Synthesis and Antiviral Activities of *N*-Mono- and/or *N*,*N*′-Di- Carbamoyl and Acyl Derivatives of Symmetrical Diamines

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***N*-carbamoyl and *N*-acyl diamine derivatives were synthesized from symmetrical diamines by their addition to iso(thio)cyanates, cleavage reaction of acid anhydride, or *N*-acylation by acyl chloride. (1*R*,2*R*)-1,2-Diaminocyclohexane [(1*R*,2*R*)-1], *meso*-1,2-diaminocyclohexane (*meso*-1), (1*R*,2*R*)-1,2-diphenylethylenediamine [(1*R*,2*R*)-3], or *meso*-1,2-diphenylethylenediamine (*meso*-3) were used as the starting symmetrical diamines. The target compounds synthesized herein were evaluated for antiviral activity with herpes simplex virus type 1 (HSV-1). A few derivatives of 1,2-diaminocyclohexane [(1*R*,2*R*)-7aa and *cis*-7b] with adamantyl group(s) showed significant antiviral activity (EC_{50}=16.0, 27.0 μg/ml).**

Key words diaminocyclohexane; urea; thiourea; adamantane; anti-herpes simplex virus type 1; plaque reduction assay

In the course of our research for novel antiviral compounds, we demonstrated that some *N*-long-chain monoacylated or *N*-monocarbamoyl derivatives of 2,6-diaminopyridine (DAP) (**A**, **B**) possessed a notable antiviral activity against herpes simplex virus type 1 (HSV-1) (Chart 1).[1,2] We reported previously on the synthesis and properties of the *N*-monocarbamoyl derivatives obtained by the addition of symmetrical 1,2-diamines (**1**, **3**) to iso(thio)cyanates. By the introduction of an adamantyl group as a substitutent, we found that derivative **B** showed less cytotoxicity and comparable antiviral activity than that of compound **A**.

It is well known that adamantane derivatives possess various biological activities,[3] and among these derivatives are some of the potent antiviral agents that have been reported so far, especially those against the influenza virus.[4] Furthermore, a dramatic improvement in physical properties has been shown recently in some bioactive adamantane series.[5] These reports led us to further study molecular modification and evaluation of synthesized derivatives, aiming at the discovery of new leads with antiviral activity *via* interference with sugar chains, as postulated in our previous hypothesis.[2]

In this paper, we describe the preparation of *N*-carbamoyl and *N*-acyl derivatives from symmetrical 1,2- and 1,3-diamines (**1**—**3**) and show the results of plaque reduction assay conducted for their anti-HSV-1 activity.

Results and Discussion

Synthesis of *N*,*N*′-Dicarbamoyl and *N*-Monocarbamoyl Derivatives [(1*R*,2*R*)-7(aa—cc), *meso*-7bb, 9bb, (1*R*,2*R*)-7c, *cis*-7b, and 9b] from Symmetrical Diamines These target compounds were prepared by the reactions of the symmetrical diamines [(1*R*,2*R*)-1,2-diaminocyclohexane [(1*R*,2*R*)-**1**], *meso*-1,2-diaminocyclohexane (*meso*-**1**), or 1,3-diaminocyclohexane (**2**)] with the corresponding iso(thio)cyanate (**4a**—**c**) as shown in Chart 2. The reaction conditions and yields of the products are summarized in Table 1. In these experiments for the preparation of *N*-carbamoyl derivatives, both formations of *N*-monocarbamoyl [(1*R*,2*R*)-**7c**, *cis*-**7b**, or **9b**] and *N*,*N*′-dicarbamoyl derivative [(1*R*,2*R*)-**7cc** (15%), *meso*-**7bb** (7%), or **9bb** (21%)] always took place under reaction conditions with an equimolar ratio of diamine : iso(thio)cyanate (1 : 1) (entries 3, 7, 9, respectively). These reactions were carried out under simple stirring or refluxing in an appropriate solvent (see Table 1). The change in the ratio of diamine : iso(thio)cyanate (1 : 2.1) resulted in the formation of *N*,*N*′-dicarbamoyl derivative in excellent yields (entries 1, 2, 4, 8, 10). The formation of *N*,*N*′-dicarbamoyl derivative [*meso*-**7bb** (93%)] needed a longer reflux time [18 h in tetrahydrofuran (THF)] due to the steric hindrance of the initially introduced bulky carbamoyl group for the second addition stage. In this experiment, a small amount of by-product *N*,*N*′-di(1-adamantyl)thiourea (**8**)[6,7] was also obtained in a 4% yield (entry 8).

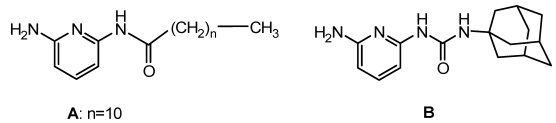

Chart 1

Synthesis of *N*-Monoacyl Derivatives [(1*R*,2*R*)-7d and (1*R*,2*R*)-10d] from Symmetrical Diamines Easy access was predicted by TLC monitoring of the reactions of diamine [(1*R*,2*R*)-**1** or (1*R*,2*R*)-**3**] with 2,3-pyrazinedicarboxylic anhydride (**5**) at room temperature in acetone or CH_3CN[8] to form *N*-monoacyl derivative [(1*R*,2*R*)-**7d** or (1*R*,2*R*)-**10d**]. However, the products were always contaminated with *N*,*N*′-diacyl derivative, resulting in decreased yields of the products by repeated purification. The yields of the pure materials (1*R*,2*R*)-**7d** and (1*R*,2*R*)-**10d** after repeated recrystallization and/or chromatography were 22 and 17%, respectively (entries 5, 11). In contrast to these experiments, the preparation of *N*-monoacyl derivative hydrochloride afforded the pure salt of mono-adduct [(1*R*,2*R*)-**7d**·HCl or (1*R*,2*R*)-**10d**·HCl] in a moderate yield (44 or 50%, respectively, entries 6, 12, see Experimental). In the case of the reaction of diamine [(1*R*,2*R*)-**3**] and acid anhydride (**5**), diacylated adduct [(1*R*,2*R*)-**10dd**] was isolated in a 14% yield. Many reactions of 1,2-diamine with acid anhydride reported previously afforded heterocyclic compounds by easy cyclization in the following stage. To the best of our knowledge, only a few examples of *N*-monoacyl derivatives have been reported.[9,10]

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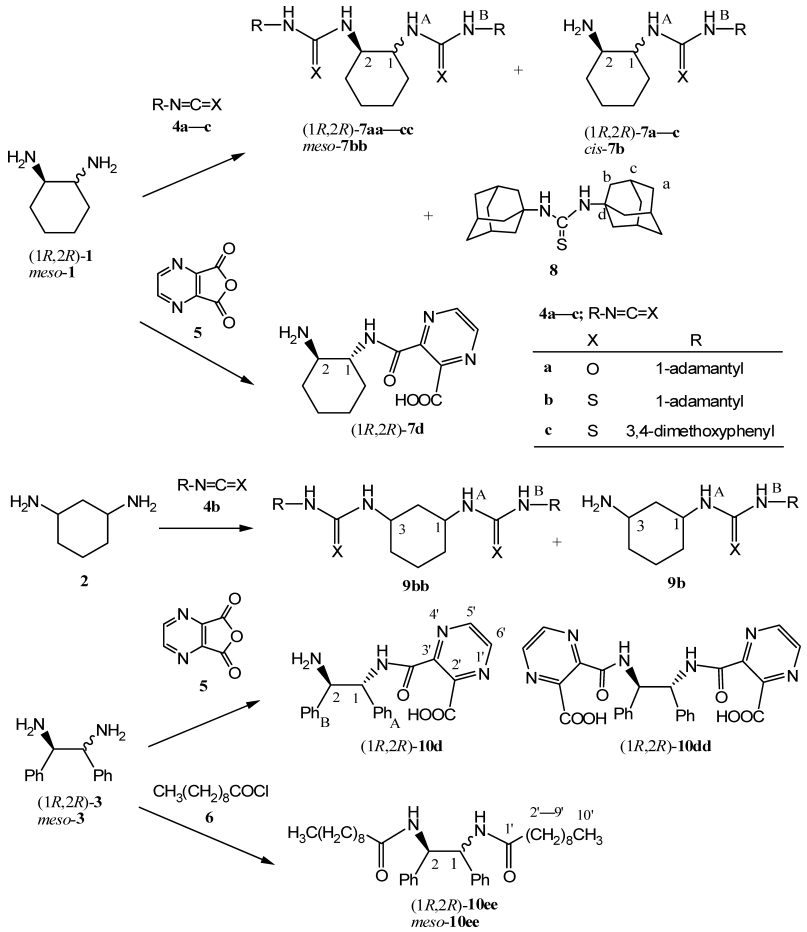

Chart 2

Table 1. Synthesis of *N*-Carbamoyl and *N*-Acyl Derivatives

Entry	Product No.	Diamine	Reagent	Conditions	Yield % (By-product)	Ratio of diamine : reagent (: additive)
1	(1*R*,2*R*)-**7aa**	(1*R*,2*R*)-**1**	**4a**	Reflux, 0.5 h, EtOH	88[a]	1 : 2.1
2	(1*R*,2*R*)-**7bb**	(1*R*,2*R*)-**1**	**4b**	Reflux, 1 h, dry THF	85[a]	1 : 2.1
3	(1*R*,2*R*)-**7c**	(1*R*,2*R*)-**1**	**4c**	rt, 1 d, CH_2Cl_2, N_2	62 (15)[b]	1 : 1
4	(1*R*,2*R*)-**7cc**	(1*R*,2*R*)-**1**	**4c**	Reflux 1 h, dry EtOH	80[a]	1 : 2.1
5	(1*R*,2*R*)-**7d**	(1*R*,2*R*)-**1**	**5**	rt 2 h, acetone	22	1 : 1
6	(1*R*,2*R*)-**7d**·HCl	(1*R*,2*R*)-**1**	**5**	i) rt, 1 h, dry CH_3CN, ii) HCl/EtOH	44	1 : 1
7	*cis*-**7b**	*meso*-**1**	**4b**	rt 5 h, dry THF, N_2	88 (7)[b]	1 : 1
8	*meso*-**7bb**	*meso*-**1**	**4b**	Reflux, 18 h, THF, N_2	93[c]	1 : 2.1
9	**9b**	**2**	**4b**	Reflux, 3 h, dry EtOH, N_2	58 (21)[b]	1 : 1
10	**9bb**			Reflux, 2 h, dry EtOH, N_2	86[a]	1 : 2.1
11	(1*R*,2*R*)-**10d**	(1*R*,2*R*)-**3**	**5**	rt 2 h, acetone	17	1 : 1
12	(1*R*,2*R*)-**10d**·HCl	(1*R*,2*R*)-**3**	**5**	i) rt, 1 h, CH_3CN, ii) HCl/EtOH, rt, 1 h	50 (14)[b]	1 : 1
13	(1*R*,2*R*)-**10ee**	(1*R*,2*R*)-**3**	**6**	Reflux, 5 h, benzene, Na_2CO_3 (additive)	63[a]	1 : 1 (: 1.5)
14	*meso*-**10ee**	*meso*-**3**	**6**	rt, 2 h, benzene, Na_2CO_3 (additive)	71[a]	1 : 2 (: 3.5)

a) Yield after recrystallization. *b*) Yield of the corresponding *N*,*N*′-di-carbamoyl or acyl derivative [(1*R*,2*R*)-**10dd**]. *c*) By-product **8** was obtained in a 4% yield.

Accordingly, the method described above could be used as a conventional procedure for preparing the *N*-monoacyl derivative as its HCl salt. Studies on synthetic applications are now in progress.

Synthesis of *N*,*N*′-Diacyl Derivatives [(1*R*,2*R*)-10ee, and *meso*-10ee] from Symmetrical Diamines These compounds were easily synthesized from the reactions of 1,2-diphenylethylenediamine [(1*R*,2*R*)-**3** or *meso*-**3**] with *n*-decanoyl chloride (**6**). In the case of the reaction with (1*R*,2*R*)-**3**, the ratio of diamine : acyl chloride (1 : 1) in the presence of Na_2CO_3 (1.5 eq), analytical sample of the *N*,*N*′-diacyl derivative (1*R*,2*R*)-**10ee** was isolated in a 65% yield merely by recrystallization from EtOH (entry 13). The reaction of *meso*-**3** and acyl chloride (**6**) also gave *N*,*N*′-diacyl derivative (*meso*-**10ee**) in excellent yield under the reaction conditions of the diamine : acyl chloride : Na_2CO_3 (1 : 2 : 3.5) (entry 14).

Table 2. Physical Data of *N*-Carbamoyl and *N*-Acyl Derivatives (**7**—**10**)

Compd.	mp (°C) (Recryst solvent) Character	Formula	Analysis (%) Calcd (Found) C	H	N	Formula HR-MS *m/z* Calcd (Found)	IR (cm^{-1}) (KBr)
(1*R*,2*R*)-**7bb**	192—194 (EtOH) Colorless needles	$C_{28}H_{44}N_4S_2$	67.15 (67.10	8.86 8.89	11.19 11.17)	$C_{28}H_{45}N_4S_2$ $(M+H)^+$ 501.3086 (501.3101)	3245 (NH) 1545, 1525, 1510, 1300, 1230, 1090 (>N–C=S)
(1*R*,2*R*)-**7c**	168—170 (CH_2Cl_2) Colorless powder	$C_{15}H_{23}N_3O_2S \cdot 0.4H_2O$	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) 1510, 1340, 1235, 1020 (>N–C=S)
(1*R*,2*R*)-**7cc**	112—114 (EtOH) Colorless needles	$C_{24}H_{33}N_4O_4S_2 \cdot 0.3H_2O$	56.51 (56.53	6.44 6.38	10.98 10.95)	$C_{24}H_{33}N_4O_4S_2$ $(M+H)^+$ 505.1943 (505.1929)	3315, 3205 (NH) 1540, 1510, 1235, 1025 (>N–C=S)
(1*R*,2*R*)-**7d**	265—266 (H_2O–EtOH) White powder	$C_{12}H_{16}N_4O_3 \cdot 0.2H_2O$	53.80 (54.05	6.17 6.14	20.91 20.79)	$C_{12}H_{17}N_4O_3$ $(M+H)^+$ 265.1301 (265.1294)	3255, 3100 br (NH, OH) 1655, 1620, 1570, 1500, 1370 (CONH, COOH, Ar)
(1*R*,2*R*)-**7d**·HCl	214—218 (MeOH) White powder	$C_{12}H_{16}N_4O_3 \cdot HCl$	47.92 (47.72	5.70 5.70	18.63 18.44)	$C_{12}H_{17}N_4O_3$ $(M+H)^+$ 265.1301 (265.1308)	*ca.* 3000—2600 br (NH, OH) 1750, 1670, 1545, 1350 (CONH, COOH, Ar)
cis-**7b**	168—170 (MeOH) Colorless crystals	$C_{17}H_{29}N_3S$	66.40 (66.41	9.51 9.57	13.67 13.61)	$C_{17}H_{30}N_3S$ $(M+H)^+$ 308.2160 (308.2159)	3360, 3225 (NH) 1525, 1300, 1230, 1090 (>N–C=S)
meso-**7bb**	172—175 (CH_3CN) Colorless amorphous	$C_{28}H_{44}N_4S_2 \cdot 0.2H_2O$	66.67 (66.57	8.87 8.72	11.11 11.36)	$C_{28}H_{45}N_4S_2$ $(M+H)^+$ 501.3086 (501.3069)	3270 (NH) 1515, 1300, 1230, 1090 (>N–C=S)
8[a]	166—168 — Colorless crystals	$C_{21}H_{32}N_2S \cdot 0.2H_2O$	72.44 (72.56	9.38 9.24	8.05 7.78)	$C_{21}H_{33}N_2S$ $(M+H)^+$ 345.2364 (345.2354)	3435, 3255 (NH) 1545, 1510, 1300, 1220, 1095 (>N–C=S)
9b	163—169 (CH_3CN) White powder	$C_{17}H_{29}N_3S$	66.40 (66.32	9.51 9.54	13.67 13.73)	$C_{17}H_{30}N_3S$ $(M+H)^+$ 308.2160 (308.2159)	3250 (NH) 1535, 1525, 1300, 1220, 1100 (>N–C=S)
9bb	225—226 ($CHCl_3$) White shiny powder	$C_{28}H_{44}N_4S_2 \cdot 0.7H_2O$	65.50 (65.56	8.91 8.80	10.91 10.91)	$C_{28}H_{45}N_4S_2$ $(M+H)^+$ 501.3086 (501.3101)	3295 br (NH) 1535, 1530, 1305, 1250, 1090 (>N–C=S)
(1*R*,2*R*)-**10d**	175—176 (H_2O) Colorless crystals	$C_{20}H_{18}N_4O_3 \cdot 2.0H_2O$	60.29 (60.17	5.57 5.53	14.06 13.87)	$C_{20}H_{19}N_4O_3$ $(M+H)^+$ 363.1457 (363.1466)	3400 br (OH), *ca.* 3000 (NH_3^+) *ca.* 1625, 1380 (COO^-) 1560, 1520 (NH_3^+)
(1*R*,2*R*)-**10d**·HCl	193—197 (EtOH) White solid	$C_{20}H_{18}N_4O_3 \cdot HCl \cdot 0.6H_2O$	58.64 (58.73	4.97 5.08	13.68 13.40)	$C_{20}H_{19}N_4O_3$ $(M+H)^+$ 363.1457 (363.1458)	3440 (OH), *ca.* 3000 br (NH_3^+) 1725 (COOH), 1655 (CONH) 1655, 1520 (NH_3^+)
(1*R*,2*R*)-**10ee**	192.5—193 (EtOH) Colorless needles	$C_{34}H_{52}N_2O_2$	78.41 (78.37	10.06 10.07	5.38 5.36)	$C_{34}H_{53}N_2O_2$ $(M+H)^+$ 521.4107 (521.4111)	3310 (NH), 1640 (CONH) 1540 (CONH)
meso-**10ee**	188—189 (EtOH) White powder	$C_{34}H_{52}N_2O_2$	78.41 (78.36	10.06 10.13	5.38 5.39)	$C_{34}H_{53}N_2O_2$ $(M+H)^+$ 521.4107 (521.4106)	3310 (NH), 1640 (CONH) 1540 (CONH)

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

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

Evaluation of Antiviral Activities The anti-HSV-1 activities of the target compounds were estimated by using a plaque reduction assay in Vero cells.[11] Among these compounds, *N*-monocarbamoyl derivatives (*cis*-**7b**) from 1,2-diaminocyclohexane (**1**) showed more potent anti-HSV-1 activity (EC_{50}=27.0) than the original lead (1*R*,2*R*)-**7a**[1] (EC_{50}=42.0) reported previously.[1] Compound *cis*-**7b**, however, showed a greater cytotoxic property than (1*R*,2*R*)-**7a** and had a much lower selectivity index (CC_{50}/EC_{50}=1.4). The compound **9b** derived from 1,3-diaminocyclohexane (**3**) showed no significant antiviral activity at the concentration of 40 μg/ml.

Among the synthesized compounds, *N*,*N*′-dicarbamoyl derivative (1*R*,2*R*)-**7aa** showed the highest activity (EC_{50}= 16.0 μg/ml), however, this compound showed cytotoxicity (CC_{50}=64.0 μg/ml). In terms of the selectivity index, the compounds (1*R*,2*R*)-**7a** and (1*R*,2*R*)-**7aa** were nearly identical (CC_{50}/EC_{50}=4, 6). The other *N*,*N*′-dithiocarbamoyl derivatives of adamantane didn't show antiviral activity under a dose of 20—40 μg/ml. The *N*-acyl derivatives synthesized above, unfortunately, also showed no significant antiviral activities under a dose of 20—40 μg/ml. The introduction of a pyrazine nucleus as a part of the acyl groups in the target

Table 3. ^{13}C-NMR Spectral Data of *N*-Carbamoyl and *N*-Acyl Derivatives (**7**—**10**)

C No.		(1*R*,2*R*)-**7bb**	(1*R*,2*R*)-**7c**	(1*R*,2*R*)-**7cc**	(1*R*,2*R*)-**7d**	(1*R*,2*R*)-**7d**·HCl	*cis*-**7b**	*meso*-**7bb**	**8**	**9b**	**9bb**	(1*R*,2*R*)-**10d**	(1*R*,2*R*)-**10d**·HCl	(1*R*,2*R*)-**10ee**	*meso*-**10ee**
Cycylohexane	C1	55.55	60.1	59.04	53.92	50.47	56.21	51.68		52.09	50.19	58.09	56.68	59.35	59.37
ring or ethylene	C2	55.55	53.94	59.04	58.14	52.74	49.37	51.68		38.45	38.81	59.27	57.70	59.35	59.37
chain	C3	31.92	34.60	32.24	32.34	29.28	32.97	28.38		47.06	50.19				
	C4	24.13	24.53 (or 24.68)	24.60	26.65	24.04	19.85	21.77		30.84	31.60				
	C5	24.13	24.68 (or 24.53)	24.60	26.17	23.33	23.39	21.77		18.73	22.63				
	C6	31.92	31.23	32.24	33.41	30.75	27.05	28.38		33.57	31.60				
–NHCO–					170.34	162.96						164.44	162.92	174.10	174.10
–NHCS–		179.93	180.52	180.62			178.92	180.29	179.14	178.64	179.52				
C of adamantyl, phenyl, pyrazynyl ring,[e] or alkyl chain		28.97 (c), 35.82 (a), 41.18 (b), 52.77 (d)	109.17 (C2′) 112.03 (C5′), 116.08 (C6′), 132.38 (C1′), 146.11 (C4′), 148.62 (C3′)	110.14 (C2′), 111.91 (C5′), 118.78 (C6′), 128.44 (C1′), 148.67 (C4′), 149.95 (C3′)	146.72 (C6′), 147.60 (C2′), 148.59 (C5′), 151.93 (C3′)	142.52 (C2′), 143.71 (C6′), 145.78 (C5′), 147.15 (C3′)	29.37 (c), 36.02 (b), 41.91 (a), 53.54 (d)	28.95 (c), 35.91 (b), 41.18 (a), 52.78 (d)	29.56 (c), 36.25 (a), 42.26 (b), 54.26 (d)	29.37 (c), 36.00 (a), 41.76 (b), 53.48 (d)	28.96 (c), 35.93 (a), 41.23 (b), 52.57 (d)	127.10 (*p*-Ph$_A$), 127.71 (*o*-Ph$_B$), 127.81 (*p*-Ph$_B$), 127.91, 127.93, 128.17 (*m*-Ph$_A$ & $_B$, *o*-Ph$_A$), 138.50 (on C2), 139.51 (on C1), 142.26 (C6′), 143.80 (C2′), 145.01 (C5′), 150.62 (C3′)	127.71 (*p*-Ph$_A$), 127.92 (*o*-Ph$_A$), 128.23 (*m*-Ph$_A$), 128.39 (*o*-Ph$_B$), 128.53 (*m*-Ph$_B$), 128.81 (*p*-Ph$_B$), 134.67 (on C2), 138.18 (on C1), 142.07 (C2′), 143.92 (C5′), 146.17 (C6′), 147.54 (C3′)	14.04 (C10′), 22.63 (C9′), 25.77 (C3′), 29.26, 29.32, 29.38, 29.46 (C4′—7′), 31.85 (C8′), 36.74 (C2′), 127.52 (*o*-Ph), 127.69 (*p*-Ph), 128.50 (*m*-Ph), 138.90 (on C1, 2)	14.04 (C10′), 22.64 (C9′), 25.77 (C3′), 29.27, 29.32, 29.38, 29.46 (C4′—7′), 31.86 (C8′), 36.74 (C2′), 127.52 (*o*-Ph), 127.70 (*p*-Ph), 128.51 (*m*-Ph), 138.90 (on C1, 2)
–OCH_3			55.64, 55.89	56.11, 55.30											
–COOH					173.91	166.73						168.47	166.79		
Solvent		DMSO-d_6	DMSO-d_6	$CDCl_3$	D_2O	DMSO-d_6	$CDCl_3$	DMSO-d_6	$CDCl_3$	$CDCl_3$	DMSO-d_6	DMSO-d_6	DMSO-d_6	$CDCl_3$	$CDCl_3$

Table 4. ^{1}H-NMR Spectral Data (*J* in Hz) of *N*-Carbamoyl and *N*-Acyl Derivatives (**7**—**10**)

H No.		(1*R*,2*R*)-**7bb**	(1*R*,2*R*)-**7c**	(1*R*,2*R*)-**7cc**	(1*R*,2*R*)-**7d**	(1*R*,2*R*)-**7d**·HCl	*cis*-**7b**	*meso*-**7bb**	**8**	**9b**	**9bb**	(1*R*,2*R*)-**10d**	(1*R*,2*R*)-**10d**·HCl	(1*R*,2*R*)-**10ee**	*meso*-**10ee**
Cycylohexane ring or	H1	4.12 br s	3.87 br s	4.39 m	4.07 dt (11.0, 4.3)	3.90 m	4.19 br s	4.42 br s		4.47 br s	3.99 m	5.53 dm (7.6)	5.51 dd (10.7, 9.2)	5.27 m	5.27 m
ethylene chain	H2	4.12 br s	2.56 m	4.39 m	3.25 dt (11.0, 4.3)	3.19 m	3.08 d (4.3)	4.42 br s		*ca.* 2.0[c] m	0.87[c] q (11.6), 2.19[c] br d (11.6)	4.43 s	4.99 d (10.7)	5.27 m	5.27 m
	H3	1.06—1.08 m, 2.11 m	1.17[c] m, 1.84 m	*ca.* 1.2 m, 2.07 d (12.1)	1.60[c] m 2.17 m	1.44 m, 2.09 d (13.1)	*ca.* 1.7[c] m	1.55 m		3.21 br s	3.99 m				
	H4	1.21 m, 1.61 m	1.17[c] m, 1.62[c] m	*ca.* 1.3 m, *ca.* 1.7 m	1.41 m, 1.85 m	1.24 m, 1.71 m	1.41[c] m, 1.47 m	1.34 m, 1.44[c] m		*ca.* 1.78[c] m	0.94[c] dq (12.3, 3.3), 1.89 d (12.3)				
	H5	1.21 m, 1.61 m	1.17[c] m, 1.62[c] m	*ca.* 1.3 m, *ca.* 1.7 m	1.41 m, 1.85 m	1.26 m, 1.73 m	1.39[c] m, 1.60 m	1.34 m, 1.44[c] m		1.42[c] m, 1.76[c] m	1.24 qm (13.1), 1.69 dm (13.7)				
	H6	1.06—1.08 m, 2.11 m	1.17[c] m, 2.01 m	*ca.* 1.2 m, 2.07 d (12.1)	1.52[c] m, 2.07 m	1.52 m, 1.83 m	1.39[c] m, 2.0[c] m	1.55 m		1.40[c] m, 1.76[c] m	0.94[c] dq (12.3, 3.3), 1.89 d (12.3)				
NH_2, or NH_3^+			*ca.* 3.5[c]			8.06[d] br s	*ca.* 1.6[c] br s			*ca.* 1.65[a,c] m		3.87[d] br s	*ca.* 8.7[a] br s		
Ch or Et–NH_ACXNH_B–[b],	H_A	7.07[a] br d (*J*=5.2)	7.4[a] br s	6.36[a] br s		8.96[a] d (9.2)	6.84[a] br s	6.94[a] br s	*ca.* 5.6 br s	7.42[a] br s	7.06[a] d (7.9)	9.36[a] dm (7.6)	9.77[a] d (9.2)	7.02[a] br s	7.00[a] br s
NHC=O	H_B	6.91[a] s	9.3[a] br s	7.58[a] br s			6.06[a] br s	7.17[a] br s		5.88[a] br s	6.72[a] br s				
H of adamantyl, phenyl, pyrazynylring, or alkyl chain		1.61 br s (a),[c] 2.01 br s (c), 2.11 br s (b)[c]	6.84 dd (8.5, 2.4) (H6′), 6.89 d (8.9) (H5′), 7.08 d (2.1) (H2′)	6.83 br s (H2′), 6.86—6.90 m (H5′,6′)	8.70 d (2.6) (H6′), 8.76 d (2.6) (H5′)	8.83 d (2.4) (H6′), 8.86 d (2.4) (H5′)	1.69[c] m (b), 2.02[c] br t (14.5) (a), 2.11[c] br s (c)	1.62 br s (a), 2.02 br s (c), 2.14 br s (b)	1.69 br s (a), 2.11 br s (c,b)	*ca.* 1.67[c] m (a), 2.02[c] m (b), 2.11[c] br s (c)	1.61 br s (a), 2.01 br s (c), 2.14[c] d (2.4)	7.12 tm (7.3) (*p*-Ph_A), 7.15—7.19 m (*p*-Ph_B, *m*-Ph_A & B), 7.23 m (*o*-Ph_B), 7.29 m (*o*-Ph_A), 8.57 d (2.4) (H6′), 8.64 d (2.4) (H5′)	7.12 t (7.0) (*p*-Ph_A), 7.16 t (7.0) (*m*-Ph_A), 7.25—7.79 m (*o*-Ph_A, *p*,*m*-Ph_B), 7.34 dd (7.9, 1.5) (*o*-Ph_B), 8.86 d (2.4) (H5′), 8.87 d (2.4) (H6′)	0.88 t (7.0) (H10′), 1.24 m (H4′—9′), 1.59 m (H3′), 2.19 t (7.8) (H2′), 7.12 m (7.0) (*o*-Ph), 7.15 m (*p*,*m*-Ph)	0.88 t (7.0) (H10′), 1.24 m (H4′—9′), 1.59 m (H3′), 2.19 t (7.6) (H2′), 7.12 m (7.0) (*o*-Ph), 7.15 m (*p*,*m*-Ph)
$-OCH_3$			3.73 s, 3.74 s	3.88 s (on C3′), 3.89 s (on C4′)											
–COOH						13.5 br s[a]									
Solvent		DMSO-d_6	DMSO-d_6	$CDCl_3$	D_2O	DMSO-d_6	$CDCl_3$	DMSO-d_6	$CDCl_3$	$CDCl_3$	DMSO-d_6	DMSO-d_6	DMSO-d_6	$CDCl_3$	$CDCl_3$

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

Table 5. Antiviral Activity against HSV-1

	EC_{50} (μg/ml)	CC_{50} (μg/ml)	CC_{50}/EC_{50}
(1*R*,2*R*)-**7a**[a]	42.0	250	6
cis-**7b**	27.0	37.4	1.4
9b	>40	48.1	—
(1*R*,2*R*)-**7aa**	16.0	64.0	4
meso-**7bb**	>40	>320	—
Aciclovir[b]	0.2—0.9	>800	—

a) This compound (1*R*,2*R*)-**7a** has been reported.[1] *b*) Data were taken from ref. 13.

molecule imparts at least a reversed physical property (an increasing hydrophilic character) to the *N*-acylated product of diamines. However, the trial compounds prepared in this study [(1*R*,2*R*)-**7d** and (1*R*,2*R*)-**10d**] also possessed no significant antiviral activity under the concentration of 40 μg/ml. Some of the antiviral active compounds by plaque reduction assay are shown in Table 5. The *N*,*N*′-dicarbamoyl derivative [(1*R*,2*R*)-**7aa**] possesses two adamantyl urea moieties and has no basic primary amine functionality in the molecule. In comparison with antiviral activity in adamantyl derivatives such as 1-aminoadamantane[4] and *N*-(1-adamantyl)thiourea,[12] this fact is quite interesting for the search of antiviral compounds (leads), providing useful information for molecular modification of adamantane series. However, since the plaque reduction assay applied in this study offers no advantage in finding detailed information of the mode of action of the antiviral compounds, we need to make more precise experiments for further discussion.

Experimental

The melting points were determined using a micro melting point apparatus (Yanagimoto MP-S3) without correction. IR spectra were measured by a Shimadzu FTIR-8100 IR spectrophotometer. Low- and high-resolution mass spectra (LR-MS and HR-MS) were taken by the 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) and dimethyl sulfoxide (DMSO)-d_6 (39.50 ppm)] for ^{13}C-NMR. In D_2O, the chemical shifts were referenced to 3-(trimethylsilyl)propionic acid-d_4 sodium salt (TSP-d_4). 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 or flash column chromatography was performed on silica gel (Able-Biott or Fuji Silysia FL40D, respectively) with a UV detector. Commercially available starting materials were used without further purification.

General Procedure for Addition of Iso(thio)cyanate to Diamines A solution of a diamine (2—5 mmol) and an appropriate iso(thio)cyanate (molar ratios listed in Table 1) in an appropriate solvent (20—60 ml) was stirred at room temperature or refluxed for the indicated time (Table 1). Except for the synthesis of (1*R*,2*R*)-**7c**, after the evaporation of the solvent of the reaction mixture, purification of the residue by recrystallization or flash chromatography afforded the pure product. Physical and spectroscopic data are listed in Tables 2—4.

***N*,*N*″-(1*R*,2*R*)-1,2-Cyclohexanediylbis[*N*′-tricyclo[3.3.1.1^{3,7}]dec-1-ylurea] [(1*R*,2*R*)-7aa]** This compound was prepared from diamine (1*R*,2*R*)-**1** and isocyanate **4a** at a ratio of 1 : 2.1 and purified by recrystallization from EtOH (entry 1 in Table 1). The physical and spectroscopic data for this compound have already been reported.[1]

***N*,*N*″-(1*R*,2*R*)-1,2-Cyclohexanediylbis[*N*′-tricyclo[3.3.1.1^{3,7}]dec-1-ylthiourea] [(1*R*,2*R*)-7bb]** This product was obtained by recrystallization from EtOH (entry 2).

***N*-[(1*R*,2*R*)-2-Aminocyclohexyl]-*N*′-(3,4-dimethoxyphenyl)thiourea [(1*R*,2*R*)-7c]** After the reaction was completed, the precipitated crude product of the title compound was filtrated and purified by recrystallization from EtOH. After concentration of the filtrate, separation by centrifugal chromatography (SiO_2, 2—5% v/v CH_2Cl_2 in EtOH) also gave additional pure products of (1*R*,2*R*)-**7c** and *N*,*N*′-dithiocarbamoyl derivative (1*R*,2*R*)-**7cc** (entry 3).

***N*,*N*″-(1*R*,2*R*)-1,2-Cyclohexanediylbis(*N*′-3,4-dimethoxyphenylthiourea) [(1*R*,2*R*)-7cc]** After evaporation of the solvent, recrystallization from EtOH afforded a pure product [(1*R*,2*R*)-**7cc**] (entry 4).

***N*-(*cis*-2-Aminocyclohexyl)-*N*′-tricyclo[3.3.1.1^{3,7}]dec-1-ylthiourea (*cis*-7b)** After evaporation of the solvent, flash column chromatography (SiO_2, CH_2Cl_2/EtOH/28% NH_4OH, 980 : 15 : 5, v/v) gave pure products (*cis*-**7b** and *meso*-**7bb**) (entry 7). An analytical sample was obtained by recrystallization from MeOH.

***N*,*N*″-(1*R*,2*S*)-rel-1,2-cyclohexanediylbis(*N*′-tricyclo[3.3.1.1^{3,7}]dec-1-ylthiourea) (*meso*-7bb) and *N*,*N*′-Bis(tricyclo[3.3.1.1^{3,7}]dec-1-yl)thiourea (8)** After evaporation of the solvent, flash column chromatography (SiO_2, CH_2Cl_2/EtOH/28% NH_4OH, 980 : 15 : 5, v/v) gave pure products (*meso*-**7bb** and **8**) (entry 8). Recrystallization from CH_3CN gave an analytical sample of *meso*-**7bb**.

***N*-(3-Aminocyclohexyl)-*N*′-tricyclo[3.3.1.1^{3,7}]dec-1-ylthiourea (9b)** After evaporation of the solvent, flash column chromatography (SiO_2, CH_2Cl_2/EtOH/28% NH_4OH, 980 : 15 : 5, v/v) gave pure products of **9b** and *N*,*N*′-dithiocarbamoyl derivative **9bb**. For analytical use of **9b**, recrystallization from CH_3CN was employed (entry 9).

***N*,*N*″-1,3-Cyclohexanediylbis[*N*′-tricyclo[3.3.1.1^{3,7}]dec-1-ylthiourea] (9bb)** After evaporation of the solvent, recrystallization from $CHCl_3$ gave a pure product **9bb** (entry 10).

Preparation of 3-[[[(1*R*,2*R*)-2-Aminocyclohexyl]amino]carbonyl]-pyrazinecarboxylic Acid [(1*R*,2*R*)-7d] A solution of acid anhydride **5** (4.5 mmol) in acetone (20 ml) was added dropwise to a stirred solution of diamine (1*R*,2*R*)-**1** (4.5 mmol) in acetone (20 ml) under the conditions shown in Table 1 (entry 5). After cooling, the reaction mixture was filtrated to afford a crude product. Recrystallization twice from EtOH–H_2O gave a pure product (1*R*,2*R*)-**7d**.

Preparation of [(1*R*,2*R*)-7d]·HCl The reaction was carried out for (1*R*,2*R*)-**7d** in a similar manner as that described above except for using dry CH_3CN as a solvent. The filtrated crude product from the reaction mixture was suspended in MeOH (50 ml), added with 1 M HCl/EtOH (7 ml), and evaporated under reduced pressure. The resulting material was recrystallized from MeOH to give the desired [(1*R*,2*R*)-**7d**]·HCl (entry 6).

Preparation of 3-[[[(1*R*,2*R*)-2-Amino-1,2-diphenylethyl]amino]carbonyl]pyrazinecarboxylic Acid [(1*R*,2*R*)-10d] This compound was also prepared in a similar manner to the compound (1*R*,2*R*)-**7d**. Crude product was purified by recrystallization from H_2O twice to give an analytically pure sample (1*R*,2*R*)-**10d** (entry 11).

Preparation of [(1*R*,2*R*)-10d]·HCl A solution of acid anhydride **5** (2 mmol) in dry CH_3CN (10 ml) was added to a stirred solution of diamine (1*R*,2*R*)-**3** (2 mmol) in dry CH_3CN (20 ml) under the conditions shown in Table 1 (entry 12). The resulting mixture was filtrated to give crude mixed products of (1*R*,2*R*)-**10d** and *N*,*N*′-diacyl derivative (1*R*,2*R*)-**10dd**, the mixture of which was suspended in EtOH (12 ml) and added with 1 M HCl/EtOH (16 ml) with stirring for 1 h. At the beginning of the addition of HCl, the reaction mixture became a transparent solution, and then precipitation gradually occurred. After cooling the mixture, filtration of the precipitated material gave (1*R*,2*R*)-**10dd** in a 14% yield. The filtrate was evaporated and the residue was purified by recrystallization to afford the product (1*R*,2*R*)-**10d** (entry 12).

3,3′-[[(1*R*,2*R*)-1,2-Diphenyl-1,2-ethanediyl]bis(iminocarbonyl)]bispyrazine-carboxylic Acid [(1*R*,2*R*)-10dd] White solid: mp 174—175 °C (from HCl–EtOH); HR positive ion FAB-MS, Calcd for $C_{26}H_{21}N_6O_6$ $(M+H)^+$: 513.1523. Found: 513.1526. IR (KBr) cm^{-1}: *ca.* 3400 br (OH), 3295 (CONH), *ca.* 3000—2500 br (COOH), 1720 (COOH), 1650 (CONH). ^{1}H-NMR (DMSO-d_6) δ: 5.70 (2H, dt, *J*=8.5, 2.1 Hz, PhCH), 7.15 (2H, dt, *J*=7.3, 1.2 Hz, *p*-Ph), 7.22 (4H, dt, *J*=7.3, 1.2 Hz, *m*-Ph), 7.39 (4H, dd, *J*=7.3, 1.2 Hz, *o*-Ph), 8.78 (2H, d, *J*=2.4 Hz, H6′), 8.81 (2H, d, *J*=2.4 Hz, H5′), 9.67 (2H, d, *J*=8.4 Hz, NH). ^{13}C-NMR (DMSO-d_6) δ_C: 57.08 (PhCH), 127.15 (*p*-Ph), 127.53 (*o*-Ph), 128.02 (*m*-Ph), 139.63 (4′-Ph), 142.69 (C2′), 143.96 (C6′), 145.93 (C5′), 147.57 (C3′), 162.94 (CONH), 166.63 (COOH). *Anal.* Calcd for $C_{26}H_{20}N_6O_6 \cdot 1.8H_2O$: C, 57.31; H, 4.37; N, 15.42. Found: C, 57.37; H, 4.37; N, 15.19.

Preparation of *N*,*N*′-[(1*R*,2*R*)-1,2-Diphenyl-1,2-ethanediyl]bisdecanamide [(1*R*,2*R*)-10ee] *n*-Decanoyl chloride **6** (2 mmol) was added to a stirred mixture of diamine (1*R*,2*R*)-**3** (2 mmol) and Na_2CO_3 (3.5 mmol) at room temperature under an N_2 atmosphere. After refluxing for 5 h, the reaction mixture received addition of saturated aqueous $NaHCO_3$ (30 ml), ex-

tracted with CH_2Cl_2 (3×30 ml) and washed with brine (30 ml), and the organic layer was dried ($MgSO_4$), filtered, and evaporated to give a crude product, which was purified by recrystallization from EtOH to give this product, (1*R*,2*R*)-**10ee** (entry 13).

Preparation of *N*,*N*′-[(1*S*,2*S*)-1,2-Diphenyl-1,2-ethanediyl]bis(decanamide) [*meso*-10ee] This compound was prepared in a similar manner to the above compound (1*R*,2*R*)-**10ee**, except for the mole ratio of reagents as shown in Table 1 (entry 14).

The physical and spectroscopic (^{1}H- and ^{13}C-NMR) data for the prepared compounds (**7**–**10**) are summarized in Tables 2—4.

Antiviral Activity Assay and Cytotoxicity of Target Compounds (7—10) The antiviral activities of synthesized compounds were measured by using a plaque reduction assay[11] as described in our previous paper.[1] For some of the active compounds in this study, the calculated EC_{50} and cytotoxicity (CC_{50}) values are summarized in Table 5. Other compounds showed no significant anti-HSV-1 activity under the dose of 20—40 μg/ml. The cytotoxicity of the compounds *cis*-**7b**, **9b**, (1*R*,2*R*)-**7aa**, and *meso*-**7bb** were determined by the method described in a previous paper.

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References and Notes

1) Mibu N., Yokomizo K., Miyata T., Sumoto K., *Chem. Pharm. Bull.*, **55**, 1406—1411 (2007) and related references cited therein.
2) Mibu N., Yokomizo K., Kashige N., Miake F., Miyata T., Uyeda M., Sumoto K., *Chem. Pharm. Bull.*, **55**, 111—114 (2007).
3) Spasov A. A., Khamidova T. V., Bugaeva L. I., Morozov I. S., *Pharm. Chem. J.* (translation of *Khimiko-Farmatsevticheskii Zhurnal*), **34**, 1—7 (2000).
4) Setaki D., Tataridis D., Stamatiou G., Kolocouris A., Foscolos G. B., Fytas G., Kolocouris N., Padalko E., Neyts J., De Clercq E., *Bioorg. Chem.*, **34**, 248—273 (2006).
5) For example, see: Kim I., Tsai H., Nishi K., Kasagami T., Morisseau C., Hammock B. D., *J. Med. Chem.*, **50**, 5217—5226 (2007).
6) Stetter H., Wulff C., *Chem. Ber.*, **95**, 2302—2304 (1962).
7) Herr R. J., Kuhler J. L., Meckler H., Opalka C. J., *Synthesis*, **2000**, 1569—1574 (2000).
8) Within 1 h, disappearance of the starting compounds of diamine and acid anhydride could be detected by TLC monitoring.
9) Salakhov M. S., Umaeva V. S., Salakhova Ya. S., Idrisova S. Sh., *Russ. J. Org. Chem.* (translation of *Zhurnal Organicheskoi Khimii*), **35**, 397—401 (1999).
10) Hassan M. A., Zayed S. E., El-Gaziri W. N., Metwally S. A., *Arch. Pharm.*, **324**, 185—187 (1991).
11) Schinazi R. F., Peters J., Williams C., Chance D., Nahmias A., *Antimicrob. Agents Chemother.*, **22**, 499—507 (1982).
12) Nishimura T., Toku H., Fukuyasu H., Kawakami M., Kazuno Y., Sekizawa Y., *Kitasato Arch. Exp. Med.*., **52**, 15—21 (1979).
13) Yamanaka G., Tuomari A. V., Hagen M., McGeever-Rubin B., Terry B., Haffey M., Bisacchi G. S., Field A. K., *Mol. Pharmacol.*, **40**, 446—453 (1991).