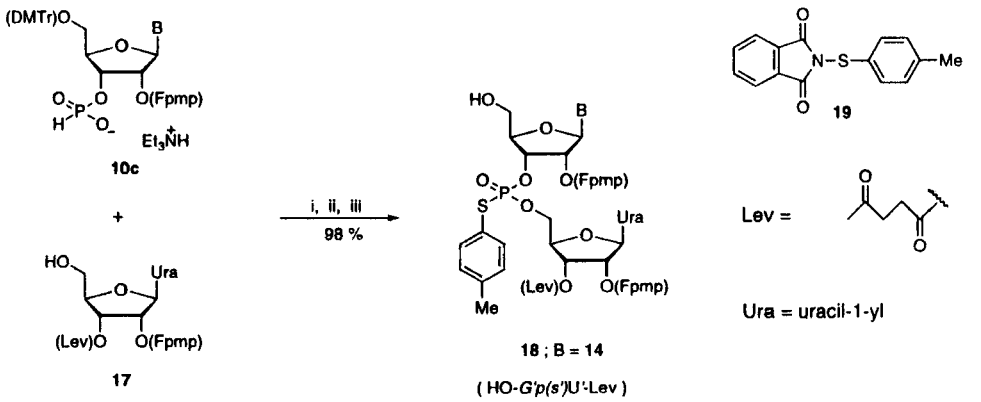
15

r(ApCpGpUp)

16

In an initial study, it was decided to prepare the tetraribonucleoside triphosphate **15** and the cyclic tetraribonucleotide **16**. The coupling procedure used in the synthesis of oligoribonucleotides was identical to that used previously[1] in oligodeoxyribonucleotide synthesis. Thus, the guanosine-derived *H*-phosphonate building block **10c** (1.2 mol equiv.) and 2'-*O*-(Fpmp)-3'-*O*-levulinoyluridine[6] **17** (1.0 mol equiv.) were allowed to react (Scheme 3) with di-(2-chlorophenyl) phosphorochloridate **5** (3.0 mol equiv.) in dry pyridine-dichloromethane (9 : 1

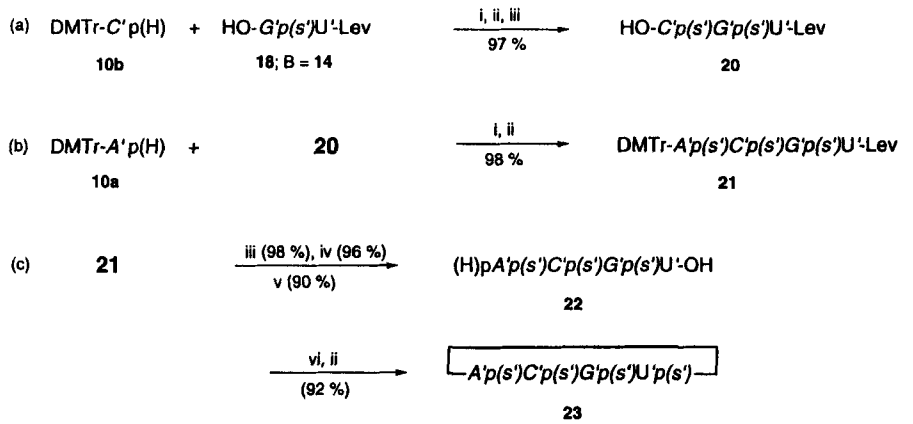
v/v) at -40°C. After 10 min, *N*-[(*p*-tolyl)sulfanyl]phthalimide[7] **19** (2.0 mol equiv.) was added while the reactants were maintained at -40°C. After 15 min, the products were worked-up and treated with trifluoroacetic acid to remove the 5'-*O*-(DMTr) protecting group. Following its chromatographic purification, the partially-protected dinucleoside phosphorothioate **18**; B = **14** was isolated as a colourless solid in 98% overall yield. A number of years ago, one of us introduced[8] a system of abbreviations for protected oligoribonucleotides in which ribonucleoside residues are italicized (as in *A*, *C* and *G*) if their base residues are protected and a prime is added (as in *A'*, *C'*, *G'* and U') if their 2'-hydroxy functions are protected. The protecting groups used in this study are indicated in Scheme 2. Internucleotide linkages are also italicized if they are protected. Thus, in this study, *-p(s')-* represents an *S*-(*p*-tolyl)-protected phosphorothioate internucleotide linkage (as in **18**). A terminal *H*-phosphonate (i.e. -p(H)) is not italicized as it is not protected. Using this system, the partially-protected diribonucleoside phosphorothioate **18**; B = **14** is abbreviated to HO-*G'p(s')*U'-Lev.



Scheme 3 *Reagents and conditions*: i, **5**, C_5H_5N, CH_2Cl_2, -40 °C, 10 min; ii, **19**, C_5H_5N, CH_2Cl_2, -40 °C, 15 min; iii, CF_3CO_2H, CH_2Cl_2, room temp., 1 min

The three step procedure (Scheme 3) used for the preparation of the partially-protected diribonucleoside phosphorothioate **18**; B = **14** was also used successfully in its conversion (Scheme 4(a)) to the partially-protected triribonucleoside diphosphorothioate **20**, which was isolated as a colourless solid in 97% yield. The fully-protected tetramer, DMTr-*A'p(s')C'p(s')G'p(s')*U'-Lev **21** was similarly prepared (Scheme 4(b)) from the latter intermediate **20** in 98% isolated yield. Finally, the five step procedure for the conversion of DMTr-*A'p(s')C'p(s')G'p(s')*U'-Lev **21** into the fully-protected cyclic tetramer **23** is indicated in outline in Scheme 4(c). The strategy adopted, which involved the intramolecular coupling of a 5'-*H*-phosphonate with a 3'-hydroxy function, was chosen as it seemed possible that, at high dilution, phosphorylation of a 5'-hydroxy function (but not of a much more hindered 3'-hydroxy function)

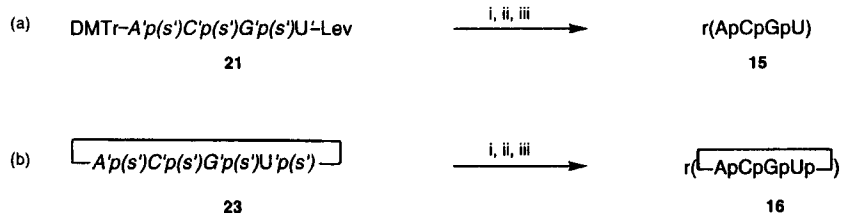
with di-(2-chlorophenyl) phosphorochloridate **5** might, to some extent, compete with the desired cyclization reaction. Following the removal of the 5'-*O*-(DMTr) group, the intermediate HO-*A'p(s')C'p(s')G'p(s')*U'-Lev was converted (step iv, 96% yield) into its 5'-*H*-phosphonate. Relatively large excesses of triethylammonium *p*-tolyl *H*-phosphonate[5] **11** (*ca.* 8 mol equiv.) and pivaloyl chloride (*ca.* 10 mol equiv.) were used. The least satisfactory step (step v, 90% yield) was the removal of the 3'-*O*-levulinoyl group as this reaction[9] could not easily be monitored by thin layer chromatography. Cyclization was effected at high dilution by adding a solution of (H)p*A'p(s')C'p(s')G'p(s')*U'-OH **22** in dichloromethane dropwise over a period of 15 min to a solution of di-(2-chlorophenyl) phosphorochloridate **5** (20 mol equiv.) in pyridine at -40°C. The final concentration of tetramer in the reaction medium was *ca.* 0.004 mol dm^{-3}. Following treatment of the intermediate cyclic *H*-phosphonate diester with *N*-[(*p*-tolyl)sulfanyl]phthalimide **19**, the fully-protected cyclic tetramer **23** was isolated as a colourless solid in 92% yield.



Scheme 4 *Reagents and conditions*: i, **5**, CH_2Cl_2 - C_5H_5N (1 : 9 v/v), -40 °C, 10 min; ii, **19**, CH_2Cl_2, C_5H_5N, -40 °C, 15 min; iii, CF_3CO_2H, CH_2Cl_2, room temp., 1 min; iv, a, **11**, Me_3C·COCl, C_5H_5N, -30 °C, 30 min, b, H_2O,-30 °C to room temp., 1 h; v, N_2H_4·H_2O, C_5H_5N - AcOH (4 : 1 v/v), room temp., 10 min; vi, **5**, CH_2Cl_2 - C_5H_5N (1 : 5 v/v), -40 °C, 20 min

Small quantities (0.025 g) of the fully-protected tetramer **21** and the fully-protected cyclic tetramer **23** were unblocked by a three step process (Scheme 5). Treatment with *E*-2-nitrobenzaldoxime **7** and TMG **8** in acetonitrile solution at room temperature (step i) led smoothly to the conversion of the phosphorothioate triester groups (as in **18**) into standard phosphodiester internucleotide linkages, and to the removal[10] of the 6-*O*-(2,5-dichlorophenyl) protecting group from the guanine residue. The *N*-acyl protecting groups were then removed from the adenine, cytosine and guanine residues by heating with concentrated aqueous ammonia at 50°C (step ii). This treatment also led to the removal of the 3'-*O*-levulinoyl group from the acyclic tetramer. Finally, the 2'-*O*-(Fpmp) protecting groups were removed from all of the sugar residues by

treatment with pH 4.0 sodium acetate buffer at 40°C (step iii). This also led to the removal of the 5'-*O*-(DMTr) protecting group from the acyclic tetramer. Both the tetraribonucleoside triphosphate **15** and the cyclic tetraribonucleotide **16** were isolated as their pure triethylammonium salts following chromatography on DEAE Sephadex A25. The isolated yield of tetraribonucleoside triphosphate **15** was 210 A_{260} units, starting from *ca.* 7.7 μmol of fully-protected material **21**, and the isolated yield of cyclic tetranucleotide **16** was 230 A_{260} units, starting from *ca.* 8.3 μmol of fully-protected material **23**. The ^{31}P NMR spectra and reverse phase HPLC profiles of both fully-unblocked tetramers **15** and **16** are illustrated in Figure 1.



Scheme 5 *Reagents and conditions*: i, **7**, **8**, MeCN, room temp., 12 h; ii, conc. aq. NH_3 (*d* 0.88), 50 °C, 15 h; iii, 0.5 mol dm^{-3} aq. NaOAc buffer (pH 4.0), 40 °C, 5 h

Confirmation of the constitutions of the tetraribonucleoside triphosphate **15** and the cyclic tetraribonucleotide **16** was provided by HPLC analysis of their enzymatic digests. Both tetramers were completely converted to their constituent nucleosides by digestion with snake venom phosphodiesterase and bacterial alkaline phosphatase. The adenosine : cytidine : guanosine : uridine ratios for the digests obtained from **15** and **16** were estimated by reverse phase HPLC to be 1.00 : 1.00 : 1.08 : 1.00 and 0.98 : 1.00 : 1.01 : 0.97, respectively. Digestion of the tetranucleoside triphosphate **15** with ribonuclease A gave two components with R_t [250 x 4.6 mm Hypersil ODS 5μ column, gradient eluted with 0.1 mol dm^{-3} aq. triethylammonium acetate -

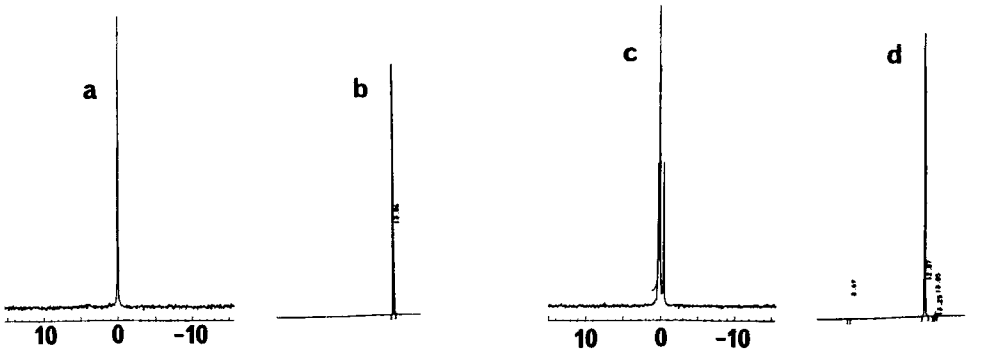


Figure 1 (a) ^{31}P NMR spectrum (D_2O) of the tetraribonucleoside triphosphate **15**, (b) reverse phase HPLC profile of **15**, (c) ^{31}P NMR spectrum (D_2O) of the cyclic tetraribonucleotide **16**, and (d) reverse phase HPLC profile of **16**.

acetonitrile (3 : 97 to 15 : 85 v/v) over 15 min] 11.2 and 11.5 min. Ribonuclease A digestion of the cyclic tetranucleotide **16** gave two components with R_t 10.2 and 11.2 min. When **15** and **16** were digested with ribonuclease A and bacterial alkaline phosphatase, the same two components (R_t 11.5 and 12.5 min) were obtained. These digestion products were identified as r(GpU) and r(ApC), respectively, by comparison with authentic material, purchased from the Sigma - Aldrich Company. The components with R_t 10.2 and 11.2 min were assumed to be r(GpUp) and r(ApCp), respectively.

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