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ORIGINAL PAPER

An effective and convenient synthesis of cordycepin from adenosine

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Abstract Cordycepin is a purine nucleoside analog with potent and diverse biological activities. Herein, we designed two methods to synthesize cordycepin. One method mainly converted the 3′-OH group into an iodide group and further dehalogenation to yield the final product. Although this method presented a short synthetic procedure, the synthesis had a low overall yield, resulting in only 13.5% overall yield. To improve the overall yield of cordycepin, another synthetic route was studied, which consisted of four individual steps: (1) 5′-OH protection (2) esterification (3) -*O*-tosyl (-OTs) group removal (4) deprotection. The key step in the synthetic method involved the conversion of 5′-*O*-triphenylmethyladenosine to 3′-*O*-tosyl-5′-*O*-triphenylmethyladenosine, using $LiAlH_4$ as reducing agent. The main advantages of this route were an acceptable total product yield and the commercial availability of all starting materials. The optimal reaction conditions for each step of the route were identified. The overall yield of cordycepin obtained from adenosine as the starting material was 36%.

Keywords Adenosine · Deoxyadenosine · Cordycepin · Synthesis

Introduction

A large number of medicinal fungi are currently being used in various health care applications as well as in disease prevention and treatment. The Chinese fungus Dong-Chong-Xia-Cao, which translates to the Latin technical term Cordyceps sinensis, is an entomogenous fungus that grows on the larvae or pupae of insects. The medicinal Cordyceps species exhibits various clinical health effects. In traditional Chinese medicine, the alcoholic extracts of Cordyceps species have been used for thousands of years as a tonic to reach longevity and vitality (Zhou et al. 2008). As early as 1950, a nucleoside substance, namely cordycepin, was isolated from the original liquid of *Cordyceps militaris* by Cunningham et al. (1950). Shortly thereafter, cordycepin has been shown to be the main component of Cordyceps sinensis (Bentley et al. 1951).

Cordycepin (compound **1,** Fig. 1), with the systematic IUPAC name 3′-deoxyadenosine or 9-(3-deoxy-β-D-ribofuranosyl)adenine, represents a nucleoside analogue that proves to be structurally similar to adenosine, except for the lack of a 3′-hydroxyl group. Therefore, it has been shown to competitively inhibit the processes of synthesis and metabolism of DNA and RNA (Holbein et al. 2009; Tuli et al. 2013) and may further interfere with the activity of adenosine deaminase (Vodnala et al. 2013) (ADA) and the mTOR signaling pathway (Wong et al. 2010). Therefore, cordycepin exhibits a variety of pharmacological activities including immunological (Zhou et al. 2002), anticancer (Hunter et al. 2008; Noh et al. 2010), antioxidant, anti-inflammatory (Rao et al. 2010), anti-microbial (Sugar and Mccaffrey 1998; Ahn et al. 2000), antiviral (Müller et al. 1991), hypolipidemic (Guo et al. 2010) and hypoglycemic properties (Ma et al. 2015). Furthermore, some research groups have also shown that *Cordyceps*

Shen Huang and Hui Liu contributed equally to this work.

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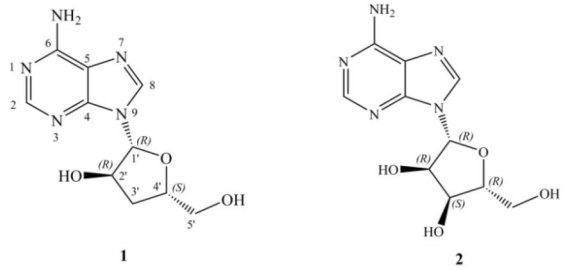


Fig. 1 Chemical structures of cordycepin (**1**) and adenosine (**2**)

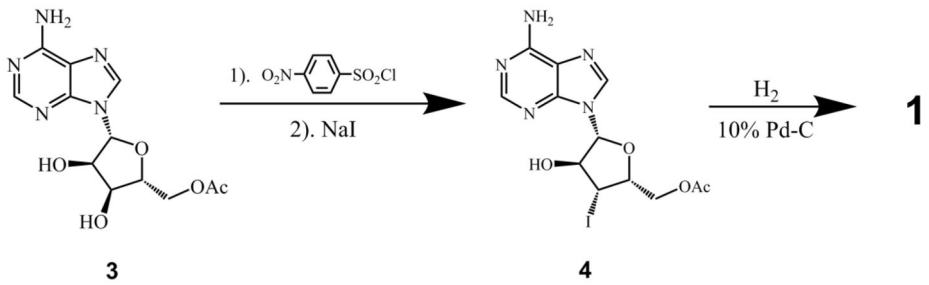
militaris seems to be suitable for the treatment of influenza A viral infections (Ohta et al. 2011).

Due to the large amount of applications and over-exploitation, natural Cordyceps and cordycepin are becoming more sparse. As a consequence, in the last 10 years the market price of cordycepin has increased almost 20-fold, approximately \$12,000 kg^{-1} in 2006 (Ni et al. 2009), with gram quantities now sold by some suppliers at a price of more than \$2200. Therefore, artificial synthetic methods are now being developed to produce cordycepin. In traditional procedures, cordycepin is mainly obtained by extraction from *Cordyceps militaris*. However, this extraction process is not suitable to satisfy market needs due to the low amount of cordycepin in *Cordyceps militaris* of only 2–3 mg/kg. So far, chemical synthesis has gradually replaced *Cordyceps militaris* extractions as a way to produce cordycepin.

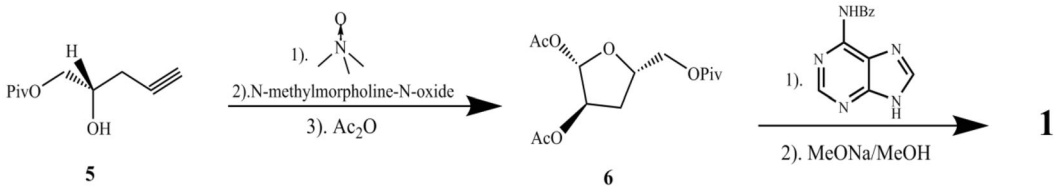
Several semisynthetic and total synthetic methods have been reported for the preparation of cordycepin. A synthetic pathway of cordycepin was first reported by Todd and Ulbricht (Todd and Ulbricht 1960) in 1960. In this method (Scheme 1), **3** was used as a starting material. The 3′-hydroxyl group (3′-OH) was first esterified by *p*-nitrobenzenesulfonyl chloride and the obtained ester was then reacted with sodium iodide to yield **4**. Finally, cordycepin **1** was obtained by deiodination and deprotection reactions. All materials were commercially available at the time and the synthetic procedure was short, however, the overall yield in the last reaction step is reported to be only 17%, resulting in a low overall yield.

McDonald and Gleason (1996) reported a route for the synthesis of cordycepin starting from **5** to obtain the 3′-deoxyribose derivative **6** (Scheme 2). This method represents the first total synthesis of cordycepin. However, **5** (synthesized from allyl alcohol, 21% yield) and TMSOTf were not commercially available at the time, and a low overall yield (14% total yield) was obtained.

Aman et al. (2000) developed another method to produce cordycepin **1** with adenosine **2** as starting material (Scheme 3). The 3′-OH group in adenosine was first converted into a 3′-bromo intermediate **7** by selectively protecting other hydroxyl groups and using 2-acetoxyisobutyryl bromide as a reagent. The target compound cordycepin was synthesized by debromination and deprotection reactions. In this process, cordycepin was obtained as the main product. However, two features limit the overall applicability of this route to cordycepin: (1) 2-acetoxyisobutyryl bromide is no longer available from commercial sources and (2) the purification process is complicated.

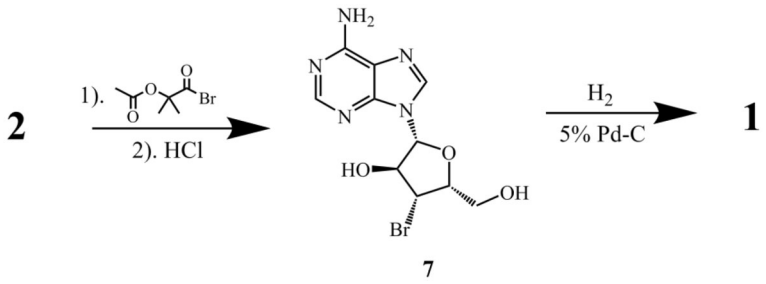


Scheme 1 Synthetic route of cordycepin reported by Todd and Ulbricht

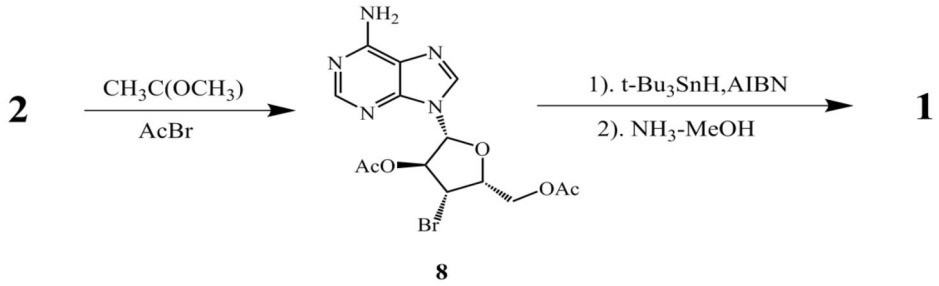


Scheme 2 Synthetic route of cordycepin designed by McDonald

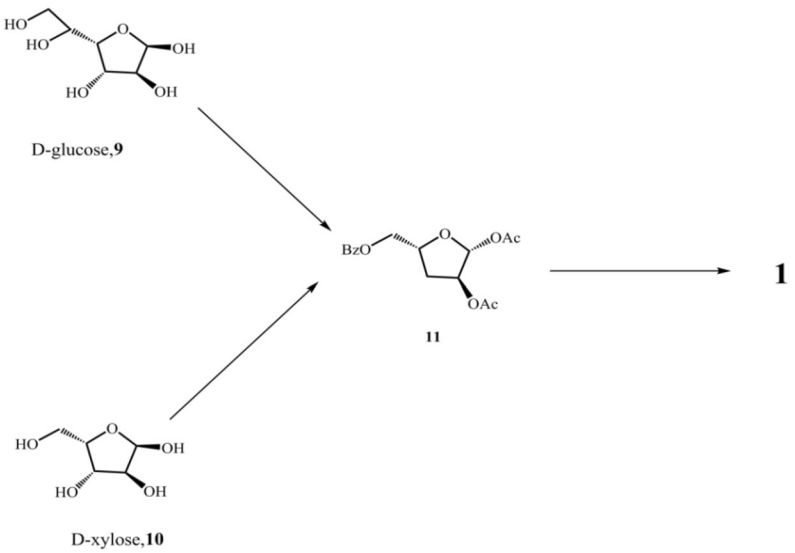
Scheme 3 Synthetic route of cordycepin designed by Aman



Scheme 4 Synthetic route of cordycepin designed by Christelle Moreau



Scheme 5 Total synthesis of cordycepin from D-glucose **8** and D-xylose **9** designed by Li et al. (2013)



Moreau et al. (2013) reported another method for the synthesis of cordycepin (Scheme 4). In this method, the 2′-OH and 3′-OH group in adenosine were cyclized by trimethyl orthoacetate. Then, the intermediate was brominated with acetyl bromide to yield **8**. Finally, cordycepin was obtained by debromination and deprotection reactions. The overall procedure included only three steps and all materials were commercially available. However, the yield of the bromination step was only 29%, resulting in a low overall yield of approximately 20%. In any case, to date this synthetic route represents the most common synthetic pathway used to produce cordycepin (Katayama et al. 2006; Shiragami et al. 1992; Takamatsu et al. 2006).

Meanwhile, Li et al. (2013) have developed a total synthesis for the preparation of cordycepin (Scheme 5). In this method, **9** and **10** were used as starting materials. The key step in the process involved was to remove 3-hydroxyl group via Barton–McCombie reaction to obtain the 3′-deoxyribose derivative **11**, followed by a total of eight and seven steps, respectively, with an overall yield of 37–40%. If further optimized, this method may be used for the large-scale production of cordycepin.

Experimental

General information

All starting materials, reagents and solvents were of analytical reagent (AR) grade, from commercial sources, and

were used without further purification, except for dimethylsulfoxide (DMSO). Calcium hydride (CaH2) was added to DMSO to remove water. The mixture was stirred at room temperature for 48 h, and anhydrous DMSO was obtained by reduced pressure distillation. Analytical samples were obtained by column chromatographic purification on silica gel (200–300 mesh). Nuclear magnetic resonance (NMR, ^{1}H NMR and ^{13}C NMR) spectra were recorded on an Avance III 500 M using tetramethylsilane (TMS) as an internal standard. DMSO-d_6 or $CDCl_3$ were used as the NMR solvents and in the case of benzotrifluoride as the internal standards. All reactions were monitored by thin-layer chromatography and the compounds were analyzed on TLC using UV-light for visualization. Adenosine **2** was used as a starting material with a purity of 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (s, 1H, 2-H), 8.14 (s, 1H, 8-H), 7.38 (s, 2H, –NH_2), 5.88 (d, $J = 6.2$ Hz, 1H, 1′-H), 5.45 (d, $J = 6.4$ Hz, 1H, 2′-OH + 5′-OH), 5.20 (s, 1H, 3′-OH), 4.63–4.59 (m, 1H, 2′-H), 4.14 (s, 1H, 3′-H), 3.98–3.95 (m, 1H, 4′-H), 3.67 (dd, $J = 12.1$, 3.7 Hz, 1H, 5′-H), 3.57–3.54 (m, 1H, 5′-H).^{13}C NMR (101 MHz, DMSO) δ 156.61 (6-C), 152.84 (2-C), 149.49 (4-C), 140.41 (8-C), 119.81 (5-C), 88.36 (4′-C), 86.36 (1′-C), 73.88 (2′-C), 71.14 (3′-C), 62.14 (5′-C).

Synthesis of *N*-acetyl-2′,5′-*O*-diacetyl-3′-iodo-3′-deoxyadenosine (12)

Under nitrogen atmosphere, $TsOH{\cdot}H_2O$ (1.0 g) was added to a solution of adenosine **2** (2.67 g, 10 mmol) and trimethyl orthoacetate (4.0 mL, 30 mmol) in anhydrous DMSO (30 mL). The reaction mixture was stirred at room temperature for 12 h. Then, the mixture was neutralized with a 1 M methanolic MeONa solution. Chloroform was added to dissolve the crude product and the solution was washed with brine until no adenosine **2** could be detected in the organic layer as judged by TLC. The organic layer was dried with anhydrous sodium sulfate, filtered, and concentrated in vacuo.

The crude product, sodium iodide (2.25 g, 15 mmol), and acetic acid (40 mL) were placed in a 100 mL round-bottom flask. The reaction mixture was stirred at 50 °C for 8 h, and was then concentrated in vacuo. The crude product was neutralized with sodium bicarbonate solution, extracted with dichloromethane (3 × 20 mL), and the organic phases were combined, dried with anhydrous sodium sulfate, filtered and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel using dichloromethane/methanol (20:1) as eluents to afford neat compound **12** (2.17 g, 43.1%) as an orange liquid with the purity of 98.6% (HPLC). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.36 (s, 1H), 8.17 (s, 1H), 7.39 (s, 1H), 6.21 (d, $J = 5.5$ Hz, 1H), 6.04 (t, $J = 5.7$ Hz, 1H), 5.63 (dd, $J = 5.9$, 4.8 Hz, 1H), 4.42 (dd, $J = 11.8$, 3.8 Hz, 1H), 4.38–4.34 (m, 1H), 4.24 (dd, $J = 11.8$, 5.5 Hz, 1H), 2.12 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H).^{13}C NMR (126 MHz, $CDCl_3$) δ 170.36 (–CONH–)169.60 (–COO–), 169.40 (–COO–), 155.57 (6-C), 153.36 (2-C), 149.77 (4-C), 138.80 (8-C), 120.12 (5-C), 86.21 (4′-C), 80.25 (1′-C),77.28 (2′-C) 70.65 (5′-C), 63.13 (3′-C), 20.78 (–CH_3), 20.56 (–CH_3), 20.43 (–CH_3).

Synthesis of 2′, 3′-*O*-isopropylideneadenosine (14)

In a 250 mL, three-neck round-bottom flask were placed adenosine **2** (4.01 g, 15 mmol) and acetone (40 mL). Trimethyl orthoacetate (6 mL) was added to the reaction mixture upon stirring and thionyl chloride ($SOCl_2$, 3.3 mL) was added dropwise to the solution for acidification. After addition of thionyl chloride, the mixture was stirred at room temperature for another 6 h. After reaction completion, the mixture was filtered and the residue was added into saturated sodium bicarbonate solution to adjust the pH value to 7 or 8. After 30 min of standing, the crude product was obtained by filtration and was dried at 50 °C in a vacuum drying oven. Recrystallization from water afforded pure **14** as a light yellow solid (3.23 g, 70.1%) with the purity of 98.7% (HPLC). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H, 2-H), 8.16 (s, 1H, 8-H), 7.40 (s, 2H, –NH_2), 6.13 (d, $J = 3.1$ Hz, 1H, 1′-H), 5.35 (dd, $J = 6.2$, 3.1 Hz, 1H, 2′-H), 5.25 (s, 1H, 5′-OH), 4.97 (dd, $J = 6.2$, 2.5 Hz, 1H, 3′-H), 4.23–4.21 (m, 1H, 4′-H), 3.58–3.51 (m, 2H, 5′-H), 1.55 (s, 3H, –CH_3), 1.33 (s, 3H, –CH_3).^{13}C NMR (101 MHz, DMSO) δ 156.60 (6-C), 153.10 (2-C), 149.25 (4-C), 140.17 (8-C), 119.56 (5-C), 113.49 (quaternary carbon), 90.09 (1′-C), 86.80 (4′-C), 83.67 (2′-C), 81.82 (3′-C), 62.05 (5′-C), 27.54 (–CH_3), 25.64 (–CH_3).

Synthesis of 2′-*O*-*p*-toluenesulfonyladenosine (16)

Adenosine **2** (2.67 g, 10 mmol) and dibutyltin oxide (2.99 g, 12 mmol) were added to methanol (50 mL) in a 100 mL round-bottom flask. The mixture was heated under reflux for 2 h and was then concentrated in vacuo. Then, *p*-toluenesulfonyl chloride (*p*-TsCl, 2.86 g, 15 mmol), 1,4-dioxane (50 mL) and triethylamine (2 mL) were added to the mixture. The solution was stirred at room temperature for 24 h. After reaction completion, the mixture was dropped slowly into methanol (25 mL) and the resulting solid was filtered off. The solid was washed with methanol for a total of three times and the precipitate was dried at 60 °C in a vacuum drying oven to obtain pure **16** (3.43 g, 81.4%) as a white solid with the purity of 98.3% (HPLC). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.15 (s, 1H, 8-H), 7.97 (s, 1H, 2-H), 7.41 (s, 2H, Ph-H), 7.38–7.34 (m, 2H, –NH_2), 6.98 (d, $J = 8.0$ Hz, 2H, Ph-H), 6.09–6.05 (m, 2H, 1′-

H + 5′-OH), 5.79 (dd, J = 8.6, 3.9 Hz, 1H, 3′-OH), 5.45 (dd, J = 7.4, 4.9 Hz, 1H, 2′-H), 4.36–4.32 (m, 1H, 3′-H), 4.07–4.04 (m, 1H, 4′-H), 3.67–3.62 (m, 1H, 5′-H), 3.58–3.51 (m, 1H, 5′-H), 2.26 (s, 3H, –CH_3).^{13}C NMR (126 MHz, DMSO) δ 156.53 (6-C), 152.36 (2-C), 148.28 (4-C), 145.42 (Ph-C), 140.28 (8-C), 131.68 (Ph-C), 129.79 (Ph-C), 127.12 (Ph-C), 119.97 (5-C), 87.79 (4′-C), 85.33 (1′-C), 79.37 (2′-C), 70.27 (3′-C), 62.03 (5′-C), 21.54 (–CH_3).

Synthesis of 2′-deoxyadenosine (17)

Under nitrogen atmosphere, 2′-*O*-*p*-toluenesulfonyladenosine **16** (2.11 g, 5 mmol) and anhydrous tetrahydrofuran (THF, 35 mL) were placed in a 100 mL round-bottom flask. Lithium aluminum hydride in anhydrous THF ($LiAlH_4$/THF, 1 M, 25 mL) was slowly dropped to the solution and the resulting mixture was stirred at 0–5 °C for 4 h and was then continued to stir at room temperature for 6 h. After reaction completion, the mixture was carefully quenched with 10 mL of methanol, concentrated in vacuo, and recrystallization from ethanol to afford pure **17** (1.06 g, 84.6%) as a white powder with the purity of 98.3% (HPLC). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (s, 1H, 8-H), 8.13 (s, 1H, 2-H), 7.32 (s, 2H, –NH_2), 6.35 (dd, J = 7.9, 6.1 Hz, 1H, 1′-H), 5.31 (dd, J = 4.0, 1.3 Hz, 1H, 3′-OH), 5.25 (dd, J = 6.6, 4.8 Hz, 1H, 5′-OH), 4.43–4.39 (m, 1H, 3′-H), 3.90–3.87 (m, 1H, 4′-H), 3.65–3.60 (m, 1H, 5′-H), 3.55–3.49 (m, 1H, 5′-H), 2.77–2.70 (m, 1H, 2′-H), 2.28–2.23 (m, 1H, 2′-H). ^{13}C NMR (126 MHz, DMSO) δ 156.56 (6-C), 152.84 (2-C), 149.34 (4-C), 140.03 (8-C), 119.72 (5-C), 88.46 (4′-C), 84.42 (1′-C), 71.45 (3′-C), 62.37 (5′-C), 39.44 (2′-C).

Synthesis of 5′-*O*-triphenylmethyladenosine (18)

In a 100 mL round-bottom flask, adenosine **2** (1.34 g, 5 mmol) and anhydrous N,N-dimethylformamide (30 mL) was placed. Triphenylmethyl chloride (4.20 g, 15 mmol) and triethylamine (10 mL) were added to the reaction mixture while upon stirring at 45 °C for 24 h. Ethyl acetate (50 mL) was added to the reaction mixture to dissolve the crude product and washed with saturated salt water. The organic layer was dried with anhydrous sodium sulfate and concentrated in vacuo to afford the crude product **18** as an orange liquid. The crude product was purified by flash chromatography on silica gel using dichloromethane/methanol (30:1) as eluents to afford pure **18** (2.16 g, 75.0%) as a light yellow solid with the purity of 97.9% (HPLC). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (s, 1H, 2-H), 8.10 (s, 1H, 8-H), 7.38–7.26 (m, 17H, Ph-H + –NH_2), 5.93 (d, J = 4.5 Hz, 1H, 1′-H), 5.56 (d, J = 5.7 Hz, 1H, 2′-OH), 5.23 (d, J = 5.9 Hz, 1H, 3′-OH), 4.72–4.68 (m, 1H, 2′-H), 4.34–4.30 (m, 1H, 3′-H), 4.09–4.05 (m, 1H, 4′-H), 3.27–3.18 (m, 2H, 5′-H).^{13}C NMR (126 MHz, DMSO) δ 156.54 (6-C), 153.06 (2-C), 149.76 (4-C), 144.09 (Ph), 140.07 (8-C), 128.71 (Ph), 128.31 (Ph), 127.47 (Ph),119.66 (5-C), 88.43 (4′-C), 86.51 (1′-C), 83.33 (2′-C), 73.42 (Ph_3C-), 70.73 (3′-C), 64.36 (5′-C).

Synthesis of 3′-*O*-tosyl-5′-*O*-triphenylmethyladenosine (19)

5′-*O*-triphenylmethyladenosine **18** (2.55 g, 5 mmol) and anhydrous DMF (30 mL) were placed in a 100 mL round-bottom flask. Triethylamine (10 mL) and *p*-toluenesulfonyl chloride (1.91 g, 10 mmol) were added to the reaction mixture while stirring. The reaction mixture was stirred at 30 °C for 24 h, which was sampled and showed no or very low amounts of 5′-*O*-triphenylmethyladenosine 18 being detected by TLC, and it showed two new points on the thin-layer. Then, ethyl acetate (50 mL) was added to the reaction mixture to dissolve the crude product and the solution was washed with saturated salt water for three times. The organic layer was dried with anhydrous sodium sulfate, concentrated in vacuo to afford the crude product **19** as a yellow liquid. The crude product was purified by flash chromatography on silica gel using dichloromethane/methanol (40:1) as eluents to afford pure **19** (2.35 g, 70.7%) as a white powder with the purity of 97.5% (HPLC). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (s, 1H, 8-H), 7.86 (s, 1H, 2-H), 7.47 (d, J = 8.4 Hz, 2H, Ts-H), 7.39–7.35 (m, 7H, Tr-H + –NH_2), 7.32–7.25 (m, 10H, Tr-H), 7.06 (d, J = 8.1 Hz, 2H, Ts-H), 6.11 (d, J = 6.0 Hz, 1H, 1′-H), 6.06 (d, J = 5.9 Hz, 1H, 2′-OH), 5.75 (d, J = 6.2 Hz, 1H, 2′-H), 4.55–4.51 (m, 1H, 3′-H), 4.15–4.11 (m, 1H, 4′-H), 3.33 (dd, J = 10.4, 4.3 Hz, 1H, 5′-H), 3.17 (dd, J = 10.5, 5.0 Hz, 1H, 5′-H), 2.28 (s, 3H, –CH_3).^{13}C NMR (101 MHz, DMSO) δ 156.36 (6-C), 152.66 (2-C), 148.90 (4-C), 145.48 (Ph), 143.93 (Ph), 140.36 (8-C), 132.11 (Ph), 129.89 (Ph), 128.68 (Ph), 128.35 (Ph), 127.54 (Ph), 127.36 (Ph), 119.64 (5-C), 86.62 (4′-C), 85.32 (1′-C), 84.19 (Ph_3C-), 79.11 (2′-C), 69.30 (3′-C), 63.35 (5′-C), 21.55 (–CH_3).

Synthesis of 5′-*O*-triphenylmethyl-3′-deoxyadenosine (20)

3′-*O*-tosyl-5′-*O*-triphenylmethyladenosine **19** (3.32 g, 5 mmol) and THF (30 mL) were placed in a 100 mL round-bottom flask. Lithium aluminum hydride in anhydrous THF ($LiAlH_4$/THF, 1 M, 25 mL) was slowly dropped to the solution and the resulting mixture was stirred at 0–5 °C for 4 h. After reaction completion, the reaction mixture was kept standing until the temperature reached room temperature. Then, the mixture was carefully

quenched with 10 mL methanol. Ethyl acetate (50 mL) was added to the reaction mixture to dissolve the crude product and the solution was washed with brine for three times. The organic layer was dried with anhydrous sodium sulfate, and was concentrated in vacuo to afford the crude product **20** as a light yellow liquid with the purity of 94.6% (HPLC). The crude product was purified by flash chromatography on silica gel using dichloromethane/methanol (60:1) as eluents to afford pure **20** (1.98 g, 75.3%) as a lightly yellow powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.24 (s, 1H, 8-H), 8.07 (s, 1H, 2-H), 7.36–7.32 (m, 6H, Tr-H + –NH_2), 7.30–7.22 (m, 11H, Tr-H), 6.38–6.34 (m, 1H, 1′-H), 5.38 (d, J = 4.5 Hz, 1H, 2′-OH), 4.48 (dd, J = 6.1, 4.3 Hz, 1H2′-H), 4.00–3.97 (m, 1H, 4′-H), 3.18 (d, J = 5.1 Hz, 2H, 5′-H), 2.90–2.85 (m, 1H, 3′-H), 2.40–2.25 (m, 1H, 3′-H).^{13}C NMR (126 MHz, DMSO) δ 156.50 (6-C), 152.94 (2-C), 149.53 (4-C), 144.11 (Ph), 139.91(8-C), 128.68 (Ph), 128.27 (Ph), 127.43 (Ph), 119.72 (5-C), 86.44 (1′-C), 86.10 (4′-C), 83.77 (2′-C), 71.08 (5′-C), 64.67 (3′-C).

Synthesis of cordycepin (1)

Method A

N-acetyl-2′,5′-*O*-diacetyl-3′-iodo-3′-deoxyadenosine **12** (2.52 g, 5 mmol), toluene (40 mL) and 2,2-azobisisobutyronitrile (AIBN, 0.1 g, 0.6 mmol) were placed in a 100 mL round-bottom flask. Lithium aluminum hydride in anhydrous THF ($LiAlH_4$/THF, 1 M, 20 mL) was slowly dropped to the solution. The stirred reaction mixtures were then heated to reflux for 12 h. After reaction completion, the reaction mixture was cooled to room temperature and was then poured into petroleum ether. The resulting precipitate was collected by filtration and further washed with petroleum ether. The residue was used directly in the next step. The residue was added into a 1 M methanolic MeONa solution (25 mL) and the mixture was stirred for 6 h at 60 °C. After reaction completion, the reaction mixture was cooled to room temperature and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel using dichloromethane/methanol (10:1) as eluents to afford pure **1** (0.39 g, 31.3%, 13.5% overall yield) as a white solid with the purity of 99.1% (HPLC). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.37 (s, 1H, 8-H), 8.15 (s, 1H, 2-H), 7.32 (s, 2H, –NH_2), 5.87 (d, J = 2.4 Hz, 1H, 1′-H), 5.67 (d, J = 4.3 Hz, 1H, 2′-OH), 5.17 (s, 1H, 5′-OH), 4.59–4.57 (m, 1H, 2′-H), 4.37–4.34 (m, 1H, 4′-H), 3.71–3.68 (m, 1H, 5′-H), 3.52 (d, J = 12.1 Hz, 1H, 5′-H), 2.28–2.23 (m, 1H, 3′-H), 1.94–1.90 (m, 1H, 3′-H). ^{13}C NMR (101 MHz, DMSO) δ 156.49 (6-C), 152.88 (2-C), 149.29 (4-C), 139.52 (8-C), 119.52 (5-C), 91.24 (1′-C), 81.14 (4′-C), 75.05 (2′-C), 63.06 (5′-C), 34.50 (3′-C).

Compound **13** (56 mg, 4.8%) was obtained as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H, 8-H), 8.13 (s, 1H, 2-H), 7.25 (s, 2H, –NH_2), 6.22 (dd, J = 5.9, 4.7 Hz, 1H, 1′-H), 5.06 (t, J = 5.6 Hz, 1H, OH), 4.14–4.09 (m, 1H, 4′-H), 3.66–3.61 (m, 1H, 5′-H), 3.53–3.49 (m, 1H, 5′-H), 2.43–2.39 (m, 2H, 3′-H), 2.09–2.02 (m, 2H, 2′-H).^{13}C NMR (126 MHz, DMSO) δ 156.46 (6-C), 152.83 (2-C), 149.27 (4-C), 139.46 (8-C), 119.56 (5-C), 84.88 (1′-C), 82.12 (4′-C), 63.41 (5′-C), 32.18 (3′-C), 26.18 (2′-C).

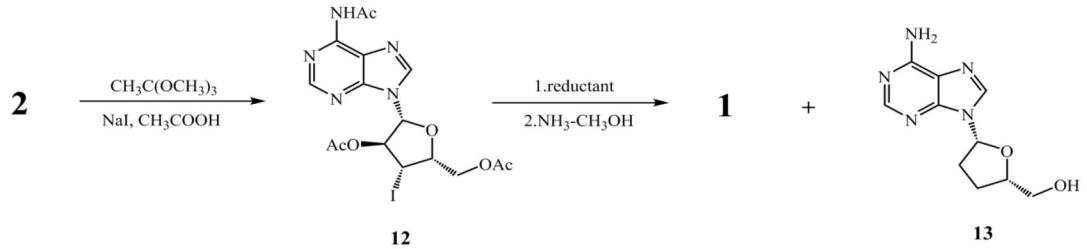
Method B

5′-*O*-triphenylmethyl-3′-deoxyadenosine **20** (2.47 g, 5 mmol) was dissolved in a mixture of dichloromethane (30 mL) and methanol (20 mL), and the pH of the reaction mixture was adjusted to ~4 using acetic acid. After stirring the reaction mixture at room temperature for 4 h, the pH of the reaction mixture was adjusted to 7–8 by addition of a 1 M methanolic CH_3ONa solution. After removal of all solvents in vacuo, the residue was purified by column chromatography on silica gel using a gradient of dichloromethane/methanol (15:1–8:1) as eluents to afford the target product **1** (1.13 g, 90.0%, 35.9% overall yield) as a white solid with the purity of 99.1% (HPLC).

Results and discussion

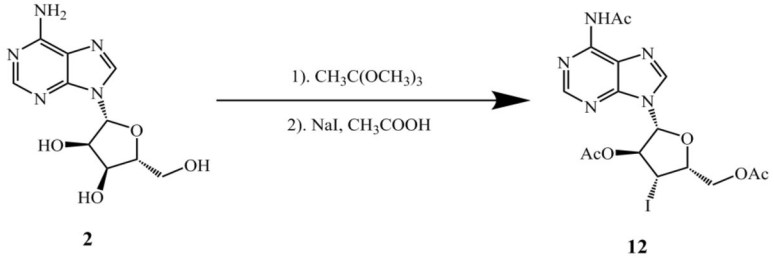
Considering the structural similarity between cordycepin and adenosine, and considering the fact that adenosine represents a cheap and commercially readily available material, adenosine was selected as the starting material in the synthesis of cordycepin. Only the removal of the 3′-OH group in adenosine to afford cordycepin needs to be considered for this synthesis. Initially, reactions involving the 3′-OH group were considered to be most feasible, thought to improve the synthetic route originally designed by Christelle Moreau et al. To conduct the dehalogenation reaction in a convenient fashion, the formation of an iodine group was carried out in the second step (Scheme 6). According to a literature procedure, the 2′-OH and 3′-OH groups in adenosine were cyclized by trimethyl orthoacetate and were reacted with sodium iodide in acetic acid to yield **12**. Finally, cordycepin **1** was obtained by deiodination and deprotection reactions as follows:

Our study first focused on optimizing the synthesis of **12** via reaction of **2** with trimethyl orthoacetate and NaI. The results are shown in Table 1. However, only a miniscule amount of **12** was obtained. This poor result can be mostly attributed to the fact that NaI does not completely dissolve in the reaction mixture, leading to a less than ideal heterogenous solid–liquid system. However, hydrolysis was found to occur when NaI dissolved in water was added to the reaction



Scheme 6 Improved synthetic route based on the route of Christelle Moreau

Table 1 Reaction of adenosine with trimethyl orthoacetate and sodium iodide



Entry[c]	Solvent 1	Solvent 2	Temp[a] (°C)	Yield of **12**[b] (%)
1	DMSO	Acetic acid	25	40.1
2	DMSO	CH_3CN	25	32.6
3	Acetic acid	Acetic acid	25	24.8
4	Acetic acid	CH_3CN	25	28.8
5	DMSO	Acetic acid	40	42.3
6[d]	DMSO	Acetic acid	50	43.1
7[e]	DMSO	Acetic acid	25	38.5

[a] Temperature in the reaction between **2** and $CH_3C(OCH_3)_3$

[b] Isolated yield after column chromatography

[c] Standard conditions: **2** (10 mmol), trimethyl orthoacetate (30 mmol), solvent 1 (30 mL), r.t., 12 h, sodium iodide (15 mmol), solvent 2 (40 mL), 50 °C, 8 h

[d] The optimum condition

[e] Sodium iodide was increased to 20 mmol

mixture. Unfortunately, a large amount of unreacted adenosine was found to be present in the reaction mixture as detected by TLC, leading to a low overall yield of **12.**

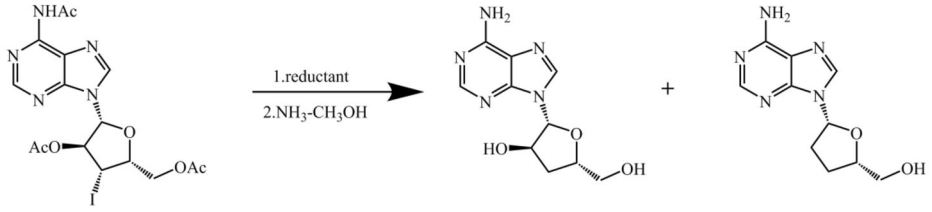
Some polar impurities like adenosine were found to be present as detected by TLC and these impurities could not be removed easily, negatively affecting the purity of **12**.

To improve this synthetic route (Table 1), the intermediate synthesized by **2** with $CH_3C(OCH_3)_3$ was replaced by **14** obtained by reacting adenosine **2** with acetone (Townsend et al. 2009). However, we found that the formation of compound **14** did not take place upon addition of NaI into the reaction mixture. The corresponding process is shown in Scheme 7.

With **12** in hand, the selection of a suitable reducing agent was considered for removal of the iodine group. To be specific, reaction of **12** with tributyltin hydride ($n\text{-}C_4H_9)_3SnH$ or lithium aluminum hydride (LAH) was attempted. In addition, different conditions were screened for this reaction, e.g., different solvents and reaction times, and the influence of the deprotection reaction in different bases was tested. The corresponding results are shown in Table 2.

The produced amount of **1** was found to be reduced by replacing $LiAlH_4$ with $(n\text{-}C_4H_9)_3SnH$ (entry 4) and by replacing toluene with DCM or THF (entries 1-3). Furthermore, the obtained results show that the produced amount of **1** was reduced and **13** was increased by increasing the molar ratio of the reducing agent (entries 1 and 4–7). To assess the effect of $LiAlH_4$, we monitored the reaction time from 4 to 12 h. When the reaction was continued for at least 6 h, compound **13** could be detected by TLC analysis.

Table 2 Synthesis of cordycepin via deiodination and deprotection reaction



12 1 13

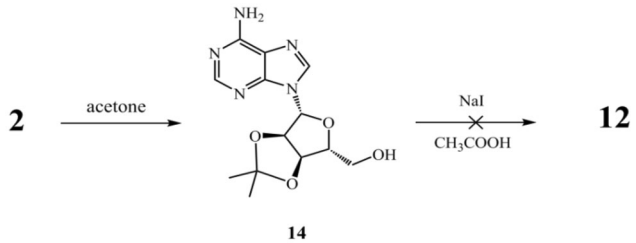
Entry[b]	Solvent	Reducing agent	Time (h)	Base	Yield of **1**[a] (%)	Yield of **13**[a] (%)
1[d]	Toluene	$LiAlH_4$	4	CH_3ONa–CH_3OH	30.2	0[c]
2	DCM	$LiAlH_4$	4	CH_3ONa–CH_3OH	28.1	7.4
3	THF	$LiAlH_4$	4	CH_3ONa–CH_3OH	23.2	2.3
4	Toluene	$(n\text{-}C_4H_9)_3SnH$	4	CH_3ONa–CH_3OH	25.6	0[c]
5	Toluene	$LiAlH_4$	6	CH_3ONa–CH_3OH	27.7	2.5
6	Toluene	$LiAlH_4$	8	CH_3ONa–CH_3OH	30.6	3.8
7	Toluene	$LiAlH_4$	12	CH_3ONa–CH_3OH	31.3	4.8
8	Toluene	$LiAlH_4$	12	NaOH–H_2O	25.8	8.5

[a] Isolated yield after column chromatography

[b] Standard conditions: **12** (5 mmol), solvent (40 mL), AIBN (0.6 mmol), reducing agent (20 mmol), 12 h, reflux, base (1 M, 25 mL) 6 h, 60 °C

[c] Compound **13** was observed by TLC

[d] The optimum condition

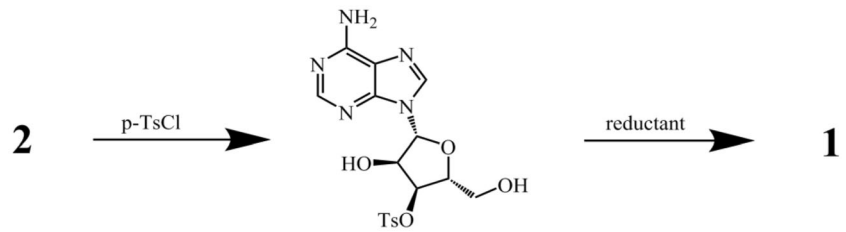


Scheme 7 Attempted synthesis of **12** from **2** via intermediate **14**

As –OTs represents a good leaving group and given the fact that tosylates of secondary alcohols in particular may undergo elimination reaction, the reaction between 3′-OH group of adenosine and *p*-toluenesulfonyl chloride (*p*-TsCl) was also considered to furnish **15** (Grouiller et al. 1987). Then, a reducing agent can be used to remove the –OTs group to produce cordycepin **1**. This process is shown in Scheme 8 below.

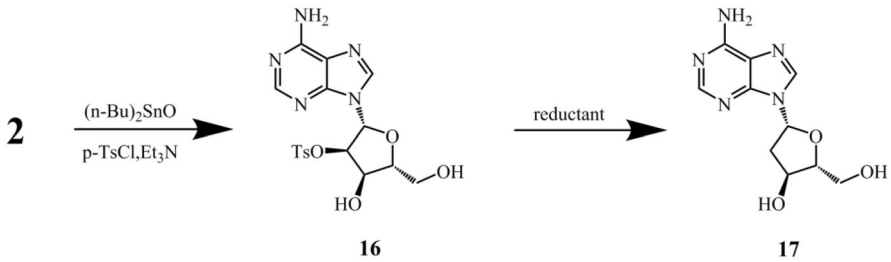
Adenosine was used and directly reacted with *p*-TsCl. This process exhibited unsuitable selectivity characteristics as judged by TLC. Most likely, the reactivity of all 2′-OH, 3′-OH and 5′-OH groups when exposed to *p*-TsCl is fairly similar, resulting in the production of various compounds that prove to be difficult to be separated from each other. However, upon using $(n\text{-}C_4H_9)_2SnO$, **16** has been shown to be the main product (Wagner et al. 1974) (Scheme 9). Upon removal of the –OTs group from **16**, a product structurally similar to cordycepin(2′-deoxyadenosine) **17** was obtained.

In an effort to reduce the amount of byproducts formed, the 5′-OH group in adenosine was protected by triphenylmethyl chloride (TrCl) before the reaction of the 3′-OH group with *p*-TsCl. Then, the 3′-OH group was reacted with *p*-TsCl to reduce 19 (Morio and Masakatsu 1967) and a reducing agent could be used to remove the –OTs group to obtain **20**. Finally, acetic acid and dichloromethane



Scheme 8 Synthesis of cordycepin via **15**

Scheme 9 Synthesis of **17** from adenosine **2**



Scheme 10 Improved synthesis of cordycepin

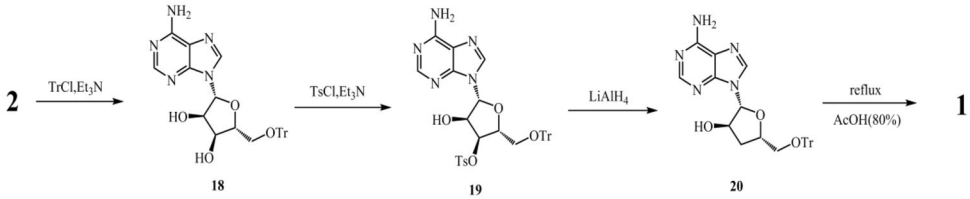
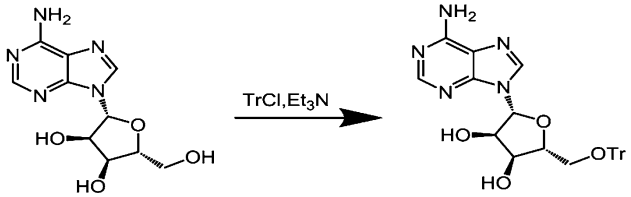


Table 3 Reaction of adenosine **2** with TrCl and triethylamine



Entry[b]	Solvent	Temperature (°C)	Reaction time (h)	Yield of **18**[a] (%)
1	DMF	30	24	71.6
2	CH_2Cl_2	30	24	65.4
3	Pyridine	30	24	69.8
4	DMF	45	24	73.5
5	DMF	60	24	73.8
6	DMF	30	36	72.0
7	DMF	30	48	73.1
8[c]	DMF	45	24	75.0

[a] Isolated yields after column chromatography

[b] Standard conditions: **2** (5 mmol), TrCl (10 mmol), Et_3N (10 mL)

[c] The optimum condition where TrCl was increased to 15 mmol

solution were used to remove the triphenylmethyl protecting group to obtain cordycepin **1** (Scheme 10).

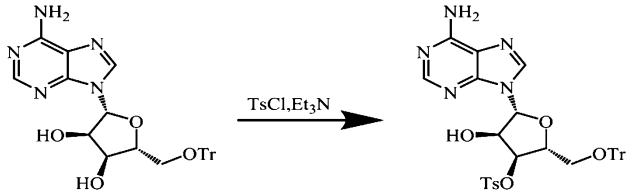
The synthetic pathway first focused on the synthesis of **18** from **2** using triethylamine as base. In an effort to achieve an improved yield, several other solvents and temperatures were screened as shown in Table 3.

As shown in Table 3, The reaction for 18 can get a best overall yield of 75.0% with an [c]optimized reaction temperature of 45 °C, an [c]optimum ratio of reactants (**2** and TrCl) at 1: 3, using triethylamine as acid neutralizing agent, DMF as solvent and continuous stirring for 24 h.

Similarly, reaction conditions for the transformation of intermediate **18–19** were also screened for optimization. Several solvents, temperature ranges and reaction times were investigated in an effort to obtain a higher product yield (Table 4). As listed in Table 4, the reaction temperature did not have a significant effect on the product yield of **19** (entries 1 and 4–5). The same proved to be the case for modifications in reaction time (entries 1 and 6–7) and molar ratio of **18** and *p*-TsCl. Thus, the temperature was set at 30 °C and the molar ratio of the reactants was 1:2, using triethylamine as acid neutralizing agent, DMF as solvent and continuous stirring for 24 h were chosen as reaction [c]conditions.

Furthermore, we evaluated the synthetic progress of **20** under several experimental conditions as shown in Table 5.

Table 4 Reaction of **18** with *p*-toluenesulfonyl chloride and triethylamine



18 **19**

Entry[b]	Solvent	Temperature (°C)	Reaction time (h)	Yield of **19**[a] (%)
1[c]	DMF	30	24	70.7
2	CH_2Cl_2	30	24	62.9
3	Pyridine	30	24	66.3
4	DMF	45	24	71.1
5	DMF	60	24	71.8
6	DMF	30	36	71.5
7	DMF	30	48	72.0
8[d]	DMF	30	24	71.4

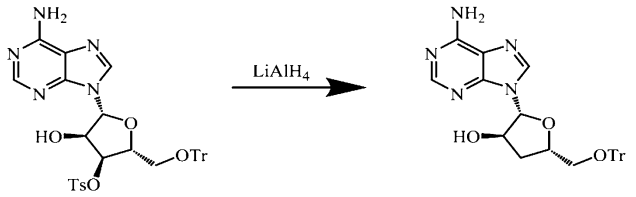
[a] Isolated yield from column chromatography

[b] Standard conditions: **18** (5 mmol), *p*-TsCl (10 mmol), solvent (30 mL), triethylamine (10 mL)

[c] The optimum condition

[d] *p*-TsCl was increased to 15 mmol

Table 5 Reaction of **19** with lithium aluminum hydride



19 **20**

Entry[b]	Solvent	Reducing agent	Reaction time (h)	Yield of **20**[a] (%)
1	DMSO	$LiAlH_4$	4	76.3
2	DMF	$LiAlH_4$	4	75.1
3[c]	THF	$LiAlH_4$	4	75.3
4	CH_2Cl_2	$LiAlH_4$	4	62.8
5	THF	$LiBH(CH_2CH_3)_3$	4	65.4
6	THF	Bu_3SnH	4	73.4
7	THF	$LiAlH_4$	8	75.9
8	THF	$LiAlH_4$	12	76.5
9[d]	THF	$LiAlH_4$	4	70.9

[a] Isolated yield from chromatography

[b] Standard conditions: **19** (5 mmol), Reducing agent(1 M), solvent (30 mL), temperature at 0–5 °C

[c] The optimum condition

[d] $LiAlH_4$/THF was increased to 1 M, 25 mL

When the reaction was considered complete as judged by TLC analysis, product **20** could be purified by column chromatography. As shown in Table 5, the choice of reducing agent was explored, considering the toxicity and cumbersome post-treatment of Bu_3SnH, we prefer $LiAlH_4$ as the reducing agent. The reaction using an incremental amount of $LiAlH_4$ provided a lower yield (entry 9). Meanwhile, the influences of the reaction time were explored. However, increasing the yield of **20** was not obvious (entries 1 and 7–8) upon increasing the reaction time. The isolated yield reached an optimum value upon treatment of **19** with 5 equivalents of $LiAlH_4$ at room temperature, with an adopted reaction time of 4 h.

Finally, **20** was converted into the final product, cordycepin **1** in acetic acid and dichloromethane solution via deprotection reaction.

Conclusions

We designed two independent methods for the synthesis of cordycepin. One method we developed for the production of cordycepin proves to be shorter, however, suffers from a relatively low yield of 13.5%. The other synthetic method we developed proves to be more suitable for the production of cordycepin, and leads to the production of cordycepin both in higher product quality and yield. This synthetic route consists of four steps with a total product yield of 35.9%. The key step in the synthetic process is the conversion from **18** to **19**. Since all starting materials are commercially available and produce cordycepin in a high overall yield, this synthetic method proves to be superior over other pathways reported in the literature.

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