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SYNTHESIS, CHEMICAL PROPERTIES, AND BIOLOGICAL EVALUATIONS OF SOME TWIN-DRUG TYPE C_2-SYMMETRICAL DERIVATIVES

Fumiko Fujisaki, Haruka Usami, Saya Nakashima, Sho Nishida, Toshihiro Fujioka, Nobuhiro Kashige, Fumio Miake, and Kunihiro Sumoto*

Faculty of Pharmaceutical Sciences, Fukuoka University, Nanakuma, Jonan-Ku, Fukuoka 814-0180, Japan; E-mail: kunihiro@adm.fukuoka-u.ac.jp

Abstract – We describe the synthesis and chemical properties of newly designed C_2-symmetrical twin-drug type aminoguanidines or 4-aminomethyloxazolidinone derivatives (**4**-**7**) in which a long chain alkyl group [-$(CH_2)_{10}$-] was used as a linker. Synthesis of some triplet-drug type symmetrical oxazolidinones (**8**) is also described. Among the tested compounds, the aminoguanidine derivative **4a** showed the highest α-glucosidase inhibition activity (IC_{50} = 76.3 μmol/L).

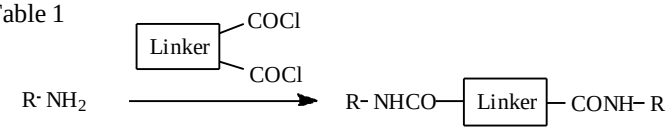
Extensive studies on molecular recognition properties of biomolecules have been carried out, and it is well known that many types of receptors or functionalized proteins in the native state often have a high order of interface symmetry. Molecular recognition of C_2- or C_3-symmetrical macromolecules is an interaction that is often found in several important biological processes.[1-7] We have therefore designed some symmetrical target molecules for the purpose of developing new biologically active substances that interfere with a sugar recognition process or a sugar chain function. From this view point, we have already reported a few examples of the design and synthesis of C_2- or C_3-symmetrical and other symmetrical molecules and results of their biological evaluations.[8-10] We previously found that two C_2-symmetrical compounds (**1a** and **1b**) (Table 1) in which a biphenyl group was used as a linker showed significant antibacterial activity.[8]

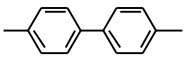
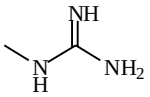
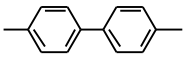
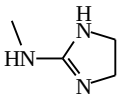
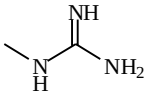
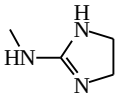
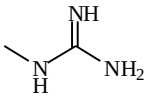
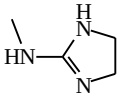
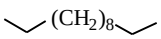
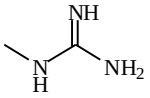
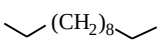
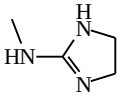
As an extension of our molecular modification studies on these symmetrical molecules for finding new bioactive leads, we attempted further additional synthesis of some new C_2-symmetrical twin-drug type aminoguanidine or 4-aminomethyloxazolidinone[11] derivatives in which a long chain alkyl [-$(CH_2)_{10}$-] group was used as a linker in the designed molecules.[12] In this article, we describe the synthesis and chemical properties of newly designed twin-drug type symmetrical derivatives. We also present the result of biological evaluations of the synthesized compounds.

SYNTHESIS OF TARGET TWIN-DRUG TYPE MOLECULES

In our previous works on twin-drug type molecules, we obtained some synthetic C_2-symmetrical molecules (**1a**,**b**-**3a**,**b**) by reactions of dicarboxylic acid derivatives and corresponding aminoguanidines[8] (Table 1).

Table 1



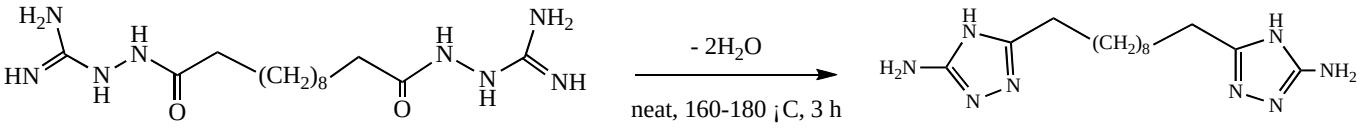
Compd.	Linker	R	Yield (%)
1a[a)]			69.3
1b[a)]			89.0
2a[a)]			50.9
2b[a)]			54.5
3a[a)]			27.9
3b[a)]			53.9
4a			62.8
4b			15.5

a) Ref. 8

New twin-drug type symmetrical molecules (**4a** and **4b)** that used a longer methylene chain alkyl [-$(CH_2)_{10}$-] group as a linker between two terminal aminoguanidine moieties were also obtained from the reaction of dicarboxylic acid dichloride with a corresponding aminoguanidine derivative in 62.8% and 15.5% yields, respectively.

The obtained *N*-acyl aminoguanidine derivative **4a** was easily cyclized to form the corresponding triazole derivative **5** in 25.6% yield by simply heating at 160—180 °C for 3 h **(**Scheme 1). A similar cyclization stepwise to a triazole ring system by intramolecular dehydration of *N*-acyl aminoguanidines **2a** was reported in our previous paper.[8]

The C_2-symmetrical structures of the products (**4a** and **5**) were confirmed by ^{13}C-NMR spectroscopic analysis. The two twin-drug type molecules showed magnetically equivalent signals assignable to half of the symmetrical molecules, indicating a C_2-symmetrical molecular feature in solution.



4a **5** (25.6%)

Scheme 1

The protocol for preparation of new twin-drug type aminoguanidine derivatives (**4a** and **4b)** and a symmetrical triazole derivative **5** and their physical and spectroscopic data are shown in detail in the Experimental section.

With reference to the structure of a twin-drug type compound **4b**, the obtained evidence, especially that from ^{13}C-NMR measurements, is not fully consistent with a single structure of an expected C_2-symmetrical molecule **4b** in solution, showing the characteristic two sets of ^{13}C-NMR signal patterns (161.4, 167.4 and 158.4, 172.3 ppm, respectively) ascribable to two quaternary carbons (guanidinium carbons and C=O) for the terminal *N*-acylaminoguanidine moieties (see Experimental). This probably arises from the existence of two types of C_2-symmetrical molecular features in solution. We speculated that the formation of intramolecular 5-membered hydrogen bonds in the terminal *N*-acylaminoguanidine moieties including two imidazoline rings is involved in the tautomer-like conversion {[**4bA**] $\rightleftharpoons$ [**4bB**]}. The broadening of the ^{13}C-signal at the C(2) position in the imidazoline ring supports this tautomer-like conversion[13] (see Figure 1).

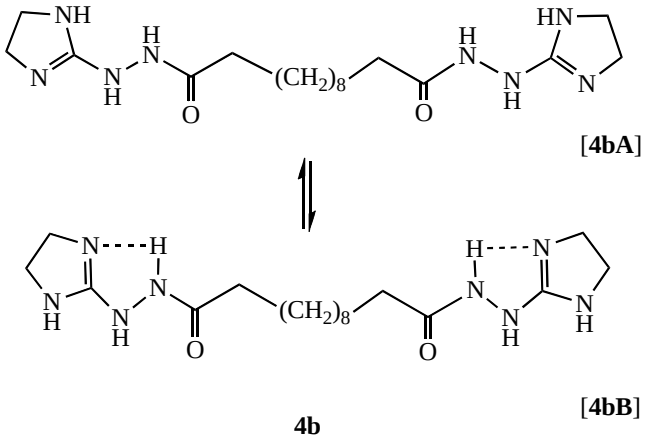
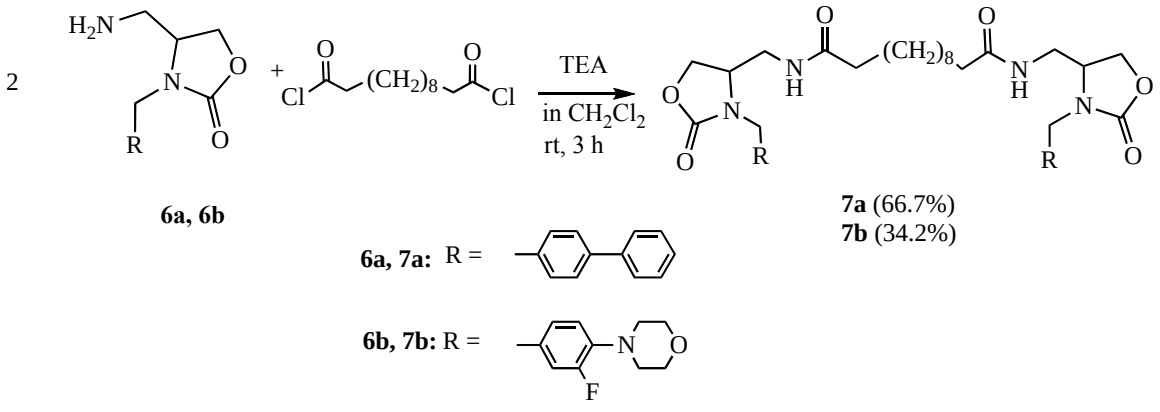


Figure 1

As part of our structural modification studies on a new class of bioactive leads, we have further aimed to prepare two twin-drug type oxazolidinone derivatives,[11] **7a** and **7b**, having two *N*(3)-substituted oxazolidinone rings[14-18] (Scheme 2).

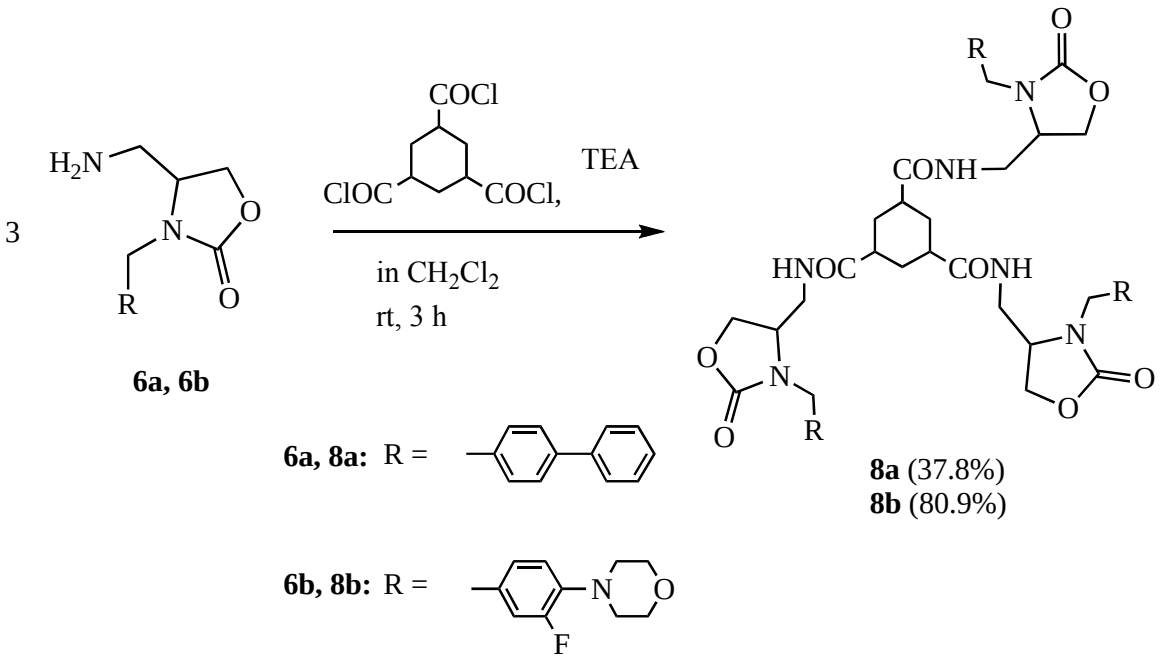


Scheme 2

Synthesis of these new twin-drug type oxazolidinone analogues (**7a** and **7b)** was also achieved from the reaction of dicarboxylic dichloride and corresponding 4-aminomethyloxazolidinones **6**[16] (see Experimental for details).

The obtained twin-drug type compound (**7a** or **7b)** is expected to be a mixture of three twin-drug type molecules, i.e., a Cs-symmetrical *meso* compound and two enantiomeric C_2-symmetrical molecules, that have the same absolute configuration regarding two C(4)-substituted oxazolidin-2-one rings in each molecule. All of the obtained diastereomeric mixtures showed very simple symmetrical ^{13}C-NMR in DMSO-d_6, and we could not distinguish the above two diastereomers from their ^{13}C-NMR spectra. However, three stereoisomeric components (1:2:1) in product **7a** could be detected by HPLC enantioseparation.[19]

In this study, newly designed triplet-drug type symmetrical oxazolidinone derivatives **(8a** and **8b)** using a 1,3,5-*cis*-cyclohexanetricarbonyl framework were also prepared for comparison of biological activities (Scheme 3).



Scheme 3

The fragments of oxazolidinone moieties incorporated in the designed triplet-drug type molecules **8a** and **8b** are based on previous biological results of this series mimicking the molecule of linezolid.[20]

The symmetrical structures of these triplet-drug type compounds were easily confirmed by ^{13}C-NMR spectroscopic analysis. The two triplet-drug type molecules (**8a** and **8b**) showed magnetically equivalent signals ascribable to a third of the molecules.[21] Elemental analyses for both triplet-drug type compounds were carried out by high-resolution FAB-MS spectra (see Experimental).

BIOLOGICAL ASSAYS AND DISCUSSION

Regarding antibacterial activities of the synthesized C_2-symmetrical derivatives, none of the new C_2-symmetrical twin-drug type aminoguanidine or oxazolidinone derivatives that have a long chain alkyl group [-$(CH_2)_{10}$-] as a linker in the molecule showed significant antibacterial activities against either gram-negative (*E. coli*) or gram-positive (*S. aureus*) bacteria at a dose of >128 μg/mL. These results indicate that a long chain alkyl [-$(CH_2)_{10}$-] linker seems to be less effective for enhancement of antibacterial activity than a linker having a biphenyl group (**1a** or **1b**).[8]

In this study, we also evaluated the activities of new synthesized C_2-symmetrical aminoguanidines as well as some of the previously obtained twin-drug type C_2-symmetrical aminoguanidine derivatives for inhibition of α-glucosidase.

Some compounds in this series (**1a**, **1b** and **4a**) that have been tested showed significant inhibitory activity against α-glucosidase (IC_{50} = 76.3~279.6 μmol/L). Among the evaluated samples, compound **4a** in which a long chain alkyl group [-$(CH_2)_{10}$-] was introduced as a linker showed the highest inhibitory activity against α-glucosidase (IC_{50} = 76.3 μmol/L). Compounds (**1a** and **1b)** that have a biphenyl group as a linker showed weaker inhibitory activity (IC_{50} = 279.6 and 259.9 μmol/L, respectively) than that of compound **4a**. The other compounds showed no remarkable inhibitory activity. This results indicate that twin-drug type aminoguanidines that have a longer linker such as biphenyl or [-$(CH_2)_{10}$-] groups in the molecule are more favorable for inhibitory activity against α-glucosidase than those having a benzene or pyridine ring as a linker.

Based on these results of bioassays, we are studying further synthetic molecular modifications for C_2- or C_3-symmetrical or other types of symmetric derivatives by introduction of different linkers into target molecules in order to find useful bioactive leads.

EXPERIMENTAL

Melting points are uncorrected. IR spectra were measured by a Shimadzu FT/IR-8100 spectrometer. ^{1}H- and ^{13}C-NMR spectra were obtained by a JEOL JNM A-500 at rt. The chemical shifts were expressed in δ ppm downfield from an internal tetramethylsilane (TMS) signal. The signal assignments were confirmed by ^{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) spectra. High FAB-MS spectra were obtained by a JEOL JMS-HX110 mass spectrometer. The following abbreviations in parentheses were used for cyclohexane ring (Cyc), morpholine ring (Mor), oxazolidinone ring (Oxaz).

2,2'-Dodecanedioylbis(hydrazinecarboximidamide) Dihydrochloride (4a)

Aminoguanidine hydrochloride (0.85 g, 7.70 mmol) was added portionwise to dodecanedioyl dichloride

(1.00 g, 3.74 mmol) with stirring at rt, and the resulting mixture was heated at 150—160 °C for 15 min. After cooling, the mixture was triturated with EtOH and isolated insoluble material was collected by filtration. The crystalline substance was washed with AcOEt to give **4a** as a white solid (0.98 g, 62.8%). Mp 219—221 °C (dec). IR (KBr) cm^{-1}: 3320, 3170, 1695, 1671. FAB-MS (positive) *m/z*: 343 $(M+H)^+$. ^{1}H-NMR (DMSO-d_6) δ: 1.26 (12H, s, dodecane H-4, H-5, H-6, H-7, H-8, H-9), 1.51 (4H, t, *J* = 6.5 Hz, dodecane H-3, H-10), 2.17 (4H, t, *J* = 7.5 Hz, dodecane H-2, H-11), 7.0—8.2 {8H, br, C=(N^+H_2)NH_2}, 9.53 (2H, s, CONHNH), 10.03 (2H, s, CONHNH). ^{13}C-NMR (DMSO-d_6) δ: 24.3 (dodecane C-3, C-10), 28.5, 28.6, 28.8, 28.8 (dodecane C-4, C-5, C-6, C-7, C-8, C-9), 33.0 (dodecane C-2, C-11), 158.7 {NHC(=NH)NH_2}, 172.6 (CO). *Anal*. Calcd for $C_{14}H_{30}N_8O_2$ · 2HCl: C, 40.48; H, 7.77; N, 26.98. Found: C, 40.58; H, 7.70; N, 26.71.

N'^1,N'^{12}-Bis(4,5-dihydro-1*H*-imidazol-2-yl)dodecanedihydrazide (4b)

A slurry of dodecanedioyl dichloride (1.00 g, 3.74 mmol) and 2-hydrazinoimidazoline hydrobromide (1.35 g, 7.46 mmol) in DMF (1 mL) was heated at 160 °C for 1 h. After cooling, the precipitated material was washed with AcOEt and dissolved in water (10 mL). This aqueous solution was made alkaline (*ca.* pH 11) with K_2CO_3 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel) with EtOH/NH_3 (28% aq) (95:5) as a solvent to give **4b** as a white solid (0.23 g, 15.5%). Mp 174—190 °C (dec). IR (KBr) cm^{-1}: 3264, 2925, 1662, 1613. FAB-MS (positive) *m/z*: 395 $(M+H)^+$. ^{1}H-NMR (DMSO-d_6) δ: 1.23—1.24 (12H, s, dodecane H-4, H-5, H-6, H-7, H-8, H-9), 1.44—1.50 (4H, br, dodecane H-3, H-10), 1.98 (4H x 3/4, t, *J* = 7.5 Hz, dodecane H-2, H-11), 2.21 (4H x 1/4, t, *J* = 7.5 Hz, dodecane H-2, H-11), 3.27 (8H, s, imidazole H-4, H-5), 5.86—6.22 (4H, br, NH), 7.5—9.0 (2H, br, NH). ^{13}C-NMR (DMSO-d_6) δ: 24.2, 25.4, 25.4, 28.7, 28.7, 28.8, 28.8, 28.9, 28.9, 31.3, 34.1, 34.1 (dodecane C), 42.2 (br, imidazole C-4, C-5), 158.4, 161.4 (imidazole C-2), 167.4, 172.3 (CO). *Anal*. Calcd for $C_{18}H_{34}N_8O_2$ • 0.6 H_2O: C, 53.34; H, 8.75; N, 27.65. Found: C, 53.34; H, 8.47; N, 27.93.

5,5'-(Decane-1,10-diyl)bis(4*H*-1,2,4-triazol-3-amine) Dihydrochloride (5)

Aminoguanidine hydrochloride (1.65 g, 14.9 mmol) was added portionwise to dodecanedioyl dichloride (1.00 g, 3.74 mmol) with stirring at rt, and the resulting mixture was heated at 160—180 °C for 3 h. After cooling, the mixture was washed with water to give **5** as a white solid (0.38 g, 25.6%) as a solid material. Mp >188 °C. IR (KBr) cm^{-1}: 1698. FAB-MS (positive) *m/z*: 307 $(M+H)^+$. ^{1}H-NMR (DMSO-d_6) δ: 1.25—1.27 (12H, m, decane H-3, H-4, H-5, H-6, H-7, H-8), 1.59—1.63 (4H, m, decane, H-2, H-9), 2.51 (4H, t, *J* = 1.8 Hz, decane H-1, H-10), 8.0 (4H, br, NH_2), 13.6 (2H, br, triazole NH). ^{13}C-NMR (DMSO-d_6) δ: 24.8 (decane C-1, C-10), 26.1 (decane C-2, C-9), 28.2, 28.5, 28.8 (decane C-3, C-4, C-5, C-6, C-7, C-8), 151.0 (triazole C-3, C-5, C-3', C-5'). *Anal.* Calcd for $C_{14}H_{26}N_8$ · 2HCl · H_2O: C, 42.32; H, 7.61; N, 28.20. Found: C, 42.22; H, 7.42; N, 28.08.

N^1,N^{12}-Bis((3-([1,1'-biphenyl]-4-ylmethyl)-2-oxooxazolidin-4-yl)methyl)dodecanediamide (7a)

A solution of dodecanedioyl dichloride (0.122 g, 0.457 mmol), 4-(aminomethyl)-3-(3-biphenyl-4-ylmethyl)oxazolidin-2-one (**6a**)[16] (0.258 g, 0.914 mmol) and TEA (0.092 g, 0.911 mmol) in CH_2Cl_2 was stirred at rt for 3 h. The reaction mixture was washed with water, 1 *N* HCl, and then with 5% aqueous Na_2CO_3. After removal of the solvent under reduced pressure, the residue was crystallized by the addition of CH_3CN (5 mL, 50 °C). Compound **7a** (0.232 g, 66.7%) was obtained by filtration as a white solid. Mp 123—124 °C. IR (KBr) cm^{-1}: 1739, 1666. FAB-MS (positive) *m/z*: 759 $(M+H)^+$. ^{1}H-NMR (DMSO-d_6) δ: 1.22—1.24 (12H, m, dodecane H-4, H-5, H-6, H-7, H-8, H-9), 1.45—1.48 (4H, m, dodecane H-3, H-10), 2.07 (4H, dd, *J* = 8.0, 7.0 Hz, dodecane H-2, H-11), 3.27—3.37 (4H, m, CONHC<u>H</u>$_2$), 3.68—3.72 (2H, m, Oxaz H-4), 4.05 (2H, dd, *J* = 9.0, 6.0 Hz, Oxaz H-5), 4.27 (2H, d, *J* = 15.5 Hz, C<u>H</u>H-Ph), 4.31 (2H, d, *J* = 9.0 Hz, Oxaz H-5), 4.62 (2H, d, *J* = 15.5 Hz, CH<u>H</u>-Ph), 7.36—7.38 (6H, m, Ar-H), 7.44—7.48 (4H, m, Ar-H), 7.64—7.67 (8H, m, Ar-H), 7.97 (2H, t, *J* = 6.0 Hz, CON<u>H</u>CH$_2$). ^{13}C-NMR (DMSO-d_6) δ: 25.2 (dodecane C-3, C-10), 25.6, 28.7, 28.8, (dodecane C-4, C-5, C-6, C-7, C-8, C-9), 35.3 (dodecane C-2, C-11), 38.1 (CONH<u>C</u>H$_2$), 44.6 (<u>C</u>H$_2$-Ph), 53.6 (Oxaz C-4), 64.9 (Oxaz C-5), 126.6, 126.9 (Ar C), 127.4 (Ar C-4'), 128.3, 128.9 (Ar C), 135.6 (Ar C-1), 139.4 (Ar C-4 or Ar C-1'), 139.7 (Ar C-1' or Ar C-4), 157.7 (Oxaz C-2), 173.0 (<u>C</u>ONH). *Anal.* Calcd for $C_{46}H_{54}N_4O_6$: C, 72.80; H, 7.17; N, 7.16. Found: C, 72.88; H, 7.32; N, 7.16.

N^1,N^{12}-Bis((3-(3-fluoro-4-morpholinobenzyl)-2-oxooxazolidin-4-yl)methyl)dodecanediamide (7b)

A solution of dodecanedioyl dichloride (0.060 g, 0.225 mmol), 4-(aminomethyl)-3-(3-fluoro-4-morpholinobenzyl)oxazolidin-2-one (**6b**)[16] (0.150 g, 0.485 mmol) and TEA (0.046 g, 0.455 mmol) in CH_2Cl_2 was stirred at rt for 3 h. After removal of the solvent, the residue was purified by column chromatography (SiO_2/*i*-PrOH→EtOH) to give **7b** as a white solid (0.068 g, 34.2%). Mp 116—118 °C. IR (KBr) cm^{-1}: 1736, 1637. FAB-MS (positive) *m/z*: 835 $(M+Na)^+$. ^{1}H-NMR (DMSO-d_6) δ: 1.23 (12H, s, dodecane H-4, H-5, H-6, H-7, H-8, H-9), 1.45—1.48 (4H, m, dodecane H-3, H-10), 2.05—2.08 (4H, m, dodecane H-2, H-11), 2.98 (8H, d, *J* = 4.5 Hz, Mor H-2, H-6), 3.24 (2H, dd, *J* = 14.5, 4.5 Hz, CONHC<u>H</u>H), 3.45 (2H, dd, *J* = 14.5, 7.0 Hz, CONHCH<u>H</u>), 3.64—3.66 (2H, m, Oxaz H-4), 3.72—3.74 (8H, m, Mor H-3, H-5), 4.01—4.04 (2H, m, Oxaz H-5), 4.15 (2H, d, *J* = 16.0 Hz, C<u>H</u>H-Ph), 4.26—4.29 (2H, t, *J* = 9.0 Hz, Oxaz H-5), 4.50 (2H, d, *J* = 16.0 Hz, CH<u>H</u>-Ph), 7.00—7.08 (6H, m, Ar-H), 7.98 (2H, t, *J* = 6.0 Hz, CON<u>H</u>CH$_2$). ^{13}C-NMR (DMSO-d_6) δ: 25.2 (dodecane C-3, C-10), 28.6, 28.7, 28.8 (dodecane C-4, C-5, C-6, C-7, C-8, C-9), 35.3 (dodecane C-2, C-11), 38.1 (CONH<u>C</u>H$_2$), 44.0 (<u>C</u>H$_2$-Ph), 50.4 (Mor C-2, C-6), 53.6 (Oxaz C-4), 64.9 (Oxaz C-5), 66.1 (Mor C-3, C-5), 115.4 (d, *J* = 21 Hz, Ar C-2), 119.1 (d, *J* = 3 Hz, Ar C-5), 124.2 (d, *J* = 3 Hz, Ar C-6), 131.0 (d, *J* = 7 Hz, Ar C-1), 139.0 (d, *J* = 8 Hz, Ar C-4), 154.7 (d, *J* = 245 Hz, Ar C-3), 157.7 (Oxaz C-2), 173.0 (<u>C</u>ONH). *Anal.* Calcd for $C_{42}H_{58}F_2N_6O_8$ · 2HCl · $0.5H_2O$: C, 56.37; H, 6.87; N, 9.39. Found: C, 56.55; H, 6.84; N, 9.13.

***N*^{1},*N*^{3},*N*^{5}-Tris((3-([1,1'-biphenyl]-4-ylmethyl)-2-oxooxazolidin-4-yl)methyl)cyclohexane-1,3,5-tricarboxamide (8a)**

A solution of 1,3,5-*cis*-cyclohexanetricarbonyl chloride (0.103 g, 0.379 mmol), 4-(aminomethyl)-3-(3-biphenyl-4-ylmethyl)oxazolidin-2-one (**6a**) (0.030 g, 0.106 mmol) and TEA (0.033 g, 0.327 mmol) in CH_2Cl_2 was stirred at rt for 3 h. After removal of the solvent under reduced pressure, the residue was purified by centrifugal chromatography (silica gel) with EtOH as a solvent to give a crystalline product. This material was washed with water followed by AcOEt to afford the described compound **8a** as a white solid (0.042 g, 37.8%). Mp 139 °C (with dec). IR (KBr) cm^{-1}: 1734, 1653. FAB-MS (positive) *m/z*: 1009 $(M+H)^+$. ^{1}H-NMR (DMSO-d_6) δ: 1.43—1.46 (3H, m, Cyc H_A-2, H_A-4, H_A-6), 1.70—1.73 (3H, m, Cyc H_B-2, H_B-4, H_B-6), 2.25 (3H, t-like, *J* = 12.0 Hz, Cyc H-1, H-3, H-5), 3.26—3.29 (3H, m, CONHC<u>H</u>H), 3.34—3.39 (3H, m, CONHCH<u>H</u>), 3.71—3.73 (3H, s, Oxaz H-4), 4.04—4.09 (3H, m, Oxaz H-5), 4.26 (3H, d, *J* = 16.0 Hz, C<u>H</u>H-Ph), 4.28—4.32 (3H, m, Oxaz H-5), 4.62 (3H, d, *J* = 16.0 Hz, CH<u>H</u>Ph), 7.34—7.41 (9H, m, Ar H), 7.44—7.47 (6H, m, Ar H), 7.65—7.68 (12H, m, Ar H), 8.00—8.01 (3H, br, N<u>H</u>). C^{13}-NMR (DMSO-d_6) δ: 31.2 (Cyc C-2, C-4, C-6), 38.2 (CONH<u>C</u>H_2), 42.8 (Cyc C-1, C-3, C-5), 44.6 (<u>C</u>H_2-Ph), 53,6 (Oxaz C-4), 65.0 (Oxaz C-5), 126.6, 127.0 (Ar C), 127.4 (Ar C-4'), 128.3, 128.9 (Ar C), 135.7 (Ar C-1), 139.4 (Ar C-4 or Ar C-1'), 139.7 (Ar C-1' or C-4), 157.7 (Oxaz C-2), 174.9 (<u>C</u>ONHCH$_2$). HR-MS *m/z:* 1009.4496. (Calcd for $C_{60}H_{61}N_6O_9$: 1009.4500).

***N*^{1},*N*^{3},*N*^{5}-Tris((3-(3-fluoro-4-morpholinobenzyl)-2-oxooxazolidin-4-yl)methyl)cyclohexane-1,3,5-tricarboxamide (8b)**

A solution of 1,3,5-*cis*-cyclohexanetricarbonyl chloride (0.034 g, 0.125 mmol), 4-(aminomethyl)-3-(3-fluoro-4-morpholinobenzyl)oxazolidin-2-one (**6b**) (0.140 g, 0.453 mmol) and TEA (0.038 g, 0.376 mmol) in CH_2Cl_2 was stirred at rt for 3 h. After removal of the solvent, the white residue was purified by column chromatography using EtOH as a solvent. The obtained crude material was washed with water and then AcOEt to give **8b** as a white solid **(**0.110 g, 80.9%). Mp 148—152 °C. IR (KBr) cm^{-1}: 1737, 1655. FAB-MS (positive) *m/z*: 1112 $(M+Na)^+$. ^{1}H-NMR (DMSO-d_6) δ: 1.41—1.43 (3H, m, Cyc H_A-2, H_A-4, H_A-6), 1.64—1.73 (3H, m, Cyc H_B-2, H_B-4, H_B-6), 2.20—2.25 (3H, m, Cyc H-1, H-3, H-5), 2.98 (12H, t, *J* = 4.5 Hz, Mor H-2, H-6), 3.25—3.40 (6H, m, CONHC<u>H</u>$_2$), 3.66—3.68 (3H, m, Oxaz H-4), 3.73 (12H, t, *J* = 4.5 Hz, Mor H-3, H-5), 4.00—4.04 (3H, m, Oxaz H-5), 4.14 (3H, d, *J* = 16.0 Hz, C<u>H</u>H-Ph), 4.28 (3H, t, *J* = 9.0 Hz, Oxaz H-5), 4.49 (3H, d, *J* = 16.0 Hz, CH<u>H</u>-Ph), 7.00—7.08 (9H, m, Ar H-2, H-5, H-6), 7.96—7.97 (3H, m, CON<u>H</u>CH$_2$). ^{13}C-NMR (DMSO-d_6) δ: 31.3 (Cyc C-2, C-4, C-6), 38.1 (CONH<u>C</u>H_2), 42.7 (Cyc C-1, C-3, C-5), 44.0 (<u>C</u>H_2-Ph), 50.4 (Mor C-2, C-6), 53.5 (Oxaz C-4), 64.9 (Oxaz C-5), 66.1 (Mor C-3, C-5), 115.4 (d, *J* = 21 Hz, Ar-2), 119.1 (d, *J* = 3 Hz, Ar-5), 124.2 (d, *J* = 3 Hz, Ar-6), 131.0 (d, *J* = 7 Hz, Ar-1), 139.0 (d, *J* = 9 Hz, Ar-4), 154.7 (d, *J* = 245 Hz, Ar-3), 157.7 (Oxaz C-2), 174.9 (<u>C</u>ONH). HR-FAB-MS (positive) *m/z*: 1112.4685 (Calcd for $C_{54}H_{66}O_{12}N_9F_3Na$: 1112.4681).

BIOLOGICAL ASSAY

α-Glucosidase Inhibition Assay: α-Glucosidase inhibition assays for the synthesized compounds were performed by the modified method of Shibano *et al.*[22] Dimethyl sulfoxide (DMSO) was used as a solvent to dissolve the test samples. Phosphate buffer (pH = 7.0) was used as a solvent for the enzyme α-glucosidase or the substrate *p*-nitrophenyl-α-D-glucopyranoside. After incubation of the reaction mixture for 30 min at 37 °C and interruption of the reaction by addition of aqueous sodium carbonate, the amount of generated *p*-nitrophenol was measured colorimetrically at 405 nm, and α-glucosidase inhibition activity (IC_{50}) was determined.

Assay for Antibacterial Activity: We used gram-negative bacteria (*E. coli* NIHJ) and gram-positive bacteria (*S. aureus* ATCC6538P) as target organisms for the assay of antibacterial activities of the synthesized symmetrical compounds. The bioassay for antibacterial activity was carried out by the authentic method described in our previous report[8] to obtain the minimum inhibitory concentration (MIC) values. Dimethyl sulfoxide (DMSO) was used as a solvent to dissolve the target test compounds.

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11. It has been reported that some oxazolidine derivatives show molecular recognition properties against monosaccharides.[23] In addition, oxazolidinone moieties are common and useful components for

various biological active compounds.[24] Since we have already established a conventional procedure for the preparation of new 4-aminomethyloxazolidinones,[16] we incorporated this oxazolidinone unit into designed target symmetrical molecules in order to develop new types of biologically active leads.

12. Regarding two-fold or three-fold (homooligometric) type macromolecules of cell surface receptors, it is difficult to anticipate the exact distance between two recognition domains (or functional groups in two active sites). However, some references[6,7] are available for investigation of molecular designs. For example, the reported distance (17.2 Å) between two equilateral triangles formed by α-amino acid residues in the homotrimeric CD40L is particularly interesting (see reference 6). The linker length as a –CO-$(CH_2)_{10}$-CO- functionality in our designed molecules is attractive and is consistent with the distance shown above.
13. Tautomer-like conversion {[**4bA**] $\rightleftharpoons$ [**4bB**]} is one of the possible interconversions of the molecule **4b**. Many types of the tautomer-like conversion involving terminal functional groups are possible because of the possibilities of E/Z isomers of amide or hydrazine moiety in addition to imidazoline ring tautomerization. 5-Membered hydrogen interaction between the terminal imidazoline ring and E/Z isomers of the hydrazine group is also conceivable. So far, we have no spectroscopic evidence to define the two C_2-symmetrical molecular structures in solution.
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19. Three components in the product **7a** were detected by using CHIRALPAC IA® as a chiral stationary phase and *n*-hexane/*i*-PrOH/EtOH (7:2:1 <v/v>) as an eluant.
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21. We used a racemic oxazolidin-2-one derivative **6** (*N*-4-(aminomethyl)-3-(3-substituted-4-ylmethyl)oxazolidin-2-one) as a starting material in the synthesis of triplet-drug type compounds (**8a** and **8b**). The obtained compounds exhibited very simple symmetrical ^{13}C-NMR in DMSO-d_6, indicating little difference with respect to the signals assignable to substituted oxazolidin-2-one rings and a linker cyclohexane ring. From the viewpoint of stereochemistry, however, the obtained product can be considered to be a mixture of four triplet-drug type molecules, i.e., two C_3-symmetrical molecules that have the same absolute configuration regarding three introduced oxazolidin-2-ones (*R*,*R*,*R* or *S*,*S*,*S*) in the molecules and two enantiometric molecules (*R*,*R*,*S* or

S,S,R). We distinguished the presence of three predominant stereoisomers in the product **8a** by an HPLC method with a solvent of MeOH/EtOH/DEA (50:50:0.1 <v/v>), and the ratio of these three peaks was 1.5:1:1. The largest peak probably consists of two stereoisomers, but, so far, our trials to separate two isomers in this peak have been unsuccessful.

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