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1,1,1,3,3,3-Hexafluoro-2-propanol for the Removal of the 4,4′-Dimethoxytrityl Protecting Group from the 5′-Hydroxyl of Acid-Sensitive Nucleosides and Nucleotides

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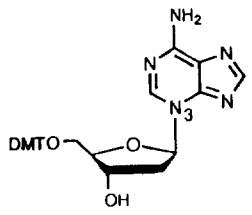
Abstract: 1,1,1,3,3,3-Hexafluoro-2-propanol is introduced as a suitable reagent and solvent for the detritylation of 5′-*O*-(4,4′-dimethoxytrityl)-nucleosides and -deoxynucleosides, especially those that are susceptible to *N*-glycosyl cleavage under more strongly acidic conditions.

Cleavage of the 4,4′-dimethoxytrityl (DMT) group[1] from its protective attachment at 5′-hydroxyl loci in nucleoside and deoxynucleoside units during oligonucleotide synthesis is usually accomplished efficiently with trichloroacetic or dichloroacetic acid in dichloromethane[2-4] or with 80% acetic acid.[5] Certain substrates, however, may suffer *N*-glycosyl cleavage under these detritylation conditions, even though the length of time allotted for the reaction is very short. We encountered some *N*-glycosyl cleavage of 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-3-isoadenosine (**1**) alone or in combinations when we used the normally prescribed conditions. Accordingly, we sought even milder conditions for detritylation that would preserve intact the 2′-deoxy-3-isoadenosine unit and would also be applicable to other acid-labile substrates.



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Because of our favorable experience with the use of fluorinated alcohols in conversions during which *N*-ribosyl and *N*-deoxyribosyl groups were satisfactorily retained[6,7] and because of the known weak acidity of the fluorinated alcohols, we selected 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP),[8] pK_a 9.3,[9,10] as an appropriate reagent and solvent. We examined first the detritylation of 5′-*O*-DMT-3-isoadenosine in HFIP and followed the reaction by the appearance of the bright orange color (λ_{max} 498 nm) of the DMT carbocation. The detritylation was approximately 80% complete in one hour at room temperature. The reaction was followed by TLC on

silicagel with $CHCl_3$-CH_3OH as the solvent, which could separate DMT-ribonucleoside, ribonucleoside, and, if formed, any depurinated product. The DMT-ribonucleoside spot was identified by addition of a drop of 3% dichloroacetic acid in dichloromethane and appearance of the orange color. The other components were identified by parallel chromatography of authentic samples, and in representative cases by physical and spectroscopic properties. The times required for disappearance of the original protected riboside varied with the substrates and were shorter in general for the adenosine than for the cytidine compounds. Our finding is consistent with the statement of Atkinson and Smith relating to 3% dichloroacetic acid in dichloromethane, that 5′-*O*-(DMT)-deoxyadenosine residues require the shortest exposure to acid and deoxycytidine residues, the longest.[11]

In our experiments, each detritylation with HFIP was run (100 mg/ml) in direct comparison with that of 5′-*O*-DMT-adenosine. The extent of detritylation of each DMT derivative was determined at hourly intervals; accordingly, the percent conversions in Table 1, which represent averages of several runs, are relative, but they

Table 1. Detritylation with $(CF_3)_2CHOH$

5′-*O*-DMT Derivative of -	Time, h	Conversion, %
Adenosine	1	87
2′-Deoxyadenosine	2	75
N^6-Allyloxycarbonyl-2′-deoxyadenosine	1	85
N^6-Benzoyl-2′-deoxyadenosine	1	90
3′-*O*-TBDMS-2′-deoxyadenosine	1	90
3-Isoadenosine	1	80
2′-Deoxy-3-isoadenosine (**1**)	1	77
Cytidine	3[a]	91
2′-Deoxycytidine	3	76
N^4-Benzoyldeoxycytidine	3	78
Guanosine	3	87
2′-Deoxyguanosine	3	80
N^2-Isobutyryl-2′-deoxyguanosine	3	80
Inosine	3	84
Thymidine	3	78
Uridine	3	73

[a] 50% Conversion after 1 h.

are repeatable under our laboratory conditions. The percent conversions were obtained by addition of methanol followed by steps of concentration in vacuo, trituration with ether, filtration, and drying or recrystallization. The relative purity of the HFIP-detritylated product was checked by NMR. Included in Table 1 are selected entries of 5′-*O*-DMT derivatives of ribonucleosides, deoxyribonucleosides, and representatives of both with additional substitution that did not interfere. The times and conversions are intended as approximate guides for the course of the detritylation. The percent conversions of a third of the entries are respectable -- for solution chemistry -- after one hour. A three-hour reaction time is ample for detritylation of almost all of the remaining entries. The relative

ease of cleavage noted in Table 1 does not follow an obvious dependence upon the ionization of the base moiety under the reaction conditions. Nevertheless, certain generalities are apparent. The 5′-*O*-DMT-substituted adenosine derivatives undergo detritylation readily with HFIP in the range of 75-90% conversion, as do the 3-isoadenosine derivatives. The 5′-*O*-DMT-substituted cytidine, guanosine, inosine, and uridine derivatives require longer time for the same degree of conversion at room temperature. *N*-Acyl substitution does not interfere with the detritylation, nor does tert-butyldimethylsilyl substitution. The HFIP method is offered for those cases where side reactions can occur when dichloroacetic acid or trichloroacetic acid in dichloromethane is used for the purpose of detritylation.[12]

In order to show the generality of DMT cleavage in non-nucleoside cases, we turned to simple ethers containing the 4,4′-dimethoxytrityl group as one of the substituents on oxygen. Thus, 4,4′-dimethoxytrityl methyl ether and 4,4′dimethoxytrityl ethyl ether were subjected to reaction with 1,1,1,3,3,3-hexafluoro-2-propanol under the conditions described above for the substituted nucleosides. The reaction was followed by NMR, and cleavage was about 80% complete within 3 h. The brilliant orange color that developed was consistent with the appearance of the DMT carbocation. 4,4′-Dimethoxytrityl alcohol was recovered following concentration and preparative TLC on silica gel with chloroform as the solvent. The alcohol, which was characterized by NMR and FAB MS, resulted from adsorbed and adventitious water. The FAB mass spectra of 4,4′-dimethoxytrityl ethyl ether, methyl ether, chloride, and alcohol all showed *m/e* 303 as the major peak, which is consistent with fragmentation to the carbocation. The cleavage of the DMT ethers with HFIP is assumed to follow the general mechanism of the protonation of the ether, which proceeds then with scission to form the stable carbocation associated with $(CF_3)_2CHO^-$. 4,4′-Dimethoxytrityl alcohol itself develops the orange color of the carbocation when dissolved in HFIP.

While the illustrations above have been limited to removal of the DMT group, it is predictable that HFIP[13] can be applied to effect mild acid hydrolysis of other selected functions and may also be useful in differential solvolysis.

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13. See a related function: Williamson, S.; Belisle, C. M.; Singaram, B. Abstracts of Papers, 209th American Chemical Society National Meeting, Anaheim, California, April 2-6, 1995, ORGN 498, used trifluoroethanol as a cosolvent with THF to catalyze the reduction of α,β-unsaturated esters with $NaBH_4$ / $NiCl_2$.

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