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# A FACILE DETRITYLATION METHOD FOR ZIDOVUDINE, AN ANTI-RETROVIRAL DRUG

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**ABSTRACT**

A facile detritylation method was developed for zidovudine drug substance by sodium hydrogen sulphate supported on silicagel, without any side reactions. This method is also applicable to detritylation of similar type of molecules with a similar procedure.

**Key words**: Sodium hydrogen sulphate supported on silicagel and Trityl deprotection.



## INTRODUCTION

Zidovudine is chemically known as 3'-azido-3'-deoxythymidine (1a, Azidothymidine, AZT) and first clinically approved drug for treating acquired immuno deficiency syndrome (AIDS)[1]. Zidovudine is a nucleoside reverse transcriptase inhibitor and structurally it is very close to thymidine. It has activity against retroviruses including HIV and is used in the management HIV associated infections. Zidovudine is used alone as well as in combination with other antiviral drugs to treat AIDS patients. Zidovudine has been licensed as Retrovir.

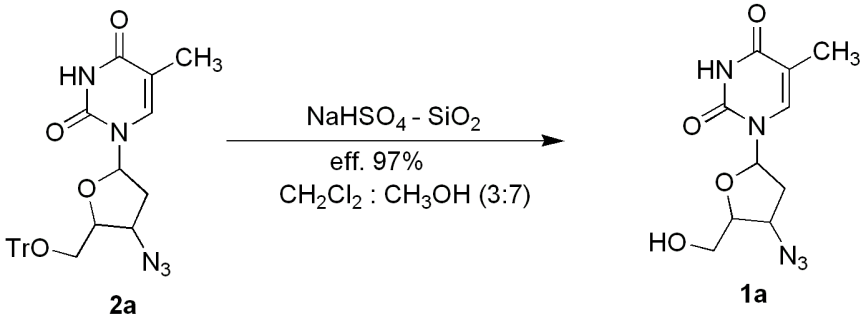
In recent years, the use of silica-supported catalysts has gained considerable attention both in industrial and academic research due to their unique properties. The triphenylmethyl (Trityl) moiety is a valuable protecting group for the hydroxyl, amine and thiol functionalities[2]. Deprotection of trityl group using silica supported acid is advocated in the present work (Scheme-1).

There are several synthetic methods reported in literature for preparation of zidovudine (**1a**). The methods for the preparation of zidovudine involves detritylation of 3'-azido-3'-deoxy-5'-O-trityl thymidine (trityl zidovudine **2a**)[3-7]. The reported methods were used different detritylation reagents such as 50% aqueous formic acid[3], 80% aqueous acetic acid[4], trifluoroacetic acid on a silicagel column[5], dilute hydrochloric acid in methanol [6] and dilute hydrochloric acid in chloroform[7].

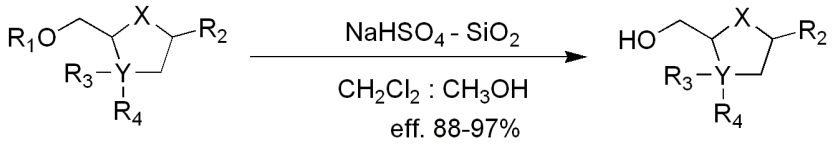
Most of these methods are straight forward and these are associated with some of disadvantages. 50% aqueous formic acid method [3], end of the completion of reaction, reaction mass pH adjusted to ~ 8.2 with aqueous ammonia solution. This leads to the formation of ammonium formate salt, which sticks to the product and lowered chemical assay of zidovudine. Trityl deprotection by trifluoro acetic acid on silica gel column method[5] is very simple, however in commercialization point view, column chromatography is a drawback. Hydrochloric acid in methanol[6] and dilute hydrochloric acid in chloroform [7] methods generate the ~2% chloro zidovudine which is genotoxic impurity and removal of this impurity demands additional purification step resulting in lower yields. We have developed a simple method for conversion of trityl zidovudine (**2a)** to zidovudine (**1a**) by using sodium hydrogen sulphate supported on silicagel, without any side reactions. This method also applied to its analogues.

## EXPERIMENTAL

Solvents and reagents were obtained from commercial source and used without purification. The IR spectra (ϑmax, $cm^{-1}$) were recorded in solid state KBr dispersion using Perkin Elmer FT-IR spectrometer. The $^{1}H$ NMR and $^{13}C$ NMR spectra were recorded on Bruker-Avance 300 MHz spectrometer. The chemical shifts were reported in δ (ppm) relative to TMS. The mass spectra were recorded on API 2000 Perkin Elmer PE-Sciex mass spectrometer. The reactions were monitored by Thin layer chromatography (TLC) associated with high performance liquid chromatography. Melting points were determined by polmon melting point apparatus (Model No.: MP 96), open capillary method and are uncorrected. The compounds (**1b-1f)** are known in literature [9-13]. We synthesized all these compounds as described below detritylation method.




Scheme-1: Structures of Trityl Zidovudine (**2a**) and Zidovudine (**1a**)




Scheme-2: Detritylation reaction

### Typical procedure

***Preparation of 3'-azido-3'-deoxythymidine (Azidothymidine, AZT, Zidovudine, 1a)***

3'-Azido-3'-deoxy-5'-O-tritylthymidine **2a** (tritylzidovudine) (100 g, 196.46 mmol) was dissolved in mixture of dichloromethane: methanol (3:7, 500 mL) at 25-30 °C. The solution was stirred at room temperature for 10 min to obtain a clear solution. Sodium hydrogen sulphate supported on silicagel was added (3 g, 3%w/w). Resulted heterogeneous mass stirred for 2 h at 25-30 °C, during reaction maintenance methyl triphenylmethyl ether (byproduct) precipitated and filtered off byproduct along with heterogeneous catalyst and washed with methanol (50 mL, 2-8 °C). Filtrate was subjected to carbon treatment and concentrated to get oily residue. Added ethyl acetate (220 mL) to the above oily residue and heated to ~ 45 °C to obtain a clear solution. The solution was cooled to ~ 20 °C, white crystals of the product separated out. Filtered the product and washed with chilled ethyl acetate (30 mL, 2-8 °C) to yield compound **1a** (50.8 g, 97%); m.p.123 °C (lit.[4] 120-122 °C ($H_2O$)); Anal. Calcd. for $C_{10}H_{13}N_5O_4$: C, 44.95; H, 4.90; N, 26.21. Found: C, 45.05; H, 4.80; N, 25.90; IR (KBr,$cm^{-1}$): 3463 (N-H, str.), 2116 ($N_3$, str.), 1687 (C=O, str.), 1467 (C-H, bend.), 1281 (C-N, str.), 1090 (C-O str.); $^{1}H$ NMR (300MHz, DMSO-$d_6$): δ (ppm) 1.78 (*s,* 3H), 2.27-2.36 (*m,* 2H), 3.60-3.65 (*m,* 2H), 3.80-3.83 (*m,* 1H), 4.38-4.42 (*m,* 1H), 5.22 (*t* , *J*= 9.0 Hz,1H), 6.10 (*t*, *J*= 15.0 Hz, 1H), 7.68 (*s,* 1H), 11.32 (*s,* 1H); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) : δ (ppm) 13.1, 37.1, 61.0, 61.7, 84.3, 84.8, 110.4, 136.9, 151.3, 164.6; MS *m/z*: 266.0 [$(M-H)^-$].

**Spectral data of the products**

***1-(2-Deoxy-β-D-ribofuranosyl)-5-methyluracil (β-thymidine, 1b)***

This compound was prepared in similar way to **1a**, using 5'-O- Trityl thymidine **2b** ( 10.0g, 20.66 mmol) and crystallization from ethyl acetate, as white crystals (4.6g, 92%); m.p. 184-186 °C, ( lit.[9] 186-187 °C ) ; Anal. calcd. for $C_{10}H_{14}N_2O_5$: C, 49.59; H, 5.82; N, 11.56. Found: C, 49.53; H, 5.79; N, 11.51; IR (KBr,$cm^{-1}$) 3314 (N-H, str.), 1700, 1659 (C=O, str.), 1477 (C-H, bend.), 1274 (C-N, str.), 1066 (C-O, str.); $^1$H NMR (300MHz, DMSO-$d_6$): δ (ppm) 1.77 (*s,* 3H), 2.02-2.15 (*m,* 2H), 3.52-3.62 (*m,* 2H), 3.76-3.77 (*m,* 1H), 4.24 (*brs,* 1H), 5.02 (*t* , *J*=9.0 Hz, 1H), 5.23 (*d, J*= 6.0 Hz, 1H), 6.17 (*t* , *J*=12.0 Hz, 1H) 7.70 (*s,* 1H), 11.27 (*s,* 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) : δ (ppm) 12.2, 61.3, 70.4, 83.7, 87.2, 109.3, 136.1, 150.4, 163.7; MS *m/z* : 241.3[$(M-H)^-$].

Table-1: Detritylation reactions of trityl ethers to alcohols with $NaHSO_4$-$SiO_2$

| Entry no. | Substitutions | | | | | | yields (%) [a] |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | X | Y | |
| 2a | Tr | T | H | $N_3$ | O | C | 97 |
| 2b | Tr | T | H | OH | O | C | 92 |
| 2c | Tr | C | - | - | O | S | 91 |
| 2d | Tr | U | - | - | O | S | 89 |
| 2e | Tr | C | O | - | O | S | 88 |
| 2f | Tr | C | - | O | O | S | 90 |

a = Isolated yields.

Tr = Triphenylmethyl, T = Thymine, C = Cytosine, U = Uracil

Table-2: Effect of different quantity of $NaHSO_4 – SiO_2$ on detritylation reactions

| Entry no | % of $NaHSO_4 – SiO_2$ (w/w) | Temperature ($^0$C) | Time (hr) | yields (%) [a] |
|---|---|---|---|---|
| 1 | 50 | 25-30 | 0.5 | 48 |
| 2 | 25 | 25-30 | 1.0 | 60 |
| 3 | 10 | 25-30 | 1.4 | 73 |
| 4 | 5 | 25-30 | 2.0 | 85 |
| 5 | 3 | 25-30 | 2.0 | 97 |
| 6 | 3 | 38- 42 | 1.0 | 82 |
| 7 | 1 | 38- 42 | 12.0 | 37 |

a = Isolated yields.

***4-Amino-1-[(2R, 5S)-2-(Hydroxymethyl-1, 3-oxathiolan-5-yl)-(1H)]-pyrimidin-2-one (Lamivudine, 1c)***

This compound was prepared in similar way to **1a**, using Trityl lamivudine **2c** (10.0g, 21.23 m mol) and crystallization from aqueous isopropyl alcohol, as white neddle shape crystals, (4.4g 91%); m.p. 127-130 °C (lit.[10] 135 °C ); Anal. Calcd. for $C_8H_{11}N_3O_3S$: C, 41.91; H, 4.84; N, 18.33; S, 13.99. Found: C, 41.74; H, 4.81; N, 18.25; S, 14.10; IR(KBr,$cm^{-1}$): 3236, 3370 (N-H, O-H, str.), 3076 - 2928 (Aromatic C-H, str.), 1643, 1613 (C=O, str.), 1493, 1433, 1404, 1356 (C-C, C-H, C-N ring str.), 1065 (C-O, str.), 803, 787 (Out of plane aromatic C-H bend.); $^1$H NMR (300MHz, DMSO-$d_6$): δ (ppm) 3.06 (*dd*, *J*= 12.0 Hz,1H), 3.33 (*dd, J*= 12.0 Hz, 1H), 3.69-3.75 (*m*, 2H), 5.17 (*t, J*=9.3 Hz, 1H) 5.32 (*t, J*= 12.0 Hz, 1H), 5.73 (*d, J*= 12.0 Hz, 1H), 6.20 (*t, J*= 12.0 Hz, 1H), 7.20, 37.24 (2*brs,* 2H), 7.81 (*d, J*= 9.0 Hz, 1H); $^{13}$C NMR ( 75 MHz, DMSO-$d_6$): δ (ppm) 36.4, 62.9, 85.9, 86.6, 94.0, 141.0, 154.8, 165.7; MS *m/z*: 229.9 [$(M+H)^+$], 252.2 [$(M+H+Na)^+$].

***Cis-2-hydroxymethyl-5-(uracil-1'-yl)-1,3-oxathiolane (1d)***

This compound was prepared in similar way to **1a**, using **2d** (10.0g, 21.18 mmol) and crystallization from ethyl acetate, as off-white powder (4.3g, 89%); m.p. 144-145 °C (lit. [11] Foam); Anal. Calcd. for

$C_8H_{10}N_2O_4S$: C, 41.73; H, 4.37; N, 12.17; S, 13.93. Found: C, 41.60; H, 4.35; N, 12.11; S, 13.90; IR (KBr, $cm^{-1}$ ): 3441 (N-H, str.), 1697, 1678 (C=O, str.), 1464, 1412 (C-H, bend.), 1265 (C-N, str.), 1058 (C-O, str.); $^1$H NMR (300MHz, DMSO-$d_6$): δ (ppm) 3.23 & 3.45 (*2m,* 2H), 3.74 (*brm,* 2H), 5.18 (*t, J*= 9.0 Hz, 1H), 5.36 (*brs,* 1H), 5.63 (*d, J*= 9.0 Hz, 1H), 6.20 (*t, J*= 9.0 Hz, 1H), 7.90 (*d, J*= 9.0 Hz, 1H), 11.39 (*brs,* 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) : δ (ppm) 35.9, 62.6, 85.6, 86.2, 101.5, 140.4, 150.2, 163.0; MS *m/z*: 229.1 [(M-H)$^-$].

Table-3: The detritylation reaction in presence of recycled $NaHSO_4 – SiO_2$

| Entry no | Cycle | Time (hr) | yields (%)[a] |
|---|---|---|---|
| 1 | 1st recycle | 2.0 | 97 |
| 2 | 2nd recycle | 3.0 | 96 |
| 3 | 3rd recycle | 5.0 | 94 |
| 4 | 4th recycle | 7.0 | 91 |

a = Isolated yields

Table-4: Effect of solvent ratio of dichloromethane: methanol

| Entry no | Ratio of dichloromethane:methanol | Reaction Time (hr) | % of conversion[b] |
|---|---|---|---|
| 1 | 100% methanol | 18.0 | 18 |
| 2 | 100% dichloromethane | 18.0 | 2 |
| 3 | 1:1 | 6.0 | 82 |
| 4 | 3:7 | 2.0 | 99 |
| 5 | 7:3 | 6.0 | 91 |
| 6 | 4.6 | 6.0 | 89 |

b = Conversion of alcohol from trityl ether

***Cis-2-hydroxymethyl-5-(cytosin-1'-yl)-3(R)-oxo-1, 3-oxathiolane (1e)***

This compound was prepared in similar way to **1a**, using **2e** (10.0g, 20.53 mmol) and crystallization from water, as white powder (4.4g, 88%); m.p. 256-259 °C (lit.[12] > 270 °C); Anal. Calcd. for $C_8H_{11}N_3O_4S$: C, 39.18; H, 4.52; N, 17.13; S, 13.07. Found: C, 39.08; H, 4.50; N, 17.11; S, 13.04; IR (KBr,$cm^{-1}$): 3424, 3348 (N-H, O-H, str.), 1651, 1619 (C=O, str.), 1404 (C-H, bend.), 1360 (S=O, str.), 1257 (C-N, str.), 1056 (C-O, str.); $^1$H NMR (300 MHz, DMSO-$d_6$): δ (ppm) 3.08 & 3.38 (*2m,* 2H), 3.75 (*m,* 1H), 3.92 (*m,* 1H), 4.66 (*m,* 1H), 5.49 (*t, J*= 9.0 Hz, 1H), 5.08 (*d, J*= 9.0 Hz , 1H), 6.71 (*dd, J*= 9.0 Hz, 1H), 7.37 (*brs,* 2H), 7.71 (*d, J*= 7.5Hz, 1H ); MS *m/z*: 246.1 [$(M+H)^+$], 268.2 [$(M+H+Na)^+$].

***Cis-2-hydroxymethyl-5-(cytosin-1'-yl)-3(S)-oxo-1, 3-oxathiolane (1f)***

This compound was prepared in similar way to **1a**, using **2f** (10.0g, 20.53 mmol) and crystallization from aqueous acetone (4.5g, 90%); m.p. 219-221 °C (lit.[12] > 220 °C); Anal. Calcd. for $C_8H_{11}N_3O_4S$: C, 39.18; H, 4.52; N, 17.10: S, 13.07. Found: C, 39.15; H, 4.52; N, 17.10; S, 13.01; IR (KBr, $cm^{-1}$): 3328 (N-H, str.), 1655, 1613 (C=0, str.), 1405 (C-H, bend.), 1304 (S=O, str.), 1258 (C-N, str.), 1063 (C-O, str.); $^1$H NMR(300MHz, DMSO-$d_6$): δ (ppm) 3.20 (*dd, J*= 15.0 Hz, 1H ), 3.68 (*dd, J*= 15.0 Hz, 1H), 3.80 (*dd, J*= 12.0 Hz, 2H), 4.67 (*t, J*= 15.0 Hz, 1H), 5.47 (*t, J*= 12.0 Hz, 1H), 5.80 (*d, J*= 6.0Hz, 1H), 6.69 (*dd, J*= 9.0 Hz, 1H ), 7.30 (*brs,*2H ), 7.79 (*d, J*= 9.0 Hz, 1H); MS *m/z*: 246.1[$(M+H)^+$], 268.1 [$(M+H+Na)^+$].

## RESULTS AND DISCUSSION

In our reaction procedure, reaction operations are very simple and reliable because of trityl zidovudine **(2a**) and zidovudine **(1a)** are soluble in dichloromethane-methanol mixture (3:7) and insolubility of catalyst and byproduct (Methyl tritylphenylmethyl ether). Moreover we are using catalytic amount of sodium hydrogen sulphate supported on silicagel ($NaHSO_4$-$SiO_2$) reagent (~3%). Product was isolated

from the filtrate by stripping the solvents under reduced pressure and residue crystallized from ethyl acetate to obtain pure zidovudine **(1a)**. The product was free from chloro zidovudine, dimeric impurities and salts. We also checked our method with similar type of molecules (Scheme-2 & Table1). Sodium hydrogen sulphate supported on silicagel ($NaHSO_4$-$SiO_2$) reagent prepared by known literature method[8]. We studied the effect of quantity of catalyst ($NaHSO_4$-$SiO_2$) in different amounts (%,w/w) on 3'-azido-3'-deoxy-5'-O-trityl thymidine (trityl zidovudine **2a**) at 25-42 °C. The results are summarized in table-2. It indicates the best results were obtained when the reaction was carried out in the presence of 3% catalyst ($NaHSO_4$-$SiO_2$) at 25-30 °C. Ease recycling of catalyst is the most significant advantage of our method. In our detritylation reaction, no significant loss of the product was observed when $NaHSO_4$-$SiO_2$ was reused after three times of recycling for compound **1a** (See Table-3). We also studied the different solvent ratio of dichloromethane and methanol to enhance the rate of detritylation, the results are tabulated in table – 4. Table-4 indicates, the best results were observed when the reaction performed in ratio (3:7, dichloromethane: methanol). The compounds (**1b-1d)** are reported as potential anti HIV agents [4, 10-11] and compounds (**1e-1f)** are metabolites of compound **1c**[13].

## CONCLUSION

In summary, we have developed a facile, highly efficient and inexpensive detritylation procedure for zidovudine **(1a)** from tritylzidovudine **(2a)** by using sodium hydrogen sulfate supported on silicagel. Therefore this method is cost-effective and affords the product in higher yield and quality. This method of approach is general and it will be useful for similar type of anti- retroviral drugs, their intermediates and metabolites (**1a-f**) in good yields.

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