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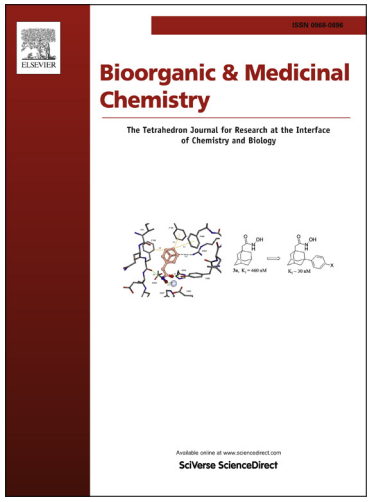

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Discovery of novel series of 6-benzyl substituted 4-aminocarbonyl-1,4-diazepane-2,5-diones as human chymase inhibitors using structure-based drug design

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ABSTRACT

A novel series of 6-benzyl substituted 4-aminocarbonyl-1,4-diazepane-2,5-diones were explored as human chymase inhibitors using structure-based drug design according to the X-ray cocrystal structure of chymase and compound **1**. The optimization focused on the prime site led to the attainment of compounds that showed potent inhibitory activity, and among them, **18R** shows a novel interaction mode.

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1. Introduction

Chymase is a chymotrypsin-like serine protease stored in mast cell granules[1] that hydrolyzes a variety of physiological substrates, including angiotensin I.[2] It is known to play a role in the pathogenesis of atopic dermatitis; for example, it has been reported that an intradermal injection of chymase to mouse skin elicits edema as well as inflammatory cell accumulation,[3,4] and a correlation between a particular single nucleotide polymorphism of the chymase gene and incidence of atopic dermatitis has been observed.[5–7] In a previous paper,[8,9] we reported on novel, orally active human chymase inhibitors with a 6-benzyl substituted 4-aminocarbonyl-1,4-diazepane-2,5-dione scaffold, which demonstrated selectivity against other serine proteases. Oral administration of the representative compound SUN13834 reduced skin inflammation in NC/Nga mice[10] that spontaneously develop dermatitis under conventional conditions,[11,12] in addition to significantly decreasing the amount of scratching induced by DNFB challenge in the mouse model.[13] SUN13834 also showed promising results when tested on humans with atopic dermatitis.[14]

In order to increase the chances of successfully launching our chymase inhibitor with lower dosage and/or different pharmacokinetic profile onto the market, a new class of the compound, which showed enhanced inhibitory activity and/or different physical property, was explored using a structure-based drug design approach. Heteroaromatic derivatives and amino acid derivatives were designed by referring to the cocrystal structure of chymase and compound **1** which is SUN13834 series compound (Figure 1). In this paper, the approach used in the design, in addition to the structure activity relationships, is described. In the course of our study, various derivatives were synthesized that showed potent inhibitory activity, comparable to SUN13834.

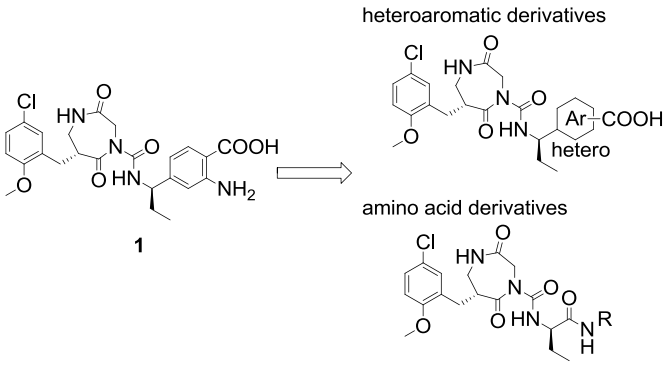

Figure1. Approach to obtaining new inhibitors

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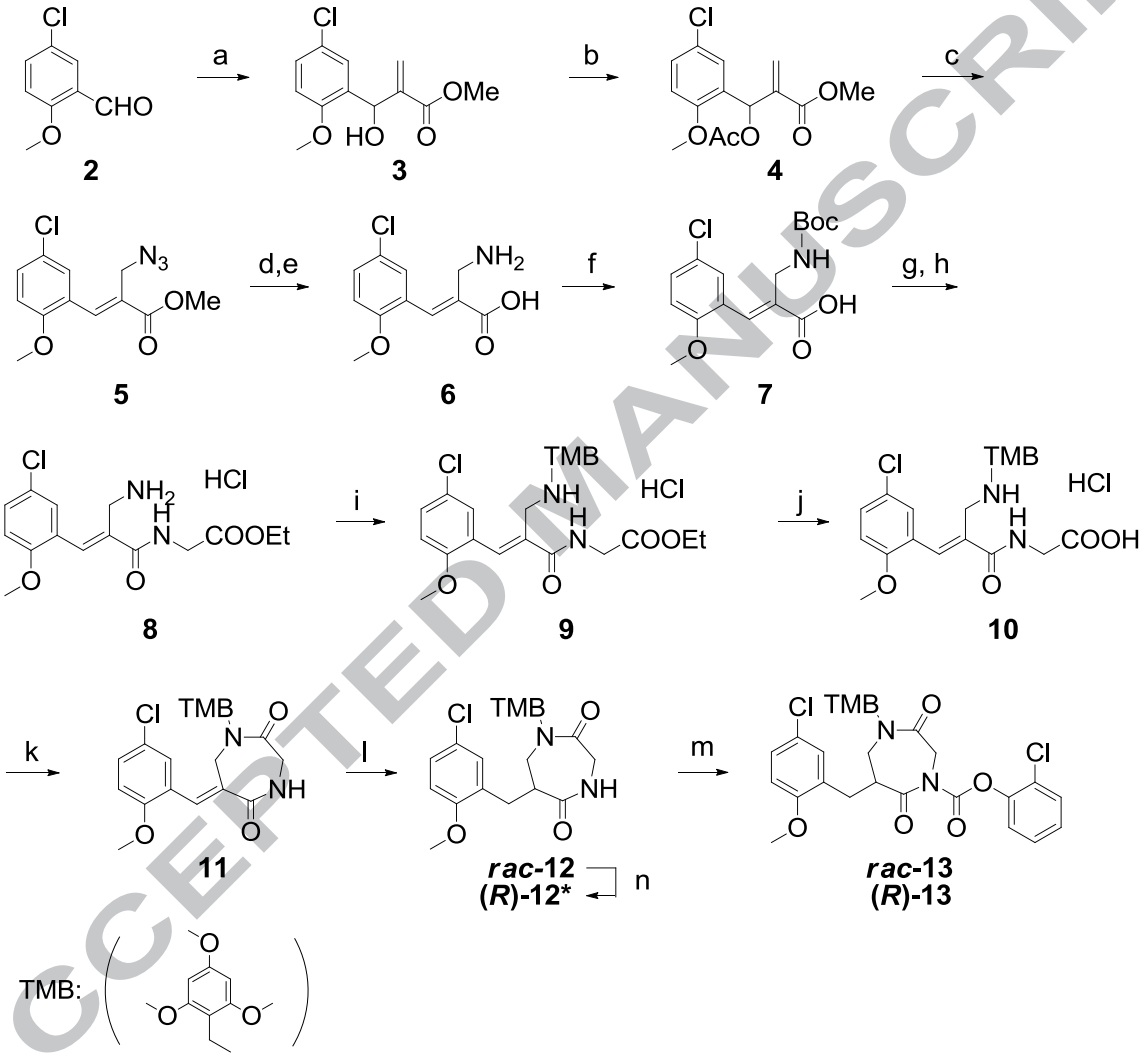
2. Chemistry

***Rac*-13** (racemic mixture) and **(*R*)-13** (*R* form at 6-position of 1,4-diazepane core; the configuration was selected by referring to the structure of compound **1**), which were used as a common intermediate for both derivatives, were prepared from 5-chloro-2-methoxybenzaldehyde (**2**), as shown in Scheme 1. The Baylis–Hillman reaction of **2** with methyl acrylate, followed by acetylation, gave adduct **4**. SN2′-type substitution of the acetoxy group of **4** by sodium azide, and subsequent reduction of the azide group and hydrolysis of the methyl ester, led to β-amino acid **6**, which was then converted to *N*-Boc derivative **7** using standard methods. Condensation of **7** with glycine ethyl ester hydrochloride, followed by deprotection of the Boc group, provided amine **8**. Introduction of a 2,4,6-trimethoxybenzyl (TMB) group to **8** by reductive alkylationand subsequent hydrolysis of the ester moiety, gave amino acid **10**. Lactamization of **10**, followed by catalytic hydrogenation, afforded the seven-membered lactam ***rac*-12**. **(*R*)-12**[15] was prepared by optical resolution of ***rac*-12** using a chiral HPLC column (CHIRALCEL OD). Following this, ***rac*-12** and **(*R*)-12** were converted to the carbamate ***rac*-13** and **(*R*)-13**, respectively, by sequential treatment with *n*-butyllithium and 2-chlorophenylchloroformate.



Scheme 1.Reagents and conditions: (a) methyl acrylate, DABCO, $La(OTf)_3$, $N(CH_2CH_2OH)_2$, rt; (b) AcCl, Et_3N, CH_2Cl_2, 0°C; (c) NaN_3, DMSO, rt; (d) PPh_3, H_2O, THF, rt; (e) aq NaOH, MeOH, rt; (f) $(Boc)_2O$, aq NaOH, THF, 0°C; (g) glycine ethyl ester HCl, EDCI HCl, HOBt, Et_3N, CH_2Cl_2, rt; (h) 4M HCl/EtOAc, rt; (i) 2,4,6-$(MeO)_3$PhCHO, $NaBH(OAc)_3$, THF, rt; (j) aq NaOH, MeOH, rt; (k) EDCI HCl, HOBt, Et_3N, DMF, rt; (l) H_2, 2%Pt(sulfide).C, THF, rt; (m) *n*-BuLi, THF, -78°C (for***rac*-13**) or -20°C (for**(R)-13**), then 2-ClPhOCOCl, -78°C (for***rac*-13**) or -20°C (for**(R)-13**); (n) optical resolution using chiral HPLC (CHIRALCEL OD). *THF solvate

As shown in Scheme 2, the heteroaromatic derivatives listed in Table1 were prepared from optically active carbamate **(*R*)-13** as a common intermediate. Coupling reactions of **(*R*)-13** with the corresponding amines furnished compounds **15A–F** and **H**, which were deprotected to yield heteroaromatic derivatives **16A–F** and **H**. Compound **16G** was synthesized in a similar manner from deprotected carbamate **(*R*)-14**.

As shown in Scheme 3, the amino acid derivatives listed in Table 2 and 3 were prepared from ***rac*-13** as a common intermediate. A coupling reaction of ***rac*-13** with *tert*-butyl 2-aminobutylate, followed by deprotection of the TMB group, provided ***rac*-17**. Amino acid derivatives **18A–R** were furnished by condensation of ***rac*-17** with the corresponding amines and/or deprotection. The optically active compounds **(*R*)-15** and **(*R*)-16R**, which were used for cocrystallization with human chymase for X-ray structure analysis, were synthesized in a similar manner from **(*R*)-13** via the deprotected carbamate **(*R*)-14**.

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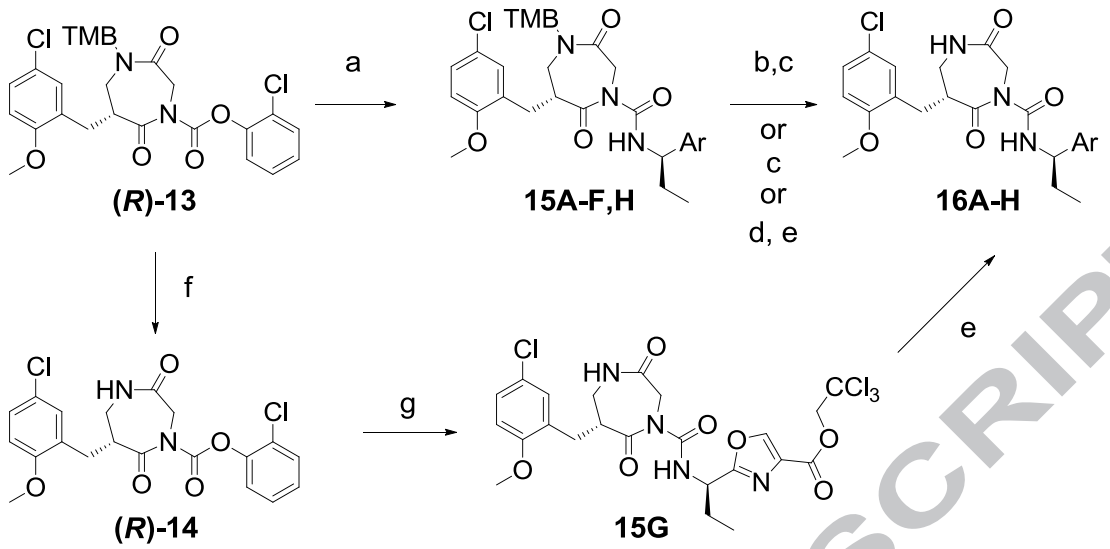
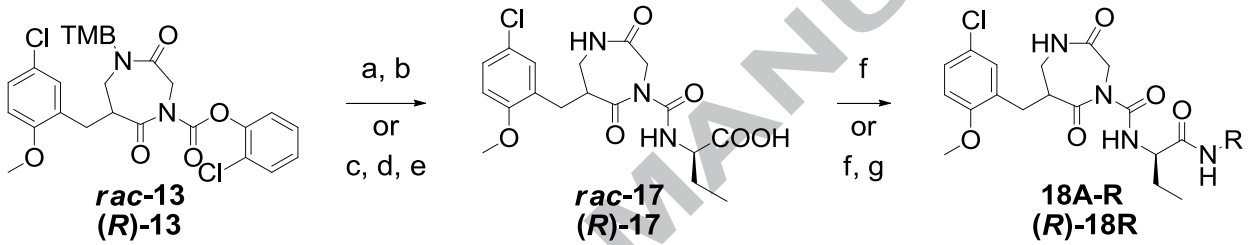


Scheme 2. Reagents and conditions: (a) amines, Et_3N, DMAP, DMF, 0°C; (b) DDQ, CH_2Cl_2, H_2O, rt; (c) 1 M HCl/AcOH, rt (d) 1 M HCl/AcOH, anisole, rt; (e) Zn, AcOH, rt; (f) TFA, anisole, rt.; (g) **42**, DIPEA, DMAP, DMF, 0°C.



Scheme 3. Reagents and conditions: (a) (*R*)-*tert*-butyl 2- aminobutanoate, Et_3N, DMF, 0°C; (b) TFA, CH_2Cl_2, rt; (c) TFA, anisole, rt; (d) (*R*)-*tert*-butyl 2-aminobutanoate, DIPEA, DMAP, DMF, 0°C; (e) 4 M HCl/EtOAc, rt; (f) amines *n*-propyl phosphoric acid anhydride, Et_3N, CH_2Cl_2, rt; (g) 1 M HCl/AcOH, rt.

Amines for preparation of **16A–C** were synthesized from the corresponding aromatic halide **19a–c**, as shown in Scheme 4. *tert*-Butyl esterification of **19a–c** with *O-tert*-butyl *N*,*N*′-diisopropylisourea, followed by palladium-catalyzed cyanation, provided aromatic cyanide **21a–c**. The cyanide group was then converted into an ethyl carbonyl moiety. Asymmetric reduction[16] of the carbonyl group, followed by the Mitsunobu reaction with phthalimide, provided **24a–c**. After hydrazinolysis of the phthalimide moiety, tartrate salts **25a–c**[17] were formed from the obtained amines.

As shown in Scheme 5, the amine for preparation of **16D** was synthesized from 5-formyl-2-furancarboxylic acid (**26**), which was converted to secondary alcohol **28** by sequential manipulation (protection of carboxyl group and enantioselective addition of Et_2Zn[18] to the aldehyde). The desired amine **31**[17] was then synthesized in a similar manner to Scheme 4 from **28**, shown in Scheme 5.

As demonstrated in Scheme 6, amines for the preparation of **16E** and **16F** were synthesized from 3-carboxyl furan (**32a**) and 3-carboxyl thiophene (**32b**), respectively. Friedel-Crafts reaction using indium(III) triflate as a Lewis acid,[19] followed by *tert*-butyl esterification with *O-tert*-butyl *N*,*N*′-diisopropylisourea, provided aryl ethyl ketones **33a,b**. The desired amines **36a**,**b**[17] were then synthesized in a similar manner to Scheme 4 from the obtained ketones shown in Scheme 6.

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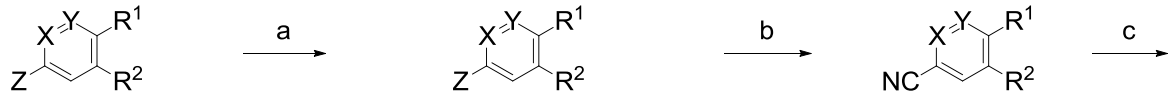
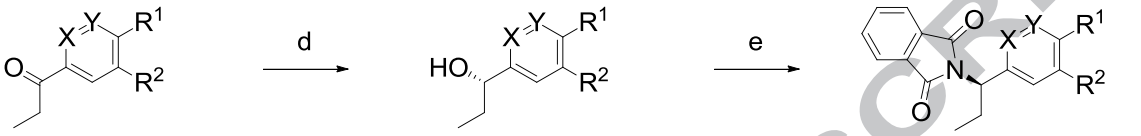


19a (X = N, Y = CH, Z = Cl
R^1 = COOH, R^2 = H)
19b (X = N, Y = CH, Z = Cl
R^1 = H, R^2 = COOH)
19c (X = CH, Y = N, Z = Br
R^1 = H, R^2 = COOH)

20a (X = N, Y = CH, Z = Cl
R^1 = COO^{t}Bu, R^2 = H)
20b (X = N, Y = CH, Z = Cl
R^1 = H, R^2 = COO^{t}Bu)
20c (X = CH, Y = N, Z = Br
R_1 = H, R_2 = COO^{t}Bu)

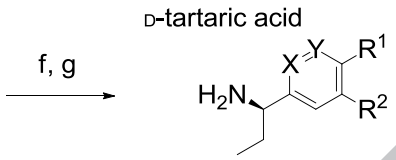
21a (X = N, Y = CH,
R^1 = COO^{t}Bu, R^2 = H)
21b (X = N, Y = CH,
R^1 = H, R^2 = COO^{t}Bu)
21c (X = CH, Y = N,
R^1 = H, R^2 = COO^{t}Bu)



22a (X = N, Y = CH,
R^1 = COO^{t}Bu, R^2 = H)
22b (X = N, Y = CH,
R^1 = H, R^2 = COO^{t}Bu)
22c (X = CH, Y = N,
R^1 = H, R^2 = COO^{t}Bu)

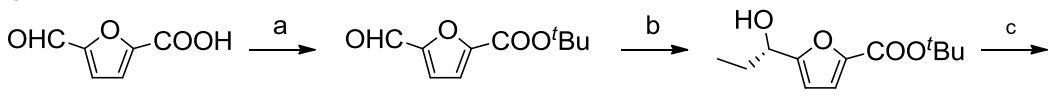
23a (X = N, Y = CH,
R^1 = COO^{t}Bu, R^2 = H)
23b (X = N, Y = CH,
R^1 = H, R^2 = COO^{t}Bu)
23c (X = CH, Y = N,
R^1 = H, R^2 = COO^{t}Bu)

24a (X = N, Y = CH,
R^1 = COO^{t}Bu, R^2 = H)
24b (X = N, Y = CH,
R^1 = H, R^2 = COO^{t}Bu)
24c (X = CH, Y = N,
R^1 = H, R^2 = COO^{t}Bu)

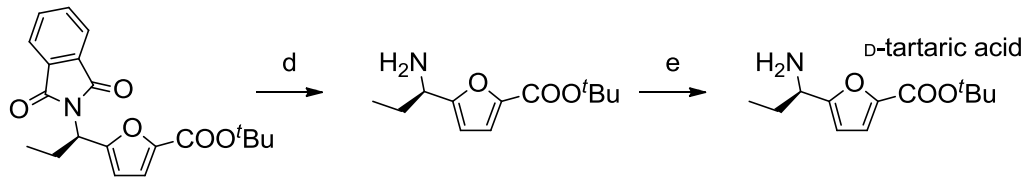


25a (X = N, Y = CH,
R^1 = COO^{t}Bu, R^2 = H)
25b (X = N, Y = CH,
R^1 = H, R^2 = COO^{t}Bu)
25c (X = CH, Y = N,
R^1 = H, R^2 = COO^{t}Bu)

Scheme 4. Reagents and conditions: (a) *O*-tert- butyl *N*,*N*′-diidopropylisourea, CH_2Cl_2, reflux; (b) $Zn(CN)_2$,$Pd_2(dba)_3$, dppf,Zn,$AcNMe_2$, 120°C; (c) CuI, EtMgBr, THF, 0°C; (d) (-)-Ipc_2BCl, THF, -20°C; (e) DEAD, PPh_3,THF, phthalimide, rt; (f) $NH_2NH_2.H_2O$, MeOH, 60°C; (g) D-tartaric acid, EtOH, EtOAc.



26 **27** **28**



29 **30** **31**

Scheme 5. Reagents and conditions: (a) *O-tert-* butyl *N*,*N*′-diidopropylisourea, CH_2Cl_2, reflux; (b) (1*S*,2*R*)-2-(dibutylamino)-1-phenylpropan-1-ol, Et_2Zn, hexane, toluene, 0°C; (c) DEAD, PPh_3, THF, phthalimide, rt; (d) $NH_2NH_2H_2O$, MeOH, 60°C; (e) D-tartaric acid, EtOH, EtOAc.

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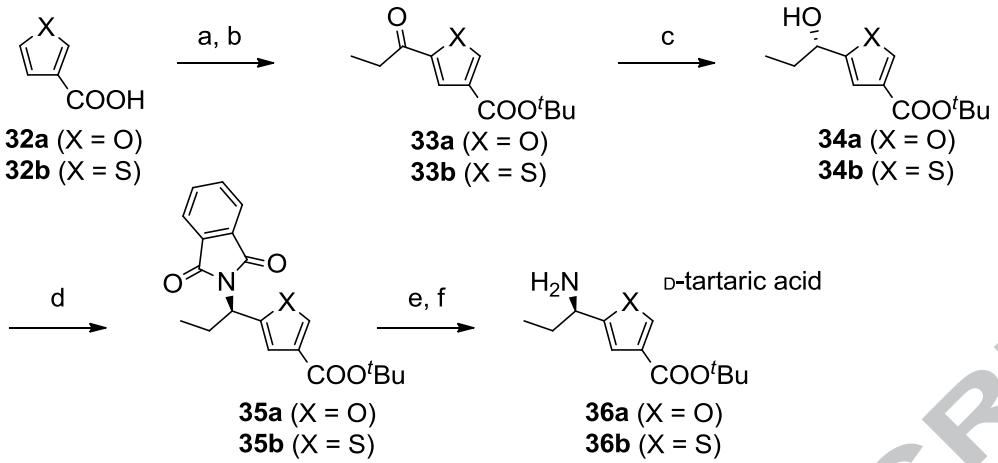
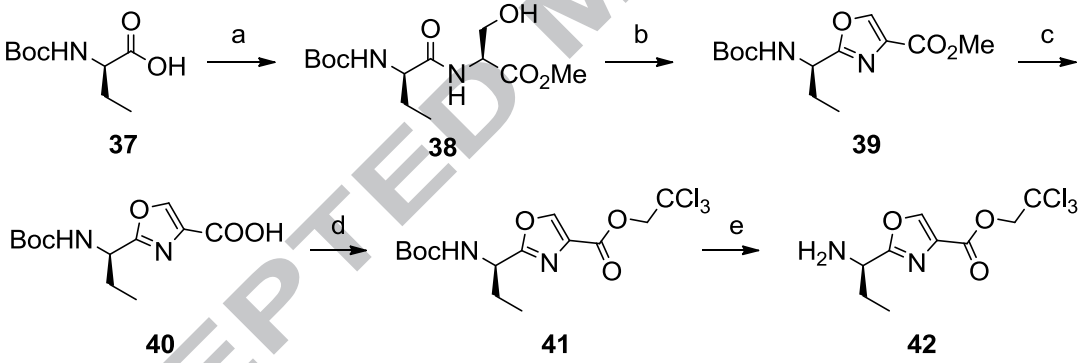


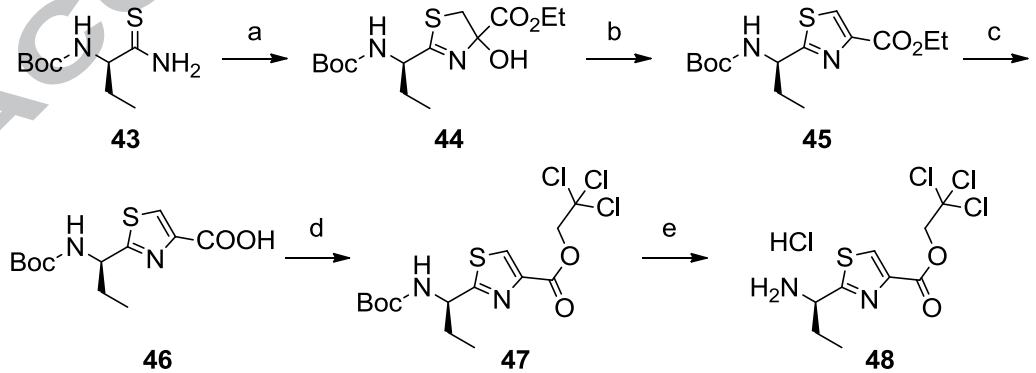
Scheme 6. Reagents and conditions: (a) $In(OTf)_3$,$LiClO_4$, $(EtCO)_2O$,$MeNO_2$, 50°C; (b) *O-tert-* butyl *N,N'*-diidopropylisourea, CH_2Cl_2, reflux; (c) (-)-Ipc_2BCl, THF, -20°C; (d)DEAD, PPh_3, THF, phthalimide, rt; (e) $NH_2NH_2H_2O$, MeOH, 60°C; (f) D-tartaric acid, EtOH, EtOAc.

The amine for the preparation of **16G** was synthesized from (*R*)-2-(*tert*-butoxycarbonylamino)butyric acid (**37**), as shown in Scheme 7. Condensation of **37** with (L)-serine methyl ester hydrochloride, followed by cyclization using DAST[20] and dehydrogenation using bromotrichloromethane, provided **39**. After hydrolysis of the methyl ester moiety, obtained carboxylic acid **40** was condensed with trichloroethanol to obtain **41**. Deprotection of the Boc group provided desired amine **42**.

The amine used the for preparation of **16H** was synthesized from (*R*)-1-amino-1-thioxobutane-2-yl carbamic acid *tert*-butyl ester (**43**), as shown in Scheme 8. Condensation of **43** with ethyl bromopyruvate, followed by dehydroxylation using TFAA, provided **45**. The desired amine was then synthesized in a similar manner to Scheme 7 from **45**, as shown in Scheme 8.



Scheme 7. Reagents and conditions: (a) (*L*)-serine methyl ester HCl, EDCI HCl, HOBt, DIPEA, CH_2Cl_2, rt; (b) DAST, CH_2Cl_2, -20°C, then bromotrichloromethane, DBU, 0°C; (c) aq. NaOH, MeOH, rt; (d) 2,2,2-trichloroethanol, EDCI HCl, 4-DMAP, CH_2Cl_2, rt; (e) 4M HCl/EtOAc, rt.



Scheme 8. Reagents and conditions: (a) ethyl bromopyruvate, $KHCO_3$, DME, rt; (b)TFAA, pyridine, DME, 0°C; (c) aq. NaOH, EtOH, rt; (d) 2,2,2-trichloroethanol, EDCI HCl, 4-DMAP, CH_2Cl_2, rt; (e) 4M HCl/EtOAc, rt.

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3. Results and discussion

The X-ray crystal structure of compound **1** within the structure of human chymase is shown in Figure 2. In this configuration, the benzyl unit and the ethyl unit are placed in S1 site and S1′ site, respectively. There is a hydrogen bond between the amide nitrogen of the seven-membered ring and the carbonyl oxygen of Ser214.The anthranilic acid unit is located in the prime site. The aromatic ring interacts hydrophobically with the cleft, and the carboxyl group and the amino group interact with Arg143 by ionic interaction and with the carbonyl oxygen of Phe41 by hydrogen-bonding, respectively. This ionic interaction could be responsible for the increase in affinity to human chymase that we previously reported.[9] However, the electron density of the carboxyl group is unclear, as shown in Figure 3. This was thought to be due to its flexibility within the prime site. We therefore considered that there was still room for improvement in the interaction of human chymase with compound **1**, especially in the prime site. In order to achieve this, we focused on modification of that particular site, and designed heteroaromatic derivatives and amino acid derivatives based on the X-ray crystal structure.

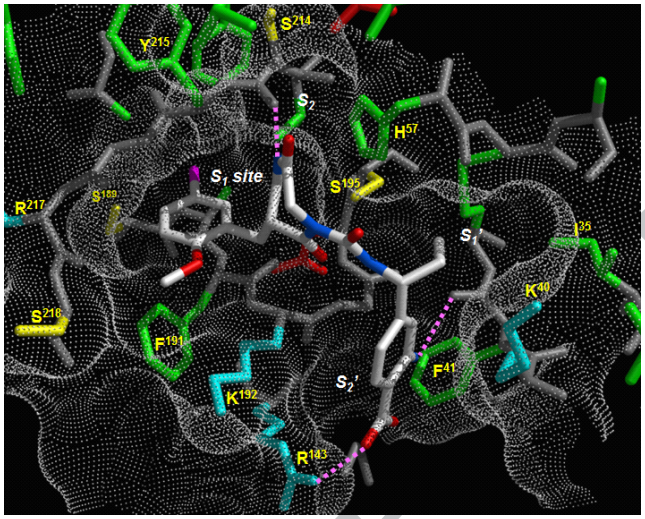

Figure 2. X-ray crystal structure of compound **1**in the active site of human chymase.

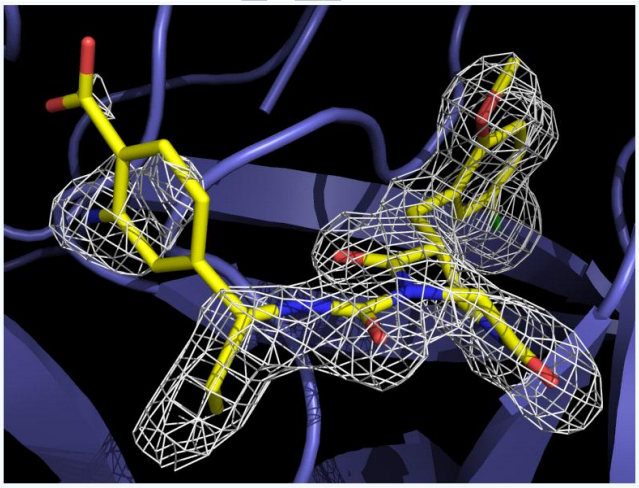

Figure 3.Electron density map of compound **1** on the crystal with human chymase(contoured at 1 sigma).

It was first considered that conversion to a heteroaromatic ring from a benzene ring in the prime site would lead to an additional interaction of the heteroatom with the enzyme, for example, with the carbonyl oxygen of Phe41. This would increase the affinity of the inhibitor for the chymase. Table 1 shows the inhibitory activities of the heteroaromatic derivatives thus designed.

Compounds **16C**, **16D**, and **16F** showed potent activity, whereas, the other derivatives were less effective. It was speculated that the heteroaromatic rings did not interact directly with the chymase in the manner that was predicted, but rather fixed the configuration of the carboxyl group, leading to increased activity due to an interaction with Lys40 and/or Arg143 in the S2′ site.

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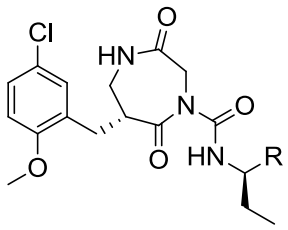
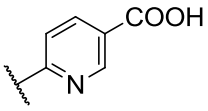
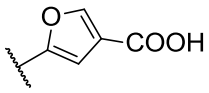
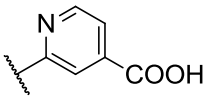
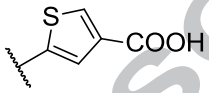
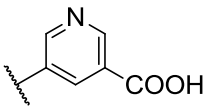
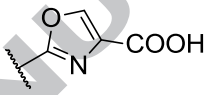
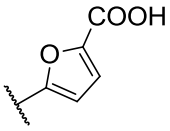
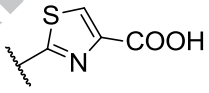
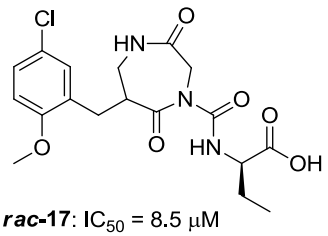
Table 1. Inhibitory activity of heteroaromatic derivatives against recombinant human chymase.



compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
16A		1.0	**16E**		1.2
16B		1.3	**16F**		0.52
16C		0.38	**16G**		2.77
16D		0.41	**16H**		1.64

It was next considered that an effective interaction with Lys40 would provide increased affinity compared with an interaction with Arg143, as in compound **1**.[9] Therefore, we investigated amino acid derivative ***rac*-17** which also might show improvement of pharmacokinetics profile due to smaller size. Figure 4 shows the chymase inhibitory activity of ***rac*-17** and the X-ray cocrystal structure of **(*R*)-17** (*R* form at 6-position of the 1,4-diazepane core) with human chymase. In the S1 and S1′ sites, the benzyl unit and the ethyl unit show similar modes of interaction to **1**. From a standpoint of stereochemistry effect, *R* form at 6-position of 1,4-diazepane core was more efficient than *S* form for our inhibitor because **(*R*)-17** showed more potent inhibitory activity than ***rac*-17**. Referring to X-ray crystal structure shown in Figure 4, it was supposed that if the benzyl unit of S form derivative made favorable interaction with S1 site, 1,4-diazepane and prime site could not interact with enzyme well. Furthermore, it was speculated that the carboxyl group interacted with the amino group of Lys40; however, it was considered that the configuration was not sufficient and that the interaction did not compensate for the large desolvation penalty, and so, did not contribute to an increase in affinity to human chymase.

It was therefore considered that increasing the enzyme–inhibitor affinity by enhancing the interaction between Lys40 and the amino acid unit was not a useful strategy. A different approach was subsequently investigated that involved extension from the carboxyl group by amide bond formation. This was hypothesized to cause an increase in affinity owing to hydrophobic and/or ionic interaction with the S2′ site, as previously described for compound **1**. Therefore, **18A–H** were designed and synthesized, and the inhibitory activities of the compounds are summarized in Table 2. **18A–H** showed moderate activity; however, no further increase in activity was demonstrated. These results indicated that both the hydrophobic and ionic interactions were needed to achieve potent activity.



***rac*-17**: IC_{50} = 8.5 μM
(*R*)-17*: IC_{50} = 3.9 μM
*R form at 6-position of the 1,4-diazepane core

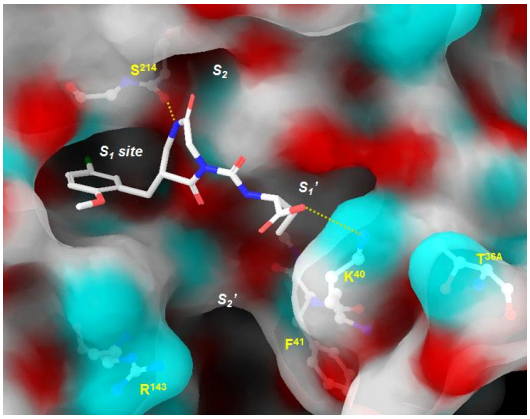


Figure 4. Inhibitory activity against recombinant human chymase of ***rac*-17** (left); X-ray crystal structure of **(R)-17** in the active site of human chymase (right).

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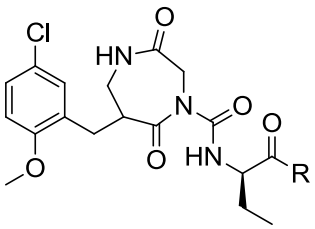
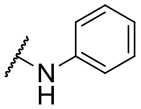
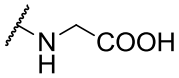
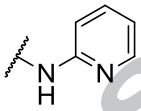
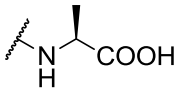
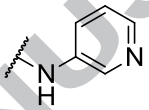
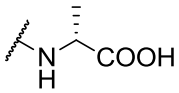
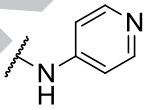
Table 2. Inhibitory activity of amino acid derivatives against recombinant human chymase.



compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
18A		5.2	**18E**		1.6
18B		4.2	**18F**		2.8
18C		2.6	**18G**		3.1
18D		3	**18H**		2.0

The introduction of a carboxyl group on the phenyl group of **18E** was next attempted, with the intention of enhancing interaction with the S2′site. Additionally, introduction of an amino group to the phenyl group was also undertaken, similar to another interaction of compound **1** with the S2′ site. As shown in Table 3, **18I–M**, which were all designed according the aforementioned strategies, showed improvements in activity, with **18I** being the only exception. With the aim of achieving a further enhancement in activity, structures that combined each of the important interactions that had been identified were designed (**18N–Q**). These compounds all exhibited potent activity; in particular, **18P** showed comparable activity to **1**.

In a separate approach, replacemant of the carboxyl group with a tetrazolyl group, a known bioisostere, was carried out, producing **18R**, which showed comparable activity to **1**. According to the X-ray crystal structure shown in Figure 5, in the case of **(*R*)-18R** (*R* form of **18R** at 6-position of 1,4-diazepane core), the tetrazolyl group is not placed in a cleft of the prime site, making contact with the hydrophobic side chain of Lys40 and the methyl group of Thr36. The potent activity of **18R** was assumed to be due to a different type of interaction in the prime site compared to compounds **1** and **18P**.

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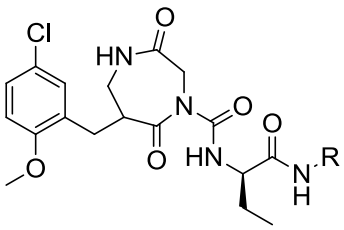
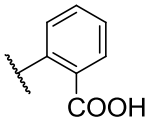
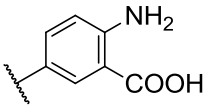
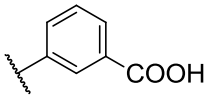
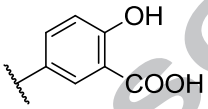
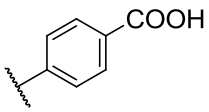
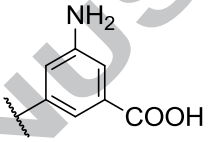
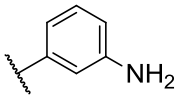
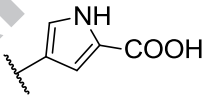
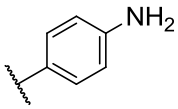
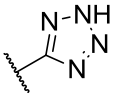
Table 3. Inhibitory activity of amino acid derivatives against recombinant human chymase.



compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
18I		3.7	**18N**		0.73
18J		0.57	**18O**		0.57
18K		0.77	**18P**		0.38
18L		0.66	**18Q**		0.85
18M		0.79	**18R**		0.26

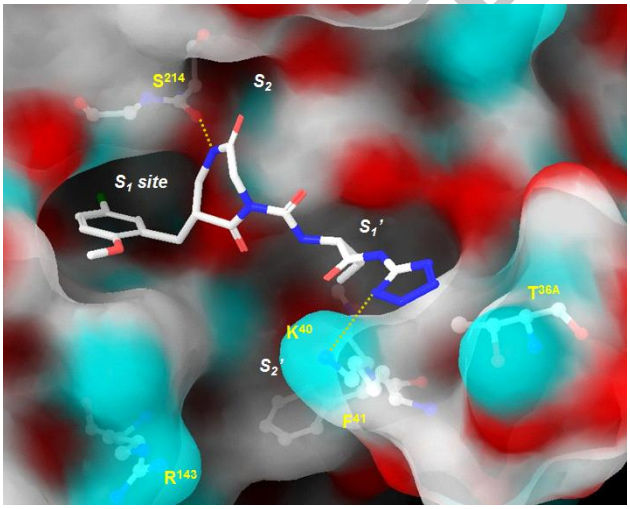


Figure 5. X-ray crystal structure of **(*R*)-18R** in the active site of human chymase.

Several potent activity inhibitors were obtained overall, and furthermore, they showed selectivity against other serine proteases, including bovine α-chymotrypsin, human cathepsin G, and human elastase, as shown in Table 4.

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Table 4. IC_{50} (μM) values of inhibition of human chymase and other serine proteinases for **1**, **16C**, **16D**, **18J**, **18L**, and **18R**.

Enzyme	1	16C	16D	18J	18L	18R
Human Chymase	0.30	0.38	0.41	0.57	0.66	0.26
Bovine α-chymotrypsin	>100	88	>100	>10	>10	>10
Human cathepsin G	16.0	7.8	16	8.5	>10	>10
Human elastase	>100	>100	>100	>10	>10	>10

4. Conclusion

A novel set of human chymase inhibitors were explored, which included a heteroaromatic moiety and an amino acid moiety, and their structure activity relationships and interaction modes were investigated using X-ray crystal structure analysis. Several active and selective inhibitors were obtained, with (*R*)-18R exhibiting potent inhibitory activity comparable to SUN13834, which has previously shown efficacy in treating humans with atopic dermatitis, and in addition, demonstrating a new mode of interaction.

5. Experimental section

5.1. Chemistry

5.1.1. Genaral

Proton nuclear magnetic resonance spectra (^{1}H NMR) and was recorded on Brucker ARX-400 or Brucker Avance III (400 MHz) spectrometer in the indicated solvent. Chemical shifts (d) are reported in parts per million relative to the internal standard tetramethylsilane. Electro-spray ionization (ESI) mass spectra were recorded on Agilent G1956A MSD spectrometer system. Other chemical reagents and solvents were purchased from Aldrich, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, Kanto Kagaku or Nacalai tesque and used without purification. Flash column chromatography was performed using Merck Silica Gel 60 (230-400 mesh) or Purif-Pack_ SI 30um supplied by Shoko Scientific. Preparative TLC was performed using Merck PTLC Glass Plates Si 60 20 x 20cm, 0.5mm or 1mm thick.

5.1.2. Methyl2-[(5-chloro-2-methoxyphenyl)(hydroxy)methyl]propenoate (3)

A reaction mixture of 5-chloro-2-methoxybenzaldehyde (7 g), methyl acrylate (6 ml), 1,4-diazabicyclo[2.2.2]octane (4.6 g), lanthanum trifluoromethanesulfonate (1.2 g), and diethanol amine (2.7 ml) was stirred at room temperature for 60 hours. Distilled water and saturated potassium hydrogensulfate aqueous solution were added to the reaction solution, and the mixture was extracted with ethyl acetate. The organic layer was successively washed with distilled water and saturated saline, dried over with anhydrous sodium sulfate, then concentrated. The residue was dried in vacuo to obtain the title compound (11.1 g). ^{1}H NMR ($CDCl_3$) *δ*: 7.37 (1H, *d*, J = 2.9 Hz), 7.22 (1H, dd, *J* = 8.7, 2.9 Hz), 6.8 (1H, d, *J* = 8.7 Hz), 6.31 (1H, m), 5.83 (1H, d, *J* = 5.8 Hz), 5.69 (1H, m), 3.81 (3H, s), 3.77 (3H, s); MS (ESI): 239 (M-OH)$^+$

5.1.3. Methyl2-[(5-chloro-2-methoxyphenyl)(acetoxy)methyl]propenoate (4)

To the compound **3** (11 g) in methylene chloride (100 ml) solution, pyridine (3.5 ml) and acetylchloride (3.1 ml) were added under ice cooling and the mixture was stirred at that temperature for 1 hour. Distilled water was added to the reaction solution, methylene chloride was distilled off in vacuo, and the remaining aqueous layer was extracted with ethyl acetate. The organic layer was successively washed with saturated potassium hydrogensulfate aqueous solution, distilled water, saturated sodium hydrogencarbonate aqueous solution, and saturated saline, dried over with anhydrous sodium sulfate, then concentrated. The residue was dried in vacuo to obtain the title compound (11.8 g). ^{1}H NMR ($CDCl_3$) *δ*: 7.21-7.26 (m, 2H), 6.99 (s, 1H), 6.81 (d, *J* = 8.53 Hz, 1H), 6.41 (s, 1H), 5.62-5.66 (m, 1H), 3.81 (s, 3H), 3.75 (s, 3H), 2.11 (s, 3H); MS (ESI): 298 (M+H)$^+$

5.1.4. (2*E*)-2-(Aminomethyl)-3-(5-chloro-2-methoxyphenyl)-2-propenoic acid (6)

To the compound **4** (11.8 g) in dimethylsulfoxide (70 ml) solution, sodium azide (3.9 g) was added and the mixture was stirred at room temperature for 30 minutes. Distilled water was added to the reaction solution, and the mixture was extracted with diethylether. The organic layer was successively washed with distilled water and saturated saline, dried over with anhydrous sodium sulfate, and concentrated. The residue was dried in vacuo to obtain the compound **5** (10.1 g). To the compound **5** (10 g) in tetrahydrofuran (70 ml) solution, triphenylphosphine (9.4 g) and distilled water (1 ml) were added and the mixture was stirred at room temperature for 15 hours. Next, tetrahydrofuran was distilled off in vacuo, methanol (70 ml) and 2N sodium hydroxide aqueous solution (35 ml) were added to the remaining mixture, and the mixture was stirred at room temperature for 2 hours. Next, the methanol was distilled off in vacuo and the remaining aqueous layer was washed with ethyl acetate. Further, the aqueous layer was neutralized by hydrochloric acid, then precipitate was collected by filtration, was washed with diethylether, and was dried in vacuo to obtain the compound **6** (6.3 g). ^{1}H NMR (Acetic acid-*d*4) *δ*: 8.07 (1 H, s), 7.41 (1H, dd, *J* = 2.6, 8.8 Hz), 7.34 (1H, d, *J* = 2.6 Hz), 7.03 (1H, d, *J* = 8.8 Hz), 4.07 (2H, s); MS (ESI): 242 (M+H)$^+$

5.1.5. (2*E*)-2-{[(*tert*-Butoxycarbonyl)amino]methyl}-3-(5-chloro-2-methoxyphenyl)-2-propenoic acid (7)

To the compound **6** (15 g) in tetrahydrofuran (300 ml) suspension, 2N sodium hydroxide aqueous solution (70 ml) and di-tert-butyldicarbonate (15 g) were added and the mixture was stirred at room temperature for 1 hour. Next, tetrahydrofuran was distilled off in vacuo, the obtained aqueous mixture was acidified by adding saturated potassium hydrogensulfate aqueous solution, then the mixture was extracted with ethyl acetate. The organic layer was washed with saturated saline and dried over with anhydrous sodium sulfate and concentrated. The residue was recrystallized by ethyl acetate/hexane to obtain the title compound (19.8 g). ^{1}H NMR ($CDCl_3$) *δ*: 7.93 (0.5H, br), 7.78 (0.5H, br), 7.42 (0.5H, br), 7.30 (1H, dd, *J* = 8.8, 2.4 Hz), 7.19 (0.5H, br), 6.84 (1H, *d*, J= 8.8 Hz), 6.76 (0.5H, br), 5.12 (0.5H, br), 4.14 (2H, br), 3.84 (3H, s), 1.55-1.15 (9H, m); MS: 364 (M+Na)$^+$

5.1.6. [((2*E*)-3-(5-Chloro-2-methoxyphenyl)-2-{[(2,4,6-trimethoxybenzyl)amino]methyl}-2-propenyl)amino]ethyl acetate hydrochloride (9)

To the compound **7** (123 g) in methylene chloride (400 ml) solution, glycine methyl ester hydrochloride (51 g), 1-hydroxybenzotriazole (49 g), triethylamine (53 ml), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (76 g) were added under ice cooling and the mixture was stirred at room temperature for 2 hours. The reaction solution was diluted with distilled water, then the precipitate was filtered out and the filtrate was extracted with ethyl acetate. The organic layer was successively washed with saturated potassium hydrogensulfate aqueous solution, distilled water, saturated sodium hydrogencarbonate aqueous solution, and saturated saline, dried over with anhydrous sodium sulfate, then concentrated. The

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residue was recrystallized by hexane/ethyl acetate. A mixed solution of the obtained compound (131.5 g) and a 4M hydrogen chloride/ethyl acetate solution (350 ml) was stirred at room temperature for 20 minutes. The reaction solution was diluted with diethylether, then the precipitate was collected by filtration to obtain the compound **8** (109.3 g). To the compound **8** (55.8 g) in tetrahydrofuran (800 ml) solution, 2,4,6-trimethoxybenzaldehyde (30.5 g) was added and the mixture was stirred at room temperature for 20 minutes. Next, sodium triacetoxy borohydride (50 g) was added to the reaction solution and the mixture was stirred at room temperature for 1 hour. Distilled water was added to the reaction solution, then tetrahydrofuran was distilled off in vacuo. The remaining aqueous layer was basified by a sodium hydroxide aqueous solution, then the mixture was extracted with ethyl acetate. The organic layer was washed with saturated saline, dried over with anhydrous sodium sulfate, and partially distilled off in vacuo. A 4M hydrogen chloride/ethyl acetate solution was added to the remaining solution and the mixture was stirred under ice cooling for 30 minutes. The precipitate was collected by filtration to obtain the title compound (75.7 g). ^{1}H NMR (DMSO-*d*6) δ: 9.12 (1H, br), 8.58 (1H, br), 7.59 (1H, s), 7.48 (1H, d, *J* = 8.9 Hz), 7.32 (1H, s), 7.13 (1H, d, *J* = 8.9 Hz), 6.23 (2H, s), 4.13 (2H, q, *J* = 7.1 Hz), 3.96 (2H, d, *J* = 5.7 Hz), 3.84-3.70 (16H, m), 1.21 (3H, t, *J* = 7.1 Hz); MS (ESI): 507 $(M+H)^+$

5.1.7. (6*E*)-6-(5-Chloro-2-methoxybenzylidene)-1-(2,4,6-trimethoxybenzyl)-1,4-diazepan-2,5-dione (11)

To the compound **9** (148.5 g) in methanol (300 ml) solution, 2N sodium hydroxide aqueous solution (300 ml) was added and the mixture was stirred at room temperature for 2 hours. The reaction solution was neutralized by 6M hydrochloric acid (100 ml), then the methanol was distilled off in vacuo. Crystal nuclei were added to the remaining solution and the mixture was stirred under ice cooling for 30 minutes. The precipitate was collected by filtration to obtain the compound **10** (143.1 g). A solution of the compound **10** (53 g) and 1-hydroxybenzotriazole (14 g) in N,N-dimethylformamide (1000 ml) was added dropwise to a solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (24 g) and triethylamine (17 ml) in N,N-dimethylformamide (500 ml) over 2 hours. After dropping, the insoluble compound was filtered out, then the filtrate was concentrated. Ethyl acetate and 1N hydrochloric acid were added to the residue and the mixture was stirred for 30 minutes. The insoluble compound was collected by filtration and washed with distilled water and ethyl acetate. Ethyl acetate and 1N sodium hydroxide aqueous solution were added to the obtained solid and stirred for 30 minutes. The insoluble compound was collected by filtration and washed with distilled water and ethyl acetate. A mixed solvent of tetrahydrofuran/methanol=1/1 was added to the obtained solid and the mixture was stirred under heating and reflux for 30 minutes. The solution was allowed to cool to room temperature, then the mixed solution was filtered by sellite and the filtrate was concentrated. The residue was recrystallized by ethyl acetate to obtain the title compound (22.1 g). ^{1}H NMR ($CDCl_3$) δ: 7.6 (1H, s), 7.29-7.19 (1H, m), 6.76-6.69 (2H, m), 6.05 (1H, br), 5.75 (2H, s), 4.52 (2H, s), 4.23 (2H, s), 4.05 (2H, d, *J* = 6.1 Hz), 3.80-3.74 (6H, m), 3.52 (6H, s)

5.1.8. 6-(5-Chloro-2-methoxybenzyl)-1-(2,4,6-trimethoxybenzyl)-1,4-diazepan-2,5-dione (*rac*-12)

To the compound **11** (16.3 g) in tetrahydrofuran (600 ml) solution, 2% platinum carbon (sulfur poisoned catalyst) (7.3 g) was added and the mixture was stirred under hydrogen atmosphere at room temperature for 60 hours. Next, the catalyst was filtered out and the filtrate was concentrated. The residue was purified by silica gel column chromatography (chloroform/ethyl acetate/methanol=7/1/0.5) to obtain the title compound (13.1 g). ^{1}H NMR ($CDCl_3$) δ: 7.14 (1H, dd, *J* = 8.7, 2.5 Hz), 6.88 (1H, d, *J* = 2.5 Hz), 6.69 (1H, d, *J* = 8.7 Hz), 5.98 (2H, s), 5.94 (1H, br), 4.8 (1H, d, *J* = 13.7 Hz), 4.30-4.20 (2H, m), 3.83 (3H, s), 3.78-3.69 (4H, m), 3.63 (6H, s), 3.38 (1H, dd, *J* = 15.4, 12.2 Hz), 3.11 (1H, dd, *J* = 13.0, 3.2 Hz), 2.94 (1H, dd, *J* = 15.4, 4.9 Hz), 2.5 (1H, dd, *J* = 13.0, 10.7 Hz); MS (ESI): 461 $(M+H)^+$

5.1.9. (6*R*)-6-(5-Chloro-2-methoxybenzyl)-1-(2,4,6-trimethoxybenzyl)-1,4-diazepan-2,5-dione tetrahydrofuran solvate((*R*)-12)

The compound ***rac*-12** was separated using CHIRALCEL OD-H (Daicel Chemical Industries) (movement phase: acetonitrile/trifluoroacetic acid = 100/0.1) .The obtained compound was diluted with tetrahydrofuran, and the resultant suspension was stirred at 60 °C until the solution became clear. Then, the solution was gradually cooled to room temperature, and the appeared precipitate was collected by filtration to obtained the title compound which contained 82% free form. ^{1}H NMR ($CDCl_3$) δ: 7.14 (1H, dd, *J* = 2.4, 8.5 Hz), 6.88 (1H, d, *J* = 2.4 Hz), 6.70 (1H, d, J=8.5 Hz), 5.98 (2H, s), 4.81 (1H, d, *J* = 13.4 Hz), 4.27 - 4.20 (2H, m), 3.83 (3H, s), 3.77 - 3.73 (4H, m), 3.72 (3H, s), 3.63 (6H, s), 3.39 (1H, dd, *J* = 12.4, 15.2 Hz), 3.12 (1H, dd, J=3.4, 13.2 Hz), 2.94 (1H, dd, *J* = 4.7, 15.2 Hz), 2.54 - 2.38 (2H, m), 1.89 - 1.82 (4H, m); MS (ESI): 461 $(M+H)^+$

5.1.10. 2-Chlorophenyl 6-(5-chloro-2-methoxybenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-carboxylate (*rac*-13)

To the compound ***rac*-12** (4.07 g) in tetrahydrofuran (200 ml) solution, a 1.59M hexane solution of n-butyllithium (6 ml) was added at -78°C and the mixture was stirred at that temperature for 20 minutes. Next, 2-chlorophenyl chlorocarbonate (1.4 ml) was added to the reaction solution at -78°C and the mixture was stirred at that temperature for 20 minutes. The reaction solution was diluted with saturated potassium hydrogensulfate aqueous solution and distilled water, and the mixture was extracted with ethyl acetate. The organic layer was successively washed with saturated saline and distilled water, dried over with anhydrous sodium sulfate, then concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate/methanol = 1/2/0 to 3/3/1) to obtain the title compound (4.21 g). ^{1}H NMR ($CDCl_3$) δ: 7.44-7.40 (1H, m), 7.33-7.12 (4H, m), 6.93 (1H, d, *J* = 2.6 Hz), 6.73 (1H, d, *J* = 8.8 Hz), 6.05 (2H, s), 5.06 (1H, d, *J* = 17.6 Hz), 4.83 (1H, d, *J* = 13.7 Hz), 4.44 (1H, d, *J* = 17.6 Hz), 4.34 (1H, d, *J* = 13.7 Hz), 3.83 (3H, s), 3.77 (3H, s), 3.7 (6H, s), 3.57-3.45 (1H, m), 3.29-3.14 (2H, m), 3.07 (1H, dd, *J* = 14.1, 3.8 Hz), 2.38 (1H, dd, *J* = 14.1, 9.8 Hz); MS (ESI): 617 $(M+H)^+$

5.1.11. (6*R*)- 2-Chlorophenyl 6-(5-chloro-2-methoxybenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-carboxylate ((*R*)-13)

To the compound **(*R*)-12** (8.2g: net weight of free form) in dimethoxyethane (200 ml) solution cooled at -20°C, n-BuLi (12.2 ml)(1.60M hexane solution) was added , and the mixture was stirred at that temperature for a few minutes. Then, 2-chlorophenyl chloroformate (2.7 ml) was added to the mixture at -20°C, and the resultant mixture was subsequently stirred at that temperature for 1 hour. Then, saturated ammonium chloride aqueous solution was added to the reaction solution, the resultant solution was extracted with ethyl acetate. Then, the organic layer was successively washed with saturated saline, dried over with

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anhydrous sodium sulfate, then concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1/2, then ethyl acetate/methanol = 10/1) to obtain the title compound (1.29 g). ^{1}H NMR ($CDCl_3$) δ: 7.46 - 7.42 (1H, m), 7.32 - 7.25 (2H, m), 7.25 - 7.17 (2H, m), 6.95 (1H, d, *J* = 2.4 Hz), 6.76 (1H, d, *J* = 8.9 Hz), 6.07 (2H, s), 5.08 (1H, d, *J* = 17.9 Hz), 4.84 (1H, d, *J* = 13.8 Hz), 4.46 (1H, d, *J* = 17.0 Hz), 4.36 (1H, d, *J* = 13.8 Hz), 3.85 (3H, s), 3.79 (3H, d, *J* = 5.3 Hz), 3.72 (6H, s), 3.55 - 3.46 (1H, m), 3.28 - 3.16 (2H, m), 3.12 - 3.05 (1H, m, *J* = 3.7 Hz), 2.40 (1H, dd, *J* = 9.7, 13.8 Hz)

5.1.12. (6*R*)- 2-Chlorophenyl 6-(5-chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-carboxylate ((*R*)-14)

Anisole (2.3 ml) and trifluoroacetic acid (10 ml) were added to the compound **(*R*)-13** (1.29 g), and the mixture was stirred at room temperature for 2 hours. Then, the reaction mixture was concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl = 1/1 to 1/99). The obtained solid was suspended with diethyl ether, and subsequently collected by filtration to obtaine the title compound (595 mg). ^{1}H NMR ($CDCl_3$) δ: 7.91 (1H, d, *J* = 4.5 Hz), 7.63 - 7.60 (1H, m), 7.45 - 7.41 (2H, m), 7.39 - 7.32 (2H, m), 7.28 (1H, dd, *J* = 2.6, 8.7 Hz), 7.03 (1H, d, *J* = 8.9 Hz), 4.83 (1H, d, *J* = 17.5 Hz), 4.50 (1H, d, J = 17.5 Hz), 3.89 - 3.83 (1H, m), 3.81 (3H, s), 3.30 - 3.22 (1H, m), 3.13 - 2.96 (2H, m), 2.63 (1H, dd, J = 8.9, 14.2 Hz); MS (ESI): 438 $(M+H)^+$

5.1.13. 6-[(1*R*)-1-({[(6*R*)-6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)propyl]nicotinic acid (16A)

To the compound **(*R*)-13** (509m g) in *N*,*N*-dimethylformamide (2.5 ml) solution, the compound **25a** (319 mg) , triethylamine (0.23 ml) and 4-dimethylaminopyridine (101 mg) were added under ice cooling and the mixture was stirred at that temperature for 16 hours. Then, saturated ammonium chloride aqueous solution and ethylacetate were added to the reaction mixture, the organic layer and the aqueous layer were separated. Then, the organic layer was successively washed with saturated saline, dried over with anhydrous sodium sulfate, then concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1/3) to obtain the compound **15A** (0.58 g). To the compound **15A** (0.57 g) in dichloromethane (11 ml) solution, water (1.1 ml) and DDQ (0.36 g) were added , and the mixture was stirred at room temperature for 20 hours. Then, the mixture was filtrated through NH-silica gel (ethyl acetate), and the filtrate was concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl =1/2 to 0/1). To the obtained compound (221mg), 1M HCl/AcOH solution (2.2ml) was added, and the mixture was stirred at room temperature for 4 hours. Then, the mixture was concentrated, and acetone and ethyl acetate were added to the residue. The appeared precipitate was collected by filtration to obtain the title compound (108 mg). ^{1}H NMR (DMSO-*d*6) δ: 9.75(1H, d, *J* = 7.5 Hz), 9.04 (1H, d, *J* = 2.1Hz), 8.25 (1H, dd, *J* = 8.1, 2.1 Hz), 7.70 (1H, brd, *J* = 3.9 Hz), 7.53 (1H, d, *J* = 8.1 Hz), 7.34 (1H, d, *J* = 2.7 Hz), 7.27 (1H, dd, *J* = 8.7, 2.7 Hz), 7.01 (1H, d, *J* = 8.7 Hz), 4.94 (1H, dd, *J* = 14.1, 7.0 Hz), 4.77 (1H, d, *J* = 17.3 Hz), 4.51 (1H, d, *J* = 17.3 Hz), 3.94-3.82 (1H, m), 3.80 (3H, s), 3.17 (1H, t, *J* = 12.5 Hz), 3.08-2.98 (2H, m), 2.72-2.64 (1H, m), 1.92-1.80 (2H, m), 0.80 (3H, t, *J* = 7.4 Hz); MS (ESI): 489 $(M+H)^+$

5.1.14. 2-[(1*R*)-1-({[(6*R*)-6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)propyl]isonicotinic acid (16B)

Instead of the starting material compound of 5.1.13, that is, the compound **25a**, the compound **25b** was used for the similar procedure as in 5.1.13 to obtain the title compound. Yield: 34.3%; ^{1}H NMR (DMSO-*d*6) δ: 9.75 (1H, d, *J* = 7.6 Hz), 8.75 (1H, d, *J* = 4.9 Hz), 7.81 (1H, brs), 7.75-7.68 (2H, m), 7.34 (1H, d, *J* = 2.7 Hz), 7.27 (1H, dd, *J* = 8.8, 2.7 Hz), 7.01 (1H, d, *J* = 8.8 Hz), 4.96 (1H, dd, *J* = 14.1, 7.0 Hz), 4.77 (1H, d, *J* = 17.2 Hz), 4.50 (1H, d, *J* = 17.2 Hz), 3.94-3.80 (1H, m), 3.79 (3H, s), 3.17 (1H, t, *J* = 13.0 Hz), 3.07-2.97 (2H, m), 2.72-2.64 (1H, m), 1.92-1.80 (2H, m), 0.80 (3H, t, *J* = 7.4 Hz); MS (ESI): 489 $(M+H)^+$

5.1.15. 5-[(1*R*)-1-({[(6*R*)-6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)propyl]nicotinic acid (16C)

To the compound **(*R*)-13** (500 mg) in *N*,*N*-dimethylformamide (2.5 ml) solution, the compound **25c** (313 mg) , triethylamine (113 μl) and 4-dimethylaminopyridine (99 mg) were added under ice cooling and the mixture was stirred at that temperature for 20 hours. Then, distilled water and ethyl acetate were added to the reaction mixture, the organic layer and the aqueous layer were separated. Then, the organic layer was successively washed with saturated saline, dried over with anhydrous sodium sulfate, then concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1/1 to 1/2) to obtain the compound **15C** (518 mg). To the compound **15C** (516 mg), 1M hydrogen chloride/acetic acid solution (5 ml) was added , the mixture was stirred at room temperature for 14 hours. Then, the mixture was concentrated, the residue was purified by silica gel column chromatography (ethyl acetate/acetic acid/methanol = 20/1, then ethyl acetate/acetic acid/methanol = 20/1/2) to obtain the title compound (118 mg). ^{1}H NMR (DMSO-*d*6) δ: 9.48 (1H, d, *J* = 7.1 Hz), 8.93 (1H, d, *J* = 1.8 Hz), 8.71 (1H, brs), 8.17 (1H, s), 7.67 (1H, brd, *J* = 3.8 Hz), 7.34 (1H, d, *J* = 2.7 Hz), 7.27 (1H, dd, *J* = 8.7, 2.7 Hz), 7.01 (1H, d, *J* = 8.7 Hz), 4.79 (1H, dd, *J* = 14.2, 7.0 Hz), 4.69 (1H, d, *J* = 17.1 Hz), 4.49 (1H, d, *J* = 17.1 Hz), 3.92-3.80 (1H, m), 3.79 (3H, s), 3.17 (1H, t, *J* = 12.9 Hz), 3.07-2.97 (2H, m), 2.78-2.65 (1H, m), 1.95-1.78 (2H, m), 0.87 (3H, t, *J* = 7.3 Hz); MS (ESI): 489 $(M+H)^+$; Melting point: 138-140°C

5.1.16. 5-[(1*R*)-1-({[(6*R*)-6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)propyl]-2-furan carboxylic acid (16D)

To the compound ***rac*-13** (2.8 g) in *N*,*N*-dimethylformamide (14 ml) solution, the compound **31** (1.7 g) , triethylamine (0.63 ml) and 4-dimethylaminopyridine (0.55 g) were added under ice cooling and the mixture was stirred at that temperature for 16 hours. Then, distilled water and ethyl acetate were added to the reaction mixture, the organic layer and the aqueous layer were separated. Then, the organic layer was successively washed with saturated saline, dried over with anhydrous sodium sulfate, then concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate =1/2) to obtain the compound **15D** (1.02 g). To the compound **15D** (1.02 g), 1M hydrogen chloride/acetic acid solution (10 ml) was added , the mixture was stirred at room temperature for 14 hours. Then, the mixture was concentrated, the residue was purified by silica gel column chromatography (chloroform/ethyl acetate/methanol/acetic acid = 4/4/1/0.1) to obtain the title compound (284 mg). ^{1}H NMR (DMSO-*d*6) δ: 9.35 (1H, d, *J* = 7.9 Hz), 7.69-7.60 (1H, br), 7.30 (1H, s), 7.24 (1H, d, *J* = 8.7 Hz), 7.11 (1H, d, *J* = 3.4 Hz), 6.97 (1H, d, *J* = 8.7 Hz), 6.46 (1H, d, *J* = 3.4 Hz), 4.85 (1H, dd, *J* = 14.4, 7.1 Hz), 4.74 (1H, d, *J* = 17.3 Hz), 4.50 (1H, d, *J* = 17.3 Hz), 3.89-3.78 (1H, m), 3.76 (3H, s), 3.17-2.88 (3H, m), 2.65-2.57 (1H, m), 1.89-1.77 (2H, m), 0.84 (3H, t, *J* = 7.3 Hz); MS (ESI): 478 $(M+H)^+$

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5.1.17. 5-[(1*R*)-1-({[(6*R*)-6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)propyl]-3-furan carboxylic acid (16E)

Instead of starting material compound of 5.1.13, that is, the compound **25a**, the compound **36a** was used for the similar procedure as in 5.1.13. to obtain the title compound. Yield: 40.6%; ^{1}H NMR (DMSO-*d*6) *δ*: 12.71-12.59 (1H, br), 9.36 (1H, d, *J* = 8.1 Hz), 8.20 (1H, brs), 7.69 (1H, brd, *J* = 3.9 Hz), 7.33 (1H, d, *J* = 2.6 Hz), 7.27 (1H, dd, *J* = 8.8, 2.6 Hz), 7.01 (1H, d, *J* = 8.8 Hz), 6.54 (1H, s), 4.85 (1H, dd, *J* = 14.7, 7.1 Hz), 4.78 (1H, d, *J* = 17.1 Hz), 4.52 (1H, d, *J* = 17.1 Hz), 3.91-3.81 (1H, m), 3.79 (3H, s) 3.15 (1H, t, *J* = 12.5 Hz), 3.07-2.92 (2H, m), 2.68-2.57 (1H, m), 1.89-1.75 (2H, m), 0.86 (3H, t, *J* = 7.3 Hz); MS (ESI): 478 $(M+H)^+$

5.1.18. 5-[(1*R*)-1-({[(6*R*)-6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)propyl]-3-thiophencarboxylic acid (16F)

Instead of starting material compound of 5.1.13, that is, the compound **25a**, the compound **36b** was used for the similar procedure as in 5.1.13. to obtain the title compound. Yield: 44.3%; ^{1}H NMR (DMSO-*d*6) *δ*: 12.80-12.65 (1H, br), 9.42 (1H, d, *J* = 7.8 Hz), 8.09 (1H, brs), 7.69 (1H, brd, *J* = 3.5 Hz), 7.33 (1H, d, *J* = 2.6 Hz), 7.30-7.24 (2H, m), 7.01 (1H, d, *J* = 8.8 Hz), 4.97 (1H, dd, *J* = 14.3, 7.1 Hz), 4.77 (1H, d, *J* = 17.1 Hz), 4.53 (1H, d, *J* = 17.1 Hz), 3.93-3.84 (1H, m), 3.79 (3H, s) 3.15 (1H, t, *J* = 12.5 Hz), 3.07-2.94 (2H, m), 2.68-2.58 (1H, m), 1.94-1.85 (2H, m), 0.89 (3H, t, *J* = 7.3 Hz); MS (ESI): 494 $(M+H)^+$

5.1.19. 2-[(1*R*)-1-({[(6*R*)-6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)propyl]-4-oxazolcarboxylic acid (16G)

To the compound **14** (173 mg) in *N*,*N*-dimethylformamide (0.4 ml) solution cooled at 0°C, the compound **42** (134 mg) , *N*, *N*-diisopropylethylamine (69 μl) and 4-dimethylaminopyridine (37 mg) were added under ice cooling and the mixture was stirred at that temperature for 15 hours. Then, distilled water was added to the reaction solution, the resultant mixture was extracted with ethyl acetate. The organic layer was successively washed with saturated potassium hydrogensulfate aqueous solution, saturated sodium hydrogencarbonate aqueous solution and saturated saline, and dried over with anhydrous sodium sulfate, then concentrated. The residue was purified by silica gel column chromatography (chloroform/ethyl acetate = 1/1) to obtain the compound **15G** (188 mg). To the compound **15G** (97 mg) in acetic acid (2 ml) solution, zinc (200 mg) was added , and the mixture was stirred at room temperature for 3 hours. Then, insoluble compound was removed by filtration, and the filtrate was subsequently concentrated. The residue was purified by preparative TLC (chloroform/ethyl acetate/methanol/acetic acid = 5/5/1/0.1) to obtain the title compound (7 mg). ^{1}H NMR ($CDCl_3$) *δ*: 9.53 (1 H, d, *J* = 6.9 Hz), 7.69 (1H, s), 7.34 (1H, s), 7.27 (1H, d, *J* = 8.5 Hz), 7.01 (1H, d, *J* = 8.5 Hz), 4.99 - 4.92 (1H, m), 4.76 (1H, d, *J* = 15.8 Hz), 4.56 (1H, d, *J* = 17.0 Hz), 3.92 - 3.84 (1H, m), 3.79 (4H, s), 3.50 - 3.36 (1H, m), 3.25 - 3.10 (1H, m), 3.04 - 2.92 (2H, m), 2.69 - 2.60 (1H, m), 1.99 - 1.80 (2H, m), 0.90 - 0.81 (3H, m); MS (ESI): 479 $(M+H)^+$

5.1.20. 2-[(1*R*)-1-({[(6*R*)-6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)propyl]-4-thiazolcarboxylic acid (16H)

To the compound **(*R*)-13** (163 mg) in *N*,*N*-dimethylformamide (5 ml) solution cooled at 0°C, the compound **48** (93 mg) ,triethylamine (37 μl) and 4-dimethylaminopyridine (32 mg) were added under ice cooling and the mixture was stirred at that temperature for 3 days. Then, distilled water was added to the reaction solution, the resultant mixture was extracted with ethyl acetate. The organic layer was successively washed with saturated potassium hydrogensulfate aqueous solution, saturated sodium hydrogencarbonate aqueous solution and saturated saline, and dried over with anhydrous sodium sulfate, then concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1/1 to 0/100) to obtain the compound **15H** (46 mg). 1M hydrogen chloride acetic acid solution (2 ml) and anisole (89 ml) were added to the compound **15H**, the mixture was stirred at room temperature for 40 hours. Then, the mixture was concentrated, the residue was purified by silica gel column chromatography (chloroform/ethyl acetate/methanol = 15/15/1). To the obtained compound (42 mg) in acetic acid (0.8 ml) solution, zinc (80 mg) was added, and the mixture was stirred at room temperature for 25 hours. Then, insoluble compound was removed by filtration, and the filtrate was subsequently concentrated. The residue was purified by preparative TLC (chloroform/ethyl acetate/methanol = 10/10/1) to obtain the title compound (14 mg). ^{1}H NMR ($CDCl_3$) *δ*: 9.62 - 9.53 (1H, m), 7.71 (1H, brs), 7.35 (1H, brs), 7.28 (1H, dd, *J* = 2.4, 8.5 Hz), 7.01 (1H, d, *J* = 8.5 Hz), 5.19 - 5.04 (1H, m), 4.80 - 4.67 (1H, m), 4.67 - 4.50 (1H, m), 3.94 - 3.84 (1H, m), 3.80 (3H, s), 3.48 - 3.37 (1H, m), 3.23 - 3.15 (1H, m), 3.03 - 2.95 (2H, m), 2.74 - 2.62 (1H, m), 2.08 - 1.91 (2H, m), 0.94 - 0.89 (3H, m); MS (ESI): 495 $(M+H)^+$

5.1.21. (2*R*)-2-({[6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)butanoic acid (*rac*-17)

To the compound ***rac*-13** (3 g) in *N*,*N*-dimethylformamide (75 ml) solution, (*R*)-tert-butyl 2-aminobutanoate acetate (0.70 g) and triethylamine (0.45 ml) were added under ice cooling and the mixture was stirred at that temperature for 3 hours. Then, distilled water was added to the reaction mixture, and the resultant mixture was extracted with a mixed solution of Hexane/EtOAc (1/1). Then, the organic layer was successively washed with saturated saline, dried over with anhydrous sodium sulfate, then concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1/1 to 1/99) to obtain tert-Butyl (2*R*)-2-({[6-(5-chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)butanoate (1.20 g). To the compound (1.20 g) in CH_2Cl_2 cooled at 0°C, TFA (15 ml) was added, and the reaction mixture was stirred at room temperature for 7.5 hours. Then, the mixture was concentrated, and the residue was purified with Florisil column to give a reddish brown oil. The oil was diluted with ethyl acetate, and the solution was washed with distilled water and saturated sodium hydrogencarbonate aqueous solution. The sodium hydrogencarbonate aqueous layer was acidified with HCl aq., and the resultant solution was extracted with ethyl acetate. The organic layer was washed with saturated saline, dried over with anhydrous sodium sulfate, then concentrated to obtain the title compound (638 mg). ^{1}H NMR ($CDCl_3$) *δ*: 9.45 (0.5H, d, *J* = 6.7 Hz), 9.44 (0.5H, d, *J* = 6.5 Hz), 7.24-7.20 (1H, m), 7.19-7.10 (0.5H, m), 7.15 (0.5H, d, *J* = 2.4 Hz), 7.11 (0.5H, d, *J* = 2.5 Hz), 6.95 (0.5H, br), 6.817 (0.5H, d, *J* = 8.8 Hz), 6.813 (0.5H, d, *J* = 8.7 Hz), 5.38-5.29 (1H, m), 4.48-4.41 (1H, m), 4.23-4.15 (1H, m), 3.84 (3H, s), 3.78-3.65 (1H, m), 3.40-3.33 (2H, m), 3.27-3.15 (1H, m), 2.69-2.58 (1H, m), 2.05-1.80 (2H, m), 1.09-1.00 (3H, m)); A NMR spectrum of the compound ***rac*-17** was assigned as mixture of two diastereomers. If each signal of correspond protons was separated, the number of proton was assigned as 0.5. NMR spectra of following diastereomers was assigned in the same manner.; MS (ESI): 411 $(M+H)^+$

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5.1.22. (2*R*)-2-({[(6*R*)-6-(5-chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)butanoic acid ((*R*)-17)

To the compound **(*R*)-14** (60 mg) in *N*,*N*-dimethylformamide (0.14 ml) solution cooled at 0°C, (*R*)-tert-butyl 2-aminobutanoate acetate (27 mg), 4-dimethylaminopyridine (17 mg) and *N*, *N*-diisopropylethylamine (0.024 ml) were added under ice cooling and the mixture was stirred at that temperature for 15 hours. Then, distilled water was added to the reaction mixture, and the resultant mixture was extracted with ethyl acetate. Then, the organic layer was successively washed with saturated potassium hydrogensulfate aqueous solution, saturated sodium hydrogencarbonate aqueous solution and saturated saline, dried over with anhydrous sodium sulfate, then concentrated. The residue was purified by silica gel column chromatography (chloroform/ethyl acetate = 1/1) to obtain tert –Buthyl (2*R*)-2-({[(6*R*)-6-(5-chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)butanate (51 mg). 4M hydrogen chloride ethyl acetate solution (2 ml) was added to the compound (50 mg), and the mixture was stirred at room temperature for 4 hours. Then, the mixture was concentrated. The residue was purified by silica gel column chromatography (chloroform/ethyl acetate/methanol/acetic acid = 5/5/1/0.1) to obtain the title compound (21 mg). ^{1}H NMR (DMSO-*d*6) δ: 9.39 (2H, d, *J* = 6.5 Hz), 7.69 (1H, br), 7.34 (2H, br), 7.27 (2H, d, *J* = 9.3 Hz), 7.01 (2H, d, *J* = 8.9 Hz), 4.81 (1H, d, *J* = 17.0 Hz), 4.54 (1H, d, *J* = 17.5 Hz), 4.24 - 4.18 (1H, m), 3.89 (1H, br), 3.80 (3H, s), 3.14 (1H, br), 3.09 - 2.93 (2H, m), 2.70 - 2.58 (1H, m), 1.88 - 1.64 (2H, m), 0.86 (3H, t, *J* = 7.1 Hz); MS (ESI): 412 $(M+H)^+$

5.1.23. 6-(5-Chloro-2-methoxybenzyl)-*N*-{(1*R*)-1-[(methylamino)carbonyl]propyl}-3,7-dioxo-1,4-diazepan-1-carboxamide (18A)

To the compound ***rac*-17** (54 mg) in methylene chloride (3 ml) solution, methylamine (30% ethyl acetate solution) (30 μl), triethylamine (0.27 ml), and n-propyl phosphoric acid anhydride (25% ethyl acetate solution) (0.39 ml) were added and the mixture was stirred at room temperature for 4 hours. The reaction solution was concentrated, then ethyl acetate was added to the residue. The obtained solution was successively washed with distilled water, saturated potassium hydrogensulfate aqueous solution, saturated saline, saturated sodium hydrogencarbonate aqueous solution, and saturated saline, dried over with anhydrous sodium sulfate, then concentrated. The precipitate was washed with diethylether/hexane and collected by filtration to obtain the title compound (34 mg). ^{1}H NMR ($CDCl_3$) δ: 9.40 (0.5H, d, *J* = 7.2 Hz), 9.35 (0.5H, d, *J* = 7.2 Hz), 7.24-7.20 (1H, m), 7.14 (0.5H, d, *J* = 2.6 Hz), 7.11 (0.5H, d, *J* = 2.5 Hz), 6.81 (1H, d, *J* = 8.8 Hz), 6.06 (1H, br), 5.77 (1H, br), 5.35 (0.5H, d, *J* = 17.7 Hz), 5.32 (0.5H, d, *J* = 17.7 Hz), 4.28-4.18 (1H, m), 4.14 (1H, d, *J* = 17.7 Hz), 3.83 (3H, s), 3.76-3.67 (1H, m), 3.38-3.30 (2H, m), 3.25-3.18 (1H, m), 2.83 (1.5H, s), 2.82 (1.5H, s), 2.66-2.57 (1H, m), 2.00-1.90 (1H, m), 1.83-1.73 (1H, m), 1.03-0.94 (3H, m); MS (ESI): 425 $(M+H)^+$

5.1.24. {[2-({[6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)butanoyl]amino}acetic acid (18B)

To the compound ***rac*-17** (50 mg) in methylene chloride (3 ml) solution, glycine tert-butyl ester hydrochloride (41 mg), triethylamine (0.29 ml), and n-propyl phosphoric acid anhydride (25% ethyl acetate solution) (0.37 ml) were added and the mixture was stirred at room temperature for 3 hours. The reaction solution was concentrated, then ethyl acetate was added to the residue. The obtained solution was successively washed with distilled water, saturated potassium hydrogensulfate aqueous solution, saturated saline, saturated sodium hydrogencarbonate aqueous solution, and saturated saline, dried over with anhydrous sodium sulfate, then concentrated. The precipitate was washed with diethylether/hexane and collected by filtration to obtain {[2-({[6-(5-chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)butanoyl]amino}acetic acid tert-butyl ester (41.6 mg). A mixed solution of the compound (33 mg) and 1N hydrogen chloride/acetic acid solution (2 ml) was stirred at room temperature for 5 hours. The reaction solution was concentrated, then the residue was diluted with ethyl acetate. The insoluble compound was filtered out, then the filtrate was concentrated. The residue was recrystallized by hexane/ethyl acetate to obtain the title compound (18 mg). ^{1}H NMR ($CDCl_3$) δ: 9.42 (0.5H, *J* = d, 7.4 Hz), 9.39 (0.5H, d, *J* = 6.9 Hz), 7.25-7.05 (3.5H, m), 6.80 (1H, d, *J* = 8.9 Hz), 6.36 (0.5H, br), 5.28-5.16 (1H, m), 4.41-3.90 (5H, m), 3.83 (3H, s), 3.78-3.68 (1H, m), 3.38-3.30 (2H, m), 3.22-3.14 (1H, m), 3.67-3.55 (1H, m), 2.00-1.90 (1H, m), 1.85-1.74 (1H, m), 1.05-0.99 (3H, m); MS (ESI): 469 $(M+H)^+$

5.1.25. (2*S*)-2-{[(2*R*)-2-({[6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)butanoyl]amino}propanoic acid (18C)

Instead of the starting material compound of 5.1.16., that is, the glycine tert-butyl ester hydrochloride, L-alanine tert-butyl ester hydrochloride was used for the similar procedure as in 5.1.16. to obtain the title compound. Yield: 80.6%; ^{1}H NMR (DMSO-*d*6) δ: 12.79-12.19 (1H, br), 9.40 (0.5H, d, *J* = 7.5 Hz), 9.35 (0.5H, d, *J* = 7.7 Hz), 8.45 (0.5H, d, *J* = 8.0 Hz), 8.43 (0.5H, d, *J* = 8.0 Hz), 7.64 (1H, br), 7.307 (0.5H, s), 7.301 (0.5H, s), 7.24 (0.5H, d, *J* = 8.8 Hz), 7.23 (0.5H, d, *J* = 8.8 Hz), 6.97 (1H, d, *J* = 8.8 Hz), 4.78 (0.5H, d, *J* = 17.0 Hz), 4.76 (0.5H, d, *J* = 17.0 Hz), 4.48 (1H, d, *J* = 17.0 Hz), 4.35-4.28 (1H, m), 4.25-4.15 (1H, m), 3.89-3.80 (1H, m), 3.76 (3H, s), 3.15-3.08 (1H, m), 3.03-2.91 (2H, m), 2.65-2.58 (1H, m), 1.72-1.57 (2H, m), 1.23 (3H, d, *J* = 7.3 Hz), 0.82-0.75 (3H, m); MS (ESI): 483 $(M+H)^+$

5.1.26. (2*R*)-2-{[(2*R*)-2-({[6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)butanoyl]amino}propanoic acid (18D)

Instead of the starting material compound of 5.1.24., that is, the glycine tert-butyl ester hydrochloride, D-alanine tert-butyl ester hydrochloride was used for the similar procedure as in 5.1.24. to obtain the title compound. Yield: 77.5%; ^{1}H NMR (DMSO-*d*6) δ: 12.75-12.21 (1H, br), 9.37 (0.5H, d, *J* = 7.4 Hz), 9.33 (0.5H, d, *J* = 7.7 Hz), 8.41 (0.5H, d, *J* = 7.6 Hz), 8.39 (0.5H, d, *J* = 8.0 Hz), 7.64 (1H, br), 7.30 (0.5H, s), 7.29 (0.5H, s), 7.24 (0.5H, d, *J* = 8.8 Hz), 7.23 (0.5H, d, *J* = 8.8 Hz), 6.97 (1H, d, *J* = 8.8 Hz), 4.77 (0.5H, d, *J* = 17.1 Hz), 4.76 (0.5H, d, *J* = 17.1 Hz), 4.48 (1H, d, *J* = 17.1 Hz), 4.35-4.26 (1H, m), 4.23-4.13 (1H, m), 3.89-3.80 (1H, m), 3.76 (3H, s), 3.16-3.05 (1H, m), 3.04-2.91 (2H, m), 2.67-2.57 (1H, m), 1.75-1.55 (2H, m), 1.25 (1.5H, d, *J* = 7.4 Hz), 1.24 (1.5H, d, *J* = 7.3 Hz), 0.88-0.81 (3H, m); MS (ESI): 483 $(M+H)^+$

5.1.27. *N*-[(1*R*)-1-(Anilinocarbonyl)propyl]-6-(5-chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-carboxamide (18E)

Instead of the starting material compound of 5.1.23., that is, methylamine, aniline was used for the similar procedure as in 5.1.23. to obtain the title compound. Yield: 41.5%; ^{1}H NMR ($CDCl_3$) δ: 9.51 (0.5H, d, *J* = 7.3 Hz), 9.43 (0.5H, d, *J* = 6.9 Hz), 8.20 (0.5H, s), 8.15 (0.5H, s), 7.53 (2H, d, *J* = 8.0 Hz), 7.34-7.29 (2H, m), 7.21 (1H, dd, *J* = 8.7, 2.6 Hz), 7.15-7.00 (2H, m), 6.81 (0.5H, d, *J* = 8.7 Hz), 6.80 (0.5H, d, *J* = 8.7 Hz), 5.85-5.78 (1H, m), 5.36 (0.5H, d, *J* = 17.4 Hz), 5.33 (0.5H, d, *J* = 17.4 Hz), 4.41-

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4.33 (1H, m), 4.16 (0.5H, d, *J* = 17.4 Hz), 4.15 (0.5H, d, *J* = 17.4 Hz), 3.84 (1.5H, s), 3.83 (1.5H, s), 3.77-3.65 (1H, m), 3.37-3.30 (2H, m), 3.25-3.16 (1H, m), 2.65-2.55 (1H, m), 2.10-1.99 (1H, m), 1.91-1.79 (1H, m), 1.10-1.00 (3H, m); MS (ESI): 487 $(M+H)^+$

5.1.28. *N*-{(1*R*)-1-[(*tert*-Butoxyamino)carbonyl]propyl}-6-(5-chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-carboxamide (18F)

Instead of the starting material compound of 5.1.23., that is, methylamine, 2-aminopyridine was used for the similar procedure as in 5.1.23. to obtain the title compound. Yield:33.2%; ^{1}H NMR ($CDCl_3$) δ: 9.34 (0.5H, d, *J* = 7.4 Hz), 9.28 (0.5H, d, *J* = 7.4 Hz), 8.42 (1H, s), 7.19 (1H, dd, *J* = 8.8, 2.5 Hz), 7.12 (0.5H, d, *J* = 2.5 Hz), 7.09 (0.5H, d, *J* = 2.5 Hz), 6.794 (0.5H, d, *J* = 8.8 Hz), 6.790 (0.5H, d, *J* = 8.8 Hz), 5.69 (1H, br), 5.31 (0.5H, d, *J* = 17.6 Hz), 5.28 (0.5H, d, *J* = 17.6 Hz), 4.15-4.01 (1H, m), 4.12 (1H, d, *J* = 17.6 Hz), 3.82 (1.5H, s), 3.81 (1.5H, s), 3.74-3.62 (1H, m), 3.55-3.25 (2H, m), 3.23-3.12 (1H, m), 2.63-2.53 (1H, m), 2.02-1.91 (1H, m), 1.83-1.72 (1H, m), 1.25 (9H, s), 1.01-0.95 (3H, m); MS (ESI): 483 $(M+H)^+$

5.1.29. 6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-*N*-{(1*R*)-1-[(3-pyridylamino)carbonyl]propyl}-1,4-diazepan-1-carboxamide (18G)

Instead of the starting material compound of 5.1.23., that is, methylamine, 3-aminopyridine was used for the similar procedure as in 5.1.23. to obtain the title compound. Yield: 41.5%; ^{1}H NMR ($CDCl_3$) δ: 9.51 (0.5H, d, *J* = 7.2 Hz), 9.42 (0.5H, d, *J* = 6.8 Hz), 8.63-8.58 (2H, m), 8.31 (0.5H, s), 8.30 (0.5H, s), 8.15-8.10 (1H, m), 7.24-7.18 (2H, m), 7.13 (0.5H, d, *J* = 2.6 Hz), 7.10 (0.5H, d, *J* = 2.6 Hz), 6.795 (0.5H, d, *J* = 8.8 Hz), 6.791 (0.5H, d, *J* = 8.7 Hz), 5.88 (1H, br), 5.32 (0.5H, d, *J* = 17.5 Hz), 5.29 (0.5H, d, *J* = 17.5 Hz), 4.44-4.32 (1H, m), 4.17 (1H, d, *J* = 17.5 Hz), 3.82 (1.5H, s), 3.81 (1.5H, s), 3.75-3.65 (1H, m), 3.35-3.26 (2H, m), 3.22-3.15 (1H, m), 2.65-2.55 (1H, m), 2.10-1.98 (1H, m), 1.90-1.77 (1H, m), 1.08-0.99 (3H, m); MS (ESI): 488 $(M+H)^+$

5.1.30. 6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-*N*-{(1*R*)-1-[(4-pyridylamino)carbonyl]propyl}-1,4-diazepan-1-carboxamide (18H)

Instead of the starting material compound of 5.1.23., that is, methylamine, 4-aminopyridine was used for the similar procedure as in 5.1.23. to obtain the title compound. Yield: 40.9%; ^{1}H NMR ($CDCl_3$) δ: 9.50 (0.5H, d, *J* = 7.1 Hz), 9.41 (0.5H, d, *J* = 6.8 Hz), 8.79 (0.5H, s), 8.76 (0.5H, s), 8.46 (2H, d, *J* = 6.0 Hz), 7.48 (1H, d, *J* = 6.0 Hz), 7.47 (1H, d, *J* = 6.0 Hz), 7.20 (1H, dd, *J* = 8.7, 2.5 Hz), 7.13 (0.5H, d, *J* = 2.5 Hz), 7.11 (0.5H, d, *J* = 2.5 Hz), 6.797 (0.5H, d, *J* = 8.7 Hz), 6.792 (0.5H, d, *J* = 8.7 Hz), 5.85 (1H, brd, *J* = 8.4 Hz), 5.32 (0.5H, d, *J* = 17.4 Hz), 5.29 (0.5H, d, *J* = 17.4 Hz), 4.39-4.30 (1H, m), 4.17 (1H, d, *J* = 17.4 Hz), 3.82 (1.5H, s), 3.81 (1.5H, s), 3.80-3.65 (1H, m), 3.35-3.30 (2H, m), 3.22-3.12 (1H, m), 2.66-2.56 (1H, m), 2.09-1.98 (1H, m), 1.89-1.79 (1H, m), 1.08-1.00 (3H, m); MS (ESI): 488 $(M+H)^+$

5.1.31. 2-{[(2*R*)-2-({[6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)butanoyl]amino}benzoic acid (18I)

Instead of the starting material compound of 5.1.24., that is, the glycine tert-butyl ester hydrochloride, 2-aminobenzoic acid tert-butyl ester was used for the similar procedure as in 5.1.24. to obtain the title compound. Yield:21.3%; ^{1}H NMR (DMSO-*d*6) δ: 13.78-13.22 (1H, br), 11.4 3(0.5H, s), 11.37 (0.5H, s), 9.53 (0.5H, d, *J* = 6.5 Hz), 9.46 (0.5H, d, *J* = 6.6 Hz), 8.46-8.40 (1H, m), 7.95 (1H, d, *J* = 7.5 Hz), 7.70-7.60 (1H, m), 7.57 (1H, t, *J* = 7.5 Hz), 7.35-7.31 (1H, m), 7.28-7.22 (1H, m), 7.15 (1H, t, *J* = 7.5 Hz), 6.99 (1H, d, *J* = 8.8 Hz), 4.77 (0.5H, d, *J* = 17.3 Hz), 4.73 (0.5H, d, *J* = 17.3 Hz), 4.53 (1H, d, *J* = 17.3 Hz), 4.31-4.25 (1H, m), 3.95-3.84 (1H, m), 3.77 (3H, s), 3.15-3.06 (1H, m), 3.05-2.91 (2H, m), 2.69-2.61 (1H, m), 1.94-1.72 (2H, m), 0.95-0.89 (3H, m); MS (ESI): 531 $(M+H)^+$

5.1.32. 3-{[(2*R*)-2-({[6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)butanoyl]amino}benzoic acid (18J)

Instead of the starting material compound of 5.1.24., that is, the glycine tert-butyl ester hydrochloride, 3-aminobenzoic acid tert-butyl ester was used for the similar procedure as in 5.1.24. to obtain the title compound. Yield: 77.3%; ^{1}H NMR (DMSO-*d*6) δ: 13.10-12.47 (1H, br), 10.35 (0.5H, s), 10.33 (0.5H, s), 9.48 (0.5H, d, *J* = 7.2 Hz), 9.43 (0.5H, d, *J* = 7.3 Hz), 8.22-8.20 (1H, br), 7.78 (1H, d, 7.9), 7.65 (1H, s), 7.61 (1H, d, *J* = 7.9 Hz), 7.41 (1H, t, *J* = 7.9 Hz), 7.31 (1H, s), 7.24 (0.5H, d, 8.8Hz), 7.23 (0.5H, d, *J* = 8.8 Hz), 6.98 (1H, d, *J* = 8.8 Hz), 4.78 (0.5H, d, *J* = 17.2 Hz), 4.77 (0.5H, d, *J* = 17.2 Hz), 4.51 (1H, d, *J* = 17.2 Hz), 4.49-4.39 (1H, m), 3.90-3.81 (1H, m), 3.77 (3H, s), 3.18-3.07 (1H, m), 3.05-2.91 (2H, m), 2.69-2.60 (1H, m), 1.89-1.67 (2H, m), 0.92-0.80 (3H, m); MS (ESI): 531 $(M+H)^+$

5.1.33. 4-{[(2*R*)-2-({[6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)butanoyl]amino}benzoic acid (18K)

Instead of the starting material compound of 5.1.24., that is, the glycine tert-butyl ester hydrochloride, 4-aminobenzoic acid tert-butyl ester was used for the similar procedure as in 5.1.24. to obtain the title compound. Yield: 50.8%; ^{1}H NMR (DMSO-*d*6) δ: 12.88-12.35 (1H, br), 10.47 (0.5H, s), 10.45 (0.5H, s), 9.47 (0.5H, d, *J* = 7.2 Hz), 9.43 (0.5H, d, *J* = 7.3 Hz), 7.87 (2H, d, *J* = 8.6 Hz), 7.68 (2H, d, *J* = 8.6 Hz), 7.67 (1H, br), 7.31 (1H, s), 7.248 (0.5H, d, *J* = 8.8 Hz), 7.242 (0.5H, d, *J* = 8.8 Hz), 6.98 (1H, d, *J* = 8.8 Hz), 4.77 (1H, d, *J* = 17.1 Hz), 4.51 (1H, d, *J* = 17.1 Hz), 4.48-4.40 (1H, m), 3.92-3.81 (1H, m), 3.77 (3H, s), 3.18-3.08 (1H, m), 3.05-2.92 (2H, m), 2.65-2.59 (1H, m), 1.85-1.65 (2H, m), 0.90-0.85 (3H, m); MS (ESI): 531 $(M+H)^+$

5.1.34. *N*-{(1*R*)-1-[(3-Aminoanilino)carbonyl]propyl}-6-(5-chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-carboxamide (18L)

Instead of the starting material compound of 5.1.24., that is, the glycine tert-butyl ester hydrochloride, 1-tert-butoxycarbonyl-1,3-phenylenediamine was used for the similar procedure as in 5.1.24. to obtain the title compound. Yield: 58.8%; ^{1}H NMR (DMSO-*d*6) δ: 9.83 (0.5H, s), 9.81 (0.5H, s), 9.45 (0.5H, d, *J* = 7.4 Hz), 9.41 (0.5H, d, *J* = 7.4 Hz), 7.65 (1H, d, *J* = 2.9 Hz), 7.316 (0.5H, s), 7.311 (0.5H, s), 7.24 (0.5H, d, *J* = 8.8 Hz), 7.23 (0.5H, d, *J* = 8.8 Hz), 6.98 (1H, d, *J* = 8.8 Hz), 6.91-6.87 (2H, m), 6.64 (1H, d, *J* = 7.8 Hz), 6.23 (1H, d, *J* = 7.8 Hz), 5.02 (2H, s), 4.78 (0.5H, d, *J* = 17.1 Hz), 4.77 (0.5H, d, *J* = 17.1 Hz), 4.50 (1H, d, *J* = 17.1 Hz), 4.45-4.37 (1H, m), 3.92-3.81 (1H, m), 3.77 (3H, s), 3.19-3.08 (1H, m), 3.07-2.91 (2H, m), 2.68-2.60 (1H, m), 1.82-1.65 (2H, m), 0.89-0.82 (3H, m); MS (ESI): 502 $(M+H)^+$

5.1.35. *N*-{(1*R*)-1-[(4-Aminoanilino)carbonyl]propyl}-6-(5-chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-carboxamide (18M)

Instead of the starting material compound of 5.1.24., that is, the glycine tert-butyl ester hydrochloride, 1-tert-butoxycarbonyl-1,4-phenylenediamine was used for the similar procedure as in

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5.1.24. to obtain the title compound. Yield:56.9%; ^{1}H NMR (DMSO-*d*6) *δ*: 9.72 (0.5H, s), 9.70 (0.5H, s), 9.43 (0.5H, d, *J* = 7.4 Hz), 9.39 (0.5H, d, *J* = 7.5 Hz), 7.65 (1H, d, *J* = 3.2 Hz), 7.31 (0.5H, s), 7.30 (0.5H, s), 7.24 (0.5H, d, *J* = 8.9 Hz), 7.23 (0.5H, d, *J* = 8.9 Hz), 7.17 (2H, d, *J* = 8.6 Hz), 6.98 (1H, d, *J* = 8.9 Hz), 6.48 (1H, d, *J* = 8.6 Hz), 6.46 (1H, d, *J* = 8.6 Hz), 4.847 (1H, s), 4.842 (1H, s), 4.79 (0.5H, d, *J* = 17.1 Hz), 4.77 (0.5H, d, *J* = 17.1 Hz), 4.50 (1H, d, *J* = 17.1 Hz), 4.39-4.32 (1H, m), 3.91-3.81 (1H, m), 3.77 (3H, s), 3.18-3.07 (1H, m), 3.03-2.92 (2H, m), 2.68-2.60 (1H, m), 1.80-1.62 (2H, m), 0.89-0.81 (3H, m); MS (ESI): 502 $(M+H)^+$

5.1.36. 2-Amino-5-{[(2*R*)-2-({[6-(5-chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)butanoyl]amino}benzoic acid hydrochloride (18N)

Instead of the starting material compound of 5.1.24., that is, the glycine tert-butyl ester hydrochloride, tert-butyl 2,5-diaminobenzoate was used for the similar procedure as in 5.1.24. to obtain the title compound. Yield: 38.8%; ^{1}H NMR (DMSO-*d*6) *δ*: 9.92 (0.5H, s), 9.89 (0.5H, s), 9.45 (0.5H, d, *J* = 7.4 Hz), 9.40 (0.5H, d, *J* = 7.4 Hz), 7.95-7.92 (1H, m), 7.66 (0.5H, s), 7.61 (0.5H, s), 7.427 (0.5H, d, *J* = 8.7 Hz), 7.421 (0.5H, d, *J* = 8.7 Hz), 7.31 (1H, s), 7.24 (0.5H, d, *J* = 8.8 Hz), 7.23 (0.5H, d, *J* = 8.8 Hz), 6.98 (1H, d, *J* = 8.8 Hz), 6.71 (1H, d, *J* = 8.7 Hz), 4.78 (0.5H, d, *J* = 17.1 Hz), 4.77 (0.5H, d, *J* = 17.1 Hz), 4.50 (1H, d, *J* = 17.1 Hz), 4.39-4.32 (1H, m), 3.91-3.79 (1H, m), 3.77 (3H, s), 3.17-3.07 (1H, m), 3.03-2.92 (2H, m), 2.68-2.60 (1H, m), 1.83-1.65 (2H, m), 0.89-0.81 (3H, m); MS (ESI): 546 $(M+H)^+$

5.1.37. 5-{[(2*R*)-2-({[6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)butanoyl]amino}-2-hydroxybenzoic acid (18O)

Instead of the starting material compound of 5.1.24., that is, the glycine tert-butyl ester hydrochloride, tert-butyl 4-amino-2-hydroxybenzoate was used for the similar procedure as in 5.1.24. to obtain the title compound. Yield: 56.7%; ^{1}H NMR (DMSO-*d*6) *δ*: 13.78-13.22 (1H, br), 11.43 (0.5H, s), 11.37 (0.5H, s), 9.53 (0.5H, d, *J* = 6.5 Hz), 9.46 (0.5H, d, *J* = 6.6 Hz), 8.46-8.40 (1H, m), 7.95 (1H, d, *J* = 7.5 Hz), 7.70-7.60 (1H, m), 7.57 (1H, t, *J* = 7.5 Hz), 7.35-7.31 (1H, m), 7.28-7.22 (1H, m), 7.15 (1H, t, *J* = 7.5 Hz), 6.99 (1H, d, *J* = 8.8 Hz), 4.77 (0.5H, d, *J* = 17.3 Hz), 4.73 (0.5H, d, *J* = 17.3 Hz), 4.53 (1H, d, *J* = 17.3 Hz), 4.31-4.25 (1H, m), 3.95-3.84 (1H, m), 3.77 (3H, s), 3.15-3.06 (1H, m), 3.05-2.91 (2H, m), 2.69-2.61 (1H, m), 1.94-1.72 (2H, m), 0.95-0.89 (3H, m); MS (ESI): 547 $(M+H)^+$

5.1.38. 3-amino-5-{[(2*R*)-2-({[6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)butanoyl]amino}benzoic acid (18P)

Instead of the starting material compound of 5.1.24., that is, the glycine tert-butyl ester hydrochloride, tert-butyl 5-amino-3-tert-butoxycarbonylaminobenzoate was used for the similar procedure as in 5.1.24. to obtain the title compound. Yield:54.8%; ^{1}H NMR (DMSO-*d*6) *δ*: 12.73-11.90 (1H, br), 10.1 (0.5H, s), 10.0 (0.5H, s), 9.46 (0.5H, d, *J* = 7.3 Hz), 9.42 (0.5H, d, *J* = 7.4 Hz), 7.65 (1H, d, *J* = 3.2 Hz), 7.32-7.22 (3H, m), 7.10 (0.5H, s), 7.09 (0.5H, s), 6.99 (0.5H, s), 6.97 (0.5H, s), 6.87 (0.5H, s), 6.86 (0.5H, s), 5.32 (2H, brs), 4.78 (1H, d, *J* = 17.3 Hz), 4.50 (1H, d, *J* = 17.1 Hz), 4.42-4.38 (1H, m), 3.90-3.82 (1H, m), 3.77 (3H, s), 3.17-3.07 (1H, m), 3.04-2.93 (2H, m), 2.67-2.62 (1H, m), 1.85-1.60 (2H, m), 0.88-0.81 (3H, m); MS (ESI): 546 $(M+H)^+$

5.1.39. 4-{[(2*R*)-2-({[6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl]carbonyl}amino)butanoyl]amino}-1H-pyrrole-2-carboxylic acid (18Q)

Instead of the starting material compound of 5.1.24., that is, the glycine tert-butyl ester hydrochloride, 4-aminopyrrole-2-carboxylic acid tert-butyl ester was used for the similar procedure as in 5.1.24. to obtain the title compound. Yield:53.3%; ^{1}H NMR (DMSO-*d*6) *δ*: 12.30-12.10 (1H, br), 11.46 (1H, s), 10.10 (0.5H, s), 10.07 (0.5H, s), 9.44 (0.5H, d, *J* = 7.4 Hz), 9.39 (0.5H, d, *J* = 7.4 Hz), 7.659 (0.5H, s), 7.651 (0.5H, s), 7.315 (0.5H, s), 7.310 (0.5H, s), 7.24 (0.5H, d, *J* = 8.9 Hz), 7.23 (0.5H, d, *J* = 8.9 Hz), 7.15 (1H, s), 6.98 (1H, d, *J* = 8.9 Hz), 6.61 (1H, s), 4.78 (0.5H, d, *J* = 17.1 Hz), 4.76 (0.5H, d, *J* = 17.1 Hz), 4.50 (1H, d, *J* = 17.1 Hz) 4.37-4.29 (1H, m), 3.91-3.80 (1H, m), 3.77 (3H, s), 3.12 (0.5H, t, *J* = 13.2 Hz), 3.11 (0.5H, t, *J* = 12.8 Hz), 3.03-2.92 (2H, m), 2.66-2.51 (1H, m), 1.80-1.62 (2H, m), 0.86-0.80 (3H, m); MS (ESI): 520 $(M+H)^+$

5.1.40. 6-(5-Chloro-2-methoxybenzyl)-3,7-dioxo-*N*-{(1*R*)-1-[(1H-tetrazol-5-ylamino)carbonyl]propyl}-1,4-diazepan-1-carboxamide (18R)

Instead of the starting material compound of 5.1.23., that is, methylamine, 5-amino-1H-tetrazole was used for the similar procedure as in 5.1.23. to obtain the title compound. Yield:58.1%; ^{1}H NMR (DMSO-*d*6) *δ*: 15.90 (1H, brs), 12.10 (1H, brs), 9.52-9.47 (1H, m), 7.67 (1H, br), 7.31 (1H, s), 7.249 (0.5H, d, *J* = 8.8 Hz), 7.243 (0.5H, d, *J* = 8.8 Hz), 6.9 8(1H, d, *J* = 8.8 Hz), 4.75 (1H, d, *J* = 17.2 Hz), 4.56-4.46 (1H, m), 4.52 (1H, d, *J* = 17.2 Hz), 3.94-3.84 (1H, m), 3.77 (3H, s), 3.18-2.90 (3H, m), 2.68-2.58 (1H, m), 1.89-1.63 (2H, m), 0.90-0.75 (3H, m); MS (ESI): 479 $(M+H)^+$

5.1.41. (6*R*)-6-(5-chloro-2-methoxybenzyl)-3,7-dioxo-N-{(1*R*)-1-[(1H-tetrazol-5-ylamino)carbonyl]propyl}-1,4-diazepan-1-carboxamide ((*R*)-18R)

To the crude compound **(*R*)-17** in methylene chloride (5 ml) solution, 5-amino-1H-tetrazole monohydrate (180 mg), triethylamine (2 ml), and n-propyl phosphoric acid anhydride (50% ethyl acetate solution) (1.5 ml) were added and the mixture was stirred at room temperature for 15 hours. Then, 2M hydrogen chloride aqueous solution was added to the mixture at 0°C, and the resultant mixture was extracted with ethyl acetate. The organic layer was successively washed with saturated saline, dried over with anhydrous sodium sulfate, then concentrated. The residue was purified by preparative TLC (chloroform/methanol/acetic acid = 10/1/0.1). Hexane and ethyl acetate were added to obtain the compound, and the appeared precipitate was collected by filtration to obtain the title compound (15 mg). ^{1}H NMR ($CDCl_3$) *δ*: 9.46 (1H, d, *J* = 7.7 Hz), 7.71 (1H, br), 7.34 (1H, s), 7.27 (1H, d, *J* = 8.5 Hz), 7.01 (1H, d, *J* = 8.5 Hz), 4.80 (1H, d, *J* = 17.5 Hz), 4.57 - 4.45 (2H, m), 3.93 - 3.85 (1H, m), 3.80 (3H, s), 3.20 - 3.09 (1H, m, *J* = 14.2 Hz), 3.06 - 2.91 (2H, m), 2.69 - 2.60 (1H, m), 1.87 - 1.66 (2H, m, *J* = 7.3 Hz), 0.89 (3H, t, *J* = 7.1 Hz); MS (ESI): 479 $(M+H)^+$

5.1.42. (*S*)-*tert*-Butyl 6-(1-hydroxypropyl)nicotinate(23a)

To 6-chloronicotinic acid **19a** (10 g) in methylene chloride (100 ml) solution, *N*,*N'*-diisopropyl-*O*-tert-butylisourea (50 g) was added and the mixture was stirred under heating and reflux for 16 hours. The insoluble compound was filtered out, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (chloroform/ethyl acetate = 6/1) to obtain the compound **20a** (11.66 g). To the compound **20a** (6.0 g)

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in dimethylacetoamide (30 ml) solution, tris(dibenzylideneacetone)dipalladium (0.51 g), zinc cyanide (0.22 g), diphenylphosphinoferrocene (0.62 g), and zinc powder (1.98 g) were added and the mixture was stirred under argon atmosphere at 120°C for 3 hours. The reaction solution was filtered by sellite, which was then washed with ethyl acetate. Then, the filtrate was washed with distilled water and saturated saline, then the organic layer was dried over with anhydrous sodium sulfate and concentrated. The obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate = 5/1) to obtain the compound **21a** (3.59 g). To copper (I) iodide (0.93 g) in tetrahydrofuran (50 ml) suspension, ethylmagnesium bromide (0.86M tetrahydrofuran solution) (12.5 ml) was added dropwise under cooling at 0°C, the mixture was stirred at 0°C for 30 minutes, then the compound **21a** (1.0 g) obtained above in tetrahydrofuran (10 ml) solution was added at the -20°C. After stirring for 30 minutes at that temperature, 28% ammonia solution in water and ethyl acetate were added to the mixture and the solution separated. The aqueous layer was extracted with ethyl acetate, while the combined organic layer was washed with saturated saline, then was dried over with anhydrous sodium sulfate and concentrated. The obtained residue was purified by silica gel column chromatography (hexane/ethyl acetate = 8/1) to obtained the compound **22a** (0.47 g). To the compound **22a** (200 mg) in tetrahydrofuran (1 ml) solution, (-)-B-chlorodiisopinocampheylborane (65% hexane solution) (0.92 ml) was added dropwise under cooling at -20°C, then the mixture was stirred at that temperature for 18 hours. Then, ether and distilled water were added to the reaction mixture, and the solution was separated. The organic layer was extracted with distilled water and 1M hydrogen chloride. Then, the combined aqueous layer was neutralized by sodium hydrogen carbonate, and extracted with ethyl acetate. Organic layer was washed with saturated saline, then was dried over with anhydrous sodium sulfate and concentrated to obtained the title compound (150 mg, 84%ee) . The optical purity was determined using CHIRALCEL AD-H (Daicel Chemical Industries) (movement phase:hexane/ethanol = 98/2, 1.0ml/min) . ^{1}H NMR ($CDCl_3$) δ: 9.10 - 9.08 (1H, m), 8.23 (1H, dd, J = 2.2, 8.1 Hz), 7.32 (1H, d, J = 8.1 Hz), 4.78 - 4.73 (1H, m), 4.00 (1H, d, J = 5.7 Hz), 1.95 - 1.85 (1H, m), 1.80 - 1.67 (1H, m), 1.64 - 1.59 (9H, m), 0.94 (3H, t, J = 7.3 Hz)

5.1.43. *tert*-Butyl 6-[(1*R*)-1-aminopropyl]-nicotinate D-tartrate (25a)

To the compound **23a** (1.33 g) in tetrahydrofuran (27 ml) solution, triphenylphosphine (4.41 g), diethyl azodicarboxylate (2.65 ml) and phtalimide (1.24 g) were added under cooling at 0°C, then the mixture was stirred at room temperature for 1 hour. The mixture was concentrated, the residue was purified by silica gel column chromatography (chloroform/ethyl acetate = 6/1) to obtain the compound **24a** (1.34 g). To the compound **24a** (1.37 g) in methanol (14 ml), hydrazine monohydrate (0.9 ml) was added under warming at 60 °C, then the mixture was stirred at that temperature for 2 hours. Then, ethyl acetate and distilled water were added to the mixture. Then, the organic layer and the aqueous layer were separated, subsequently the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with saturated saline, then was dried over with anhydrous sodium sulfate and concentrated. To the obtained compound (0.94 g) in ethyl acetate (9.4 ml) solution, D-tartaric acid in ethanol 1M solution (3.74 ml) was added, then the appeared precipitate was collected by filtration to obtained title compound (1.28 g). ^{1}H NMR (DMSO-*d*6) δ: 9.04 (1H, d, J = 2.1 Hz), 8.29 (1H, dd. J = 8.1, 2.1 Hz), 7.62 (1H, d, J = 8.1 Hz), 4.34 (1H, t, J = 6.8 Hz), 3.84 (2H, s), 1.90-1.74 (2H, m), 1.57 (9H, s), 0.79 (3H, t, J = 7.5 Hz); MS (ESI): 237 $(M+H)^+$

5.1.44. *tert*-Butyl 2-[(1*R*)-1-aminopropyl]-isonicotinate D-tartrate (25b)

Instead of the starting material compound of 5.1.42., that is, 6-chloronicotinic acid, 2-chloroisonicotinic acid was used for the similar procedure as in 5.1.42. and 5.1.43. to obtain the title compound. ^{1}H NMR (DMSO-*d*6) δ: 8.81 (1H, d, J = 4.9 Hz), 7.90 (1H, d. J = 1.5 Hz), 7.77 (1H, dd, J = 4.9, 1.5 Hz), 4.39 (1H, t, J = 6.8 Hz), 3.83 (2H, s), 1.90-1.76 (2H, m), 1.57 (9H, s), 0.80 (3H, t, J = 7.5 Hz); MS (ESI): 237 $(M+H)^+$

5.1.45. *tert*-Butyl 5-[(1*R*)-1-aminopropyl]-nicotinate D-tartrate (25c)

Instead of the starting material compound of 5.1.42., that is, 6-bromonicotinic acid, 5-bromonicotinic acid was used for the similar procedure as in 5.1.42. and 5.1.43. to obtain the title compound. ^{1}H NMR (DMSO-*d*6) δ: 8.99 (1H, d, J = 2.0 Hz), 8.81 (1H, d, J = 2.1 Hz), 8.33 (1H, t, J = 2.0 Hz), 7.75-6.95 (3H, br), 4.31 (0.5H, d, J = 2.6 Hz), 4.18 (1H, t, J = 6.4 Hz), 4.04-3.99 (0.5H, br), 3.86 (1H, s), 1.95-1.71 (2H, m), 1.57 (9H, s), 0.78 (3H, t, J = 7.4 Hz); MS (ESI): 237 $(M+H)^+$

5.1.46. *tert*-Butyl 5-[(1*S*)-1-hydroxypropyl]-2-furancarboxylic acid (28)

To 5-formyl-2-furancarboxylic acid **26** (10 g) in methylene chloride (200 ml) solution, *N*,*N*'-diisopropyl-*O*-tert-butylisourea (42 g) was added and the mixture was stirred under heating and reflux for 12 hours. The insoluble compound was filtered out, then the filtrate was concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 6/1) to obtain the compound **27** (10.97 g). To the (1*S*,2*R*)-2-di-n-butylamino-1-phenyl-1-propanol (81 mg) in hexane (5 ml)/ toluene (5 ml) solution, **27** (1 g) was added and the mixture was stirred at room temperature for 30 minutes. Next, diethylzinc in 1N hexane solution (11.3 ml) was added to the reaction solution under ice cooling and the mixture was stirred at that temperature for 16 hours. Saturated ammonium chloride aqueous solution was added to the reaction solution, the mixture was stirred at 20 minutes, and 1N hydrochloric acid was added. The obtained mixed solution was extracted with ethyl acetate. The organic layer was dried over with anhydrous sodium sulfate, then concentrated to obtain the title compound (1.53 g, 85%ee). The optical purity was determined using CHIRALCEL AD-H (Daicel Chemical Industries) (movement phase: hexane/ethanol = 98/2, 1.0ml/min). ^{1}H NMR ($CDCl_3$) δ: 6.98 (1H, d, J = 3.4 Hz), 6.30 (1H, d, J = 3.4 Hz), 4.65 (1H, br), 1.99-1.80 (2H, m), 1.55 (9H, s), 0.96 (3H, t, J = 7.4 Hz)

5.1.47. *tert*-Butyl 5-[(1*R*)-1-aminopropyl]-2-furancarboxylate D-tartrate (31)

Instead of the starting material compound of 5.1.43., that is, the compound **23a**, the compound **28** was used for the similar procedure as in 5.1.43., to obtain the title compound. ^{1}H NMR (DMSO-*d*6) δ: 8.25-7.30 (3H, br), 7.16 (1H, d, J = 3.5 Hz), 6.59 (1H, d, J = 3.5 Hz), 4.15 (1H, t, J = 6.8 Hz), 3.95 (2H, s), 1.89-1.70 (2H, m), 1.51 (9H, s), 0.85 (3H, dt, J = 7.3, 2.3 Hz); MS (ESI): 226 $(M+H)^+$

5.1.48. *tert*-Butyl 5-[(1*S*)-1-hydroxypropyl]-3-furancarboxylate (34a)

To 3-furancarboxylic acid (1.12 g), nitromethane (10 ml), indium (III) trifluoromethane sulfonate (56 mg), lithium perchlorate (1.06 g), and propionic anhydride (1.28 ml) were added and the mixture was stirred at 50°C for 3 hours. Water was added to the reaction solution and the solution separated. The aqueous layer was extracted with ethyl acetate. The combined

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organic layer was washed with saturated saline, dried over with anhydrous sodium sulfate, and concentrated to obtain 5-propionyl-3-furancarboxylic acid (1.11 g) To 5-propionyl-3-furancarboxylic acid (1.10 g) in methylene chloride (22 ml) solution, *N*,*N*'-diisopropyl-*O*-tert-butylisourea (8 ml) was added and the mixture was stirred under heating and reflux for 3 hours. The insoluble compound was filtered out, then the filtrate was concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 5/1). Then, hexane was added to the obtained compound, and the appeared precipitate was collected by filtration to obtained the compound **33a** (0.71 g). The mother liquid was concentrated to obtain the compound **33 a** (0.37 g). The compound **34a** was synthesized in twice. To the compound **33a** (0.1 g , 0.90 g) in tetrahydrofuran (1 ml, 9 ml) solution cooled at -20°C, 65%(-)-B-chlorodiisopinocampheylborane in hexane solution (0.26 ml, 4.8 ml) was added dropwise, then the mixture was stirred at that temperature for overnight. Then, water was added to the each reaction mixture, and the obtained solutions were combined. The resultant mixture was extracted with ethyl acetate. The organic layer was washed with saturated saline, then was dried over with anhydrous sodium sulfate and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 3/1) to obtain the compound **34a**. (1.33 g, 91%ee) The optical purity was determined using CHIRALCEL AD-H (Daicel Chemical Industries) (movement phase:hexane/ethanol = 98/2, 1.0ml/min) . ^{1}H NMR ($CDCl_3$) δ: 7.86 (1H, s), 6.53 (1H, s), 4.63 - 4.55 (1H, m), 4.11 - 4.02 (1H, m), 1.75 - 1.71 (1H, m), 1.71 - 1.67 (1H, m), 1.54 (9H, s), 1.00 - 0.93 (3H, m)

5.1.49. *tert*-Butyl 5-[(1*R*)-1-aminopropyl]-3-furancarboxylate D-tartrate (36a)

Instead of the starting material compound of 5.1.43., that is, the compound **23a**, the compound **34a** was used for the similar procedure as in 5.1.43., to obtain the title compound. ^{1}H NMR (DMSO-*d*6) δ: 8.28 (1H, s), 6.65 (1H, s), 4.12 (1H, t, *J* = 6.3 Hz), 3.91 (2H, s), 1.89-1.72 (2H, m), 1.50 (9H, s), 0.82 (3H, t, *J* = 7.4 Hz); MS (ESI): 209 $(M-NH_2)^+$

5.1.50. *tert*-Butyl 5-[(1*R*)-1-aminopropyl] -3-thiophenecarboxylate D-tartrate (36b)

Instead of the starting material of 5.1.48., that is, 3-furancarboxylic acid, 3-thiophenecarboxylic acid was used for the similar procedure as with 5.1.48. and 5.1.49. to obtain the title compound. ^{1}H NMR (DMSO-*d*6) δ: 8.17 (1H, s), 7.41 (1H, s), 4.35 (1H, t, *J* = 7.8 Hz), 3.93 (2H, s), 1.92-1.70 (2H, m), 1.51 (9H, s), 0.84 (3H, t, *J* = 7.3 Hz); MS (ESI): 225 $(M+H)^+$

5.1.51. (*R*)-2-(1-Aminopropyl)oxazole-4-carboxylic acid 2,2,2-trichloroethyl ester hydrochloride (42)

To (*R*)-2-(tert-butoxycarbonylamino)butyric acid (5 g) in methylene chloride (100 ml) solution, serine methyl ester hydrochloride (5.7 g), 1-hydroxybenzotriazole (5 g), *N*,*N*-diisopropylethylamine (6.4 ml), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (7.1 g) were added and the mixture was stirred at room temperature for 3 hours. The reaction solution was diluted with ethyl acetate, then was successively washed with distilled water, saturated potassium hydrogensulfate aqueous solution, saturated sodium hydrogencarbonate aqueous solution and saturated saline, dried over with anhydrous sodium sulfate, then concentrated. Then, the residue was successively purified by silica gel column chromatography (hexane/ethyl acetate = 4/6 to 3/7) to give the compound **38** (5.27 g). To the compound **38** (2.46 g) in methylene chloride (50 ml) solution cooled at -20°C, *N*,*N*- diethylaminosulfur trifluoride (1.17 ml) was dropwisely added, and the mixture was stirred at that temperature for 1 hour. Then, bromotrichloromethane (2.8 ml) and 1,8-diazabicyclo[5.4.0]undec-7-ene (4.2 ml) were added to the mixture. After stirring at 0°C for 6 hours, saturated sodium hydrogencarbonate aqueous solution was added to the mixture, and the resultant mixture was extracted with ethyl acetate. The organic layer was successively washed with saturated saline, dried over with anhydrous sodium sulfate, and concentrated. Then, the residue was successively purified by silica gel column chromatography (hexane/ethyl acetate = 8/2 to 7/3) to give the compound **39** (1.0 g). To the compound **39** (0.67 g) in methanol (10 ml) solution, 4M sodium hydroxide aqueous solution (1.18 ml) was added, and the mixture was stirred at room temperature for 3 hours. The methanol was removed by evaporation, and the residue was diluted water, and the resultant solution was washed with ethyl acetate. The aqueous layer was acidified by 6M hydrogen chloride aqueous solution, and the resultant solution was extracted with ethyl acetate. The organic layer was successively washed with saturated saline, dried over with anhydrous sodium sulfate, and concentrated to give the compound **40** (0.52 g). To the compound **40** (0.52 g) in methylene chloride (10 ml) solution, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.44 g), 4-dimethylaminopyridine (23 mg) and 2,2,2-trichloroethanol (0.28 ml) were added and the mixture was stirred at room temperature for 2.5 hours. The reaction solution was evaporated. The residue was diluted with ethyl acetate, then the solution was successively washed with distilled water, saturated potassium hydrogensulfate aqueous solution, saturated sodium hydrogencarbonate aqueous solution and saturated saline, dried over with anhydrous sodium sulfate, then concentrated. Then, the residue was successively purified by silica gel column chromatography (hexane/ethyl acetate = 6/1 to 3/1) to give the compound **41** (0.46 g). A 4M hydrogen chloride/ethyl acetate solution (5 ml) was added to the compound **41** (0.75 g), and the mixture stirred at room temperature for 2 hours. Then, the reaction solution was concentrated to obtain the title compound (0.51 g). ^{1}H NMR ($CDCl_3$) δ: 9.50 - 9.37 (2H, m), 8.39 (1H, s), 4.97 - 4.89 (2H, m), 4.84 - 4.75 (1H, m), 2.37 - 2.28 (2H, m), 1.11 (3H, t, *J* = 7.5 Hz,); MS (ESI): 301 $(M+H)^+$

5.1.52. (*R*)-2-(1-Aminopropyl)thiazole-4-carboxylic acid 2,2,2-trichloroethyl ester hydrochloride(48)

To (*R*)-1-amino-1-thioxobutane-2-ylcarbamic acid tert-butyl ester (2.82 g) in dimethoxyethane (60 ml) solution cooled at 0°C, potassium hydrogen carbonate (10.3 g) and ethyl bromopyruvate (4.9 ml) were added ,and the mixture was stirred at that temperature for 30 min. Then, the mixture was warmed to room temperature. After stirring at room temperature for 18hours, the reaction mixture was evaporated. The residue was diluted with $CHCl_3$, then the solution was successively washed with distilled water and saturated saline, dried over with anhydrous sodium sulfate, then concentrated to obtain the compound **44**. To the compound **44** in dimethoxyethane (60 ml) solution cooled at 0°C, trifluoroacetic anhydride (3.6 ml) and pyridine (4.7 ml) were added, and the mixture was stirred at that temperature for 1 hour. Then, the reaction mixture was evaporated, the residue was diluted with ethyl acetate. Then, the solution was successively washed with saturated potassium hydrogensulfate aqueous solution, saturated sodium hydrogencarbonate aqueous solution and saturated saline, dried over with anhydrous sodium sulfate, then concentrated. Then, the residue was successively purified by silica gel column chromatography (hexane/ethyl acetate = 9/1 to 17/3) to give the compound **45** (4.25 g). To the compound **45** (2.1 g) in ethanol (40 ml) solution , 4M sodium hydroxide aqueous solution (3.3 ml) was added , and the mixture was stirred

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at room temperature for 2 hours. Then, water and ethylacetate were added to the mixture, and the organic layer and the aqueous layer was separated. The aqueous layer was acidified by 6M hydrogen chloride aqueous solution, and the resultant solution was extracted with ethyl acetate. The organic layer was successively washed with saturated saline, dried over with anhydrous sodium sulfate, and concentrated to give the compound **46** (1.47g). To the compound **46** (1.47 g) in methylene chloride (30 ml) solution, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.18 g), 4-dimethylaminopyridine (63 mg) and 2,2,2-trichloroethanol (0.74 ml) were added and the mixture was stirred at room temperature for 3 hours. The reaction solution was evaporated. The residue was diluted with ethyl acetate, then the solution was successively washed with distilled water, saturated potassium hydrogensulfate aqueous solution, saturated sodium hydrogencarbonate aqueous solution and saturated saline, dried over with anhydrous sodium sulfate, then concentrated. Then, the residue was successively purified by silica gel column chromatography (hexane/ethyl acetate = 5/1) to give the compound **47** (1.42 g). A 4M hydrogen chloride/ethyl acetate solution (5 ml) was added to the compound **47** (1.42 g), and the mixture stirred at room temperature for 3 hours. Then, the reaction solution was concentrated to obtain the title compound (0.7 g). ^{1}H NMR ($CDCl_3$) δ: 9.43 - 9.30 (3H, m), 8.37 (1H, s), 5.00 - 4.93 (3H, m), 2.42 - 2.27 (2H, m), 1.10 (3H, t, J = 7.3 Hz); MS (ESI): 316 $(M+H)^+$

5.2. Human chymase assay

The human chymase assay was performed as follows: recombinant human chymase[4] was preincubated with the test compounds for 10 min in 50 mM Tris/HCl buffer (pH7.5) containing 1 M NaCl and 0.01% Triton X-100. The enzyme reaction was initiated with substrate Suc-Ala-Ala-Pro-Phe-MCA (Peptide Institute), supplied at 100 lM, and was stopped with 30% acetic acid after 10 minincubation. The intensity of the fluorescence of the AMC produced by chymase was measured (extinction380 nm, emission 460 nm), and the IC50 value wascalculated from the inhibition of AMC formation at eachconcentration of the tested compound.

5.3. Inhibitory effects on the enzymatic activity of serine proteases

The inhibitory effects on the enzymatic activity of serineproteases were evaluated by a similar procedure as in 5.2. The enzymes and substrates used for the assays were asfollows: Suc-Ala-Ala-Pro-Phe-MCA (Peptide Institute) for bovine pancreatic a-chymotrypsin (Sigma) and humanneutrophil cathepsin G (Calbiochem); Boc-Gln-Ala-Arg-MCA (Peptide Institute) for bovine pancreatic trypsin(Nacalai Tesque); MeOSuc-Ala-Ala-Pro-Val-pNA (Bachem) for human neutrophil elastase (Calbiochem).The assay buffer was as follows: 0.1 M Tris/HCl (pH 7.5)containing 1M KCl and 0.01% Triton X-100 for achymotrypsin;50 mM Tris/HCl buffer (pH 7.5) containing1 M NaCl and 0.01% Triton X-100 for cathepsin G andelastase; 50 mM Tris/HCl buffer (pH 7.5) containing 20 mM CaCl2 for trypsin.

5.4. Preparation of the crystal

Human chymase, which substituted Cys22 with Ser and Phe127 with Lys for high-yield production[21] and the avoidance of self-cleavage[22], respectively, was expressed by *Pichia pastoris*. The supernatant with the secretory product was loaded onto a heparin sepharose 6FF column (GE healthcare), and were eluted by NaCl. Collected sample was concentrated to 15 mg/ml, and treated with $EndoH_f$ (New England Biolabs) for the deglycosylation. Due to the removal of $EndoH_f$, the sample was re-loaded onto the same column. After the elution of the sample, the amino-terminal extra region was cleaved by enterokinase (Roche Applied Science) for the activation as the enzyme. Sample was concentrated to 15mg/ml in 20 mM MES buffer (pH 6.0) and saturated compounds. Crystallization was performed by the hanging-drop vapor diffusion method. Crystals were obtained at 20°C after a few days in 22% PEG8000, 5 mM $ZnCl_2$, and 100 mM MES buffer (pH 6.8).

5.5. X-ray data processing and structure solution

The X-ray data sets were collected at room temperature on an R-Axis IV^{++} imaging plate detector (Rigaku) using a MicroMax 007 rotating anode generator (Rigaku). Data processing was carried out with HKL2000.[23] Co-crystal structures of human chymase were solved by the molecular replacement method. The previously reported chymase structure (PDB code: 1T31[24]) was used as the search model. Phase refinement and model improvement were carried out with refmac[25] and coot.[26] Stereochemistry checks indicate that the refined protein model is in good agreement with expectations within each resolution range.

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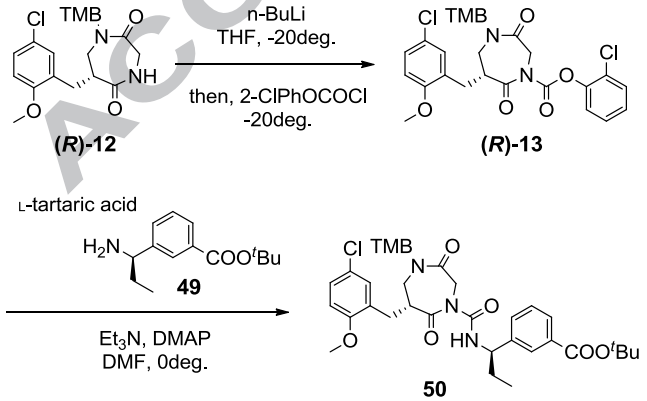

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Graphical Abstract

Discovery of novel series of 6-benzyl substituted 4-aminocarbonyl-1,4-diazepane-2,5-diones as human chymase inhibitors using structure-based drug design

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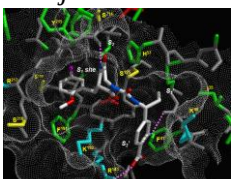

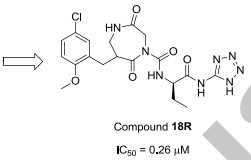

Compound **18R**

IC_{50} = 0.26 μM

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