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New 5'-Hydroxyl Protecting Groups for Rapid Internucleotide Bond Formation

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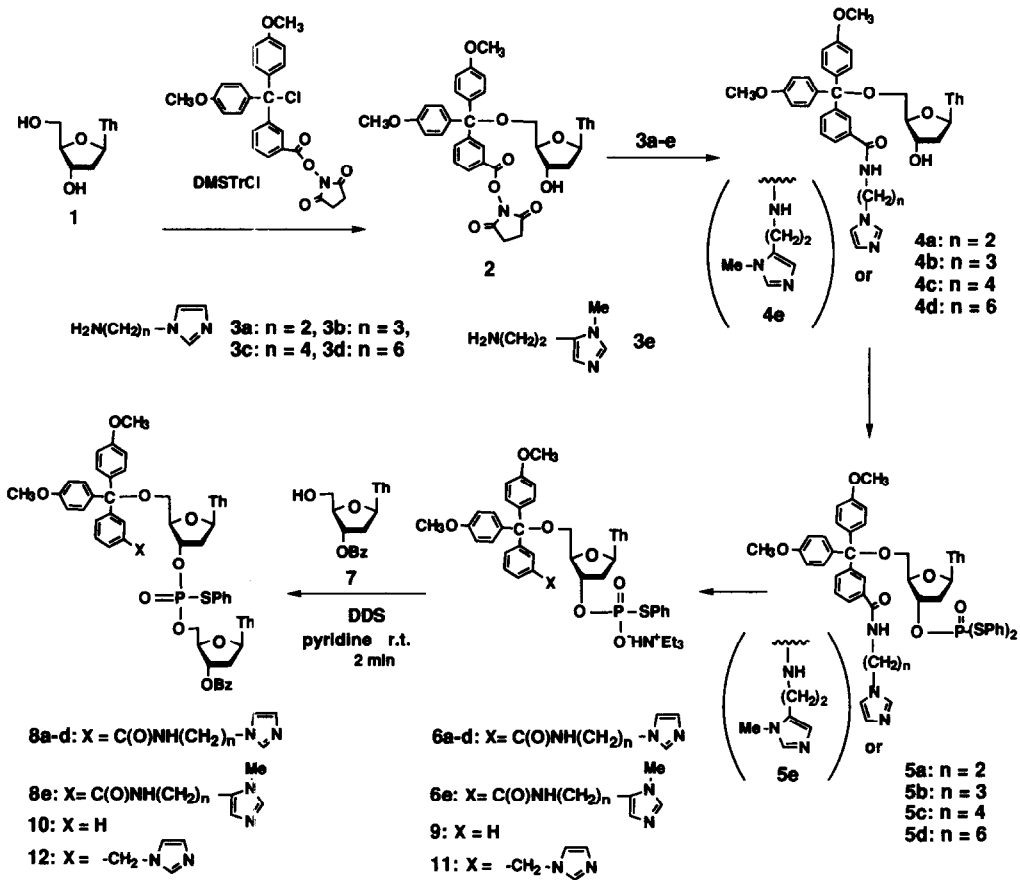
Abstract: A variety of 5'-hydroxyl protecting groups having catalytic sites for internucleotidic bond formation have been studied. Among them, a modified dimethoxytrityl group having an imidazolylethylcarbamoyl substituent has proved to be highly effective and superior to the IDTr group previously known.

In the current oligonucleotide synthesis, the phosphoramidite and H-phosphonate approaches have been utilized as most reliable methods in the solid phase synthesis.[1,2] One of the drawbacks in these approaches is to use an excess amount of synthetic unit for sufficient condensation. Recent development in antisense DNA/RNA has required large quantities of materials for gene therapy.[3] More effective conditions for internucleotidic condensation should be explored not only in the two practical approaches but also in the phosphotiester approach which has been less used in recent years. In the latter, Matteucci, [4] Efimov,[5] and we[6] have reported several improved methods by the use of protecting groups having catalytic sites such as imidazole or pyridine N-oxide residues which accelerated condensation.

In our previous paper, [6] it was reported that the use of the 3-(imidazol-1-yl)-4,4'-dimethoxytrityl (IDTr) group resulted in remarkable shortening of the time required for coupling between a diester and a 5'-OH component. This protecting group, however, was introduced to the 5'-hydroxyl of deoxyribonucleosides by the use of a trityl bistetrafluoroborate reagent under limited acidic conditions, where the amino group of deoxyadenosine should be protected with a dibenzoyl group to avoid the depurination. We have also encountered difficulty in separating the desired 5'-tritylated product from the hydrolyzed material derived from the tritylating reagent because of its inherent poor lipophilicity.[7]

In this paper, we wish to report a more effective protecting group for the 5'-hydroxyl function in oligonucleotide synthesis.

Köster and his coworkers[7] have recently developed a unique trityl type of protecting group having an active ester site at the *para* position of the phenyl group in the aim to introduce a variety of functional groups at the 5'-terminus of oligodeoxyribonucleotides. This work stimulated us to create a new "catalytic site" carring protecting group and extend our original idea.[6] Köster's two-step strategy for introduction of a functional trityl group onto the 5'-hydroxyl enabled us to make a stable tritylating reagent which could be modified at the *meta* position of the phenyl substituent of the DMTr group with various aminoalkylimidazoles after the 5'-tritylation. Thus, a new tritylating reagent, 4,4'-dimethoxy-4"-3-succinimidoxycarbonoyltrityl



chloride (DMSTrCl),[8] was prepared from 3-bromobenzoic acid in a manner similar to that described by Köster et al. in the case of the corresponding 4"-3-succinimidoxycarbonoyltrityl chloride.

Reaction of thymidine (**1**) with 1.2 equiv of DMSTrCl in pyridine for 18 h gave the corresponding tritylated product **2** in 71% yield. Five kinds of aminoalkylimidazoles (**3a-e**) were allowed to react with 1.1-1.2 equiv of **2** in CH_2Cl_2 at room temperature. Thus, the corresponding imidazole-containing thymidine derivatives **4a-e** were obtained in more than 86% yields. Phosphorylation of **4a-e** with 1.5 equiv of cyclohexylammonium *S,S*-diphenyl phosphorodithioate (PSS)[9] in the presence of 3 equiv of isodurenedisulfonyl dichloride (DDS)[10] gave the 3'-phosphorylated products **5a-e** in 79-89% yields except for **5c**, which was obtained in an overall yield of 63% from thymidine. We have noticed that these reactions proceeded rapidly within 5-10 min.

Next, we examined the relative rate of the condensation of **6a-e** with 1 equiv of 3'-*O*-benzoylthymidine (**7**) in the presence of 2 equiv of DDS as a condensing reagent. A solution of DDS in pyridine was added to a vigrously stirred mixture of **6a-e** and **7** in pyridine. After being stirred at room temperature (25° C) for 2 min, the mixture was quenched by addition of water and all trityl containing materials were completely extracted with CH_2Cl_2. The yields of the 3'-5' linked dimers **8a-e** were estimated by ^{31}P NMR analysis. [11]

Table 1. Condensation of Tritylated Thymidine Phosphorothioate Derivatives **6a-e**, **9** and **11** with **7** in the Presence of DDS

Compd	Abbrev. of Protecting Group	n	R	Concentration of **6a-e**, **9**,**11** and **7** M	Time for Pre-Activation min	DDS equiv	Product	Yield[a] %	Diastereomers ^{31}P NMR ppm A B	Ratio A : B
6a	IETr	2	Im[b]	0.05	2	2.0	**8a**	87	24.828 25.170	56:44
6b	IPTr	3	Im	0.05	2	2.0	**8b**	48	24.856 25.219	53:47
6c	IBTr	4	Im	0.05	2	2.0	**8c**	45	24.793 25.184	52:48
6d	IHTr	6	Im	0.05	2	2.0	**8d**	40	24.737 25.135	56:44
6e	IMTr	2		0.05	2	2.0	**8e**	88	24.828 25.177	63:37
6a	IETr	2	Im	0.05	20	2.0	**8a**	86	24.835 25.184	58:42
6a	IETr	2	Im	0.05	2	3.0	**8a**	96	24.863 25.198	56:44
9	DMTr + N-Methylimidazole (1equiv)			0.05	2	2.0	**10**	27	24.618 24.849	51:49
11	IDTr	-	-	0.05	2	2.0	**12**	60	24.765 25.086	85:15

a) The yields of products were estimated by ^{31}P NMR ($CDCl_3$).[11]
b) Im refers to the imidazol-1-yl group.

As a reference reaction, condensation of a 5'-dimethoxytritylated thymidine derivative **9** with **7** was also carried out in the presence of 1 equiv of *N*-methylimidazole (MeIm) in pyridine. As shown in Table 1, all protecting groups tested exhibited remarkable effects on the acceleration of the internucleotidic bond formation, giving more than 40% yields in 2 min, as compared with the yield (27%) of **10** obtained by condensation of **9** with **7** in the presence of *N*-methylimidazole. Among a series of protecting groups having imidazolylalkyl chains as depicted in general structure **6a-d**, the 4,4'-dimethoxy-3"-[*N*-(imidazolylethyl)carbamoyl]trityl (IETr) group (see Scheme 1 and Table 1 for abbreviation of all protecting groups) was found to be most effective (87%). Next, we compared the condensations of **7** with **6a** and **11** having the IETr and IDTr[6] groups, respectively. As a consequence, the IETr group was superior to the IDTr group which gave a less yield of **12** (60%) under similar conditions. Thus, it was concluded that n = 2 was most suitable as the number of methylene groups for close proximity of the imidazole residue to the 3'-phosphoryl group. Furthermore, a similar catalytic effect (88%) was also achieved when the 4,4'-dimethoxy-3"-[*N*-(1-methylimidazol-5-ylethyl)carbamoyl]trityl (IMTr) group having a *N*-methylimidazolylethyl

substituent was employed as in the case of **6e**. This is apparently rationalized by the fact that the unsubstituted nitrogen atoms of both the IETr and IMTr can approach the 3'-position equally. When the concentration each of **6a** and **7** was increased twice to 0.1 M and 3 equiv of DDS was used, the protected dimer **8a** was formed in 96% yield.

Ohtsuka et al. reported a stereoselective synthesis of dinucleoside phosphate aryl esters by the use of arenesulfonyl 5-(pyridine-2-yl)tetrazole.[12] During our study, we have also observed an interesting stereoselectivity in internucleotidic bond formation. The ratio of two diasteromers formed by condensation remarkably varied when **6a-e**, **9**, and **11** were employed. When compound **9** bearing the DMTr group was used for condensation with **7** in the presence of *N*-methylimidazole, the ratio of two diasteromers was nearly 1:1. On the other hand, the use of protecting groups having imidazolylalkyl chains gave nearly equal or slightly in favor of one diastereomer. Interestingly, however, the IMTr group having the *N*-methylimidazolylethyl substituent affected considerably the ratio of two diasteroisomers, giving a 63:37 mixture of two diasteromers . From a mechanistic point of view, we added an interesting observation that the IDTr group resulted in predominant formation of one of the two diastereomers in the ratio of 85:15. These findings would be useful for consideration of stereoselective formation of phosphotriester bonds. More detailed studies on the absolute configuration of these two diastereomers will be needed to clarify essential factors which induced the stereoselectivity observed in this study.

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