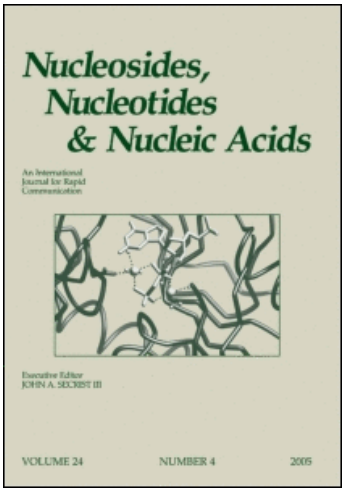

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A New Method for Removal of Modified Trityl and Pixyl Groups by Use of an Acid Species Generated by Reaction of Diethyl Oxomalonate with Methanol

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A New Method for Removal of Modified Trityl and Pixyl Groups by Use of an Acid Species Generated by Reaction of Diethyl Oxomalonate with Methanol*

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***Abstract**: Diethyl oxomalonate (DEOM) was found to exhibit unique properties as a new type of detritylating reagent in the presence of methanol. A variety of deoxyribonucleoside derivatives protected with modified trityl groups were allowed to react with DEOM. The ease of detritylation highly depended on the basicity of protected bases of adenine, guanine and cytosine. Inhibitory effects of various amines possessing the pKa values between 0.79-10.87 on the present detritylation were studied. Several mechanistic considerations were also discussed on the basis of ^{1}H and ^{13}C NMR studies of the DEOM-Methanol adduct.*

INTRODUCTION

In chemical transformation for the synthesis of natural products, primary hydroxyl functions have been frequently protected with the trityl group.[1] When acid sensitive sites were present in synthetic intermediates, modified trityls like 4-monomethoxytrityl (MMTr),[2] 4,4'-dimethoxytrityl (DMTr)[2] and 9-phenylxanthen-9-yl (Pix)[3] have widely been utilized.[4-9] Such substituted trityls have been removed by organic acids such as acetic acid,[3] dichloroacetic acid,[10,11] trichloroacetic acid,[12] trifluoroacetic acid,[13] benzenesulfonic acid,[14] toluenesulfonic acid,[15] and phenylphosphoric acid[16] or Lewis acids such as zinc bromide[17,18] and diisopropylaluminium chloride.[18] Reductive deprotection of the MMTr or α-naphthyldiphenylmethyl group with naphthalene radical anion was also reported.[19]

In oligodeoxyribonucleotide synthesis, acidic treatment for removal of the DMTr or Pix group, which was used as the 5'-hydroxyl protecting group, should be done as rapidly

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* This paper is dedicated to Prof. Morio Ikehara on the occasion of his 70th birthday.

as possible to avoid the depurination of deoxyadenosine.[9] On the other hand, in oligoribonucleotide synthesis, when acid labile protecting groups such as tetrahydropran-2-yl,[15,20-28] 4-methoxytetrahydropyran-4-yl,[29,30], tetrahydrofuran-2-yl,[31-33] and 1-ethoxy-1-methylethyl[34] were employed as the 2'-hydroxyl protecting group in combination with the 5'-DMTr or Pix group, extremely careful acidic treatment was required to achieve the selective removal of the latter for chain elongation in the 5' direction.

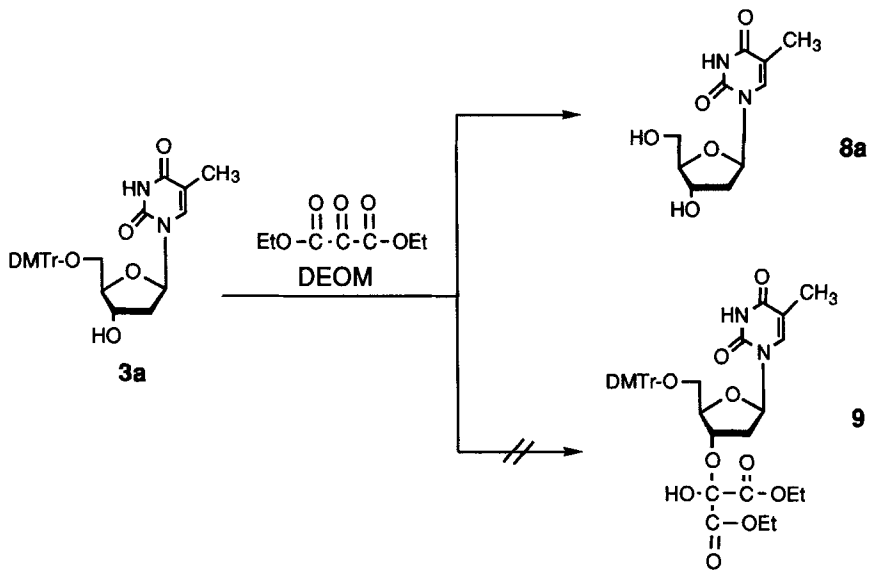
To achieve the detritylation under milder conditions, several devices have been reported.[35-37] Recently, 9-(4-methoxyphenyl)xanthen-9-yl (Mox)[4c] has been employed as the 5'-protecting group, which could be more readily removed than the Pix and DMTr groups, for pyrimidine ribonucleosides.[38] Selectivity is an essential problem in the synthesis of long oligonucleotides.

This paper deals with a new method for removal of the DMTr and related trityl groups, which were attached to the 5' hydroxyl of deoxyribonucleoside derivatives, under entirely new conditions using diethyl oxomalonate (DEOM), and also describes several unique properties of this reagent in basicity-controlled detritylation.

RESULTS AND DISCUSSION

The first clue of this study was found when reaction of 5'-*O*-dimethoxytritylthymidine (**3a**) with various kinds of electron-deficient carbonyl compounds was attempted to obtain hemiacetal derivatives which could be further protected with appropriate protecting groups.[39] In order to prepare the hemiacetal (**9**), the reaction of **3a** with DEOM was carried out in pyridine. Since no reaction occurred in this medium, solvent effects were next examined by using the aprotic solvents dimethylformamide (DMF) and tetrahydrofuran (THF). No reaction was observed in DMF either. When 10 equiv of DEOM was added to a THF solution of **3a**, an exothermic reaction occurred so that **3a** disappeared rapidly within 1 min to give **8a** as the main product. This reaction, however, was accompanied by small amounts of two unknown byproducts, which appeared between **3a** and **8a** on TLC.

The detritylation was surprisingly very rapid but it seemed to us that this was based on a considerable rate enhancement due to the reaction heat generated by solvation of DEOM with THF when it was added. Therefore, an experiment was done in such a manner where a solution of DEOM in THF, which was prepared in advance and cooled to 25 °C, was added to **3a**. As a result, it was confirmed that the detritylation proceeded under these conditions, although the reaction rate was retarded considerably. These results explicitly suggested that a new type of reaction occurred between **3a** and DEOM with cleavage of the trityl bond.



A solvent effect was observed in this detritylation. As shown in TABLE 1, protic solvents were superior to aprotic solvents. Especially, methanol was most effective and the detritylation was achieved in 10 min by use of this solvent. A similar result was obtained by use of ethanol but 2-propanol was apparently less effective. Tertiary alcohols *t*-butyl alcohol and 1,1-dimethylpropan-1-ol resulted in a considerable decrease of the reaction rate. Interestingly, these solvents gave a single product of **8a** without formation of the byproducts observed in the case of the use of THF. The other aprotic solvents examined also gave only **8a** but complete removal of the DMTr group required more than 2 h. The detritylation depended on the degree of drying of the solvent. Therefore, all solvents were distilled and used after drying over molecular sieves for several days. Next, the effect of water on the detritylation of **3a** in methanol was studied.

When an equimolar amount of water (60 equiv) relative to DEOM (2 M, 60 equiv) in methanol was added, the rate of the detritylation was considerably decreased to 1 h. On the other hand, addition of 60 equiv of acetic acid relative to DEOM (2 M, 60 equiv) in methanol resulted in remarkably rapid removal of the DMTr group from **3a**. The detritylation was completed within 1 min. Contrary to this fact, a 2 M solution of acetic acid in methanol did not show such a strong ability for detritylation, and in this medium the DMTr group of **3a** was removed very slowly ($T_{1/2}$ = 3 d, T_∞ = 3 w). Likewise, acetic acid (2 M) was ineffective for removal of the DMTr group from **3a** when either CH_2Cl_2 ($T_{1/2}$ = 15 h, T_∞= 4 d) or acetonitrile ($T_{1/2}$ = 3 d, T_∞ = 3 w) was used as the solvent.

Addition of an equimolar amount of water (60 equiv) to DEOM (2 M, 60 equiv) in dry CH_2Cl_2 resulted in a remarkable change in the initial rate of detritylation of **3a**. In this

TABLE 1. Solvent Effects of Detritylation of 5'-*O*-(4,4'-Dimethoxytrityl)-thymidine (3a) with DEOM (2 M, 60 equiv) at 25 °C

solvent	MeOH	EtOH	*i*-PrOH	CH_3CN	dioxane	*t*-BuOH	*t*-AmOH
$T_{1/2}$	1 min	2 min	15 min	20 min	2 h	1 h	1 h
T_∞	10 min	12 min	1.5 h	2 h	5 h	5 h	5 h

THF	toluene	CH_2Cl_2	$CHCl_3$	AcOEt	acetone	CH_3NO_2	DMF	pyridine
1 h	1.5 h	1.5 h	1.5 h	2 h	2 h	5 h	n. r.	n. r.
6 h	9 h	10 h	10 h	12 h	12 h	36 h		

case, $T_{1/2}$ was within 1 min but the reaction did not come to completion even after 30 min. Interestingly, the TLC pattern did not change after 1 min. A similar phenomenon was observed when an equimolar amount of methanol (60 equiv) to DEOM (2 M, 60 equiv) in acetonitrile was added. In this case, $T_{1/2}$ was ca. 1 min but similarly **3a** remained even after 30 min. It was likely that equilibrium mixtures of **3a** and **8a** were obtained in such aprotic solvents because the DEOM-water or DEOM-methanol adduct catalyzed the reverse reaction, *i.e.*, the 5'-*O*-dimethoxytritylation of thymidine once generated.

To test if the detritylation activity of a 2 M solution of DEOM in methanol can be maintained on its storage at room temperature, the same reaction of **3a** with DEOM was conducted two days after its preparation. Consequently, almost the same reactivity was confirmed.

Next, the scope and limitation of the present detritylation procedure have been examined using a series of tritylated thymidines (**1a-7a**). The reaction of these compounds with DEOM in methanol at 25 °C was carried out and monitored by TLC. These results are summarized in TABLE 2.

The reaction rate of **4a** was determined using lesser amounts of DEOM. The results are summarized in TABLE 3. The use of 60 equiv of DEOM at a concentration of 2 M gave rapid removal of the Pix group from **4a**. Therefore, we used these conditions for the present detritylation.

The ease of elimination was in the following order: MOX > Pix > DMTr > MMTr > TBTr > Tr > IDTr where TBTr, and IDTr refers to 4,4',4"-tris(benzoyloxy)trityl[40] and 3-(imidazol-1-ylmethyl)-4,4'-dimethoxytrityl[41], respectively. This order was in agreement with that of the stability of the corresponding trityl cations except for the TBTr and IDTr

TABLE 2. Detritylation of 5'-*O*-Protected Thymidine Derivatives with DEOM (2M, 60 equiv) in Methanol

compd		7a	1a		2a	3a	4a	5a	6a
R		TBTr	Tr		MMTr	DMTr	Pix	Mox	IDTr
		25 °C	70 °C	25 °C 70 °C	25 °C	25 °C	25 °C	25 °C	70 °C
$T_{1/2}$ min	a	3	4 h	5	10	1	1	< 0.5	1.5 h
T_{∞} min	a	30	30 h	25	60	10	10	1	5 h

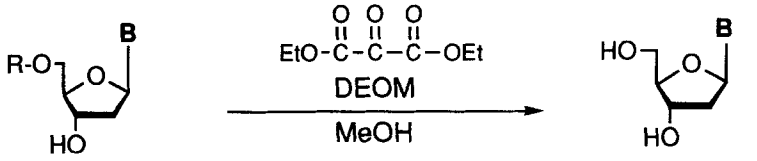
[a]At this temperature this compound was insoluble in methanol.

TABLE 3. Effects of DEOM on Removal of the Pixyl Group from 5'-*O*-Pixylthymidine (4a) with 1 M and 2 M solutions of DEOM (60 equiv) in Methanol at 25 °C

conc. of DEOM	equiv of DEOM	10	20	40	60
1.0	$T_{1/2}$	5 h (20%)	30 min	10 min	4 min
	T_{∞}	-	5 h	2 h	30 min
2.0	$T_{1/2}$	-	-	-	1 min
	T_{∞}	-	-	-	10 min

groups. The TBTr group is known to be more resistant to 80% acetic acid than the Tr group.[41] The IDTr group could be removed by trifluoroacetic acid under conditions similar to those used for the DMTr group.

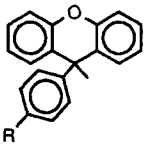
The difficulty in trityl ether bond cleavage of the IDTr group is explained in terms of the presence of the basic imidazolylmethyl residue of the side chain. Therefore, to clarify this inhibitory effect, the detritylation of **3a** was attempted by treatment with a 2 M solution of DEOM (60 equiv) in methanol in the presence of 1 equiv of *N*-methylimidazole which has the *pKa* value of 7.06 but no reaction occurred. It was clear that the imidazolylmethyl group simply inhibited removal of the IDTr group from **6a**. This result led us to study inhibitory effects of various amines having the *pKa* values of 0.79 to 10.87 on the detritylation of **3a** in more detail. These results are summarized in TABLE 4.



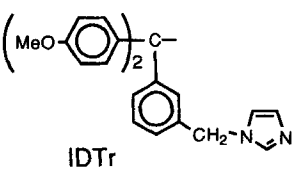
8

1: R = Tr
2: R = MMTr
3: R = DMTr
4: R = Pixyl
5: R = Mox
6: R = IDTr
7: R = TBTr

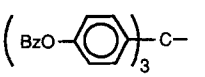
a: B = Th
b: B = Th^{bz}
c: B = Cy^{bz}
d: B = Cy^{an}
e: B = Cy^{pnb}
f : B = Cy^{bz_2}
g: B = Cy^{dmf}
h: B = Ad^{bz}
i: B = Ad^{bz_2}
j: B = G^{ibu}
k: B = $G^{dpc,pro}$



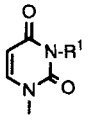
Pixyl : R = H
Mox : R = MeO



IDTr

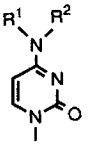


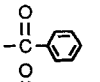
TBTr

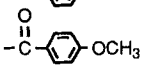


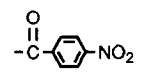
Th : R^1 = H

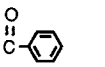
Th^{bz} : R^1 = 



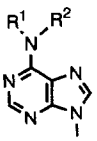
Cy^{bz} : R^1 = H, R^2 = 

Cy^{an} : R^1 = H, R^2 = 

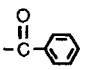
Cy^{pnb} : R^1 = H, R^2 = 

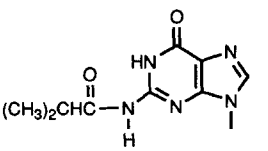
Cy^{bz_2}: R^1 = R^2 = 

Cy^{dmf} : R^1 = R^2 = $\succ$CH·N$(CH_3)_2$

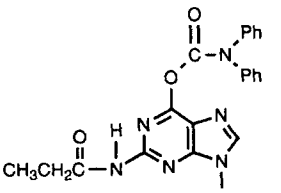


Ad^{bz} : R^1 = H, R^2 = 

Ad^{bz_2} : R^1 = R^2 = 



Gu^{ibu}



$Gu^{dpc\cdot pro}$

TABLE 4. Inhibitory Effects of Amines (1 equiv) on Detritylation of 5'-*O*-(4,4'-Dimethoxytrityl)thymidine (3a) with DEOM (2 M, 60 equiv) in Methanol at 25 °C

base	*pKa*	$T_{1/2}$	T_∞	base	*pKa*	$T_{1/2}$	T_∞
none	-	1 min	10 min	nicotine amide	3.3	n. r.	
Et_3N	10.87	n. r.[a]		isonico-tineamide	3.26	n. r.	
N-methyl-imidazole	7.06	n. r.		pyridazine	2.33	1.75 h	6 h
imidazole	7.03	n. r.		3-cyano-pyridine	1.45	2 min	25 min
pyridine	5.18	n. r.		pyrimidine	1.30	1 h	5 h
$Me_2NC_6H_5$	5.15	n. r.		2-chloro-pyridine	0.72	1 min	15 min
quinoline	4.88	n. r.		pyridine N-oxide	0.79	1.25 h	6 h

[a]n.r. refers to no reaction.

The amines more basic than isonicotineamide inhibited completely the detritylation. In the case of pyridazine, the detritylation proceeded but the rate was considerably decreased. 3-Cyanopyridine, which was a little weaker base than pyridazine, affected this detritylation at a slightly slower rate. The weakest bases, 2-chloropyridine and pyridine *N*-oxide, did not affect the reaction. These results suggested apparently that the DEOM-catalyzed detritylation was highly dependent on the basicity of substrates.

In order to see if the new deprotection technique can be applied to other nucleoside derivatives, various *N*-protected 5'-*O*-(4,4'-dimethoxytrityl)deoxyribonucleoside derivatives (**3a-k**) were synthesized and allowed to react with a 2 M solution of DEOM (60 equiv) in methanol. As shown in TABLE 5, all the compounds were detritylated at 25 °C by this reagent. Among them, *N*-unprotected thymidine derivative **3a** and doubly protected deoxyguanosine derivative (**3k**) were rapidly detritylated at the same rate in 10 min. In the case of 6-*N*-benzoyldeoxycytidine and 6-*N*-benzoyldeoxyadenosine derivatives (**3c** and **3h**), removal of the DMTr group required 6 h. During this time, competitive depurination was observed in the latter case as shown in TABLE 5.

Protection of the thymine residue with the benzoyl group resulted in a slight decrease of the rate of detritylation. When an additional benzoyl group was introduced to the 6-*N*-position of 6-*N*-benzoyldeoxyadenosine derivative **3h**, a considerable rate enhancement of

TABLE 5. Removal of the DMTr Group from 5'-*O*-Trityldeoxyribonucleoside Derivatives (3, 4, and 5) by Use of DEOM (2 M) in Methanol

			base residue										
R			T	T^{bz}	C^{bz}	C^{an}	C^{pnb}	C^{bz_2}	C^{dmf}	A^{bz}	A^{bz_2}	G^{ibu}	$G^{dpc\text{-}pro}$
	compd		**3a**	**3b**	**3c**	**3d**		**3f**[b]	**3g**	**3h**[b]	**3i**[b]	**3j**[b]	**3k**[b]
DMTr min	25 °C	$T_{1/2}$	1	1	45	45	-	2	n.r.	2 h	0.5	10	0.5
		T_∞	10	20	6 h	6 h	-	60 (30%)[c]	n.r.	6 h (60%)[c]	10 (<5%)[c]	80 (<5%)[c]	10 (<5%)[c]
	50 °C	$T_{1/2}$	<0.5	<0.5	-	-	-	<0.5	-	5	<0.5	-	<0.5
		T_∞	<0.5	2	40	-	-	2 (20%)[c]	-	25 (90%)[c]	2 (30%)[c]	-	3 (35%)[c]
	compd		**4a**	**4b**	**4c**			**4f**[b]		**4h**[b]	**4i**[b]		**4k**[b]
Pix min	25 °C	$T_{1/2}$	1	2	a	-	-	0.5	-	-	0.5	-	<0.5
		T_∞	10	20	a	-	-	50 (30%)[c]	-	-	5 (<5%)[c]	-	5 (<1%)[c]
	50 °C	$T_{1/2}$	-	-	3	-	-	-	-	-	-	-	-
		T_∞	<0.5	2	30	-	-	-	-	-	-	-	-
	compd		**5a**	**5b**		**5d**	**5e**	**5f**[b]					
MOX min	25 °C	$T_{1/2}$	<0.5	<0.5	-	25	20	0.5	-	-	-	-	-
		T_∞	1	3	-	80	80	5 (<5%)[c]	-	-	-	-	-

[a]This compound was insoluble at this temperature. Therefore, no reaction took place.

[b]In the case of these coumpounds, competitive depurination occurred. $T_{1/2}$ and T_∞ refer to the times when half the starting nucleoside and all, respectively, disappeared on TLC.

[c]The values in parentheses are the approximate ratio (±5%) of depurination / or depyrimidination / detritylation at the time of T_∞ which was estimated by TLC.

detritylation was observed. The DMTr group could be removed from the 6-*N*,6-*N*-dibenzoyldeoxyadenosine derivative (**3i**) at a rate similar to those of **3a** and **3k**. The 6-*N*,6-*N*-dibenzoyl group might serve as an electron-withdrawing group which diminished the basicity of the purine base involving a heterocyclic structure like *N*-methylimidazole as shown in TABLE 4.

Extremely slow detritylations were observed in the case of N^4-monoacyldeoxycytidine derivatives **3c** and **3d**. This is because the 4-acylamino group behaves as a faint electron-releasing group so that the cytosine ring exhibits the weakly basic property of pyrimidine which significantly inhibited the detritylation of **3a** as shown in TABLE 4. On the other hand, double protection of the cytosine residue with two benzoyl groups[42] resulted in a significant rate enhancement of detritylation as seen in the case of **3f**. However, the glycosyl bond of **3f** was simultaneously cleaved to a considerable degree. Our recent study has disclosed that 4-*N*,4-*N*-dibenzoyldeoxycytidine exists in an equilibrium mixture with 4-*N*,N^3-dibenzoyldeoxycytidine which undergoes facile depyrimidination upon treatment with 80% acetic acid.[42] In attempts to remove selectively the 5'-protecting group, compounds **4f** and **5f** having the Pix and Mox groups, respectively, were synthesized and subjected to the DEOM mediated detritylation. Consequently, the competitive depyrimidination could not be avoided even in the case of **5f**.

As expected from these considerations mentioned above, a deoxycytidine derivative (**3g**) possessing a basic protecting group of the amidine type remained unchanged when allowed to react with DEOM (2 M, 60 equiv) in methanol.

Compared with 2-*N*-isobutyrylated deoxyguanosine derivative **3j**, doubly 6-*O*,2-*N*-protected deoxyguanosine derivative **3k** was rather rapidly detritylated by DEOM as shown in TABLE 5. This difference was also explained in terms of decrease of the basicity of the heterocyclic ring owing to the diphenylcarbamoyloxy group which served as an electron-withdrawing group. At an elevated temperature of 50 °C the DMTr groups of "fully base-protected" deoxyribonucleoside derivatives **3b**, **3f**, **3i**, and **3k** were rapidly removed within 3 min.

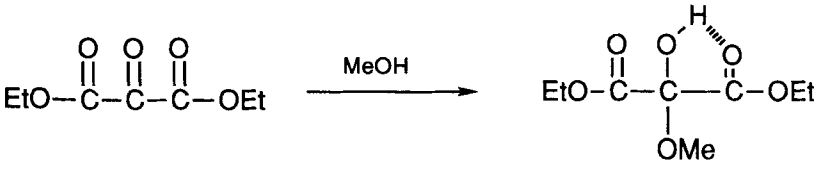
In a similar manner, detritylation of a series of 5'-*O*-Pix and Mox derivatives with DEOM was examined. These results are summarized in TABLE 5. As shown in TABLE 5, the use of the Pix or Mox group for protection of the 5'-hydroxyl group resulted in more rapid detritylation than the DMTr group. However, deprotection of 5'-*O*-tritylated deoxycytidine derivatives was rather slow even in the case of the MOX group at 50 °C (data not shown). The utility of the present reaction depends on this improvement of the detritylation of deoxycytidine derivatives.

As mentioned before, it was difficult to remove selectively the trityl function from 4-*N*,4-*N*-dibenzoyldeoxycytidine derivatives **3f**, **4f**, and **5f**. Therefore, effects of organic

salts on the detritylation of **5f** were examined in the hope that the protonation of the inhibitory basic sites by organic salts which consisted of strong acids and weak bases would enhance the 5'-detritylation. The weak bases should be similar to or weaker than 2-chloropyridine since it did not affect the detritylation as shown in TABLE 4. Since only the basic site should be neutralized, an equimolar amount of salt to the cytidine derivative **5f** was added to the detritylating system. As shown in TABLE 6, ammonium hydrochloride resulted in a slight increase of the reaction rate. Pyridinium chloride or pyridinium 3-nitrobenzenesulfonate did not affect this reaction. In the presence of 2-chloropyridinium hydrochloride, the Mox group was rapidly removed within 15 min. In this case, however, the detritylation proceeded *via* the protonation by the pyridinium salt itself. This is based on the fact that, in an independent experiment using one equiv of 2-chloropyridinium hydrochloride to **5f** in methanol, the same rate was observed regardless of the presence or absence of DEOM. It is also interesting that the detritylation can be done by such organic salts.

Since the ease of removal of trityl groups attached to the 5' hydroxyl depended essentially on the basicity of base moieties, it was suggested that the present detritylation proceeded *via* protonation of the trityl ether bond by means of an acidic species generated by the reaction of DEOM with methanol. This was supported by the fact that depurination, which should be promoted by acids, occurred to a significant extent in the tritylated deoxyadenosine **3h** and deoxyguanosine derivatives **3j** and **3k**.

In aprotic solvents, 5'-*O*-(4,4'-dimethoxytrityl)thymidine **3a** underwent relatively slow detritylation as mentioned before. Sterically hindered tertiary alcohols were also ineffective as the solvents compared with primary and secondary alcohols. These results also indicated that a hemiacetal (**10**) as depicted below should be an acidic source which catalysed the trityl ether bond cleavage.



10

The hemiacetal has a proton which can coordinate with an oxygen atom of one of two ethoxycarbonyl groups through a five-membered ring intermediate. A similar hemithioacetal formation between DEOM and thiols has also been reported by Field.[43]

The new type of acid species **10** was confirmed explicitly by ^{1}H NMR and ^{13}C NMR. The solution of DEOM in $CDCl_3$ exhibited two signals at 160.21 and 178.26 ppm, which

TABLE 6. Effects of Pyridinium Salts of Detritylation of 5'-*O*-[9-(4-Methoxyphenyl)xanthen-9-yl]-4-*N*-benzoyldeoxycytidine (5f)

	none	pyridinium hydrochloride	2-chloropyridinium hydrochloride	pyridinium 3-nitrobenzene-sulfonate
$T_{1/2}$ min	25	5	5	20
T_{∞} min	80	15	15	75

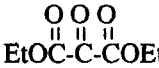
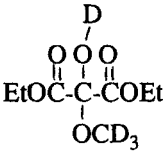
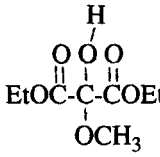
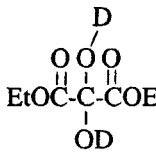
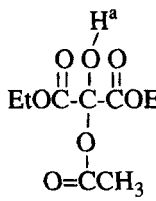
are corresponding to the electron deficient centeral carbonyl carbon and the esteric carbonyl carbon, respectively. Addition of an equimolar amount of methanol to this solution resulted in appearance of a hemiacetal type (HO-C-O) of peak at 94.77 and an esteric carbonyl carbon at 167.35 ppm with complete disappearance of the peaks at 160.21 and 178.26 ppm. A new peak at 51.28 ppm was also observed. This peak corresponds to the methoxy carbon of the hemiacetal.

The ^1H NMR spectrum of DEOM showed significant high magnetic field shifts of the ethyl group upon addition of methanol. The acidic proton was also observed at 5.15 ppm. The ^1H NMR and ^{13}C NMR spectra of DEOM in CD_3OD also exhibited similar signals but upon standing the peaks of ethanol were increasingly observed and the peaks of the hemiacetal central carbon and esteric carbonyl carbon became split. This is probably due to the self-acid catalyzed ester exchange reaction.

In D_2O, DEOM gave ^{13}C resonance signals at 93.91 and 171.26 ppm, which were similar to those of the adduct of DEOM-methanol. The ^{13}C NMR spectrum of the mixture, which was obtained by addition of an equimolar amount of acetic acid to the 2 M solution of DEOM in $CDCl_3$, revealed that the adduct was in equilibrium with DEOM and acetic acid in the ratio of 1:1. The center carbon of HO-C-OAc appeared at 90.99 ppm. The ^{13}C NMR spectra of DEOM adducts are summarized in TABLE 7.

The 0.1 M aqueous solution of DEOM exhibited acidic properties showing a pH of 2.52. This pH value was near the pH value of 2.70 which was measured when acetic acid was dissolved in water at the same concentration as 0.1 M. The 0.1 M aqueous solution of trifluoroacetic acid showed pH 1.77. These results implied that in aqueous solutions DEOM existed as a hydrate compound[44] which has an acidic proton similar to that of the DEOM-MeOH adduct.

TABLE 7. ^{13}C and ^{1}H NMR Spectra of DEOM Adducts with Methanol, Water, and Acetic Acid.

DEOM adduct	solvent	O-C-O	C-O	CH_2	CH_3	others (C, H)
				(^{1}H NMR, δ, ppm) (J_{H-H} value)		
	$CDCl_3$		160.21	63.49 (4.45)	13.95 (1.40) (J = 7.26)	[C=O] 178.26
	CD_3OD	96.25	168.20	63.12 (4.25)	14.24 (1.28) (J = 7.06)	
	$CDCl_3$	94.77	167.35	63.00 (4.33)	13.98 (1.32) (J = 7.06)	[OCH_3] [OH] 51.28 (3.43) (5.15)
	D_2O	93.91	171.26	66.04 (4.32)	15.68 (1.29) (J = 7.06)	
	$CDCl_3$	90.99	168.72	63.63 (4.32)	13.94 (1.31) (J = 7.12)	[C=O] [CH_3] 177.51 20.67 (2.09)

[a]This acidic proton was not clearly observed probably because of the rapid exchange of the acidic protons between acetic acid and the adduct, which were in equilibrium.

In the case of detritylation using methanol as the solvent, the parent deoxyribonucleosides **8a,b,c,i,k** were isolated as single nucleosidic products in 76-88% yields and the structure was confirmed by comparison of their ^{1}H NMR spectra with authentic samples. The other products derived from the DMTr group were also isolated in each case and found to be 4,4'-dimethoxytritanol (**11**) and its methyl ether (**12**). In the case of **3a**, **11** and **12** were obtained in 5% and 85% yield, respectively.

DEOM is known as a potential electrophilic reagent for organic synthesis. For example, reaction of DEOM with malononitrile gave electron deficient ethylene derivatives.[45] Friedel-Crafts type substitutions of DEOM with phenols proceeded in the presence of acid or Lewis acid catalysts to give 4- and 2-substituted phenols.[46,47] DEOM underwent 4+2 cycloaddition with dienes at elevated temperatures to give six-membered ring products.[48] During this study, fortunately, no side reactions associated with base and sugar moieties were observed except for the reaction of **3a** with DEOM in dry THF. It was likely that DEOM behaved as an acid rather than as an electrophile in methanol. DEOM is commercially available (Tokyo Kasei) but there are reported several methods for the preparation of DEOM.[49-52] Recently, an effective method that enabled us to obtain DEOM on a large scale was reported by Salomon.[53]

CONCLUSION

The present new detritylation led us to know how significantly the cleavage of trityl ether bonds depended on the basicity of protecting groups and the parent nucleoside bases. This is a clue for finding a more effective access to the selective and mild detritylation of 5'-protected nucleosides. Several suggestive results were obtained since 6-*O*-protection of the guanine residue or *N,N*-diacylation of the *exo* amino groups of adenine and cytosine bases led to marked rate enhancement of removal of trityl groups. Our preliminary experiments suggested that decrease of the basicity of base moieties by introduction of electron withdrawing protecting groups resulted in much faster removal of the DMTr group or Pix group from the corresponding 5'-tritylated nucleosides when ordinary carboxylic acids such as dichloroacetic acid and trifluoroacetic acid were used as detritylating agents. More detailed studies concerning application of the present finding to oligoribonucleotide synthesis should be necessary.

In a general sense, it has been recognized that the DMTr group attached to the 5'-hydroxyl group of purine deoxyribonucleosides (dG and dA) were distinguishably more rapidly removed than that of pyrimidine deoxyribonucleotides by treatment with organic acids and Lewis acids.[54] However, our results were inconsistent with these observations. In particular, *N*-protected deoxyadenosine derivatives were apparently more resistant than thymidine derivatives. This is a remarkable difference between DEOM and other agents.

A different mechanism should be considered. In conclusion, it should be emphasized that a species generated by mixing DEOM with methanol is a new type of acid source which will provide a new tool to hydrolysis of a variety of trityl ether bonds.

EXPERIMENTAL

General Remarks. ^{1}H NMR spectra were obtained at 60 MHz on a Hitachi 24B spectrometer and at 270 MHz on a JEOL GX-270 spectrometer. ^{13}C NMR spectra were recorded at 67.8 MHz on a JEOL GX-270 spectrometer. All solvents were distilled and dried over molecular sieves 3A. DEOM was purchased from Tokyo Kasei Co. Ltd. The detritylation was monitored by TLC using Merck silica gel plates (Art No. 5715) with the following solvent systems: A, CH_2Cl_2-MeOH (9:1, v/v); B, CH_2Cl_2-MeOH (20:1, v/v); C, hexane-ether (4:1, v/v). Compounds **1a**,[55] **2a**,[56] **3a**,[56] **3b**,[57] **3c**,[56] **3d**,[56] **3f**,[42] **3g**,[58] **3h**,[56] **3i**,[59] **3J**,[56] **3k**,[60] **4a**,[4] **4c**,[4] **4h**,[61] **4k**,[62] **4f**,[42] **5f**,[42] **6b**,[41], and **7a**[40] were synthesized by the literature method.

General Procedure for the 5'-*O*-Tritylation of Deoxyribonucleotide Derivatives (8). An appropriately protected deoxyribonucleoside (**8b**, **8c**, **8e**, or **8i**, 2 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine and finally dissolved in dry pyridine (40 mL). The solution was cooled to 0 °C and pixyl chloride (3 mmol) or mox chloride (2.4 mmol) was added portionwise. The resulting mixture was stirred at 0 °C for 30 min to 1.5 h and then methanol (0.5 mL) was added. Extraction was performed with CH_2Cl_2. The extracts were combined, dried over Na_2SO_4, filtered, and evaporated. The residue was chromatographed on a column of silica gel (30 g) with CH_2Cl_2-MeOH (100:0 - 95:5, v/v) in the presence of 0.5% pyridine to give **4b**, **4i**, **5b**, **5c**, and **5e** in 55%, 51%, 71%, 78%, and 57% yields, respectively.

5'-*O*-(9-Phenylxanthen-9-yl)-*N*^{3}-benzoylthymidine (4b). mp 130-132 °C; ^{1}H NMR($CDCl_3$-DMSO-d_6, 1:1, v/v) δ 1.56 (s, 3H, 5-CH_3), 2.31 (m, 2H, 2'-H), 3.16 (m, 2H, 5'-H), 3.91 (m, 1H, 4'-H), 4.33 (m, 1H, 3'-H), 6.20 (t, 1H, 1'-H), 6.50-8.00 (m, 18H, ArH), 7.52 (s, 1H, 6-H). Anal. $C_{36}H_{30}O_7N_2$: C, 71.75; H, 5.02; N, 4.65. Found: C, 72.16; H, 5.09; N, 4.86.

5'-*O*-(9-Phenylxanthen-9-yl)-6-*N*,6-*N*-dibenzoyldeoxyadenosine (4i). ^{1}H NMR($CDCl_3$-DMSO-d_6, 1:1, v/v) δ 2.60 (m, 2H, 2'-H), 3.30 (m, 2H, 5'-H), 3.97 (m, 1H, 4'-H), 4.44 (m, 1H, 3'-H), 6.36 (t, 1H, J = 6 Hz, 1'-H), 7.13 (m, 16H, ArH), 7.67 (m, 3 H, ArH), 8.37 (s, 1H, ArH). Anal. $C_{43}H_{33}O_6N_5 \cdot 1/2H_2O$: C, 71.26; H, 4.73; N, 9.66. Found: C, 71.42; H, 4.60; N, 9.56.

5'-*O*-[9-(4-Methoxyphenyl)xanthen-9-yl-*N*^{3}-benzoylthymidine (5b). 1H NMR($CDCl_3$-DMSO-d_6, 1:1, v/v) δ 1.58 (s, 3H, 5-CH_3), 2.34 (m, 2H, 2'-H), 3.18 (m, 2H, 5'-H), 3.68 (s, 3H, O-CH_3), 3.90 (m, 1H, 4'-H), 4.31 (m, 1H, 3'-H), 6.18 (t, 1H,

1'-H), 6.70 (d, 2H, ArH), 6.90-7.95 (m, 15H, ArH), 7.52 (s, 1H, 6-H). Anal. $C_{37}H_{32}O_8N_2$: C, 70.24; H, 5.10; N, 4.43. Found: C, 70.16; H, 5.10; N, 4.24.

5'-*O*-[9-(4-Methoxyphenyl)xanthen-9-yl-*N*^{4}-benzoyldeoxycytidine (5c). ^{1}H NMR($CDCl_3$-DMSO-d_6, 1:1, v/v) δ 2.34 (m, 2H, 2'-H), 3.19 (m, 2H, 5'-H), 3.70 (s, 3H, O-CH_3), 3.96 (m, 1H, 4'-H), 4.22 (m, 1H, 3'-H), 5.13 (d, *J* =4 Hz, 1 H, 5-H), 6.08 (t, *J* = 5 Hz, 1'-H), 6.71 (d, *J* = 9 Hz, ArH), 7.21 (m, 15 H, ArH), 7.97 (m, 3 H, ArH and 6-H). Anal. $C_{36}H_{31}O_7N_3 \cdot 1/4H_2O$: C, 69.50; H, 5.10; N, 6.75. Found: C, 69.40; H, 4.79; N, 6.66.

5'-*O*-[9-(4-Methoxyphenyl)xanthen-9-yl-4-*N*-(4-nitrobenzoyl)deoxycytidine (5e). ^{1}H NMR($CDCl_3$-DMSO-d_6, 1:1, v/v) δ 2.33 (m, 2H, 2'-H), 3.21 (m, 2H, 5'-H), 3.74 (s, 3H, O-CH_3), 3.94 (m, 1H, 4'-H), 4.23 (m, 1H, 3'-H), 5.18 (d, *J* = 5 Hz, 6-H), 6.73 (t, J = 5.5 Hz, 1'-H), 6.71 (d, *J* = 8 Hz, 2H, ArH), 7.11 (m, 10H, ArH), 8.13 (m, 5H, ArH and 6-H). Anal. $C_{36}H_{30}O_9N_4 \cdot 1/4H_2O$: C, 64.81; H, 4.61; N, 8.40. Found: C, 64.80; H, 4.26; N, 8.37.

5'-*O*-[9-(4-Methoxyphenyl)xanthen-9-ylthymidine (5a). To a suspension of 9-(4-Methoxyphenyl)xanthen-9-ol 1/2 benzene adduct (756 mg, 2.2 mmol) in dry hexane (5 mL) was added acetyl chloride (5 mL). The mixture was refluxed for 20 min, and the solvent and the reagent were evaporated under reduced pressure. The residue was evaporated two times from hexane (10 mL) and then dissolved in pyridine (10 mL). A solution of thymidine (484 mg, 2 mmol) in pyridine, which was coevaporated three times with dry pyridine, was added. After being stirred for 40 min, the solution was partitioned between CH_2Cl_2 and water. The organic extract was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Addition of toluene to the residue gave crystalline **5a** (961 mg, 91%): mp 142-145 °C; ^{1}H NMR($CDCl_3$-DMSO-d_6, 1:1, v/v) δ 1.53 (s, 3H, 5-CH_3), 2.28 (m, 2H, 2'-H), 3.11 (m, 2H, 5'-H), 3.66 (s, 3H, O-CH_3), 3.86 (m, 1H, 4'-H), 4.21 (m, 1H, 3'-H), 6.21 (t, *J* = 7.0 Hz, 1H, 1'-H), 6.66 (d, J = 8.5 Hz, ArH), 7.09 (m, 10H, ArH), 7.44 (s, 1H, 6-H). Anal. $C_{30}H_{28}O_7N_2$: C, 68.17; H, 5.34; N, 5.30. Found: C, 68.57; H, 5.55; N, 5.02.

General Procedure for Removal of Trityl or Its Modified Trityl Groups from the Corresponding 5'-*O*-Tritylated Deoxyribonucleoside Derivatives. Analytical scale: A standard 2 M solution of DEOM in dry methanol was prepared as follows. DEOM (915 μL, 6 mmol) was added dropwise to dry methanol (1.5 mL) with cooling. The solution was diluted to a 3.0 mL volume by addition of dry methanol. This solution (300 μL) was added to an appropriately protected deoxyribonucleoside derivative (10 μmol) put in an eppendorf tube and the mixture was kept at 25 °C, 50 °C, or 70 °C for the time listed in Tables. An aliquot was taken at appropriate intervals and analyzed by TLC. To judge the half time ($T_{1/2}$) as precisely as possible, a solution of each protected

deoxyribonucleoside (5 μmol) in dry methanol (300 μL) was prepared in advance, put on TLC, and the spot was developed along with a spot from the reaction mixture. Both the solutions were spotted in the same amount. Preparative Scale: The reaction was carried out on a 1 mmmol scale by using **3a**, **3b**, **3c**, **3h**, **3i**, or **3k**. After the reaction was completed, pyridine (5 mL)-CH_2Cl_2 (400 mL) was added. The CH_2Cl_2 solution was applied to a column of silica gel. Elution was performed with CH_2Cl_2-MeOH (100:0 - 90:10, v/v) to give the detritylated product (**8a**, 76%; **8b**, 79%; **8c**, 85%; **8h**, 80%; **8i**, 81 %; **8k**, 88%).

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