Note

Antiviral Activity and Molecular Geometry of Some New Symmetrical Tris(aminoalkyl)amine Derivatives

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We report the preparation of new tripodal receptor-type C_3- and C_S-symmetrical molecules constructed on a tris(2-aminoethyl)amine (TAEA) template. Both the anti-herpes simplex virus type 1 (anti-HSV-1) activity and cytotoxic activity of synthesized receptor-type derivatives were evaluated in order to find a characteristic structural feature for these bioactivities of compounds. Among the compounds of synthesized symmetrical TAEA-related derivatives, compound 13k showed high anti-HSV-1 activity (50% effective concentration (EC_{50})=16.7 μM) and low cytotoxicity (50% cytotoxic concentration (CC_{50})=>200 μM). The presence of a hydrogen bond donor proton in the molecule is thought to be an important structural factor for expressing potential anti-HSV-1 activities.

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

It is well known that many types of receptors or functionalized proteins on membranes in a native state often have a high order of symmetrical interfaces.[1] C_3- or C_2-symmetrical molecules have frequently been found in various synthetic biologically active compounds.[2] Aiming to develop new synthetic bioactive compounds, we targeted such symmetric geometrical molecules constructed on a symmetrical template.[3–5]

In our previous studies on bioactive compounds in the tripodal receptor-type tris(2-aminoethyl)amine (TAEA) series, we found that some of the new C_3- and C_S-symmetrical derivatives synthesized previously showed significant bioactivities against herpes simplex virus type 1 (HSV-1) or cytotoxic activities to Vero cells.[6] Regarding amide-type TAEA derivatives, we have reported that some symmetrical molecules have selective carbohydrate recognition properties.[7] These mid-size molecules that show lectin-like carbohydrate recognition properties might have interesting functions as new ligands or drug candidates. Considering the formation of strong drug-host interaction for host sugar recognition by multivalent molecules, attempts to synthesize such non-peptide mid-size molecules are also thought to be valid in the search for bioactive new seeds.[5,8]

Since it is well known that many amide functionalities usually have the ability to form a strong hydrogen bond, and it has also often been reported that some molecules derived from TAEA behave as acceptors for an ion or a small molecule,[11,12] we introduced a few amide functionalities into a tripodal receptor-type TAEA template. These molecular modifications may lead to new bioactive symmetrical molecules that have good properties for non-covalent interaction and bioactivities.

Here, we describe the synthesis of some new non-peptide molecules with amine or amide-relating functionalities constructed on a symmetrical tris(2-aminoethyl)amine (TAEA) template and on a spread tris(3-aminopropyl)amine (TAPA) template. The results of biological evaluations of the synthesized derivatives are also described (Fig. 1).

Results

Synthesis of New Target Derivatives of Symmetrical Tris(aminoalkyl)amines (TAEA and TAPA) Our strategies for the synthesis of target TAEA and TAPA derivatives by using TAEA (**1**) or TAPA (**17**) as a starting material are summarized in Chart 1.

The pathway for the synthesis of C_3-symmetrical tripodal receptor-type TAEA derivatives (**3**, **5**, **7**, **8**) is shown in Chart 1. All reductive amination reactions with various aryl aldehydes (**2**) were performed as one-pot reactions, and the desired amine-type TAEA derivatives (**3**) were obtained in a manner similar to that for the preparation of compound **3a**.[13] Acetylation of compounds **3** with acid anhydride (**4**) gave new amide-type TAEA derivatives (**5**). Urea-type TAEA derivatives (**7**, **8**) were also obtained by addition reactions of amine-type derivatives (**3**) or TAEA (**1**) to iso(thio)cyanate (**6**). All of these target C_3-symmetrical TAEA derivatives (**3**, **5**, **7**, **8**) were obtained in moderate to excellent yields (44–95%) (see

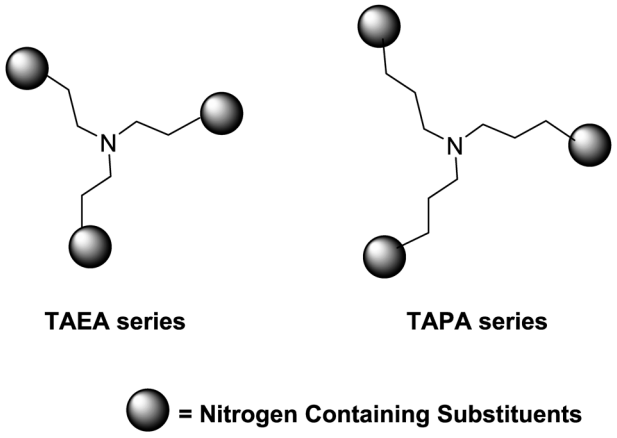

Fig. 1. Symmetrical Target Molecules Constructed on Triaminoalkyl-Substituted Amines

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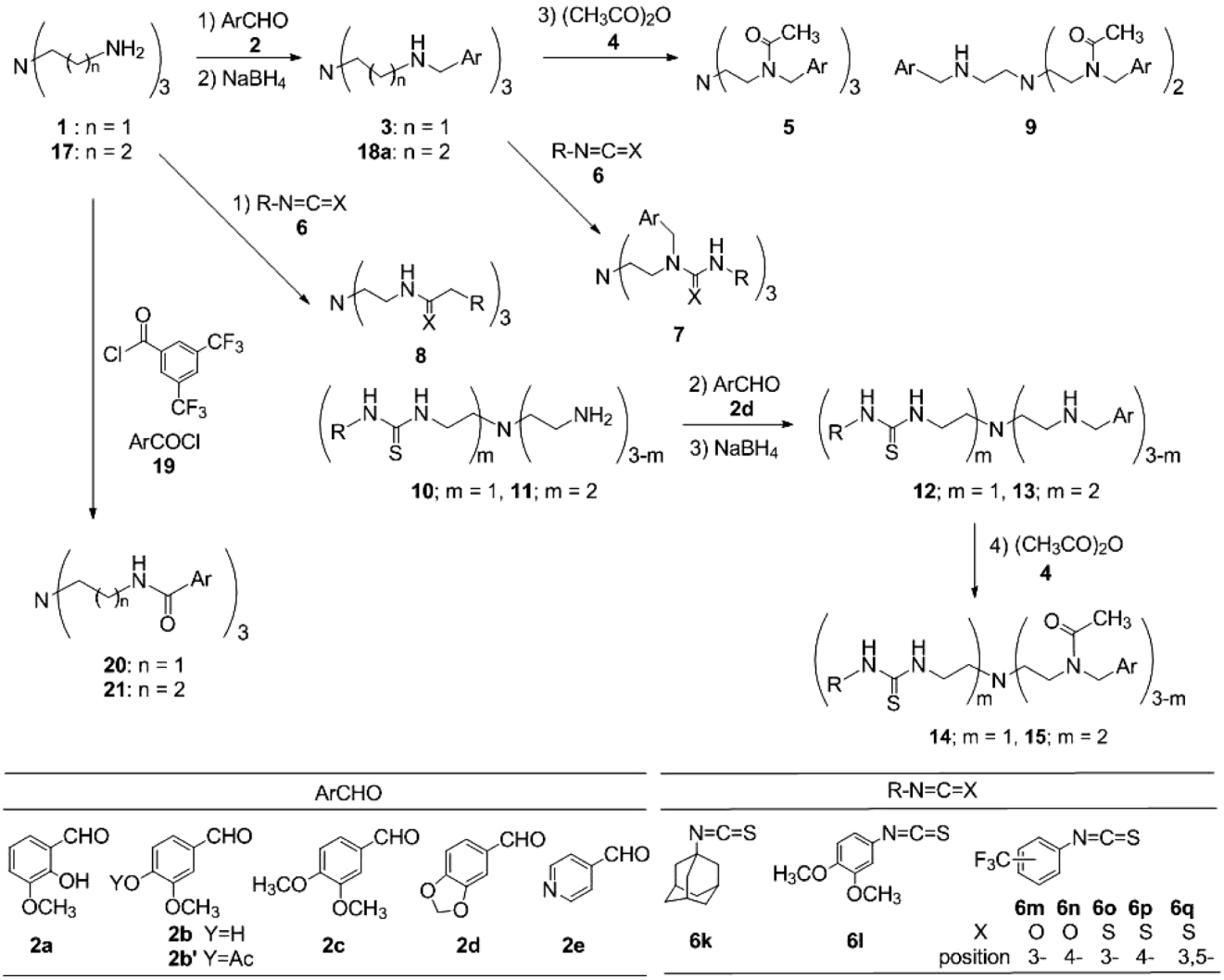


Chart 1. Synthesis of Symmetrical TAEA-Related Derivatives

Tables S1–S3 for Supplementary materials of the online version for details).

Synthesis of targeted C_S-symmetrical TAEA derivatives (**12**, **13**) was achieved from addition reactions of the starting TAEA (**1**) to isothiocyanate (**6**) with different ratios of two reagents (**1**:**6**=1:2 and **1**:**6**=2:1), and the intermediate adducts (**10**, **11**) that were formed were further used for reductive amination with an aldehyde (**2d**) and $NaBH_4$ in a one-pot reaction to give amine-urea-type TAEA derivatives (**12**, **13**) (see Table S4 for Supplementary materials). The yields of targeted derivatives (**12**, **13**) were about 32–55%. The results indicated that control of the first step of the addition reaction is not easy. The high reactivity of isothiocyanate to amine is thought to be the reason for obtaining such experimental results. Amide-urea-type TAEA derivatives (**14**, **15**) from the acylations of these isolated compounds (**12**, **13**) with acetic anhydrate (**4**) were obtained in good to excellent yields (68–92%) (see Table S5 for Supplementary materials).

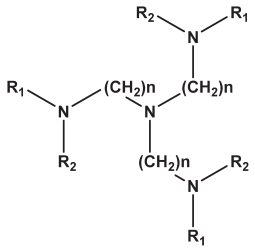
For the synthesis of some new additional C_3-symmetrical tris(3-aminopropyl)amine (TAPA) derivatives, we used the same procedure as that described for the preparation of targeted C_3-symmetrical TAEA derivatives. Thus, reductive amination of TAPA as a starting material with aldehyde (**2a**) afforded the corresponding amine-type TAPA derivative (**18a**) in 65% yield, and an amide-type C_3-symmetrical TAPA derivative (**21**) was also obtained after acylation of TAPA with 3,5-ditrifluoromethylbenzoyl chloride (**19**) in 66% yield (see Experimental).

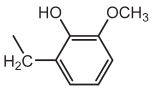
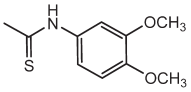
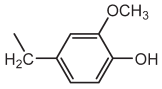
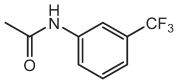
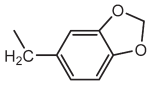
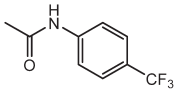
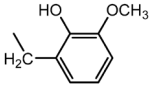
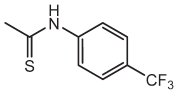
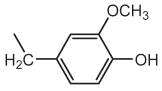
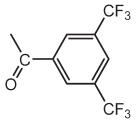
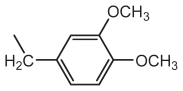
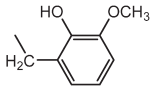
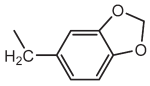
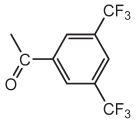
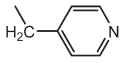
All of the structures of the synthesized symmetrical tripodal receptor-type compounds were confirmed by spectroscopic and analytical data. The geometries of the obtained symmetrical TAEA and TAPA derivatives described above were confirmed from ^{13}C-NMR spectroscopic data[14] (see Experimental for details).

Biological Activity and Discussion

In many synthetic receptor-type molecules mimicking the supramolecular interaction of biomolecules, it is expected that the ability to form multiple hydrogen bonds plays an important role in guest-molecule recognition. Considering the concept called molecular tectonics,[15,16] it is also thought that a convergent orientation of incorporated functional groups is an effective array to produce a multiple hydrogen bonding interaction between host and guest molecules. From this point of view, we prepared title tripodal receptor-type C_3- or C_S-geometrical molecules (**3**, **5**, **8**, **18a**, **21**) that have donors or acceptors for hydrogen bonds on a symmetrical TAEA or TAPA template as new target molecules (Table 1).

Table 1. Anti-HSV-1 Activity (EC_{50}) and Cytotoxicity (CC_{50}) against Vero Cells of C_3-Symmetrical TAEA and TAPA Derivatives



Compound	*n*	R_1	R_2	EC_{50} (μM)	CC_{50} (μM)	Compound	*n*	R_1	R_2	EC_{50} (μM)	CC_{50} (μM)
3a ·H_2O	2	H		>200	>200	**8l** ·0.8H_2O	2	H		>100	>200
3b ·0.875$CHCl_3$	2	H		>100	>200	**8m**	2	H		>100	>200
3d ·0.5NH_3	2	H		20.5	35.9	**8n**	2	H		>100	>200
5a ·0.9H_2O	2	Ac		>100	>200	**8p** ·2-PrOH	2	H		>100	57.3
5b ·HCl ·2-PrOH·H_2O	2	Ac		>100	>200	**20**[a]	2	H		N.D.	17.7
5c ·H_2O	2	Ac		>100	>200	**18a** ·1.2H_2O	3	H		>200	>200
5d ·0.7H_2O	2	Ac		90.7	>200	**21**	3	H		>200	>200
5e ·0.5H_2O	2	Ac		>100	>200						

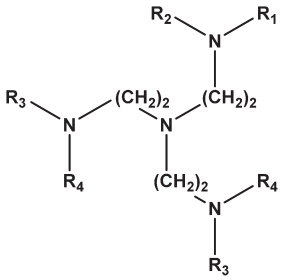
a) Data were taken from ref. 6.

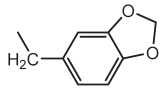
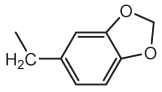
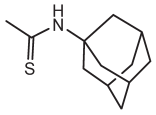
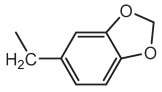
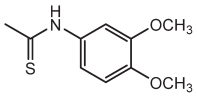
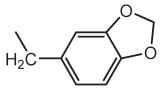
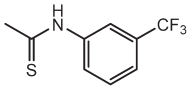
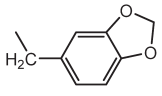
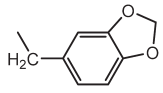
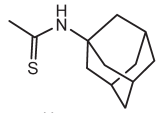
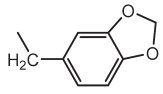
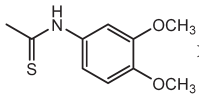
Biological evaluations of anti-HSV-1 activities 50% effective concentration (EC_{50}) by plaque reduction assays[17] and cytotoxicities against Vero cells 50% cytotoxic concentration (CC_{50}) of synthesized TAEA and TAPA derivatives (Table 1) showed that many C_3-symmetrical amine-type or urea-type TAEA derivatives (**3** or **8**) had no significant anti-HSV-1 (EC_{50}=>100 μM) and cytotoxic (CC_{50}=>200 μM) activities. Among the tested amine-type C_3-symmetrical derivatives (**3**) shown in Table 1, only compound **3d** showed both anti-HSV-1 (EC_{50}=20.5 μM) and cytotoxic (CC_{50}=35.9 μM) activities. Compound **8p** with a large log*P* value[19] (9.94) showed moderate cytotoxic activity (CC_{50}=57.3 μM). Among the C_3-symmetrical compounds listed in Table 1 and also the C_S-symmetrical derivatives listed in Table 2, there were few distinct correlations between log *P* values and anti-HSV-1 activities (EC_{50} values).

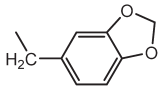
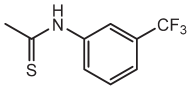
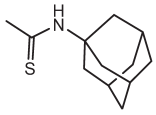
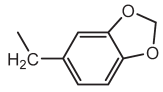
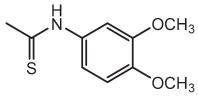
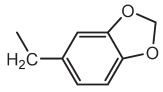
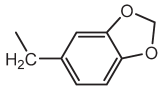
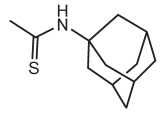
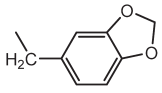
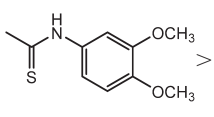
All of the C_3-symmetrical amide-type TAEA derivatives (**5**) with loss of hydrogen bond donor protons by acylation reactions of an amine (–NH–) functionality, except for a very low level of anti-HSV-1 activity of compound **5d** (EC_{50}=90.7 μM), also showed no significant anti-HSV-1 (EC_{50}=>100 μM) and cytotoxic (CC_{50}=>200 μM) activities.[14] Neither of the two prepared TAPA derivatives **18a** and **21**, which correspond to TAEA derivatives **3a** and **20**, respectively, showed significant anti-HSV-1 (EC_{50}=>100 μM) and cytotoxic (CC_{50}=>200 μM) activities.

The most important information from the above-described results is considered to be the fact that all of the *N*-acetylated *cis–trans* mixtures (compounds **5a**–**d**) listed in Table 1 showed almost no anti-HSV-1 and cytotoxic activities at doses of <100 μM and <200 μM, respectively. Thus, the obtained biological results indicate at least that the loss of hydrogen atoms as hydrogen bond donors by *N*-acetylation of secondary amine moieties in amine-type molecules has a greater influence than the geometrical changes of these tripodal receptor-type TAEA

Table 2. Anti-HSV-1 Activity (EC_{50}) and Cytotoxicity (CC_{50}) against Vero Cells of C_S-Symmetrical TAEA Derivatives



Compound	R_1	R_2	R_3	R_4	EC_{50} (μM)	CC_{50} (μM)
9d ·2H_2O	H		Ac		>100	250
12k ·0.5H_2O	H		H		120.9	>200
12l ·0.9H_2O	H		H		>100	>200
12o ·0.6H_2O	H		H		>100	57.2
13k ·0.5H_2O	H		H		16.7	>200
13l ·1.2H_2O	H		H		>100	>200

Compound	R_1	R_2	R_3	R_4	EC_{50} (μM)	CC_{50} (μM)
13o ·EtOH	H		H		16.1	21.0
14k ·0.9H_2O	H		Ac		>100	>200
14l ·0.5H_2O	H		Ac		>100	>200
15k ·0.4H_2O	Ac		H		>100	>200
15l	Ac		H		>100	250
Aciclovir[a]					1.1	>444

a) Data were taken from ref. 18.

derivatives on the expression of anti-HSV-1 activities.

On the other hand, regarding C_S-symmetrical molecules (as shown in Table 2), many amine-urea-type TAEA derivatives (**12k**, **l**, **o**) that have one urea functionality showed no anti-HSV-1 activity (EC_{50}=>100 μM); however, two C_S-symmetrical amine-urea-type TAEA derivatives (**13k**, **o**) that have two urea functionalities showed considerably high levels of anti-HSV-1 activity (EC_{50}=16.7, 16.1 μM, respectively). With regard to the molecules containing an adamantylthiourea group(s), we consider that the presence of two adamantylthiourea groups (**13k**) is a desirable structural feature in these amine-urea-type derivatives because of almost no anti-HSV-1 activity of the same geometric compound **12k**. Since *N*-acetylated compounds **14k** and **15k**, which correspond to C_S-symmetrical compounds **12k** and **13k**, respectively, showed no anti-HSV-1 activity, the presence of a benzylamino group as a hydrogen bond donor in the molecule **13k** is also required. Compound **13o** possessing a high level of cytotoxic activity (CC_{50}=21.0 μM) had large calculated log *P* values[19] over 6.0. We consider that both the structure and lipophilic property of the molecule contribute to its overall biological activity, especially its cytotoxic activity. In some C_3-type molecules such as **8p** and **20**, a similar tendency for cytotoxic activity[19] was observed (see Table 1). Regarding anti-HSV-1 activities (EC_{50}) of these C_S-symmetrical derivatives, amine-urea-type molecules with *N*-acetylation of secondary amine moieties (see compounds **14k, l, 15k**, **l**) listed in Table 2 also showed almost no anti-HSV-1 activity at a dose of <100 μM, indicating that the loss of hydrogen atoms as hydrogen bond donors by *N*-acetylation of secondary amine moieties in amine-urea-type molecules has a greater influence than the geometrical features of these tripodal receptor-type TAEA molecules on expression of potential anti-HSV-1 activities.

As can be seen in the current geometrical and functional group modifications for the targeted TAEA derivatives [**3d** (C_3)→**12k** (C_S)→**13k** (C_S)→**8k** (C_3)[6,20]→**14k** (C_S)→**15k** (C_S)], compound **13k** (C_S) revealed the maximum magnitude of anti-HSV-1 activity in these typical examples.

On the basis of the obtained structural information on biological activities in this TAEA series, we are investigating further molecular modifications by incorporating a few other new functional groups that work as hydrogen bonding donors into a TAEA framework in order to confirm the importance of the presence of hydrogen bonding donors and hopefully to

find new promising bioactive seeds.

Experimental

Melting points were determined using a micro melting point apparatus (Yanaco 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 and ECG600R (only for compounds **7cm**, **9d** and **16o**). Chemical shifts were expressed in δ ppm downfield from an internal TMS signal for ^{1}H-NMR and the carbon signal of the corresponding solvent [$CDCl_3$ (77.00 ppm), dimethyl sulfoxide (DMSO)-d_6 (39.50 ppm)] for ^{13}C-NMR. The abbreviations dd=double doublets, dt=double triplets, and dm=double multiplets are used for the multiplicity of ^{1}H-NMR data. The signal assignments were confirmed by two-dimensional (2D)-NMR analyses: ^{1}H–^{1}H 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). Open and flash column chromatography or centrifugal chromatography separations of the reaction products were performed on silica gel (Kanto 60N or Able-Biott) with a UV detector. Commercially available starting materials were used without further purification, and dry solvents were used in all reactions.

General Procedure for the Preparation of C_3-Symmetrical Amine-Type TAEA Derivatives (3): Example: Synthesis of 4,4′,4″-[Nitrilotris(2,1-ethanediyliminomethylene)]tris(2-methoxyphenol) (3b) (Entry 2) (Step 1) To a solution of vanillin (**2b**, 2.28 g, 15.0 mmol) in methanol (MeOH, 6 mL) was added TAEA (**1**, 731 mg, 5.00 mmol) in MeOH (3 mL) at room temperature (r.t.) under an N_2 atmosphere. After stirring for 1 h, the yellow solution changed to a yellow sticky suspension.

(Step 2) After dilution with MeOH (12 mL) and ice-cooling, compound **1** (1.84 g, 10.0 mmol) in dry benzene (20 mL) was added dropwise with stirring. After stirring for 20 min at r.t., the reaction mixture was refluxed for 1 h. After cooling to r.t., a small amount of $NaBH_4$ (945 mg, 25.0 mmol) was added to the mixture and the mixture was stirred for 2.5 h at 0°C. It was then diluted with aqueous ammonium chloride (NH_4Cl, 10%, 100 mL) and extracted with CH_2Cl_2 (3×200 mL). The combined organic layer was washed with brine (50 mL) and dried over anhydrous magnesium sulfate ($MgSO_4$). Evaporation of the solvent gave crude **3b** (2.43 g, 88%). An analytical sample of **3b** was obtained by recrystallization from chloroform ($CHCl_3$) as an off-white solid.

3b: mp 64–69°C (from $CHCl_3$). IR (KBr) cm^{-1}: 1279, 1034, 1125 (C–O). ^{1}H-NMR (DMSO-d_6) δ: 2.5–2.7 (12H, m, H1′, 2′), 3.21 (6H, brs, NH, OH), 3.54 (6H, brs, Hα), 3.71 (9H, brs, OCH_3), 6.60–6.73 (6H, m, H5, 6), 6.84 (3H, d, J=1.5 Hz, H3), 8.30 (<1H, s, $CHCl_3$). ^{13}C-NMR (DMSO-d_6) δ: 46.56 (C1′), 52.75 (Cα), 54.00 (C2′), 55.44 (OCH_3), 79.08 ($CHCl_3$), 112.07 (C3), 114.99 (C6), 120.14 (C5), 131.60 (C4), 145.09 (C1), 147.31 (C2). Positive-ion FAB-MS m/z: 555 (M+H$^+$). HR-FAB-MS m/z: 555.3185 (Calcd for $C_{30}H_{43}N_4O_6$: 555.3183). *Anal.* Calcd for $C_{30}H_{42}N_4O_6\cdot 0.875CHCl_3$: C, 56.26; H, 6.56; N, 8.50. Found: C, 56.29; H, 6.62; N, 8.45.

2,2′,2″-[Nitrilotris(2,1-ethanediyliminomethylene)]tris[6-methoxyphenol] (3a) (Entry 1) Compound **3a** was prepared from the reaction of **1** with *o*-vanillin (**2a**) under the conditions shown in Table S1. Separation of the products by centrifugal chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=90 : 9.5 : 0.5→85 : 13 : 2) gave **3a** (1.73 g, 62%) as a reddish purple solid.

3a: mp 35–38°C (mp 42–45°C).[15] IR (KBr) cm^{-1}: 3395 (OH), 3305 (NH), 1590, 1475 (C=C of Ar), 1235, 1075 (C–N and C–O). ^{1}H-NMR ($CDCl_3$) δ: 2.55 (6H, m, H2′), 2.68 (6H, m, H1′), 3.83 (9H, brs, CH_3O–), 3.97 (6H, brs, Hα), 6.27 (6H, brs, NH, OH), 6.60 (3H, d, J=7.6 Hz, H3), 6.71 (3H, t, J=7.6 Hz, H4), 6.78 (3H, d, J=7.6 Hz, H5). ^{13}C-NMR ($CDCl_3$) δ: 45.78 (C1′), 51.76 (Cα), 53.90 (C2′), 55.76 (CH_3O–), 110.75 (C5), 118.52 (C4), 120.69 (C3), 122.69 (C2), 147.10 (C1), 147.83 (C6). Positive-ion FAB-MS m/z: 555 (M+H)$^+$. HR-FAB-MS m/z: 555.3202 (Calcd for $C_{30}H_{43}N_4O_6$: 555.3183). *Anal.* Calcd for $C_{30}H_{42}N_4O_6\cdot H_2O$: C, 62.92; H, 7.74; N, 9.78. Found: C, 62.93; H, 7.71; N, 9.57.

***N*^{2}-[(3,4-Dimethoxyphenyl)methyl]-*N*^{1},*N*^{1}-bis[2-[[(3,4-dimethoxyphenyl)methyl]amino]ethyl]-1,2-ethanediamine (3c) (Entry 3)** Compound **3c** was prepared from the reaction of **1** with 3,4-dimethoxybenzaldehyde (**2c**) under the conditions shown in Table S1. Separation of the products by flash chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=93 : 6.6 : 0.4→85 : 14 : 1) gave **3c** (5.00 g, 84%) as a sticky yellow oil.

3c: ^{1}H-NMR ($CDCl_3$) δ: 2.75–2.77 (12H, m, H1′, H2′), 3.84 (6H, s, Hα), 3.85 (9H, s, OCH_3 on C3), 3.90 (9H, s, OCH_3 on C4), 4.36 (3H, brs, NH), 6.79 (3H, d, J=8.2 Hz, H5′), 6.86 (3H, dd, J=8.2, 2.1 Hz, H6′), 7.13 (3H, d, J=2.1 Hz, H2′). ^{13}C-NMR ($CDCl_3$) δ: 45.21 (C1′), 52.24 (Cα), 53.02 (C2′), 55.92 (OCH_3 on C4), 56.22 (OCH_3 on C3), 111.16 (C5), 112.58 (C2), 121.45 (C6), 127.88 (C1), 149.02 (C3), 149.39 (C4). Positive-ion FAB-MS m/z: 597 (M+H$^+$). HR-FAB-MS m/z: 597.3660 (Calcd for $C_{33}H_{49}N_4O_6$: 597.3652). *Anal.* Calcd for $C_{33}H_{48}N_4O_6\cdot 2.4H_2O$: C, 61.93; H, 8.32; N, 8.45. Found: C, 61.99; H, 8.17; N, 8.72.

***N*^{2}-(1,3-Benzodioxol-5-ylmethyl)-*N*^{1},*N*^{1}-bis[2-[(1,3-benzodioxol-5-ylmethyl)amino]ethyl]-1,2-ethanediamine (3d) (Entry 4)** Compound **3d** was prepared from the reaction of **1** (1.46 g, 10.0 mmol) with piperonal (**2d**) under the conditions shown in Table S1. Separation of the products by centrifugal chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=85 : 14 : 1) gave **3d** (0.760 g, 14%) as a pale yellow oil.

(Entry 5) This compound was also prepared as follows: To a solution of **2d** (2.25 g, 15.0 mmol) in benzene (40 mL) were added compound **1** (731 mg, 5.0 mmol) and *p*-toluenesufonic acid (TsOH, 66 mg, 0.35 mmol) and the resulting mixture was refluxed. The resultant H_2O was removed with a Dean–Stark distillation apparatus for 18 h under an N_2 atmosphere. After evaporation of the solvent, the resulting mixture was diluted with MeOH (30 mL) and then a small amount of $NaBH_4$ (1.57 mg, 41.5 mmol) was added and the mixture was stirred for 3 h at 0°C under an N_2 atmosphere. After evaporation of the solvent, $CHCl_3$ (20 mL), brine (5 mL), and 5% $NaHCO_3$ (5 mL) were added to the obtained yellow solid, and then the resulting mixture was vigorously stirred for 1 h. After separation of the organic layer, the aqueous layer was re-extracted with $CHCl_3$ (2×20 mL). The combined organic layer

was dried over $MgSO_4$, and evaporation of the solvent gave a brown oil. The product was purified by flash chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=73 : 25 : 2→65 : 32 : 3) to give **3d** (1.38 g, 50%) as a yellow oil. An analytical sample of **3d** was obtained as a pale yellow solid by centrifugal chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=80 : 19.5 : 0.5).

3d: mp 215°C (dec.). IR (NaCl) cm^{-1}: 3300 (NH of amine), 2825 (CH_2 of methylenedioxy), 1245, 1040 (=C–O–C–), 930 (C–O). ^{1}H-NMR (DMSO-d_6) δ: 2.76 (6H, t, J=5.2 Hz, H2′), 3.06 (6H, t, J=5.2 Hz, H1′), 4.11 (6H, s, Hα), 6.04 (6H, s, H2), 6.93 (3H, d, J=7.9 Hz, H7), 7.07 (3H, dd, J=7.9, 1.5 Hz, H6), 7.27 (3H, d, J=1.5 Hz, H4), 9.48 (3H, brs, NH). ^{13}C-NMR (DMSO-d_6) δ: 43.28 (C1′), 49.54 (C2′ or Cα), 49.62 (Cα or C2′), 101.16 (C2), 108.13 (C7), 110.42 (C4), 124.24 (C6), 125.14 (C5), 147.22 (C3a), 147.58 (C7a). ^{1}H-NMR ($CDCl_3$) δ: 2.17 (3H, brs, NH), 2.56 (6H, t, J=5.8 Hz, H2′), 2.64 (6H, t, J=5.8 Hz, H1′), 3.64 (6H, s, Hα), 5.90 (6H, s, H2), 6.70 (6H, brs, H6, 7), 6.79 (3H, s, H4). ^{13}C-NMR ($CDCl_3$) δ: 46.88 (C1′), 53.57 (Cα), 54.29 (C2′), 100.76 (C2), 107.95 (C7), 108.55 (C4), 121.08 (C6), 134.14 (C5), 146.41 (C7a), 147.62 (C3a). Positive-ion FAB-MS m/z: 549 $(M+H)^+$. HR-FAB-MS m/z: 549.2728 (Calcd for $C_{30}H_{37}N_4O_6$: 549.2713). *Anal.* Calcd for $C_{30}H_{36}N_4O_6 \cdot 0.5NH_3$: C, 64.67; H, 6.78; N, 11.31. Found: C, 64.42; H, 6.81; N, 11.04.

N^1-(4-Pyridinylmethyl)-N^2,N^2-bis[2-[(4-pyridinylmethyl)-amino]ethyl]-1,2-ethanediamine (3e) (Entry 6) Compound **3e** was prepared from the reaction of **1** with isonicotinaldehyde (**2e**) under the conditions shown in Table S1. Separation of the products by centrifugal chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=73 : 15 : 2→65 : 32 : 3) gave **3e** (3.97 g, 95%) as a yellow oil.

3e: ^{1}H-NMR ($CDCl_3$) δ: 2.00 (3H, brs, NH), 2.61 (6H, t, J=5.2 Hz, H2′), 2.67 (6H, t, J=5.2 Hz, H1′), 3.76 (6H, s, Hα), 7.20 (6H, dd, J=4.6, 1.5 Hz, H3, 5), 8.50 (6H, dd, J=4.6, 1.5 Hz, H2, 6). ^{13}C-NMR ($CDCl_3$) δ: 47.17 (C1′), 52.73 (Cα), 54.20 (C2′), 122.78 (C3, 5), 149.33 (C4), 149.80 (C2, 6). KM4-4-1 Positive-ion FAB-MS m/z: 420$(M+H^+)$. HR-FAB-MS m/z: 420.2873 (Calcd for $C_{24}H_{34}N_7$: 420.2876).

General Procedure for the Preparation of C_3-Symmetrical Amide-Type TAEA Derivatives (5): Example: Synthesis of *N*,*N*′,*N*″-(Nitrilotri-2,1-ethanediyl)tris[*N*-(3,4-dimethoxyphenylmethyl)acetamide] (5c) (Entry 9) To a solution of compound **3c** (2.85 g, 5.00 mmol) in benzene (8 mL) was added dropwise a solution of acetic anhydride (Ac_2O, **4**, 1.55 g, 15.0 mmol) in benzene (2 mL) at r.t., and then the mixture was stirred for 2 h. After removal of the solvent by evaporation, 1 M NaOH solution (20 mL) was added to the resulting residue and then the mixture was extracted with EtOAc (3×40 mL). The organic layer was washed with brine (25 mL) and dried with $MgSO_4$ and then evaporated to give a pale yellow oil, which was purified by flash chromatography (EtOH : EtOAc=4 : 6) to give **5c** (2.88 g, 80%) as a colorless semisolid.

5c: mp 34–39°C. IR (KBr) cm^{-1}: 1646 (C=O), 1261, 1233 (C–O of aromatic ether), 1137, 1025 (C–O of aliphatic ether). ^{1}H-NMR ($CDCl_3$) δ: 2.07, 2.09*, 2.11, 2.15 (9H, s, $COCH_3$), 2.05, 2.52, 2.58*, 2.63 (6H, t, J=7.3 Hz, H2′), 3.17, 3.23, 3.34*, 3.37 (6H, t, J=7.3 Hz, H1′), 3.83, 3.84, 3.86, 3.86* (18H, s, OCH_3), 4.39, 4.43*, 4.47, 4.50 (6H, s, Hα), 6.63–6.79 (6H, m, H2, 6), 6.80–6.86 (3H, m, H5). (The signals of the predominant conformer were asterisked.) ^{13}C-NMR ($CDCl_3$) δ: 21.64 ($COCH_3$), 44.28 (C1′), 52.03 (C2′), 52.50 (Cα), 55.98 (OCH_3), 109.76 (C2), 111.61 (C5), 118.51 (C6), 129.12 (C1), 148.74 (C3), 149.60 (C4), 170.87 (C=O). (Signals of only the predominant conformer were assigned.) Positive-ion FAB-MS m/z: 723 $(M+H^+)$. HR-FAB-MS m/z: 723.3967 (Calcd for $C_{39}H_{55}N_4O_9$: 723.3969). *Anal.* Calcd for $C_{39}H_{54}N_4O_9 \cdot H_2O$: C, 63.22; H, 7.62; N, 7.56. Found: C, 63.18; H, 7.70; N, 7.61.

***N*,*N*′,*N*″-[2,2′,2″-Nitrilotris(ethane-2,1-diyl)]tris[*N*-(2-hydroxy-3-methoxybenzyl)acetamide] (5a) (Entry 7)** Compound **5a** was prepared by acylation of **3a** with Ac_2O (**4**) under the conditions shown in Table S2. After work-up, crude compound **5a** (1.59 g, 75%) was obtained as a white solid. Recrystallization from AcOEt gave an analytically pure product **5a** as colorless crystals.

5a: mp 107–109°C (from AcOEt). IR (KBr) cm^{-1}: 3150 (OH), 1625 (C=O), 1070 (C–O of phenol, ether). ^{1}H-NMR (DMSO-d_6) δ: 1.98, 1.99, 2.02, 2.03* (9H, s, CH_3CO–), 2.36–3.47 (3H, m, H2′), 2.50–2.58 (3H, m, H2′), 3.13–3.24 (6H, m, H1′), 3.74*, 3.78 (9H, s, CH_3O–), 4.38, 4.40, 4.42* (6H, brs, Hα), 6.57–6.76 (6H, m, H3, 4), 6.83–6.88 (3H, m, H5), 8.76, 8.79, 8.82, 8.95, 8.96*, 8.98 (3H, brs, OH). (The signals of the predominant conformer were asterisked.) ^{13}C-NMR (DMSO-d_6) δ: 20.64, 20.86, 20.90*, 20.93, 21.28, 21.32* ($\underline{C}H_3CO$–), 43.05, 43.31, 43.37, 43.52, 43.77 (Cα, C1′), 46.16, 46.24*, 46.40 (C1′), 47.39, 47.51* (Cα), 51.07, 51.32*, 51.83, 52.07* (C2′), 55.70*, 55.79* (CH_3O–), 110.98, 111.01, 111.10, 111.17*, 111.27 (C5), 118.61, 118.82*, 119.51 (C4), 119.61, 119.79, 121.25* (C3), 123.78, 123.86, 124.09* (C2), 143.99, 144.06, 144.50* (C1), 147.38*, 147.61, 147.64, 147.68 (C6), 169.78, 169.84, 170.43, 170.54*, 170.67 (C=O). (Signals of only the predominant conformer were asterisked.) Positive-ion FAB-MS m/z: 681 $(M+H)^+$. HR-FAB-MS m/z : 681.3517 (Calcd for $C_{36}H_{49}N_4O_9$: 681.3500). *Anal.* Calcd for $C_{36}H_{48}N_4O_9 \cdot 0.9\ H_2O$: C, 62.04; H, 7.20; N, 8.04. Found: C, 62.09; H, 7.15; N, 7.85.

***N*,*N*′,*N*″-(Nitrilotri-2,1-ethanediyl)tris[*N*-(4-hydroxy-3-methoxyphenylmethyl)acetamide] Hydrochloride (5b·HCl) and [[(Nitrilotri-2,1-Ethanediyl)tris(acetylazanediyl)]tris-(methylene)]tris(2-methoxybenzene-4,1-diyl) Triacetate (5b′) (Entry 8)** Compound **5b**′ was prepared by acylation of compound **3b** (0.943 g, 1.70 mmol) with Ac_2O (**4**, 1.39 g, 13.6 mmol) under the conditions shown in Table S2. After work-up crude compound **5b**′ (987 mg, 72%) was obtained as a white solid. After dilution of this material with EtOH (4 mL) and addition of 1 M HCl (in EtOH), evaporation of the solvent and azeotropic evaporation with dry benzene were repeated. Then the white semisolid material was recrystallized from 2-PrOH to give an analytically pure salt of the deacylated target compound **5b**·HCl as colorless crystals.

5b·HCl: mp 69–73°C (from 2-PrOH). IR (KBr) cm^{-1}: *ca.* 3200 (OH), *ca.* 2600 (NH^+), 1636 (C=O), 1279, 1032, 1125 (C–O). ^{1}H-NMR (DMSO-d_6) δ: 1.04 (6H, d, J=6.1 Hz, CH_3 of 2-PrOH), 2.10*, 2.11 (9H, s, $COCH_3$), 3.17, 3.24*, 3.33 (6H, brs, H2′), 3.39 (4H, brs, OH) 3.66*, 3.75 (6H, brs, H1′), 3.75, 3.76, 3.77*, 3.78 (9H, s, OCH_3), 3.80 (1H, q, J=6.1 Hz, CH of 2-PrOH), 4.38, 4.48* (9H, brs, Hα), 6.65* (0.8H, dd, J=7.9, 1.8 Hz, H5), 6.68–6.73 (0.4H, m, H5, 6), 6.79* (0.8H, d, J=7.9 Hz, H6), 6.82* (0.8H, brs, H3), 6.88–6.93 (0.2H, m, H3), 8.96*, 9.85, 10.88, 11.73 (3H, brs, NH, OH). (The signals of the predominant conformer were asterisked.) ^{13}C-NMR (DMSO-d_6) δ: 21.40 ($COCH_3$), 25.39 (CH_3 of 2-PrOH), *ca.* 40 (C1′), 50.33 (C2′), 51.29 (Cα), 55.64 (OCH_3), 61.93 (CH of 2-PrOH), 111.52 (C3), 115.64 (C6), 119.23 (C4), 127.56 (C5), 146.01 (C1), 147.77 (C2), 171.38 (C=O). (Signals of only the

predominant conformer were assigned.) Positive-ion FAB-MS *m/z*: 681 ($M+H^+$). HR-FAB-MS *m/z*: 681.3491 (Calcd for $C_{36}H_{49}N_4O_9$: 681.3500). *Anal.* Calcd for $C_{36}H_{48}N_4O_9 \cdot HCl \cdot 2\text{-}PrOH \cdot H_2O$: C, 58.89; H, 7.48; N, 7.04. Found: C, 58.75; H, 7.70; N, 6.84.

5b′: Positive-ion FAB-MS *m/z*: 807 ($M+H^+$). HR-FAB-MS *m/z*: 807.3812 (Calcd for $C_{42}H_{55}N_4O_{12}$: 807.3816). Both ^{1}H- and ^{13}C-NMR in $CDCl_3$ were too complicated and we could not provide full assignments of the observed signals.

***N*,*N*′,*N*″-(Nitrilotri-2,1-ethanediyl)tris[*N*-(1,3-benzodioxol-5-ylmethyl)acetamide] (5d) and *N*,*N*′-[[[2-[(1,3-Benzodioxol-5-ylmethyl)amino]ethyl]imino]di-2,1-ethanediyl]bis[*N*-(1,3-benzodioxol-5-ylmethyl)acetamide] (9d) (Entry 10)** Compound **5d** was prepared by acylation of **3d** with Ac_2O (**4**) under the conditions shown in Table S2. Separation of the products by flash chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=95 : 4.7 : 0.3→93 : 6.6 : 0.4) gave **5d** (2.67 g, 53%) as a colorless semisolid and **9d** (0.94 g, 20%) as a colorless oil. Compound **9d** was purified by centrifugal chromatography (EtOAc : EtOH=99.5 : 0.5) to give an analytically pure **9d** as a hygroscopic white semisolid.

5d: mp 46–65°C. IR (KBr) cm^{-1}: 1644 (C=O), 1248, 1037 (=C–O–C–), 923 (C–O of methylenedioxy). ^{1}H-NMR ($CDCl_3$) δ: 2.09*, 2.10, 2.11, 2.14 (9H, s, CH_3), 2.40–2.70 [6H, 2.43 (brs), 2.49, 2.55* (t, J=7.0 Hz), 2.65 (brs), H2′], 3.10–3.45 [6H, 3.16, 3.22, 3.31* (t, J=7.0 Hz), 3.37 (brs), H1′], 4.36, 4.39*, 4.44, 4.46 (6H, s, Hα), 5.91, 5.91, 5.94*, 5.95, 5.96 (6H, s, H2), 6.57–6.80 (9H, m, ArH). (The signals of the predominant conformer were asterisked.) ^{13}C-NMR ($CDCl_3$) δ: 21.54 (CH_3), 44.06 (C1′), 51.84 (C2′), 52.50 (Cα), 101.15 (C2), 106.79 (C4), 108.45 (C7), 119.57 (C6), 130.41 (C5), 147.14 (C3a or 7a), 148.27 (C7a or 3a), 170.71 (C=O). (Signals of only the predominant conformer were assigned.) Positive-ion FAB-MS *m/z*: 675 ($M+H^+$). HR-FAB-MS *m/z*: 675.3027 (Calcd for $C_{36}H_{43}N_4O_9$: 675.3030). *Anal.* Calcd for $C_{36}H_{42}N_4O_9 \cdot 0.7H_2O$: C, 62.91; H, 6.36 ; N, 8.15. Found: C, 62.90; H, 6.57; N, 8.01.

9d: mp 76–81°C. IR (KBr) cm^{-1}: 1638(C=O), 1251, 1037 (=C–O–C–), 924 (C–O of methylenedioxy). ^{1}H-NMR (DMSO-d_6) δ: 1.44 (1H, brs, NH), 1.98*, 1.99 (5H, s, CH_3), 2.02 (0.6H, s, CH_3), 2.06 (0.4H, s, CH_3), 2.29–2.47 (4H, m, H2′), 2.50–2.57 (1H, m, H2″), 2.61–2.69 (1H, m, H2″), 3.05–3.31 (6H, m, H1′, 1‴), 3.45–5.57 (1H, m, Hα′), 3.83–3.89 (1H, m, Hα′), 4.32*, 4.36, 4.39, 4.44 (4H, s, Hα), 5.98*, 5.99 (4H, s, H2), 6.01*, 6.05 (2H, s, H2‴), 6.62–6.70 (2H, m, H6), 6.73–6.77 (2H, m, H4), 6.79–6.92 (4H, m, H7, 6‴, 7‴), 7.03–7.08 (1H, m, H4‴). ^{13}C-NMR (DMSO-d_6) δ: 21.50 (CH_3), 43.30 (C1″), 45.57 (C1′), 47.55 (Cα), 49.99 (C2″), 51.56 (C2′), 58.48 (Cα′), 100.91 (C2‴), 101.09 (C2), 108.06 (C4), 108.14 (C7‴ or C7), 108.36 (C7 or C7‴), 110.18 (C4‴), 119.79 (C6), 123.82 (C6‴), 128.34 (C5‴), 132.05 (C5), 146.32 (C7a), 147.05 (C7a‴), 147.26 (C3a‴), 147.72 (C3a), 169.78 (C=O). (Signals of only the predominant conformer were assigned.) Positive-ion FAB-MS *m/z*: 633 ($M+H^+$). HR-FAB-MS *m/z*: 633.2918 (Calcd for $C_{34}H_{41}N_4O_8$: 633.2924). *Anal.* Calcd for $C_{34}H_{40}N_4O_8 \cdot 2H_2O$: C, 61.07; H, 6.63 ;N, 8.38. Found: C, 61.01; H, 6.76; N, 8.13.

***N*,*N*′,*N*″-(Nitrilotri-2,1-ethanediyl)tris[*N*-(pyridin-4-ylmethyl)acetamide] (5e) (Entry 11)** Compound **5e** was prepared by acylation of compound **3e** with Ac_2O (**4**) under the conditions shown in Table S2. Separation of the products by flash chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=90 : 9.5 : 0.5→85 : 14 : 1) gave **5e** (2.51 g, 61%) as a pale yellow sticky oil.

5e: IR (KBr) cm^{-1}: 1636 (C=O), 796 (CH of pyridine). ^{1}H-NMR ($CDCl_3$) δ: 2.02, 2.04*, 2.07, 2.17, 2.21 (9H, s, CH_3), 2.50–2.70 (6H, m, H2′), 3.18–3.45 (6H, m, H1′), 4.45, 4.50*, 4.55, 4.56, 4.58 (6H, s, Hα), 7.05–7.17 (6H, m, H3, 5), 8.52, 8.60* (6H, t, J=6.1 Hz, H2, 6). (The signals of the predominant conformer were asterisked.) ^{13}C-NMR ($CDCl_3$) δ: 21.46 (CH_3), 44.55 (C1′), 51.59 (C2′), 51.78 (Cα), 121.15 (C3, 5), 145.92 (C4), 150.41 (C2, 6), 170.94 (C=O). (Signals of only the predominant conformer were assigned.) Positive-ion FAB-MS *m/z*: 546 ($M+H^+$). HR-FAB-MS *m/z*: 546.3194 (Calcd for $C_{30}H_{40}N_7O_3$: 546.3193). *Anal.* Calcd for $C_{30}H_{39}N_7O_3 \cdot 0.5H_2O$: C, 64.96; H, 7.27; N, 17.68. Found: C, 65.00; H, 7.20; N, 17.46.

General Procedure for the Preparation of *C*$_3$-Symmetrical Urea-Type TAEA Derivatives (7, 8): Example: Synthesis of *N*,*N*″,*N*-(Nitrilotri-2,1-ethanediyl)tris[*N*′-(3,4-dimethoxyphenyl)thiourea] (8l) (Entry 14) To a solution of 3,4-dimethoxyphenyl isothiocyanate (**6l**, 2.93 g, 15.0 mmol) in CH_2Cl_2 (20 mL) was added TAEA (**1**, 731 mg, 5.00 mmol) at r.t. After stirring for 1 h, the resulting white solid was filtrated to obtain crude compound **8l** (3.35 g, 91%). Recrystallization from MeOH gave analytically pure compound **8l** as a white solid.

8l: mp 161–163°C (from MeOH). IR (KBr) cm^{-1}: 1513 (C=S), 1258, 1235, 1134, 1027 (C–O). ^{1}H-NMR ($CDCl_3$) δ: 2.67 (6H, t, J=6.9 Hz, H2′), 3.48–3.55 (6H, m, H1′), 3.72 (9H, s, –OCH_3), 3.73 (9H, s, –OCH_3), 6.79 (3H, dd, J=8.5, 2.1 Hz, H6), 6.90 (3H, d, J=8.5 Hz, H5), 6.96 (3H, d, J=2.1 Hz, H2), 7.36 (3H, brs, Hβ), 9.37 (3H, brs, Hα). ^{13}C-NMR ($CDCl_3$) δ: 41.97 (C1′), 52.19 (C2′), 55.45 (–OCH_3), 55.70 (–OCH_3), 109.23 (C2), 111.97 (C5), 116.28 (C6), 131.62 (C1), 146.27 (C4), 148.64 (C3), 180.28 (C=S). Positive-ion FAB-MS *m/z*: 732 ($M+H^+$). HR-FAB-MS *m/z*: 723.2682 (Calcd for $C_{33}H_{46}N_7O_6S_3$: 732.2672). *Anal.* Calcd for $C_{33}H_{45}N_7O_6S_3 \cdot 0.8H_2O$: C, 53.10; H, 6.29 ; N, 13.14. Found: C, 53.08; H, 6.09; N, 13.22.

***N*,*N*″,*N*‴-(Nitrilotri-2,1-ethanediyl)tris[*N*-(3,4-dimethoxybenzyl)-*N*′-[3-(trifluoromethyl)phenyl]urea] (7cm) (Entry 12)** Compound **7cm** was prepared from **3c** (282 mg, 0.473 mmol) and 3-(trifluoromethyl)phenyl isocyanate (**6m**, 266 mg, 1.41 mmol) under the conditions shown in Table S3. Separation of the product by flash chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=97 : 2.7 : 0.3→95 : 4.7 : 0.3) gave **7cm** (242 mg, 44%) as a colorless oil.

7cm: ^{1}H-NMR ($CDCl_3$) δ: 1.64 (3H, s, NH), 2.79 (6H, brt, J=6.6 Hz, H2′), 3.55 (6H, brs, H1′), 3.82 (9H, s, MeO on C3), 3.84 (9H, s, MeO on C4), 4.74 (6H, s, Hα), 6.75–6.80 (9H, m, H2, 5, 6), 7.19 (3H, brd, J=7.5 Hz, H4″), 7.23 (3H, t, J=7.5 Hz, H5″), 7.36 (3H, brd, J=7.5 Hz, H6″), 7.58 (3H, brs, H2″). ^{13}C-NMR ($CDCl_3$) δ: 45.44 (C1′), 51.03 (Cα), 52.68 (C2′), 55.89 (CH_3O), 55.92 (CH_3O), 110.24 (C2), 111.31 (C5), 116.40 (q, J=4.3 Hz, C2″), 119.28 (q, J=4.3 Hz, C4″), 122.83 (C6″), 123.89 (q, J=271.6 Hz, CF_3), 129.07 (C1), 129.19 (C5″), 131.02 (q, J=31.8 Hz, C3″), 139.72 (C1″), 148.92 (C4), 149.66 (C3), 155.87 (C=O). Positive-ion FAB-MS *m/z*: 1158 ($M+H^+$). HR-FAB-MS *m/z*: 1158.4369 (Calcd for $C_{57}H_{61}F_9N_7O_9$: 1158.4387).

***N*,*N*″,*N*‴-(Nitrilotri-2,1-ethanediyl)tris[*N*-(pyridin-4-ylmethyl)-*N*′-[4-(trifluoromethyl)phenyl]thiourea] (7ep) (Entry 13)** Compound **7ep** was prepared from **3e** (242 mg, 0.577 mmol) and 4-(trifluoromethyl)phenyl isothiocyanate (**6p**, 352 mg, 1.73 mmol) under the conditions shown in Table S3. After evaporation of the solvent, recrystallization of the result-

ing mixture from 2-PrOH gave **7ep** (296 mg, 50%) as a white solid.

7ep: mp 122–127°C (from 2-PrOH). ^{1}H-NMR (DMSO-d_6) δ: 2.95 (6H, t, J=6.7 Hz, H2′), 3.88 (6H, brs, H1′), 5.10 (6H, brs, Hα), 7.18 (6H, d, J=5.6 Hz, H3, 5), 7.48 (6H, d, J=8.4 Hz, H2″, 6″), 7.59 (6H, d, J=8.4 Hz, H3″, 5″), 8.50 (6H, d, J=5.6 Hz, H2, 6), 9.84 (3H, brs, NH). ^{13}C-NMR (DMSO-d_6) δ: 49.65 (C1′), 51.25 (C2′), 53.10 (Cα), 121.69 (C3, 5), 124.40 (q, J=32.1 Hz, C4″), 124.74 (q, J=271.1 Hz, CF_3), 124.94 (q, J=4.1 Hz, C3″, 5″), 125.23 (C2″, 6″), 144.35 (C1″), 145.99 (C4), 149.56 (C2, 6), 182.21 (C=S). Positive-ion FAB-MS m/z: 1029 (M+H$^+$). HR-FAB-MS m/z: 1029.2927 (Calcd for $C_{48}H_{46}F_9N_{10}S_3$: 1029.2925).

***N*,*N*″,*N*-(Nitrilotri-2,1-ethanediyl)tris[*N*′-[3-(trifluoromethyl)phenyl]urea] (8m) (Entry 15)** Compound **8m** was prepared from **1** (292 mg, 2.00 mmol) and 3-(trifluoromethyl)-phenyl isocyanate (**6m**, 1.12 g, 6.00 mmol) under the conditions shown in Table S3. After filtration of the resulting precipitates, recrystallization from propionitrile (EtCN) gave **8m** (1.19 g, 84%) as colorless crystals.

8m: mp 187–190°C (from EtCN). IR (KBr) cm^{-1}: 3334 (NH), 1645 (CONH), 1340 (CF_3). ^{1}H-NMR (DMSO-d_6) δ: 2.63 (6H, t, J=6.7 Hz, H2′), 3.17–3.25 (6H, m, H1′), 6.25 (3H, t, J=5.5 Hz, NH$_\beta$), 7.20 (3H, dd, J=7.6, 0.9 Hz, H4), 7.41 (3H, t, J=7.9 Hz, H5), 7.49 (3H, d, J=8.5, H6), 7.93 (3H, s, H2), 8.87 (3H, s, NH$_\alpha$). ^{13}C-NMR (DMSO-d_6) δ: 37.53 (C1′), 53.72 (C2′), 113.54 (q, J=4.1 Hz, C2), 117.08 (q, J=4.1 Hz, C4), 121.06 (C6), 124.16 (q, J=272.1 Hz, CF_3), 129.34 (q, J=31.0, C3), 129.52 (C5), 141.24 (C1), 155.03 (C=O). Positive-ion FAB-MS m/z: 708 (M+H$^+$). HR-FAB-MS m/z: 708.2352 (Calcd for $C_{30}H_{31}F_9N_7O_3$: 708.2345). *Anal.* Calcd for $C_{30}H_{30}F_9N_7O_3$: C, 50.92; H, 4.27; N, 13.86. Found: C, 50.84; H, 4.07; N, 13.89.

***N*,*N*″,*N*-(Nitrilotri-2,1-ethanediyl)tris[*N*′-[4-(trifluoromethyl)phenyl]urea (8n) (Entry 16)** Compound **8n** was prepared from **1** (292 mg, 2.00 mmol) and 4-(trifluoromethyl)-phenyl isocyanate (**6n**, 1.12 g, 6.00 mmol) under the conditions shown in Table S3. After filtration of the resulting precipitates, recrystallization from EtOH gave **8n** (1.24 g, 88%) as colorless crystals.

8n: mp 237–238°C (from EtOH). IR (KBr) cm^{-1}: 1649 (CONH), 1329 (CF_3). ^{1}H-NMR (DMSO-d_6) δ: 2.63 (6H, t, J=6.4 Hz, H2′), 3.22 (6H, dt, J=6.4, 6.1 Hz, H1′), 6.28 (3H, t, J=5.5 Hz, NH$_\beta$), 7.52 (6H, d, J=8.9 Hz, H3, 5), 7.56 (6H, d, J=8.9 Hz, H2, 6), 8.91 (3H, s, NH$_\alpha$). ^{13}C-NMR (DMSO-d_6) δ: 37.52 (C1′), 53.65 (C2′), 117.20 (C2, 6), 120.89 (q, J=32.1 Hz, C4), 124.54 (q, J=271.0 Hz, CF_3), 125.74 (q, J=4.1 Hz, C3, 5), 144.09 (C1), 154.84 (C=O). Positive-ion FAB-MS m/z: 708 (M+H$^+$). HR-FAB-MS m/z: 708.2352 (Calcd for $C_{30}H_{31}F_9N_7O_3$: 708.2345). *Anal.* Calcd for $C_{30}H_{30}F_9N_7O_3$: C, 50.92; H, 4.27; N, 13.86. Found: C, 50.88; H, 4.20; N, 13.92.

***N*,*N*″,*N*-(Nitrilotri-2,1-ethanediyl)tris[*N*′-[4-(trifluoromethyl)phenyl]-thiourea] (8p) (Entry 17)** Compound **8p** was prepared from **1** (292 mg, 2.00 mmol) and 4-(trifluoromethyl)phenyl isothiocyanate (**6p**, 1.12 g, 6.00 mmol) under the conditions shown in Table S3. Filtration of the resulting precipitates gave the crude product **8p** (1.12 g, 74%). Recrystallization from 2-PrOH gave analytically pure **8p** as colorless crystals.

8p: mp 198–200°C (from 2-PrOH). IR (KBr) cm^{-1}: 3275 (NH), 1548, 1542 (S=C–N), 1329 (CF_3), 1111, 1067 (C=S). ^{1}H-NMR (DNSO-d_6) δ: 2.80 (6H, t, J=6.7 Hz, H2′), 3.57–3.90 (6H, m, H1′), 7.62 (6H, d, J=8.4 Hz, H3, 5), 7.70 (6H, d, J=8.4 Hz, H2, 6), 7.93 (3H, brs, NH$_\beta$), 9.88 (3H, s, NH$_\alpha$). ^{13}C-NMR (DMSO-d_6) δ: 41.81 (C1′), 51.86 (C2′), 121.77 (C2, 6), 123.34 (q, J=32.1 Hz, C4), 124.28 (q, J=271.0 Hz, CF_3), 125.50 (q, J=3.1 Hz, C3, 5), 143.19 (C1), 180.22 (C=S). Positive-ion FAB-MS m/z: 756 (M+H$^+$). HR-FAB-MS m/z: 756.1658 (Calcd for $C_{30}H_{31}F_9N_7S_3$: 756.1659). *Anal.* Calcd for $C_{30}H_{30}F_9N_7S_3$·2-PrOH : C, 48.58; H, 4.69; N, 12.02. Found: C, 48.54; H, 4.59; N, 11.96.

General Procedure for the Preparation of *C*$_S$-Symmetrical Amine-Urea-Type TAEA Derivatives (12, 13): Example: Synthesis of *N*-[2-[Bis[2-[(1,3-benzodioxol-5-ylmethyl)amino]ethyl]amino]ethyl]-*N*′-(tricyclo[3.3.1.13,7]-dec-1-yl)thiourea (12k) (Entry 18) (Step 1) To a solution of TAEA (**1**, 1.46 g, 10.0 mmol) in CH_2Cl_2 (160 mL) was added 1-adamantyl isothiocyanate (**6k**, 2.93 g, 15.0 mmol) in CH_2Cl_2 (20 mL) and the mixture was stirred for 1 h at r.t. Evaporation of the solvent gave a pale yellow oil.

(Step 2) To a solution of the resulting oil in MeOH (5 mL) was added a solution of piperonal (**2d**, 3.00 g, 20.0 mmol) in MeOH (10 mL) at r.t. under an argon atmosphere and the mixture was stirred for 20 h.

(Step 3) To the resulting yellow solution was added MeOH (80 mL) and $NaBH_4$ (2.04 g, 54.0 mmol) at 0°C under an argon atmosphere with stirring for 4 h, and then stirring was continued at r.t. for 15 h. After evaporation of the solvent, aqueous ammonium acetate (NH_4OAc, 10%) was added to the resulting white solid and the mixture was extracted with $CHCl_3$ (3×200 mL). The separated organic layer was dried over $MgSO_4$ and filtrated, and evaporation of the solvent gave a pale yellow oil. Separation of the products by flash chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=950 : 47 : 3→73 : 25 : 2) gave **16k** (403 mg, 17%) as a white solid, **13k** (365 mg, 6%) as a white solid, and **12k** (2.03 g, 33%) as a colorless semisolid.

12k: mp 46–52°C. IR (KBr) cm^{-1}: 3275 (NH of *sec*-amine), 1489 (C=S), 1247, 1038, 929 (C–O). ^{1}H-NMR ($CDCl_3$) δ: 1.58–1.67 (6H, m, Ha), 1.99 (6H, brs, Hb), 2.02 (3H, brs, Hc), 2.35 (3H, brs, NH), 2.61 (4H, brt, J=6.1 Hz, H2′), 2.66–2.71 (6H, m, H1′, 2″), 3.67 (4H, s, Hα), 5.92 (4H, s, H2), 6.09 (1H, brs, NH), 6.75 (4H, brs, H4, 6), 6.81 (2H, s, H7). ^{13}C-NMR ($CDCl_3$) δ: 29.44 (Cc), 36.11 (Ca), 42.03 (Cb), 43.06 (C1″), 46.63 (C1′), 53.43, 53.55, 53.62, 53.72 (C2′, 2″, α, d), 100.91 (C2), 108.14 (C4), 108.67 (C7), 121.37 (C6), 133.34 (C5), 146.72 (C3a), 147.78 (C7a), 180.73 (C=S). Positive-ion FAB-MS m/z: 608 (M+H$^+$). HR-FAB-MS m/z: 608.3273 (Calcd for $C_{33}H_{46}N_5O_4S$: 608.3271). *Anal.* Calcd for $C_{33}H_{45}N_5O_4S$·0.5H_2O: C, 64.26; H, 7.52; N, 11.35. Found: C, 64.32; H, 7.64; N, 11.25.

16k: ^{1}H-NMR ($CDCl_3$) δ: 1.69 (6H, brs, Ha), 2.13 (3H, brs, Hc), 2.15 (6H, brs, Hb), 2.70 (2H, dt, J=5.5, 0.3 Hz, H5), 3.62–3.68 (2H, m, H4), 6.49 (0.5H, brs, NH), 7.28 (0.5H, brs, NH). ^{13}C-NMR ($CDCl_3$) δ: 29.54 (Cc), 36.19 (Ca), 42.11 (Cb), 43.01 (C4), 53.53 (C5), 54.25 (Cd), 180.49 (C=S). Positive-ion FAB-MS m/z: 237 (32, M+H$^+$). HR-FAB-MS m/z: 237.1417 (Calcd for $C_{13}H_{21}N_2S$: 237.1425).

***N*,*N*″-[[[2-[(1,3-Benzodioxol-5-ylmethyl)amino]ethyl]-imino]di-2,1-ethanediyl]bis[*N*′-(tricyclo[3.3.1.1^{3,7}]dec-1-yl)-thiourea] (13k) (Entry 19)** Compound **13k** was prepared from **1** (1.46 g, 10.0 mmol) with **6k** (3.87 g, 20.0 mmol) and **2d** (1.50 g, 10.0 mmol) under the conditions shown in Table S4. Separation of the products by flash chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=95 : 4.7 : 0.3→93 : 6.6 : 0.4) gave

13k (2.12 g, 32%) as a white solid and **12k** (0.903 g, 15%) as a pale yellow oil.

13k: mp 48–50°C. IR (KBr) cm^{-1}: 3276 (NH of *sec*-amine), 1539 (C=S), 1247, 1038, 933 (C–O). ^{1}H-NMR ($CDCl_3$) δ: 1.67 (12H, brs, Ha), 2.09 (19H, brs, Hb, Hc, NH), 2.63–2.68 (2H, m, H2′), 2.68–2.74 (6H, m, H1′, 2″), 3.56–3.64 (4H, m, H1″), 3.72 (2H, s, Hα), 5.93 (2H, s, H2), 6.08 (2H, brs, NH), 6.54 (2H, brs, NH), 6.73–6.79 (2H, m, H4, 6), 6.84 (1H, d, J=1.2 Hz, H7). ^{13}C-NMR ($CDCl_3$) δ: 29.51 (Cc), 36.16 (Ca), 42.12 (Cb), 42.95 (C1″), 46.72 (C1′), 53.11 (C2″), 53.62 (Cα), 53.80 (C2′), 54.07 (Cd), 100.88 (C2), 108.16 (C4), 108.73 (C7), 121.41 (C6), 133.61 (C5), 146.65 (C3a), 147.74 (C7a), 180.77 (C=S). Positive-ion FAB-MS m/z: 667 (5, M+H$^+$). HR-FAB-MS m/z: 667.3824 (Calcd for $C_{36}H_{55}N_6O_2S_2$: 667.3828). (YO2-6) *Anal.* Calcd for $C_{36}H_{54}N_6O_2S_2\cdot0.5H_2O$: C, 63.96; H, 8.20; N, 12.43. Found: C, 63.99; H, 8.19; N, 12.16.

***N*-[2-[Bis[2-[(1,3-benzodioxol-5-ylmethyl)amino]ethyl]-amino]ethyl]-*N*′-(3,4-dimethoxyphenyl)thiourea (12l) and 1-(3,4-Dimethoxyphenyl)-2-imidazolidinethione (16l) (Entry 20)** Compound **12l** was prepared from **1** (1.46 g, 10.0 mmol) with **6l** (1.95 g, 10.0 mmol) and **2d** (3.00 g, 20.0 mmol) under the conditions shown in Table S4. Separation of the products by flash chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=93 : 6.6 : 0.4→85 : 14 : 1) gave **16l** (trace) as a white solid, **13l** (1.50 g, 22%) as a white solid, and **12l** (2.77 g, 45%) as a white oil.

12l: IR (KBr) cm^{-1}: 3290 (NH of *sec*-amine), 1513 (C=S), 1242, 1034, 928 (C–O). ^{1}H-NMR ($CDCl_3$) δ: 2.76 (6H, t, J=5.8 Hz, H2″, 2′), 2.82 (4H, t, J=5.8 Hz, H1′), 3.75 (2H, t, J=5.8 Hz, H1″), 3.82 (3H, s, OCH_3 on C4‴), 3.82 (2H, s, Hα), 3.83 (3H, s, OCH_3 on C3‴), 4.48 (3H, brs, NH), 5.92 (4H, s, H2), 6.73 (2H, d, J=7.9 Hz, H7), 6.76 (1H, d, J=8.5 Hz, H5‴), 6.85 (2H, dd, J=7.9, 1.5 Hz, H6), 6.91 (2H, d, J=1.5 Hz, H4), 6.93 (1H, dd, J=8.5, 2.4 Hz, H6‴), 7.26 (1H, brs, H2‴), 9.06 (1H, brs, NH). ^{13}C-NMR ($CDCl_3$) δ: 42.12 (C1″), 45.25 (C1′), 51.92 (Cα), 52.28 (C2′), 54.39 (C2″), 55.99, 56.08 (OCH_3), 101.31 (C2), 108.55 (C7), 109.10 (C2‴), 109.60 (C4), 111.19 (C5‴), 116.53 (C6‴), 123.14 (C6), 127.58 (C5), 132.14 (C1‴), 146.79 (C3‴), 147.93 (C7a), 148.14 (C3a), 148.90 (C4‴), 181.62 (C=S). Positive-ion FAB-MS m/z: 610 (M+H$^+$). HR-FAB-MS m/z: 610.2714 (Calcd for $C_{31}H_{40}N_5O_6S$: 610.2699). *Anal.* Calcd for $C_{31}H_{39}N_5O_6S\cdot0.9H_2O$: C, 59.48; H, 6.57; N, 11.19. Found: C, 59.51; H, 6.50; N, 11.09.

16l: ^{13}C-NMR ($CDCl_3$) δ: 42.98 (C4), 53.24 (C5), 56.02, 56.09 (OCH_3), 109.47 (C2′), 111.70 (C5′), 117.53 (C6′), 130.34 (C1′), 147.69 (C4′), 149.41 (C3′), 181.30 (C=S). (Signals of only the predominant conformer were assigned.) Positive-ion FAB-MS m/z: 239 (M+H$^+$). HR-FAB-MS m/z: 239.0850 (Calcd for $C_{11}H_{15}N_2O_2S$: 239.0854).

***N*,*N*″-[[[2-[(1,3-Benzodioxol-5-ylmethyl)amino]ethyl]-imino]di-2,1-ethanediyl]bis[*N*′-(3,4-dimethoxyphenyl)thiourea] (13l) (Entry 21)** Compound **13l** was prepared from **1** (731 mg, 5.00 mmol) with **6l** (1.95 g, 10.0 mmol) and **2d** (751 mg, 5.00 mmol) under the conditions shown in Table S4. Separation of the products by flash chromatography (CH_2Cl_2: 95% EtOH : 28% NH_3=95 : 4.7 : 0.3→93 : 6.6 : 0.4) gave **8l** (1.11 g, 30%) as a white solid and **13l** (1.15 g, 34%) as a colorless semisolid.

13l: mp 65–69°C. IR (KBr) cm^{-1}: 3264 (NH of *sec*-amine), 1512 (C=S), 1236, 1026, 927 (C–O). ^{1}H-NMR ($CDCl_3$) δ: 1.81 (2H, brs, NH), 2.59 (4H, brs, H1′, 2′), 2.66 (4H, t, J=6.1 Hz, H2″), 3.56 (2H, s, Hα), 3.63 (2H, brs, H1″), 3.82 (6H, s, CH_3O on C3‴), 3.84 (6H, s, CH_3O on C4‴), 5.87 (2H, s, H2), 6.66–6.71 (2H, m, H7, 6), *ca.* 6.7 (1H, brs, NH), 6.73 (1H, brs, H4), 6.76 (2H, dd, J=8.2, 2.1 Hz, H6‴), 6.82 (2H, d, J=8.2 Hz, H5‴), 6.83 (2H, brs, H2‴), 7.88 (2H, brs, NH). ^{13}C-NMR ($CDCl_3$) δ: 43.48 (C1″), 46.77 (C1′), 53.36 (Cα), 53.43 (C2′ or 2″), 53.62 (C2″ or 2′), 56.09, 56.12 (OCH_3), 100.89 (C2), 108.11 (C7 or 4), 108.59 (C4 or 7), 109.83 (C2‴), 111.65 (C5‴), 118.04 (C6‴), 121.25 (C6), 129.69 (C1‴), 133.50 (C5), 146.61 (C3a or 7a), 147.72 (C4‴), 148.14 (C7a or 3a), 149.67 (C3‴), 181.27 (C=S). Positive-ion FAB-MS m/z: 671 (M+H$^+$). HR-FAB-MS m/z: 671.2684 (Calcd for $C_{32}H_{43}N_6O_6S_2$: 671.2686). *Anal.* Calcd for $C_{32}H_{42}N_6O_6S_2\cdot1.2H_2O$: C, 55.50; H, 6.46; N, 12.14. Found: C, 55.54; H, 6.24; N, 11.86.

***N*-[2-[Bis[2-[(1,3-benzodioxol-5-ylmethyl)amino]ethyl]-amino]ethyl]-*N*′-[3-(trifluoromethyl)phenyl]thiourea (12o), *N*,*N*′-[[[2-[(1,3-Benzodioxol-5-ylmethyl)amino]ethyl]imino]-di-2,1-ethanediyl]bis[*N*′-[3-(trifluoromethyl)phenyl]thiourea] (13o) and 1-[3-(Trifluoromethyl)phenyl]-2-imidazolidinethione (16o) (Entry 22)** Compounds **12o** and **13o** were prepared from **1** (1.46 g, 10.0 mmol) with **6o** (2.03 g, 10.0 mmol) and **2d** (3.00 g, 20.0 mmol) under the conditions shown in Table S4. Separation of the products by flash chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=93 : 6.6 : 0.4→85 : 14 : 1) gave **16o** (466 mg, 19%) as a pale yellow semi-solid, **13o** (1.51 g, 22%) as a pale yellow semi-solid, and **12o** (3.42 g, 55%) as a pale yellow oil.

12o: IR (KBr) cm^{-1}: 3260 (NH of *sec*-amine), 1490 (C=S), 1332 ($ArCF_3$), 1249, 1038, 930 (C–O). ^{1}H-NMR ($CDCl_3$) δ: *ca.* 2.3 (4H, brs, NH), 2.58 (4H, t, J=5.5 Hz, H2′), 2.65–2.70 (6H, m, H1′, 2″), 3.60 (4H, s, Hα), 3.60 (2H, brs, H1″), 5.87 (4H, s, H2), 6.67–6.71 (4H, m, H6, 7), 6.74 (2H, s, H4), 7.34–7.37 (2H, m, H4‴, 5‴), 7.56 (2H, brs, H2‴, 6‴). ^{13}C-NMR ($CDCl_3$) δ: 43.24 (C1″), 46.66 (C1′), 52.57 (C2″), 53.46 (Cα), 53.95 (C2′), 100.95 (C2), 108.19 (C7), 108.57 (C4), 120.36 (q, J=4.1 Hz, C2‴), 121.30 (C6), 121.49 (q, J=4.1 Hz, C4‴), 125.96 (q, J=272.1 Hz, CF_3), 127.05 (C6‴), 129.22 (C5‴), 131.21 (q, J=33.1 Hz, C3‴), 133.20 (C5), 139.29 (C1‴), 146.77 (C3a), 147.83 (C7a), 181.05 (C=S). Positive-ion FAB-MS m/z: 135 (100), 247 (14), 618 (6, M+H$^+$). HR-FAB-MS m/z: 618.2363 (Calcd for $C_{30}H_{35}F_3N_5O_4S$: 618.2362). *Anal.* Calcd for $C_{30}H_{34}F_3N_5O_4S\cdot0.6H_2O$: C, 57.33; H, 5.65; N, 11.14. Found: C, 57.35; H, 5.65; N, 10.94.

13o: IR (KBr) cm^{-1}: 3256 (NH of *sec*-amine), 1490 (C=S), 1332 ($ArCF_3$), 1251, 1038, 930 (C–O). ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, t, J=7.0 Hz, CH_3 of EtOH), *ca.* 2.15 (3H, brs, NH and/or OH), 2.66–2.71 (2H, m, H2′), *ca.* 2.7 (1H, brs, NH or OH), 2.71–2.78 (6H, m, H1′, 2″), 3.57 (2H, s, Hα), 3.69 (4H, brs, H1″), 3.70 (2H, q, J=7.0 Hz, CH_2 of EtOH), *ca.* 3.7 (1H, brs, NH), 5.78 (2H, s, H2), 6.57–6.68 (3H, m, H6, 7, 4), *ca.* 6.65 (1H, brs, NH), 7.22–7.31 (4H, m, H4‴, 5‴), 7.36 (2H, brd, J=7.6 Hz, H6‴), 7.59 (2H, s, H2‴). ^{13}C-NMR ($CDCl_3$) δ: 18.40 (CH_3 of EtOH), 43.36 (C1″), 46.82 (C1′), 53.29 (C2′), 53.59 (Cα), 53.66 (C2″), 58.34 (CH_2 of EtOH), 100.99 (C2), 108.33 (C7), 108.61 (C4), 120.45 (q, J=4.1 Hz, C2‴), 121.56 (C6), 121.86 (q, J=4.1 Hz, C4‴), 123.68 (q, J=272.1 Hz, CF_3), 127.07 (C6‴), 129.25 (C5‴), 131.07 (q, J=30.0 Hz, C3‴), 132.27 (C5), 138.90 (C1‴), 146.93 (C3a), 147.85 (C7a), 181.20 (C=S). Positive-ion FAB-MS m/z: 135 (100), 247 (61), 687 (22, M+H$^+$). HR-FAB-MS m/z: 687.2006 (Calcd for $C_{30}H_{33}F_6N_6O_2S_2$: 687.2011). *Anal.* Calcd for $C_{30}H_{32}F_6N_6O_2S_2\cdot$EtOH: C, 52.45; H,

5.23; N, 11.47. Found: C, 52.44; H, 5.00; N, 11.46.

16o: Positive-ion FAB-MS *m/z*: 135 (57), 247 (100, M+H$^+$). HR-FAB-MS *m/z*: 247.0515 (Calcd for $C_{10}H_{10}F_3N_2S$: 247.0517). ^{1}H-NMR ($CDCl_3$) δ: 2.70 (2H, t, J=4.8 Hz, H5), 3.80 (2H, brd, J=4.8 Hz, H4), 7.20–7.24 (1H, m, H5′), 7.26 (1H, d, J=6.8 Hz, H4′), 7.50 (1H, brd, J=6.9 Hz, H6′), 7.91 (1H, brs, NH), 7.97 (1H, brs, H2′). ^{13}C-NMR ($CDCl_3$) δ: 41.97 (C4), 52.57 (C5), 119.65 (brs, C2′), 121.11 (C4′), 123.79 (q, J=273.1 Hz, CF_3), 126.27 (C6′), 129.11 (C5′), 130.66 (q, J=33.2 Hz, C3′), 139.58 (C1′), 180.88 (C=S).

General Procedure for the Preparation of C_S-Symmetrical Amide-Urea-Type TAEA Derivatives (14, 15): Example: Synthesis of *N*-(1,3-Benzodioxol-5-ylmethyl)-*N*-[2-[Bis[2-[[thioxo(tricyclo[3.3.1.1^{3,7}]dec-1-ylamino)methyl]amino]-ethyl]amino]ethyl]acetamide (15k) (Entry 24) (Step 1) To a pale yellow suspension of compound **13k** (1.88 g, 2.82 mmol) in tetrahydrofuran (THF) (5 mL) was added Ac_2O (**4**, 288 mg, 2.82 mmol) and the mixture was stirred for 30 min at r.t. The crude precipitated solid was obtained as a white substance **15k** (1.83 g, 92%). Recrystallization from THF gave an analytically pure sample as a white powder.

15k: mp 188–192°C (from THF). IR (KBr) cm^{-1}: 1627 (C=O), 1540 (C=S), 1357, 1238, 1038 (C–O). ^{1}H-NMR ($CDCl_3$) δ: 1.68 (12H, brs, Ha), 2.11 (6H, brs, Hc), 2.14 (12H, brs, Hb), 2.19 (3H, s, CH_3), 2.63–2.68 (6H, m, H2′, 2″), 3.43 (2H, t, J=5.5 Hz, H1′), 3.55 (4H, q, J=5.2 Hz, H1″), 4.53 (2H, brs, Hα), 5.96 (2H, s, H2), 6.32 (2H, brs, NH), 6.62 (2H, brs, NH), 6.63 (1H, d, J=7.9 Hz, H6), 6.64 (1H, brs, H4), 6.7–6.8 (1H, d, J=7.9 Hz, H7). ^{13}C-NMR ($CDCl_3$) δ: 22.26 (CH_3), 29.60 (Cc), 36.31 (Ca), 42.17 (Cb), 42.86 (C1″), 43.89 (C1′), 51.53 (Cα), 52.53 (C2′), 53.19 (C2″), 53.94 (Cd), 101.27 (C2), 106.75 (C4), 108.65 (C7), 119.63 (C6), 129.72 (C5), 147.30 (C3a), 148.42 (C7a), 172.52 (C=O), 181.19 (C=S). Positive-ion FAB-MS *m/z*: 709 (M+H$^+$). HR-FAB-MS *m/z*: 709.3948 (Calcd for $C_{38}H_{57}N_6O_3S_2$: 709.3934). *Anal*. Calcd for $C_{38}H_{56}N_6O_3S_2\cdot 0.4H_2O$: C, 63.72; H, 7.99; N, 11.73. Found: C, 63.69; H, 8.13; N, 11.47.

***N*,*N*′-[[[2-[[Thioxo(tricyclo[3.3.1.1^{3,7}]dec-1-ylamino)methyl]amino]ethyl]imino]di-2,1-ethanediyl]bis[*N*-(1,3-benzodioxol-5-ylmethyl)acetamide] (14k) (Entry 23)** Compound **14k** was prepared from **12k** (1.22 g, 2.00 mmol) and Ac_2O (**4**, 408 mg, 4.00 mmol) under the conditions shown in Table S5. Separation of the product by flash chromatography (EtOAc : EtOH=99 : 1) gave **14k** (934 mg, 68%) as a white solid.

14k: mp 66–68°C. IR (KBr) cm^{-1}: 3324 (NH), 1637 (C=O), 1490 (C=S), 1248, 1038, 924 (C–O). ^{1}H-NMR ($CDCl_3$) δ: 1.66 (6H, brs, Ha), 2.06 (3H, brs, Hc), 2.12*, 2.16 (6H, s, CH_3), 2.08, 2.20* (6H, brs, Hb), 2.6–2.55 (4H, m, H2′), 2.6–2.7 (2H, m, H2″), 3.2–3.25, 3.3–3.4* (4H, m, H1′), 3.4–3.5, 3.5–3.6* (2H, m, H1″), 4.36, 4.45*, 4.48 (4H, s, Hα), 5.92, 5.96*, 5.97 (4H, s, H2), 6.34 (1H, brs, NH), 6.6–6.7 (4H, m, H4, 6), 6.7–6.8 (2H, m, H7), 7.18 (1H, brs, NH). (The signals of the predominant conformer were asterisked.) ^{13}C-NMR ($CDCl_3$) δ: 21.84 (CH_3), 29.51 (Cc), 36.30 (Ca), 41.88 (Cb), 42.41 (C1″), 43.38 (C1′), 51.34 (Cα), 51.99 (C2′), 52.69 (C2″), 53.27 (Cd), 101.09 (C2), 106.66 (C4), 108.50 (C7), 119.45 (C6), 130.07 (C5), 147.05 (C3a), 148.23 (C7a), 171.50 (C=O), 180.82 (C=S). (Signals of only the predominant conformer were assigned.) Positive-ion FAB-MS *m/z*: 692 (M+H$^+$). HR-FAB-MS *m/z*: 692.3488 (Calcd for $C_{37}H_{50}N_5O_6S$: 692.3482). *Anal*. Calcd for $C_{37}H_{49}N_5O_6S\cdot 0.9H_2O$: C, 62.76; H, 7.23; N, 9.89. Found: C, 62.87; H, 7.33; N, 9.64.

***N*,*N*′-[[[2-[[Thioxo(3,4-dimethoxyphenylamino)methyl]-amino]ethyl]imino]di-2,1-ethanediyl]bis[*N*-(1,3-benzodioxol-5-ylmethyl)acetamide] (14l) (Entry 25)** Compound **14l** was prepared from **12l** (1.53 g, 2.51 mmol) and **4** (0.512 g, 5.02 mmol) under the conditions shown in Table S5. Separation of the product by centrifugal chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=95 : 4.7 : 0.3→93 : 6.6 : 0.4) gave **14l** (1.39 g, 80%) as a white solid.

14l: mp 54–58°C. IR (KBr) cm^{-1}: 1639 (C=O), 1512, 1490 (C=S), 1237, 1036, 923 (C–O). ^{1}H-NMR ($CDCl_3$) δ: 2.03, 2.08, 2.10*, 2.11 (6H, s, CH_3CO), 2.45–2.6 (4H, m, H2′), 2.6–2.70 (2H, m, H2″), *ca*. 2.65 (1H, brs, OH), 3.05–3.15, 3.24–3.3, 3.3–3.35* (4H, m, H1′), 3.57 (2H, brs, H1″), 3.80, 3.82*, 3.83 (6H, s, OCH_3), 4.33, 4.42*, 4.44 (4H, s, Hα), 5.91, 5.95*, 5.95 (4H, s, H2), 6.55–6.95, 7.17–7.23 (9H, m, H6, 4, 7, 5‴, 6‴, 2‴), 7.49, 8.30, 8.63* (2H, brs, NH). (The signals of the predominant conformer were asterisked.) ^{13}C-NMR ($CDCl_3$) δ: 21.64 (CH_3CO), 42.46 (C1″), 43.61 (C1′), 51.53 (Cα), 51.88 (C2′), 52.74 (C2″), 55.71, 55.84 (OCH_3), 100.99 (C2), 106.58 (C4), 108.32 (C7), 108.91 (C2‴), 111.19 (C5‴), 116.22 (C6‴), 119.41 (C6), 129.92 (C5), 131.31 (C1‴), 146.96 (C3a or C7a), 147.72 (C4‴), 148.09 (C7a or C3a), 148.84 (C3‴), 171.32 (C=O), 181.02 (C=S). (Signals of only the predominant conformer were assigned.) Positive-ion FAB-MS *m/z*: 694 (M+H$^+$). HR-FAB-MS *m/z*: 694.2928 (Calcd for $C_{35}H_{44}N_5O_8S$: 694.2911). *Anal*. Calcd for $C_{35}H_{43}N_5O_8S\cdot 0.5H_2O$; C, 59.81; H, 6.31; N, 9.96. Found: C, 59.74; H, 6.31; N, 10.04.

***N*-(1,3-Benzodioxol-5-ylmethyl)-*N*-[2-[Bis[2-[[thioxo(3,4-dimethoxyphenylamino)methyl]amino] Ethyl]amino]ethyl]-acetamide (15l) (Entry 26)** Compound **15l** was prepared from **13l** (394 mg, 587 μmol) and **4** (59.9 mg, 587 μmol) under the conditions shown in Table S5. Separation of the product by centrifugal chromatography (CH_2Cl_2 : EtOH=95 : 5) gave **15l** (368 mg, 88%) as a white solid. An analytical sample of **15l** was obtained by recrystallization from dichloromethane (CH_2Cl_2) as an off-white solid.

15l: mp 193–194°C (from CH_2Cl_2). IR (KBr) cm^{-1}: 1616 (C=O), 1511, 1453 (C=S), 1239, 1034, 922 (C–O). ^{1}H-NMR (DMSO-d_6) δ: 1.99, 2.08* (3H, s, CH_3CO), 2.54–2.66 (6H, m, H2′, 2″), 3.18–3.27 (2H, m, H1′), 3.49 (4H, brt, J=5.5 Hz, H1″), 3.71 (6H, s, CH_3 on C3‴), 3.72 (6H, s, CH_3 on C4‴), 4.39*, 4.41 (2H, brs, Hα), 5.70*, 6.00 (2H, s, H2), 6.64–6.98 (9H, m, H6, 7, 4, 6‴, 5‴, 2‴), 7.31 (2H, brs, NHβ), 9.39 (2H, s, NHγ). (The signals of the predominant conformer were asterisked.) ^{13}C-NMR (DMSO-d_6) δ: 21.15 (CH_3CO), 41.94 (C1″), 45.93 (C1′), 47.64 (Cα), 51.71 (C2′), 52.40 (C2″), 55.44, 55.68 (OCH_3), 100.76 (C2), 107.93 (C7), 108.08 (C4), 109.30 (C2‴), 111.98 (C5‴), 116.39 (C6‴), 120.96 (C6), 131.42 (C1‴ or C5), 132.12 (C5 or C1‴), 146.27 (C3a or C7a), 146.38 (C4‴), 147.29 (C7a or C3a), 148.70 (C3‴), 169.64 (C=O), 180.26 (C=S). (Signals of only the predominant conformer were assigned.) Positive-ion FAB-MS *m/z*: 713 (M+H$^+$). HR-FAB-MS *m/z*: 713.2786 (Calcd for $C_{34}H_{45}N_6O_7S_2$: 713.2791). *Anal*. Calcd for $C_{34}H_{44}N_6O_7S_2$: C, 57.29; H, 6.22; N, 11.79. Found: C, 57.10; H, 6.17; N, 11.75.

Preparation of C_3-Symmetrical TAPA Derivatives (18a, 21) 2,2′,2″-[Nitrilotris(3,1-propanediyliminomethylene)]tris-[6-methoxyphenol] (18a) Compound **18a** was prepared starting from the reaction of TAPA (**17**, 942 mg, 5.0 mmol) and **2a** (3.04 g, 20.0 mmol) in a manner similar to that for

the preparation of compound **3a**. The reactions in both steps were conducted in EtOH instead of MeOH with the ratio of TAPA : **2a** : $NaBH_4$=1 : 4 : 8.2. Separation of the product by centrifugal chromatography ($CHCl_3$: 2-PrOH : 28% NH_3=84 : 14 : 2) gave **18a** (1.93 g, 65%) as a light-brown semi-solid.

18a: mp 31–35°C. IR (KBr) cm^{-1}: 3420 (OH), 3310 (NH), 1590, 1480 (C=C of Ar), 1235, 1075 (C–O). ^{1}H-NMR ($CDCl_3$) δ: 1.63 (6H, qu, J=6.7 Hz, H2′), 2.41 (6H, t, J=6.7 Hz, H3′), 2.64 (6H, t, J=6.7 Hz, H1′), 3.84 (9H, s, CH_3O), 3.93 (6H, s, Hα), 6.26 (6H, brs, NH, OH), 6.61 (3H, d, J=7.6 Hz, H3), 6.71 (3H, dd, J=7.9, 7.6 Hz, H4), 6.79 (3H, d, J=7.9 Hz, H5). ^{13}C-NMR ($CDCl_3$) δ: 26.74 (C2′), 47.35 (C1′), 52.21 (C3′ or Cα), 52.24 (Cα or C3′), 55.83 (CH_3O), 110.83 (C5), 118.48 (C4), 120.55 (C3), 122.69 (C2), 147.40 (C1), 147.96 (C6). Positive-ion FAB-MS m/z: 597 ($M+H^+$). HR-FAB-MS m/z: 597.3644 (Calcd for $C_{33}H_{49}N_4O_6$: 597.3652). *Anal.* Calcd for $C_{33}H_{48}N_4O_6 \cdot 1.2\ H_2O$: C, 64.10; H, 8.22; N, 9.06. Found: C, 64.15; H, 8.31; N, 8.91.

***N*,*N*′,*N*″-(Nitrilotri-3,1-propanediyl)tris[3,5-bis-(trifluoromethyl)benzamide] (21)** To a solution of **17** (942 mg, 5.0 mmol) and triethylamine (TEA, 1.77 g, 17.5 mmol) in CH_2Cl_2 (50 mL) was added a solution of 3,5-bis(trifluoromethyl)benzoyl chloride (**19**, 4.78 g, 17.3 mmol) in CH_2Cl_2 (25 mL) at 0°C under an N_2 atmosphere. After stirring for 30 min, the reaction mixture was stirred at r.t. for another 1 h. The resulting mixture was poured into water (100 mL), and then the aqueous layer was extracted with CH_2Cl_2 (2×100 mL). The combined organic layer was washed with brine (50 mL) and dried over $MgSO_4$, and the solvent was evaporated to give a yellow oil. Separation of the product by flash chromatography (CH_2Cl_2 : 95% EtOH : 28% NH_3=97 : 2.7 : 0.3) and then recrystallization from MeOH gave compound **21** (2.99 g, 66%) as colorless crystals.

21: mp 205–206°C (from MeOH). IR (KBr) cm^{-1}: 3265 (NH), 1645, 1550, 1280 (C=O), 1135 (CF_3). ^{1}H-NMR (DMSO-d_6) δ: 1.71(6H, t, J=7.0 Hz, H2′), 2.49 (6H, m, H3′), 3.35 (6H, m, H1′), 8.23 (3H, s, H4), 8.41 (6H, s, H2, H6), 8.94 (3H, t, J=5.3 Hz, NH). ^{13}C-NMR (DMSO-d_6) δ: 26.39 (C2′), 38.03 (C1′), 50.93 (C3′), 123.08 (CF_3, q, J=273.1 Hz), 124.58 (C4), 127.86 (C2, C6), 130.41 (C3, C5, q, J=33.1 Hz), 136.68 (C1), 163.13 (C=0). Positive-ion FAB-MS m/z: 909 ($M+H^+$). HR-FAB-MS m/z: 909.2101 (Calcd for $C_{36}H_{31}F_{18}N_4O_3$: 909.2109). *Anal.* Calcd for $C_{36}H_{30}F_{18}N_4O_3$: C, 47.59; H, 3.33; N, 6.17. Found: C, 47.58; H, 3.34; N, 6.22.

Antiviral Activity Assay and Cytotoxicity of Synthesized Trisubstituted Tris(aminoalkyl)amine Derivatives The anti-HSV-1 activities (EC_{50}) of the synthesized tris(aminoalkyl)-amine derivatives were assessed by using a plaque reduction assay[17] and their cytotoxicity against Vero cells (CC_{50}) was also evaluated. The results are summarized in Tables 1, 2 together with data for aciclovir.[18] Log P values for all of the compounds for which biological activities were evaluated were calculated by using CAChe v.6.1.12. There were few distinct correlations between log$P$ values and anti-HSV-1 activities (EC_{50} values) among the compounds listed in Tables 1, 2.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.
Table S1. Synthesis of C_3-Symmetrical Amine-Type TAEA Derivatives (**3**)
Table S2. Synthesis of C_3-Symmetrical Amide-Type TAEA Derivatives (**5**)
Table S3. Synthesis of C_3-Symmetrical Urea-Type TAEA Derivatives (**7**, **8**)
Table S4. Synthesis of C_S-Symmetrical Amine-Urea-Type TAEA Derivatives (**12**, **13**)
Table S5. Synthesis of C_S-Symmetrical Amide-Urea-Type TAEA Derivatives (**14**, **15**)

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8) In the field of peptide biomedicines, much attention is being paid to interesting substances known as middle-size molecules (mid-size molecules) having molecular weights of *ca.* 500–50000. New TAEA derivatives of targeted symmetrical molecules have considerably large molecular weights over 500; however, the mid-size TAEA symmetrical derivatives that we have synthesized belong to a new class of non-peptide compounds. Hitherto, we have already reported that compound **20** has a lectin-like carbohydrate recognition property and shows considerably high cytotoxic activity.[6,7] Compound **20** and its analogues may be classified into the category of non-peptide mid-size molecules (see also ref. 5). In terms of non-peptide mid-size multivalent symmetrical molecules, a few new drugs such as daclatasvir[9] and ombitasvir[10] that have recently been developed for the treatment of hepatitis C virus (HCV) infection diseases can be considered to be C_2-symmetrical geometric non-peptide mid-size molecules.
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14) In the ^{13}C-NMR spectra of *N*-acetylated C_3- and C_S-type TAEA derivatives (**5a-e**, **5b′**, **14k**, **14l**, and **15l**) except for **15k**, multiple carbon resonance signal patterns ascribable to characteristic carbons such as amide functionalities were observed. For example, theoretically four *cis–trans* rotational isomers can be obtained from full *N*-acetylation of three secondary amines in the C_3-type original amine-type molecule **5c**, *i.e.*, two C_3-symmetrical molecules that have the same conformations (*trans–trans–trans* and *cis–cis–cis*) with regard to three amide functionalities (=N–CO–CH_3) in the molecules and two C_S-symmetrical *N*-acetylated compounds having different combinations of conformational isomers (*trans–trans–cis* and *trans–cis–cis*). These expected structural properties of full *N*-acetylation reaction products are considered to be the most reasonable interpretation for the multiple splitting of carbon signals assignable to the target molecules. For these complicated ^{13}C-NMR spectroscopic data of *N*-acetylated TAEA derivatives, we could not provide a successful assignment for the obtained product. However, we detected the presence of four stereoisomers (ratio of isomers=73 : 4 : 20 : 3) in the obtained *N*-acetylated compound **5c** by the HPLC method. The data obtained from IR (absorption band of the amide group), HR-MS (correct molecular ions) and ^{1}H-NMR measurements are fully consistent with the *N*-acetylated structures shown in Tables S2 and S5. We used the obtained materials for further biological experimental study without further purification.
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19) Calculated log *P* values by using CAChe v.6.1.12 for cyctotoxic compounds **8p**, **20** and **13o** were 9.94, 8.30 and 7.40, respectively.
20) Compound **8k** showed no significant anti-HSV-1 (EC_{50}=>100 μM) and cytotoxic (CC_{50}=>200 μM) activities, and calculated log*P* value for this compound was 7.41. (see ref. 6).