N-Monocarbamoyl Derivatives of Symmetrical Diamines with Antiviral Activity

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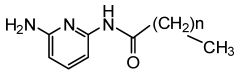
Received May 15, 2007; accepted June 25, 2007

Some new *N*-monocarbamoyl symmetrical diamines have been prepared by the addition of symmetrical amines to isocyanates or isothiocyanates. 2,6-Diaminopyridine (1), (1*R*,2*R*)-1,2-diaminocyclohexane [(1*R*,2*R*)-2], *meso*-1,2-diaminocyclohexane (*meso*-2), or (1*R*,2*R*)-1,2-diphenylethylenediamine (3) were used as the starting symmetrical diamine frameworks. All of the newly synthesized compounds were subjected to an evaluation of antiviral activity with herpes simplex virus (HSV)-1. *N*-Monocarbamoyl 2,6-diaminopyridines (5a, b) showed significant antiviral activity (EC_{50}=17.0, 6.2 μg/ml) comparable to that of *N*-monododecanoyl 2,6-diaminopyridine (A2). As a result, compound 5a showed a better selectivity index (CC_{50}/EC_{50}=*ca.* 10.0) than that of A2.

Key words symmetrical diamine; 2,6-diaminopyridine; 1,2-diaminocyclohexane; urea; anti-herpes simplex virus (HSV)-1; plaque reduction assay

The continuing search for antiviral compounds has identified *N*-monoacylated 2,6-diaminopyridines (**A1**—**3**) as a novel class of anti-herpes simplex virus (HSV)-1 agents (Chart 1).[1] Some of the derivatives of urea, thiourea, and their related molecules are known to be important structural components in biological processes[2,3] such as antiviral activity.[2,4—6] In connection with previous studies on the search for antiviral compounds, the recent focus has been on the symmetrical chiral diamines and some achiral diamine derivatives.

This paper reports the synthesis and properties of the *N*-monocarbamoyl derivatives obtained by the addition of diamines to isocyanates or isothiocyanates. In addition, this study documents the results of their antiviral [anti-herpes simplex virus (HSV)-1] activity.

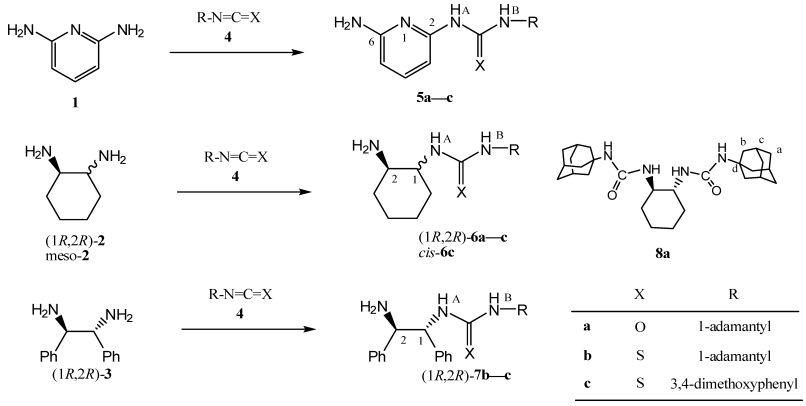


A1: n=8
A2: n=10
A3: n=12

Chart 1

Results and Discussion

Synthesis of *N*-Monocarbamoyl Derivatives of Symmetrical Diamines [(5a—c), (1*R*,2*R*)-6(a—c), *cis*-6c, (1*R*,2*R*)-7b—c] The above target compounds have been prepared by the reaction of the symmetrical diamines [2,6-diaminopyridine (DAP, **1**), (1*R*,2*R*)-1,2-diaminocyclohexane [(1*R*,2*R*)-**2**], *meso*-1,2-diaminocyclohexane (*meso*-**2**), and (1*R*,2*R*)-1,2-diphenylethylenediamine (**3**)] with the corresponding isocyanate (**4a**), or isothiocyanates (**4b**, **c**) (Chart 2). The reaction, which involved the simple refluxing of an equimolar amount of DAP (**1**) and 1-adamantyl isocyanate (**4a**) in dry tetrahydrofuran (THF) or dry pyridine required a long reaction time to generate the target compound (**5a**) and resulted in a low yield (entries 1, 2). Similarly, the reaction of DAP with an equimolar amount of 1-adamantyl isothiocyanate (**4b**) or 3,4-dimethoxyphenyl isothiocyanate (**4c**) in common organic solvents also gave the product **5b** or **5c**, in low yields (10—23%, see entries 5, 6, 8 in Table 1). In both reactions, for the preparation of **5a** (entries 1, 2), there was a gradual disappearance of the starting materials (**4a** and DAP) and the formation of *N*,*N*′-disubstituted diaminopyridine could not be detected by monitoring with thin layer chromatography (TLC). The change of the ratio of diamine : isocyanate (1 : 2) resulted in the formation of *N*-monosubstituted diaminopyri-



	X	R
a	O	1-adamantyl
b	S	1-adamantyl
c	S	3,4-dimethoxyphenyl

Chart 2

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Table 1. *N*-Monocarbamoyl Derivatives (**5**—**7**) from the Reactions of Iso(thio)cyanates with Symmetrical Diamines

Entry	Diamine	Reagent	Product	Yield %	Additive	Ratio of diamine (HCl) : reagent : additive	Conditions
1	**1**	**4a**	**5a**	27		1 : 1 : 0	Reflux, 3 d, dry THF
2	**1**	**4a**	**5a**	25		1 : 1 : 0	Reflux, 2 d, dry pyridine
3	**1**	**4a**	**5a**	69		1 : 2 : 0	Reflux, 12 d, THF
4	**1**	**4a**	**5a**	70	*n*-BuLi	1 : 1 : 2.2	rt, 1 h (pretreatment), rt, 10 min, dry THF, N_2
5	**1**	**4b**	**5b**	23		1 : 1 : 0	Reflux, 5 d, dry DME
6	**1**	**4b**	**5b**	10		1 : 1 : 0	Reflux, 2 d, toluene, N_2
7	**1**·HCl	**4c**	**5c**	61		1.4 : 1 : 0	−10—0 °C, 1 d, dry CH_2Cl_2–EtOH, N_2
8	**1**	**4c**	**5c**	17		1 : 1 : 0	rt, 1 h, reflux 18 h, CH_2Cl_2
9	(1*R*,2*R*)-**2**	**4a**	(1*R*,2*R*)-**6a**	11[a]		1 : 1 : 0	rt, 17 h, dry CH_2Cl_2
10	(1*R*,2*R*)-**2**	**4a**	(1*R*,2*R*)-**6a**	39[a]		1 : 1 : 0	rt, 1 h, reflux 1 h, dry CH_3CN
11	(1*R*,2*R*)-**2**	**4a**	(1*R*,2*R*)-**6a**	8[a]		1 : 1 : 0	rt, 0.5 h, reflux 0.5 h, dry THF
12	(1*R*,2*R*)-**2**	**4a**	(1*R*,2*R*)-**6a**	66	9-BBN	1 : 0.95 : 1	rt, 1 h (pretreatment), rt, 1 h, THF, N_2
13	(1*R*,2*R*)-**2**	**4b**	(1*R*,2*R*)-**6b**	57		1 : 0.93 : 0	rt, 1 d, dry CH_2Cl_2
14	(1*R*,2*R*)-**2**	**4c**	(1*R*,2*R*)-**6c**	67		1 : 1 : 0	rt, 1 d, CH_2Cl_2, N_2
15	*meso*-**2**	**4c**	*cis*-**6c**	91		1 : 1 : 0	rt, 1 d, CH_2Cl_2, N_2
16	(1*R*,2*R*)-**3**	**4b**	(1*R*,2*R*)-**7b**	48		1 : 1 : 0	rt, 1 d, CH_2Cl_2, N_2
17	(1*R*,2*R*)-**3**	**4c**	(1*R*,2*R*)-**7c**	76		1 : 1 : 0	rt, 17 h, CH_2Cl_2, N_2

a) Additional *N*,*N*′-disubstituted derivative (**8a**) is also obtained in 54, 26, and 60% yield in entries 9, 10, and 11, respectively.

dine (**5a**) with a 69% yield (entry 3). The reactivity of the aromatic amines as an electrophile was weaker than that of the aliphatic amines, and the steric factor (bulkiness of the adamantyl group) apparently decreased the accessibility of the electrophiles to iso(thio)cyanate functionality in the molecules of **4a** and **4b**. In order to enhance the reactivity, the protocol used in the preparation of the monobenzoylated derivatives from acylation of the symmetrical diamines according to the procedure reported by Wang *et al.*[7] was successfully applied. Therefore, one equivalent of isocyanate (**4a**) was added to the pretreated DAP with 2 eq of *n*-butyllithium to generate a dianion of DAP, at room temperature, resulting in the preferential formation of the *N*-monosubstituted product **5a** in excellent yield (70%, entry 4; Method A). DAP was also found to react fairly slowly with **4c**, similar to the cases of **4a** and **4b**. Therefore, the procedure was also attempted by starting with DAP monohydrochloride to iso(thio)cyanates to prepare *N*-monosubstituted diamines.[8] By the deactivation of one nitrogen lone pair of diamines, monoammonium salt was found to be superior in terms of the preferential formation procedure of *N*-monocarbamoyl diamines. This procedure was successful in the reaction of DAP with **4c** for the preparation of **5c** (see, entry 7; Method B). In the reactions of DAP with **4a** and **4b**, unfortunately, this procedure was not very efficient. It is possible that the poor results in these cases may be due to the steric bulkiness of the adamantyl group in **4a** and **4b**. In addition, an equilibrium state of the mono ammonium ion of the diamines between the diammonium ion in the solution may also be another factor for the deactivation of the diamine's reactivity.[9] In the case of diamine (1*R*,2*R*)-1,2-diaminocyclohexane [(1*R*,2*R*)-**2**], the reactions with iso(thio)cyanates **4b**, **c** proceeded more rapidly than those of DAP with **4b**, **c** to produce the *N*-monocarbamoyl diamines (1*R*,2*R*)-**6b** and (1*R*,2*R*)-**6c**, in a 57% and 67% yield, respectively (entries 13, 14). The reactions with isocyanate **4a** gave *N*-monocarbamoyl diamine (1*R*,2*R*)-**6a** in low yields by the above procedure, and other attempts for the preparation of *N*-monocarbamoyl diamines also resulted in low yields, associated with the formation of *N*,*N*′-dicarbamoyl diamine **8a** (entries 9—11 in Table 1, and see Experimental). In order to improve the yields of the monosubstituted products (**6**), the procedure for selective monoacylation of symmetrical diamines reported by Zhang Z. *et al.*[10] was employed. In order to form a simple associative complex of diamine (1*R*,2*R*)-**2**, one equivalent of Lewis acid [9-borabicyclo[3.3.1]nonane (9-BBN)] was added to a diamine in THF. The mixture was stirred for 1 h at room temperature. After the addition of isocyante **4a** to this pretreated solution with stirring, the resulted mixture was kept another 1 h. After workup, the desired product was obtained in 66% (entry 12; Method C). In the reactions with **4c**, the diamine *meso*-**2** proceeded more easily to generate the product *cis*-**6c** in a 91% yield (entry 15) than the diamine (1*R*,2*R*)-**2** (entry 14).[11,12] Using (1*R*,2*R*)-**3** as a diamine, the isothiourea derivatives (1*R*,2*R*)-**7b** and (1*R*,2*R*)-**7c** were also synthesized from the reactions with isothiocyanates (**4b**, **c**) in 48% and 76% yields, respectively (entries 16, 17). In order to increase the solubility in water for bioassay, the compounds (1*R*,2*R*)-**6c** and (1*R*,2*R*)-**7c** were treated with 10% HCl in ethanol to transform the corresponding hydrochlorides (see Experimental).

The structures of the target compounds were confirmed by spectroscopic and elemental analyses (Tables 2—4).

Evaluation of Antiviral Activities The anti-HSV-1 activities of the target *N*-monosubstituted diamines were estimated using a plaque reduction assay in Vero cells.[13] For the active compounds [**5a**, **b** and (1*R*,2*R*)-**6a**], their cytotoxic activities were also evaluated. The obtained 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) values for the synthesized compounds are listed in Table 5. The data for the *N*-long alkyl chain monoacylated derivative of diaminopyridine **A2** are also listed in Table 5.[1] The *N*-monosubstituted 2,6-diaminopyridines (**5a**, **b**) showed a significant anti-HSV-1 activity (EC_{50}=17.0, 6.2 μg/ml). Although compound **5a** showed lower activity against HSV-1 than that of acyclovir (ED_{50}=0.2—0.9 μg/ml),[1] it possessed a comparable potency and a lower cytotoxicity in comparison to that of **A2**, thus resulting in a better selectivity index (CC_{50}/EC_{50}=*ca.* 10.0) than **A2** (CC_{50}/EC_{50}=3.3). Compound **5b** has higher antiviral activity in comparison to **A2**, and

Table 2. Physical Data of *N*-Monocarbamoyl Derivatives (**5**—**7**) from the Reactions of Iso(thio)cyanates with Diamine Derivatives

Compd.	mp (°C) (Recryst solvent)	Formula	Analysis (%) Calcd (Found)			Formula HR-MS *m/z* Calcd (Found)	IR (cm^{-1}) (KBr)
			C	H	N		
5a	220—224 ($EtOH–CH_2Cl_2$)	$C_{16}H_{22}N_4O \cdot 0.1H_2O$	66.69 (66.61	7.76 7.76	19.44 19.35)	$C_{16}H_{23}N_4O$ $(M+H)^+$ 287.1872 (287.1873)	3475, 3380, 3220, 3085 (NH_2, NH) 1685 (C=O)
5b	209—210 (EtOH)	$C_{16}H_{22}N_4S$	63.54 (63.72	7.33 7.33	18.52 18.24)	$C_{16}H_{23}N_4S$ $(M+H)^+$ 303.1643 (303.1637)	3490, 3320, 3205 (NH_2, NH) 1450, 1240 (NH–C=S)
5c	242—244 (CH_2Cl_2)	$C_{14}H_{16}N_4O_2S$	55.25 (55.05	5.30 5.27	18.41 18.34)	$C_{14}H_{17}N_4O_2S$ $(M+H)^+$ 305.1072 (305.1074)	3480, 3365, 3295 (NH_2, NH) 1510, 1230 (NH–C=S)
(1*R*,2*R*)-**6a**	248—252 decn. (CH_2Cl_2)	$C_{17}H_{29}N_3O \cdot 0.3H_2O$	68.79 (68.72	10.05 10.00	14.16 13.90)	$C_{17}H_{30}N_3O$ $(M+H)^+$ 292.2389 (292.2392)	3855, 3355 br (NH_2, NH) 1630 (C=O)
(1*R*,2*R*)-**6b**	125—128 (EtCN)	$C_{17}H_{29}N_3S \cdot 0.9H_2O$	63.08 (62.95	9.59 9.60	12.98 13.04)	$C_{17}H_{30}N_3S$ $(M+H)^+$ 308.2160 (308.2161)	3305, 3295, 3145, 3065 (NH_2, NH) 1555, 1305 br (NH–C=S)
(1*R*,2*R*)-**6c**	168—170 (CH_2Cl_2)	$C_{15}H_{23}N_3O_2S \cdot 0.4H_2O$	56.90 (56.93	7.58 7.29	13.27 13.17)	$C_{15}H_{24}N_3O_2S$ $(M+H)^+$ 310.1589 (310.1591)	3340, 3290 (NH_2) (NH_2, NH) 1510, 1235 br (NH–C=S)
cis-**6c**	109—110 ($AcOEt–iPr_2O$)	$C_{15}H_{23}N_3O_2S$	58.22 (58.18	7.49 7.47	13.58 13.33)	$C_{15}H_{24}N_3O_2S$ $(M+H)^+$ 310.1589 (310.1587)	3360, 3340, 3280 (NH_2, NH) 1515, 1235 (NH–C=S)
(1*R*,2*R*)-**7b**	81—84[a]	$C_{25}H_{31}N_3S$	74.03 (73.82	7.70 7.69	10.36 10.56)	$C_{25}H_{32}N_3S_2$ $(M+H)^+$ 406.2317 (406.2317)	3380, 3280 (NH_2, NH) 1520, 1225 (NH–C=S)
(1*R*,2*R*)-**7c**	78—82 (EtOH)	$C_{23}H_{25}N_3O_2S \cdot 0.2H_2O$	67.19 (67.24	6.23 6.28	10.22 10.14)	$C_{23}H_{26}N_3O_2S$ $(M+H)^+$ 408.1746 (408.1736)	3345 br (NH_2, NH) 1510, 1235 (NH–C=S)

a) Analytical sample (1*R*,2*R*)-**7b** was obtained by silica-gel chromatography using acetonitrile as an eluent.

Table 3. ^{13}C-NMR Spectral Data of *N*-Monocarbamoyl 2,6-Diaminopyridine, 1,2-Diaminocyclohexane, and 1,2-Diphenylethylenediamine (**5**—**7**)

C No.		**5a**	**5b**	**5c**	(1*R*,2*R*)-**6a**	(1*R*,2*R*)-**6b**	(1*R*,2*R*)-**6c**	*cis*-**6c**	(1*R*,2*R*)-**7b**	(1*R*,2*R*)-**7c**
Pyridine ring or	C1[a]	—	—	—	56.34	61.51	61.10	55.25	64.41	63.68
cyclohexane ring	C2	152.36	152.39	152.31	56.00	56.21	53.94	49.72	59.52	59.66
or ethylene chain	C3	98.12	99.04	98.89	34.67	34.79	34.60	31.86	—	—
	C4	138.83	139.55	139.77	24.91	24.82	24.53 or 24.68	20.28	—	—
	C5	99.48	100.80	101.42	25.18	24.74	24.68 or 24.53	22.97	—	—
	C6	153.39	156.54	157.19	33.12	32.24	31.23	27.15	—	—
–NHCO–		157.43	—	—	158.1	—	—	—	—	—
–NHCS–		—	176.83	178.17	—	181.24	180.52	179.75	180.49	181.18
C of adamantyl or phenyl		28.87 (c), 36.05 (a), 41.62 (b), 49.79 (d)	28.94 (c), 35.98 (a), 40.45 (b), 53.51 (d)	109.91 (C2′), 111.55 (C5′), 117.13 (C6′), 132.48 (C1′), 146.52 (C4′), 148.20 (C3′)	29.60 (c), 36.51 (a), 42.51 (b), 50.93 (d)	29.53 (c), 36.24 (a), 42.07 (b), 54.08 (d)	109.17 (C2′), 112.03 (C5′), 116.08 (C6′), 132.38 (C1′), 146.11 (C4′), 148.62 (C3′)	109.23 (C2′), 111.63 (C5′), 117.31 (C6′), 129.78 (C1′), 147.75 (C4′), 149.59 (C3′)	29.36 (c), 35.99 (a), 41.93 (b), 53.75 (d), 126.36, 126.41 (*o*-), 127.28, 127.56 (*p*-), 128.43, 128.63 (*m*-), 140.32, 141.36 (on C1,2)	on $C_6H_3(OMe)_2$ 109.99 (C2′), 111.65 (C5′), 118.44 (C6′), 129.32 (C1′), 148.41 (C4′), 149.81 (C3′), on Ph 126.57, 126.67 (*o*-), 127.52, 127.80 (*p*-), 128.48, 128.68 (*m*-), 139.58 (on C2), 140.76 (on C1), 56.07 (on C3′), 56.11 (on C4′)
–OCH_3		—	—	55.67, 55.84	—	—	55.64, 55.89	55.99[b]	—	
Solvent		DMSO-d_6	DMSO-d_6	DMSO-d_6	$CDCl_3$	$CDCl_3$	DMSO-d_6	$CDCl_3$	$CDCl_3$	$CDCl_3$

a) Corresponding to N1 in the case of pyridine. *b*) Overlapped with the other signals.

Table 4. [1]H-NMR Spectral Data (*J* in Hz) of *N*-Monocarbamoyl 2,6-Diaminopyridine, 1,2-Diaminocyclohexane, and 1,2-Diphenylethylenediamine (**5**—**7**)

H No.		**5a**	**5b**	**5c**	(1*R*,2*R*)-**6a**	(1*R*,2*R*)-**6b**	(1*R*,2*R*)-**6c**	*cis*-**6c**	(1*R*,2*R*)-**7b**	(1*R*,2*R*)-**7c**
Pyridine ring or cycylohexane ring or ethylene chain	H1[e]	—	—	—	3.16—3.24 m	3.65 br s	3.87 br s	4.40 br s	5.45 br s	5.64 br s
	H2	—	—	—	2.44—2.50 m	2.59 dt (10.4, 0.4)	2.58—2.53 m	3.12 br s	4.40 s	4.40 d (4.3)
	H3	5.95 dd (7.9, 0.6)	6.24 d (7.3)	6.31 dd (7.9, 0.6)	*ca.* 1.30—1.4 m *ca.* 1.93—1.98 m	*ca.* 1.3 m 1.92—1.98 m	1.25—1.10 m 1.82—1.87 m	1.35—1.55 m 1.65—1.70 m	—	—
	H4	7.21 t (7.9)	7.32 t (7.9)	7.37 dd (8.2, 7.9)	*ca.* 1.20—1.35 m	*ca.* 1.25—1.35 m *ca.* 1.67—1.74 m	1.25—1.10 m *ca.* 1.72—1.75 m	1.35—1.55 m 1.60—1.67 m	—	—
	H5	6.28 dm (7.6)	6.08 d (7.9)	6.12 dd (8.2, 0.6)	*ca.* 1.20—1.35 m *ca.* 1.67—1.74 m	*ca.* 1.25—1.35 m *ca.* 1.72—1.75 m	1.25—1.10 m 1.60—1.67 m	1.35—1.55 m	—	—
	H6	—	—	—	1.10—1.25 m *ca.* 1.95—2.05 m	1.13—1.23 m *ca.* 2.15 m	1.25—1.10 m 1.98—2.03 m	1.35—1.55 m 1.80—1.90 m	—	—
NH_2		5.71[a] s	3.51[a] br s	6.29[a] s	2.68[a] br s	2.20[a] br s	[d]	2.58[a] br s	1.90[a] br s	2.38[a] br s
Py or Ch or Et-NH_ACXNH_B-[b]	H_A	8.47[a] s	9.71[a] br s	10.29[a] s	4.83[a] d (7.9)	5.96[a] br s,	7.4[a] br s,	7.08[a] br s	*ca.* 7.35[a] br s[c]	7.54[a] br s
	H_B	8.45[a] br s	11.69[a] br s	13.49[a] s	5.05[a] br s	7.1[a] br s[c]	9.3[a] br s[c]	8.22[a] br s	6.00[a] br s	7.94[a] br s
H of adamantyl or phenyl		1.64 br s (a), 1.98 br d (2.7) (b), 2.03 br s (c)	1.06 s (a), 2.07 s (c), 2.31 d (2.4) (b)	6.93 d (8.9) (H5′), 7.12 dd (8.9, 2.4) (H6′), 7.39 d (2.4) (H2′)	1.66 t (2.9) (a), 1.97 br d (2.4) (b), 2.06 br s (c)	1.69 br s (a), 2.11 br s (c), 2.15 br s (b)	6.84 dd (8.5, 2.3) (H6′), 6.89 d (8.5) (H5′), 7.08 d (2.3) (H2′)	6.81 dd (8.5, 2.3) (H6′), 6.85 d (8.5) (H5′), 6.88 br s (H2′)	1.68 br s (a), 2.00 br s (b), 2.12 br s (c), 7.30—7.23 m (*o*-), 7.31—7.37 m (*m*-, *p*-)	6.80—6.85 m (H2′,6′), 6.90 d (8.6) (H5′), 7.16—7.20 m (*o*-), 7.23—7.32 m (*p*-, *m*-)
$-OCH_3$		—	—	3.77 s[c]	—	—	3.73 s, 3.74 s	3.84 s, 3.87 s	—	3.82 s (on C3′), 3.92 s (on C4′)
Solvent		DMSO-d_6	DMSO-d_6	DMSO-d_6	$CDCl_3$	$CDCl_3$	DMSO-d_6	$CDCl_3$	$CDCl_3$	$CDCl_3$

a) These signals disappeared after treatment with D_2O. *b*) Abbreviations (Py, Ch, and Et) stand for pyridine, cyclohexane, and ethylene, respectively. *c*) Overlapped with the other signals. *d*) Signal of water in the solvent overlapped with the signal of NH_2. *e*) Corresponding to N1 in the case of pyridine.

Table 5. Antiviral Activity of *N*-Monocarbamoyl Derivatives (**5**—**7**) of Symmetrical Diamines

	EC_{50} (μg/ml)	CC_{50} (μg/ml)	CC_{50}/EC_{50}
5a	17.0	170	*ca.* 10.0
5b	6.2	15.0	2.4
5c	>40	*b)*	*b)*
(1*R*,2*R*)-**6a**	42	250	*ca.* 5.0 (6.0)
(1*R*,2*R*)-**6b**	>40	*b)*	*b)*
(1*R*,2*R*)-**6c** · HCl	>40	*b)*	*b)*
cis-**6c**	>40	*b)*	*b)*
(1*R*,2*R*)-**7b**	>40	*b)*	*b)*
(1*R*,2*R*)-**7c** · HCl	>40	*b)*	*b)*
A2[a]	15.3	50.0	3.3

a) *N*-(6-Amino-2-pyridinyl)dodecanamide (**A2**) was reported.[1] *b*) CC_{50} values and selectivity indexes (CC_{50}/EC_{50}) for these compounds were not determined.

showed a high cytotoxic activity, thus resulting in a lower selectivity index ($CC_{50}/EC_{50}=2.4$). Compound (1*R*,2*R*)-**6a** showed considerable degree of antiviral activity and had less cytotoxicity than that of **A2**, and the selectivity index was *ca.* 5.0—6.0. The other synthesized compounds listed in Table 5 were unfortunately inactive against HSV-1 at the concentration of 40 μg/ml.

These observations demonstrate that the derivatives of DAP (**5a**, **b**) possess a higher antiviral activity than the compounds [**6** and (1*R*,2*R*)-**7**] derived from aliphatic 1,2-diaminocyclohexanes and 1,2-diaminoethanes. In comparison to prototype **A2**, compound **5a** actually showed identical antiviral activities, and the replacement of the long-chain alkyl substituent in the adamantyl-amino group resulted in the attenuation of the cytotoxicity ($CC_{50}=170$ μg/ml for **5a**). Compound (1*R*,2*R*)-**6a** with an adamantyl-urea moiety showed a lower cytotoxicity and antiviral potency than compound **5a**. The sulfur analog **5b** resulted in an increased cytotoxicity as shown in Table 5. The CC_{50} values for the other *N*-monosubstituted 1,2-diamine derivatives [**5c**, (1*R*,2*R*)-**6b**, **c**, *cis*-**6c**, and (1*R*,2*R*)-**7b**, **c**] were not evaluated, because there were no significant antiviral activities over a dose of 40 μg/ml. However, these data demonstrate that the mono-substituted aliphatic 1,2-diaminocyclohexane derivatives exhibit a tendency toward lower antiviral activity in comparison to the *N*-monosubstituted aromatic diamine derivatives.

This study showed that the derivatives of DAP **5a** showed the same level of significant anti-HSV-1 activity and less cytotoxicity, providing a new candidate for antiviral leads. Further synthetic application of the above information and biological studies on related compounds are in progress.

Experimental

The melting points were determined using a 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 δ 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), 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 60F_{254} plates (E. Merck). Centrifugal, flash column, or open column chromatography was performed on silica gel (Able-Biott, Fuji Silysia FL40D, or Wako gel LP-40, respectively) with a UV detector. Commercially available starting materials were used without further purification.

General Procedure for Addition of Isocyanate or Isothiocyanate to Diamines A solution of an iso(thio)cyanate (a proper molar ratio listed in Table 1) in a proper solvent (5—10 ml) was added to a stirring solution of an appropriate diamine (**1**—**3**, 2 mmol) in the same solvent (10—20 ml), and this mixture was stirred for the indicated time and temperature (in Table 1). In some entries, the experiment was carried out under an N_2 atmosphere. After the evaporation of the solvent, purification by centrifugal or flash column chromatography (SiO_2, EtOH or EtOH–CH_2Cl_2 as solvent) produced the target products (**5**—**7**). Recrystallization from a proper solvent afforded an analytically pure sample (see Tables 1, 2). The purification of the compound (1*R*,2*R*)-**7b** was carried out by centrifugal chromatography.

Method A: Preparation of *N*-(6-Amino-2-pyridinyl)-*N*′-tricyclo-[3.3.1.1^{3,7}]dec-1-ylurea (5a) Based on the protocol reported by Wang T. *et al.*,[7] 1.6 M *n*-BuLi in *n*-hexane (1.71 ml, 2.73 mmol, 220 mol%) was added to a stirred solution of 2,6-diaminopyridine (**1**; 135 mg, 1.24 mmol, 100 mol%) in dry THF (6 ml) under N_2 atmosphere at room temperature. After stirring for 1 h, 1-adamantyl isocyanate (**4a**; 220 mg, 1.24 mmol, 100 mol%) was added to this solution, and the resulting mixture was then stirred for an additional 10 min. The reaction mixture was quenched with MeOH (4 ml) and the solvents were evaporated. The residue was extracted with EtOAc (3×25 ml). The organic layer was dried over $MgSO_4$ and concentrated to yield the crude product **5a**. Recrystallization from EtOH–CH_2Cl_2 gave analytically pure **5a** (249 mg, 70% yield).

Method B: Preparation of *N*-(3,4-Dimethoxyphenyl)-*N*′-(6-amino-2-pyridinyl)-thiourea (5c) 2,6-Diaminopyridine monohydrochloride was prepared by adding one equivalent of 10% hydrochloric acid in ethanol. Recrystallization from *i*-PrOH generated the monohydrochloride of 2,6-diaminopyridine (**1** · HCl) in 73% yield, as a green solid, mp 153—154 °C.[14,15] FAB-MS *m/z*: 110 (M^++H). IR (KBr) cm^{-1}: 3370, 3190 (NH_2), *ca.* 3000 (NH_3^+), 1645 (N–H). ^{1}H-NMR (DMSO-d_6) δ: 5.91 (2H, d, J=8.2 Hz, H3,5), 7.33 (4H, br s, NH_2), 7.48 (1H, t, J=8.2 Hz, H4), 13.04 (1H, br s, HCl). ^{13}C-NMR (DMSO-d_6) δ_C: 94.89 (C3,5), 144.68 (C4), 151.96 (C2,6). *Anal.* Calcd for $C_5H_7N_3 \cdot HCl \cdot 0.7H_2O$: C, 37.96; H, 5.99; N, 26.56. Found: C, 37.84; H, 5.89; N, 26.83. The addition of isothiocyanate **4c** to this diamine monohydrochloride was carried out according to the procedure described by Bied C. *et al.*[8] Therefore, 2,6-diaminopyridine · HCl · 0.7H_2O (**1** · HCl; 443 mg, 2.8 mmol, 140 mol%) in dry CH_2Cl_2–EtOH (50 ml, 10 ml) was placed in a round-bottomed flask under an N_2 atmosphere at −10 °C. A solution of 3,4-dimethoxyphenyl isothiocyanate (**4c**) in dry CH_2Cl_2 (15 ml) was added dropwise. After stirring overnight at −10—10 °C, the solution was then washed with aqueous Na_2CO_3 (20 ml) to remove the excess unreacted diamine hydrochloride. The organic layer was dried over $MgSO_4$ and then the solvent was evaporated. The residue was recrystallized from CH_2Cl_2 to produce the product **5c** in a 61% yield.

Method C: Preparation of *N*-[(1*R*,2*R*)-2-Aminocyclohexyl]-*N*′-tricyclo[3.3.1.1^{3,7}]dec-1-ylurea [(1*R*,2*R*)-6a] A solution of 0.4 M 9-BBN in hexanes (10 ml, 4.0 mmol, 100 mol%) was added to a stirred solution of (1*R*,2*R*)-**2** (457 mg, 4.0 mmol, 100 mol%) in THF (60 ml) at room temperature under N_2 atmosphere. After stirring for 1 h, 1-adamantyl isocyanate (**5a**; 674 mg, 3.8 mmol, 95 mol%) was added and the reaction mixture was stirred for an additional 1 h. In order to dissociate the complex with 9-BBN from product, 1 M aqueous HCl (17 ml) was added, and the mixture was stirred at room temperature overnight. Neutralization, extraction with CH_2Cl_2, and then purification by open column chromatography (SiO_2, EtOH) produced the compound (1*R*,2*R*)-**6a** in a 66% yield (see Table 1).

The physical and spectroscopic (^{1}H-, ^{13}C-NMR) data for the prepared compounds (**5**—**7**) are summarized in Tables 2—4.

The HCl salts (1*R*,2*R*)-**6c** and (1*R*,2*R*)-**7c** were also obtained by the reactions from the corresponding compounds with a large excess of 10% HCl in EtOH. In addition, recrystallization produced the desired salts.

Compound (1*R*,2*R*)-**6c** · HCl: mp 218—220 °C (from EtOH): HR positive ion FAB-MS: Calcd for $C_{15}H_{24}N_3O_2S$ $(M+H)^+$: 310.1589. Found: 310.1591. IR (KBr) cm^{-1}: 3205 br (NH_3^+ salt), 1510, 1230 (NH–C=S). ^{1}H-NMR (DMSO-d_6) δ: *ca.* 1.20 (1H, m, H5), *ca.* 1.25 (1H, m, H4), *ca.* 1.30 (1H, m, H6), *ca.* 1.43 (1H, m, H3), 1.68 (1H, m, H5), 1.70 (1H, m, H4), 1.97 (1H, d, J=13.7 Hz, H6), 2.04 (1H, d, J=13.7 Hz, H3), 3.09 (1H, dt, J=10.8, 0.4 Hz, H2), 3.73 (3H, s, OCH_3 on C3′), 3.74 (3H, s, OCH_3 on C4′), *ca.* 4.38 (1H, m, H1), 6.88 (1H, dd, J=8.5, 1.8 Hz, H6′), 6.91 (1H, d, J=8.5 Hz, H5′), 7.18 (1H, br s, H2′), 7.85 (1H, d, J=7.9 Hz, Ch-N<u>H</u>–), 7.99 (2H, br s, NH_2), 9.73 (1H, br s, Ar-N<u>H</u>–). ^{13}C-NMR (DMSO-d_6) δ_C: 23.27 (C4), 24.01 (C5), 29.42 (C3), 30.76 (C6), 52.87 (C2), 55.20 (C1), 55.57 (OCH_3 on C3′), 55.80 (OCH_3 on C4′), 109.20 (C2′), 111.87 (C5′), 116.03 (C6′), 132.02 (C1′), 146.15 (C4′), 148.46 (C3′), 180.63 (C=S). *Anal.* Calcd for

$C_{15}H_{23}N_3O_2S \cdot HCl$: C, 52.09; H, 6.99; N, 12.15. Found: C, 51.93; H, 6.91; N, 12.15.

Compound (1*R*,2*R*)-**7c**·HCl: mp 194—198 °C (from EtOH): HR positive ion FAB-MS: Calcd for $C_{23}H_{26}N_3O_2S$ $(M+H)^+$: 408.1746. Found: 408.1747. IR (KBr) cm^{-1}: 3315 (NH), 3000 br (NH_3^+ salt), 1515, 1235 (NH–C=S). ^{1}H-NMR (CD_3OD) δ: 3.73 (3H, s, OCH_3 on C3′), 3.84 (3H, s, OCH_3 on C4′), 4.81(1H, d, J=10.4 Hz, H2), 6.34 (1H, d, J=10.4 Hz, H1), 6.84 (1H, dd, J=8.4, 2.3 Hz, H6′), 6.95 (1H, d, J=8.5 Hz, H5′), *ca.* 6.96 (1H, br s, H2′), 7.17—7.20 (2H, m, *o*-Ph on C1), 7.21—7.24 (3H, m, *p*-,*m*-Ph on C1), 7.30—7.34 (5H, m, C_6H_5 on C2). ^{13}C-NMR (CD_3OD) δ_C: 56.53 (OCH_3 on C3′), 56.77(OCH_3 on C4′), 60.64 (C2), 62.86 (C1), 110.94 (C2′), 113.16 (C5′), 118.78 (C6′), 128.99 (*o*-Ph on C1), 129.23 (*o*-Ph on C2), 129.49 (*p*-Ph on C1), 129.83 (*m*-Ph on C1), 130.07 (*m*-Ph on C2), 130.47 (*p*-Ph on C2), 132.28 (C1′), 135.24 (Ph on C2), 138.02 (Ph on C1), 148.96 (on C4′), 150.48 (on C3′), 183.46 (C=S). *Anal.* Calcd for $C_{23}H_{25}N_3O_2S \cdot HCl$: C, 62.22; H, 5.90; N, 9.46. Found: C, 62.08; H, 5.86; N, 9.41.

Formation of Bis-ureido: *N*,*N*″-(1*R*,2*R*)-1,2-Cyclohexanediylbis[*N*″-tricyclo[3.3.1.1^{3,7}]dec-1-ylurea] (8a) At the end of the reactions between (1*R*,2*R*)-**2** and **4a** in the dry solvent (CH_2Cl_2, CH_3CN, or THF for entries 9, 10, 11, respectively in Table 1) the precipitated material formed in the reaction mixture. The crude material was then filtered and purified by recrystallization from EtOH or flash chromatography (SiO_2, EtOH) to produce **8a** as a white powder; mp 303—304 °C (from EtOH): HR positive ion FAB-MS: Calcd for $C_{28}H_{45}N_4O_2$ (M^+): 469.3543. Found: 469.3545. IR (KBr) cm^{-1}: 3355, 3325 (NH), 1630 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.1—1.3 (4H, m, H3—6), 1.66 (12H, br s, a in adamantyl), 1.65—1.75 (2H, m, H4,5), 1.95 (12H, d, J=2.8 Hz, b in adamantyl), 1.9—2.05 (2H, m, H3,6), 2.06 (6H, br s, c in adamantyl), 3.3—3.4 (2H, m, H1,2), 4.5 (2H, br s, NH), 5.1 (2H, br s, NH). ^{13}C-NMR ($CDCl_3$) δ_C: 24.99 (C4,5), 29.60 (c in adamantyl), 33.23 (C3,6), 36.49 (a in adamantyl), 42.54 (b in adamantyl), 51.07 (d in adamantyl), 54.78 (C1,2), 158.29 (C=O). *Anal.* Calcd for $C_{28}H_{44}N_4O_2$: C, 71.76; H, 9.46; N, 11.95. Found: C, 71.56; H, 9.65; N, 11.92. The filtrate was evaporated under reduced pressure. The purification of the residue gave the compound (1*R*,2*R*)-**6a** in the yields listed in Table 1 (entries 9—11).

Antiviral Activity Assay and Cytotoxicity of Target Compounds (5—7) The antiviral activities of the compounds were measured using a plaque reduction assay[13] as described in a previous paper.[1] The calculated EC_{50} values for the tested compounds are summarized in Table 5. The cytotoxicity of the compounds **5a**, **b**, and (1*R*,2*R*)-**6a** were determined by the method described in a previous paper. The calculated cytotoxicity (CC_{50}) values for the tested compounds are summarized in Table 5.

Acknowledgments We thank Ms. Chie Fujita and Ms. Sayaka Ueno for their valuable technical assistance.

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