# *N*-Long-chain Monoacylated Derivatives of 2,6-Diaminopyridine with Antiviral Activity

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***N*-Monoacyl-2,6-diaminopyridines (2a—c) and *N*,*N*′-diacyl-2,6-diaminopyridines (3a—c) were synthesized from 2,6-diaminopyridine by acylation with the corresponding acyl halide or by dehydration with the corresponding carboxylic acid using 1,3-dicyclohexylcarbodiimide (DCC). The antiviral activities of *N*-monoacyl- and *N*,*N*′-diacyl-2,6-diaminopyridines (2a—c and 3a—c) were estimated using plaque reduction assay with HSV-1. All *N*-monoacyl derivatives (2a—c) showed significant anti-herpes simplex virus (HSV)-1 activity ($EC_{50}$=15.3—18.5 $\mu$g/ml). The $CC_{50}$ values of 2a—c measured using Vero cells ranged at 37.5—50.0 $\mu$g/ml. These compounds showed no significant antibacterial activities with *Escherichia coli* or *Staphylococcus aureus* even at a concentration of 1 mg/ml. The *N*,*N*′-diacyl derivatives (3a—c) showed no significant anti-HSV-1 activity.**

**Key words** 2,6-diaminopyridine; acylation; antiviral activity; anti-herpes simplex virus (HSV)-1; plaque reduction assay; antibacterial activity

The structure of 2,6-diaminopyridine (DAP) (**1**) has an unique symmetrical feature. It possesses three nitrogen lone pairs that display the remarkable characteristics of a Lewis base. This compound's *N*-acyl derivatives have also attracted considerable attention, in particular, due to their hydrogen-bonding motifs[1] and macrocyclic synthetic receptors.[2,3]

Derivatization of oligosaccharides (sugar chains) is an important research tool for studying the biochemistry of glycoconjugates.[4] A typical example of such derivatization is biotinylated 2,6-diaminopyridine,[5] which can be used to tag oligosaccharides. It is well known that in early-stage viral fusion some oligosaccharide "spikes" are essential to the invasion of a virus into a host cell.[6]

In connection with our search for antiviral compounds,[7,8] we synthesized a new type of compounds, *N*-monoacylated 2,6-diaminopyridines (**2**) and *N*,*N*′-diacylated 2,6-diaminopyridines (**3**) (Chart 1). These functional groups play an important role in molecular recognition through hydrogen bonding[1—3] and in fluorogenic oligosaccharide derivatization *via* the formation of a Schiff base including the 2-aminopyridyl moiety.[5] Furthermore, the introduction of long-chain acyl groups may enhance the affinity of these molecules to bind to the biological membranes made up of lipid bilayers. The primary objective of this study was to develop a new antiviral lead that operates by molecular recognition or modification of oligosaccharides within the glycoprotein "spikes" of the viral envelope.

In this paper, we report the synthesis and properties of long-chain *N*-monoacylated 2,6-diaminopyridines and *N*,*N*′-diacylated 2,6-diaminopyridines, together with the results of our examination of their antiviral [anti-herpes simplex virus (HSV)-1] activity.

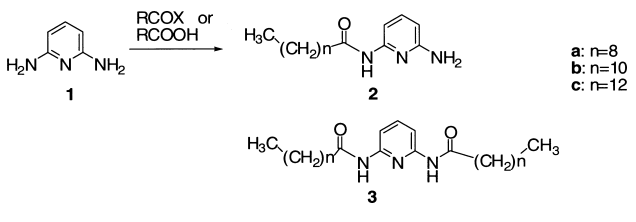


Chart 1

## Results and Discussion

**Synthesis of *N*-Monoacylated Diaminopyridines (2a—c) and *N*,*N*′-Diacylated Diaminopyridine Derivatives (3a—c)** The target compounds, *N*-monoacylated diaminopyridines (**2a**—**c**), are easily prepared by one-step acylation of commercially available 2,6-diaminopyridine (**1**) with the corresponding long-chain acyl halide, or by dehydration condensation of 2,6-diaminopyridine (**1**) with a long-chain carboxylic acid using 1,3-dicyclohexylcarbodiimide (DCC). The reaction of an equimolar amount of 2,6-diaminopyridine and an acyl halide without triethylamine (TEA) in tetrahydrofuran (THF) resulted in the predominant formation of *N*,*N*′-diacylated compounds (**3**), although a small amount of *N*-monoacylated compounds **2** was detected by TLC (see Experimental; **3a** in Method C). However, the procedure with TEA described in ref. 1 for *N*-monoacylation of 2,6-diaminopyridine was also efficient for the preparation of compounds (**2**) (see Experimental; **2a** in Method A). These compounds **2** and **3** gave the corresponding hydrochlorides on treatment with 10% HCl in ethanol (see Experimental).

The structures of the target compounds were confirmed by spectroscopic methods and elemental analysis (Tables 1—5). An interesting point of these materials **3a**—**c** is their behavior on NMR spectroscopy in solution (see Tables 3, 5). The data indicate a symmetrical molecular structure. Thus the $^{13}$C-resonances of the two acyl chains completely overlap. $^{1}$H-NMR also indicated the same symmetrical structure.

**Evaluation of Antiviral Activities** The biological properties of *N*-monoacylated 2,6-diaminopyridines (**2a**—**c**) (with 50% effective concentration, $EC_{50}$, values of anti-HSV-1 activity) are listed in Table 6. As expected, the *N*-monoacylated compounds **2a**—**c** showed significant anti-HSV-1 activity ($EC_{50}$=15.3—18.5 $\mu$g/ml). Compounds **2a**—**c** listed in Table 6 were weakly active against HSV-1 compared with aciclovir ($ED_{50}$=0.2—0.9 $\mu$g/ml).[9,10] However, this is the first observation of antiviral activity for this class of com-

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Table 1. Yields and Physical Data of *N*-Monoacylated and *N*,*N*′-Diacylated 2,6-Diaminopyridines (**2**, **3**) and their Hydrochlorides (**2a**—**c**·HCl, **3a**—**c**·HCl)

| Compd. | Yield (%) (Method)[a] | mp (°C) (Recryst solvent) | Formula | Analysis (%) Calcd (Found) | | | Formula HR-MS *m*/*z* Calcd (Found) | IR ($cm^{-1}$) (KBr) |
|---|---|---|---|---|---|---|---|---|
| | | | | C | H | N | | |
| **2a** | 71 (A) | 50—51[b] (*n*-Pentane) | $C_{15}H_{25}N_3O$ | 68.40 (68.49) | 9.57 9.59 | 15.95 15.98) | $C_{15}H_{26}N_3O$ $(M+H)^+$ 264.2076 (264.2077) | 3275 (NH) 1670 (C=O) |
| **2b** | 67 (A) | 67.5—68.5 (*n*-Heptane) | $C_{17}H_{29}N_3O$ | 70.06 (70.34 | 10.03 10.23 | 14.42 14.25) | $C_{17}H_{30}N_3O$ $(M+H)^+$ 292.2389 (292.2387) | 3275 (NH) 1670 (C=O) |
| **2c** | 47 (B) | 68—71 ($CH_2Cl_2$) | $C_{19}H_{33}N_3O$ | 71.43 (71.51 | 10.41 10.39 | 13.15 12.94) | $C_{19}H_{34}N_3O$ $(M+H)^+$ 320.2702 (320.2701) | 3335 (NH) 1670 (C=O) |
| **2a**·HCl | Quant. | 117 (EtOH) | $C_{15}H_{25}N_3O$ ·HCl·0.4$H_2O$ | 58.68 (58.64 | 8.80 8.52 | 13.69 13.70) | $C_{15}H_{26}N_3O$ $(M+H)^+$ 264.2076 (264.2076) | 3100 br (NH) 1665 (C=O) 1615 br (NH) |
| **2b**·HCl | Quant. | 80—84 ($CH_3CN$) | $C_{17}H_{29}N_3O$ ·HCl·0.3$H_2O$ | 61.26 (61.26 | 9.25 9.48 | 12.61 12.54) | $C_{17}H_{30}N_3O$ $(M+H)^+$ 292.2389 (292.2388) | 3100 br (NH) 1655 (C=O) 1615 br (NH) |
| **2c**·HCl | Quant. | 107—110 (EtOH–$H_2O$) | $C_{19}H_{33}N_3O$ ·HCl·0.8$H_2O$ | 61.62 (61.61 | 9.69 9.41 | 11.35 11.07) | $C_{19}H_{34}N_3O$ $(M+H)^+$ 320.2702 (320.2701) | 3100 br (NH) 1655 (C=O) 1615 (NH) |
| **3a** | 83 (C) | 104—105 (EtOH) | $C_{25}H_{43}N_3O_2$ | 71.9 (71.84 | 10.38 10.52 | 10.06 9.96) | $C_{25}H_{44}N_3O_2$ $(M+H)^+$ 418.3434 (418.3434) | 3400, 3320 (NH) 1705, 1670 (C=O) 1595 (C=C) |
| **3b** | 77 (A′) | 102—103.5[c] (EtOH) | $C_{29}H_{51}N_3O_2$ | 73.52 (73.41 | 10.85 10.90 | 8.87 8.84) | $C_{29}H_{52}N_3O_2$ $(M+H)^+$ 474.4060 (474.4054) | 3395, 3320 (NH) 1705, 1670 (C=O) 1595 (C=C) |
| **3c** | 55 (A′) | 112—112.5 ($CH_2Cl_2$–EtOH) | $C_{33}H_{59}N_3O_2$ | 74.81 (74.66 | 11.22 11.40 | 7.93 7.86) | $C_{33}H_{60}N_3O_2$ $(M+H)^+$ 530.4686 (530.4691) | 3395, 3320 (NH) 1705, 1670 br (C=O) 1600 (C=C) |
| **3a**·HCl | 45% | 125—132 ($CH_3CN$) | $C_{25}H_{43}N_3O_2$ ·HCl | 66.13 (66.37 | 9.77 9.86 | 9.25 9.30) | $C_{25}H_{44}N_3O_2$ $(M+H)^+$ 418.3434 (418.3434) | 3400 br (NH) 1705, 1650 (C=O) 1570 (C=C) |
| **3b**·HCl | 44% | 138—143 ($CH_3CN$) | $C_{29}H_{51}N_3O_2$ ·HCl·0.5$H_2O$ | 67.09 (67.12 | 10.29 10.40 | 8.09 8.30) | $C_{29}H_{52}N_3O_2$ $(M+H)^+$ 474.4060 (474.4054) | 3430 br (NH) 1700, 1650 (C=O) 1570 (C=C) |
| **3c**·HCl | 47% | 127—136 (MeOH) | $C_{33}H_{59}N_3O_2$ ·HCl | 69.99 (70.12 | 10.68 10.81 | 7.42 7.13) | $C_{33}H_{60}N_3O_2$ $(M+H)^+$ 530.4686 (530.4687) | 3400 br (NH) 1705, 1650 (C=O) 1570 (C=C) |

*a*) Method A: DAP : RCOCl : TEA = 1 : 1 : 1, 0 °C THF. Method A′: DAP : RCOCl : TEA = 1 : 2 : 2, rt THF. Method B: DAP : RCOOH : DCC : HOBT = 1 : 0.67 : 1 : 0.67, rt $CH_2Cl_2$–DMF. Method C: DAP : RCOCl = 1 : 1, rt THF. *b*) Melting point of 54.3—55.7 °C has been reported.[11] *c*) Melting point of 110—111 °C ($CHCl_3$–hexane) has been reported.[15]

Table 2. $^{13}$C-NMR Spectral Data of *N*-Monoacylated 2,6-Diaminopyridines (**2a**—**c**) and their Hydrochlorides (**2a**—**c**·HCl)[a]

| C No. | | **2a** | **2b**[c] | **2c** | **2a**·HCl | **2b**·HCl | **2c**·HCl |
|---|---|---|---|---|---|---|---|
| Pyridine ring | C-2 | 149.24 | 149.59 | 149.1 | 144.08 | 144.07 | 144.07 |
| | C-3 | 103.16 | 103.27 | 103.16 | 98.66 | 98.65 | 98.66 |
| | C-4 | 140.71 | 140.50 | 140.89 | 144.74 | 144.72 | 144.71 |
| | C-5 | 104.19 | 104.14 | 104.19 | 105.37 | 105.38 | 105.39 |
| | C-6 | 156.48 | 156.73 | 156.33 | 152.77 | 152.78 | 152.78 |
| –NHCO– | | 171.64 | 171.67 | 171.63 | 174.68 | 174.66 | 174.66 |
| –CO–CH$_2$– | C2′ | 37.86 | 37.83 | 37.90 | 35.86 | 35.86 | 35.86 |
| –(CH$_2$)$_n$– | | 31.83 (C8′), 29.18, 29.22, 29.31, 29.38 (C4′—7′), 25.36 (C3′), 22.62 (C9′) | 31.87 (C10′), 29.57,[b] 29.43, 29.32, 29.29, 29.19 (C4′—9′), 25.39 (C3′), 22.64 (C11′) | 31.91 (C12′), 29.65, 29.62,[b] 29.59, 29.44, 29.32,[b] 29.19 (C4′—11′), 25.36 (C3′), 22.66 (C13′) | 31.14 (C8′), 28.73, 28.63, 28.53 (C5—7′), 28.33 (C4′), 24.27 (C3′), 21.96 (C9′) | 31.16 (C10′), 28.87, 28.85, 28.76, 28.61, 28.57 (C5′—9′), 28.31 (C4′), 24.27 (C3′), 21.95 (C11′) | 31.17 (C12′), 28.93, 28.90, 28.88,[b] 28.77, 28.63, 28.58 (C5′—11′), 28.32 (C4′), 24.27 (C3′), 21.96 (C13′) |
| –CH$_3$ | | 14.03 | 14.04 | 14.07 | 13.80 | 13.79 | 13.80 |

*a*) Measured in $CDCl_3$ or DMSO-$d_6$ for **2a**—**c** or **2a**—**c**·HCl, respectively. *b*) The overlapping of the signals for two carbons was confirmed by a quantitative operation mode [no nuclear Overhauser effct (NNE) method]. *c*) NMR data of this compound in the same solvent have been reported without details about the spectrometer and radio frequency energy.[13]

Table 3. $^{13}$C-NMR Spectral Data of *N*,*N*′-Diacylated 2,6-Diaminopyridines (**3a**—**c**) and their Hydrochlorides (**3a**—**c**·HCl)[a]

| C No. | | **3a** | **3b** | **3c** | **3a**·HCl | **3b**·HCl | **3c**·HCl |
|---|---|---|---|---|---|---|---|
| Pyridine ring | C-2,6 | 149.08 | 153.03 | 152.85 | 145.35 | 146.70 | 151.25 |
| | C-3,5 | 109.2 | 110.82 | 110.63 | 107.81 | 108.67 | 110.36 |
| | C-4 | 141.21 | 141.67 | 141.49 | 145.71 | 148.09 | 144.05 |
| –NHCO– | | 171.8 | 173.17 | 173.00 | 176.80 | 177.30 | 174.88 |
| –CO–$\underline{C}H_2$– | C2′ | 37.78 | 39.01 | 38.83 | 37.12 | 37.75 | 38.98 |
| –$(CH_2)_n$– | | 31.83 (C8′), 29.37, 29.31, 29.22, 29.17 (C4′—7′), 25.28 (C3′), 25.61 (C9′) | 34.22 (C10′), 31.96, 31.93, 31.86, 31.79, 31.64, 31.59 (C4′—9′), 27.52 (C3′), 24.90 (C11′) | 34.05 (C12′), 31.81, 31.80, 31.79, 31.77, 31.68, 31.62, 31.47, 31.42 (C4′—11′), 25.34 (C3′), 24.73 (C13′) | 31.83 (C8′), 29.37, 29.33, 29.23, 29.15 (C4′—7′), 24.67 (C3′) 22.61 (C9′) | 33.03 (C10′), 30.67,[b] 30.52, 30.41, 30.36, 30.11 (C4′—9′), 25.74 (C3′), 23.68 (C11′) | 34.24 (C12′), 32.00,[b] 31.98, 31.96, 31.87, 31.78, 31.66, 31.55 (C4′—11′), 27.37 (C3′), 24.91 (C13′) |
| –$CH_3$ | | 14.03 | 15.76 | 15.59 | 14.03 | 14.39 | 15.77 |

*a*) Measured in $CDCl_3$, THF-$d_8$, or $CD_3OD$ for **3a** and **3a**·HCl, **3b**—**c** and **3c**·HCl, or **3b**·HCl, respectively. *b*) The overlapping of the signals for two carbons was confirmed by a quantitative operation mode (NNE method).

Table 4. $^1$H-NMR Spectral Data (*J* in Hz) of *N*-Monoacylated 2,6-Diaminopyridines (**2a**—**c**) and their Hydrochlorides (**2a**—**c**·HCl)[a]

| H No. | | **2a** | **2b**[c] | **2c** | **2a**·HCl | **2b**·HCl | **2c**·HCl |
|---|---|---|---|---|---|---|---|
| Pyridine ring | H-3 | 7.54 d (7.9) | 7.55 d (7.6) | 7.55 d (7.9) | 6.67 d (7.9) | 6.67 d (7.9) | 6.67 d (7.8) |
| | H-4 | 7.46 t (7.9) | 7.45 t (7.9) | 7.47 t (7.9) | 7.81 dd (8.6, 7.9) | 7.81 dd (8.5, 7.9) | 7.81 dd (8.5, 7.8) |
| | H-5 | 6.26 dd (7.9, 0.6) | 6.24 dd (7.9, 0.9) | 6.26 d (7.9) | 6.61 d (8.6) | 6.61 d (8.5) | 6.61 dd (8.5, 0.6) |
| $NH_2$ | | 4.43[b] br s | 4.38[b] br s | 4.51[b] br s | 8.24[b] br s | 8.23[b] br s | 8.2[b] br s |
| –NHCO– | | 7.94[b] br s | 7.98[b] br s | 7.95[b] br s | 12.10[b] br s | 12.09[b] br s | 12.10[b] br s |
| –CO–$\underline{C}H_2$– | H2′ | 2.35 t (7.5) | 2.34 t (7.6) | 2.35 t (7.5) | 2.49 t (7.3) | 2.49 t (7.3) | 2.49 t (7.3) |
| –$CH_2$– | H3′ | 1.70 quintet (7.5) | 1.70 quintet (7.6) | 1.70 quintet (7.5) | 1.61 quintet (7.3) | 1.61 quintet (7.3) | 1.61 quintet (7.3) |
| –$(CH_2)_n$– | | 1.2—1.4 m (H4′—9′) | 1.2—1.4 m (H4′—11′) | 1.2—1.45 m (H4′—13′) | 1.2—1.35 m (H4′—9′) | 1.2—1.4 m (H4′—11′) | 1.2—1.35 m (H4′—13′) |
| –$CH_3$ | | 0.88 t (7.0) | 0.88 t (6.9) | 0.88 t (7.0) | 0.85 t (6.9) | 0.85 t (7.0) | 0.85 t (6.9) |

*a*) Measured in $CDCl_3$ or DMSO-$d_6$ for **2a**—**c** or **2a**—**c**·HCl, respectively. *b*) These signals disappeared after treatment with $D_2O$. *c*) NMR data of this compound in the same solvent have been reported minus details on the spectrometer and radio frequency energy.[13]

Table 5. $^1$H-NMR Spectral Data of *N*,*N*′-Diacylated 2,6-Diaminopyridines (**3a**—**c**) and their Hydrochlorides (**3a**—**c**·HCl)[a]

| H No. | | **3a** | **3b**[c] | **3c** | **3a**·HCl | **3b**·HCl | **3c**·HCl |
|---|---|---|---|---|---|---|---|
| Pyridine ring | H-3,5 | 7.86 d (7.9) | 7.87 d (8.2) | 7.87 d (7.9) | 7.62 d (7.9) | 8.22 t (8.2) | 7.76 m |
| | H-4 | 7.70 t (7.9) | 7.58 t (8.2) | 7.58 t (7.9) | 7.91 br s | 7.05 d (8.2) | 7.74 m |
| –NHCO– | | 7.85[b] br s | 8.76[b] br s | 8.78[b] br s | 12.66[b] br s | | 10.37[b] br s |
| –CO–$\underline{C}H_2$– | H2′ | 2.39 t (7.3) | 2.32 t (7.5) | 2.32 t (7.3) | 2.71 t (7.5) | 2.55 t (7.5) | 2.46 t (7.3) |
| –$CH_2$– | H3′ | 1.72 quintet (7.3) | 1.66 quintet (7.5) | 1.66 quintet (7.3) | 1.78 quintet (7.5) | 1.75 quintet (7.5) | 1.69 quintet (7.3) |
| –$(CH_2)_n$– | | 1.25—1.4 m (H4′—9′) | 1.25—1.4 m (H4′—11′) | 1.25—1.4 m (H4′—13′) | 1.2—1.4 m (H4′—9′) | 1.29—1.41 m (H4′—11′) | 1.25—1.4 m (H4′—13′) |
| –$CH_3$ | | 0.88 t (7.0) | 0.88 t (6.7) | 0.88 t (6.9) | 0.88 t (7.0) | 0.89 t (7.0) | 0.88 t (6.9) |
| $NH^+Cl^-$ | | | | | 1.68[b] br s | | |

*a*) Measured in $CDCl_3$, THF-$d_8$, or $CD_3OD$ for **3a** and **3a**·HCl, **3b**—**c** and **3c**·HCl, or **3b**·HCl, respectively. *b*) These signals disappeared after treatment with $D_2O$. *c*) $^1$H-NMR data (500 MHz) have been reported.[15]

pounds. The 50% cytotoxic concentration ($CC_{50}$) values measured using Vero cells are also listed in Table 6. The activities of these compounds were at a similar level. The observed $CC_{50}$ values are close to the $EC_{50}$ values of anti-HSV-1 activities shown in Table 6. Interestingly, the diaminopyridine derivatives (**2a**—**c**) had no significant antibacterial activity against *Escherichia coli* (Gram-negative bacterium) or *Staphylococcus aureus* (Gram-positive bacterium) even at high concentration (1 mg/ml). All hydrochlorides of *N*,*N*′-diacyl-2,6-diaminopyridines (**3a**—**c**·HCl) showed no significant anti-HSV-1 ($EC_{50}$>20 $\mu$g/ml) and antibacterial ($EC_{50}$> 1 mg/ml) activities. From these observations, it is apparently indicated that the functional group of an aromatic primary amine may play an important role for the antiviral activities.

Table 6. Antiviral Activity of *N*-Monoacylated 2,6-Diaminopyridines (**2a**—**c**)

| | **2a** | **2b** | **2c** |
|---|---|---|---|
| $EC_{50}$ ($\mu$g/ml) | 16.0 | 15.3 | 18.5 |
| $CC_{50}$ ($\mu$g/ml) | 38.8 | 50.0 | 37.5 |

It is noteworthy that the simple heterocycles showed significant anti-HSV-1 activity and cytotoxic effects with Vero cells but no antibacterial activity. Although we currently have no evidence for modification of sugar chains with compounds **2a**—**c**, the ease of molecular modification by combination of the starting materials with this simple amide struc-

ture indicates that it has a wide range of applications. Further synthetic and biological studies on related compounds are in progress.

## Experimental

Melting points were determined by micro melting point apparatus (Yanagimoto MP-S3) without correction. IR spectra were measured by Shimadzu FTIR-8100 IR spectrophotometer. Low- and high-resolution mass spectra (LR-MS and HR-MS) were taken by JEOL JMS HX-110 double-focusing model equipped with a FAB ion source interfaced with a JEOL JMA-DA 7000 data system. $^1$H- and $^{13}$C-NMR spectra were obtained by JEOL JNM A-500. Chemical shifts were expressed in $\delta$ ppm downfield from an internal tetramethylsilane (TMS) signal for $^1$H-NMR and the carbon signal of the corresponding solvent [$CDCl_3$ (77.00 ppm), THF-$d_8$ (68.60 ppm), $CD_3OD$ (49.00 ppm), and dimethyl sulfoxide (DMSO)-$d_6$ (39.50 ppm)] for $^{13}$C-NMR. Microanalyses were performed with a Yanaco MT-6 CHN corder. Routine monitoring of reactions was carried out using precoated Kieselgel 60$F_{254}$ plates (E. Merck). Centrifugal chromatography was performed on silica gel (Able-Biott) with a UV detector. Commercially available starting materials were used without further purification.

**Method A: Preparation of *N*-(6-Amino-2-pyridinyl)-decanamide (2a)[11,12] and *N*-(6-Amino-2-pyridinyl)-dodecanamide (2b)[13,14]** 2,6-Diaminopyridine (**1**; 327 mg, 3.0 mmol, 100 mol%) and triethylamine (304 mg, 3.0 mmol, 100 mol%) were dissolved in dry trifluoroacetic acid (TFA) (6 ml), and the solution was cooled to 0 °C in an ice bath. Under $N_2$ atmosphere, a solution of decanoyl chloride or dodecanoyl chloride (601 or 689 mg, 3.15 mmol, 105 mol%) in dry THF (2 ml) was added dropwise over a period of 20 min, then the reaction mixture was kept at 0 °C for another 3 h. After warming to room temperature, the mixture was stirred overnight. The reaction mixture was filtered, evaporated to dryness, and purified by centrifugal chromatography ($SiO_2$, 1% EtOH in $CH_2Cl_2$) to afford **2a** and **2b** in 71% and 67% yield, respectively (Table 1).

**Method A′: Preparation of *N*,*N*′-2,6-Pyridinediylbisdodecanamide (3b)[15] and *N*,*N*′-2,6-Pyridinediylbistetradecanamide (3c)** Compounds **3b** and **3c** were prepared in a similar manner as that reported by Tamura N. *et al.*[15] Reactions with molar ratio of 2,6-diaminopyridine (100 mol%) and each acyl chloride (200 mol%) and triethylamine (200 mol%) afforded the desired **3b** and **3c** in 77% and 55% yield, respectively (Table 1).

**Method B: Preparation of *N*-(6-Amino-2-pyridinyl)-tetradecanamide (2c)** 1,3-Dicyclohexyl carbodiimide (DCC) (1.0 M solution in dichloromethane; 4.0 ml, 4.0 mmol, 100 mol%) was added to a stirred solution of 2,6-diaminopyridine (**1**; 436 mg, 4.0 mmol, 100 mol%), 1-hydroxybenzotriazole (HOBt) (362 mg, 2.68 mmol, 67 mol%), and tetradecanoic acid (612 mg, 2.68 mmol, 67 mol%) in $CH_2Cl_2$/DMF (10 ml, 10 : 1 v/v). After stirring overnight the reaction mixture was filtered through celite and concentrated under reduced pressure. The resulting material was diluted with AcOEt (20 ml), washed with saturated aqueous $NaHCO_3$ (2×10 ml) and brine (2×10 ml), and the organic layer dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a viscous oil, which was purified by centrifugal chromatography ($SiO_2$: 2% EtOH in $CH_2Cl_2$) to give **2c** in 47% yield.

**Method C: Preparation of *N*,*N*′-2,6-Pyridinediylbisdecanamide (3a)[16]** Compound **3a** was prepared smilarly to the procedure in Method A in the absence of triethylamine. Thus 2,6-diaminopyridine (**1**; 327 mg, 3.0 mmol, 100 mol%) was dissolved in dry THF (6 ml) and the solution was cooled to −10 °C. Under $N_2$ atmosphere, a solution of decanoyl chloride (601 mg, 3.15 mmol, 105 mol%) in dry THF (2 ml) was added dropwise, then the resulting mixture was allowed to stand at ambient temperature with stirring for 5 h. The reaction mixture was filtered, evaporated to dryness and purified by centrifugal chromatography ($SiO_2$, 2% EtOH in $CH_2Cl_2$). Compound **3a** was obtained in 83% yield from decanoyl chloride (Table 1).

The HCl salts (**2a**—**c**·HCl, **3a**—**c**·HCl) were obtained by reaction of each compound (**2a**—**c**, **3a**—**c**) in EtOH with large excess of 10% HCl in EtOH. Recrystallization from a proper solvent (Table 1) gave the desired salts.

The physical and spectroscopic ($^1$H- and $^{13}$C-NMR) data are summarized in Tables 1—5.

**Antiviral Activity Assay** The antiviral activities of the compounds were measured by plaque reduction assay.[17] Confluent monolayers of Vero cells (*ca.* $1\times10^6$ cells/well) in 6-well plastic plates were infected with 100 PFU of HSV-1 (KOS). After a 1 h adsorption period at 37 °C, the cultures were overlaid with 2 ml of Dulbecco's modified Eagle's minimum essential medium (DMEM) containing 2% heat-inactivated fetal calf serum, 2% $\gamma$-globulin, and various concentrations of the target compounds. The cultures inoculated with HSV-1 were incubated in a $CO_2$ incubator, fixed with formalin, and stained with crystal violet in methanol at 3 d postinoculation. After washes with water and drying, the plaques were enumerated. Calculated $EC_{50}$ values for **2a**—**c** in the tested compounds are summarized in Table 6. Those for **3a**—**c** were >20 $\mu$g/ml, and exact values were not determined.

**Cytotoxicity Assay** The antiviral activities of the compounds were examined as described below. Confluent monolayers of Vero cells were seeded in 96-well plastic plates at $5\times10^4$ cells per well. After 1 d, the cells were re-fed with 100 $\mu$l of DMEM containing 5% fetal calf serum and various concentrations of the target compounds. After 69-h incubation, 10 $\mu$l of AlamarBlue reagent was added to each culture, then the plates were reincubated for 4 h. The optical density of each culture at 570 nm was determined by spectrophotometer using a reference wavelength of 630 nm.[18] Calculated cytotoxicity ($CC_{50}$) values for the tested compounds **2a**—**c** are summarized in Table 6.

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## References and Notes

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