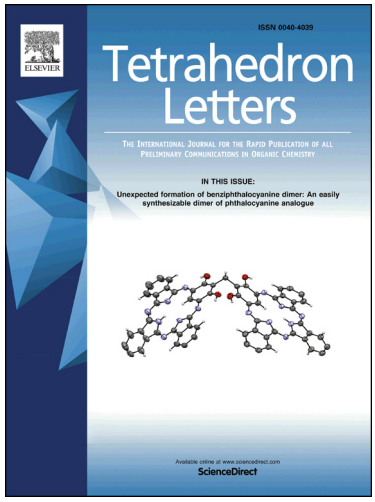

DDQ mediated regiospecific protection of primary alcohol and deprotection under neutral conditions: Application of new *p*-methoxy benzyl-pixyl ether as reagent of choice for nucleoside protection

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Graphical Abstract

DDQ mediated regiospecific protection of primary alcohol and deprotection under neutral conditions: Application of new *p*-methoxy benzyl-pixyl ether as reagent of choice for nucleoside protection

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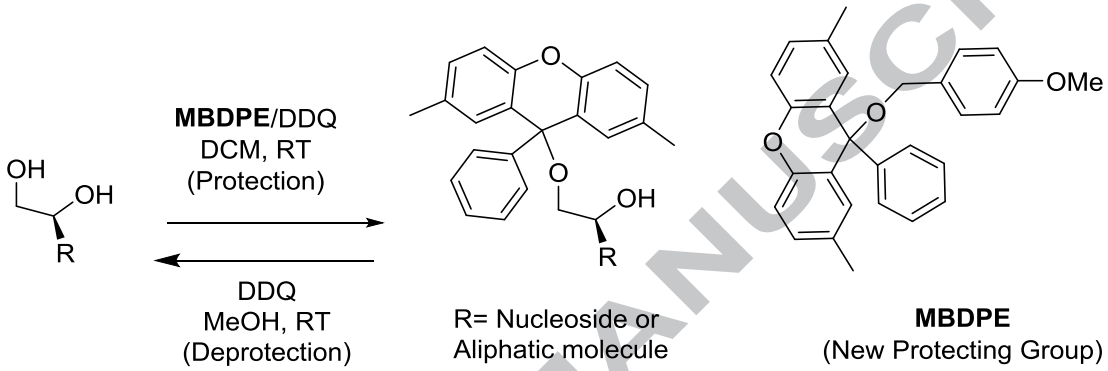

Simple and efficient - Regioselective - Mild neutral conditions - Broad scope - Gram-scale

Tetrahedron Letters

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DDQ mediated regiospecific protection of primary alcohol and deprotection under neutral conditions: Application of new *p*-methoxy benzyl-pixyl ether as reagent of choice for nucleoside protection

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ABSTRACT

A simple and efficient protocol is described for regiosepecific protection of primary hydroxyl group both in nucleosides and other molecules with *p*-methoxy-benzyl 2,7-dimethyl pixylether (MBDPE) in presence of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). Furthermore, swift deprotection of 2, 7-dimethylpixyl (DMPx) is accomplished with DDQ in MeOH. Both procedures are successfully implemented on gram-scale synthesis of modified nucleosides. This protocol offers mild and neutral conditions for selective protection and deprotection of DMPx group while compatible in presence of other conventional protecting groups such as benzoyl, benzyl, THP, TBDPS and acetonide.

Introduction

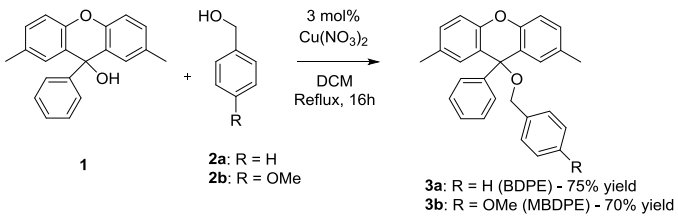
Hydroxyl group protection and deprotection is one of the most critical steps during oligonucleotide synthesis.[1,2] Given the recent FDA approval of modified oligonucleotides as therapeutics, it has become furthermore important to develop improved routes for synthesis of these molecules and their building-blocks. Although 4,4'-dimethoxytrityl (DMT) group is widely used as a 5'-hydroxyl protecting group for oligonucleotide synthesis, 2,7-dimethyl-phenylxanthene-9-yl (DMPx) has emerged as an alternative protecting group due to its crystalline state of products and ease of deprotection.[3-11] In this pursuit we have reported the use of $B(C_6F_5)_3$ as a Lewis acid catalyst[12] for selective protection of primary hydroxyl group in nucleoside with DMPx.[13] This effort led to a modest yield (10-60%) of DMPx-protected nucleosides where cleavage of DMPx was achieved by acid treatment.In order to develop improved synthesis protocols for the next generation of chemically modified oligonucleotides,[14,15] we wish to protect and deprotect the DMPx-group under neutral conditions. This desire triggered our search for a protection and deprotection protocol which we can execute at neutral *p*H. Literature search revealed that tritylation of alcohol was accomplished by using benzyl tritylether or *p*-methoxy benzylether in the presence of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).[16] Additionally, protection of alcohol by trityl, monomethoxy trityl and dimethoxy trityl groups using benzyl triphenylmethyl ethers and various cerium salt as catalyst have been reported.[17] These reports encouraged us to explore the possibility of using DDQ for the installation of DMPx group on 5'-hydroxyl group of nucleosides.

Herein, we report the synthesis of *p*-methoxy-benzyl 2,7-dimethyl pixylether (MBDPE) as a new reagent for the protection of a hydroxyl group in the presence of DDQ. Furthermore, efficient deprotection of DMPx-group has been accomplished with DDQ in methanol at room temperature in good yield.

Results and discussions

Earlier, we have reported on the synthesis of DMPx-OH **1** in excellent yield by treatment of di-*p*-tolylether with benzoic acid in the presence of zinc chloride and phosphorus oxychloride.[13] To date, no synthesis of benzyl or *p*-methoxybenzyl derivative of DMPx alcohol has been reported. Our prior experience with the application of Lewis acids for hydroxyl group protection motivated us to try $Cu(NO_3)_2$ as catalyst for the synthesis of both benzyl and *p*-methoxybenzyl derivatives BDPE **3a** and MBDPE **3b**, respectively.[18] We were delighted to note that the reaction of benzyl alcohol (**2a**) and *p*-methoxybenzyl alcohol (**2b**) with **1** in presence of catalytic $Cu(NO_3)_2$ in dichloromethane (DCM) as solvent under reflux for 16h furnished **3a** and **3b**, respectively, in high yields (Scheme 1). Both benzyl ether **3a** and *p*-methoxy ether **3b** were isolated as white crystalline solid and found to be stable at room temperature.

Scheme-1

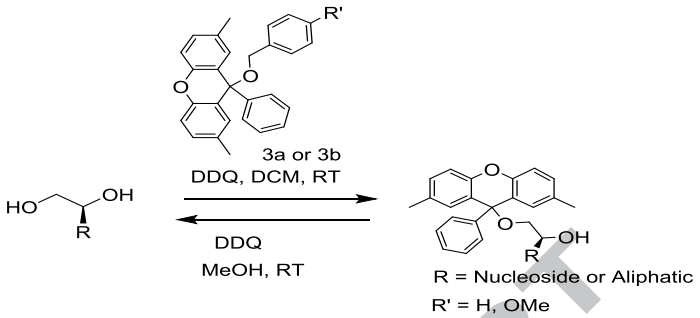


1

2a: R = H
2b: R = OMe

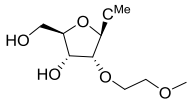
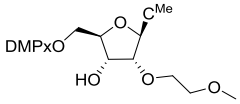
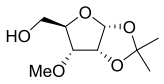
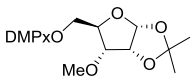
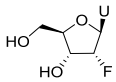
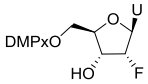
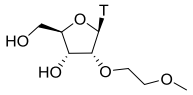
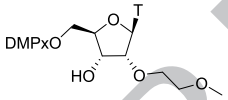
3a: R = H (BDPE) - 75% yield
3b: R = OMe (MBDPE) - 70% yield

With these two reagents in hand, we evaluated their potential for protection of a hydroxyl group. This screening was carried out with four substrates and the data is summarized in Table 1. Interestingly both reagents were capable of installing DMPx group by ether transposition reaction with slight excess of DDQ in DCM at room temperature within an hour. These results show expeditious protection of the primary hydroxyl group in modest yield with BDPE **3a** and good yield with MBDPE **3b** (Scheme 2).



Scheme-2

Table 1: Comparison of yields for both MBDPE and BDPE as dimethylpixyl (DMPx) protection of primary alcohols

Entry	Substrates	Products[a]	With MBDPE (% yield) [b]	With BDPE (% yield) [b]	Reaction Time (in minute)	m.p. (^{o}C)
1	 **4a**	 **5a**	86	60	40	125-127
2	 **4b**	 **5b**	80	50	30	150-152
3	 **4c**	 **5c**	65	45	60	113-115
4	 **4d**	 **5d**	87	70	40	90-92

Reaction conditions: Substrate (1.0 mmol), MBDPE/BDPE (1.2 mmol) and DDQ (1.5 mmol) in DCM (5 mL) at room temperature. Notes: Other solvents like acetonitrile, dichloroethane, tetrahydrofuran and DMF, among these DCM worked best. The solubility of substrate in DCM is necessary for successful protection.

C^{Me} = N^4-Bz-5-Me-cytidine, U = Uracil, T = Thymine

[a] All products were characterized by ^{1}H NMR, ^{13}C NMR and MS.

[b] Yields refer to isolated yields after column chromatography.

The relative rate of the reaction and regioselectivity was monitored by HPLC which clearly indicated that the reaction with *p*-methoxy benzyl ether **3b** was faster than the reaction with benzyl ether **3a**. The enhanced rate of reaction is attributed to the higher oxidation potential of the MBDPE over BDPE. High regioselectivity for primary hydroxyl over secondary hydroxyl group was observed for the three nucleosides **4a, c, d** tested and confirmed by LCMS as single product.

To further elaborate the application of MBDPE as a reagent of choice for the installation of DMPx under neutral conditions. We elected a dozen substrates with diverse functionality and structures. The optimized protection protocol was easily transferrable to six nucleosides **4e-j** furnishing DMPx-protected **5e-j** (Table 2). This reaction was also utilized to synthesize sugar analogs **5k** and **5l** in 65% and 86% yields, respectively. All of the foregoing examples were of conformationally constrained molecules that may prefer protection of the primary hydroxyl group. In order to determine the selectivity for the protection of primary hydroxyl group over secondary hydroxyl group, this protocol was tested with simple aliphatic diols **4m-o**. Gratifyingly, only the primary hydroxyl group was protected with DMPx resulting in the isolation of **5m-o** as crystalline products (Table 2; entries 9-11). Similarly, diol **4p** was converted into **5p** in 40 min furnishing 70% isolated yield. It is noteworthy to mention that a variety of protecting groups such as benzyl, benzoyl, THP, TBDPS and acetonide were present in these molecules and remained unchanged. The results summarized in Table 2 exhibited the broad utility of the MBDPE as a simple, stable and efficient reagent for transferring DMPx to a hydroxyl group under extremely mild conditions.

Scheme-3: Plausible reaction mechanism

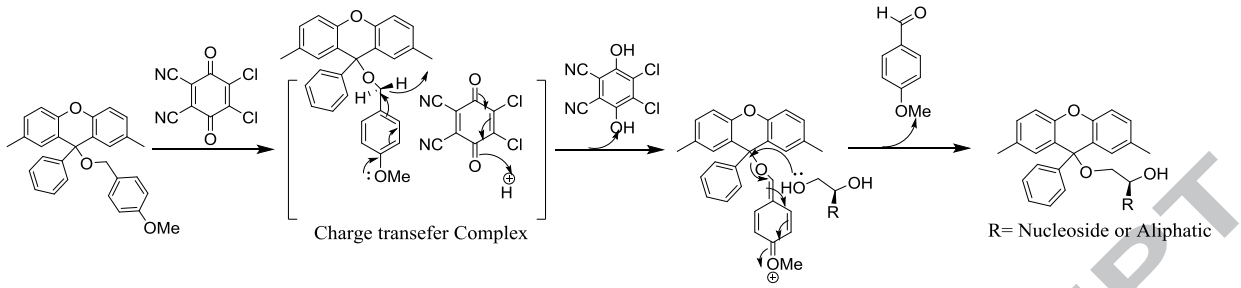
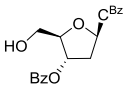
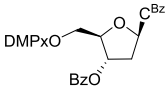
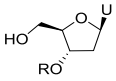
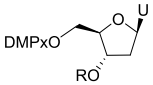
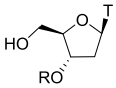
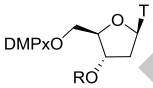
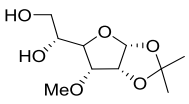
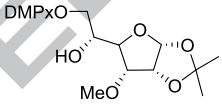
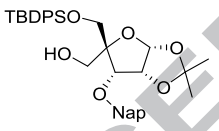
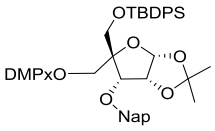
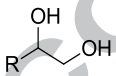
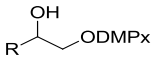
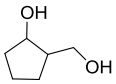
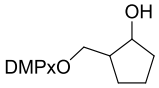


Table-2: DMPx protection with MBDPE and DDQ for structurally diverse substrates

Entry	Substrates	Products[a]	% Yield[b]	Time (in minutes)	m.p (°C)
1	 **4e**	 **5e**	86	25	160-164
2	 R = H **4f**	 R = H **5f**	40	95	140-146
3	R = Bz **4g**	R = Bz **5g**	70	40	130-133
4	 R = Bz **4h**	 R = Bz **5h**	83	35	138-142
5	R = THP **4i**	R = THP **5i**	88	20	92-96
6	R = TBDPS **4j**	R = TBDPS **5j**	85	25	93-95
7	 **4k**	 **5k**	65	35	92-95
8	 **4l**	 **5l**	86	30	136-138
9	 R = CH_3 **4m**	 R = CH_3 **5m**	55	120	115-117
10	R = C_2H_5 **4n**	R = C_2H_5 **5n**	62	90	120-123
11	R = CH_2NHBz **4o**	R = CH_2NHBz **5o**	75	70	93-95
12	 **4p**	 **5p**	70	40	122-125

Reaction conditions: Substrate (1.0 mmol), MBDPE (1.2 mmol) and DDQ (1.5 mmol) in DCM (5 mL) at room temperature.
C^{Bz} = N^4-Bz-cytidine, U = Uracil, T = Thymine
[a] All the products were characterized by ^{1}H NMR, ^{13}C NMR and mass spectral analysis
[b] Yields refer to isolated yields after column chromatography

A plausible oxidative reaction mechanism of the present pixylation protocol is proposed based on an earlier report where tritylation was performed using benzyl tritylether and DDQ.[16, 17] As shown in Scheme 3, the DDQ mediated oxidative reaction triggers abstraction of a benzylic proton of MBDPE generating a DMPx ether cation which reacts with the primary alcohol furnishing desired DMPx-protected molecule and *p*-methoxy benzaldehyde as byproduct.

The conventional protocol for the cleavage of DMPx is accomplished by acidic hydrolysis with a 3% solution of

dichloroacetic acid. Under these conditions, other acid labile protecting group such as THP is also partially cleaved. Also, during the synthesis of long oligonucleotides, depurination is the primary cause for lower yield of the full-length product.[7] Therefore, it is desirable to develop deprotection protocol that would work under neutral conditions. To the best of our knowledge no protocol has been reported for the cleavage of DMPx-group under neutral conditions. In this vein, we decided to examine the possibility of using DDQ for the deprotection of DMPx-group. Upon screening, various reaction parameters including choice of solvent, we discovered that DDQ in methanol at room temperature offers facile and effective cleavage of DMPx-group. Deprotection of DMPx-group from five molecules was accomplished within an hour in high yield.[20] The results are summarized in Table 3.

Table 3: Deprotection of pixyl protecting group with DDQ in methanol:

Entry	Substrates	Products[a]	Time (in minutes)	% Yield[b]
1	**5a**	**4a**	45	80
2	**5b**	**4b**	30	90
3	**5c**	**4c**	40	91
4	**5e**	**4e**	60	95
5	**5l**	**4l**	60	95

Conditions: Substrate (1.0 mmol), and DDQ (0.5 mol %) in methanol (5 mL).
Note: We tried different protic solvents like ethanol, isopropanol, and isobutanol, among these methanol worked best.
[a] All the products were characterized by ^{1}H NMR, D_2O Exc. and mass spectral analysis.
[b] Yields refer to isolated yields after column chromatography.

General Procedure

To a mixture of alcohol (1.0 mmol) and *p*-methoxy benzyl-pixyl ether **l** (MBDPE) (1.2 mmol) and activated molecular sieves 4Å (0.3 g) in anhydrous DCM (5 ml) under nitrogen was added DDQ (1.5 mmol) and the reaction mixture was stirred at room temperature for the given time (Table 1 and 2). After completion of the starting material (monitored by TLC), the reaction mixture was quenched with sat. $NaHCO_3$ (5 ml). The layers were separated and the aqueous layer extracted with DCM (2 x 5 ml), the combined organic layers were washed with water (2 x 5 ml) and brine (1 x 5 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under vacuum. The crude compound was purified by silica gel column chromatography to afford the DMPx ether in good to excellent yields.

Summary

In summary, we have developed the synthesis of MBDPE as a new reagent useful for regioselective installation of DMPx-group onto a hydroxyl group of diverse molecules, including several nucleosides under neutral conditions. Both protection and deprotection of DMPx group was achieved using DDQ. This method is expected to widen the use of DMPx as a hydroxyl protecting group offering several advantages such as the ability to synthesize crystalline products, ease of installation and cleavage under neutral conditions, introducing hydrophobic handle for separation and the enhanced UV profile for detection.

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Supplementary Material

Supplementary data (Details of the preparation and characterization of the products) associated with this article can be found, in the online version.

DDQ mediated regiospecific protection of primary alcohol and deprotection under neutral conditions: Application of new *p*-methoxy benzyl-pixyl ether as reagent of choice for nucleoside protection

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Highlight:

- p-Methoxy benzyl-pixyl ether, a reagent for DMPx protection of alcohol.
- Protection and deprotection of DMPx group using DDQ under neutral conditions.
- Regiospecific protection of primary alcohol over secondary alcohol.

Graphical Abstract

DDQ mediated regiospecific protection of primary alcohol and deprotection under neutral conditions: Application of new *p*-methoxy benzyl-pixyl ether as reagent of choice for nucleoside protection

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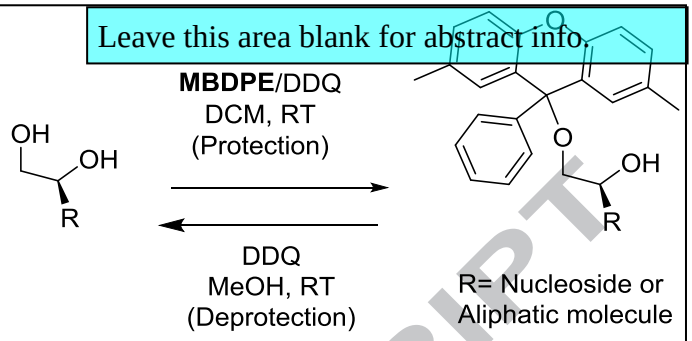

Simple and efficient - Regioselective - Mild neutral conditions -