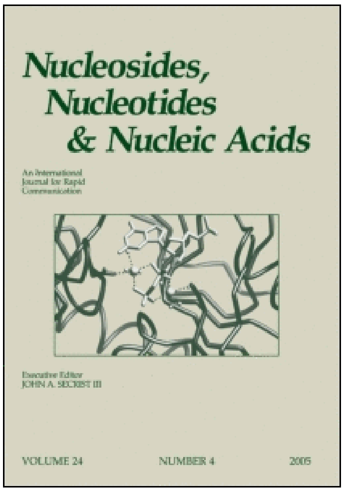


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of 2-Alkynylcordycepins and Evaluation of Their Vasodilating Activity

Hiroki Kumamoto[a]; Hiroyuki Hayakawa[a]; Hiromichi Tanaka[a]; Satoru Shindoh[a]; Keisuke Kato[a]; Tadashi Miyasaka[a]; Kazuki Endo[a]; Haruhiko Machida[a]; Akira Matsuda[a]
[a] School of Pharmaceutical Sciences, Showa University, Tokyo, Japan

To cite this Article Kumamoto, Hiroki , Hayakawa, Hiroyuki , Tanaka, Hiromichi , Shindoh, Satoru , Kato, Keisuke , Miyasaka, Tadashi , Endo, Kazuki , Machida, Haruhiko and Matsuda, Akira(1998) 'Synthesis of 2-Alkynylcordycepins and Evaluation of Their Vasodilating Activity', Nucleosides, Nucleotides and Nucleic Acids, 17: 1, 15 — 27

To link to this Article: DOI: 10.1080/07328319808005154

URL: http://dx.doi.org/10.1080/07328319808005154

NUCLEOSIDES & NUCLEOTIDES, 17(1-3), 15-27 (1998)

SYNTHESIS OF 2-ALKYNYLCORDYCEPINS AND EVALUATION OF THEIR VASODILATING ACTIVITY

Hiroki Kumamoto,[1] Hiroyuki Hayakawa,[1] Hiromichi Tanaka,[1]* Satoru Shindoh,[1]
Keisuke Kato,[1] Tadashi Miyasaka,[1] Kazuki Endo,[2] Haruhiko Machida,[2]
and Akira Matsuda[3]

[1]*School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan,* [2]*Research & Development Division, Yamasa Corporation, 2-10-1 Araoicho, Chosi, Chiba 288, Japan, and* [3]*Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan*

ABSTRACT: Based on the recently developed lithiation-mediated stannyl migration of 6-chloropurine derivatives, 2-iodocordycepin was prepared from cordycepin. The reaction of this compound with terminal alkynes was carried out to synthesize a series of 2-alkynyl derivatives. The vasodilating effect of these compounds was evaluated.

Among the vast number of nucleoside antibiotics,1) compounds having adenine as a nucleobase constitute the largest family. Synthesis of their 2-carbon-substituted analogues has previously relied on either condensation between the base and sugar or ring closure of imidazole nucleoside precursors,2) due to the lack of a general method for the modification at the 2-position of purine nucleosides.3) Nair *et al.* reported an elegant method for the preparation of 6-chloro-2-iodopurine nucleoside,4) which can be used in further functionalization leading to 2-carbon-substituted derivatives.5,6) The preparation of this versatile compound, however, necessitates guanine precursors for generating the purin-2-yl radical.

During our studies on the lithiation of purine nucleosides,7) we recently found that although initial lithiation of 6-chloropurine ribonucleoside takes place at the 8-position, reaction of the lithiated species with Bu_3SnCl yields the corresponding 2-stannylated product *via* an anionic migration of a stannyl group from the 8- to the 2-position.8) This

---

* Hiromichi Tanaka: phone 81-3-3784-8187, fax 81-3-3784-8252.

This paper is dedicated to the late Professor Tsujiaki Hata who made a great contribution to nucleic acid chemistry.

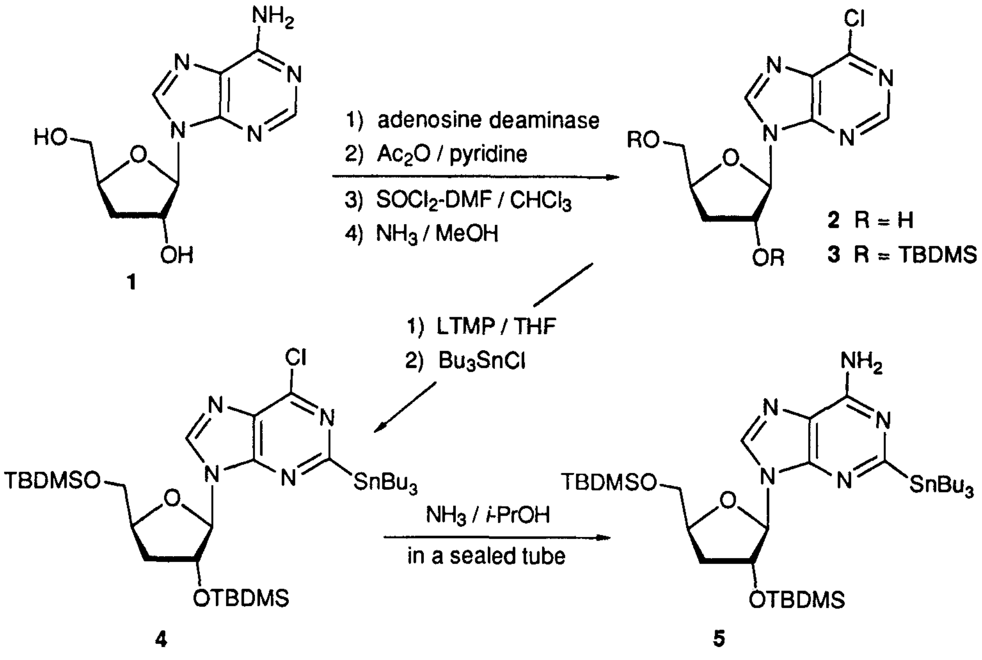
finding combined with further manipulation of the 2-stannyl group, has provided a new entry to various types of 2-carbon-substituted purine ribonucleosides.8) In the present study, by using cordycepin (**1**) as a starting material, we demonstrate that this approach is particularly useful for the synthesis of 2-substituted analogues of adenine nucleoside antibiotics.

CHEMISTRY

The 6-chloropurine derivative **2** was prepared from **1** by a sequence of reactions, as shown in SCHEME 1. Although the first two steps proceeded in quantitative yield, some depurination occurred in the third step, chlorination of 2',5'-di-*O*-acetyl-3'-deoxyinosine, to form 6-chloropurine (detected by TLC, R_f 0.70 in $CHCl_3$/MeOH = 3/1). Compound **2** was obtained in 60% yield after deacetylation. Protection of the two hydroxyl groups in **2** with *tert*-butyldimethylsilyl (TBDMS) group gave **3**, which serves as a substrate for lithiation-based stannyl migration.

When **3** was lithiated with lithium 2,2,6,6-tetramethylpiperidide (LTMP, 5 equiv) in THF below –70 °C and then reacted with Bu_3SnCl (5 equiv), the 2-stannylated product **4** was obtained in 90% yield after silica gel column chromatography. It should be mentioned that the reaction mixture contains not only **4** but also the 2,8-bis(tributylstannyl) derivative.8) However, the latter product then undergoes protonolysis of the 8-stannyl group to form **4** during the column chromatographic purification. Treatment of **4** with NH_3/MeOH at 100 °C in a sealed tube gave a considerable amount of destannylated product which presumably could be formed by attack of methoxide ion on the tin atom. The use of *i*-PrOH as a solvent in this ammonolysis on the other hand suppressed such an undesired reaction pathway to give the 2-stannylcordycepin **5** in 91% yield.

Compound **5** is a versatile synthetic intermediate for 2-substituted derivatives, which is illustrated in SCHEME 2 by the preparation of **6**, **7**, and **8**. The 2-benzyl analogue **6** was prepared by the Stille reaction9) using $PhCH_2Br$ and $Pd(PPh_3)_4$/CuI in refluxing THF (70% yield). Fluorination leading to **7** (55% yield) was carried out according to the published procedure in which XeF_2 was used as an electrophilic fluorine source in combination with AgOTf and 2,6-di-(*tert*-butyl)-4-methylpyridine.10) Iodination of **5** can be performed simply by reacting with iodine in THF at room temperature to give **8** in quantitative yield, which was further used for the synthesis of 2-alkynylcordycepins. In TABLE 1 are listed purine ring ^{13}C NMR chemical shifts of compounds **5**-**8**. Apart from the chemical shifts of C2 which vary significantly depending on the electronic nature of the substituents, only a small difference can be seen in other carbon resonances. Among these other carbon atoms, C5 resonates at the highest field, reflecting the fact that it is the sole carbon atom bound to only one electronegative atom. In HMBC (heteronuclear multiple

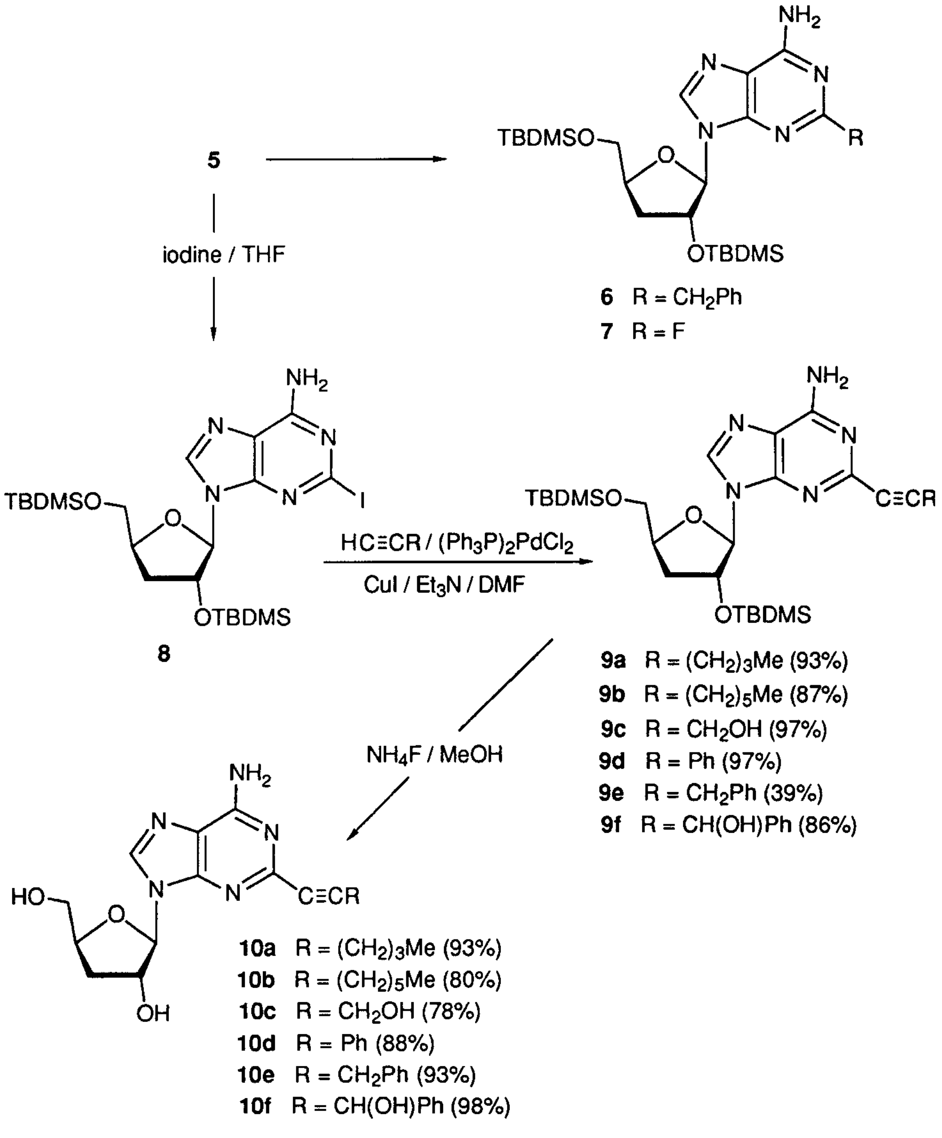


SCHEME 1

bond connectivity) spectra of **5**-**8**, a correlation of $^{3}J_{C,H}$ was observed between the purine ring proton and C5. This observation confirmed the regiochemistry of these compounds.

Since adenosine shows coronary vasodilating activity in mammalian system,11) its 2-substituted derivatives have been synthesized and it has been shown that these compounds act as analogues with increased duration of activity.12) It has also been reported recently that 2-alkynyladenosines serve as potent antihypertensive agents.6) These 2-alkynyl derivatives were synthesized through palladium-catalyzed cross-coupling of terminal alkynes13) with 2-iodoadenosine which was prepared by an improved method of the aforementioned protocol of Nair. In the present study, **8** was used for this coupling reaction.

The coupling reactions of **8** leading to **9a**-**f** (yields are given in parentheses in SCHEME 2) were carried out in DMF at 70-75 °C by using the respective terminal alkyne (2.0 equiv) in the presence of $(Ph_3P)_2PdCl_2$ (10 mol%), CuI (5 mol%) and Et_3N (1.2 equiv). Although the 2-alkynyl derivatives **9** were obtained mostly in high yields,14) only the reaction of $HC{\equiv}CCH_2Ph$ gave **9e** in a significantly lower yield. Attempted isolation by HPLC of any byproduct formed in this reaction failed, due to the presence of many minor


5
iodine / THF
TBDMSO
OTBDMS
NH$_2$
R
6 R = CH$_2$Ph
7 R = F
8
I
HC≡CR / (Ph$_3$P)$_2$PdCl$_2$
CuI / Et$_3$N / DMF
C≡CR
9a R = (CH$_2$)$_3$Me (93%)
9b R = (CH$_2$)$_5$Me (87%)
9c R = CH$_2$OH (97%)
9d R = Ph (97%)
9e R = CH$_2$Ph (39%)
9f R = CH(OH)Ph (86%)
NH$_4$F / MeOH
HO
OH
10a R = (CH$_2$)$_3$Me (93%)
10b R = (CH$_2$)$_5$Me (80%)
10c R = CH$_2$OH (78%)
10d R = Ph (88%)
10e R = CH$_2$Ph (93%)
10f R = CH(OH)Ph (98%)

SCHEME 2

TABLE 1. Purine ring ^{13}C NMR chemical shifts of compounds **5**-**8**.

Compd.	C2-Substituent	Chemical shift (δ ppm) C2	C4	C5	C6	C8
5	$SnBu_3$	180.4	148.8	119.2	153.3	138.3
6	CH_2Ph	163.9	150.1	119.5	155.1	138.9
7	F	159.0*	150.7*	118.2*	156.9*	139.3*
8	I	120.0	149.5	119.2	155.0	138.9

* appeared as a doublet: $J_{C2,F}$ = 210.0 Hz, $J_{C4,F}$ = 19.7 Hz, $J_{C5,F}$ = 4.1 Hz, $J_{C6,F}$ = 19.6 Hz, and $J_{C8,F}$ = 3.1 Hz.

products. The Stille reaction, on the other hand, appeared to be suitable for the preparation of **9e**. Thus, by converting the terminal alkyne to $Bu_3SnC{\equiv}CCH_2Ph$ and by reacting it with **8** in refluxing THF in the presence of $Pd(PPh_3)_4$/CuI, **9e** was obtained in an improved yield of 80%. Deprotection of **9** was performed with NH_4F[15)] in refluxing MeOH to give **10** (yields are given in parentheses).

BIOLOGICAL ACTIVITY

The 2-alkynylcordycepins (**10**) synthesized in this study were evaluated for their vasodilating activity in the isolated rat femoral vein and artery. The procedure to measure the activity has been described previously.[16)] As shown in FIGURE 1, all compounds induced concentration-dependent relaxations in venous rings precontracted with 10^{-6} M of 5-hydroxytryptamine. In contrast, these compounds uniformly induced only a little vasodilation in the artery at concentrations as high as 10^{-5} M. Compound **10f** showed a vasodilation greater than 90% at a concentration of 10^{-6} M (EC_{50} = 30 nM), which was the most potent vasodilative action observed in the present assay. The 2-octynyl analogue **10b** (EC_{50} = 232.9 nM) was found to be much less active than a promising antihypertensive agent 2-octynyladenosine (EC_{50} = 3.88 nM).[16)] 2-Octynyladenosine has been reported to dilate preferentially the femoral vein rather than the femoral artery of rats, as a result of adenosine A_2 receptor activation.[16)] The structural similarity of **10** to 2-octynyladenosine suggests that the observed vasodilating activity of **10** may fall into a similar mechanism of action.

EXPERIMENTAL

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. ^1H NMR spectra were measured at 23 °C (internal standard, Me_4Si) with

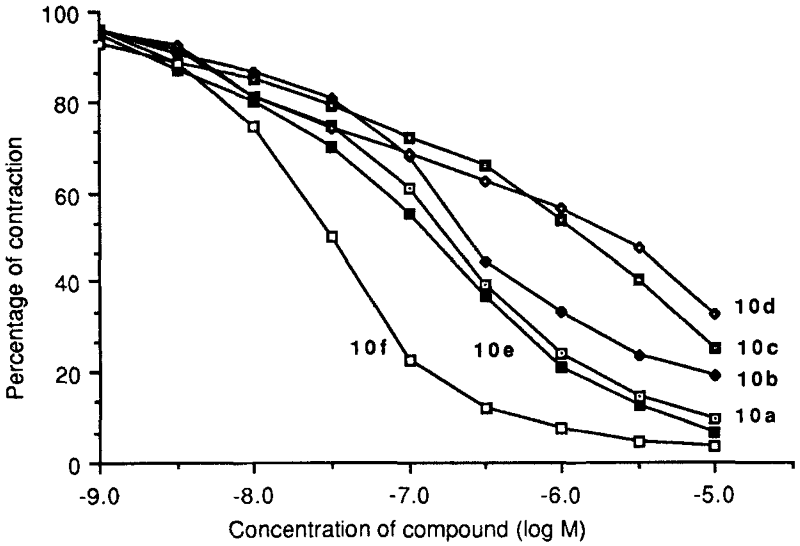


FIG. 1. Vasodilating effect of compounds **10a-f** in isolated rat femoral vein. Each point represents the mean of four experiments.

either a JEOL JNM-GX 400 or a JEOL JNM-LA 500 spectrometer. Mass spectra (MS) were taken on a JEOL SX-102A (in FAB mode, *m*-nitrobenzyl alcohol as a matrix) spectrometer. For compounds containing Cl and/or Sn, ion peaks corresponding to ^{35}Cl and/or ^{120}Sn are shown. Ultraviolet spectra (UV) were recorded on a JASCO Ubest-55 spectrophotometer. Column chromatography was performed on silica gel (Silica Gel 60, Merck). Thin layer chromatography (TLC) was carried out on silica gel (precoated silica gel plate F_{254}, Merck). Adenosine deaminase and cordycepin were purchased from Amano Pharmaceutical Corp. and Yamasa Corp., respectively

6-Chloro-9-(3-deoxy-β-D-ribofuranosyl)purine (2). Adenosine deaminase (4.0 g) in H_2O (30 mL) was added to a solution of cordycepin (10.0 g, 39.8 mmol) in H_2O (800 mL). The mixture was stirred at 45 °C for 5 h with occasional addition of dilute aqueous HCl to adjust the solution to *ca.* pH 6. The mixture was evaporated and then treated with Ac_2O (50 mL)/pyridine (300 mL) at room temperature overnight. After evaporation, the whole reac-tion mixture was chromatographed on a silica gel column (10-15% MeOH in $CHCl_3$) to give 2',5'-di-*O*-acetyl-3'-deoxyinosine (13.3 g, 100%). To a suspension of the acetate (5.0 g, 15 mmol) in $CHCl_3$ (50 mL), DMF (3.5 mL, 45 mmol) and $SOCl_2$ (3.3 mL, 45 mmol) was added, and the whole mixture was refluxed for 4 h. The reaction mixture was washed with saturated aqueous $NaHCO_3$. The $CHCl_3$ solution was dried ($MgSO_4$) and evaporated. The residue was treated with NH_3/MeOH (100 mL) at 4 °C overnight. Evaporation of the solvent gave a precipitate which was crystallized from EtOH to give **2** (2.41 g, 60%, mp 180-182 °C): UV (MeOH) λ_{max} 264 nm (ε 8400), λ_{min}

226 nm (ε 2500); ^1H NMR (DMSO-d_6, after addition of D_2O) δ 1.92 (1H, ddd, *J*= 11.5, 5.9, and 2.2 Hz, H-3'), 2.25 (1H, ddd, *J*= 11.5, 9.7, and 5.2 Hz, H-3'), 3.55 (1H, dd, *J*= 12.2 and 3.7 Hz, H-5'), 3.76 (1H, dd, *J*= 12.2 and 3.1 Hz, H-5'), 4.41-4.46 (1H, m, H-4'), 4.60 (1H, ddd, *J*= 5.2, 2.2, and 1.5 Hz, H-2'), 6.03 (1H, d, *J*= 1.5 Hz, H-1'), 8.78 and 8.93 (2H, each as s, H-8 and H-2); FAB-MS *m/z* 271 (M^++H). Anal. Calcd for $C_{10}H_{11}ClN_4O_3$: C, 44.37; H, 4.10; N, 20.70. Found: C, 44.48; H, 4.01; N, 20.58.

9-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-β-D-ribofuranosyl]-6-chloropurine (3). A mixture of **2** (4.02 g, 14.9 mmol), TBDMSCl (6.72 g, 44.6 mmol), and imidazole (5.05 g, 74.2 mmol) in DMF (10 mL) was stirred for 45 min at room temperature. The reaction mixture was partitioned between EtOAc and H_2O. The organic layer was dried ($MgSO_4$), evaporated, and chromatographed on a silica gel column (hexane/EtOAc = 30/1) to give **3** (7.04 g, 100%) as an oil: UV (MeOH) λ_{max} 263 nm (ε 6400), λ_{min} 227 nm (ε 1600); ^1H NMR ($CDCl_3$) δ 0.09, 0.12, and 0.13 (12H, each as s, SiMe), 0.90 and 0.93 (18H, each as s, SiBu-*t*), 1.84-1.89 and 2.21-2.28 (2H, each as m, H-3'), 3.77 (1H, dd, *J*= 12.6 and 2.5 Hz, H-5'), 4.15 (1H, dd, *J*= 12.6 and 2.2 Hz, H-5'), 4.57-4.59 (1H, m, H-4'), 4.59-4.62 (1H, m, H-2'), 6.07 (1H, d, *J*= 1.1 Hz, H-1'), 8.70 and 8.72 (2H, each as s, H-8 and H-2); FAB-MS *m/z* 499 (M^++H). Anal. Calcd for $C_{34}H_{65}ClN_4O_3Si_2$: C, 52.93; H, 7.87; N, 11.22. Found: C, 53.15; H, 8.09; N, 11.02.

9-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-β-D-ribofuranosyl]-6-chloro-2-(tributylstannyl)purine (4). To a THF (10 mL) solution of LTMP (5.0 mmol), **3** (499 mg, 1.0 mmol) in THF (5 mL) was added dropwise at below –70 °C under a positive pressure of dry Ar. After 5 min, Bu_3SnCl (1.36 mL, 5.0 mmol) was added and the whole reaction mixture was stirred for 5 min at below –70 °C. The reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with CH_2Cl_2. Silica gel column chromatography (hexane/EtOAc = 60/1) of the extract gave **4** (678 mg, 86%) as a syrup: UV (MeOH) λ_{max} 270 nm (ε 7300), λ_{min} 237 nm (ε 6000); ^1H NMR ($CDCl_3$) δ 0.07, 0.08, 0.13, and 0.14 (12H, each as s, SiMe), 0.90 and 0.93 (18H, each as s, SiBu-*t*), 0.88 (9H, t, *J*= 8.3 Hz, $Sn(CH_2)_3C\underline{H}_3$), 1.13-1.29, 1.29-1.39, and 1.55-1.67 (18H, each as m, $Sn(C\underline{H}_2)_3CH_3$), 1.55-1.67 (6H, m, $SnC\underline{H}_2(CH_2)_2CH_3$), 1.86-1.90 (1H, m, H-3'), 2.28-2.33 (1H, m, H-3'), 3.78 (1H, dd, *J*= 11.7 and 2.4 Hz), 4.13 (1H, dd, *J*= 11.7 and 2.4 Hz), 4.56-4.60 (1H, m, H-4'), 4.62 (1H, d, *J*= 4.6 Hz, H-2'), 6.11 (1H, d, *J*= 1.0 Hz, H-1'), 8.57 (1H, s, H-8); FAB-MS *m/z* 789 (M^++H). Anal. Calcd for $C_{34}H_{65}ClN_4O_3Si_2Sn$: C, 51.81; H, 8.31; N, 7.11. Found: C, 51.52; H, 8.58; N, 7.12.

2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-2-(tributylstannyl)cordycepin (5). A THF (5.0 mL) solution of **4** (250 mg, 0.32 mmol) and NH_3/*i*-PrOH (50 mL, saturated at 0 °C, NH_3 content *ca.* 5%) were placed in a sealed tube and heated at 100 °C for 60 h. Evaporation of the reaction mixture followed by silica gel column chromatography (hexane/EtOAc = 1/1) gave **5** (222 mg, 91%) as a syrup: UV (MeOH) λ_{max} 262 nm (ε 9100), λ_{min} 237 nm (ε 5100); ^{1}H NMR ($CDCl_3$) δ ; 0.08, 0.11, 0.12, and 0.15 (12H, each as s, SiMe), 0.88 (9H, t, *J*= 7.3 Hz, $Sn(CH_2)_3C\underline{H}_3$), 0.90 and 0.93 (18H, each as s, SiBu-*t*), 1.08-1.14, 1.26-1.36, and 1.54-1.62 (18H, each as m, $Sn(C\underline{H}_2)_3CH_3$), 1.82-1.87 and 2.22-2.33 (2H, each as m, H-3'), 3.78 (1H, dd, *J*= 11.4 and 3.2 Hz, H-5'), 4.05 (1H, dd, *J*= 11.4 and 2.6 Hz, H-5'), 4.53 (1H, m, H-4'), 4.67 (1H, d, *J*= 4.4 Hz, H-2'), 5.35 (2H, br, NH_2), 6.03 (1H, s, H-1'), 8.22 (1H, s, H-8); FAB-MS *m/z* 769 (M^++H). Anal. Calcd for $C_{34}H_{67}N_5O_3Si_2Sn$: C, 53.12; H, 8.79; N, 9.11. Found: C, 53.38; H, 9.12; N, 9.08.

2-Benzyl-2',5'-bis-*O*-(*tert*-butyldimethylsilyl)cordycepin (6). A mixture of **5** (80 mg, 0.10 mmol), $PhCH_2Br$ (32 µL, 0.13 mmol), $Pd(PPh_3)_4$ (6 mg, 0.005 mmol), and CuI (6 mg, 0.02 mmol) in THF (1 mL) was refluxed for 5 h under a positive pressure of dry Ar. The reaction mixture was partitioned between saturated aqueous $NaHCO_3$ and CH_2Cl_2. Silica gel column chromatography (hexane/EtOAc = 2/1) of the organic layer gave **6** (40 mg, 70%) as a syrup: UV (MeOH) λ_{max} 265 nm (ε 9900), λ_{min} 234 nm (ε 3700); ^{1}H NMR ($CDCl_3$) δ 0.08, 0.11, 0.12, and 0.15 (12H, each as s, SiMe), 0.90 and 0.93 (18H, each as s, SiBu-*t*), 1.81-1.86 (1H, m, H-3'), 2.14-2.23 (1H, m, H-3'), 3.76 (1H, dd, *J*= 11.4 and 3.3 Hz, H-5'), 4.07-4.08 (1H, m, H-5'), 4.10 (2H, s, $C\underline{H}_2Ph$), 4.52-4.56 (1H, m, H-4'), 4.64 (1H, d, *J*= 4.0 Hz, H-2'), 5.53 (2H, br, NH_2), 5.97 (1H, s, H-1'), 7.17 (1H, t, *J*= 7.7 Hz, Ph), 7.26 (2H, t, *J*= 7.7 Hz, Ph), 7.35 (2H, d, *J*= 7.7 Hz, Ph), 8.24 (1H, s, H-8); FAB-MS *m/z* 570 (M^++H). Anal. Calcd for $C_{29}H_{47}N_5O_3Si_2.1/4H_2O$: C, 60.64; H, 8.34; N, 12.19. Found: C, 60.69; H, 8.53; N, 11.87.

2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-2-fluorocordycepin (7). A mixture of **5** (146 mg, 0.18 mmol), XeF_2 (75 mg, 0.43 mmol), AgOTf (125 mg, 0.43 mmol), and 2,6-di-(*tert*-butyl)-4-methylpyridine (83 mg, 0.36 mmol) in CH_2Cl_2 (3 mL) was stirred at room temperature for 0.5 h under a positive pressure of dry Ar. The reaction mixture was partitioned beween saturated aqueous $NaHCO_3$ and CH_2Cl_2. Silica gel column chromatography (hexane/EtOAc = 5/1) of the organic layer gave **7** (49 mg, 55%) as a solid: UV (MeOH) λ_{max} 261 nm (ε 11700), λ_{min} 223 nm (ε 2000); ^{1}H NMR ($CDCl_3$) δ 0.10, 0.12, 0.13, and 0.15 (12H, each as s, SiMe), 0.90 and 0.93 (18H, each as s, SiBu-*t*), 1.82-1.86 (1H, m, H-3'), 2.21-2.26 (1H, m, H-3'), 3.77 (1H, dd, *J*= 11.6 and 2.2 Hz, H-5'), 4.11 (1H, dd, *J*= 11.6 and 2.2 Hz, H-5'), 4.53-4.56 (1H, m, H-4'), 4.59 (1H, m, H-2'), 5.90 (1H, s, H-1'), 6.22 (2H, br, NH_2), 8.28 (1H, s, H-8); FAB-MS *m/z* 498 (M^++H). Anal. Calcd for $C_{22}H_{40}FN_5O_3Si_2$: C, 53.09; H, 8.10; N, 14.07. Found: C, 53.19; H, 8.25; N, 13.97.

2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-2-iodocordycepin (8). A mixture of **5** (700 mg, 0.9 mmol) and iodine (250 mg, 1.1 mmol as I_2) in THF (10 mL) was stirred for 0.5 h. The reaction mixture was partitioned beween CH_2Cl_2 and saturated aqueous $Na_2S_2O_3$. Silica gel column chromatography (hexane/EtOAc = 2/1) of the organic layer gave **8** (550 mg, 100%) as a foam: UV (MeOH) λ_{max} 267 nm (ε 10100), λ_{min} 237 nm (ε 4700); ^1H NMR ($CDCl_3$) δ 0.12, 0.13, 0.14, and 0.21 (12H, each as s, SiMe), 0.91 and 0.93 (18H, each as s, SiBu-*t*), 1.78-1.82 and 2.14-2.20 (2H, each as m, H-3'), 3.77 (1H, dd, *J*= 11.6 and 2.4 Hz, H-5'), 4.14 (1H, dd, *J*= 11.6 and 2.4 Hz, H-5'), 4.54-4.59 (1H, m, H-4'), 4.60 (1H, d, *J*= 4.3 Hz, H-2'), 5.91 (1H, s, H-1'), 6.16 (2H, br, NH_2), 8.25 (1H, s, H-8); FAB-MS *m*/*z* 606 (M^++H). Anal. Calcd for $C_{22}H_{40}IN_5O_3Si_2 \cdot 1/2H_2O$: C, 42.99; H, 6.72; N, 11.39. Found: C, 43.38; H, 6.74; N, 11.12.

Synthesis of 2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-2-(1-hexyn-1-yl)-cordycepin (9a) as a typical procedure for the preparation of compounds of structure 9. A mixture of **8** (430 mg, 0.71 mmol), 1-hexyne (165 µL, 1.42 mmol), $(PPh_3)PdCl_2$ (50 mg, 72 µmol), CuI (7 mg, 36 µmol), and Et_3N (110 µL, 0.78 mmol) in DMF (3 mL) was heated at 75 °C for 1 h. The reaction mixture was diluted with $CHCl_3$, treated with H_2S by bubbling, and then filtered through celite. Silica gel column chromatography (hexane/EtOAc = 5/1) of the filtrate gave **9a** (370 mg, 93%) as a solid: UV (MeOH) λ_{max} 268 nm (ε 9400), $\lambda_{shoulder}$ 287 nm (ε 6800), λ_{min} 245 nm (ε 4400); ^1H NMR ($CDCl_3$) δ 0.11, 0.12, 0.13, and 0.18 (12H, each as s, SiMe), 0.91 and 0.93 (18H, each as s, SiBu-*t*), 1.20-1.30 (3H, m, $C{\equiv}C(CH_2)_3C\underline{H}_3$), 1.46-1.53 and 1.58-1.64 (4H, each as m, $C{\equiv}CCH_2(C\underline{H}_2)_2CH_3$), 1.79-1.84 and 2.16-2.21 (2H, each as m, H-3'), 2.43 (2H, t, *J*= 7.0 Hz, $C{\equiv}CC\underline{H}_2$), 3.76 (1H, dd, *J*= 11.6 and 2.7 Hz, H-5'), 4.12 (1H, dd, *J*= 11.6 and 2.4 Hz, H-5'), 4.53-4.56 (1H, m, H-4'), 4.59-4.60 (1H, m, H-2'), 5.88 (2H, br, NH_2), 6.00 (1H, d, *J*= 0.9 Hz, H-1'), 8.33 (1H, s, H-8); FAB-MS *m*/*z* 560 (M^++H). Anal. Calcd for $C_{28}H_{49}N_5O_3Si_2 \cdot 1/2H_2O$: C, 59.11; H, 8.86; N, 12.31. Found: C, 59.49; H, 8.89; N, 12.20.

2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-2-(1-octyn-1-yl)cordycepin (9b). This compound was obtained as a solid by the procedure described for the preparation of **9a**. The reaction was continued for 1 h: UV (MeOH) λ_{max} 269 nm (ε 9500), $\lambda_{shoulder}$ 288 nm (ε 7100), λ_{min} 245 nm (ε 4600); ^1H NMR ($CDCl_3$) δ 0.11, 0.12, 0.13, and 0.17 (12H, each as s, SiMe), 0.90 and 0.93 (18H, each as s, SiBu-*t*), 0.87-0.90 (3H, m, $C{\equiv}C(CH_2)_5C\underline{H}_3$), 1.24-1.30, 1.43-1.49, and 1.59-1.66 (8H, each as m, $C{\equiv}CCH_2$-$(C\underline{H}_2)_4CH_3$), 1.80-1.84 and 2.16-2.21 (2H, each as m, H-3'), 2.42 (2H, t, *J*= 7.0 Hz, $C{\equiv}CC\underline{H}_2$), 3.76 (1H, dd, *J*= 11.6 and 2.4 Hz, H-5'), 4.12 (1H, dd, *J*= 11.6 and 2.4 Hz, H-5'), 4.53-4.57 (1H, m, H-4'), 4.59-4.60 (1H, m, H-2'), 5.81 (2H, br, NH_2), 6.01 (1H, s, H-1'), 8.32 (1H, s, H-8); FAB-MS *m*/*z* 588 (M^++H). Anal. Calcd for $C_{30}H_{53}N_5$-O_3Si_2: C, 61.28; H, 9.08, N, 11.91. Found: C, 61.65; H, 9.37; N, 11.92.

2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-2-(3-hydroxy-1-propyn-1-yl)-cordycepin (9c). This compound was obtained as a solid by the procedure described for the preparation of **9a**. The reaction was continued for 4 h: UV (MeOH) λ_{max} 269 nm (ε 8000), $\lambda_{shoulder}$ 270 nm (ε 5800), λ_{min} 245 nm (ε 3900); ^{1}H NMR ($CDCl_3$) δ 0.09, 0.12, 0.14, and 0.15 (12H, each as s, SiMe), 0.90 and 0.94 (18H, each as s, SiBu-*t*), 1.77 (1H, br, OH), 1.82-1.86 and 2.20-2.25 (2H, each as m, H-3'), 3.76 (1H, dd, *J*= 11.6 and 2.5 Hz, H-5'), 4.12 (1H, dd, *J*= 11.6 and 2.4 Hz, H-5'), 4.51 (2H, s, $C{\equiv}C\underline{CH_2}$), 4.54-4.57 (1H, m, H-4'), 4.58-4.60 (1H, m, H-2'), 6.00 (1H, d, *J*= 1.6 Hz, H-1'), 6.03 (2H, br, NH_2), 8.37 (1H, s, H-8); FAB-MS *m*/*z* 534 (M^++H). Anal. Calcd for $C_{25}H_{43}N_5O_4Si_2$: C, 56.25; H, 8.12; N, 13.12. Found: C, 56.39; H, 8.34; N, 12.91.

2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-2-(phenyl)ethynylcordycepin (9d). This compound was obtained as a solid by the procedure described for the preparation of **9a**. The reaction was continued for 3.5 h: UV (MeOH) λ_{max} 260 nm (ε 12900), $\lambda_{shoulder}$ 300 nm (ε 9800), λ_{min} 237 nm (ε 9300); ^{1}H NMR ($CDCl_3$) δ 0.13, 0.14, 0.15, and 0.24 (12H, each as s, SiMe), 0.93 and 0.94 (18H, each as s, SiBu-*t*), 1.81-1.85 and 2.16-2.26 (2H, each as m, H-3'), 3.78 (1H, dd, *J*= 11.6 and 2.7 Hz, H-5'), 4.14 (1H, dd, *J*= 11.6 and 2.4 Hz, H-5'), 4.56-4.60 (1H, m, H-4'), 4.66 (1H, m, H-2'), 5.98 (2H, br, NH_2), 6.03 (1H, d, *J*= 0.9 Hz, H-1'), 7.33-7.39 and 7.61-7.63 (5H, each as m, Ph), 8.37 (1H, s, H-8); FAB-MS *m*/*z* 580 (M^++H). Anal. Calcd for $C_{30}H_{45}N_5O_3Si_2 \cdot 1/10H_2O$: C, 61.95; H, 7.83; N, 12.04. Found: C, 61.75; H, 7.92; N, 11.95.

2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-2-(3-phenyl-1-propyn-1-yl)-cordycepin (9e). The following procedure was used for the preparation of $Bu_3SnC{\equiv}CCH_2Ph$. To a THF (3 mL) solution containing BuLi (2.4 mmol), $HC{\equiv}CCH_2Ph$ (250 μL, 2.0 mmol) was added at below –70 °C under a positive pressure of dry Ar. After stirring for 5 min, Bu_3SnCl (600 μL, 2.4 mmol) was added and the whole mixture was stirred for 0.5 h at below –70 °C. The reaction mixture was partitioned between saturated aqueous $NaHCO_3$ and CH_2Cl_2. Silica gel column chromatography (hexane) of the organic layer gave $Bu_3SnC{\equiv}CCH_2Ph$ (812 mg, 100%) as a liquid. Compound **9e** was obtained by the following procedure. A mixture of **8** (100 mg, 0.16 mmol), $Bu_3SnC{\equiv}CCH_2Ph$ (129 mg, 0.32 mmol), $Pd(PPh_3)_4$ (9 mg, 8 μmol), and CuI (6 mg, 32 μmol) in THF (3 mL) was refluxed for 16.5 h under a positive pressure of dry Ar. The reaction mixture was partitioned between saturated aqueous $NaHCO_3$ and CH_2Cl_2. Silica gel column chromatography (hexane/EtOAc = 2/1) of the organic layer gave **9e** (76 mg, 80%) as a syrup: UV (MeOH) λ_{max} 268 nm (ε 8700), $\lambda_{shoulder}$ 287 nm (ε 5400), λ_{min} 245 nm (ε 6600); ^{1}H NMR ($CDCl_3$) δ 0.07, 0.09, 0.11, and 0.13 (12H, each as s, SiMe), 0.90 and 0.94 (18H, each as s, SiBu-*t*), 1.79-1.83 and 2.14-2.22 (2H, each as m, H-3'), 3.77 (1H, dd, *J*= 11.6 and 2.4 Hz, H-5'), 3.86 (2H, s, $\underline{CH_2}Ph$), 4.13 (1H, dd, *J*= 11.6 and 2.4 Hz, H-5'), 4.54-4.57 (1H, m, H-4'), 4.58-4.60 (1H, m, H-2'), 5.77 (2H, br, NH_2), 6.01 (1H, s, H-1'), 7.24 (1H, t, *J*= 7.2 Hz, Ph), 7.32 (2H, t, *J*= 7.2 Hz, Ph), 7.42 (2H, d, *J*= 7.2 Hz, Ph), 8.36 (1H, s, H-8); FAB-MS *m/z* 594 (M^++H). Anal. Calcd for $C_{31}H_{47}N_5O_3Si_2\cdot 1/10H_2O$: C, 62.50; H, 7.99; N, 11.76. Found: 62.22; H, 8.13; N, 11.43.

2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-2-(3-hydroxy-3-phenyl-1-propyn-1-yl)cordycepin (9f). This compound was obtained as an oil by the procedure described for the preparation of **9a**. The reaction was continued for 3.5 h: UV (MeOH) λ_{max} 269 nm (ε 9400), $\lambda_{shoulder}$ 290 nm (ε 6900), λ_{min} 247 nm (ε 6000); ^1H NMR ($CDCl_3$) δ 0.08, 0.11, 0.13, and 0.16 (12H, each as s, SiMe), 0.89 and 0.93 (18H, each as s, SiBu-*t*), 1.78-1.82 and 2.13-2.21 (2H, each as m, H-3'), 3.76 (1H, dd, *J*= 10.9 and 2.5 Hz, H-5'), 4.13 (1H, dd, *J*= 10.9 and 2.4 Hz, H-5'), 4.69 (1H, s, OH), 4.53-4.56 (1H, m, H-4'), 4.56-4.60 (1H, m, H-2'), 5.71 (1H, br, $C\underline{H}(OH)Ph$), 5.97 (1H, d, *J*= 0.9 Hz, H-1'), 8.31 and 8.34 (2H, each as br, NH_2), 7.27-7.30 (1H, m, Ph), 7.32-7.36 (2H, m, Ph), 7.60-7.62 (2H, m, Ph), 8.35 (1H, s, H-8); FAB-MS *m/z* 610 (M^++H). Anal. Calcd for $C_{31}H_{47}N_5O_4Si_2$: C, 61.05; H, 7.77; N, 11.48. Found: C, 60.94; H, 8.03; N, 11.27.

Preparation of 2-(1-hexyn-1-yl)cordycepin (10a) as a typical procedure for desilylation. A mixture of **9a** (332 mg, 0.59 mmol) and NH_4F (330 mg, 8.9 mmol) in MeOH (5 mL) was heated at 60-65 °C for 20 h. After evaporation, the reaction mixture was chromatographed on a silica gel column ($CHCl_3$/MeOH = 10/1) to give **10a** (183 mg, 93%) as a solid: UV (MeOH) λ_{max} 270 (ε 9000), $\lambda_{shoulder}$ 287 nm (ε 6500), λ_{min} 245 nm (ε 4300); ^1H NMR (DMSO-d_6, after addition of D_2O) δ 0.94 (3H, t, *J*= 7.3 Hz, $(CH_2)_3C\underline{H}_3$), 1.43-1.50 and 1.53-1.60 (4H, each as m, $(C\underline{H}_2)_2CH_3$), 1.89-1.92 and 2.19-2.24 (2H, each as m, H-3'), 2.40 (2H, t, *J*= 7.4 Hz, $C{\equiv}CC\underline{H}_2$), 3.52 (1H, dd, *J*= 12.2 and 3.3 Hz, H-5'), 3.73 (1H, dd, *J*= 12.2 and 2.7 Hz, H-5'), 4.35-4.40 (1H, m, H-4'), 4.47-4.49 (1H, m, H-2'), 5.82 (1H, d, *J*= 1.8 Hz, H-1'), 8.36 (1H, s, H-8); FAB-MS *m/z* 332 (M^++H). Anal. Calcd for $C_{16}H_{21}N_5O_3\cdot 1/5H_2O$: C, 57.37; H, 6.44; N, 20.91. Found: C, 57.08; H, 6.43; N, 20.54.

2-(1-Octyn-1-yl)cordycepin (10b). This compound was obtained from **9b** as a solid by the procedure described for the preparation of **10a**: UV (MeOH) λ_{max} 270 (ε 7200), $\lambda_{shoulder}$ 288 nm (ε 5000), λ_{min} 245 nm (ε 3300); ^1H NMR (DMSO-d_6, after addition of D_2O) δ 0.89 (3H, t, *J*= 7.0 Hz, $(CH_2)_5C\underline{H}_3$), 1.24-1.30, 1.38-1.44, and 1.51-1.57 (8H, each as m, $(C\underline{H}_2)_4CH_3$), 1.87-1.92 and 2.19-2.24 (2H, each as m, H-3'), 3.52 (1H, dd, *J*= 12.1 and 3.3 Hz, H-5'), 3.70 (1H, dd, *J*= 12.1 and 2.8 Hz, H-5'), 4.34-4.37 (1H, m, H-4'), 4.50 (1H, dd, *J*= 2.4 and 1.5 Hz, H-2'), 5.83 (1H, d, *J*= 1.5 Hz, H-1'), 8.38 (1H, s, H-8); FAB-MS *m/z* 360 (M^++H). Anal. Calcd for $C_{18}H_{25}N_5O_3$: C, 60.15; H, 7.01; N, 19.49. Found: C, 59.95; H, 7.17; N, 19.21.

2-(3-Hydroxy-1-propyn-1-yl)cordycepin (10c). This compound was obtained from **9c** by the procedure described for the preparation of **10a**: mp 254-256 °C (MeOH-H_2O); UV (MeOH) λ_{max} 269 nm (ε 8800), $\lambda_{shoulder}$ 290 nm (ε 6400), λ_{min} 246 nm (ε 4300); ^1H NMR (DMSO-d_6, after addition of D_2O) δ 1.85-1.88 and 2.17-2.22 (2H, each as m, H-3'), 3.53 (1H, dd, *J*= 12.2 and 3.6 Hz, H-5'), 3.73 (1H, dd, *J*= 12.2 and 3.0 Hz, H-5'), 4.26 (2H, s, C$\underline{H}_2$OH), 4.35-4.38 (1H, m, H-4'), 4.46-4.48 (1H, m, H-2'), 5.84 (1H, d, *J*= 1.8 Hz, H-1'), 8.39 (1H, s, H-8); FAB-MS *m/z* 307 (M^++H). Anal. Calcd for $C_{13}H_{15}N_5O_4 \cdot 1/4H_2O$: C, 50.40; H, 5.04; N, 22.61. Found: C, 50.13; H, 5.23; N, 22.34.

2-(Phenyl)ethynylcordycepin (10d). This compound was obtained from **9d** as a solid by the procedure described for the preparation of **10a**: UV (MeOH) λ_{max} 260 nm (ε 14500), $\lambda_{shoulder}$ 305 nm (ε 11000), λ_{min} 237 nm (ε 10800); ^1H NMR (DMSO-d_6, after addition of D_2O) δ 1.89-1.94 and 2.21-2.36 (2H, each as m, H-3'), 3.54(1H, dd, *J*= 12.1 and 3.5 Hz, H-5'), 3.74 (1H, dd, *J*= 12.1 and 3.1 Hz, H-5'), 4.36-4.40 (1H, m, H-4'), 4.51-4.53 (1H, m, H-2'), 5.88 (1H, d, *J*= 2.1 Hz, H-1'), 7.42-7.47 and 7.58-7.60 (5H, each as m, Ph), 8.43 (1H, s, H-8); FAB-MS *m/z* 352 (M^++H). Anal. Calcd for $C_{18}H_{17}N_5O_3 \cdot 1/10H_2O$: C, 61.22; H, 4.91; N, 19.83. Found: C, 60.96; H, 4.76; N, 19.71.

2-(3-Phenyl-1-propyn-1-yl)cordycepin (10e). This compound was obtained from **9e** as a solid by the procedure described for the preparation of **10a**: UV (MeOH) λ_{max} 270 nm (ε 8000), $\lambda_{shoulder}$ 290 nm (ε 6800), λ_{min} 246 nm (ε 4700); ^1H NMR (DMSO-d_6, after addition of D_2O) δ 1.89 (1H, ddd, *J*= 13.2, 6.1, and 3.1 Hz, H-3'), 2.21 (1H, ddd, *J*= 13.2, 8.9, and 5.5 Hz, H-3'), 3.52 (1H, dd, *J*= 12.2 and 3.7 Hz, H-5'), 3.72 (1H, dd, *J*= 12.2 and 3.0 Hz, H-5'), 3.86 (2H, s, C$\underline{H}_2$Ph), 4.34-4.38 (1H, m, H-4'), 4.47-4.49 (1H, m, H-2'), 5.83 (1H, d, *J*= 2.2 Hz, H-1'), 7.23-7.28, 7.34-7.37, and 7.39-7.41 (5H, each as m, Ph), 8.38 (1H, s, H-8); FAB-MS *m/z* 366 (M^++H). Anal. Calcd for $C_{19}H_{19}N_5O_3 \cdot 3/4H_2O$: C, 60.23; H, 5.45; N, 18.48. Found: C, 60.36; H, 5.41; N, 18.45.

2-(3-Hydroxy-3-phenyl-1-propyn-1-yl)cordycepin (10f). This compound was obtained from **9f** by the procedure described for the preparation of **10a**: mp 130-133 °C (MeOH-H_2O); UV (MeOH) λ_{max} 269 nm (ε 10600), $\lambda_{shoulder}$ 285 nm (ε 8300), λ_{min} 247 nm (ε 7400); ^1H NMR (DMSO-d_6, after addition of D_2O) δ 1.86-1.90 and 2.18-2.23 (2H, each as m, H-3'), 3.54 (1H, dd, *J*= 12.2 and 2.0 Hz, H-5'), 3.71 (1H, dd, *J*= 12.2 and 3.1 Hz, H-5'), 4.35-4.40 (1H, m, H-4'), 4.49-4.51 (1H, m, H-2'), 5.59 (1H, s, C$\underline{H}$(OH)Ph), 5.85 (1H, d, *J*= 2.1 Hz, H-1'), 7.30-7.34 (1H, m, Ph), 7.34-7.41 (2H, m, Ph), 7.52-7.54 (2H, m, Ph), 8.42 (1H, s, H-8); FAB-MS *m/z* 382 (M^++H). Anal. Calcd for $C_{19}H_{19}N_5O_4 \cdot 1/10H_2O$: C, 59.55; H, 5.05; N, 18.28. Found: C, 59.56; H, 5.16; N, 18.00.

ACKNOWLEDGEMENT Part of this work has been supported by the Grant-in-Aid for Scientific Research (to H. T., No. 08672445) from the Ministry of Education, Science, and Culture, Japan.

REFERENCES AND NOTES

1) For a review concerning nucleoside antibiotics, see: Isono, K. *J. Antibiot.* **1988,** *41*, 1711-1739.
2) For a review, see: Srivastava, P. C.; Robins, R. K.; Meyer, R. B., Jr. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B. Ed.; Plenum Press, New York, 1988; Vol. 1, pp. 113-281.
3) For the synthesis of 2-substituted derivatives from purine nucleosides, see: a) Maeda, M.; Nushi, K.; Kawazoe, Y. *Tetrahedron* **1974,** *30*, 2677-2682. b) Matsuda, A.; Nomoto, Y.; Ueda, T. *Chem. Pharm. Bull.* **1979,** *27*, 183-192.
4) a) Nair, V.; Richardson, S. G. *Synthesis* **1982**, 670-672. b) Nair, V.; Young, D. A. *J. Org. Chem.* **1985,** *50*, 406-408.
5) Nair, V. *Nucleosides Nucleotides* **1989,** *8*, 699-708 and references cited therein.
6) a) Matsuda, A.; Shinozaki, M.; Miyasaka, T.; Machida, H.; Abiru, T. *Chem. Pharm. Bull.* **1985,** *33*, 1766-1769. b) Matsuda, A.; Shinozaki, M.; Yamaguchi, T.; Homma, H.; Nomoto, R.; Miyasaka, T.; Watanabe, Y.; Abiru, T. *J. Med. Chem.* **1992,** *35*, 241-252.
7) a) Tanaka, H.; Uchida, Y.; Shinozaki, M.; Hayakawa, H.; Matsuda, A.; Miyasaka, T. *Chem. Pharm. Bull.* **1983,** *31*, 787-790. b) Hayakawa, H.; Haraguchi, K.; Tanaka, H.; Miyasaka, T. *Chem. Pharm. Bull.* **1987,** *35*, 72-79. c) Hayakawa, H.; Tanaka, H.; Haraguchi, K.; Mayumi, M.; Nakajima, M.; Sakamaki, T.; Miyasaka, T. *Nucleosides Nucleotides* **1988**, *7*, 121-128. d) Hayakawa, H.; Tanaka, H.; Sasaki, K.; Haraguchi, K.; Saitoh, T.; Takai, F.; Miyasaka, T. *J. Heterocycl. Chem.* **1989,** *26*, 189-192.
8) a) Kato, K.; Hayakawa, H.; Tanaka, H.; Kumamoto, H.; Miyasaka, T. *Tetrahedron Lett.* **1995,** *36*, 6507-6510. b) Kato, K.; Hayakawa, H.; Tanaka, H.; Kumamoto, H.; Shindoh, S.; Shuto, S.; Miyasaka, T., submitted for publication.
9) For a review concerning the Stille reaction, see: Mitchell, T. N. *Synthesis* **1992,** 803-815.
10) a) Tius, M. A.; Kawakami, J. K. *Synth. Commun.* **1992,** *22*, 1461-1471. b) Tius, M. A. *Tetrahedron* **1995,** *51*, 6605-6634.
11) Winbury, M. M.; Papierski, D. H.; Hemmer, M. L.; Hambourger, W. E. *J. Pharmacol. Exp. Ther.* **1953,** *109*, 255-260.
12) a) Maguire, M. H.; Nobbs, D. M.; Einstein, R.; Middleton, J. C. *J. Med. Chem.* **1971,** *14*, 415-420. b) Marumoto, R.; Yoshioka, Y.; Miyashita, O.; Shima, S.; Imai, K.; Kawazoe, K.; Honjo, M. *Chem. Pharm. Bull.* **1975,** *23*, 759-774.
13) a) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, 4467-4470. b) Heck, R. F. *Acc. Chem. Res.* **1979,** *12*, 146-151.
14) Although the ^{1}H NMR spectrum of **9f** measured in $CDCl_3$ was seemingly that of a single isomer, this compound is assumed to be a mixture of two diastereoisomers, as the reagent used was a racemic modification.
15) Zhang, W.; Robins, M. J. *Tetrahedron Lett.* **1992,** *33*, 1177-1180.
16) Abiru, T.; Endo, K.; Machida, H. *Eur. J. Pharmacol.* **1995,** *281*, 9-15.