Communications

3-(Imidazol-1-ylmethyl)-4',4''-dimethoxytrityl: A New Functionalized 5'-Hydroxyl Protecting Group Capable of Rapid Internucleotidic Bond Formation in the Phosphorothio Ester Approach

Summary: A remarkable rate enhancement of internucleotidic bond formation in DNA synthesis has been achieved by use of 3-(imidazol-1-ylmethyl)-4',4''-dimethoxytrityl as a new 5'-hydroxyl protecting group which has a potential catalytic site for condensation.

Sir: Recently, two remarkable improvements in the phosphotriester approach have been reported by Efimov[1] and Matteucci.[2] The former group showed that pyridine *N*-oxide (PNO) derivatives served as potential catalysts for internucleotidic bond formation. The latter explored 2-(1-methylimidazol-2-yl)phenyl (IMP) as a new type of phosphate protecting group whereby remarkable acceleration of condensation was accomplished. However, in the PNO approach,[1] there still remained a serious problem, i.e., the 5'-sulfonation which took place to the same degree as observed in the case of the usual condensing agents such as 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT). Moreover, the IMP group required somewhat drastic conditions for its removal compared with the 2-chlorophenyl group.[2,3] On the other hand, we have developed a series of functionalized trityls as protecting groups for primary hydroxyls.[4-7] This paper describes 3-(imidazol-1-ylmethyl)-4',4''-dimethoxytrityl (IDTr) as a promising protecting group of the 5'-hydroxyl of deoxyribonucleosides.

Matteucci[2] suggested explicitly that neighboring group participation of the imidazole residue involved in the IMP group was essential for rapid condensation of phosphodiesters with hydroxyl components. As an alternative to utilization of the neighboring group participation, we constructed several CPK models to see if the imidazole residue can approach the 3'-hydroxyl group when it was introduced to the 4,4'-dimethoxytrityl (DMTr) group at an appropriate position. As a consequence, it was expected that introduction of the (imidazol-1-ylmethyl) group at the 3-position of the DMTr group might be favorable for our purpose.

Therefore, we tried to synthesize an introducing agent, 3-(imidazol-1-ylmethyl)-4,4'-dimethoxytritanol (IDTrOH). This compound was obtained easily as a crystalline benzene adduct[8] by a three-step reaction from methyl *m*-toluate[9] and was converted to the corresponding trityl chloride (IDTrCl) by using acetyl chloride in the usual manner.[7] However, this reagent failed to react with N-protected deoxyribonucleotides (**1a-d**)[11] under the usual conditions using pyridine as the solvent. IDTrCl was rapidly dimerized in this solvent to give quantitatively a *N*-tritylimidazolium chloride derivative. This problem, however, was overcome by the following new procedure for tritylation. Treatment of IDTrOH with tetrafluoroboric acid in acetic anhydride followed by precipitation into ether gave a brownish reagent (IDTr-BBF). This reagent was allowed to react in situ with **1a-d** in DMF to give the tritylated compounds (**2a-d**) in ca. 70% yields. The reactions proceeded very rapidly within 10 min. Nevertheless, highly selective 5'-tritylation was accomplished. During tritylation, the glycosyl bonds of **3a-d** were stable.[12]

The phosphorylation of **2a-d** with cyclohexylammonium *S,S*-diphenyl phosphorodithioate (PSS)[15] in the presence of mesitylenedisulfonyl chloride (MDS)[16] gave the fully protected deoxyribonucleotide units (**3a-d**) in 80-90% yields. It was noteworthy that these phosphorylations were also very rapid and were completed in 10 min. Since the 3'-phosphorylation of the corresponding 5'-O-DMTr deoxyribonucleosides under the same conditions required 5-6 h, this remarkable acceleration should be ascribed to the neighboring group assistance of the imidazole residue as expected.

The phosphodiester units (**4a-d**) were readily prepared in quantitative yields by treatment of **3a-d** with a 5 M phosphinic acid (PSA, 60 equiv) pyridine solution-triethylamine (2:1, v/v) for 5-10 min. This smooth dephenylthiolation was also explained in terms of intramolecular imidazole-mediated hydrolysis. On the other hand, the IDTr group could be removed rapidly within 30 s and selectively like the DMTr group from **3a-d** by 0.2 M dichloroacetic acid in CH_2Cl_2 at 22 °C or 1% trifluoroacetic acid (TFA) in CH_2Cl_2 at 22 °C to give the 5'-hydroxyl components (**5a-d**). Under these conditions, depurination did not occur within several hours.

(1) Efimov, V. A.; Chakhmakheva, O. G.; Ovchinnikov, Y. A. *Nucleic Acids Res.* **1985**, *13*, 3651.
(2) Froehler, B. C.; Matteucci, M. D. *J. Am. Chem. Soc.* **1985**, *107*, 270.
(3) Sproat, B. S.; Rider, P.; Beijer, B. *Nucleic Acids Res.* **1986**, *14*, 1811.
(4) Sekine, M.; Hata, T. *J. Org. Chem.* **1983**, *48*, 3011.
(5) Sekine, M.; Hata, T. *J. Am. Chem. Soc.* **1984**, *106*, 5763.
(6) Sekine, M.; Hata, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 336.
(7) Sekine, M.; Hata, T. *J. Am. Chem. Soc.* **1986**, *108*, 4581.
(8) mp 71-73 °C (benzene); ^1H NMR ($CDCl_3$) δ 3.72 (s, 6 H, OMe), 4.93 (s, 2 H, CH_2), 6.65-7.45 (m, 18 H, Ar H). Anal. Calcd for $C_{25}H_{24}N_2O_3 \cdot {}^1/_2C_6H_6$: C, 76.52, H, 6.19, N, 6.37. Found: C, 76.35, H, 6.15, N, 6.32.
(9) Methyl *m*-toluate was converted to methyl 3-(bromomethyl)-benzoate by the method reported by Sankaran, V.; Marvel, C. S. *J. Polym. Sci.* **1980**, *18*, 1835. The benzoate was allowed to react in situ with 2 equiv of imidazole in acetone under reflux for 2 h to give methyl 3-imidazol-1-ylbenzoate in a 55% overall yield. The Grignard reaction of this product with *p*-anisylmagnesium bromide gave IDTrOH in 58% yield.
(10) Smith, M.; Rammler, D. H.; Goldberg, I. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1962**, *84*, 430.
(11) **1a**: Matsuzaki, J.; Hotoda, H.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1984**, *25*, 4019. **1b**: Schaller, G.; Weimann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821. **1c**: Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316. **1d**: Kamimura, T.; Tsuchiya, M.; Koura, K.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1983**, *24*, 2775.
(12) When N^6-benzoyldeoxyadenosine was used in place of **2c**, depurination was observed to a considerable extent during the reaction. The retarding effect of N^6-diacyldeoxyadenosine derivatives on depurination has been reported by us[13] and Takaku.[14]
(13) Kume, A.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1982**, *23*, 4365. Kume, A.; Iwase, R.; Sekine, M.; Hata, T. *Nucleic acids Res.* **1984**, *12*, 8525.
(14) Takaku, H.; Morita, K.; Sumiuchi, T. *Chem. Lett.* **1983**, 1661.
(15) Sekine, M.; Hamaoki, K.; Hata, T. *J. Org. Chem.* **1979**, *44*, 2325; *Bull. Chem. Soc. Jpn.* **1981**, *54*, 3815. Yamaguchi, K.; Honda, S.; Hata, T. *Chem. lett.* **1978**, 507.
(16) Sekine, M.; Matsuzaki, J.; Hata, T. *Tetrahedron Lett.* **1981**, *22*, 3209. Sekine, M.; Matsuzaki, J.; Hata, T. *Tetrahedron* **1985**, *42*, 5279.

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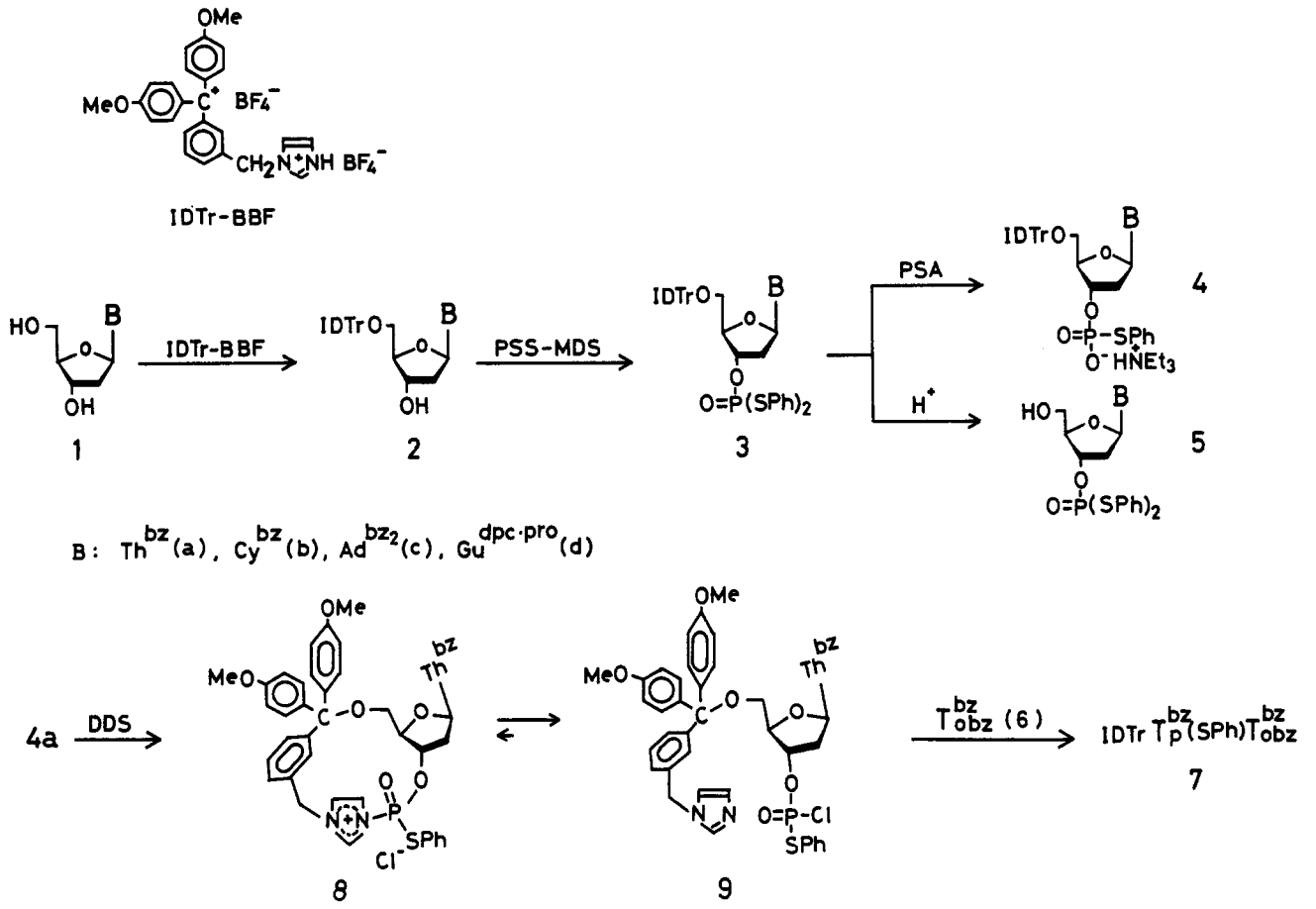

In order to examine the effect of the IDTr group on condensation, 0.9–1.2 equiv of **4a** was coupled with 3,3′-*N*,*O*-dibenzoylthymidine (**6**) in the presence of 3 equiv of isodurenedisulfonyl chloride (DDS)[16] to **4a** in pyridine. The reactions were completed in 2–5 min depending on the ratio of **4a** to **6** and the dimer (**7**) was obtained in more than 96% yields. It was noteworthy that the condensation proceeded rapidldy and predominantly over the 5′-sulfonation so that the 5′-sulfonated byproducts were not detected during the condensation. Therefore, a fully protected heptamer d(CpGpGpCpApTpT) was synthesized in 64% overall yield (average coupling yield 95%) from **6** by stepwise condensation (room temperature, 3–5 min) with 1.2 equiv of phosphodiester components (**4b–d**) in which the intermediate oligomers were roughly purified by silica gel column chromatography and treated in situ with 2% TFA in CH_2Cl_2 at 0 °C for 5 min for chain elongation. The unprotected heptamer was obtained in 51% yield by treatment with 0.5 M bis(tributyltin) oxide (15 equiv per one PhS) in pyridine at room temperature for 1 h[17] followed by the usual workup and characterized by enzyme assay.

The ^{31}P NMR spectrum of the mixture obtained by the reaction of **4a** with DDS showed no signals corresponding to the tetrasubstituted pyrophosphate derivative of **4a** but a set of two major sharp signals at 31.33 and 30.96 ppm. There is no evidence that these peaks are derived from the two diastereomers of **8** because the ^{31}P NMR of N-phosphorylated imidazole derivatives is unknown. Zarytova[18] described that similar *N*-(bis(aryloxy)phosphoryl)-pyridinium chloride derivatives, which are positively charged and delocalized by electron-donating groups, appear at higher magnetic fields by 3–8 ppm than diaryl phosphorochloridates or diaryl phosphates. Very recently, Garegg[19] and we[20] reported that *S*-phenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidine 3′-phosphorochloridothioate derivatives were detected as doublet peaks at the region of 30 ppm. Since the major peaks were similar to the phosphorochloridate intermediates, it seemed that the present reaction proceeded via a phosphorochloridate intermediate (**9**) which existed predominantly over **8** that should be in equilibrium with **9**. However, we feel that further study must be required.

Finally, we should add the promising fact that the condensation was accelerated so remarkably by addition of diisopropylethylamine[21] (2 equiv to DDS) that the reaction of **4a** with **6** was completed *within 30 s*[22] to give *almost quantitatively* the dimer **7**.

(17) Sekine, M.; Tanimura, H.; Hata, T. *Tetrahedron Lett.* **1985**, *26*, 4621.

(18) Knorre, D. G.; Zarytova, V. F. *Phosphorus Chemistry Directed Towards Biology*; Stec, W. J., Ed.; Pergamon Press: New York, 1980; pp 13–31. Zarytova, V. F.; Knorre, D. G. *Nucleic Acids Res.* **1984**, *12*, 2091.

(19) Garegg, P. J.; Regberg, T.; Stawinski, J.; Stromberg, R. *Tetrahedron lett.* **1986**, *23*, 2665. This paper gave the chemical shifts (29.8 and 29.6 ppm) of *S*-phenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidine 3′-phosphorochloridothioate (DMTrTp(SPh)Cl).

(20) Matsuzaki, J.; Hotoda, H.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1986**, *27*, 5645. In this paper, DMTrTp(SPh)Cl appeared at 30.00 and 29.85 ppm in pyridine–$CDCl_3$ and $DMTrT^{bz}p(SPh)(O^-HNEt_3)$ at 11.24 ppm. On the other hand, IDTrTp(SPh)(O^-HNEt_3) (**4a**) appeared at 12.52 ppm in pyridine–C_6D_6 (3:1, v/v). In our present paper all the chemical shifts in the low magnetic field are described with the plus sign.

(21) Van Boom reported that the tertiary base was effective for his coupling system using hydroxybenzotriazole intermediates: Marugg, J. E.; Mclaughlin, L. W.; Piel, N.; Tromp, M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1983**, *24*, 3989.

(22) After **4a** was treated with 3 equiv of DDS in pyridine for 5 min, the mixture was added to **6** in the presence of $(i\text{-Pr})_2NEt$ with vigorous stirring. This homogeneous reaction reached dramatically rapid condensation (30 s). When DDS was added to a mixture of **4a** and **6** in the presence of $(i\text{-Pr})_2NEt$, it took 1–2 min. This is apparently because DDS required ca. 1 min for its dissolution in pyridine.

In conclusion, the present method provides a mild and rapid method for the DNA synthesis during the whole synthetic procedure in the phosphothio ester approach using the phenylthio group.

Registry No. **1a**, 94189-75-0; **1b**, 4836-13-9; **1c**, 6711-37-1; **1d**, 86979-56-8; **2a**, 106502-50-5; **2b**, 106469-69-6; **2c**, 106469-70-9; **2d**, 106469-71-0; **3a**, 106469-72-1; **3b**, 106502-51-6; **3c**, 106502-52-7; **3d**, 106502-53-8; **4a**, 106502-55-0; **4b**, 106502-57-2; **4c**, 106502-59-4; **4d**, 106502-61-8; **5a**, 94189-78-3; **5b**, 106469-73-2; **5c**, 106469-74-3; **5d**, 87007-25-8; **6**, 94189-81-8; **7**, 106521-95-3; IDTrOH, 106502-46-9; IDTr-BBF, 106502-49-2; fully protected d(CpGpGpCpApTpT), 106587-87-5; d(CpGpGpCpApTpT), 106469-75-4.

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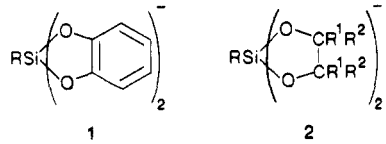
Reduction of Carbonyl Compounds with Pentacoordinate Hydridosilicates[1]

Summary: Bis(diolato)hydridosilicates, derived from trichlorosilane and catechol or 2,2′-dihydroxybiphenyl in THF, can reduce aldehydes and ketones without any catalyst in very high yield. Chemo- and stereoselectivity as well as the structure and reactivity relationship in the reduction is demonstrated.

Sir: Hydrosilanes such as alkoxyhydrosilanes[2] and phenyldimethylhydrosilane[3] are capable of reducing carbonyl compounds by nucleophilic catalysis with fluoride ions. Pentacoordinate hydridosilicates $[R_3SiHF]^-$ are postulated frequently as reactive species, although there is no structural proof of the intermediate. Perrozzi and Martin[4] have reported the formation of a pentacoordinate hydridosilicate by the reaction of trichlorosilane ($HSiCl_3$) with the dilithio derivative of hexafluorocumyl alcohol as a hygroscopic high-melting solid which was not analytically pure.

Reduction of carbonyl compounds with pentacoordinate hydridosilicates seemed interesting from both a mechanistic and practical point of view. We report herein a new entry of such species which appeared very useful and interesting.

Among numerous pentacoordinate silicon compounds,[5] Frye was the first to prepare bis(*o*-arenediolato)organosilicate (**1**) and other 1,2-diolato derivatives.[6]

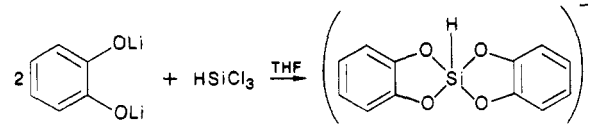


1 **2**

R = alkyl or Aryl

Later, Holmes et al. studied detailed structures of a variety of bis(1,2-diolato)silicates,[7] but these works have not extended to the corresponding hydridosilicates. We thought that bis(1,2-diolato)hydridosilicates could be prepared similarly and might behave as a typical pentacoordinate hydridosilicate.

Dilithium catecholate (**3**), prepared by the reaction of catechol and butyllithium in THF, was used to prepare bis(1,2-benzenediolato)hydridosilicate (**4**). Trichlorosilane



3 **4**

and **3** were mixed first at −78 °C to give a heterogeneous viscous slurry which was warmed gradually to a slightly turbid solution at about 0 °C. It became a clear solution when it was dissolved in a large excess of THF.

The solution was unstable at room temperature and all attempts to isolate **4** failed to result in the formation of bis(1,2-phenyldioxy)silane. However, it turns out that **4**

$$R^1COR^2 \xrightarrow[2.\ H_2O]{1.\ 4} R^1R^2CHOH$$

in the solution reduces aldehydes and ketones without any catalyst.

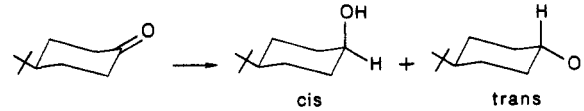
Besides catechol (**5**), 2,2′-dihydroxybiphenyl (**6**) also provides a ligand which produces an effective reducing agent. However, aliphatic diols such as 1,2-ethanediol and pinacol produce reducing agents which are less effective and monoalcohols were totally uneffective to reduce ketones under the conditions.

After standing for 2 h at room temperature, the hydridosilicate (**7**) derived from **6** and trichlorosilane gave cyclohexanol in 96% yield by the reaction with cyclohexanone, while **4** gave the product in 60% yield under the same conditions.

Yields of primary and secondary alcohols are generally excellent, but esters such as methyl benzoate were not reduced. Results are listed in Table I.

Both **4** and **7** predominantly afforded 2-cyclohexenol as the 1,2-reduction product from 2-cyclohexenone. Chemoselectivity in the reduction was also demonstrated by the competitive reduction of a mixture of pentanal and cyclohexanone. The ratios of primary and secondary alcohols were 75/25 for **4** at 0 °C and 79/21 for **7** at room temperature. These are fairly larger than the corresponding value (63/37) from a mixture of butanal and methyl ethyl ketone reduced by $LiAlH_4$ at 25 °C[8] but smaller than those from a mixture of hexanal and cyclohexanone with $LiAlH(O\text{-}t\text{-}Bu)_3$ at 0 °C (87/13)[9] and $LiAlH(OCEt_3)_3$ at 0 °C (94/6).[9]

Stereoselectivity in the reduction of 4-*tert*-butylcyclohexanone was examined with **4** and **7**. Cis and trans alcohols were obtained in ratios of 44/56 and 67/33 with **4** and **7**, respectively.



cis trans

It is known that the ratio of the cis alcohol to the trans increases when the steric bulkiness of reducing reagents increases. Thus a simple reducing reagent, $LiAlH_4$, gave

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