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Note

Facile detritylation of nucleoside derivatives by using trifluoroacetic acid*

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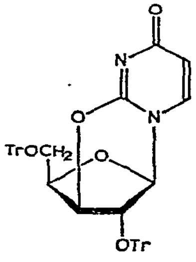
The triphenylmethyl (trityl) ether is one of the most commonly used, acid-labile protecting groups for primary hydroxyl functions in carbohydrate and nucleoside chemistry[1,2]. Previous methods for deprotection have included 80% acetic acid at reflux[3], hydrogen bromide in acetic acid[4], hydrogen chloride in various organic solvents[5–7], sodium in liquid ammonia[8], catalytic hydrogenation[3,9], and absorption onto silica gel[10].

On using these procedures, difficulties have arisen in certain instances; for example, detritylation of 2,3′-anhydro-1-(2,5-di-*O*-trityl-β-D-xylofuranosyl)uracil (**1**) with hydrogen chloride in ethanol to yield 2,3′-anhydro-1-(β-D-xylofuranosyl) uracil[7] gives as much as 34% of 1-(5-chloro-5-deoxy-β-D-xylofuranosyl)uracil as a by-product, because of a rearrangement of the anhydro ring, followed by nucleophilic opening by chloride ion[11–12]. In the hydrogenolysis of trityl derivatives of nucleosides, the reaction has been shown to be somewhat sluggish and unsatisfactory[13].



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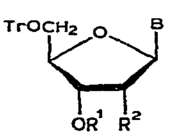

2 B = adenine, R^1 = H, R^2 = H
3 B = adenine, R^1 = H, R^2 = OH
4 B = uracil, R^1 = H, R^2 = OH
5 B = thymine, R^1 = H, R^2 = H
6 B = adenine, R^1 = Ac, R^2 = H

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The main drawback to the use of the trityl protecting group has been with purine deoxynucleosides, where the glycosyl bond is especially susceptible to acid hydrolysis, and concomitant cleavage of the base usually occurs[14]. The presence of an electron-withdrawing group on the sugar has been shown to stabilize the glycosyl bond to acid hydrolysis[15,16], and detritylation without excessive glycosyl cleavage has been found possible for purine deoxynucleosides having 3'-*O*-trifluoroacetyl[15], 3'-*O*-acetyl[16,17], or 3'-*O*-tosyl substituents[17]. Usually, it has been necessary to resort to the more-labile mono-(*p*-methoxyphenyl)diphenylmethyl or di-(*p*-methoxyphenyl) phenylmethyl ethers to permit deprotection without cleavage of the base[14]. Benzenesulfonic acid has recently been shown to be effective for deblocking such derivatives[18]. However, these substituted trityl groups usually show lower selectivity for the primary hydroxyl group during their introduction[2].

A mixture of 1-butanol–formic acid–toluene has been used to deprotect various "methoxytrityl"-protected, aminoacyl nucleoside derivatives[19], and aqueous trifluoroacetic acid (TFA) has been used for the hydrolysis of sugar acetals[20]. This report describes the use of 1-butanol–TFA for hydrolyzing trityl ethers under exceptionally mild conditions.

In our initial studies to find a better procedure, the detritylation of **1** was accomplished with 90% (v/v) aqueous TFA for 2 min at room temperature to yield 2,3'-anhydro-1-(β-D-xylofuranosyl)uracil[7] as the sole u.v.-absorbing product. This result is in direct contrast to previous procedures[7,12], which gave rise to various proportions of by-products. A simple isolation (see Experimental) gave 67% of the crystalline deprotected product, with no attempt being made to reprocess the mother liquors. When these conditions were applied to 2'-deoxy-5'-*O*-trityladenosine (**2**), excessive glycosyl-bond cleavage occurred. This could be minimized by using TFA diluted with 1-butanol and by terminating the hydrolysis by rapid neutralization of the mixture with Bio-Rad 1 × 2 (OH^-) resin prior to evaporation of volatile materials. An overall yield of 79% of 2'-deoxyadenosine (sole u.v.-absorbing product) could be obtained from **2** by this procedure, thus demonstrating the first detritylation of an unsubstituted purine deoxyribonucleoside in good yield. It should be noted that any adenine formed during this hydrolysis would be retained on the resin.

The use of the ion-exchange resin to effect rapid neutralization of the mixture was not necessary for the deprotection of 5'-*O*-trityladenosine (**3**), 5'-*O*-trityluridine (**4**), and 5'-*O*-tritylthymidine (**5**), all of which are more stable to glycosyl bond-cleavage under acid conditions. In these instances, the mixtures were processed by dilution with 1-butanol and evaporation of volatile products *in vacuo*, followed by partition between water and ether to yield the chromatographically pure, deprotected nucleoside in the aqueous layer. When these procedures were used for deprotecting **2**, various proportions of adenine were produced. The mode of isolation was shown, by subjecting **3** to two parallel reactions, to influence the time needed for reaction. In one instance, the reaction was terminated by evaporation of volatile materials, and in the other it was terminated after the same length of time by rapid neutralization with Bio-Rad 1 × 2 (OH^-) resin. The former gave a quantitative

TABLE I

DETRITYLATION WITH 1-BUTANOL–TRIFLUOROACETIC ACID

Starting material (ref.)	*Product*[a]	*1-Butanol–TFA (by volume)*	*Time (min)*	*Yield*[b] *(%)*
1 (7)	2,3′-anhydro-1-(β-D-xylofuranosyl)uracil	9:1[c]	2	67[d]
2 (13)	2′-deoxyadenosine	1.86:1	3	79
3[e]	adenosine	3:1	30	100
4[e]	uridine	3:1	30	100
5[e]	thymidine	3:1	30	100
6 (17)	3′-*O*-acetyl-2′-deoxyadenosine	3:1	30	93[f]

[a]Compared with authentic samples. [b]Estimated by u.v., purity checked by t.l.c. (see Experimental). [c]H_2O–TFA. [d]Crystallized yield (from ethanol). [e]Purchased from Sigma. [f]Product contained ~5% adenine.

yield of adenosine, but the latter yielded ~62% of unreacted **3**. This difference is presumably due to concentration of the acid and/or the continuation of the reaction during the evaporation process.

The increased stability of the glycosyl bond in 3′-*O*-acetyl-2′-deoxy-5′-*O*-trityladenosine (**6**) relative to **2** (refs. 16 and 17) was again demonstrated by the fact that, in the case of **6**, the reaction could be terminated by evaporation of volatiles with the accompaniment of only a small extent (~5%) of glycosyl bond-cleavage. The optimum conditions and yields for **1**–**6** are shown in Table I.

Finally, subjecting 2′,3′-*O*-isopropylideneuridine to the same conditions as used for the detritylation of **3**–**6** showed (t.l.c.) no deblocking, indicating that, if desired, the selective removal of a trityl group in the presence of an isopropylidene group should be feasible. Also, the dinucleoside monophosphate, uridylyl-(3′ → 5′)-uridine (UpU), was treated with 90% aqueous TFA under the identical conditions that caused complete deblocking of **1**. Examination of the crude product by n.m.r. spectroscopy (220 MHz) showed no detectable isomerization of the internucleotide linkage[21,22].

EXPERIMENTAL

General procedure. — The tritylated nucleoside was dissolved in the appropriate solution of trifluoroacetic acid (see Table I) to a concentration of 1–10 mM and kept for the time indicated at room temperature. The reaction was monitored by t.l.c. [Eastman Silica Gel sheets no. 13181 with either (A) the upper phase of 4:1:2 (by volume) ethyl acetate–1-propanol–water, or (B) 1:49 (by volume) methanol–chloroform, as developing solvent]. The reaction was terminated by the addition of 1-butanol (2 vols) and evaporation to dryness *in vacuo* (temp. <40°). This residue was separated between water and ether (washing the aqueous layer well with ether) to yield the deprotected nucleoside as the sole component in the aqueous layer.

For compound **2**, a sample (0.028 mmol) in 9 ml of 1-butanol–TFA (1.86:1) was treated by the foregoing procedure and the reaction was rapidly terminated after 3 min by addition of the mixture to a cooled (−10°), well-stirred suspension of Bio-Rad 1 × 2 (OH^-) resin (120 ml) in methanol. The resin was filtered off, washed well with methanol, and the filtrate evaporated to dryness. This residue was separated between water (25 ml) and ether (25 ml), and the aqueous layer was washed with ether (2 × 50 ml) to yield 2′-deoxyadenosine (0.022 mmol; 79%) in the aqueous phase, as the sole u.v.-absorbing product.

Glycosyl bond-cleavage was evaluated in all cases by t.l.c. [System A for **1**, **4**–**6** and Cellulose sheets no. 13254, with 4:3:2:1 (by volume) tert-butanol–butan-2-one water–ammonium hydroxide as developing solvent, for **2** and **3**.]

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