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THE SYNTHESIS OF 2'-5' LINKED OLIGORIBONUCLEOTIDES*

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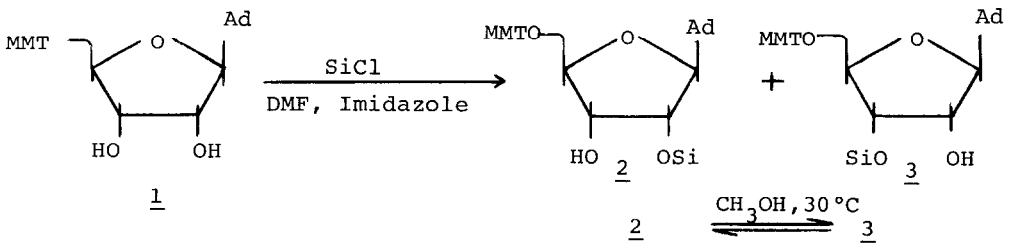
The use of alkylsilyl protecting groups provides a rapid route to 3',5'-diprotected ribonucleosides which are easily coupled via the phosphorodichloridite procedure to produce 2'-5' linked ribonucleotides.

It has recently been demonstrated[1-3] that 2'-5' linked oligoadenylic acids act as inhibitors of cell-free protein synthesis. These 2'-5' linked nucleotides of which the trimer is most active are formed from ATP by an enzyme that is activated in interferon-treated cell extracts. We wish to demonstrate that our recent developments in the synthesis of oligoribonucleotides[4,5] permit the rapid synthesis of 2'-5' linked oligoribonucleotides. Specifically this report deals with the synthesis of Ap(2'-5')Ap(2'-5')A (7).

The major advance of our procedure is in the use of the alkylsilyl protecting groups for the rapid synthesis of protected ribonucleosides which can be directly coupled by the chlorophosphite procedure[6] to form ribonucleotides. In this example 5'-monomethoxytrityladenosine (MMT-A, 1) can be converted in 30 min to a mixture of the 2'-silyl and 3'-silyl derivatives 2 and 3. The procedure in DMF requires imidazole (3 eq) and t-butyldimethylsilyl chloride (Si-Cl, 1.5 eq). The isolated yields of 2 and 3 are 35% and 42% respectively. Compound 2 can be used directly in the synthesis of natural 3'-5' linked nucleotides while 3 leads directly to 2'-5' linked compounds.

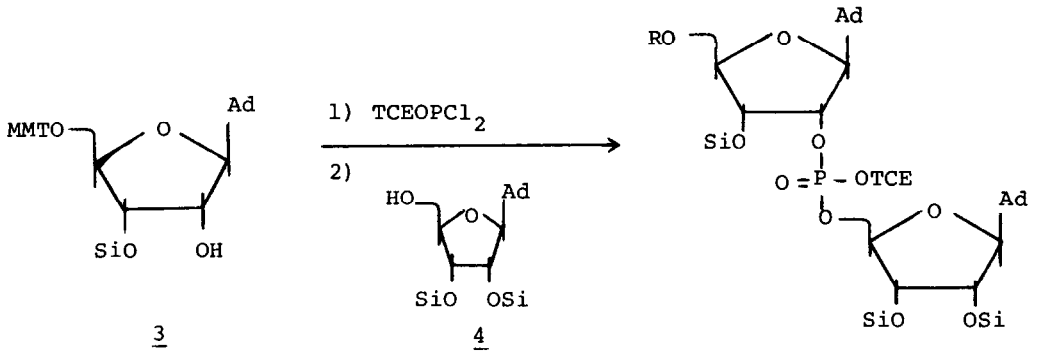


1 2 3

An important observation is that while the silyl groups are stable in most solvents, isomerization between 2 and 3 occurs in methanol. For example the addition of pure 2 to methanol leads to a mixture of 55% 2 and 45% 3 after 24 h at 30°C. Consequently the effective yield of either 2 or 3 from 1 can be greatly increased.

The synthesis of the trinucleotide 7 is rapidly accomplished. Compound 3 (0.71 mmole) dissolved in dry THF (1.05 ml) was added over a 10 min period to trichloroethylphosphorodichloridite ($TCEOPCl_2$, 0.71 mmole) dissolved in dry THF (1.05 ml) containing collidine (3 mmole) at -78°C. The solution was stirred at -78°C for a total of 45 min. At this point 2',3'-di-t-butyldimethylsilyladenosine[5] (4, 0.64 mmole) in THF (2 ml) was added and stirring was continued at -78°C for 1 h. The solution was allowed to warm to room temperature and iodine (1.3 mmole) in THF-H_2O (4.5 ml, 2:1, containing a few drops of pyridine) was added. After 5 min at room temperature the solution was concentrated at reduced pressure and the residue was dissolved in chloroform. The chloroform solution was washed with an aqueous sodium bisulfite solution (4 ml of saturated sodium bisulfite solution in 20 ml of water). The aqueous extracts were back-extracted twice with chloroform and the combined chloroform extracts were concentrated to a small volume and the residue was applied to preparative silica gel TLC plates (20 x 20 cm) which were developed three times in ether-chloroform-methanol (5:4:1). In this solvent the two diastereomers of MMT-A^{P}_{Si}(TCE)A^{Si}_{Si} (5a) were obtained in 37% (faster R_f 0.62) and 31% (slower R_f 0.52) yields for a total yield of 68%. Both isomers had the same UV spectrum (λ^{EtOH}_{max} 257 and 235 nm). The faster R_f isomer had a melting point of 112-117°C while the slower R_f isomer had a melting point of 142-148°C. These diastereomers do not pose a problem for synthesis since they have similar chromatographic† properties in a number of solvents (e.g. R_f^{THF} 0.80). On detritylation the higher R_f isomer of 5a gave 5b, mp 134-138°C while the lower R_f isomer of 5a gave 5b, mp 108-112°C. These two diastereomers of 5b have very similar properties in a number of solvents (e.g. R_f^{EtOAc} 0.17 and 0.14 respectively). Thus they can be combined or used separately for chain elongation. In all practical syntheses we combine the diastereomers. On this occasion we also used only the higher melting isomer of 5b for chain elongation.

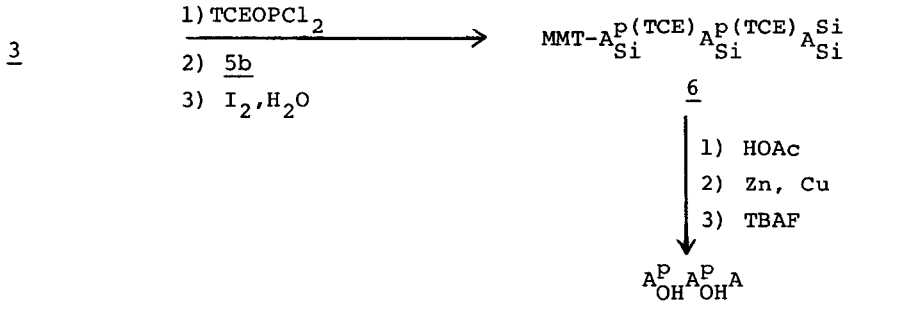
†Thin layer chromatographic data were recorded on Eastman chromagram sheets 13181. Paper chromatography was carried out on Whatman 3MM sheets in solvent F (n-propanol-concentrated ammonium hydroxide-water (55:10:35)).



3 4

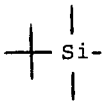
5a R = MMT($MMT\text{-}A^{p(TCE)}_{Si}A^{Si}_{Si}$)

5b R = H



7 Ap(2'-5')Ap(2'-5')A

MMT = monomethoxytrityl

Si = 

Compound 3 (0.3 mmole) was treated as above with $TCEOPCl_2$ followed by the high melting isomer of 5b (0.21 mmole, 0.7 eq) and worked up as above except that TLC plates were developed first in chloroform-methanol (9:1) and then twice in ether-chloroform-methanol (5:4:1). The yield of the fully protected trinucleotide 6 was 66% (R_f^{THF} 0.79). It was again

possible to separate the two diastereomers arising from the new phosphotriester. These two diastereomers had R_f^{EtOAc} of 0.11 and 0.06 with melting points of 129-133°C and 175-180°C respectively. The UV spectra for the compounds 6 showed λ_{max}^{EtOH} at 259 and a weak shoulder at 236 nm. Compound 6 was deprotected using standard procedures of first 80% HOAc followed by zinc-copper couple in DMF at 50°C for 3 h and finally tetrabutylammonium fluoride[5] in THF (20 min). The product 7 (Ap(2'-5')Ap(2'-5')A) was the sole nucleotidic product and showed R_f^F 0.34 and $R_M^{Tp,\ pH\ 7.5}$ 0.55. The trinucleotide was degraded by snake venom phosphodiesterase (which degrades 2'-5' linked nucleotides[7]) to adenosine and adenosine 5'-phosphate in the correct ratio.

This manuscript demonstrates the remarkable versatility of the silylated ribonucleosides in the synthesis of oligonucleotides possessing any desired linkage. The procedure works equally well with uridine, cytidine and guanosine without modification. The synthesis of 5'-5', 2'-2' and 3'-3' linkages will be described elsewhere[8] and are even easier to obtain than the 2'-5 and 3'-5' linkages which have now been described.

Acknowledgements

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References

This manuscript represents Part IV in a series on oligoribonucleotides and Part IX in a series on silyl groups in nucleoside and nucleotide chemistry. For the previous article in each series see reference 9.

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