New Nucleoside−Sugar Conjugates: 6-*N*-Glycosyloxyphosphorylated Adenosine Derivatives as Partial Structures of Agrocin 84

Tomohisa Moriguchi, Takeshi Wada, and Mitsuo Sekine*

Department of Life Science, Tokyo Institute of Technology, Nagatsuta, Midoriku, Yokohama 226, Japan

Received August 1, 1996[⊗]

We report the first successful synthesis of 6-*N*-[(glucofuranos-1-yloxy)phosphoryl]adenosine as a partial structure of Agrocin 84 *via* a two-step phosphorylation of 2′,3′,5′-tri-*O*-benzoyladenosine with a 2,3,5,6-tetra-*O*-acetylglucofuranoside 1-*O*-phosphoramidite derivative that has a 2-(trimethylsilyl)ethyl group as the phosphate protecting group. A similar nucleoside−sugar conjugate, 6-*N*-[(ribofuranos-1-yloxy)phosphoryl]adenosine, was also synthesized. The stabilities of these 6-*N*-[(glycos-1-yloxy)phosphoryl]adenosine derivatives under acidic, basic, and thermal conditions are described. In particular, we found that the P−O bond of these sugar−nucleoside conjugates was selectively cleaved by treatment with 0.1 M NaOH to give 6-*N*-phosphoryladenosine, while acidic treatment gave directly adenosine with cleavage of the P−N bond.

Introduction

Agrocin 84 is a nucleotide bacteriocin that inhibits the growth of sensitive strains of *Agrobacterium radiobacter* and is produced by *Agrobacterium tumefaciens* K84[1] to biologically control the plant cancer crown gall disease.[2,3] This antibiotic was first believed to inhibit the DNA, RNA, and protein syntheses and amino acid transport.[4] Later, Das *et al.*[5] suggested that Agrocin 84 inhibits thymidine uptake by Agrocin-sensitive *A. tumefaciens* so that DNA synthesis is regulated. Studies of its biosynthesis[6] and sensitivity[7] and biological control of crown gall[8,9] have also been reported.

Agrocin 84 has a unique structure in which the 1-OH group of glucofuranose is connected to the 6-amino group of the adenine (Figure 1).[10] Nucleotide−sugar conjugates such as UDP−Glc[11] and CMP−Neu-5-Ac[12] play a very important role in living cells and act as glycosyl donors for the biosynthesis of oligosaccharides. In these conjugates, the 1-OH group of sugars is linked to nucleoside 5′-mono- or diphosphate derivatives by a phosphate diester bond. In contrast to these conjugates, Agrocin 84 has another linkage in which the 1-OH group of glucofuranose binds to the amino group of the adenine base *via* a phosphoramidate bond.

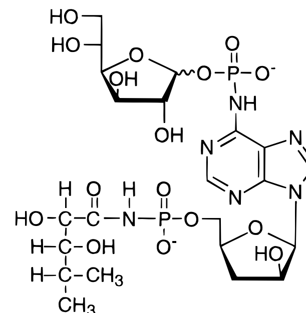


Figure 1. Structure of Agrocin 84.

Despite the early discovery of this natural product, only two papers have been presented on the structure−function relationship of Agrocin 84.[13,14] The 5′-phosphoramidate substituent is essential for the biological activity of Agrocin 84, while the 6-*N*-[(glucofuranos-1-yloxy)phosphoryl]adenine moiety is responsible for the strain-specific uptake of Agrocin 84. Several attempts to synthesize a core nucleoside, 9-(3′-deoxy-*β*-D-*threo*-pentofuranosyl)adenine, which was expected to have both antiviral and antitumor properties, have been reported.[15] However, the two phosphoramidate bonds have not yet been synthesized successfully. This is probably due to the extreme difficulty in constructing the O−P−N bond, which is generally considered to be "too unstable". The stereoselective introduction of either an unprotected or protected phosphoryl group into the anomeric center of glucofuranose[16] is particularly difficult because such a 1-*O*-phosphate functionality is extremely labile.[17] In addition, the anomeric configuration of the glucofuranose

⊗ Abstract published in *Advance ACS Abstracts,* November 15, 1996.

(1) (a) Kerr, A.; Htay, H. *Physiol. Plant Pathol.* **1974**, *4*, 37. (b) Murphy, P. J.; Roberts, W. P. *J. Gen. Microbiol.* **1979**, *114*, 207. (c) Holmes, B.; Roberts, W. P. *J. Appl. Bacteriol.* **1981**, *50*, 443.
(2) New, P. B.; Kerr, A. *J. Appl. Bacteriol.* **1972**, *35*, 279.
(3) Lopez, M. M.; Gorris, M. T.; Salcedo, C. I.; Montojo, A. M.; Miro, M. *Appl. Environ. Microbiol.* **1989**, *55*, 741.
(4) McCardell, B. A.; Pootjes, C. F. *Antimicrob. Agents Chemother.* **1976**, *10*, 498.
(5) Das, P. K.; Basu, M.; Chatterjee, G. C. *J. Antibiot.* **1978**, *31*, 490.
(6) (a) Ryder, M. H.; Slota, J. E.; Scarim, A.; Farrand, S. K. *J. Bacteriol.* **1987**, *169*, 4184. (b) Law, I. J.; Strijdom, B. W. *Arch. Macrobiol.* **1989**, *152*, 463.
(7) (a) Van Zyl, F. G. H.; Strijdom, B. W.; Staphorst, J. L. *Appl. Environ. Microbiol.* **1986**, *52*, 234. (b) Cooksey, D. A. *Plasmid* **1986**, *16*, 222. (c) Hayman, G. T.; Farrand, S. K. *J. Bacteriol.* **1988**, *170*, 1759. (d) Hayman, G. T.; Farrand, S. K. *Mol. Gen. Genet.* **1990**, *223*, 465.
(8) (a) Jones, D. A.; Ryder, M. H.; Clare, B. G.; Farrand, S. K.; Kerr, A. *Mol. Gen. Genet.* **1988**, *212*, 207. (b) Webster, J.; Thomson, J. *Mol. Gen. Genet.* **1988**, *214*, 142. (c) Lopez, M. M.; Gorris, M. T.; Salcedo, C. I.; Montojo, A. M.; Miro, M. *Appl. Environ. Microbiol.* **1989**, *55*, 741.
(9) Kerr, A.; Tate, M. E. *Microbiol. Sci.* **1984**, *1*, 1.
(10) (a) Roberts, W. P.; Tate, M. E.; Kerr, A. *Nature* **1977**, *265*, 379. (b) Tate, M. E.; Roberts, W. P.; Kerr, A. *Nature* **1979**, *280*, 697.
(11) Caputto, R.; Leloir, L. F.; Cardini, C. E.; Paladini, A. C. *J. Biol. Chem.* **1950**, *184*, 333.
(12) Schauer, R. *Adv. Carbohydr. Chem. Biochem.* **1982**, *40*, 131.
(13) Murphy, P. J.; Tate, M. E.; Kerr, A. *Eur. J. Biochem.* **1981**, *115*, 539.
(14) Thompson, R. R.; Hamilton, R. H.; Pootjes, C. F. *Antimicrob. Agents Chemother.* **1979**, *16*, 293.
(15) (a) Murray, D. H.; Prokop, J. *J. Pharm. Sci.* **1965**, *54*, 1468. (b) Martinez, A. P.; Lee, W. W.; Goodman, L. *J. Org. Chem.* **1966**, *31*, 3263. (c) Hansske, F.; Robins, M. J. *J. Am. Chem. Soc.* **1983**, *105*, 6736. (d) Nyilas, A.; Chattopadhyaya, J. *Synthesis* **1986**, 196. (e) Kawana, M.; Kuzuhara, H. *Tetrahedron Lett.* **1987**, *28*, 4075. (f) Herdewijn, P.; Balzarini, J.; Pauwels, R.; Janssen, G.; Van Aerschot, A.; De Clercq, E. *Nucleosides Nucleotides* **1989**, *8*, 1231. (g) Herdewijn, P. *Tetrahedron* **1989**, *45*, 6563.

 © 1996 American Chemical Society

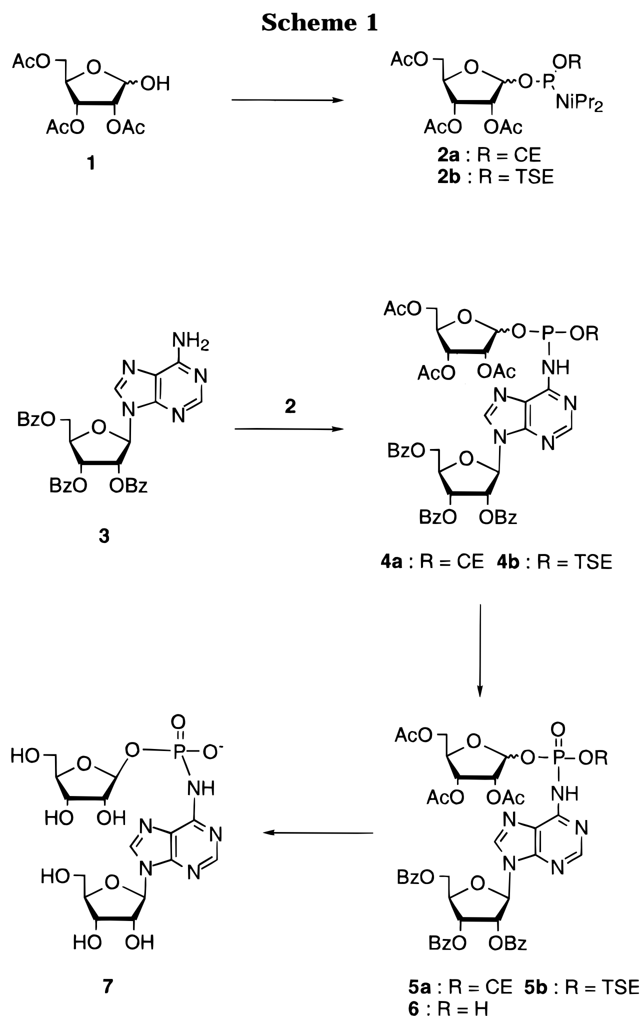
moiety of Agrocin 84 has not yet been clearly determined. Therefore, it is important to find effective methods for synthesizing this partial structure of Agrocin 84. Quite recently, Robins and his co-workers reported the synthesis of a nucleoside–sugar conjugate, 6-*N*-[[(1-*O*-methylglucopyranos-6-yl)oxy]phosphoryl]adenosine, which has an O(6″)–P–N(6) linkage, and also described the difficulty in forming the O(1″)–P–N(6) bond in connection with the synthesis of Agrocin 84.[18]

Recently, we reported the synthesis of *N*-phosphorylated nucleosides *via* a two-step reaction involving phosphorylation using phosphoramidite reagents and subsequent oxidation.[19a] This successful result led us to extend the two-step strategy to the synthesis of 6-*N*-glycosyloxyphosphorylated adenosine derivatives that have an O(1″)–P–N(6) bond. We report here the synthesis and properties of β-isomers of 6-*N*-[(glucofuranos-1-yloxy)phosphoryl]adenosine (**12**) that comprise Agrocin 84 and 6-*N*-[(ribofuranos-1-yloxy)phosphoryl]adenosine (**7**) as an analog with a similar O(1″)–P–N(6) linkage.

Results and Discussion

Synthesis of 6-*N*-[(Glycosyloxy)phosphoryl]adenosine Derivatives. To synthesize 6-*N*-glycosyloxyphosphorylated adenosine derivatives, the stepwise condensation of sugars and adenosine was performed. *N*-Phosphorylation of several *O*-protected adenosine derivatives was studied using highly reactive phosphoramidite reagents. First, we studied the synthesis of **7** in detail prior to the synthesis of **12**. A β-isomer-rich 9:1 anomeric mixture of 2,3,5-tri-*O*-acetyl-D-ribofuranose (**1**), which was synthesized by selective deacetylation of 1,2,3,5-tetra-*O*-acetylribofuranoside with tributyltin methoxide,[20] was phosphitylated by treatment with 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite[21] in the presence of diisopropylammonium tetrazolide as an activating reagent (Scheme 1). The resulting phosphoramidite **2a** was allowed to react *in situ* with 2′,3′,5′-tri-*O*-benzoyladenosine (**3**) to give the phosphite intermediate **4a**, which was further oxidized with *tert*-butyl hydroperoxide to give the fully protected 6-*N*-[(ribofuranos-1-yloxy)phosphoryl]adenosine derivative **5a**. However, **5a** was very unstable and decomposed with cleavage of the P–N bond to the starting material **3** during purification by silica gel column chromatography. Several experiments were performed to control the inherent instability of the P–N bond in **5a**. Consequently, we found that when the cyanoethyl group was eliminated from **5a** by treatment with bases such as diisopropylamine or DBU, the dissociated phosphoramidate bond of the resulting product **6** could be considerably stabilized. However, we encountered a serious problem due to the competitive formation of some deacylated byproducts that hampered the purification of **6**, which was ultimately obtained as only a crude material.

Scheme 1

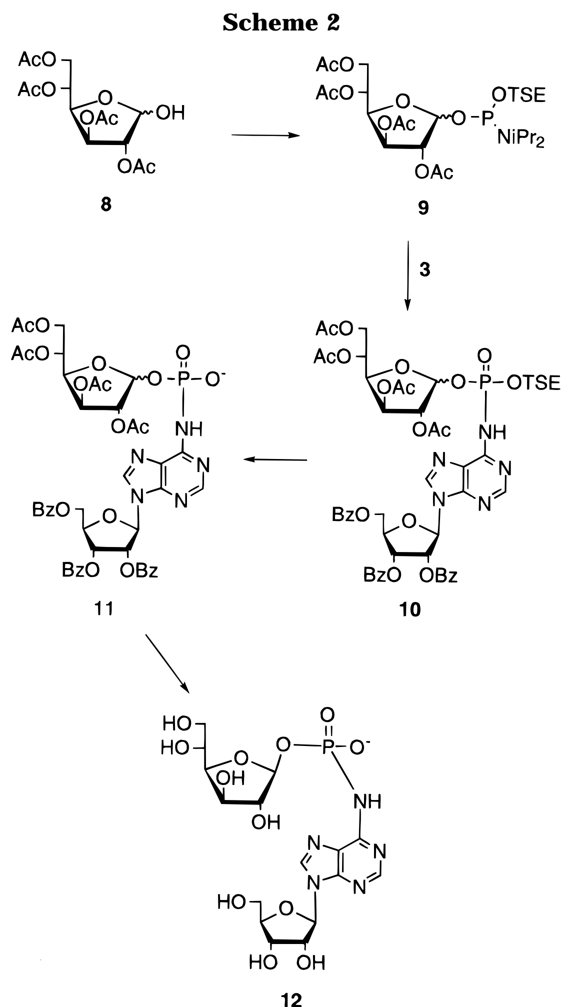


Finally, the 2-(trimethylsilyl)ethyl group was used in place of the 2-cyanoethyl group to avoid the competitive deacylation in the above decyanoethylation. The former can be used as a phosphate protecting group in DNA synthesis, as reported by Wada and Sekine,[22] since it can be easily removed by treatment with fluoride ion. Thus, the 1-*O*-phosphoramidite derivative **2b** was prepared *in situ* by a similar *O*-phosphitylation of 2,3,5-tri-*O*-acetyl-D-ribose with 1.5 equiv of 2-(trimethysilyl)ethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite in CH_2Cl_2 at rt for 1 h. Condensation of **3** with 2 equiv of **2b** in the presence of 4 equiv of 1*H*-tetrazole in acetonitrile for 1 h gave the phosphite intermediate **4b**, which was in turn oxidized with *tert*-butyl hydroperoxide to give the diester **5b**. Treatment of **5b** with 4 equiv of Bu_4NF monohydrate in THF at rt for 12 h gave the pure dissociated species **6** in 62% yield without elimination of the other protecting groups. Finally, all of the acyl protecting groups were removed by treatment with aqueous ammonia to give the desired product **7** as the sole β isomer in 69% yield. The anomeric configuration of **12** was confirmed by the ^{13}C NMR chemical shift of 1-C (δ_{1-C} 97.8 ppm).[23] It is likely that this treatment induced the decomposition of **7**-α with the formation of ribofuranose 1,2-cyclic phosphate, so that no α isomer of **7** emerged.

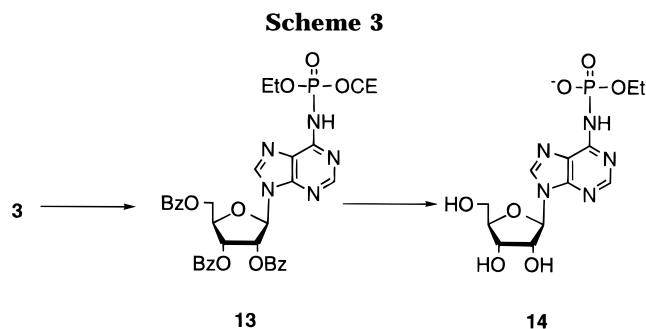
Next, the synthesis of **12**, which has the actual partial structure of Agrocin 84, was attempted by a series of

(16) (a) Wright, R. S.; Khorana, H. G. *J. Am. Chem. Soc.* **1955**, *77*, 3423. (b) Tener, G. M.; Wright, R. S.; Khorana, H. G. *J. Am. Chem. Soc.* **1956**, *78*, 506. (c) Aspinall, G. O.; Cottrell, I. W.; Matheson, N. K. *Can. J. Biochem.* **1972**, *50*, 574.
(17) Bunton, C. A.; Humeres, E. *J. Org. Chem.* **1969**, *34*, 572.
(18) Vinayak, R.; Hansske, F.; Robins, M. J. *J. Heterocycl. Chem.* **1993**, *30*, 1181.
(19) (a) Wada, T.; Moriguchi, T.; Sekine, M. *J. Am. Chem. Soc.* **1994**, *116*, 9901. (b) Hata, T.; Yamamoto, I.; Sekine, M. *Chem. Lett.* **1976**, 601–604.
(20) Nudelman, A.; Herzig, J.; Gottlieb, H. E. *Carbohydr. Res.* **1987**, *162*, 145.
(21) Caruthers, M. H. *J. Am. Chem. Soc.* **1986**, *108*, 2041.
(22) Wada, T.; Sekine, M. *Tetrahedron Lett.* **1994**, *35*, 757.
(23) Maryanoff, B. E.; Reitz, A. B.; Nortey, S. O. *Tetrahedron* **1988**, *44*, 3093.

Scheme 2



Scheme 3



reactions similar to those described above. A 1:1 anomeric mixture of 2,3,5,6-tetra-*O*-acetyl-D-glucofuranose (**8**) was synthesized in 78% yield by tributyltin methoxide-mediated deacylation of the fully acetylated derivative.[24] Compound **8** was allowed to react with 2-(trimethylsilyl)ethyl *N,N,N',N'*-tetraisopropylphosphorodiamidite to give 1-*O*-phosphoramidite **9** (Scheme 2). Further condensation of **3** with **9** gave a fully protected 6-*N*-[(glucofuranos-1-yloxy)phosphoryl]adenosine derivative **10**. A similar treatment of **10** with Bu_4NF monohydrate in THF gave **11**. The ^{31}P NMR spectrum of **11** showed two main signals derived from the anomeric isomers of glucofuranose at −7.40 and −6.68 ppm with the same peak intensity in $CDCl_3$. When 2-*O*-acetyl-3,5,6-tri-*O*-benzoyl-D-glucofuranose or 2,3,5,6-tetra-*O*-benzoyl-D-glucofuranose was used as the starting material at an early stage, the corresponding dissociated phosphoramidate could not be obtained in sufficiently pure form after deprotection of the phosphate protecting group, as evidenced by ^{31}P NMR. Treatment of **11** with NH_3–EtOH (3:1, v/v) at rt for 16 h gave the desired product **12** as the sole β isomer in an overall yield of 14% from **3**. The anomeric configuration of **12** was confirmed by ^1H NMR, which showed a $J_{1,2}$ value of nearly 0 Hz[25] and by the ^{13}C NMR chemical shift of 1-C (δ_{1-C} 106.4 ppm).[26]

Moreover, to study the effect of sugar substituents on the chemical properties of sugar–nucleoside conjugates, we also synthesized a simplified model of Agrocin 84, 6-*N*-(ethoxyphosphoryl)adenosine (**14**, 6-*N*-AMP-Et). Condensation of **3** with *N,N*-diisopropyl *O*-(2-cyanoethyl)-*O'*-ethylphosphoramidite gave compound **13** in 91% yield. Compound **14** was obtained in 38% yield by treatment of **13** with NH_3-dioxane (3:1, v/v) (Scheme 3).

UV Spectroscopy. The ultraviolet spectra of **7**, **12**, and **14** at three different pH regions are shown in Figure 2 and Table 1. The UV curves of the three compounds were very similar to one another in these three pH regions. The λ_{max} values of these compounds at pH 7 were between those of 6-*N*-AMP and 6-*N*-AMP-Et.[19a] The pH profiles of **7** were also similar to those of Agrocin 84 and 6-*N*-[[(2-cyanoethyl)oxy]phosphoryl]adenosine reported by Kerr.[2a] Particularly, the λ_{max} peak at pH 1.0 is shifted to a longer wavelength by about 7 nm compared to that at pH 7. These properties were not observed for any previously reported *N*-phosphorylated ribonucleoside.[19a] Compound **12** shows a similar behavior in the acidic pH region. The UV intensities of **7** and **12** at λ_{max} were much weaker at pH 1.0 than at pH 7.0 and 13. The UV spectra of 6-*N*-substituted adenosine derivatives in acidic, neutral, and alkaline solutions are quite different from those of **7**, **12**, and **14**. There was no difference in the UV profile between the 6-*N*-glycosyl substituents synthesized. These results suggest that compounds **7** and **12** have spectral properties similar to those of Agrocin 84 and are good candidates for analogs of Agrocin 84.

Stability of 6-*N*-Glycosyloxyphosphorylated Adenosine Derivatives. The stability of 6-*N*-glycosyloxyphosphorylated adenosine derivatives **7** and **12** was studied under various conditions. In general, compounds **7** and **12** were quite stable under neutral conditions, such as in 0.1 M ammonium acetate (pH 7.0). On the other hand, **7** and **12** gradually decomposed in 0.1 M HCl to give adenosine by cleavage of the P–N bonds without further decomposition. These results are summarized in Table 2.

It is well known that the P–N bond of phosphoramidate derivatives ROP(O)(NHR')(O^-) is very labile under acidic conditions.[19] The anomeric phosphate group of furanose 1-*O*-phosphate derivatives also exhibits extreme instability in acidic media.[27] Therefore, the present acid-catalyzed decomposition of **7** and **12** might occur via two pathways. The first mechanism occurs via direct cleavage of the P(O)–NH bond. The other involves predominant cleavage of the P–O bond of the anomeric phosphate

(24) Ferrier, R. J.; Haines, S. R. *J. Chem. Soc., Perkin Trans. 1* **1984**, 1675.

(25) Backinowsky, L. V.; Nepogod'ev, S. A.; Shashkov, A. S.; Kochetkov, N. K. *Carbohydr. Res.* **1985**, *138*, 41.

(26) Ritchie, R. G. S.; Cyr, N.; Korsch, B.; Koch, H. J.; Perlin, A. S. *Can. J. Chem.* **1975**, *53*, 1424.

(27) α-D-Ribofuranose 1-phosphate is hydrolyzed several hundred times faster than α-D-glucopyranose 1-phosphate under acidic conditions. See ref 17.

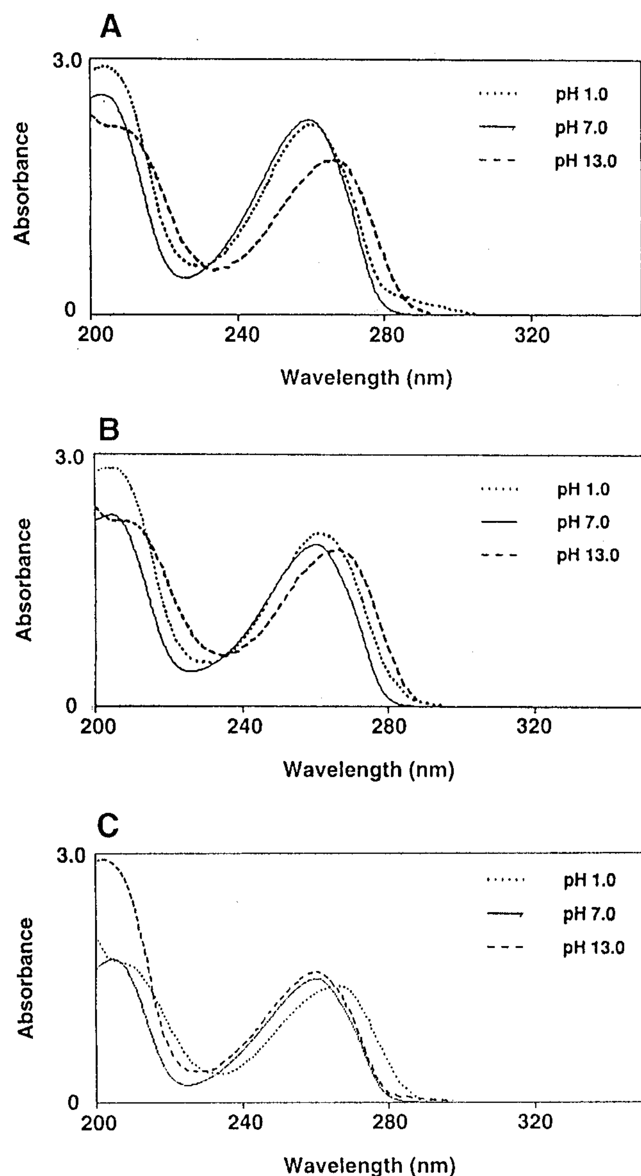


Figure 2. UV spectra of 6-*N*-glycosyloxyphosphorylated adenosine derivatives in three different pH regions: (A) compound **7**, (B) compound **12**, and (C) compound **14**.

Table 1. UV Spectral Data of *N*-Glycosyloxyphosphorylated Adenosine Derivatives

	pH	λ_{max} (nm)	$\epsilon_{max} \times 10^{-3}$	λ_{min} (nm)	$\epsilon_{min} \times 10^{-3}$
7	1.0	267.0	11.5	234.2	3.3
	7.0	259.4	14.5	225.4	3.8
	13.0	259.8	14.2	228.6	3.6
12	1.0	266.2	10.6	234.6	3.6
	7.0	260.8	11.1	225.6	2.4
	13.0	261.8	11.9	230.0	3.1
14	1.0	266.2	16.2	233.2	3.7
	7.0	260.6	16.9	225.2	2.3
	13.0	260.2	17.8	228.0	3.8

function followed by cleavage of the P–N bond of the resulting 6-*N*-AMP. Previously, we reported that the half-life of 6-*N*-AMP under these acidic conditions is 4 h. However, we did not observe any 6-*N*-AMP from the outset in these hydrolytic reactions. Therefore, we concluded that the above acidic hydrolysis of **7** and **12** proceeds mainly via the former mechanism.

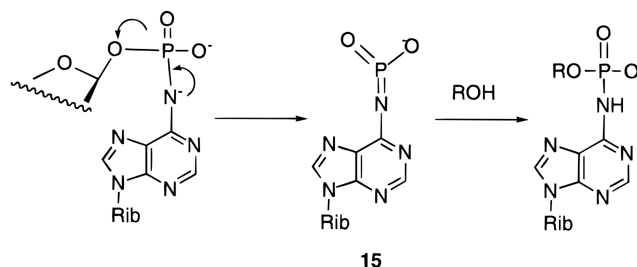
Compounds **7** and **12** also decomposed in 0.1 M NaOH. Interestingly, basic conditions gave 6-*N*-AMP via cleavage of P–O bonds but not of P–N bonds. This is a characteristic feature of **7** and **12**. This unexpected predominant P–O bond cleavage under basic conditions was also observed in related reactions: When **11** was treated with 1 M NaOH–EtOH (1:1, v/v) in an attempt to remove all of the acyl protecting groups, 6-*N*-(ethoxyphosphoryl)adenosine was obtained as the main product. On the other hand, treatment of **5a** with DBU in pyridine led to not only very rapid deprotection of the 2-cyanoethyl group within 10 min but also to decomposition of the resulting phosphoramidate **6** to give **3** with cleavage of the P–O bond. Strong bases such as DBU and hydroxide ion produce P–O bond cleavage probably via deprotonation of the P(O)–NH group, giving rise to an azamethaphosphate derivative **15** that can react with ethanol or water to give 6-*N*-(ethoxyphosphoryl)adenosine or 6-*N*-AMP, as depicted in Scheme 4.

Table 2. Stability of 6-*N*-Glycosyloxyphosphorylated Adenosine Derivatives[a]

	0.1 M HCl (25 °C)		0.1 M NH_4OAc (25 °C)		0.1 M NaOH (25 °C)		pH 5.0,[b] 90 °C		pH 7.0,[c] 90 °C	
	$t_{1/2}$ (h)	t_{comp} (h)	$t_{1/2}$ (h)	t_{comp} (h)	$t_{1/2}$ (h)	t_{comp} (h)	$t_{1/2}$ (h)	t_{comp} (h)	$t_{1/2}$ (h)	t_{comp} (h)
7	5	30	stable		2	12	2.2	13	6	35
12	7	40	stable			<10 min	1.5	8	2	15

[a] Compound **7** or **12** (1.0 A_{254}) was dissolved in a buffer. [b] 0.1 M NaH_2PO_4. [c] 0.1 M NH_4OAc.

Scheme 4



In the acidic hydrolysis of **7** and **12**, similar decomposition rates ($t_{1/2}$) of 5 and 7 h, respectively, were observed. However, there was an obvious difference in the rate of alkali-mediated hydrolysis between **7** and **12**. The time required for complete hydrolysis of **7** was 12 h. On the other hand, compound **12** showed extremely rapid decomposition within 10 min under the same conditions. This can be explained only in terms of the participation of the proximal 3-hydroxyl group, which can approach the 1-*O*-phosphoryl function with the β-configuration so that glucofuranose 1,3-(cyclic)phosphate might be formed with the release of adenosine.

Compounds **7** and **12** decomposed in 0.1 M ammonium acetate at 90 °C with cleavage of the P–N bonds to give adenosine, but depurination of adenosine derivatives was observed at the same time. Kerr *et al.* reported that the P–N bond of Agrocin 84 was completely decomposed by brief heat treatment at 100 °C for 15 min.[2b] However, the decomposition rates of compounds **7** and **12** were slower than that of Agrocin 84, as shown in Table 2. These results cannot be directly compared to those of Kerr *et al.*, since the details of the buffer and pH used for thermolysis were not given in their paper. Detailed NMR data for Agrocin 84 are unfortunately lacking since only a small amount of Agrocin 84 can be obtained from culture cells.[27] The absolute configuration of the anomeric carbon of Agrocin 84 will ultimately be determined by comparison of the NMR data of **12** with those of Agrocin 84, if a sufficient amount of Agrocin 84 is available.

Conclusions

We have succeeded in synthesizing for the first time the β-isomers of 6-*N*-glycosyloxyphosphorylated adenosine derivatives with an O(1″)–P–N(6) linkage from 1-OH sugar derivatives in several steps. The 6-*N*-[(1-*O*-glucofuranosyloxy)phosphoryl]adenine moiety in Agrocin 84 is known to be very important in controlling the strain-specific uptake of Agrocin 84 into target cells.[5] Studies directed toward the total synthesis of Agrocin 84 are currently underway.

Experimental Section

General Procedures. CH_2Cl_2 and MeCN were distilled from CaH_2 after being refluxed for several hours and stored over 4A molecular sieves. Pyridine was distilled after being refluxed over *p*-toluenesulfonyl chloride for several hours, redistilled from CaH_2, and stored over 4A molecular sieves. *tert*-Butyl hydroperoxide (containing 20% di-*tert*-butyl peroxide) was purchased from Merck & Co., Inc. ^{1}H NMR spectra were obtained at 270 MHz with tetramethylsilane as an internal standard in $CDCl_3$ and with sodium 4,4-dimethyl-4-silapentanesulfonate (DSS) as an external standard in D_2O. ^{13}C NMR spectra were obtained at 67.8 MHz with tetramethylsilane as an internal standard and with DSS as an external standard in D_2O. ^{31}P NMR spectra were obtained at 109.25 MHz using 85% H_3PO_4 as an external standard. Preparative HPLC was performed on a Shimadzu 6A system with a μBondapak column (Waters, C18–100 Å, 7.8 × 300 mm) using a linear gradient of 0–30% acetonitrile in 0.1 M NH_4OAc (pH 7.0) for 30 min at a flow rate of 3.0 mL/min at 50 °C. Thin-layer chromatography was performed on precoated glass plates of Kieselgel 60 F_{254} (Merck, No. 5715). Silica gel column chromatography was carried out using Wakogel C-200. Reversed-phase column chromatography was performed using μBondasphere C18 (Waters).

Triethylammonium Salt of 6-*N*-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranos-1-yloxy)phosphoryl)-2′,3′,5′-tri-*O*-benzoyladenosine (6). To a solution of 2,3,5-tri-*O*-acetyl-D-ribose (**1**) (635 mg, 2.30 mmol) in dry CH_2Cl_2 (20 mL) was added diisopropylammonium tetrazolide (197 mg, 1.15 mmol) and 2-(trimethylsilyl)ethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (332 mg, 3.45 mmol). After being stirred at rt for 1 h, the mixture was diluted with CH_2Cl_2. The CH_2Cl_2 was washed three times with 5% $NaHCO_3$(aq), and the aqueous layer was back-extracted with CH_2Cl_2. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was dissolved in dry MeCN (20 mL), and 2′,3′,5′-tri-*O*-benzoyladenosine (666 mg, 1.15 mmol) and 1*H*-tetrazole (322 mg, 4.60 mmol) were added to this solution. After being stirred at rt for 1 h, the mixture was treated with *tert*-butyl hydroperoxide (1.44 mL, 11.5 mmol) at rt for 30 min. The mixture was diluted with CH_2Cl_2, and the CH_2Cl_2 solution was washed once with water and twice with 5% $NaHCO_3$(aq). The aqueous layer was back-extracted with CH_2Cl_2. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was dissolved in dry THF (20 mL), and tetrabutylammonium fluoride monohydrate (1.20 g, 4.60 mmol) was added to this mixture. After being stirred at rt for 12 h, the solvent was evaporated under reduced pressure. The residue was diluted with CH_2Cl_2, and the CH_2Cl_2 solution was washed three times with 0.5 M triethylammonium hydrogen carbonate. The aqueous layer was back-extracted with CH_2Cl_2. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with CH_2Cl_2 containing 2% triethylamine, using a gradient of methanol (0–5%). Fractions that contained **6** were combined and concentrated to give **6** (662 mg, 62%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ −7.25; ^{1}H NMR ($CDCl_3$) δ 1.28, 1.97, 1.99, 2.07, 3.01, 3.67, 4.23, 4.70, 4.79, 4.87, 5.11, 5.17, 5.46, 5.88, 6.23, 6.36, 6.44, 7.33–7.61, 7.91–8.12, 7.97, 8.44; ^{13}C NMR ($CDCl_3$) δ 8.61, 20.7, 20.7, 21.0, 45.6, 62.0, 63.9, 65.6, 67.0, 68.7, 71.6, 73.9, 80.7, 86.6, 94.7, 128.5, 128.6, 128.8, 129.4, 129.8, 129.9, 133.4, 133.7, 139.4, 149.7, 153.3, 153.9, 165.1, 165.3, 166.2, 169.6, 169.7, 170.2. Anal. Calcd for $C_{48}H_{55}N_6O_{17}P\cdot3.5H_2O$: C, 53.28; H, 5.29; N, 7.77. Found: C, 53.31; H, 5.37; N, 7.63.

Triethylammonium Salt of 6-*N*-[(β-D-Ribofuranos-1-yloxy)phosphoryl]adenosine (7). Compound **6** (102 mg, 0.1 mmol) was treated with NH_3–pyridine (4:1, v/v, 20 mL) at rt for 8 h. The solution was evaporated under reduced pressure, and the residue was dissolved in water. After being washed five times with ether, the aqueous layer was concentrated to a small volume. The concentrate was applied to a column of Sephadex G-10 (300 × 15 mm) and eluted with water. Fractions that contained **7** were combined and lyophilized to give **7** (40 mg, 69%) as a white powder: ^{31}P NMR (D_2O) δ −5.54; ^{1}H NMR (D_2O) δ 1.31, 3.18, 3.62, 3.76, 3.82, 3.92–3.97, 4.05, 4.28, 4.43, 4.82, 5.57, 6.10, 8.42, 8.45; ^{13}C NMR (D_2O) δ 7.6, 53.2, 62.5, 65.5, 66.2, 69.4, 71.5, 71.6, 71.6, 74.7, 86.8, 89.4, 97.8, 122.0, 122.5, 142.5, 150.3, 152.3, 154.1; HRMS (FAB$^-$) 500.0800 [M − H]$^-$ calcd for $C_{15}H_{21}N_5NaO_{11}P$ 500.0795.

2,3,5,6-Tetra-*O*-acetyl-D-glucofuranose (8). To a solution of 1,2,3,5,6-penta-*O*-acetyl-D-glucofuranose (4.12 g, 10.6 mmol) in dry 1,2-dichloroethane (100 mL) was added tributyltin methoxide (4.56 mL, 15.9 mmol), and the mixture was refluxed for 1 h. The reaction mixture was cooled to rt and evaporated to a small volume. The concentrate was purified by silica gel column chromatography (hexane/EtOAc 7:3) to give 2.89 g (78% yield, $\alpha/\beta = 1:1$) of **8** as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 2.01, 2.02, 2.07, 2.09, 2.09, 2.13, 2.16, 3.67, 4.10–4.24, 4.41–4.51, 4.53–4.64, 5.00, 5.02, 5.16–5.23, 5.29–5.35, 5.42, 5.51, 5.66; ^{13}C NMR ($CDCl_3$) δ 20.5, 20.6, 20.7, 20.7, 63.0, 63.3, 68.0, 68.6, 73.4, 74.4, 74.8, 77.1, 78.3, 80.7, 95.6, 101.4, 169.4, 169.8, 170.8. Anal. Calcd for $C_{14}H_{20}O_{10}$: C, 48.28; H, 5.79. Found: C, 47.96; H, 5.84.

Sodium Salt of 6-*N*-[(β-D-Glucofuranos-1-yloxy)phosphoryl]adenosine (12). To a solution of 2,3,5,6-tetra-*O*-acetyl-D-glucofuranose (**8**) (255 mg, 0.73 mmol) in dry CH_2Cl_2 (7 mL) were added diisopropylethylamine (255 μL, 1.46 mmol) and *N,N*-diisopropylamino [2-(trimethylsilyl)ethyl]phosphorochloridite (312 mg, 1.10 mmol). After being stirred at rt for 30 min, the reaction mixture was diluted with $CHCl_3$. The solution was washed three times with 5% $NaHCO_3$(aq), and the aqueous layer was back-extracted with $CHCl_3$. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was dissolved in dry MeCN (4 mL), and to this solution were added 2′,3′,5′-tri-*O*-benzoyladenosine (**3**) (212 mg, 0.37 mmol) and 1*H*-tetrazole (51 mg, 0.73 mmol). After being stirred at rt for 1 h, the mixture was treated with *tert*-butyl hydroperoxide (229 μL, 1.83 mmol) at rt for 30 min. The mixture was diluted with $CHCl_3$ and washed once with water and twice with 5% $NaHCO_3$(aq), and the aqueous layer was back-extracted with $CHCl_3$. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was dissolved in dry THF (5 mL), and a mixture of tetrabutylammonium fluoride monohydrate (479 mg, 1.83 mmol) and acetic acid (105 μL, 1.83 mmol) in dry THF (5 mL) was added. After being stirred at rt for 12 h, the solvent was evaporated. The residue was diluted with $CHCl_3$ and washed three times with 0.5 M triethylammonium hydrogen carbonate, and the aqueous layer was back-extracted with $CHCl_3$. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was applied to a silica gel column, and elution was performed with CH_2Cl_2 containing 2% triethylamine, using a gradient of methanol (0–4%). Fractions that contained **11** were combined and concentrated to give crude **11** as a colorless foam. Crude **11** was treated with NH_3–pyridine (3:1, v/v, 20 mL) at rt for 16 h. The ammonia and pyridine were removed by evaporation, and the residue was dissolved in water. The aqueous solution was washed five times with ether and concentrated to a small volume. The residue was purified by preparative reversed-phase HPLC. Fractions that contained **12** were combined and

lyophilized. The residue was dissolved in a small volume of water and applied to a column of cation-exchange resin (Dowex 50Wx8, Na^+ form, 300 × 15 mm), and eluted with water. The eluate was lyophilized to give **12** (28 mg, 14%) as a white powder: ^{31}P NMR (D_2O) δ −6.40; ^{1}H NMR (D_2O) δ 3.09, 3.19, 3.44, 3.80, 3.89, 4.05, 4.15, 4.26, 4.27, 4.41, 4.79, 5.64, 6.08, 8.38, 8.41; ^{13}C NMR (D_2O) δ 64.1, 65.3, 71.4, 73.2, 76.3, 77.3, 82.9, 84.6, 88.3, 91.0, 106.4, 130.9, 131.4, 144.2, 151.9, 154.8, 155.7; HRMS (FAB$^-$) 530.0933 [M − H]$^-$ calcd for $C_{16}H_{23}N_5NaO_{12}P$ 530.0900.

6-*N*-[[(2-Cyanoethyl)oxy](ethyloxy)phosphoryl]-2′,3′,5′-tri-*O*-benzoyladenosine (13). 2′,3′,5′-Tri-*O*-benzoyladenosine (325 mg, 0.56 mmol) and 1*H*-tetrazole (79 mg, 1.12 mmol) were dried by repeated coevaporation with dry pyridine followed by dry MeCN and dissolved in dry MeCN (3 mL). To the mixture of 2′,3′,5′-tri-*O*-benzoyladenosine and 1*H*-tetrazole in MeCN was added 2-cyanoethyl ethyl *N*,*N*-diisopropylphosphoramidite (207 mg, 0.84 mmol) in MeCN (3 mL). After the mixture was stirred at rt for 1 h, *tert*-butyl hydroperoxide (351 μL, 2.80 mmol) was added, and the resulting mixture was stirred at rt for 15 min. The reaction mixture was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$(aq), and the aqueous layer was back-extracted with $CHCl_3$. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was applied to a silica gel column. Chromatography was performed with CH_2Cl_2, using a gradient of methanol (0−2%). Fractions that contained **13** were combined and concentrated to give **13** (379 mg, 91%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ −1.17; ^{1}H NMR ($CDCl_3$) δ 1.37, 1.38, 2.81, 4.27−4.35, 4.36−4.50, 4.69−4.93, 6.22−6.30, 6.37−6.46, 7.33−7.67, 7.91−8.23, 8.50. Anal. Calcd for $C_{36}H_{33}N_6O_6P \cdot 3/4H_2O$: C, 57.33; H, 4.61; N, 11.14. Found: C, 57.29; H, 4.24; N, 11.18.

Sodium Salt of 6-*N*-(Ethoxyphosphoryl)adenosine (14). Compound **13** (102 mg, 0.138 mmol) was treated with concentrated NH_3-dioxane (3:1, v/v, 20 mL) at rt for 12 h. Ammonia and dioxane were removed by evaporation, and the residue was dissolved in water and washed five times with ether. The aqueous layer was concentrated to a small volume and applied to a column of cation-exchange resin (Dowex 50Wx8, Na^+ form, 300 × 15 mm) and eluted with water. Fractions that contained **14** were combined and lyophilized to give **14** with slight impurity. Crude **14** was purified on a C18 reversed-phase column (40 × 25 mm) and eluted with water, using a gradient of MeCN (0−10%). Fractions that contained **14** were combined and lyophilized to give **14** (25 mg, 38%) as a white powder: ^{31}P NMR (D_2O) δ −3.25; ^{1}H NMR (D_2O) δ 1.20, 3.82, 3.90, 3.99, 4.27, 4.42, 4.78, 6.08, 8.37, 8.41; ^{13}C NMR (D_2O) δ 18.1, 64.0, 65.0, 73.2, 76.2, 88.3, 91.0, 123.6, 144.0, 151.8, 154.9, 155.8. Anal. Calcd for $C_{12}H_{17}N_5O_7PNa \cdot 1.2H_2O$: C, 34.41; H, 4.67; N, 16.72. Found: C, 34.40; H, 4.76; N, 16.51.

Acknowledgment. We thank Dr. M. E. Tate for his helpful suggestions regarding the structural analysis of Agrocin 84. This work was supported by a Grant-in Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, Sports and Culture, Japan.

Supporting Information Available: ^{1}H, ^{13}C, and ^{31}P NMR spectra and characterization data (16 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO961489A