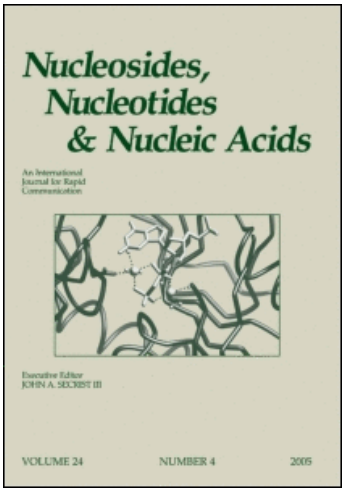

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Biological Properties of New Phosmidosine Analogs Having an *N*-Acylsulfamate Linkage

Haruhiko Taguchi[ab]; Akihiro Ohkubo[ab]; Mitsuo Sekine[ab]; Kohji Seio[bc]; Hideaki Kakeya[d]; Hiroyuki Osada[d]; Takuma Sasaki[e]
[a] Department of Life Science, Tokyo Institute of Technology, Nagatsuta, Midoriku, Yokohama, Japan [b] CREST, JST (Japan Science and Technology Agency), Yokohama, Japan [c] Frontier Collaborative Research Center, Tokyo Institute of Technology, Nagatsuta, Midoriku, Yokohama, Japan [d] Discovery Research Institute, RIKEN, Wako, Saitama, Japan [e] School of Pharmacy, Aichi Gakuin University, Nagoya, Japan

To cite this Article Taguchi, Haruhiko , Ohkubo, Akihiro , Sekine, Mitsuo , Seio, Kohji , Kakeya, Hideaki , Osada, Hiroyuki and Sasaki, Takuma(2006) 'Synthesis and Biological Properties of New Phosmidosine Analogs Having an *N*-Acylsulfamate Linkage', Nucleosides, Nucleotides and Nucleic Acids, 25: 4, 647 — 654
To link to this Article: DOI: 10.1080/15257770600686360
URL: http://dx.doi.org/10.1080/15257770600686360

Nucleosides, Nucleotides, and Nucleic Acids, 25:647–654, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770600686360

Taylor & Francis
Taylor & Francis Group

SYNTHESIS AND BIOLOGICAL PROPERTIES OF NEW PHOSMIDOSINE ANALOGS HAVING AN *N*-ACYLSULFAMATE LINKAGE

Haruhiko Taguchi, Akihiro Ohkubo, and Mitsuo Sekine □ *Department of Life Science, Tokyo Institute of Technology, Nagatsuta, Midoriku, Yokohama, Japan, and CREST, JST (Japan Science and Technology Agency), Yokohama, Japan*

Kohji Seio □ *Frontier Collaborative Research Center, Tokyo Institute of Technology, Nagatsuta, Midoriku, Yokohama 226-8503, Japan, and CREST, JST (Japan Science and Technology Agency), Yokohama, Japan*

Hideaki Kakeya and Hiroyuki Osada □ *Discovery Research Institute, RIKEN, Wako, Saitama, Japan*

Takuma Sasaki □ *School of Pharmacy, Aichi Gakuin University, Nagoya, Japan*

□ *A new phosmidosine analog* **10**, *in which the proline and 8-oxoadenosine moieties were linked by an* N*-acyl sulfamate linkage, was successfully synthesized by the sulfamoylation of an 8-oxoadenosine derivative* **5** *followed by coupling with an* L*-proline derivative* **8**. *An* L*-alanine-substituted derivative* **13** *and its derivative* **14** *without the alanyl residue were also synthesized. The morphological reversion activity of these synthetic compounds in v-src^{ts} NRK cells and their antitumor activity in L1210 and KB cells were studied. As the result, neither* L*-proline- nor* L*-alanine-substituted phosmidosine analogs* **10** *and* **13** *showed any antitumor activity. Contrary to these results, the derivative* **14** *lacking the amino acid residue showed potent antitumor activities against cancer cells.*

Keywords *N*-acyl sulfamate linkage; Phosmidosine analogs; Morphological reversion activity; Antitumor activity

Received 23 January 2006; accepted 21 February 2006.

This work was financially supported by CREST of JST (Japan Science and Technology Agency). This study was partially supported by Industrial Technology Research Grant Program in '05 from New Energy and Industrial Technology Development Organization (NEDO) of Japan. The authors would like to thank R. Onose for helpful assistance.

This article is dedicated to Professor Eiko Ohtsuka on the occasion of her 70th birthday.

Address correspondence to Haruhiko Taguchi, Department of Life Science, Tokyo Institute of Technology, Nagatsuta, Midoriku, Yokohama, 226-8501, Japan. E-mail: msekine@bio.titech.ac.jp

INTRODUCTION

A number of artificial aminoacyl adenylate derivatives having an *N*-acylsulfamate linkage have been synthesized and their biological properties have been studied.[1–4] Alanyl-, arginyl-, prolyl-, and asparaginyl adenylate analogs were synthesized. The chemical stability of the *N*-acylsulfamate linkage of aminoacyl adenylate derivatives is higher than that of the corresponding *N*-acylphosphoramidate linkage under physiological conditions. Therefore, a series of aminoacyl adenylate analogs containing *N*-acylsulfamate linkages have been used as aminoacyl-tRNA synthetase inhibitors.[1–3] Nucleocidin, which has an *N*-acylsulfamate linkage lacking the aminoacyl residue, is known to be highly toxic and to act as a highly potent inhibitor of protein synthesis.[4,5] Ascamycin, which was isolated from *Xanthomonas spp.* in 1984,[6] is a nucleoside derivative possessing an *N*-acylsulfamate linkage and a 2-chloroadenine residue as the nucleobase. Isono et al. reported the biological properties of ascamycin and its analogs substituted with other amino acids, showing that these compounds have highly potent antibacterial activities.[7] They also reported that a dealanylascamycin called AT-256, which was produced by Xc-aminopeptidase-promoted hydrolysis of ascamycin,[8] inhibited protein synthesis. Aminoacyl adenylate derivatives having an *N*-acylphosphoramide linkage have also been studied and their chemical and biological properties have been clarified. The P–N bond of the *N*-acylphosphoramide linkage is more stable than the corresponding P–O bond of an *O*-acylphosphoramide linkage and these modified nucleosides showed antitumor activities. A naturally occurring antibiotic, phosmidosine, has proved to possess potent antitumor activities against various human cancer cells.[9–11] McCloskey reported that phosmidosine was decomposed by treatment with 0.2 M NaOH to produce a proline moiety-lacking compound and rearranged compounds.[12] Recently, we have studied the synthesis of phosmidosine and its analogs.[13–17] In our continuous studies of the structure-activity relationship of a series of phosmidosine derivatives, we found that the 8-oxoadenine base and the proline moiety were essential for inhibition of the cancer cell growth.[15] These results prompted us to synthesize new phosmidosine analogs having an *N*-acylsulfamate linkage.

RESULTS AND DISCUSSION

A general procedure for construction of *N*-acylsulfamoyl linkage has been developed to obtain a series of aminoacyl adenylate derivatives containing an *N*-acylsulfamoyl linkage.[1–4] Therefore, we applied this method to the synthesis of our new phosmidosine analogs.

Thus, the *O*-selective reaction of an appropriately protected 8-oxoadenosine derivative **5** with sulfamoyl chloride,[2,18] which was prepared from chlorosulfonyl isocyanate and formic acid, was studied. As the result,

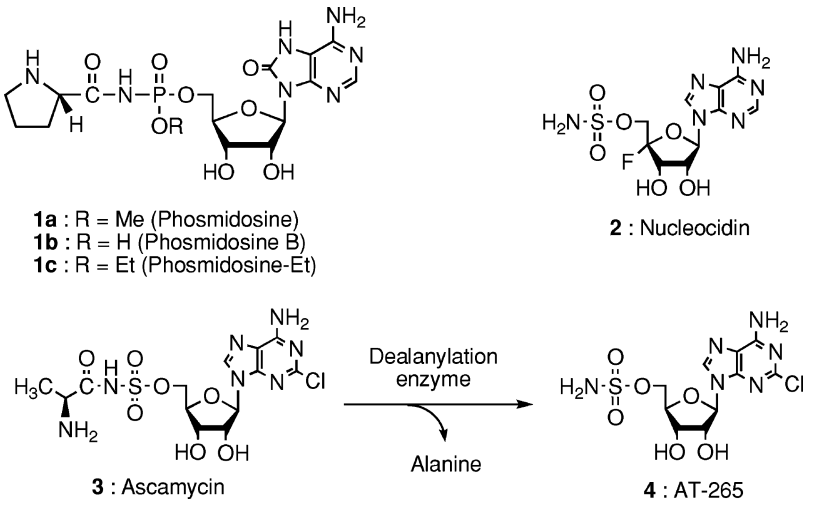

FIGURE 1 Structure of several aminoacyl adenylate derivatives.

the desired sulfamoylation proceeded to afford the 5′-*O*-sulfamoyl-8-oxoadenosine derivative **6**. In this reaction, no reactions occurred on the 7-position or the 6-amino group of the 8-oxoadenine moiety. In an attempt to obtain an *N*-acyl sufamate derivative **9**, an L-proline derivative was activated by treatment with *N*, *N*′-carbonyldiimidazole and the resulting acylimidazole derivative **7** was allowed to react with **6**. However, no prolylated compounds were obtained. In contrast to this result, when the *O*-succsinyl-L-proline derivative **8**[2] was used, the reaction gave the desired product **9** in a moderate yield. The yield of **9** was increased to 80% by the choice of

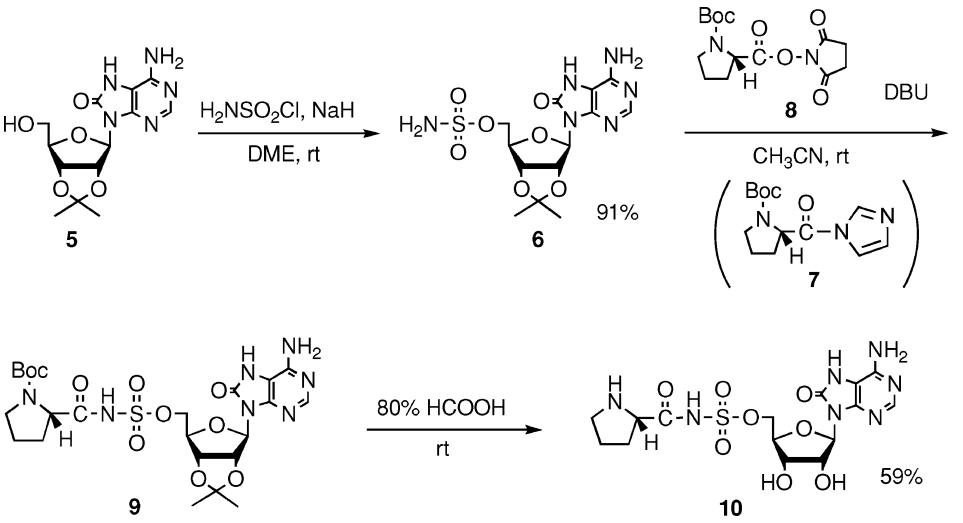

FIGURE 2 Synthesis of phosmidosine analogs.

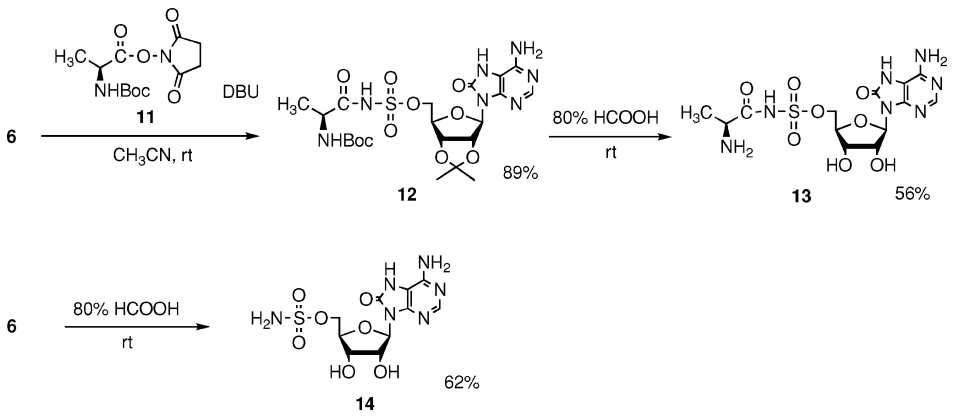

FIGURE 3 Synthesis of ascamycin mimics.

acetonitrile as the solvent. The product was treated with 80% formic acid for 12 h to give the product **10** as an amorphous white solid in 59% yield.

In a similar manner, we also synthesized an ascamycin mimic **13**, which was obtained by the reaction of **6** with the ester **11** followed by acidic treatment of the product **12**. The compound **14**, which does not have the alanyl residue, was synthesized by treatment of **6** with 80% HCOOH.

The morphological reversion activity of these synthetic compounds in v-src^{ts}NRK cells and their antitumor acttivity in L1210 and KB cells were studied. These results are shown in Table 1.

TABLE 1 The Biological Properties of Compounds **1c**, **10**, **13**, and **14**

	Morphological reversion activity (μg/ml)						
Aminoacyl nuclesides	100	30	10	3	1	0.3	0.1
Morphological reversion activity of phosmidosine analogs in v-src^{ts} NRK cells							
L-Pro-Sulfamoyl-8-oxoA : **10**	−	−	−	−	−	−	−
H-Sulfamoyl-8-oxoA : **14**	+++	+++	+++	+++	+	−	−
Phosmidosine-Et : **1C**	nt	nt	+++	+++	+	nt	nt

	L1210 μM	KB μM
IC_{50} Values of phosmidosine analogs in L1210 and KB cells		
L-Pro-Sulfamoyl-8-oxoA : **10**	218<	218<
L-Ala-Sulfamoyl-8-oxoA : **13**	231<	231<
H-Sulfamoyl-8-oxoA : **14**	0.91	4.86
Phosmidosine-Et : **1C**	3.62	3.44

$^{+++}$:More than 75% of cancer cells were morphologically reversed.
$^{++}$:25–75% of cancer cells were morphologically reversed.
$^{+}$:ca. 25% oc cancer cells were morphologically reversed.
$^{-}$:no activity; nt: not tested.

As shown in Table 1, the phosmidosine ethyl ester **1c** was used as a control sample. In the morphological reversion activity assay, the L-proline-substituted phosmidosine analog **10** did not show any morphological reversion activities. However, compound **14** showed morphological reversion activity at a low concentration. Even when the concentration was 3 μg/ml, morphological reversion activity was significantly observed. Next, we studied the antitumor activity of these compounds against L1210 and KB cells. The results were similar to those obtained in the case of the morphological reversion activity assay. Only compound **14** inhibited the growth of L1210 and KB cells. The structure of compound **14** is similar to those of nucleocidin **2** and AT-265 **4**.

In conclusion, we have successfully synthesized a new phosmidosine analog having an *N*-acylsulfamoyl linkage. An L-alanine-substituted derivative **10** and its derivative **14** were also synthesized in a similar manner. The biological properties of these new compounds were studied and it was found that 5′-*O*-sulfamoyl-8-oxo-adenosine **14** showed potent activity against human cancer cells. Further studies on the mode of action of these compounds are in progress.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were obtained at 270 and 68 MHz, respectively. The chemical shifts were measured from DMSO-d_6 (2.49 ppm) and 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP-d_4) (0 ppm) for ^{1}H NMR and from DMSO-d_6 (39.7 ppm) for ^{13}C NMR. Column chromatography was performed with silica gel C-200. Reverse-phase column chromatography was performed by use of 37-55 μm C_{18} particle. Mass spectra were measured by use of an ESI-mass spectrophotometer. *In vitro* analysis of the antitumor activity in cancer cell lines was carried out by the literature method reported by Carmichael[19] and us.[15] The morphological reversion activity test was conducted according to the literature method.[10] Compound **5** was synthesized according to our previous paper.[15]

*2′,3′-O-Isopropylidene-5′-O-sulfamoyl-8-oxoadenosine (***6***).* Under argon atmosphere, compound **5** (1.51 g, 5 mmol) was coevaporated three times with dry pyridine and dissolved in dry DME (25 ml). To this DME solution was added sodium hydrate (60%, 480 mg, 12 mmol), and the mixture was stirred at 0°C for 30 min. A DME (25 ml) solution of sulfamoyl chloride (1.16 g, 10 mmol) was added to the mixture. After being stirred at room temperature for 10 h, the mixture was quenched by addition of 20 ml of methanol and evaporated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$:methanol = 95:5, v/v) to give compound **6** (1.79 g, 89%): ^{1}H NMR (DMSO-d_6) δ 1.31 (3H, s), 1.51 (3H, s), 4.05–4.32 (3H, m), 4.98 (1H, d, $J_{2',3'} = 6.3$ Hz), 5.39 (1H, d, $J_{2',3'} = 6.3$ Hz), 5.93 (1H, s), 7.15 (2H, bs), 7.54 (2H, bs), 8.17 (1H, s), 10.94 (1H, bs); ^{13}C NMR

(DMSO-d_6)δ 25.1, 26.9, 68.3, 79.2, 81.6, 82.4, 84.3, 86.2, 103.5, 113.1, 146.0, 147.2, 151.0, 151.1. ESI-mass m/z cald for $C_{13}H_{19}N_6O_7S$ 403.1036; observed [M + H] 403.1042.

*2′,3′-O-Isopropylidene-5′-O-[N-(boc-L-prolyl)sulfamoyl]-8-oxoadenosine (***9***).* Under argon atmosphere, compound **6** (805 mg, 2 mmol) was coevaporated three times with dry pyridine, and *N*-Boc-L-proline *N*-hydroxysuccinimide ester (750 mg, 2.4 mmol) was added. The mixture was dissolved in dry acetonitrile (20 ml). To this solution, DBU (0.72 ml, 4.8 mmol) was slowly added. After being stirred at room temperature for 4 h, the mixture was diluted by addition of methanol (10 ml) and evaporated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$:methanol = 95:5, v/v) to give compound **9** (983 mg, 82%): ^{1}H NMR (DMSO-d_6) δ 1.25 (9H, s), 1.29 (3H, s), 1.34 (3H, s), 1.60–2.05 (3H, m), 3.07–3.52 (4H, m), 3.79–3.90 (2H, m), 4.00–4.10 (3H, m), 4.14–4.23 (2H, m), 4.87–4.93 (1H, m), 5.41 (1H, m), 5.86 (1H, s$J_{1',2'}$ = 5.6 Hz), 6.95 (2H, bs), 7.99 (1H, s), 11.26 (1H, bs). ESI-mass m/z calcd for $C_{23}H_{34}N_7O_{10}S$ 600.2088; observed [M + H] 600.2080.

*5′-O-[N-(L-prolyl)sulfamoyl]-8-oxoadenosine (***10***).* Compound **9** (599 mg, 1 mmol) was dissolved in 80% formic acid (10 ml). After being stirred at room temperature for 12 h, the mixture was diluted by addition of water and extracted with ethyl acetate. The aqueous layer was collected in a flask and evaporated under reduced pressure. The residue was purified by reverse-phase column chromatography (H_2O:methanol = 100:0–97:3, v/v) to give compound **10** as an amorphous white solid (271 mg, 59%): ^{1}H NMR (D_2O) δ 1.98–2.15 (4H, m), 2.40–2.55 (1H, m), 3.34–3.53 (2H, m), 4.31 (1H, m), 4.37–4.52 (2H, m), 4.83–4.90 (1H, m), 5.01–5.06 (1H, m), 6.09 (1H, s), 8.45 (1H, s). ESI-mass m/z calcd for $C_{15}H_{22}N_7O_8S$ 460.1251; observed [M + H] 460.1245.

*2′,3′-O-Isopropylidene-5′-O-[N-(boc-L-alanyl)sulfamoyl]-8-oxoadenosine (***12***).* A reaction similar to that described for the synthesis of **9** by use of **6** (805 mg, 2 mmol) and *N*-Boc-L-alanine *N*-hydroxysuccinimide ester **11** (687 mg, 2.4 mmol) gave compound **12** (1.02 g, 89%): ^{1}H NMR (DMSO-d_6) δ 1.10 (9H, s), 1.16 (3H, d, J = 5.9 Hz), 1.28 (3H, s), 1.38 (3H, s), 3.81–3.89 (2H, m), 4.00–4.11 (3H, m), 4.16–4.21 (1H, m), 4.85–5.01 (2H, m), 5.90 (1H, s), 7.00 (2H, bs), 7.86 (1H, s), 11.23 (1H, bs). ESI-mass m/z calcd for $C_{21}H_{32}N_7O_{10}S$ 574.1931; observed [M + H] 574.1944.

*5′-O-[N-(L-alanyl)sulfamoyl]-8-oxoadenosine (***13***).* A reaction similar to that described for the synthesis of **10** by use of **12** (217 mg, 0.5 mmol) gave compound **13** (134 mg, 62%): ^{1}H NMR (D_2O) δ 1.02 (3H, d, J = 5.9 Hz), 3.79–3.86 (1H, m), 4.17–4.22 (1H, m), 4.37–4.43 (1H, m), 4.50–4.60 (1H, m), 4.75–4.90 (2H, m), 5.98 (1H, s), 8.33 (1H, s); ESI-mass m/z calcd for $C_{13}H_{20}N_7O_8S$ 434.1094; observed [M + H] 434.1082.

*5′-O-Sulfamoyl-8-oxoadenosine (***14***).* Compound **6** (599 mg, 1 mmol) was dissolved in 80% formic acid (10 ml). After being stirred at room temperature for 12 h, the mixture was diluted by addition of water and extracted with ethyl acetate. The aqueous layer was collected in a flask and evaporated under reduced pressure. The residue was purified by reverse-phase column chromatography (H_2O:methanol = 100: 0–97:3, v/v) to give compound **14** as an amorphous white solid (271 mg, 62%): ^1H NMR (DMSO-d_6) δ 3.98–4.11 (2H, m), 4.22–4.37 (2H, m), 4.80–4.89 (1H, m), 5.28 (1H, d, $J_{2',3'} = 5.1$ Hz), 5.40 (1H, d, $J_{2',3'} = 5.1$ Hz), 5.69 (1H, d, $J_{1',2'} = 4.6$ Hz), 6.59 (2H, bs), 7.50 (2H, bs), 8.02 (1H, s), 10.50 (1H, bs). ESI-mass m/z calcd for $C_{10}H_{15}N_6O_7S$ 363.0723; observed [M + H] 363.0715.

REFERENCES

1. Koroniak, L.; Ciustea, M.; Gutierrez, J.A.; Richards, G.J. Synthesis and characterization of an *N*-acylsulfonamide inhibitor of human asparagine synthetase. Organic Letters **2003**, 5, 2033.
2. Heacock, D.; Forsyth, C.J.; Shiba, K.; Musier-Forsyth, K. Synthesis and aminoacyl-tRNA synthetase inhibitory activity of prolyl adenylate analogs. Bioorganic & Medicinal Chemistry **1996**, 24, 273.
3. Yu, X. Y.; Hill, J. M.; Yu, G.; Wang, W.; Kluge, A. F.; Wendler, P.; Gallant, P. Synthesis and structure-activity relationships of a series of novel thiazoles as inhibitors of aminoacyl-tRNA synthetases. Bioorganic & Medicinal Chemistry Letters **1999**, 9, 375.
4. Florini, J.R.; Bird, H.H.; Bell, P.H. Inhibition of protein synthesis *in vitro* and *in vivo* by nucleocidin, an antitrypanosomal antibiotic. Journal of Biological Chemistry **1966**, 241, 1091.
5. Jenkins, I.D.; Verheyden, J.P.H.; Moffatt, J.G. 4′-substituted nucleosides 2. Synthesis of the nucleoside antibiotic nucleocidin. Journal of the American Chemical Society **1976**, 98, 3346.
6. Isono, K.; Uramoto, M.; Kusakabe, N.; Miyata, T.; Koyama, M.; Ubukata, M.; Sethi, K.; McCloskey, J.M. Ascamycin and dealanylascamycin, nucleoside antibiotics from *Streptomyces* sp. Journal of Antibiotics **1984**, 37, 670.
7. Ubukata, M.; Osada, H.; Magae, J.; Isono, K. Synthesis Biological activity of aminoacyl analogs of ascamycin. Agricultural & Biological Chemistry **1988**, 52, 1117.
8. Sudo, T.; Shinohara, K.; Dohmae, N.; Takio, K.; Usami, R.; Horikoshi, K.; Osada, H. Isolation and characterization of the gene encoding an aminopeptidase involved in the selective toxicity of ascamyscin toward *Xanthomonas campestris pv. Citri.* Biochemical Journal **1996**, 319, 99.
9. Uramoto, M.; Kim, C.J.; Shin-ya, K.; Kusakabe, H.; Isono, K.; Philips, D.R.; McCloskey, J.A. Isolation and characterization of phosmidosine, a new antifungal nucleotide antibiotic. Journal of Antibiotics **1991**, 44, 375.
10. Matsuura, N.; Onose, R.; Osada, H. Morphology reversion activity of phosmidosine and phosmidosine B, a newly isolated derivative, on src transformed NRK cells. Journal of Antibiotics **1996**, 49, 361.
11. Kakeya, H.; Onose, R.; Liu, P.C.C.; Onozawa, C.; Matsumura, F.; Osada, H. Inhibition of cyclin D1 expression and phosphorylation of retinoblastoma protein by phosmidosine, a nucleotide antibiotic. Cancer Research **1998**, 58, 704.
12. Phillips, D.R.; Uramoto, M.; Isono, K.; McCloskey, J.A. Structure of the antifungal nucleotide antibiotic phosmidosine. Journal of Organic Chemistry **1993**, 58, 854.
13. Moriguchi, T.; Asai, N.; Wada, T.; Seio, K.; Sasaki, T.; Sekine, M. Synthesis and antitumor activities of phosmidosine A and its *N*-acetylated derivative. Tetrahedron Letters **2000**, 41, 5881.
14. Moriguchi, T.; Asai, N.; Okada, K.; Seio, K.; Sasaki, T.; Sekine, M. First synthesis and anticancer activity of phosmidosine and its related compounds. Journal of Organic Chemistry **2002**, 67, 3290.
15. Sekine, M.; Okada, K.; Seio, K.; Kakeya, H.; Osada, H.; Obata, T.; Sasaki, T. Synthesis of chemically stabilized phosmidosine analogues and the structure-activity relationship of phosmidosine. Journal of Organic Chemistry **2003**, 69, 314.

16. Sekine, M.; Okada, K.; Seio, K.; Kakeya, H.; Osada, H.; Sasaki, T. Structure-activity relationship of phosmidosine: Importance of the 7, 8-dihydro-8-oxoadenosine residue for antitumor activity. Bioorganic & Medicinal Chemistry **2004**, 12, 5193.
17. Sekine, M.; Okada, K.; Seio, K.; Sasaki, T.; Kakeya, H.; Osada, H. Synthesis of a biotin-conjugate of phosmidosine *O*-ethyl ester as a G1 arrest antitumor drug. Bioorganic & Medicinal Chemistry **2004** 12, 6343.
18. Appel, R.; Berger, G. Hydrazinesulfonic acid amide. I. hydrazodisulfamide. Chemische Berichte **1958**, 91, 1339.
19. Carmichael, D.W.G.; Gazar, A.F.; Minna, J.D.; Mitchell, J.B. Evaluation of a tetrazolium-based semi-automated colorimetric assay: Assessment of chemosensitivity testing. Cancer Research **1987**, 47, 936.