Molecular Symmetry and Biological Activities of New Symmetrical Tris(2-aminoethyl)amine Derivatives

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In terms of molecular symmetry and bioactivity, new C_3- and C_S-symmetrical derivatives based on the tris(2-aminoethyl)amine scaffold were designed and synthesized. The synthesized compounds were evaluated for antiviral activity with herpes simplex virus type 1 (HSV-1) by a plaque reduction assay and for cytotoxic activity with Vero cells. Most of the compounds showed no significant anti-HSV-1 activity, but some of the symmetrical derivatives showed high levels of cytotoxic activitiy.

Key words tris(2-aminoethyl)amine; tripodal; anti-herpes simplex virus type 1; C_3 symmetry; C_S symmetry; plaque reduction assay

There have been many reports on the molecular recognition properties of symmetrical molecules,[1] and it is known that the symmetrical feature is frequently observed in many biological stages. Two-fold and three-fold symmetrical macromolecular structures are common features in specialized biological functions.[2—4] Regarding the molecular symmetry, small symmetrical molecules with a C_2-symmetrical or C_3-symmetrical structure have often been found in various synthetic biologically active compounds.[5—7]

In the course of our work on new antiviral compounds, we have presented a new class of antiviral candidates. For example, we have already reported that most of the triarylmethane derivatives showed a wide range of antiviral activities against herpes simplex virus type 1 (HSV-1).[8,9] In continuation with our work for finding potent antiviral derivatives, synthetic molecular modifications directed our attention to the molecular symmetry for the biological activity. Our recent studies on heteroaryl-substituted triarylmethane derivatives indicated that compounds bearing a prochiral symmetric feature (possessing C_S symmetry) showed a higher level of antiviral activity than that of the corresponding C_3-symmetrical molecules[9,10] (see Fig. 1). In the area of molecular recognition, a tripodal arrangement of cell-specific ligands is an ideal recoginition motif for cell surfaces, and it is known that a number of receptors share a common binding motif with three-fold symmetry.[7,11—14] In order to find new promising candidates, we have chosen tris(2-aminoethyl)amine (TREA) as a new scaffold. TREA derivatives have been studied extensively in the field of coordination chemistry; however, little is known about their biological activities. To our knowledge, some TREA derivatives have been reported to work as membrane anion transporters[15,16] or as HIV-1 protease inhibitors.[17] In addition to these previous results, the flexibility of a TREA scaffold may enable recognition of the sites of target biological molecules, and this possibility prompted us to select TREA as a template for target tripodal-type molecules.

Here, we describe the synthesis of new symmetrical tri-substiuted TREA derivatives and the results of biological evaluation of the synthesized compounds for anti-HSV-1 activity.

Results and Discussion

Synthesis The synthetic strategies for two classes of target symmetrical compounds are outlined in Chart 1. C_3-symmetrical compounds are considered to be easily obtained by the reaction of TREA and electrophiles. Therefore, we planned the synthesis of two types of molecules, which contain amide or ureido functionality, by acylation of TREA with acylating reagents or addition of TREA to an isothiocyanate, respectively. The results of the synthesis of C_3-symmetrical compounds are shown in Chart 2 and Table 1. The results for C_S-symmetrical compounds are also shown in Chart 3 and Table 2. Thus, C_3-symmetrical compounds **6a**[18,19] and **6b** were easily obtained by the reactions of TREA with the corresponding acyl chloride **2a** or **2b** using triethylamine (TEA) (Entries 1 and 2 in Table 1). In case of the reaction with nicotinoyl chloride·HCl (**2c**·HCl), preparation by a similar procedure resulted in a low yield of **6c** (10%, Entry 3). Change of the solvent and the reagent stoichiometry resulted in improved yield of **6c** to 70% (Entry 4). The preparation of compound **6c** was also achieved by condensation with carboxylic acid **2c′**, though in a low yield (33%, Entry 5). Compound **7** was prepared by condensation with cinnamic acid derivative **3** (35%, Entry 6). The addition reaction of TREA to isothiocyanate **5** gave the target compound **11** quantitatively (Entry 7).

For the synthesis of C_S-symmetrical compounds **9** from compound **1**, we first planned a two-step method *via* diacylated TREA **8** as shown in Chart 3. In the first step, acylation of TREA by acyl chloride **2a** gave tri-acylated derivative **6a** predominantly even in a 1 : 0.1 ratio of TREA : **2a** (Entry 8). Acylation using the free carboxylic acid **2a′** gave a better yield (49%) of the intermediate **8** (Entry 9). The second step

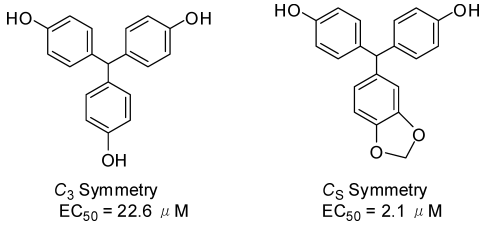

Fig. 1. Structures of Antiviral Compounds with C_3 and C_S Symmetry

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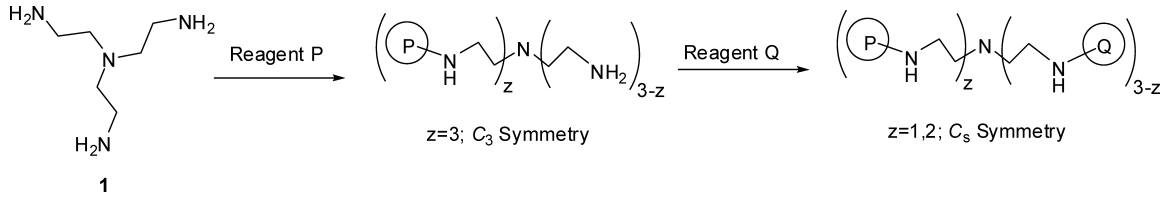
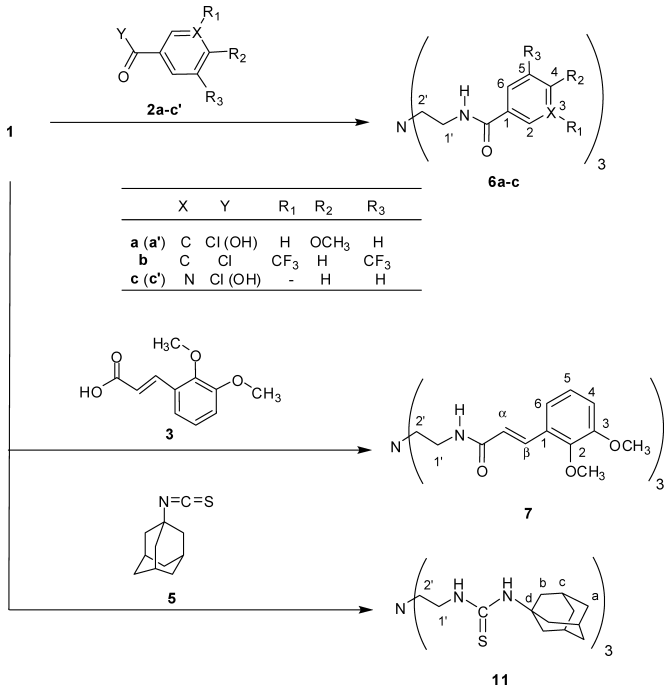


Chart 1. Synthetic Outline for Target TREA Derivatives



	X	Y	R_1	R_2	R_3
a (a')	C	Cl (OH)	H	OCH_3	H
b	C	Cl	CF_3	H	CF_3
c (c')	N	Cl (OH)	-	H	H

Chart 2. Synthesis of C_3-Symmetrical TREA Derivatives

of the reaction leading to the desired **9** by the reaction of **8** with **5** gave a moderate yield (Entry 10). The total yield under the above reaction conditions of the steps through compound **8** (Entries 9 and 10) was 28%. Another method conducted in a one-pot reaction with the inverse order of addition of reagents (**5**, **2a**) resulted in an increased yield of **9** (45%) together with a byproduct **10** in 11% yield (Entry 11). In the same method, we changed the ratio of reagents (**5**:**2a**) from 1:2 to 2:1 for the synthesis of **10**, and we obtained **10** (23%) and tri-acylated compound **11** (28% in Entry 12).

For the synthesis of an intermediate for the target compound **13**, acylation of TREA by acid anhydride **4** resulted in the formation of bis-acylated compound **12** (25%) together with tri-acylated compound **15** (11% in Entry 13). In a manner identical to that for the synthesis of **9**, the product **13** (34%) was obtained together with a byproduct **14** (7% in Entry 14). As a result, we recognized that it seems to be difficult to achieve high reaction selectivity for bis- or mono-substituted TREA derivatives. Furthermore, the method using a one-pot reaction with the combination of **5** and **2a** or **5** and **4** gave C_S-symmetrical **9** or **13** in a moderate yield.

The C_3- or C_S-symmetrical structures of the target products were easily confirmed by ^{13}C-NMR spectroscopic analysis. All of the structures of the synthesized compounds were also easily confirmed by spectroscopic and elemental analyses except for the elemental analyses data of compounds **8** and **14** (see Experimental).

Evaluation of Antiviral Activity and Discussion The anti-HSV-1 activities of all of the synthesized TREA deriva-

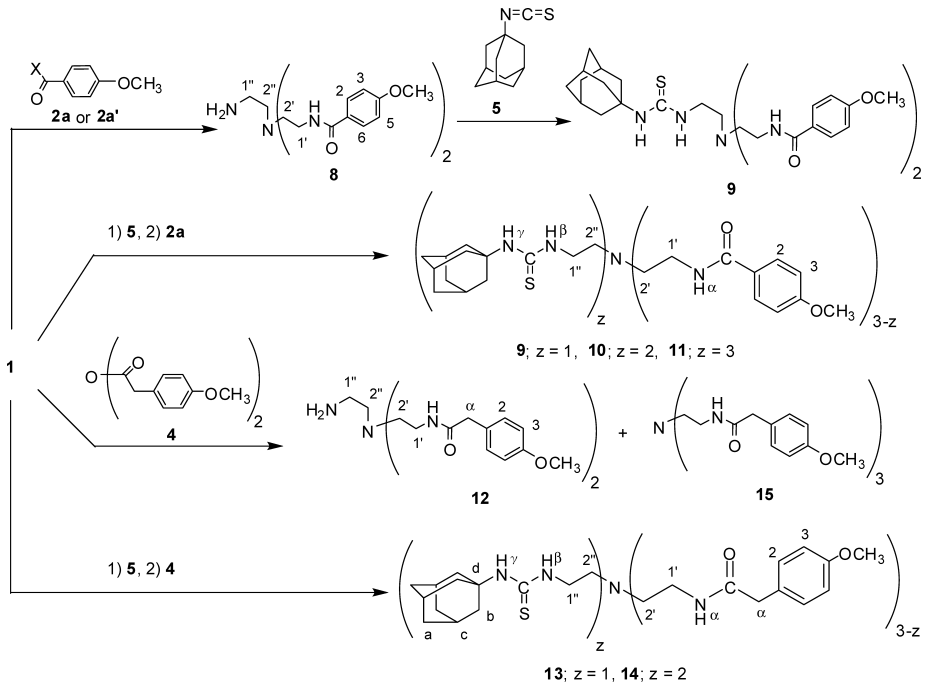


Chart 3. Synthesis of C_S-Symmetrical TREA Derivatives

tives (except for **8**, **12**, **14**) were evaluated by plaque reduction assays,[20] and the derivatives were evaluated for cytotoxicity with Vero cells. The results for compounds **6a**—**c**, **7**, **9**—**11**, **13**, and **15** are summarized in Table 3, and aciclovir[21] is also listed as a positive control. All compounds evaluated in this study showed no distinct inhibitory concentration (EC_{50}) values for antiviral activity less than the dose of 100 μM. Among the compounds tested, compounds **6b** and **13** showed high levels of cytotoxicity against Vero cells (IC_{50}=17.7, 21.5 μM), and compound **9** also showed some cytotoxicity (IC_{50}=60.9 μM).[22]

It is well known that lipophilicity of the compound as the logarithm of the partition coefficient (log P value) is one of the important factors for its biological activities.[23,24] It is interesting that compound **6b**, which has the largest log P (shown in Table 3),[25] showed a high level of cytotoxicity (IC_{50}=17.7 μM). In contrast, other C_3-symmetrical compounds (**6a**, **6c**, **7**, **11**, **15**), which have lower log P values (<7.41) than the value of highly lipophilic **6b** (log P=8.30), showed low levels of cytotoxicity against Vero cells.

In addition, two compounds (**9** and **13**) showed considerably high levels of cytotoxicity (IC_{50}=60.9, 21.5 μM, respectively).[26—28] It is of great interest that the calculated log P

Table 1. Synthesis of C_3-Symmetrical TREA Derivatives

Entry	Reagent	Conditions	Ratio of TREA : Reagent : TEA or (TPP)	Product (yield %)
1	**2a**	0°C 1 h, rt 0.5 h, dry CH_2Cl_2, N_2	1 : 3.3 : 3.3	**6a** (68)
2	**2b**	0°C 1 h, rt 0.5 h, dry CH_2Cl_2, N_2	1 : 3.5 : 3.5	**6b** (66)
3	**2c**·HCl	0°C 1 h, dry CH_2Cl_2, N_2	1 : 3.3 : 3.3	**6c** (10)
4	**2c**·HCl	0°C 1 h, dry THF, N_2	1.2 : 1 : 1.4	**6c** (70)[b]
5	**2c′**	i) **1**, **2c′**, 70°C 1 h, ii) TPP, reflux 1 d, pyridine	1 : 3 : (3)	**6c** (33)
6	**3**	i) **3**, NHS, rt 0.5 h, N_2, ii) DCC, rt 3 h, N_2, iii) **1**, rt 1 d, dry THF, N_2	1 : 3 : 3 : 3[a]	**7** (35)
7	**5**	Rt 1 d, dry CH_2Cl_2	1 : 4	**11** (99)

a) Ratio of **1** : **3** : NHS : DCC=1 : 3 : 3 : 3. *b*) The yield was based on the starting compound **2c**·HCl.

Table 2. Synthesis of C_S-Symmetrical TREA Derivatives

Entry	Reagent	Conditions	Ratio of TREA : Reagent : TEA or (CDI)	Product (yield %)
8	**2a**	0°C 0.5 h, rt 3 h, dry CH_2Cl_2, N_2	1 : 0.1 : 0.1	**6a** (80), **8** (10)[a]
9	**2a′**	i) **2a′**, CDI, reflux 2 h, ii) **1**, reflux 1 d, dry CH_2Cl_2, N_2	1 : 2 : (2)	**6a** (10), **8** (49)[a]
10[b]	**5**	Rt 3 h, dry CH_2Cl_2	1 : 3.3	**9** (58)
11	i) **5**, ii) **2a**	i) **1**, **5**, rt 2.5 h, ii) TEA, **2a**, 0°C 0.5 h, dry CH_2Cl_2, N_2	1 : 1 : 2 : 2[c]	**9** (45), **10** (11)
12	i) **5**, ii) **2a**	i) **1**, **5**, rt 2.5 h, ii) TEA, **2a**, 0°C 0.5 h, dry CH_2Cl_2, N_2	1 : 2 : 1 : 1[d]	**10** (23), **11** (28)
13	**4**	−78°C 0.5 h→rt overnight, dry CH_2Cl_2, N_2	5 : 3	**12** (25), **15** (11)[e]
14	i) **5**, ii) **4**	i) **1**, **5**, rt 1 h, ii) TEA, **4**, rt 1 h, dry CH_2Cl_2, N_2	1 : 1 : 2 : 2[f]	**13** (34), **14** (7)[g]

a) The yield was based on the starting compounds **2a** or **2a′**. *b*) The starting material **8** was used in this entry. *c*) Ratio of **1** : **5** : **2a** : TEA=1 : 1 : 2 : 2. *d*) Ratio of **1** : **5** : **2a** : TEA=1 : 2 : 1 : 1. *e*) The yield was based on the starting compound **4**. *f*) Ratio of **1** : **5** : **4** : TEA=1 : 1 : 2 : 2. *g*) The yield was based on TREA (**1**).

Table 3. Antiviral Activity (EC_{50}) against HSV-1, Cytotoxicity (IC_{50}) against Vero Cells, and Calculated log P

Compd.	**6a**	**6b**	**6c**	**7**	**9**	**10**	**11**	**13**	**15**	Aciclovir[a]
EC_{50} (μM)	>100	N.D.	>100	>100	N.D.	>100	>100	N.D.	181.7	1.1
IC_{50} (μM)	>200	17.7	>200	>200	60.9	579.5	>200	21.5	227.4	>444
log P	2.25	8.30	−0.93	2.71	3.97	5.69	7.41	3.83	2.04	−0.76

a) Data were taken from ref. 21.

values for these compounds were around *ca.* 3.9 (see Table 3) and that both derivatives belong to a class of compounds with C_S symmetry. In contrast, the corresponding C_3-symmetrical compounds (**6a**, **11**, **15**) showed no significant cytotoxicity (IC_{50}=>200 μM). It is also noteworthy that the cytotoxicity of C_S-symmetrical compound **10**, which consists of the same functional groups as those of C_S-symmetrical compound **9** with a different ratio of the groups, showed a surprisingly decreased cytotoxic property (IC_{50}=579.5 μM) (see represented molecular structures in Chart 3). These results apparently indicate that the factors for cytotoxicity of compounds in this series may involve not only molecular symmetry but also the nature of introduced functionalities and log*P* values, which characterizes the lipophilicity of a molecule in its natural state.

Based on the above findings in this study, we are investigating further synthetic molecular modifications for symmetrical tripodal types of compounds by introduction of more lipophilic and aromatic functional groups in order to search for promising cytotoxic lead compounds for anticancer agents. In the search for antiviral compounds, we are planning to introduce reversed polar (or hydrophilic) functionality in the designed symmetrical molecules. These results will be described in a separate paper.

Experimental

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 obtained by a JEOL JMS HX-110 double-focusing model equipped with an 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. The signal assignments were confirmed by 2D-NMR analyses, ^{1}H–^{1}H two-dimensional (2D) correlation spectroscopy (COSY), ^{1}H–^{13}C heteronuclear multiple-quantum coherence (HMQC), and ^{1}H–^{13}C heteronuclear multiple-bond connectivity (HMBC). 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/Kanto 60N) with a UV detector. Commercially available starting materials were used without further purification.

***N*,*N*′,*N*″-(Nitrilotri-2,1-ethanediyl)tris(4-methoxybenzamide) (6a)**[18,19] Entry 1: To a solution of TREA (**1**, 293 mg, 2.0 mmol) and TEA (661 mg, 6.6 mmol) in dry CH_2Cl_2 (20 mL) was added a solution of 4-methoxybenzoyl chloride (**2a**, 1.081 g, 6.3 mmol) in dry CH_2Cl_2 (10 mL) at 0°C under N_2 atmosphere. After stirring for 1 h, the reaction mixture was allowed to warm up to room temperature and then stirred for another 30 min. The resulting mixture was poured into water (60 mL), and then the aqueous layer was extracted with CH_2Cl_2 (2×30 mL), the combined organic layer was washed with brine and dried over $MgSO_4$, and the solvent was removed *in vacuo*. The resulting solid material was recrystallized from 2-PrOH to give compound **6a** (410 mg, 0.473 mmol, 68%) as colorless needles: mp 150—152°C (from 2-PrOH). IR (KBr) cm^{-1}: 3380 (NH of amide), 2840 (ArOMe), 1610, 1505, 1255 (amide). ^{1}H-NMR (DMSO-d_6) δ: 2.70 (6H, t, *J*=6.6 Hz, H2′), 3.36 (6H, brq, H1′), 3.78 (9H, s, OCH_3), 6.68 (6H, d, *J*=8.85 Hz, H2,6), 7.75 (6H, d, *J*=8.85 Hz, H3,5), 8.11 (3H, t, *J*=5.2 Hz, NH). ^{13}C-NMR (DMSO-d_6) δ: 37.43 (C1′), 53.23 (C2′), 55.15 (OCH_3), 113.26 (C3) 126.64 (C1), 128.76 (C2), 161.30 (C4), 165.75 (C=O). Positive-ion FAB-MS *m*/*z*: 549 $(M+H)^+$. HR-FAB-MS *m*/*z*: 549.2731 (Calcd for $C_{30}H_{37}N_4O_6$: 549.2713). *Anal.* Calcd for $C_{30}H_{36}N_4O_6$: C, 65.68; H, 6.61; N, 10.21. Found: C, 65.64; H, 6.61; N, 10.22.

***N*,*N*′,*N*″-(Nitrilotri-2,1-ethanediyl)tris[3,5-bis(trifluoromethyl)benzamide] (6b)** Entry 2: In a manner similar to that for the preparation of **6a**, by the reaction of **1** (293 mg, 2.0 mmol), TEA (708 mg, 7.0 mmol), and 3,5-bis(trifluoromethyl)benzoyl chloride (**2b**, 1.936 g, 7.0 mmol), the crude product was obtained, and recrystallization from EtOH–H_2O gave compound **6b** (1.144 g, 1.32 mmol, 66%) as colorless crystals: mp 145—147°C (from EtOH–H_2O). IR (KBr) cm^{-1}: 3250 (NH of amide), 1650, 1550, 1280 (amide), 1150 (CF). ^{1}H-NMR (DMSO-d_6) δ: 2.78 (6H, t, *J*=6.3 Hz, H2′), 3.45 (6H, m, H1′), 8.14 (3H, brs, H4), 8.32 (6H, d, *J*=1.2 Hz, H2, 6), 8.84 (3H, t, *J*=5.5 Hz, NH). ^{13}C-NMR (DMSO-d_6) δ: 38.03 (C1′), 53.38 (C2′), 122.90 (CF_3, *J*=273.1 Hz), 124.33 (C4), 127.72 (C2,6), 130.13 (C3,5 *J*=33.1 Hz). Positive-ion FAB-MS *m*/*z*: 867 $(M+H)^+$. HR-FAB-MS *m*/*z*: 867.1632 (Calcd for $C_{33}H_{25}F_{18}N_4O_3$: 867.1639). *Anal.* Calcd for $C_{33}H_{24}F_{18}N_4O_3$: C, 45.74; H, 2.79; N, 6.47. Found: C, 45.74; H, 2.80; N, 6.52.

***N*,*N*′,*N*″-(Nitrilotri-2,1-ethanediyl)tris(3-pyridinecarboxamide) (6c)** Entry 3: In a manner similar to that of the preparation of **6a**, from the reaction of **1** (293 mg, 2.0 mmol), TEA (668 mg, 6.6 mmol), and nicotinoyl chloride hydrochloride (**2c**·HCl, 1.175 g, 6.6 mmol) at 0°C under N_2 atmosphere for 1 h, the obtained crude product was recrystallized from 2-PrOH to give compound **6c** (93 mg, 0.20 mmol, 10%) as colorless crystals.

Entry 4: The reaction of **1** (620 mg, 4.2 mmol), TEA (460 mg, 4.6 mmol), and **2c**·HCl (610 mg, 3.4 mmol) in dry tetrahydrofuran (THF) was held at 0°C under N_2 atmosphere for 30 min with stirring. The reaction mixture was filtrated to remove TEA·HCl salt. After evaporation of the solvent, centrifugal chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=85 : 18.5 : 0.5) gave **6c** (367 mg, 0.80 mmol, 70% from **2c**·HCl).

Entry 5: To a mixture of nicotinic acid (**2c**, 6.40 g, 46 mmol) in pyridine (15 mL) was added a solution of **1** (2.19 g, 15 mmol) in pyridine (10 mL) dropwise with stirring at room temperature. After the resulting solution had been stirred for 1 h at 70°C, triphenylphosphite (TPP, 13.96 g, 45 mmol) was added dropwise with stirring and then the solution was refluxed overnight. After cooling and evaporation of the solvent, the residue was dissolved in CH_2Cl_2 (50 mL). The solution was extracted with water (30 mL×3), and to the combined aqueous layer was added NaOH (10 g) and CH_2Cl_2 (100 mL). The resulting mixture was separated into three layers. The middle layer was isolated, and 2-PrOH and ether were added. Then the solidified crude product was separated and recrystallized from 2-PrOH to give compound **6c** (2.31 g, 5.00 mmol, 33%) as colorless crystals: mp 150—152°C (from 2-PrOH). IR (KBr) cm^{-1}: 3350 (NH of amide), 1610, 1510, 1255 (am-

ide). ^{1}H-NMR (DMSO-d_6) δ: 2.75 (6H, brt, J=6.7 Hz, H2′), 3.40 (6H, m, H1′), 7.42 (3H, ddd, J=7.9, 4.9, 0.6 Hz, H5), 8.10 (3H, ddd, J=7.9, 2.1, 1.8 Hz, H6), 8.54 (3H, dt, J= 5.5, 0.3 Hz, NH), 8.66 (3H, dd, J=4.9, 1.8 Hz, H4), 8.95 (3H, d, J=2.1 Hz, H2). ^{13}C-NMR (DMSO-d_6) δ: 37.67 (C1′), 53.16 (C2′), 123.20 (C5) 129.85 (C1), 134.63 (C6), 148.2 (C2), 151.57 (C4), 164.83 (C=O). Positive-ion FAB-MS m/z: 462 (M+H)$^+$. HR-FAB-MS m/z: 462.2255 (Calcd for $C_{24}H_{28}N_4O_3$: 462.2254). *Anal.* Calcd for $C_{24}H_{27}N_4O_3$: C, 62.46; H, 5.92; N, 21.24. Found: C, 62.20; H, 5.92; N, 21.05.

(2*E*,2′*E*,2″*E*)-*N*,*N*′,*N*″-(Nitrilotri-2,1-ethanediyl)tris[3-(2,3-dimethoxyphenyl)-2-propenamide] (7) Entry 6: A mixture of *trans*-2,3-dimethoxycinnamic acid (**3**, 1.524 g, 7.32 mmol) and *N*-hydroxysuccinimide (NHS, 0.842 g, 7.32 mmol) in dry THF (15 mL) was stirred at room temperature under N_2 atmosphere for 30 min. After addition of *N*,*N*′-dicyclohexylcarbodiimide (DCC, 1.51 g, 7.32 mmol), the reaction mixture was stirred for another 3 h. The precipitated dicyclohexylurea (DCU) was removed by filtration, and to the resulting filtrate was added a solution of **1** (357 mg, 2.44 mmol) in dry THF (2 mL) dropwise over a period of 15 min. This mixture was stirred overnight at room temperature under N_2 atmosphere. The white precipitate was filtrated and dissolved in $CHCl_3$ (30 mL). The resulting solution was washed with saturated $NaHCO_3$ (2×30 mL), dried over $MgSO_4$, and concentrated to give an amber-colored oil. Separation by flash chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=97 : 2.7 : 0.3) gave compound **7** (610 mg, 0.851 mmol, 35%). An analytical sample was obtained by recrystallization from acetonitrile as colorless crystals: mp 128—130°C (from CH_3CN). IR (KBr) cm^{-1}: 3300 (NH of amide), 1620, 1470, 1170 (amide). ^{1}H-NMR ($CDCl_3$) δ: 2.63 (6H, dt, J=5.5, 0.6 Hz, H2′), 3.45 (6H, dt, J=5.5, 4.9 Hz, H1′), 3.70 (9H, s, 2-OCH_3), 3.81 (9H, s, 3-OCH_3), 6.64 (3H, t, J=7.9 Hz, H5), 6.69 (3H, d, J=15.9 Hz, H_α), 6.75 (3H, dd, J=7.9, 1.2 Hz, H4), 6.94 (3H, dd, J=7.9, 1.2 Hz, H6), 7.15 (3H, t, J=4.9 Hz, NH), 7.89 (3H, d, J=15.9 Hz, H_β). ^{13}C-NMR ($CDCl_3$) δ: 37.80 (C1′), 53.89 (C2′), 55.85 (3-OCH_3), 61.05 (2-OCH_3), 113.27 (C4), 119.53 (C6), 122.33 (C_α) 123.94 (C5), 129.02 (C1), 135.48 (C_β), 148.24 (C2), 152.91 (C3), 167.04 (C=O). Positive-ion FAB-MS m/z: 717 (M+H)$^+$. HR-FAB-MS m/z: 717.3509 (Calcd for $C_{39}H_{49}N_4O_9$: 717.3500). *Anal.* Calcd for $C_{39}H_{48}N_4O_9$: C, 64.54; H, 6.80; N, 7.72. Found: C, 64.56; H, 6.65; N, 7.73.

***N*,*N*′,*N*″-(Nitrilotri-2,1-ethanediyl)tris[*N*‴-tricyclo-[3.3.1.1^{3,7}]dec-1-ylurea] (11)** Entry 7: To a stirred solution of 1-adamantyl isothiocyanate (**5**, 1.94 g, 10.0 mmol) in dry CH_2Cl_2 (40 mL) was added a solution of **1** (370 mg, 2.5 mmol) in dry CH_2Cl_2 (2 mL) at room temperature. After stirring for 1 d, the solvent was removed *in vacuo*. To the resulting pale yellow oil, dry benzene (50 mL) was added, and the solvent was removed again by azeotropic distillation. The solidified crude product was dissolved in CH_2Cl_2 (*ca.* 5 mL) and MeOH (*ca.* 10 mL) was added. Then the separated crystals **11** (1.50 g, 2.1 mmol, 83%) were collected by filtration. The filtrate gave additional crystals **11** (0.29 g, 0.4 mmol, 16%). The total yield was nearly quantitative. Recrystallization of the product from EtOH gave an analytical pure sample **11** as colorless crystals: mp 110—111°C (from EtOH). IR (KBr) cm^{-1}: 3285 (NH of amide), 2905 (ArOMe), 1545, 1455, 1360 (C=S–N), 1300 (C=S). ^{1}H-NMR ($CDCl_3$) δ: 1.69 (18H, brs, a), 2.15 (27H, brs, c, b), 2.70 (6H, t, J=5.5 Hz, H2′), 3.66 (6H, d, J=4.9 Hz, H1′), 6.25 (3H, brs, NH_α or NH_β), 6.42 (3H, brs, NH_β or NH_α). ^{13}C-NMR ($CDCl_3$) δ: 29.58 (Cc), 36.24 (Ca), 42.19 (Cb), 43.12 (C1′), 53.59 (C2′), 54.31 (Cd), 180.62 (C=S). Positive-ion FAB-MS m/z: 726 (M+H)$^+$. HR-FAB-MS m/z: 726.4385 (Calcd for $C_{39}H_{64}N_7S_3$: 726.4389). *Anal.* Calcd for $C_{39}H_{63}N_7S_3\cdot H_2O$: C, 62.94; H, 8.80; N, 13.18. Found: C, 62.94; H, 8.60; N, 13.09.

***N*,*N*′-[(2-Aminoethyl)imino]di-2,1-ethanediyl]bis(4-methoxybenzamide) (8)** Entry 8: This compound was obtained by a method similar to that described in Entry 1. To a stirred solution of **1** (7.45 g, 51.0 mmol) and TEA (506 mg, 5.0 mmol) in dry CH_2Cl_2 (20 mL) was added a solution of **2a** (852 mg, 5.0 mmol) in dry CH_2Cl_2 (8 mL) at 0°C under N_2 atmosphere for 30 min dropwise. The reaction mixture was stirred for 3 h, and then the solvent was removed *in vacuo*. Centrifugal chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=80 : 19.5 : 0.5) gave **6a** (732 mg, 1.33 mmol, 80%) and compound **8** (104 mg, 0.25 mmol, 10%).

Entry 9: A solution of **2a**′ and carbonyl diimidazole (CDI, 1.07 g, 5.6 mmol) in dry CH_2Cl_2 (50 mL) was refluxed for 2 h under N_2 atmosphere. To this mixture was added dropwise a solution of **1** (395 mg, 2.7 mmol) and the reaction mixture was refluxed overnight. Then the reaction mixture was cooled to room temperature and washed with 4 M NaOH (30 mL×3) after addition of CH_2Cl_2 (50 mL). The organic layer was separated, dried over $MgSO_4$, and filtered. After removal of the solvent, the resulting yellow solid was purified by centrifugal chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=73 : 25 : 2) to give **6a** (140 mg, 0.26 mmol, 10%) and **8** (560 mg, 1.35 mmol, 49%) as a pale yellow oil.

8: Positive-ion FAB-MS m/z: 415 (M+H)$^+$. HR-FAB-MS m/z: 415.2342 (Calcd for $C_{22}H_{31}N_4O_4$: 415.2345). ^{1}H-NMR ($CDCl_3$) δ: 2.61 (2H, brt, H2″), 2.69 (4H, t, J=5.5 Hz, H2′), 2.79 (2H, brt, H1″), 3.49 (4H, dt, J=5.5, 5.2 Hz, H1′), 3.54 (2H, brs, NH_2), 3.75 (6H, s, OCH_3), 6.71 (4H, dm, J=8.9 Hz, H3), 7.69 (2H, brt, J=5.2 Hz, NH), 7.71 (4H, dm, J=8.9 Hz, H2). ^{13}C-NMR ($CDCl_3$) δ: 38.08 (C1′), 38.77 (C1″), 53.65 (C2′), 54.21 (C2″), 55.19 (OCH_3), 113.45 (C3), 126.38 (C1), 128.93 (C2), 161.88 (C4), 167.57 (C=O).

***N*,*N*′-[[[(2-Tricyclo[3.3.1.1^{3,7}]dec-1-ylthioureido)ethyl]imino]di-2,1-ethanediyl]bis(4-methoxybenzamide) (9)** Entry 10: A solution of **8** (118 mg, 0.28 mmol) and **5** (180 mg, 0.93 mmol) in dry CH_2Cl_2 (3 mL) was stirred at room temperature for 3 h. After evaporation, the residue was purified by centrifugal chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=97 : 2.7 : 0.3) to give **9** (101 mg, 0.17 mmol, 58%) as colorless crystals: mp 78—81°C. IR (KBr) cm^{-1}: 3320 (NH of amide), 2900 (ArOMe), 1550, 1500 (C=S–N), 1300 (C=S), 1250 (amide). ^{1}H-NMR ($CDCl_3$) δ: 1.67 (6H, brs, a), 2.08 (3H, brs, c), 2.20 (6H, brs, b), 2.65 (4H, t, J=5.2 Hz, H2′), 2.71 (2H, dt, J=5.2, 0.3 Hz, H2″), 3.47 (4H, dt, J=5.5, 5.2 Hz, H1′), 3.62 (2H, dt, J=5.5, 5.2 Hz, H1″), 3.79 (6H, s, –OCH_3), 6.66 (1H, brs, NH_β or NH_γ), 6.73 (4H, d, J=8.9 Hz, H3), 6.75 (2H, brs, NH_α), 7.31 (1H, br s, NH_γ or NH_β), 7.53 (4H, d, J=8.9 Hz, H2). ^{13}C-NMR ($CDCl_3$) δ: 29.69 (Cc), 36.44 (Ca), 38.18 (C1′), 42.04 (Cb), 42.18 (C1″), 53.67 (Cd), 54.03 (C2″), 54.37 (C2′), 55.30 (OCH_3), 113.64 (C3), 126.15 (C1), 128.71 (C2), 162.15 (C4), 167.87 (C=O), 181.51 (C=S). Positive-ion FAB-MS m/z: 608 (M+H)$^+$. HR-FAB-MS m/z: 608.3271 (Calcd for $C_{33}H_{46}N_5O_4S$: 608.3271). *Anal.* Calcd for $C_{33}H_{45}N_5O_4S$: C, 64.45; H, 7.51; N, 11.39. Found: C, 64.51; H, 7.59; N, 11.12.

***N*-[2-Bis[2-[[[tricyclo[3.3.1.1^{3,7}]dec-1-ylamino]thioxometh-**

yl]amino]ethyl]]-4-methoxy-benzamide (10) Entry 11: To a solution of **1** (585 mg, 4.0 mmol) in dry CH_2Cl_2 (40 mL) was added a solution of **5** (773 mg, 4.0 mmol) in dry CH_2Cl_2 (10 mL) at room temperature. After stirring for 2.5 h, TEA (810 mg, 8.0 mmol) was added to the solution. Then **2a** (1.365 g, 8.0 mmol) in dry CH_2Cl_2 (15 mL) was added dropwise with stirring for 30 min at 0°C under N_2 atmosphere. After removal of the precipitated TEA·HCl salt, the residue obtained by evaporation was purified by flash column chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=97 : 2.7 : 0.3) to give **9** (1.086 g, 1.79 mmol, 45%) and **10** (290 mg, 0.43 mmol, 11%) as a colorless solid. Recrystallization of the crude **10** from *i*-Pr_2O gave an analytical pure sample **10** as colorless crystals: mp 145—147°C (from *i*-Pr_2O). IR (KBr) cm^{-1}: 3275 (NH of amide), 2910 (ArOMe), 1630 (amide), 1555, 1500 (C=S–N), 1295 (C=S). ^{1}H-NMR (DMSO-d_6) δ: 1.61 (12H, brs, a), 2.01 (6H, brs, c), 2.12 (12H, brs, b), 2.61 (4H, m, H2″), 2.65 (2H, m, H2′), 3.34 (2H, m, H1′), 3.43 (4H, m, H1″), 3.80 (3H, s, –OCH_3), 6.95 (2H, brs, NH_γ), 6.98 (2H, d, J=8.9 Hz, H3,5), 7.11 (2H, t, J=4.9 Hz, NH_β), 7.80 (2H, d, J=8.9 Hz, H2,6), 8.24 (1H, t, J=5.8 Hz, NH_α). ^{13}C-NMR (DMSO-d_6) δ: 28.94 (Cc), 35.87 (Ca), 37.35 (C1′), 41.10 (C1″), 41.15 (Cb), 52.54 (C2″), 52.57 (Cd), 53.32 (C2′), 55.23 (OCH_3), 113.37 (C3,5), 126.71 (C1), 128.81 (C2,6), 161.42 (C4), 165.92 (C=O), 180.48 (C=S). Positive-ion FAB-MS m/z: 667 $(M+H)^+$. HR-FAB-MS m/z: 667.3824 (Calcd for $C_{36}H_{55}N_6O_2S_2$: 667.3828). *Anal.* Calcd for $C_{36}H_{54}N_6O_2S_2 \cdot 0.5i\text{-}Pr_2O$: C, 65.23; H, 8.56; N, 11.70. Found: C, 65.06; H, 8.47; N, 11.62.

Entry 12: The reaction was carried out in a manner similar to that described above except for the ratio of reagents [**1** (585 mg, 4.0 mmol) : **5** (1.55 g, 8.0 mmol) : TEA (405 mg, 4.0 mmol) : **2a** (682 mg, 4.0 mmol)]=1 : 2 : 1 : 1. After evaporation of the solvent, the residue was purified by flash column chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=92 : 7 : 1) to give **10** (604 mg, 0.91 mmol, 23%) and **11** (813 mg, 1.12 mmol, 28%) as a white solid.

***N*,*N*′-[(2-Aminoethyl)imino]di-2,1-ethanediyl]bis[(4-methoxyphenyl)acetamide] (12) and *N*,*N*′,*N*″-(Nitrilotri-2,1-ethanediyl)tris[(4-methoxyphenyl)acetamide] (15)** Entry 13: To a solution of **1** (731 mg, 5.0 mmol) in dry CH_2Cl_2 (275 mL) was added dropwise a solution of 4-methoxyphenylacetic anhydride (**4**, 943 mg, 3.0 mmol) in dry CH_2Cl_2 (100 mL) at −78°C with stirring, and then stirring was continued at room temperature overnight. After the resulting reaction mixture had been washed with saturated $NaHCO_3$, the organic layer was dried over $MgSO_4$. The filtrate was evaporated and the residue was purified by centrifugal chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=93 : 6.5 : 0.5 to 73 : 25 : 2) to give **12** (163 mg, 0.37 mmol, 25% from **4**) as a pale yellow oil and **15** (62 mg, 0.104 mmol, 11% from **4**) as a colorless solid.

12: IR (KBr) cm^{-1}: 3285 (NH_2, OH), 3070, 2935, 2835 (ArOMe), 1645, 1245 (amide), 1175, 1035 (ArOMe). ^{1}H-NMR ($CDCl_3$) δ: 1.90 (2H, brs, NH_2), 2.39 (2H, dt, J=5.5, 0.6 Hz, H2″), 2.46 (4H, dt, J=5.5, 0.3 Hz, H2′), 2.57 (2H, dt, J=5.5, 0.6 Hz, H1″), 3.20 (4H, J=6.1, 5.5 Hz, H1′), 3.48 (4H, s, α-CH_2), 3.76 (6H, s, OCH_3), 6.61 (2H, brt, NH), 6.85 (4H, dm, J=8.5 Hz, H3), 7.19 (4H, dm, J=8.5 Hz, H2). ^{13}C-NMR ($CDCl_3$) δ: 37.80 (C1′), 39.57 (C1″), 42.56 (Cα), 54.02 (C2′), 55.24 (OCH_3), 56.49 (C2″), 114.18 (C3), 127.29 (C1), 130.37 (C2), 158.67 (C4), 171.85 (C=O). Positive-ion FAB-MS m/z: 443 $(M+H)^+$. HR-FAB-MS m/z: 443.2653 (Calcd for $C_{24}H_{35}N_4O_4$: 443.2658). *Anal.* Calcd for $C_{24}H_{34}N_4O_4 \cdot 0.7H_2O$: C, 63.33; H, 7.84; N, 12.31. Found: C, 63.26; H, 7.81; N, 12.15.

15: mp 115—116°C. IR (KBr) cm^{-1}: 3300 (NH of amide), 3080, 2935, 2835 (ArOMe), 1640, 1245 (amide), 1175, 1035 (ArOMe). ^{1}H-NMR ($CDCl_3$) δ: 2.42 (6H, dt, J=5.5, 0.3 Hz, H2′), 3.13 (6H, dt, J=5.8, 5.5 Hz, H1′), 3.46 (6H, s, α-CH_2), 3.75 (9H, s, OCH_3), 6.52 (3H, brt, NH), 6.83 (6H, dm, J=8.5 Hz, H3), 7.17 (6H, dm, J=8.5 Hz, H2). ^{13}C-NMR ($CDCl_3$) δ: 37.94 (C1′), 42.52 (Cα), 54.54 (C2′), 55.21 (OCH_3), 114.17 (C3), 127.21 (C1), 130.29 (C2), 158.66 (C4), 172.05 (C=O). Positive-ion FAB-MS m/z: 591 $(M+H)^+$. HR-FAB-MS m/z: 591.3176 (Calcd for $C_{33}H_{43}N_4O_6$: 591.3183). *Anal.* Calcd for $C_{33}H_{42}N_4O_6 \cdot 0.3H_2O$: C, 66.49; H, 7.20; N, 9.40. Found: C, 66.61; H, 7.11; N, 9.37.

***N*,*N*′-[[[2-Tricyclo[3.3.1.1^{3,7}]-methyl]amino]ethyl]imino]diethylene]bis[(4-methoxyphenyl)acetamide] (13) and *N*-[2-Bis[2-[[[tricyclo[3.3.1.1^{3,7}]dec-1-ylamino]thioxomethyl]-amino]ethyl]]-(4-methoxyphenyl)acetamide (14)** Entry 14: To a solution of **1** (585 mg, 4.0 mmol) in dry CH_2Cl_2 (40 mL) was added a solution of **5** (774 mg, 4.0 mmol) in dry CH_2Cl_2 (25 mL) at room temperature, and the resulting mixture was stirred for 1 h. After addition of TEA (810 mg, 8.0 mmol), a solution of **4** (2.52 g, 8.0 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise at 0°C under N_2 atmosphere. After stirring for 1 h at room temperature, the reaction mixture was washed with saturated $NaHCO_3$ (50 mL) and dried over $MgSO_4$, and the solvent was evaporated. The residue was purified by flash column chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=97 : 2.7 : 0.3 to 95 : 4.5 : 0.5) to give **13** (869 mg, 1.37 mmol, 34%) as colorless needles and **14** (202 mg, 0.30 mmol, 7%) as colorless crystals.

13: mp 55—57°C. IR (KBr) cm^{-1}: 3315 (NH of amide), 2905 (ArOMe), 1650 (amide), 1540, 1510 (C=S–N), 1360 (C=S). ^{1}H-NMR ($CDCl_3$) δ: 1.66 (6H, brs, a), 2.06 (3H, brs, c), 2.17 (6H, brs, b), 2.45 (4H, t, J=5.5 Hz, H2′), 2.58 (2H, t, J=5.5 Hz, H2″), 3.19 (4H, dt, J=5.5, 5.2 Hz, H1′), 3.46 (2H, m, H1″), 3.49 (4H, s, α), 3.78 (6H, s, –OCH_3) 6.08 (2H, brt, NH_α), 6.62 (1H, brs, NH_β or NH_γ), 6.87 (4H, d, J=8.9 Hz, H3), 6.89 (1H, brs, NH_γ or NH_β), 7.18 (4H, d, J=8.9 Hz, H2). ^{13}C-NMR ($CDCl_3$) δ: 29.66 (Cc), 36.39 (Ca), 38.23 (C1′), 41.98 (Cb), 42.21 (C1″), 42.84 (Cα), 53.58 (Cd or C2″), 53.69 (C2″ or Cd), 54.86 (C2′), 55.30 (OCH_3), 114.45 (C3), 126.75 (C1), 130.48 (C2), 158.94 (C4), 172.40 (C=O), 181.26 (C=S). Positive-ion FAB-MS m/z: 636 $(M+H)^+$. HR-FAB-MS m/z: 636.3604 (Calcd for $C_{35}H_{50}N_5O_4S$: 636.3584). *Anal.* Calcd for $C_{35}H_{49}N_5O_4S$: C, 66.11; H, 7.77; N, 11.01. Found: C, 65.92; H, 7.81; N, 10.86.

14: mp 108—110°C, ^{1}H-NMR ($CDCl_3$) δ: 1.69 (12H, brs, a), 2.11 (6H, brs, c), 2.15 (12H, brs, b), 2.54 (2H, t, J=5.5 Hz, H2′), 2.62 (4H, t, J=5.8 Hz, H2″), 3.27 (2H, m, H1′), 3.54 (4H, m, H1″), 3.57 (2H, s, α), 3.80 (3H, s, –OCH_3) 6.09 (1H, m, NH_α), 6.29 (2H, br s, NH_β or NH_γ), 6.52 (2H, brs, NH_γ or NH_β), 6.90 (2H, dm, J=8.7 Hz, H3), 7.21 (2H, dm, J=8.7 Hz, H2). ^{13}C-NMR ($CDCl_3$) δ: 29.62 (Cc), 36.30 (Ca), 38.32 (C1′), 42.16 (Cb), 42.92 (C1″, Cα), 53.50 (C2″), 54.04 (Cd), 55.33 (C2′ or OCH_3), 55.37 (OCH_3 or C2′), 114.58 (C3), 126.44 (C1), 130.65 (C2), 159.03 (C4), 173.06 (C=O), 181.10 (C=S). Positive-ion FAB-MS m/z: 681 $(M+H)^+$. HR-FAB-MS m/z: 681.3979 (Calcd for $C_{37}H_{57}N_6O_2S_2$: 681.3984).

Antiviral Activity Assay and Cytotoxicity The antiviral activities of synthesized compounds (**6a—c**, **7**, **9—11**, **13**, **15**)

were measured by using a plaque reduction assay[20] as described in our previous paper.[29] Results of antiviral activity (EC_{50}) and cytotoxicity (IC_{50}) with Vero cells are summarized in Table 3.

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10) Through our previous studies on triarylmethane derivatives, we arrived at a working hypothesis that a C_3-symmetrical molecule having a tripodal scaffold may be an efficient structural feature for molecular recognition such as recognition of sugar chains in macromolecules or receptor sites. The rigidity of the molecules and nature of functional groups (such as H-bond donors or acceptors for suprafacial interactions) in symmetrical target molecules may play important roles for its biological properties.
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