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The chemical synthesis of oligoribonucleotides VII. A comparison of condensing agents in the coupling of silylated ribonucleosides

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ABSTRACT

The t-butyldimethylsilyl group is shown to be an ideal protecting group for the 2'-hydroxyl function of ribonucleosides during the synthesis of ribonucleotides using any of nine commonly used condensing agents. The phosphite coupling procedure compares favorably with all of the widely used condensing agents and provides a most convenient route to the key intermediates in the "modified" triester strategy.

INTRODUCTION

We have been developing the alkylsilyl groups as protecting groups for the hydroxyl functions in ribonucleosides (1-4). Silylating agents react rapidly and cleanly with the hydroxyl groups in nucleosides (5) and provide a facile route to 2',5'-protected ribonucleosides (1,3,4). The silyl-protected nucleosides are perfectly compatible with the chlorophosphite procedure (6) for the formation of internucleotide linkages in ribooligonucleotides (2-4). The procedures developed allow for the synthesis of oligoribonucleotides at least as rapidly as is currently possible for oligodeoxyribonucleotides and we have begun an attempt to chemically synthesise the ribonucleotide sequence corresponding to a transfer RNA (7).

Before we concluded our strategy for the attempt on the transfer RNA sequence it was essential to evaluate the available phosphorylation procedures with the silylated ribonucleosides. We explored all of the readily accessible and proven procedures and found that in our hands the chlorophosphite procedure gave the fastest and cleanest reactions with the best yields. Since completion of our study a few reports have appeared in which other phosphorylation procedures have been briefly described for the coupling of silylated nucleosides (8-10). One of these reports (8) claimed remarkably high yields using a combination of triisopropylbenzenesulfonyl chloride (TPS-Cl) and mesitylenesulfonyl tetrazolide (MS-TET). Two other reports (9,10) have

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indicated much lower yields using TPS-Cl.

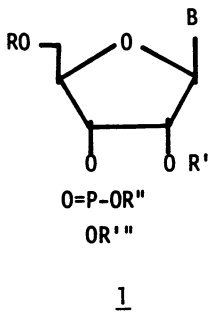
We wish to present the results of our evaluation of nine different condensing agents in addition to the chlorophosphite procedure as applied to ribonucleosides carrying the t-butyldimethylsilyl group (TBDMS) at the key 2'-position. This report will also describe the use of the chlorophosphite procedure to prepare the key intermediates (1a-c) required by the "modified" triester approach. We also wish to report the condensation of adenosine units lacking N-protection using tetrazole (MS-TET) and nitroimidazole (MS-NI) condensing agents. These latter two aspects complement and extend the recent and very useful article by Wiewiorowski et al. (11). These authors also compared three of the condensing agents discussed in this report but with ribonucleosides bearing acyl groups on the 2'-position.

DISCUSSION

Current methodology using activated phosphate reagents for internucleotide bond formation is outlined in Scheme 1. This has come to be known as the "modified" triester approach and is widely used in the deoxy and ribo areas (12-15). The key reagents in this procedure are the neutral phosphorylated nucleoside 1 and the condensing agent 3. The proposed advantage of this procedure is that the phosphorylated unit 1 can be isolated, stored, and on simple removal of one of the phosphate protecting groups (usually cyanoethyl, CE), coupled directly to the free 5'-hydroxyl of unit 2. Several combinations of R" and R"' have been described in the literature. The most common combinations include cyanoethyl (CE) plus trichloroethyl (TCE) (12,13) and cyanoethyl plus o- or p-chlorophenyl (14). The combination of o- or p-chlorophenyl and trichloroethyl has also been reported (15).

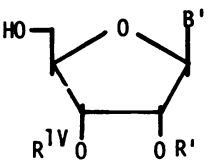
We have prepared compounds 1a-c which contain all of the above-mentioned combinations. While previously reported preparations have involved either several steps or long reaction times or both, the dichloridite approach described herein produces compounds of the type 1 rapidly and in good yields. The preparation of 1a (B=uracil) illustrates the facility of the dichloridite procedure. 5'-Monomethoxytrityl-2'-TBDMS uridine (5, B=uracil) was added to βββ-trichloroethylphosphorodichloridite in THF containing collidine at -78° over a 10 min period. Cyanoethanol was then added and after 15 min the intermediate phosphite was oxidized with iodine in water. The product 1a (B=uracil) was isolated in 70% yield from preparative TLC plates. The adenosine analogue 1a (B-adenine) was obtained in 65% yield by the same procedure. The other widely used combinations of protecting groups are represented by the synthesis

Scheme 1



1

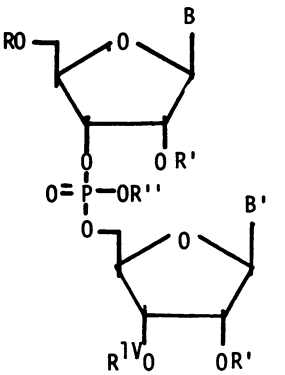
1) Remove R'"



2

$ArSO_2X$

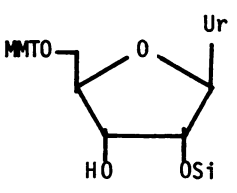
3



4

R=MMT, R'=TBDMS = R^{IV}

(a) R" = TCE, B = B' = Ur
(b) R" = pClØ-, B = B' = Ur
(c) R" = TCE, B = Ur, B' = Ad

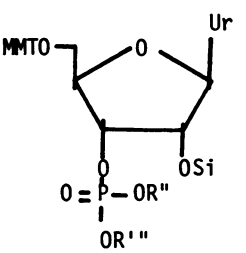


5

1) $R"OPCl_2$ 10 min

2) R'" OH 15 min

3) I_2, H_2O 5 min



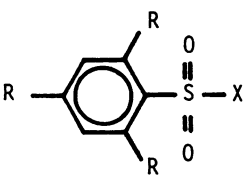
1

(a) R"=Cl_3CCH_2-; R'"=CH_2CH_2CN
(b) R"=pCl-Ø; R'"=CH_2CH_2CN
(c) R"=pCl-Ø; R'"=Cl_3CCH_2-
(d) R"=R'"=Cl_3CCH_2-

MMT = monomethoxytrityl
Si = TBDMS = t-butyldimethylsilyl
Ur = uracil, Ad - adenine

of 1b and 1c (B=uracil) in 76% and 73% yields respectively. In these latter two examples p-chlorophenylphosphodichloridite was used in the first step and cyanoethanol or trichloroethanol added in the second step. A final compound, 1d (R"=R"'=TCE), was prepared in 84% yield. This compound was shown to be stable to the conditions used in the removal of the cyanoethyl group from 1a.

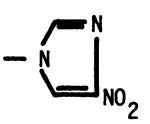
In order to show that the TBDMS group on the 2'-position was suitable for use with the modified triester approach, and to evaluate the relative utility of the currently used condensing agents, we prepared the dinucleotide 4a under a number of conditions. The condensing agents used are listed below (compounds 3). Reactions were carried out according to standard procedures and the results are collected in Table 1.



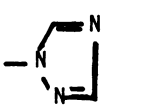
3

Compound		R	X
TPS-NI	3a	isopropyl	NI
TPS-TRI	3b	"	TRI
TPS-TET	3c	"	TET
TPS-Cl	3d	"	Cl
MS-NI	3e	methyl	NI
MS-TRI	3f	"	TRI
MS-TET	3g	"	TET
BS-NI	3h	H	NI
BS-TET	3i	H	TET

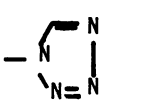
X



= NI



= TRI



= TET

From the table it is apparent that most of the yields were in the 40-60% range. These are substantially lower than the 95% yields reported by Loewen (8) using the standard triester procedure (16) and MS-TET as condensing agent for the synthesis of compounds similar to 4. In the Loewen paper the phosphate protecting group was the p-chlorophenyl group. We synthesized 4b (B=B'=Ur) using MS-TET under the best conditions from Table 1 and obtained a yield of 61%. We cannot account for the significant differences in yield between those reported by Loewen (8) and those reported herein. On the other hand van Boom has reported (10) the synthesis of a compound very similar to 4a

TABLE 1

Summary of Condensation Reactions.

Preparation of 4a (B=B'=Ur) by the "Modified" Triester Approach.

Coupling Reagent	(equivalents)	1 (R"=TCE,R'"=H)	2 (R'=R'"=Si)	time(h)	yield(%)
TPS-NI	(3 + 1.3)	1	0.5	72	46
TPS-NI	(3 + 2)	1	0.7	72	40
TPS-NI	3	1	0.8	72	37
TPS-TRI	(3 + 1)	1	0.5	72	24
TPS-TRI	3	1	0.7	72	36
TPS-TET	3	1	1.2	15	50
TPS-TET	3	1	0.5	10 - 30	54 - 60
TPS-TET	6	1	0.5	48	30 - 45
MS-NI	3	1	0.5	24	55
MS-NI	3	1	1.2	48	57
MS-TRI	3 + 2	1	0.5	96	41
MS-TET	3	1	0.7	24	70
MS-TET	3	1	1.2	13	50
MS-TET	3	1	1	18	60
BS-NI	3	1	1.2	48	40
BS-TET	2.3	1	0.5	24	60
TPS-Cl	3	1	0.5	48	33
TPS-Cl	3 + 2	1	0.5	48	55
TPS-Cl	3	1	0.7	72	41

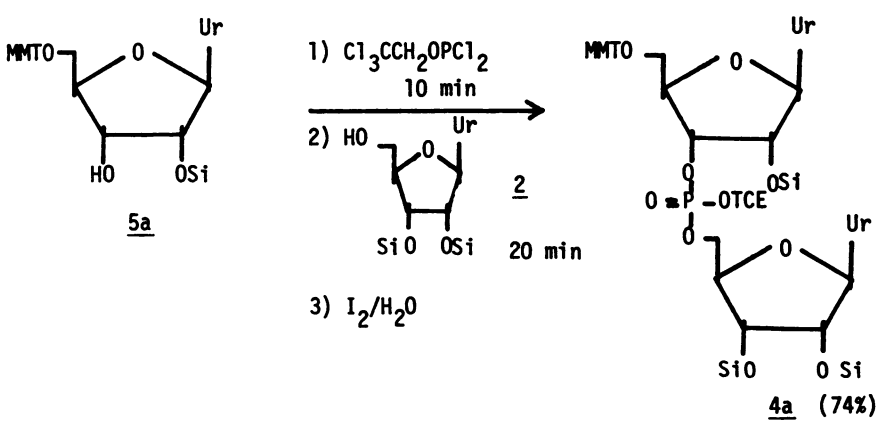
using a standard triester approach and TPS-Cl as condensing agent and reported a 27% yield when TBDMS protection was used on the 2'-hydroxyl. Pfleiderer (9) has used the cyanoethyl protecting group in preparing a dinucleotide of cytidine (with 2'-TBDMS protection) by a standard triester approach with TPS-Cl as

condensing agent and reported a 22% yield. It would thus seem that the yields we report in Table 1 are in reasonable agreement with expectations.

The times reported in Table 1 refer to the actual duration of the reactions before work-up. In many cases the reactions were over (according to TLC) in less than the total time used. In one case with MS-TET the reaction was worked-up after only 3 h of reaction time and the yield of 4a was 55%. A significant side product in all the condensations was the 5'-sulfonated derivative of 2 as has been reported by others (11).

As part of our strategy for the synthesis of oligoribonucleotides it was preferable to avoid N-protection for adenosine units. While such protection is not required when using the dichloridite procedure, it was not totally clear that N-protection could be avoided using MS-TET and MS-NI. Since completion of this work Wiewiorowski has shown that N-protection for adenosine is unnecessary when using TPS-Cl and TPS-TET (11). In our experiments 1a (R=MMT, R'=Si, R"=TCE, B=Ur) was condensed with 2b (B'=Ad, R'=R^{IV}=Si) using the "modified" triester approach to produce 4c (B=Ur, B'=Ad, R'=R^{IV}=Si, R"=TCE). The yields of 4c were 41% when using MS-NI and 50% when using MS-TET. Compound 4c was fully characterized including deprotection to the free nucleotide UpA followed by enzymatic degradation.

We have previously reported (3) in detail on the use of the dichloridite procedure for the synthesis of compounds of the type 4 in 50-70% yields in a



2 - 2½ h condensation process. We have improved upon the procedure and have cut the total time for condensation to 30 min and yields are consistently

in the 70-85% range. For example, 5 was condensed with trichloroethylphosphodichloridite for 10 min at -78°C and 2 was added and the reaction continued for 20 min. After treatment with iodine and water (5 min) and isolation on TLC plates, compound 4a was obtained in 74% yield.

CONCLUSION

It is clear from the above results and those that we have reported elsewhere (2-4) that the TBDMS group is compatible with all of the generally used procedures for the synthesis of oligoribonucleotides. We clearly prefer the dichloridite procedure for the condensations for the reasons of speed, yields and ease of work-up. Ease of work-up is a critical feature of successful procedures. In the examples of the synthesis of 4a with MS-TET and TPS-TET, nine bands appeared on TLC. In the synthesis of 4a by the dichloridite procedure only three widely separated bands appeared corresponding to 3'-3' linked dimer near the solvent front, desired product at R_f 0.7 and a band at the origin, presumably phosphorylated nucleoside (1a, R=MMT, R'=Si, R"=TCE, R'"=H, B=Ur). In our experience no other condensation procedure gives such clean results.

In this manuscript we have also demonstrated that the dichlorodite procedure offers an excellent route to the key compounds 1 used in the "modified" triester approach.

GENERAL METHODS

Descending paper chromatography was carried out using Whatman 3 MM paper. The solvent system employed was: Solvent A, isopropyl alcohol-concentrated ammonium hydroxide-water (7:1:2) prepared on a volume basis. Paper electrophoresis was performed using Whatman 3MM paper in a Savant Flat Plate electrophoretic chamber with a Savant model HB power supply operated at 2000 V for 1 h; the solution was a triethylammonium bicarbonate buffer (0.05 M, pH 7.5), prepared by making 15.15 g of triethylamine up to 3 ℓ volume with water and then bubbling 20 g of carbon dioxide through the solution. The properties of all free nucleotides (from the deprotection of 4) were compared to known samples by the above methods of chromatography and electrophoresis. In addition the deprotected nucleotides were characterized by enzymes as previously described (3).

All thin layer chromatography was conducted using Brinkman Polygram Sil G/uv 254 plastic sheets #66111709 (with fluorescent indicator) cut into

strips 2 x 10 cm. Thick-layer chromatography was carried out on glass plates (20 x 20 cm) coated with a 1-mm thick layer silica gel DSF-5 (Terrochem Laboratories). Nucleosides, nucleotides and their derivatives were detected using a uv light source (mineralite, output ∿254 nm). Compounds containing methoxytrityl groups were detected on chromatography by spraying with 10% perchloric acid solution and drying them in a stream of warm air.

The tetrazole (17) and triazole (18) reagents along with the nitroimidazole (19) reagents were prepared according to literature procedures.

General Procedure for the Preparation of Reagents 1a-d.

5'-O-Monomethoxytrityl-2'-O-TBDMS uridine (5) was dissolved in dry tetrahydrofuran (1.7 ml/mmole) and added dropwise (5-10 min) to a solution of the appropriate dichloridite in tetrahydrofuran (1.7 ml/mmole) containing collidine (7 mmole/mmole of dichloridite). After addition was complete or a total of 10 min, 4 mmole/mmole of 5 of the appropriate alcohol (cyanoethanol or trichloroethanol) was added and stirring was continued at -78°C for 30 min. A solution of iodine (2 mmole/mmole of dichloridite) in THF/Water (3:1) was added. After 2-3 min at 20°C the solution was concentrated at reduced pressure. The residue was dissolved in chloroform which was washed with a 5% aqueous sodium bisulfite solution. The chloroform solution was concentrated to a small volume and applied to TLC plates which were developed in the solvents listed in the table below. Products were eluted from the silica gel with ethyl acetate in the yields shown.

Compound		mp (°C)	R_f	λ_{max}(nm)	Yield(%)	Solvent Used on Preparative TLC
MMT-U$^{Si}_{p}$CE$_{TCE}$	1a	92-94	0.28[a]	262	70	ether:chloroform (1:1)
MMT-U$^{Si}_{p}$CE$_{pClØ}$	1b	85-86	0.15[a]	261	76	ether:chloroform (1:1)
MMT-U$^{Si}_{p}$TCE$_{pClØ}$	1c	70-75	0.75[a]	262	73	ether:chloroform (1:1)
MMT-U$^{Si}_{p}$(TCE)$_2$	1d		0.25[b]	262	84	ether
MMT-A$^{Si}_{p}$CE$_{TCE}$		82-84	0.42[c]	260	65	5% CH_3OH in chloroform

[a] ether:$CHCl_3$ (1:1) [b] ether [c] 5% CH_3OH in chloroform.

General Procedure for the Synthesis of 4 by the "Modified" Triester Procedure

This procedure follows standard practice and can be illustrated by the synthesis of 4c (B=Ur,B'=Ad). Compound 1a (0.25 mmole) was stirred with triethylamine/pyridine (1:1, 5 ml) for six hours at 20°C. Solvents were

removed at reduced pressure. Compound 2b (B=Ad, R'=R^{IV}=Si, 0.38 mmole) was added and the mixture was dried by evaporation of pyridine (4 x 2 ml). Either MS-TET (0.75 mmole) or MS-NI (0.75 mmole) was added followed by 10 ml of pyridine and the resulting solution was stirred at room temperature for 8 h in the MS-TET reaction and 48 h in the MS-NI reaction (in this reaction an addition 0.5 mmole of MS-NI was added after 24 h of reaction). The solvents were coevaporated with ethanol to dryness, and the residue extracted between $CHCl_3$ (30 ml) and H_2O (3 x 30 ml) and then applied to 20 g of silica gel in a 3 cm dia column. The products were passed quickly through this short silica gel column (∿5 x 3 cm) using 5% MeOH/$CHCl_3$ to serve as a preliminary separation. The fraction containing the desired product was concentrated and applied to preparative TLC plates developed in ethyl-acetate:chloroform:ethanol (50:45:5). Two very closely moving bands were obtained representing the desired product and 5'-mesitylenesulfonyl-2',3'-diTBDMSadenosine. These two bands were eluted together and after evaporation of solvent the residue was treated with 80% HOAc (90°C, 30 min) and the products were again separated on TLC using the above solvent. This time a clean separation occurred giving the desired product 4 (R=H, R'=R^{IV}=TBDMS, R"'=TCE, B=Ur, B'=Ad) in 50% yield (MS-TET, 41% yield MS-NI) having an R_f of 0.12 in the solvent used. The other product, the 5'-sulfonylated adenosine was obtained in 27% yield from the MS-TET reaction and 38% yield from the MS-NI reaction. (R_f 0.54)

The yields and ratios used for all other condensing agents are recorded in Table 1. Properties of 4a and 4b are summarized below.

Compound	mp	R_f	λ_{max}(nm)
MMT-U_p^{Si}(TCE)U_{Si}^{Si}	100 - 105	0.39[a]	262
MMT-U_p^{Si}(pClØ)U_{Si}^{Si}	100 - 105	0.47[a]	262
HO-U_p^{Si}(TCE)A_{Si}^{Si}	129 - 133	0.12[b]	260

[a]ether:chloroform (1:1); [b]ethyl acetate:chloroform:ethanol (50:45:5)

Preparation of 4a by the Dichloridite Procedure

Compound 5a (156 mg, 0.25 mmol) in tetrahydrofuran (1.25 ml) was added dropwise over five minutes to a solution of collidine (0.26 ml, 2 mmol) and $Cl_3CCH_2OPCl_3$ (41 µl, 0.28 mmol) in tetrahydrofuran (1 ml) maintained at -78°C. After an additional 5 min of stirring, a solution of 2',3'-di-t-butyldimethylsilyluridine (2, 1.03 mg, 0.23 mmol) in tetrahydrofuran (1.25 ml) was added dropwise during a period of 5 min. After an additional 15 min at -78°C, the

solution was warmed to room temperature and a solution of iodine (0.6 mmole) in tetrahydrofuran-water (3:1, 2 ml) was added. After 3 min the solvent was removed by evaporation. The residue was dissolved in chloroform (30 ml) and washed with 5% aqueous sodium bisulfite (30 ml) and then with water (3 x 30 ml). After drying over sodium sulfate, the solvent was removed and the residue was applied to preparative TLC plates which were developed in ether-chloroform (1:1). The desired product was eluted with ethyl acetate which on evaporation left 220 mg (74%) of 4a as a white solid.

ACKNOWLEDGEMENTS

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