Bioorganic & Medicinal Chemistry 23 (2015) 89–104

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of novel chiral diazepine derivatives as bombesin receptor subtype-3 (BRS-3) agonists incorporating an antedrug approach

Tetsuyoshi Matsufuji [a,*], Kousei Shimada [a], Shozo Kobayashi [a], Masanori Ichikawa [a], Asuka Kawamura [a], Teppei Fujimoto [a], Tsuyoshi Arita [a], Takashi Hara [b], Masahiro Konishi [b], Rie Abe-Ohya [b], Masanori Izumi [b], Yoshitaka Sogawa [b], Yoko Nagai [c], Kazuhiro Yoshida [c], Yasuyuki Abe [d], Takako Kimura [e], Hisashi Takahashi [a]

[a] Medicinal Chemistry Research Laboratories, Daiichi Sankyo Co., Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[b] Cardiovascular Metabolics Research Laboratories, Daiichi Sankyo Co., Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[c] Drug Metabolism & Pharmacokinetics Research Laboratories, Daiichi Sankyo Co., Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[d] Medicinal Safety Research Laboratories, Daiichi Sankyo Co., Ltd, 1-16-13 Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan
[e] Drug Discovery and Biomedical Technology Unit, Daiichi Sankyo RD NOVARE Co., Ltd, 1-16-13 Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan

ARTICLE INFO

Article history:
Received 9 September 2014
Revised 13 November 2014
Accepted 14 November 2014
Available online 21 November 2014

Keywords:
Anti-obesity
Antedrug
Bombesin receptor subtype-3 (BRS-3)
Brain penetration
Diazepine

ABSTRACT

Novel compounds based on the lead BRS-3 agonists from our HTS compounds **2a** and **2b** have been synthesized with the focus on obtaining peripheral BRS-3 agonists. To identify potent anti-obesity compounds without adverse effects on the central nerve system, a labile carboxylic ester with an antedrug functionality was introduced onto the terminal position. Through the extensive synthetic exploration and the pharmacokinetic studies of oral administration in mice, the phenol ester **17c** was selected due to the most suitable pharmacological profile. In the evaluation of food intake suppression in B6 mice, **17c** showed significant in vivo efficacy and no clear adverse effect on heart rate and blood pressure change in dog iv infusion. Our study paved the way for development of anti-diabetes and obesity drugs with a safer profile.

1. Introduction

Obesity is a highly prevalent major public health problem, and has been known as a chronic medical condition internationally since the World Health Organization officially defined obesity a disease in 1998.[1] Considering that related disorders such as type 2 diabetes, cardiovascular diseases and hypertension could exacerbate the condition,[2] obesity is no longer a disorder that should be resolved by self-treatment, but rather a malady that demands medical therapy. There are presently several FDA-approved drugs for chronic treatment of obesity: the lipase inhibitor, orlistat; a selective serotonin 5-HT_{2C} receptor agonist, lorcaserin; and the combination of phentermine and topiramate, Qsymia.[3] However, more efficacious and safer treatments for obesity and weight maintenance are still strongly demanded.[4] In actual fact, some patients taking currently available drugs are suffering from variable efficacy and undesirable adverse effects mainly on the central nervous system (CNS). Therefore, there are significant unmet medical needs for the treatment of obesity with safer profiles.[5]

The bombesin receptor subtype-3 (BRS-3), which is known as an orphan G-protein coupled receptor, is expressed not only in the brain, but also in peripheral organs such as the intestine, liver, lung, testes and pancreas.[6] Since BRS-3 deficient mice develop obesity and impaired glycemic regulation, finding ligands that modulate BRS-3 signaling is useful for developing a novel treatment for diabetes and obesity.[7] Although the exact physiological effects by the activation of BRS-3 are unknown since the natural ligand for BRS-3 has yet to be identified, BRS-3 not only in the CNS, but also in peripheral organs such as the intestine can be a promising drug target with an important function in regulating endocrine and metabolic processes via a vagus nerve.[8]

Recently, Guan et al. have reported a potent and brain-penetrant BRS-3 agonist as clinical candidate **1a** (MK-5046, Fig. 1) which showed anti-obesity effects in rats and dogs, while **1a** also caused side effects, such as an increase of body temperature, heart rate and blood pressure in animals.[9] In addition, it raised blood

* Corresponding author. Tel.: +81 3 3492 3131; fax: +81 3 5436 8563.
E-mail address: matsufuji.tetsuyoshi.nf@daiichisankyo.co.jp (T. Matsufuji).

http://dx.doi.org/10.1016/j.bmc.2014.11.018
0968-0896/

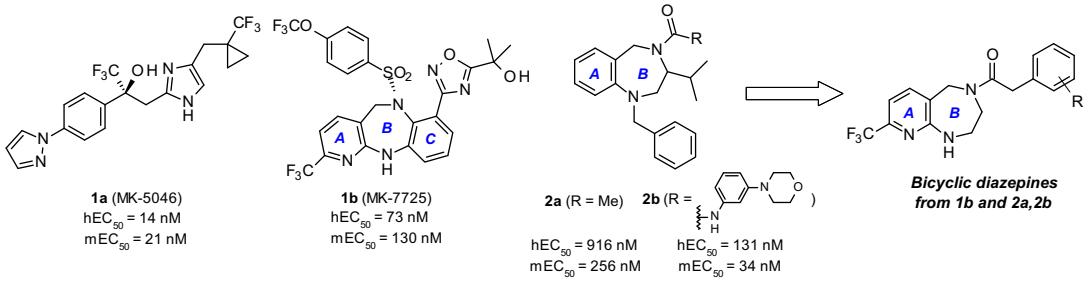


Figure 1. Brain penetrant imidazole **1a** (MK-5046), benzodiazepine sulfonamide **1b** (MK-7725) and our hit compounds **2a** and **2b** with BRS-3 EC_{50} values.

pressure in humans in the clinical trial.[10] Hence, lowering exposure of the compound to the brain is expected to avoid these side effects due to the absence of the activation of the sympathetic nervous system by stimulating BRS-3 in the CNS. In addition, to balance stimulation of BRS-3 in the intestine as a peripheral receptor and reduce brain exposure, we adopted one approach to significantly reducing systemic exposure including CNS: the 'antedrug' approach. The terms 'antedrug' and 'soft drug' were introduced to describe drugs which act topically at the site of application but that are transformed into inactive metabolites upon entry into the systemic circulation.[11] Therefore, we speculated that more potent and safer BRS-3 agonists would be obtained by use of the antedrug approach. Furthermore, our approach could be helpful to identify an unknown mechanism of BRS-3 signaling by the peripheral simulation if any physiological effect was observed.

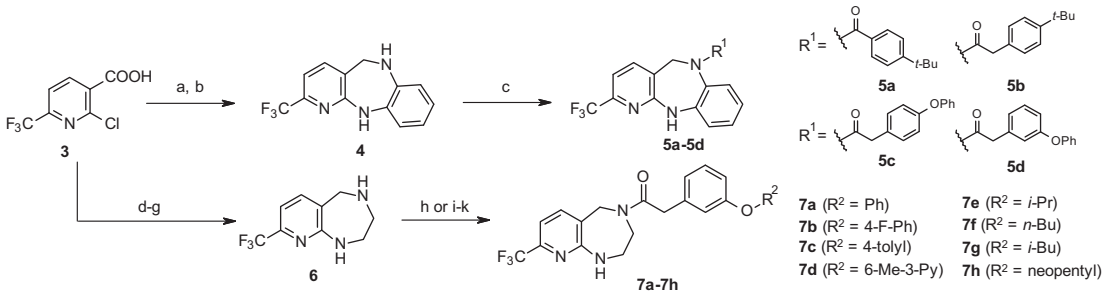
Initially, we designed and synthesized a novel benzodiazepine BRS-3 agonist based on compounds **2a**, **2b** and a benzodiazepine sulfonamide **1b**. Compounds **2a** and **2b** were identified by a high throughput screening campaign of our corporate library,[12] and **1b** was previously reported as a potent BRS-3 agonist MK-7725.[13] Inspired by a structural similarity, we surmised the sulfonyl group of **1b** could be replaced by another linker and the ring, C, in Figure 1 could be removed from the rigid benzodiazepine structure. Then, after exploring a novel potent scaffold, we planned to use an antedrug method by introducing a labile functional group onto the molecule.

2. Chemistry

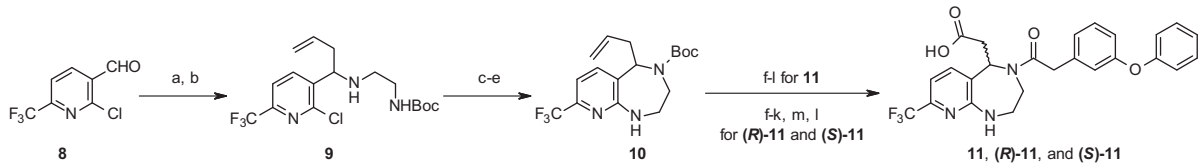
The preparation of diazepine derivatives **5a**–**5d** and **7a**–**7h** is shown in Scheme 1. The formation of a tricyclic benzodiazepine **4** was performed in a similar manner reported for **1b**.[13a] Subsequent amidation with the corresponding carboxylic acid or acyl chloride provided the desired compounds **5a**–**5d**. New scaffold compounds (**7a**–**7h**) were synthesized after synthetic examination for a formation of a 7-membered diazepine ring. Initially, we tried to obtain the diazepine **6** by the reduction of a corresponding lactam ring with lithium aluminum hydride (LAH) under heating, but failed probably because of the strong distortion of the ring. As a result, we succeeded at procuring diazepine **6** using the following 4 steps: (1) a commercially available pyridyl carboxylic acid **3** was reduced with borane–THF complex to pyridylmethyl alcohol, (2) ethylenediamine addition by S_NAr reaction upon heating, (3) oxidation with MnO_2 furnished the 7-membered imine without isolating the corresponding aldehyde in a moderate yield, and (4) hydrogenation by a Pd/C catalyst under an H_2 atmosphere in ethyl acetate yielded the desired **6**. As a final step, amidation of **6** with various carboxylic acids yielded **7a**–**7d**. Aliphatic derivatives **7e**–**7h** were synthesized via S_N2 reaction using various alkyl halides and the phenol intermediate which was prepared by direct amidation of **6** and [3-(benzyloxy)phenyl]acetic acid.

A polar derivative **11** with an acetic acid moiety on the diazepine ring was synthesized as shown in Scheme 2. First, the aldehyde **8** was condensed with *N*-Boc-ethylenediamine in anhydrous media, and the resulting imine was reacted with an allyl Grignard reagent to afford **9**. Then, a deprotection of the Boc group and an HCl salt formation by addition of 4 N HCl in ethylacetate, followed by cyclization upon heating at 160 °C and Boc protection of a nitrogen atom on the diazepine ring provided **10**. The allyl group of **10** was converted to methyl ester after oxidative cleavage to aldehyde by using an OsO_4 catalyst and $NaIO_4$ as an oxidant, Klaus oxidation reaction to carboxylic acid,[14] and methyl esterification with methyl iodide. Finally, deprotection of the Boc group of **10**, and amidation with 2-(3-phenoxyphenyl)acetic acid afforded a methyl ester of **11**. Subsequent hydrolysis under a basic condition gave **11** as a racemic compound, while chiral separation of the methyl ester of **11** using chiral column chromatography (CHIRALPAK IC) and the following hydrolysis yielded each enantiomer (***R***)-**11** and (***S***)-**11**, respectively.[15]

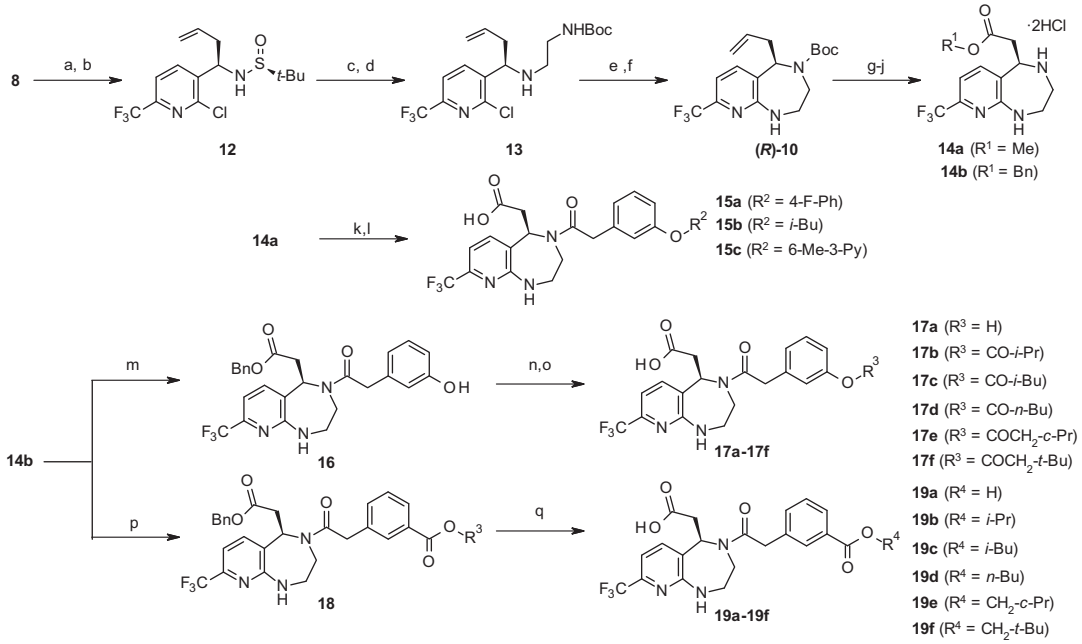
The general procedure for the synthesis of chiral diazepine derivatives **15a**–**15c**, **17a**–**17f** and **19a**–**19e** is depicted in Scheme 3. First, Ellman's chiral sulfinamide ((*S*)-*t*-BuS(O)NH_2) was adopted to stereospecifically introduce an allyl group to the benzyl position.[16]



Scheme 1. Reagents and conditions: (a) 1,2-diaminobenzene, ethylene glycol monobutylether, 160 °C, 64%; (b) BH_3–THF complex, THF, 0 °C–rt, 66%; (c) 4-*tert*-butylbenzoylchloride, Et_3N, CH_2Cl_2, or arylacetic acid, HATU, (*i*-Pr)$_2$EtN, CH_2Cl_2, 72–92% (d) BH_3–THF complex, THF, 0 °C–rt, quant.; (e) ethylenediamine (neat), 120 °C, 62%; (f) MnO_2, CH_2Cl_2/MeOH, 76%, (g) H_2, Pd–C, AcOEt, 82%; (h) arylacetic acid, HATU, (*i*-Pr)$_2$EtN, CH_2Cl_2, 34–96%; (i) [3-(benzyloxy)phenyl]acetic acid, (*i*-Pr)$_2$EtN, HATU, CH_2Cl_2, 99%; (j) H_2, Pd–C, EtOH, 98%; (k) alkylhalide, K_2CO_3, DMF, 80 °C, 31–83%. HATU: (dimethylamino)-*N*,*N*-dimethyl(3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yloxy)methaniminium hexafluorophosphate.



Scheme 2. Reagents and conditions: (a) *N*-Boc-ethylenediamine, Na_2SO_4, CH_2Cl_2, rt, 69%; (b) allylmagnesium bromide/Et_2O, THF, 0 °C, 73%; (c) 4 N HCl/AcOEt; (d) $(i\text{-}Pr)_2EtN$, NMP, 160 °C; (e) di-*tert*-butyl dicarbonate, Et_3N, CH_2Cl_2, 59%, 3 steps; (f) 2.5% OsO_4/*i*-PrOH, *N*-methylmorpholine oxide, acetone; (g) $NaIO_4$, THF/H_2O; (h) $NaClO_2$, $NaH_2PO_4{\cdot}2H_2O$, 2-methyl-2-butene, *t*-BuOH/H_2O; (i) iodomethane, K_2CO_3, DMF, 50 °C, 77%, 4 steps; (j) 4 N HCl/AcOEt, 92%; (k) 2-(3-phenoxyphenyl)acetic acid, $(i\text{-}Pr)_2EtN$, HATU, CH_2Cl_2, 91%; (l) 2 N NaOH aq, THF/MeOH, 88–97%; (m) chiral separation (CHIRALPAK IC, EtOH/hexane = 30/70). NMP: *N*-methylpyrrolidinone.



Scheme 3. Reagents and conditions: (a) (*S*)-*t*-BuS(O)NH_2, $Ti(OEt)_4$, THF, reflux, 99%; (b) allyl bromide, in, satd NaBr aq, 94%; (c) 4 N HCl/1,4-dioxane, 82%; (d) *tert*-butyl *N*-(2-oxoethyl)carbamate, $NaBH(OAc)_3$, CH_2Cl_2, quant.; (e) 4 N HCl/AcOEt; (f) $(i\text{-}Pr)_2EtN$, NMP, 160 °C, then di-*tert*-butyl dicarbonate, 59%, 3 steps; (g) 2.5% OsO_4/*i*-PrOH, *N*-methylmorpholine oxide, acetone/H_2O, 0 °C–rt; (h) $NaIO_4$, THF/H_2O; (i) $NaClO_2$, $NaH_2PO_4{\cdot}2H_2O$, 2-methyl-2-butene, *t*-BuOH/H_2O; (j) $TMSCH_2N_2$, THF/MeOH, 0 °C or BnBr, K_2CO_3, acetone, 60 °C, then 4 N HCl/1,4-dioxane, 5 steps, 64–80%; (k) arylacetic acid, $(i\text{-}Pr)_2EtN$, HATU, CH_2Cl_2; (l) 2 N NaOH aq, THF/MeOH, 69–97%, 2 steps; (m) (3-hydroxyphenyl)acetic acid, DMT-MM, MeOH, 77%; (n) alkylacyl chloride, Et_3N, CH_2Cl_2; (o) H_2, Pd–C, EtOH, 46–96%, 2 steps; (p) arylacetic acid, $(i\text{-}Pr)_2EtN$, HATU, CH_2Cl_2, (q) H_2, Pd–C, EtOH, 74–80%, 2 steps. NMP, *N*-methylpyrrolidinone. HATU: (dimethylamino)-*N*,*N*-dimethyl(3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yloxy)methaniminium hexafluorophosphate; DMT-MM: (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride *n*-hydrate.

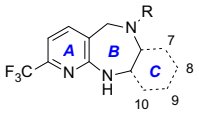
After imine formation with the aldehyde **8** and the Ellman's sulfinamide, an effective asymmetric addition of an allyl group to the imine moiety was carried out under the condition of using an indium catalyst in aqueous NaBr, which was reported by Sun et al.[17] A pure desired (*R*,*S*)-diastereomer **12** was easily isolated by silica gel column chromatography in a high yield.[18] Deprotection of the sulfoxide under an acidic condition, and the subsequent reductive amination using $NaBH(OAc)_3$ and *tert*-butyl *N*-(2-oxoethyl)carbamate gave **13**. Then, deprotection of the Boc group of **13** and cyclization in NMP at 160 °C yielded a chiral key intermediate (***R***)-**10**.[19] Next, the allyl group of (***R***)-**10** was converted to **14a** or **14b** after oxidative cleavage to carboxylic acid as depicted in Scheme 3, methyl or benzyl esterification of the carboxy group with alkylation by an S_N2 reaction, and deprotection of the Boc group in acidic solvent. The versatile phenol **16** was directly obtained by amidation with **14b** and 3-hydroxybenzene acetic acid using DMT-MM as a condensation agent.[20] The desired phenol esters **17b**–**17f** were provided by debenzylation after the corresponding acylation, whereas the benzoic acid esters of **19b**–**19f** were obtained by debenzylation after amidation of the corresponding carboxylic acid.

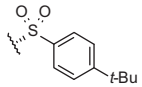
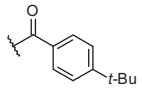
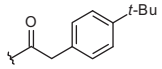
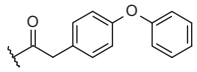
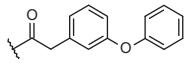
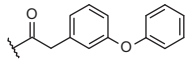
3. Results and discussions

3.1. In vitro evaluation for structure–activity relationship (SAR) study

For an efficient derivatization, we initiated the SAR study by adopting the 4-*tert*-butylphenyl group as an upper moiety by reference to the reported compound **1c** as an (*S*)-atropisomer by Liu et al.,[13a] as shown in Table 1. Although replacement of the sulfonyl group with acyl group **5a** resulted in no activity in human and mouse BRS-3 expressing cells, aryl acetyl group **5b** showed a good result. While the replacement of the *tert*-butyl group with a *para*-phenoxy group **5c** showed a sharp loss of potency, the synthesis of *meta*-phenoxy compound **5d** by reference to a partial structure of **2b** led to a dramatic improvement in both activities. Encouraged by the result, we continued further modifications of compound **5d** by removing the phenyl ring to improve its physical properties such as rigidity and lipophilicity. Consequently, this reduction turned out to further enhance the in vitro activity against both species (**7a**; human EC_{50} = 8.5 nM, mouse EC_{50} = 12.2 nM).

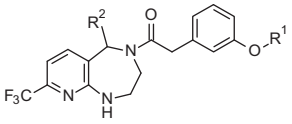
Table 1
EC_{50} values of BRS-3 agonists **1c**, **5a**–**5d** and **7a**



Compound	R	Ring C	Human BRS-3 EC_{50}[a] (nM)	Mouse BRS-3 EC_{50}[a] (nM)
1c ((*S*)-isomer)		7,8-Dimethylphenyl	97[b]	33[b]
5a		Phenyl	>10,000	>10,000
5b		Phenyl	48	227
5c		Phenyl	368	>10,000
5d		Phenyl	25	46
7a		None	8.5	12.2

[a] Data are averages of at least 3 repeated measurements.
[b] Data are cited from Ref. 13a.

Table 2
EC_{50} values of BRS-3 agonists **7a**–**7h**, **11** and **15a**–**15c**



Compound	R1	R2	Human BRS-3 EC_{50}[a] (nM)	Mouse BRS-3 EC_{50}[a] (nM)	Log*D*[b]
7a	Ph	H	8.5	12.2	4.5
7b	*p*-F-Ph	H	3.6	2.7	4.6
7c	*p*-Tolyl	H	1.3	1.0	>4.8
7d		H	9.5	2.8	3.6
7e	*i*-Pr	H	381	569	ND[c]
7f	*n*-Bu	H	59	52	ND[c]
7g	*i*-Bu	H	70	62	4.3
7h		H	199	121	ND[c]
11	Ph	CH_2CO_2H	13	15	1.2
(***R***)**-11**	Ph	(*R*)-CH_2CO_2H	1.6	3.4	1.2
(***S***)**-11**	Ph	(*S*)-CH_2CO_2H	2071	3124	1.2
15a	*p*-F-Ph	(*R*)-CH_2CO_2H	1.7	2.7	1.2
15b	*i*-Bu	(*R*)-CH_2CO_2H	17	62	0.9
15c		(*R*)-CH_2CO_2H	1.7	3.5	0.3

[a] Data are averages of repeated measurements at least 3 times.
[b] The distribution coefficients (log*D*) were measured between 1-octanol and phosphate buffered saline (pH 7.4).
[c] Not determined.

Derivatization of the right terminal phenyl ring of **7a** was focused on next, as described in Table 2, because this part was crucial for in vitro activity from the results in Table 1, and could be efficiently converted by amidation of the diazepine moiety with various carboxylic acids. Also **5b** with a 4-*tert*-butylphenyl group was relatively potent, but we did not derivatize it further in light of its rigidity, lipophilicity and synthetic variety. An increase of potency was observed by the installment of a *para*-fluoro atom on the right phenyl ring (**7b**). Also *para*-tolyl substituent **7c** indicated improved activity with an increase of the log*D* value (>4.8). Meanwhile, the combination of *para*-methyl and 3-pyridyl group **7d** provided an excellent potency with a modest lipophilicity (log*D* = 3.6). We also examined a replacement of the R1 part with aliphatic groups. Relatively small alkyl derivative **7e** decreased the activity presumably because the pharmacophore of the right terminal moiety required a certain size of lipophilic functional group.

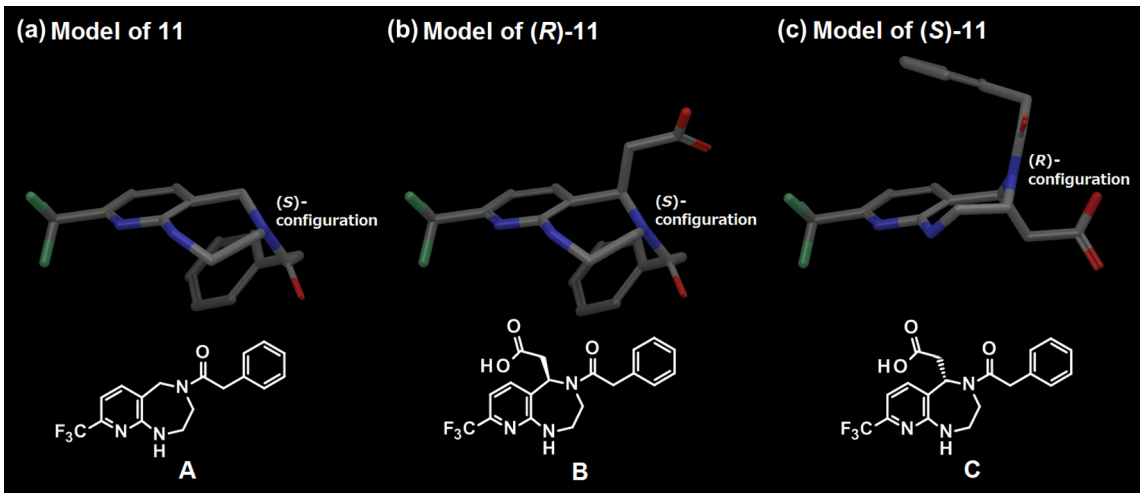


Figure 2. Calculated molecular model structures of compound **A**, **B**, and **C** for the most stable conformers (hydrogen atoms are omitted for clarity).

In fact, in vitro activity was retained in cases where the number of carbons was more than four (**7f**–**7h**). These results suggested **7g** should be excellent in terms of activity and lipophilicity at this stage.

As a next step, an introduction of a polar group onto the molecule was examined to obtain a potent compound with a lowered log*D* value aiming for low brain penetration. After a thorough synthetic examination, we succeeded at finding an acetic acid group at the R2 position that retained potency (**11**). A chiral separation was achieved by using chiral column chromatography to yield each pure enantiomer (***R***)-**11** and (***S***)-**11**. As a result, it turned out that only (***R***)-**11** retained the in vitro activity (compound (***R***)-**11**; human EC_{50} = 1.6 nM, mouse EC_{50} = 3.4 nM). The absolute configuration of (***R***)-**11** was determined by the asymmetric synthesis mentioned in Scheme 3. Sequentially, further SAR studies were pursued to improve potency and to decrease the log*D* value by the introduction of potent functional groups on the R1 position and a carboxyl unit on the R2 position. This combination enabled us to obtain **15a**–**15c** with good activity and low log*D* values, and was especially effective for **15c**, which indicated a good activity in both species with the lowest log*D* value (compound **15c**; human EC_{50} = 1.7 nM, mouse EC_{50} = 3.5 nM, log*D* = 0.3).

To elucidate a significant activity difference between (***R***)-**11** and (***S***)-**11**, we investigated the possible reason for the difference from the aspect of conformers of simplified diazepine models by using computational calculations (Fig. 2). Compound **A**, depicted as a model compound of **11** with no functional group on the 7-membered ring, showed (*S*)-configuration on the nitrogen atom as the lowest energy conformer,[21] while the ring can easily be flipped from (*S*) to (*R*)-configuration (relative potential energy for the inversion: $\Delta E = E(R) - E(S) = 0.022$ kcal/mol). Likewise, compound **B**, regarded as an active form with the (*R*)-acetic acid group on the ring, also provided (*S*)-configuration on the nitrogen atom. Given MK-7725 (**1b**), reported by Chobanian et al., also possessed the active (*S*)-atropisomer on the nitrogen atom, this configuration should be suitable for exerting a high affinity for a BRS-3. Additionally, since the ring inversion of compound **B** is more difficult to ascertain than compound **A** due to the effect of the (*R*)-acetic acid group (ΔE = 2.2 kcal/mol), the activity was supposed to be improved. In contrast, the inactive enantiomer, compound **C** with (*S*)-acetic acid group indicated (*R*)-configuration on the nitrogen atom (ΔE = −2.2 kcal/mol). Meanwhile, we assumed that some diazepine analogs existed as a mixture of conformers according to the ^{1}H NMR spectra because the ring inversion easily occurred under the ambient condition. Thus, we were successful not only at lowering lipophilicity of **7a**, but also at improving its potency by having (*S*)-configuration exist mainly due to the chiral (*R*)-acetic acid group on the neighboring carbon atom of the 7-membered ring.

Next, we decided to incorporate **15b** and **15c** into an antedrug approach to avoid the adverse effect derived from the slightest amount of their exposure to CNS. In particular, we installed various kinds of ester groups, which were easily metabolically hydrolyzed in blood, onto the right terminal moiety because it was suggested by the results of Table 2 that their pharmacophore required a certain size of lipophilic functional group. Thus, the phenol esters **17b**–**17f** and benzoic acid esters **19b**–**19f** were designed with the expectation that they would be rapidly metabolized by reaching into the blood stream after they acted on BRS-3s in the intestine in order to reduce the exposure to the brain as much as possible.

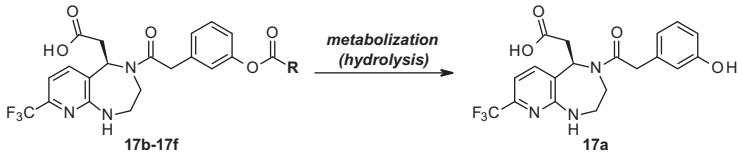
First, we confirmed that the phenol ester **17b**, which was suggested from **15b**, retained the in vitro activity (Table 3), while **17a**, a metabolite from **17b**, had no activity as expected. In contrast, we could not apply this method of ester group installment to the diaryl ether **15c** because it resulted in a complete loss of potency (data not shown). As a next step, ester compounds **17c**–**17f** with various length and bulkiness of alkyl groups were synthesized. It turned out that compounds with more than 4 carbon atoms retained the activity. Similarly, the benzoic acid ester **19b** showed excellent activity in both species, and the metabolite **19a** lost the activity (Table 4). Also, benzoic acid esters **19c**–**19f** were found to possess a similar structure–activity relationship and better potency compared to **17b**–**17f**.

3.2. Pharmacokinetic studies

Pharmacokinetic (PK) parameters of compounds **17b**–**17f** and **19b**–**19f** in mice were evaluated to select the most suitable compound for an antedrug approach: a highly active compound on peripheral BRS-3, and was rapidly metabolized before it reached the systemic circulation (Table 5).

Although the benzoic acid esters **19b**–**19f** indicated better in vitro activity than the phenol esters **17b**–**17f** on the whole, active compounds were also slightly detected (the ratio of Active/Inactive (**A**/**I**) was at least 0.014). On the other hand, the phenol esters **17b**–**17f** provided preferable results: while the active forms of **17d** (*n*-butyl ester) and **17f** (neopentyl ester) were slightly detected (C_{max} of **17d**: 0.0285 μM, **17f**: 0.0058 μM), more labile

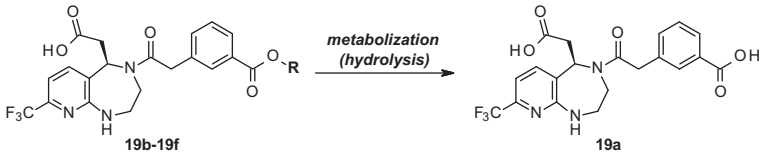
Table 3
EC_{50} values of BRS-3 agonists **15b** and **17a–17f**



Compound	R	Human BRS-3 EC_{50}[a] (nM)	Mouse BRS-3 EC_{50}[a] (nM)
15b	—	17	62
17a	—	>10,000	>10,000
17b	*i*-Pr	68	282
17c	*i*-Br	12	43
17d	*n*-Br	19	35
17e	CH_2c-Pr	42	81
17f	CH_2t-Bu	8.7	20

[a] Data are averages of repeated measurements at least 3 times.

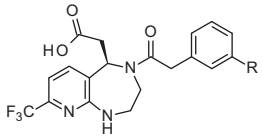
Table 4
EC_{50} values of BRS-3 agonists **19a–19f**



Compound	R	Human BRS-3 EC_{50}[a] (nM)	Mouse BRS-3 EC_{50}[a] (nM)
19a	H	>10,000	>10,000
19b	*i*-Pr	40	45
19c	*i*-Br	4.2	5.4
19d	*n*-Br	4.0	5.6
19e	CH_2c-Pr	3.4	3.7
19f	CH_2t-Bu	5.6	8.1

[a] Data are averages of repeated measurements at least 3 times.

Table 5
Mouse PK parameters of **17b–17f** and **19b–19f**



Active compound (**A**)	Inactive metabolite (**I**)	Mouse PK (po, 30 mg/kg)[a] C_{max} (μM) of **A**	C_{max} (μM) of **I**	Ratio of **A**/**I**
17b (R = O_2CiPr)	**17a** (R = OH)	0[b]	0.68	0[b]
17c (R = O_2CiBu)	**17a** (R = OH)	0[b]	1.26	0[b]
17d (R = O_2CnBu)	**17a** (R = OH)	0.0285	1.63	0.0175
17e (R = O_2CCH_2cPr)	**17a** (R = OH)	0[b]	0.607	0[b]
17f (R = O_2CCH_2tBu)	**17a** (R = OH)	0.0058	0.0325	0.179
19b (R = CO_2iPr)	**19a** (R = CO_2H)	1.24	0.918	1.35
19c (R = CO_2iBu)	**19a** (R = CO_2H)	0.0308	2.12	0.0145
19d (R = CO_2nBu)	**19a** (R = CO_2H)	0.0285	1.63	0.0175
19e (R = CO_2CH_2cPr)	**19a** (R = CO_2H)	0.0393	0.300	0.131
19f (R = CO_2CH_2tBu)	**19a** (R = CO_2H)	0.0127	0.197	0.0643

[a] Average of 2 or 3 mice dosed at 30 mg/kg po. Each dose was administered with 0.5%MC (methyl cellulose) as a solvent.
[b] Under limit of qualification.

esters **17b**, **17c** and **17e** provided no detection of active compounds. Particularly, **17c** possessed the largest amount of exposure of an inactive form among them (C_{max}: 1.26 μM). As shown in Figure 3, the PK profile of **17c** was ideal for an antedrug approach: high exposure of an inactive form and no exposure of an active form in mouse blood. On the other hand, in the case of oral administration of **17a** itself, it was hardly detected at 30 mg/kg (C_{max} = 0.04 μM).[22] Namely, **17c** existed during absorption by the gastrointestinal (GI) tract after oral administration, and then was quickly metabolized by the time it reached the systemic circulation. Therefore, we selected **17c** as a promising antedrug candidate.

Physical properties and PK parameters of compound **17c** and its metabolite **17a** in mice were summarized (Table 6). An active parent compound **17c** showed a low log *D* value, good solubility in JP2

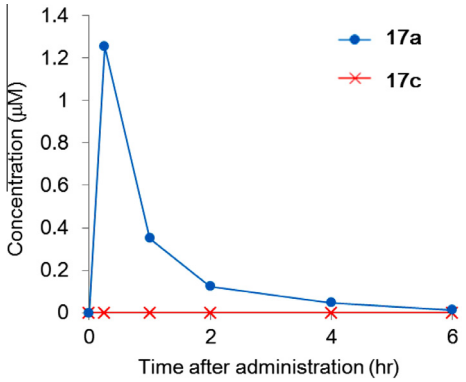


Figure 3. PK profile of **17c** (po, 30 mg/kg in mice, $n = 2$).

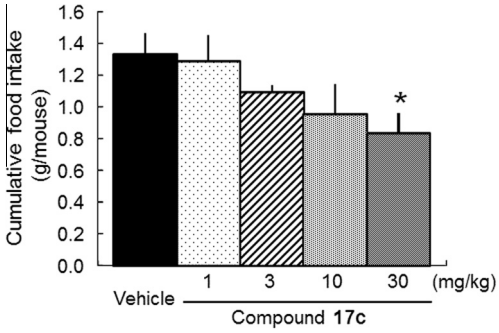


Figure 4. Effect of **17c** on food intake in C57BL/6N mice. Mice were fasted for 16 h and fed normal chow one hour after the compound administration. Cumulative food intake of each mouse over 6 h was measured. Data are mean ± SEM $n = 4$–5. $^{*}P < 0.05$, Dunnett's test.

(Japanese pharmacopoeia second test fluid, pH = 6.8), and low protein binding (PB) ability. We presumed that these properties could enhance drug efficacy of **17c** incorporating an antedrug approach as seen in Table 5. Fairly high polar surface area (PSA) values of **17a** and **17c** are also preferable to avoid acting on BRS-3 in the CNS from the aspect of lowering brain-blood barrier penetration.[23]

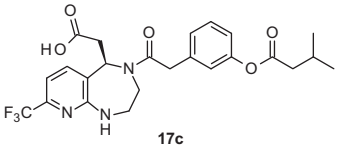
3.3. In vivo pharmacological evaluation for food-intake suppression in mice

From the most preferable PK profile of **17c** in mice as an antedrug, we evaluated the effect of **17c** on food intake in B6 mice to confirm anti-obesity efficacy as a peripheral BRS-3 agonist (Fig. 4). When 1, 3, 10, 30 mg/kg of the ester **17c** was orally administered to mice at a single dose, **17c** showed a tendency of a dose-dependent anorectic effect and the 30 mg/kg group showed a statistically significant reduction of food intake ($P < 0.05$) compared to the vehicle-treated group. We also confirmed these effects were not observed in BRS-3 deficient mice as a preliminary data. Taken together, the novel chiral diazepine **17c** was found to possess a food-intake suppression effect by stimulating peripheral BRS-3. Since a single dose of **17c** at 30 mg/kg resulted in about 30% food intake suppression, it is expected that a repeated administration at the same dose would show an anti-obesity effect from the perspective of a suppressant for energy intake, and the effect was supposed to be derived from BRS-3 activation in the intestine due to the absence of the detection of the active compound **17c** in blood. Also, this result suggested that a part of the food intake suppression observed in brain penetrant BRS-3 agonists such as **1a** (MK-5046) and **1b** (MK-7725) was comprised of the activation of BRS-3 in the intestine.

3.4. Cardiovascular safety evaluation in dogs

We turned our attention to a cardiovascular (CV) safety evaluation of **17c** using halothane anaesthetized dogs by iv infusion (Fig. 5). In this test, **15b** with low brain penetration[24] and no labile ester group as a counterpart of **17c** was also evaluated to confirm the value of an antedrug approach aiming for removing CV change including heart rate (HR) and blood pressure (BP) because CV change is used as typical and reliable index for the CNS adverse effects.[10] Each compound was administered to 2 dogs at 3 mg/kg/3 mL for 30 min by iv infusion. In case of **15b**, both dogs showed the increase of HR and BP by the time the infusion was almost finished (30 min). Since the dog shown as animal #2 especially indicated a sharp increase of HR and seemed to have difficulty with anesthetic maintenance, we had to stop the experiment at 48 min. Blood concentration of **15b** and **17c** at 30 min and 60 min was also measured. At 30 min, **15b** indicated relatively high blood concentration compared to **17c** (**15b**; animal #1 : 16.85 μM, #2 : 18.67 μM, **17b**; animal #1 : 1.73 μM, #2 : 1.39 μM), and the

Table 6
Physical properties and mouse PK parameters of **17c** and its metabolite **17a**



17c

Compound	In vitro EC_{50} (human/mouse, nM)	Log D[a]	Solubility JP1/JP2 (μM/mL)[b]	PB free[c] (%)	PSA[d]	T_{max}[e] (h)	C_{max}[e] (μg/mL)	AUC[e] (hr * μg/mL)	$T_{1/2}$[e] (h)
17c (active compound)	12/43	0.7	62/900	10.8	101.5	ND[f]	LOQ[g]	LOQ[g]	LOQ[g]
17a (inactive meabolite)	>10,000/>10,000	−0.8	750/780	>30.0	101.0	0.25[h]	1.26[h]	1.23[h]	0.25[h]

[a] The distribution coefficients (log D) were measured between 1-octanol and phosphate buffered saline (pH 7.4).
[b] JP1/JP2: Japanese pharmacopoeia first/second test fluid (pH = 1.2/6.8).
[c] Unbound fractions (%) in mouse plasma. PB: Protein Binding.
[d] Polar surface area (Å^2).
[e] Average of 2 mice dosed at 30 mg/kg po. Each dose was administered with 0.5% MC (methyl cellulose) as a solvent.
[f] Not determined.
[g] Under limit of qualification.
[h] Each PK parameter of **17a** was observed in the administration of **17c** at 30 mg/kg po.

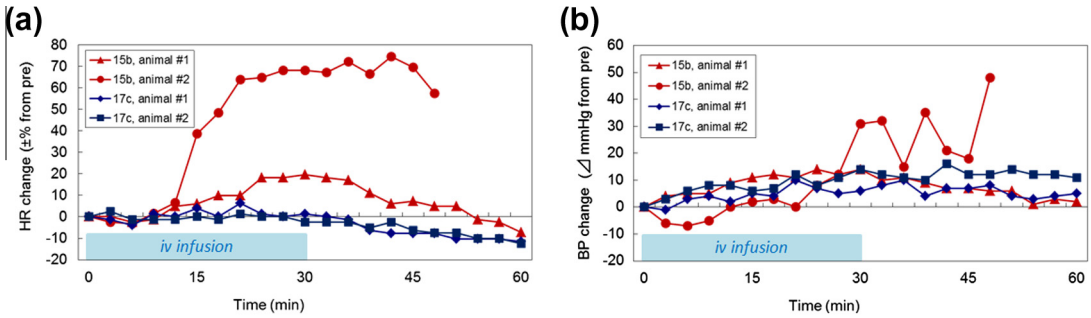


Figure 5. Effect of **15b** (animal #1, #2, red) and **17c** (animal #1, #2, blue) on (a) heart rate (HR) and (b) blood pressure (BP) in dogs (n = 2/group). The compound **15b** and antedrug **17c** were administered to halothane anaesthetized dogs at 3 mg/kg/3 mL for 30 min by iv infusion (the duration of the iv infusion is shown as a light blue zone). Cardiovascular (CV) change was normalized by the pre-administration values (at 0 min) for the heart rate and blood pressure. The observation of the animal #2 was forcibly stopped at 48 min (18 min after completing dosage) due to the difficulty of anesthetic maintenance.

measurement at 60 min provided similar results (**15b**; animal #1 : 1.01 μM, #2 : 1.28 μM, **17b**; animal #1 : 0 μM, #2 : 0 μM). This suggested that the antedrug approach installed into **17c** worked well as we expected: the terminal ester group of **17c** was rapidly hydrolyzed during and after iv infusion in dogs as well, and an unnecessary accumulation of **17c** in the systemic circulation including exposure to CNS was avoided. On the other hand, given the low brain penetration of **15b**, minimizing brain penetration alone might be insufficient for avoiding CV change. Actually, the HR was not affected at all by **17c** infusion in contrast to the abrupt increase in the case of **15b**, and also **17c** did not show a clear BP change during and after iv infusion. For these reasons, it can be said that the antedrug approach applied to **17c** is certainly effective for obtaining a potent peripheral BRS-3 agonist without an adverse effect derived from acting on CNS.

4. Conclusion

In summary, we obtained a chiral diazepine **17c** as a novel and potent BRS-3 agonist incorporating metabolic inactivation. Through a strenuous synthetic exploration, development of an efficient chiral synthetic route, and adopting the antedrug (metabolic inactivation) approach with an installment of ester groups onto an optimal position, we were able to concurrently achieve both an anorectic effect and a reduction of the risk of adverse effects derived from CNS by selective stimulation of peripheral BRS-3s. In fact, **17c** showed statistically significant food intake suppression in mice, and no clear adverse effect such as heart rate or blood pressure change, arose from the CNS in dog iv infusion. These results have provided an initial first step for developing a highly-anticipated drug with a safer profile for the treatment of diabetes and obesity, as well as valuable insight about a physiological effect due to the activation of BRS-3 in the intestine.

5. Experimental

5.1. Chemistry

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. NMR spectra were recorded on a Varian Mercury 400 or 500 spectrometer with tetramethylsilane as an internal reference. Mass spectra were recorded on a Agilent Technologies Agilent 1100 series LC/MS. Optical rotations were measured on an Autopol V Plus. TLC analysis was performed on 60F254 plates (Merck). Flash column chromatography was performed on Shoko scientific cartridge series (SI-60). The following abbreviations are used: AcOEt, ethylacetate; MeOH, methanol; EtOH, ethanol; THF, tetrahydrofurane; DMF, *N*,*N*-dimethylformamide; NMP, *N*-methylpyrrolidinone; HATU, (dimethylamino)-*N*,*N*-dimethyl(3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yloxy)methaniminium hexafluorophosphate; DMT-MM, (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride *n*-hydrate.

5.1.1. 2-(Trifluoromethyl)-6,11-dihydro-5*H*-pyrido[3,2-*c*][1,5]benzodiazepine (4)

A mixture of 2-chloro-6-(trifluoromethyl)pyridine-3-carboxylic acid **3** (400 mg, 1.77 mmol) and 1,2-diaminobenzene (196 mg. 1.81 mmol) in ethylene glycol monobutylether (5 mL) was stirred at 160 °C for 6 h. The cooled reaction mixture was diluted with water, and the resulting suspension was filtered off. The obtained solid was washed with water and dissolved in AcOEt (30 mL). The organic solution was washed with brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (hexane/AcOEt = 1:1) to provide the amide (317 mg, 64%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 8.42 (1H, d, J = 8.2 Hz), 7.54 (1H, br s), 7.24 (1H, d, J = 7.8 Hz), 7.10–7.03 (2H, m), 6.91–6.89 (1H, m), 6.87–6.85 (1H, m), 6.68 (1H, br s). A solution of borane–THF complex in THF (1.0 M, 3.3 mL, 3.3 mmol) was added to a solution of the obtained amide (307 mg, 1.10 mmol) in THF (5 mL) under N_2 atmosphere at 0 °C, and the mixture was stirred at the same temperature for 2 h. The reaction mixture was raised to room temperature and further stirred for 16 h. After addition of methanol (1 mL), the reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to provide **4** (192 mg, 66%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 7.37 (1H, d, J = 7.4 Hz), 7.22 (1H, br s), 7.00 (1H, d, J = 7.4 Hz), 6.91–6.87 (1H, m), 6.85–6.81 (2H, m), 6.76 (1H, d, J = 7.4 Hz), 4.23–4.19 (3H, m).

5.1.2. (4-*tert*-Butylphenyl)-[2-(trifluoromethyl)-5,11-dihydropyrido[3,2-*c*][1,5]benzodiazepin-6-yl]methanone (5a)

4-*tert*-Butylbenzoyl chloride (103 μL, 0.57 mmol) was added to a solution of **4** (100 mg, 0.38 mmol) and triethylamine (158 μL, 1.13 mmol) in CH_2Cl_2 (5 mL) at room temperature, and the reaction mixture was stirred for 3 h. The mixture was diluted with CH_2Cl_2 (30 mL), washed with brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (hexane/AcOEt = 3:1). The obtained solid was triturated in hexane/CH_2Cl_2, and filtered to provide **5a** (116 mg, 72%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 7.67 (1H, br s), 7.45 (1H, s), 7.19–7.09 (6H, m), 6.98 (1H, d, J = 7.8 Hz), 6.70–6.68 (2H, m), 5.89–5.85 (1H, m), 4.10–4.06 (1H, m), 1.22 (9H, s). MS (ESI$^+$) m/z: 426 ((M+H)$^+$.

5.1.3. 2-(4-*tert*-Butylphenyl)-1-[2-(trifluoromethyl)-5,11-dihydropyrido[3,2-*c*][1,5]benzodiazepin-6-yl]ethanone (5b)

4-*tert*-Butylphenylactic acid (75 mg, 0.39 mmol) was added to a solution of the diazepine **4** (80 mg, 0.30 mmol), HATU (126 mg,

0.33 mmol) and (*i*-Pr)$_2$EtN (165 μL, 0.90 mmol) in CH_2Cl_2 (5 mL) at room temperature, and the reaction mixture was stirred overnight. The mixture was diluted with CH_2Cl_2 (20 mL), washed with saturated $NaHCO_3$ aq and brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (hexane/AcOEt = 3:1). The obtained solid was triturated in hexane/CH_2Cl_2, and filtered to provide **5b** (120 mg, 91%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 7.59 (1H, d, J = 7.4 Hz), 7.22–7.16 (2H, m), 7.13–7.09 (2H, m), 7.05 (1H, d, J = 7.4 Hz), 6.99–6.95 (2H, m), 6.81 (1H, dd, J = 8.0, 1.4 Hz), 6.73–6.70 (2H, m), 5.40 (1H, d, J = 15.2 Hz), 3.86 (1H, d, J = 14.9 Hz), 3.52 (2H, s), 1.24 (9H, s). MS (ESI$^+$) m/z: 440 (M+H)$^+$.

5.1.4. 2-(4-Phenoxyphenyl)-1-[2-(trifluoromethyl)-5,11-dihydropyrido[3,2-*c*][1,5]benzodiazepin-6-yl]ethanone (5c)

Compound **5c** was prepared in a similar manner described for **5b**. Yield: 92%. ^{1}H NMR ($CDCl_3$) δ: 7.64 (1H, d, J = 7.4 Hz), 7.34–7.29 (2H, m), 7.25–7.21 (3H, m), 7.10–7.07 (2H, m), 7.03–7.01 (1H, m), 6.98–6.96 (2H, m), 6.91 (1H, dd, J = 8.0, 1.4 Hz), 6.82–6.77 (4H, m), 5.45 (1H, d, J = 14.9 Hz), 3.94 (1H, d, J = 14.5 Hz), 3.57 (2H, s). MS (ESI$^+$) m/z: 476 ((M+H)$^+$.

5.1.5. 2-(3-Phenoxyphenyl)-1-[2-(trifluoromethyl)-5,11-dihydropyrido[3,2-*c*][1,5]benzodiazepin-6-yl]ethanone (5d)

Compound **5d** was prepared in a similar manner described for **5b**. Yield: 89%. ^{1}H NMR ($CDCl_3$) δ: 7.63 (1H, d, J = 7.8 Hz), 7.32–7.28 (2H, m), 7.24–7.17 (2H, m), 7.12–7.07 (4H, m), 6.99–6.95 (1H, m), 6.92 (2H, d, J = 8.6 Hz), 6.85 (1H, d, J = 8.2 Hz), 6.80 (1H, d, J = 8.2 Hz), 6.61 (1H, d, J = 8.2 Hz), 6.48 (1H, s), 5.45 (1H, d, J = 14.9 Hz), 3.93 (1H, d, J = 14.9 Hz), 3.57 (2H, s). MS (ESI$^+$) m/z: 476 ((M+H)$^+$.

5.1.6. 8-(Trifluoromethyl)-2,3,4,5-tetrahydro-1*H*-pyrido[2,3-*e*][1,4]diazepine (6)

A solution of borane–THF complex in THF (0.9 M, 70 mL, 63 mmol) was added to a solution of 2-chloro-6-(trifluoromethyl)pyridine-3-carboxylic acid (5.10 g, 22.1 mmol) in THF (100 mL) under N_2 atmosphere at 0 °C, then the mixture was gradually raised to room temperature and stirred for 20 h. After addition of methanol (10 mL), the reaction mixture was diluted with AcOEt (100 mL), washed with brine, dried (Na_2SO_4), concentrated under reduced pressure and the residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to obtain the alcohol (4.66 g, quant.) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 8.12 (1H, d, J = 7.8 Hz), 7.69 (1H, d, J = 7.8 Hz), 4.87 (2H, d, J = 5.5 Hz), 2.06 (1H, t, J = 5.5 Hz). The obtained alcohol (42.4 g, 201 mmol) was dissolved in ethylenediamine (161 ml, 1.20 mol), and the reaction mixture was stirred at 120 °C for 12 h. The cooled mixture was diluted with brine, extracted with AcOEt (300 mL × 3). The organic layer was dried over Na_2SO_4, concentrated and purified by silica gel column chromatography (hexane/AcOEt = 3:1) to provide the amino alcohol (29.4 g, 62%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.33 (1H, d, J = 7.4 Hz), 6.85 (1H, d, J = 7.4 Hz), 6.03 (1H, br s), 4.59 (2H, s), 3.72 (2H, q, J = 5.9 Hz), 3.48 (2H, t, J = 6.3 Hz). MnO_2 (98.8 g, 1.00 mol) was added to a solution of the amino alcohol (29.4 g, 125 mmol) in CH_2Cl_2/MeOH (700 mL/100 mL), and the reaction mixture was stirred at room temperature for 2 h. The mixture was filtered through a Celite pad, concentrated to provide the imine (20.4 g, 76%). ^{1}H NMR ($CDCl_3$) δ: 8.27 (1H, t, J = 1.6 Hz), 7.75 (1H, d, J = 7.8 Hz), 7.04 (1H, d, J = 7.8 Hz), 5.99 (1H, br s), 4.14–4.11 (2H, m), 3.49–3.46 (2H, m). 10% Pd/C (8.00 g) was added to a solution of the imine (20.4 g, 94.8 mmol) in AcOEt (250 mL), and the reaction mixture was stirred under H_2 atmosphere at room temperature for 4 h. The mixture was filtered through a Celite pad, concentrated, and purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 1:8) to provide the diazepine **6** (16.9 g, 82%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.46 (1H, d, J = 7.4 Hz), 7.07 (1H, d, J = 7.4 Hz), 5.11 (1H, s), 3.91 (2H, br s), 3.49 (1H, s), 3.28–3.25 (2H, m), 3.11–3.09 (2H, m).

5.1.7. 2-(3-Phenoxyphenyl)-1-[8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-4-yl]ethanone (7a)

2-(3-Phenoxyphenyl)acetic acid (41 mg, 0.18 mmol) was added to a solution of the diazepine **6** (30 mg, 0.14 mmol), HATU (63 mg, 0.17 mmol) and (*i*-Pr)$_2$EtN (75 μL, 0.48 mmol) in CH_2Cl_2 (3 mL) at room temperature, and the reaction mixture was stirred for 15 h. The mixture was diluted with CH_2Cl_2 (20 mL), washed with brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (hexane/AcOEt = 2:3). The obtained solid was triturated in hexane/CH_2Cl_2, and filtered to provide **7a** (49 mg, 85%) as a colorless solid. ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.66–7.30 (3H, m), 7.25–7.10 (3H, m), 6.98–6.83 (5H, m), 5.21 (0.4H, br s), 5.03 (0.6H, br s), 4.61 (1.2H, s), 4.48 (0.8H, s), 3.91–3.88 (0.8H, m), 3.76–3.74 (1.2H, m), 3.73 (0.8H, s), 3.70 (1.2H, s), 3.44–3.40 (0.8H, m), 3.31–3.27 (1.2H, m). MS (ESI$^+$) m/z: 428 ((M+H)$^+$.

5.1.8. 2-[3-(4-Fluorophenoxy)phenyl]-1-[8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-4-yl]ethanone (7b)

Compound **7b** was prepared in a similar manner described for **7a**. Yield: 89%. ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.65 (0.6H, d, J = 7.3 Hz), 7.25–7.09 (2H, m), 7.05–6.88 (5.4H, m), 6.83–6.78 (2H, m), 5.21 (0.4H, br s), 5.03 (0.6H, br s), 4.61 (1.2H, s), 4.48 (0.8H, s), 3.92–3.89 (0.8H, m), 3.76–3.74 (1.2H, m), 3.74 (0.8H, s), 3.71 (1.2H, s), 3.44–3.41 (0.8H, m), 3.32–3.29 (1.2H, m). MS (ESI$^+$) m/z: 446 ((M+H)$^+$

5.1.9. 2-[3-(4-Methylphenoxy)phenyl]-1-[8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-4-yl]ethanone (7c)

Compound **7c** was prepared in a similar manner described for **7a**. Yield: 96%. ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.65 (0.6H, d, J = 7.4 Hz), 7.24–7.09 (4H, m), 7.00–6.81 (5.4H, m), 5.21 (0.4H, br s), 5.02 (0.6H, br s), 4.60 (1.2H, s), 4.47 (0.8H, s), 3.90–3.88 (0.8H, m), 3.74–3.72 (1.2H, m), 3.72 (0.8H, s), 3.72–3.70 (2H, m), 3.44–3.26 (1.2H, m), 2.34–2.33 (3H, m). MS (ESI$^+$) m/z: 442 ((M+H)$^+$.

5.1.10. 2-[3-[(6-Methyl-3-pyridyl)oxy]phenyl]-1-[8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-4-yl]ethanone (7d)

The synthesis of **7d** was prepared in a similar manner described for **7a**. Yield: 34%. ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 8.28–8.26 (1H, m), 7.64 (0.6H, d, J = 7.4 Hz), 7.24–7.09 (3.4H, m), 7.00–6.82 (4H, m), 5.22 (0.4H, br s), 5.04 (0.6H, br s), 4.62 (1.2H, s), 4.48 (0.8H, s), 3.92–3.89 (0.8H, m), 3.76–3.74 (1.2H, m), 3.72 (0.8H, s), 3.70 (1.2H, s), 3.46–3.43 (0.8H, m), 3.35–3.32 (1.2H, m), 2.54 (3H, s). MS (ESI$^+$) m/z: 443 ((M+H)$^+$.

5.1.11. 2-(3-Isopropoxyphenyl)-1-[8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-4-yl]ethanone (7e)

The diazepine **6** (230 mg, 1.06 mmol) was added to a solution of [3-(benzyloxy)phenyl]acetic acid (256 mg, 1.06 mmol), HATU (443 mg, 1.16 mmol) and (*i*-Pr)$_2$EtN (578 μL, 3.18 mmol) in CH_2Cl_2 (10 mL) at room temperature, and the reaction mixture was stirred for 15 h. The mixture was diluted with CH_2Cl_2 (50 mL), washed with saturated $NaHCO_3$ aq and brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (hexane/AcOEt = 7:3) to provide the benzyl compound (465 mg, 99%) as a colorless solid. ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.67 (0.6H, d, J = 7.8 Hz), 7.40–7.32 (5.4H, m), 7.23–7.09 (2H, m), 6.93–6.77 (3H, m), 5.19 (0.4H, s), 5.03–4.99 (2.6H, m), 4.60 (1.2H, s), 4.43 (0.8H, s), 3.91–3.89 (0.8H, m), 3.72–3.70 (3.2H, m), 3.46–3.43 (0.8H, m), 3.25–3.22 (1.2H, m). 10% Pd/C (140 mg) was added to a solution of the benzyl compound (465 mg, 1.05 mmol) in EtOH

(15 mL), and the reaction mixture was stirred under H_2 atmosphere at room temperature for 4 h. The mixture was filtered through a Celite pad and concentrated to provide the phenol compound (361 mg, 98%) as a colorless solid. ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.66 (0.6H, d, J = 7.4 Hz), 7.21–7.14 (1.4H, m), 7.08–6.97 (1H, m), 6.74–6.70 (3H, m), 5.26 (0.4H, br s), 5.04 (0.6H, br s), 4.60 (1.2H, s), 4.49 (0.8H, s), 3.91–3.88 (0.8H, m), 3.77–3.74 (1.2H, m), 3.70–3.68 (2H, m), 3.46–3.43 (0.8H, m), 3.31–3.27 (1.2H, m). The phenol compound (60 mg, 0.17 mmol) was dissolved in a mixture of 2-iodopropane (34 μL, 0.34 mmol) and K_2CO_3 (35 mg, 0.26 mmol) in DMF (2 mL), and the reaction mixture was stirred at 80 °C for 5 h. The mixture was diluted with Et_2O (30 mL), washed with brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 1:9). The obtained solid was triturated in hexane/Et_2O, and filtered to provide **7e** (28 mg, 42%) as a colorless solid. ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.68 (0.6H, d, J = 7.4 Hz), 7.17–7.13 (1.4H, m), 7.10 (0.6H, d, J = 7.4 Hz), 6.95 (0.4H, d, J = 7.4 Hz), 6.73 (3H, m), 5.20 (0.4H, br s), 4.99 (0.6H, br s), 4.61 (1.2H, s), 4.48–4.45 (1.8H, m), 3.93–3.90 (0.8H, m), 3.75–3.72 (1.2H, m), 3.72 (0.8H, s), 3.70 (1.2H, s), 3.47–3.44 (0.8H, m), 3.21–3.18 (1.2H, m), 1.29 (2.4H, d, J = 5.9 Hz), 1.25 (3.6H, d, J = 5.9 Hz). MS (ESI$^+$) m/z: 394 ((M+H)$^+$.

5.1.12. 2-(3-Butoxyphenyl)-1-[8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-4-yl]ethanone (7f)

The phenol compound obtained in the procedure of **7e** (60 mg, 0.17 mmol) was dissolved in a mixture of 1-iodobutane (29 μL, 0.16 mmol) and K_2CO_3 (35 mg, 0.26 mmol) in DMF (2 mL), and the reaction mixture was stirred at 80 °C for 3 h. The mixture was diluted with Et_2O (30 mL), washed with brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (hexane/AcOEt = 7:3). The obtained solid was triturated in hexane/Et_2O, and filtered to provide **7f** (58 mg, 83%) as a colorless solid. ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.67 (0.6H, d, J = 7.8 Hz), 7.19 (1.4H, t, J = 7.8 Hz), 7.10 (0.6H, d, J = 7.3 Hz), 6.95 (0.4H, d, J = 7.8 Hz), 6.77–6.71 (3H, m), 5.20 (0.4H, br s), 5.00 (0.6H, br s), 4.61 (1.2H, s), 4.47–4.44 (0.8H, s), 3.95–3.81 (2.8H, m), 3.76–3.73 (1.2H, m), 3.72 (0.8H, s), 3.70 (1.2H, s), 3.46–3.44 (0.8H, m), 3.24–3.21 (1.2H, m), 1.76–1.68 (2H, m), 1.50–1.41 (2H, m), 0.98–0.94 (3H, m). MS (ESI$^+$) m/z: 408 ((M+H)$^+$.

5.1.13. 2-(3-Isobutoxyphenyl)-1-[8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-4-yl]ethanone (7g)

Compound **7g** was prepared in a similar manner described for **7f**. Yield: 31%. ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.67 (0.6H, d, J = 7.4 Hz), 7.22–7.17 (1.4H, m), 7.10 (0.6H, d, J = 7.4 Hz), 6.97 (0.4H, d, J = 7.4 Hz), 6.78–6.72 (3H, m), 5.20 (0.4H, br s), 5.01 (0.6H, br s), 4.61 (1.2H, s), 4.48 (0.8H, s), 3.92–3.90 (0.8H, m), 3.75–3.73 (1.2H, m), 3.72 (0.8H, s), 3.70 (1.2H, s), 3.52–3.50 (2H, m), 3.48–3.44 (0.8H, m), 3.26–3.22 (1.2H, m), 2.02 (1H, m), 1.00 (6H, d, J = 8.2 Hz). MS (ESI$^+$) m/z: 408 ((M+H)$^+$.

5.1.14. 2-[3-(2,2-Dimethylpropoxy)phenyl]-1-[8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-4-yl]ethanone (7h)

Compound **7h** was prepared in a similar manner described for **7f**. Yield: 39%. ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.67 (0.6H, d, J = 7.4 Hz), 7.22–7.17 (1.4H, m), 7.10 (0.6H, d, J = 7.4 Hz), 6.97 (0.4H, d, J = 7.4 Hz), 6.78–6.72 (3H, m), 5.20 (0.4H, br s), 5.01 (0.6H, br s), 4.61 (1.2H, s), 4.48 (0.8H, s), 3.93–3.91 (0.8H, m), 3.76–3.74 (1.2H, m), 3.72 (0.8H, s), 3.70 (1.2H, s), 3.52–3.50 (2H, m), 3.48–3.44 (0.8H, m), 3.26–3.22 (1.2H, m), 1.01 (3.6H, s), 0.99 (5.4H, s). MS (ESI$^+$) m/z: 422 ((M+H)$^+$.

5.1.15. *tert*-Butyl *N*-[2-[1-[2-chloro-6-(trifluoromethyl)-3-pyridyl]but-3-enylamino]ethyl]carbamate (9)

2-Chloro-6-(trifluoromethyl)pyridine-3-carbaldehyde **8** (11.96 g, 57.1 mmol) was added to a solution of *N*-Boc ethylenediamine (5.83 mL, 58.2 mmol) and Na_2SO_4 (50 g) in CH_2Cl_2 (400 mL) at room temperature, and the reaction mixture was stirred for 17 h. After the reaction mixture was concentrated, the residue was purified by silica gel column chromatography (hexane/AcOEt = 3:2) to provide the imine (13.87 g, 69%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 8.67 (1H, s), 8.53 (1H, d, J = 7.8 Hz), 7.68 (1H, d, J = 7.8 Hz), 4.79 (1H, br s), 3.83 (2H, t, J = 5.5 Hz), 3.50 (2H, q, J = 5.7 Hz), 1.44 (9H, s). A solution of allylmagnesium bromide in Et_2O (0.7 M, 60 mL, 42 mmol) was added to a solution of the imine (13.44 g, 38.2 mmol) in THF (150 mL) under N_2 atmosphere at 0 °C, and the mixture was stirred at the same temperature for 45 min. Then, the reaction mixture was diluted with H_2O (200 mL), extracted with AcOEt (400 mL), washed with brine, dried (Na_2SO_4), concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 7:3) to provide **9** (10.91 g, 73%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 8.12 (1H, d, J = 8.2 Hz), 7.64 (1H, d, J = 7.8 Hz), 5.82–5.71 (1H, m), 5.17–5.11 (2H, m), 4.72 (1H, br s), 4.21–4.10 (2H, m), 3.21–3.14 (2H, m), 2.65–2.59 (1H, m), 2.57–2.52 (1H, m), 2.47–2.41 (1H, m), 2.24–2.16 (1H, m), 1.44 (9H, s).

5.1.16. *tert*-Butyl 5-allyl-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepine-4-carboxylate (10)

Compound **9** (12.22 g, 31.0 mmol) was dissolved in 4 N HCl/AcOEt (50 mL) at room temperature, and the reaction mixture was stirred for 5 h. The mixture was concentrated and triturated in (i-Pr)$_2$O (100 mL). Then, after the suspension was filtered off, the obtained solid was washed with (i-Pr)$_2$O and dried in vacuo to provide the diamine HCl salt (10.3 g). The obtained diamine HCl salt (10.3 g) was added to a solution of (i-Pr)$_2$EtN (15.0 mL, 82.2 mmol) in NMP (100 mL) at 160 °C, and the reaction mixture was stirred for 8 h. After the reaction mixture was diluted with AcOEt/Et_2O (150 mL/150 mL), washed with brine (300 mL), dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 3:7) to provide the diazepine including NMP (11.8 g) as a brown oil. Di-*tert*-butyl dicarbonate (7.98 g, 36.5 mmol) was added to a solution of the diazepine including NMP (11.8 g) and triethylamine (12.7 mL, 91.4 mmol) in CH_2Cl_2 (80 mL) at room temperature, and the reaction mixture was stirred for 15 h. Then, the reaction mixture was diluted with H_2O (200 mL), extracted with Et_2O (200 mL × 2), washed with brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (hexane/AcOEt = 4:1) to provide **10** (6.53 g, 59%, 3 steps) as a yellow oil. ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.56 (0.5H, d, J = 7.4 Hz), 7.44 (0.5H, d, J = 7.4 Hz), 7.10 (1H, d, J = 7.4 Hz), 5.68–5.59 (1H, m), 5.33 (0.5H, br s), 5.08–4.99 (3.5H, m), 4.14–4.00 (1H, m), 3.38–3.31 (2H, m), 3.19–3.08 (1H, m), 2.86–2.72 (1H, m), 2.53–2.46 (1H, m), 1.45 (9H, s).

5.1.17. 2-[4-[2-(3-Phenoxyphenyl)acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (11)

A mixture of **10** (550 mg, 1.54 mmol), 2.5% OsO_4 in i-PrOH (20 μL 20 μmol), and *N*-methylmorpholine oxide (360 mg. 3.08 mmol) in acetone (15 mL) was stirred at room temperature for 18 h. The mixture was diluted with AcOEt (30 mL), washed with brine, dried (Na_2SO_4), concentrated to provide the diol as brown oil (600 mg). A mixture of the diol (600 mg), sodium periodate (491 mg. 2.30 mmol) in THF/H_2O (9 mL/3 mL) was stirred at room temperature for 2 h. The mixture was diluted with AcOEt (30 mL), washed with brine, dried (Na_2SO_4), concentrated in vacuo to

provide the aldehyde as colorless oil (495 mg). A mixture of the obtained aldehyde, $NaH_2PO_4{\cdot}2H_2O$ (2.15 g, 13.8 mmol), $NaClO_2$ (312 mg, 3.45 mmol), 2-methyl-2-butene (7.33 mL, 69.0 mmol) in *t*-BuOH/H_2O (5 mL/5 mL) was stirred at room temperature for 2 h. After concentration, the residue was diluted with AcOEt (50 mL × 2), washed with brine, dried (Na_2SO_4), concentrated in vacuo to provide the carboxylic acid as brown oil (560 mg). A mixture of the obtained carboxylic acid, iodomethane (1.82 mL, 2.93 mmol), K_2CO_3 (303 mg, 2.20 mmol) in DMF (8 mL) was stirred at 50 °C for 4 h. Then, the reaction mixture was diluted with H_2O (30 mL), extracted with Et_2O (30 mL × 2), washed with brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (hexane/AcOEt = 2:1) to provide the methyl ester (460 mg, 77%, 4 steps) as a brown oil. ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.72–7.59 (1H, m), 7.13 (1H, d, *J* = 7.4 Hz), 5.76 (0.4H, s), 5.57 (0.6H, s), 5.02–4.98 (1H, m), 4.12–3.99 (2H, m), 3.63 (3H, s), 3.50–3.33 (2H, m), 3.18–2.99 (2H, m), 1.44 (9H, s). The methyl ester (460 mg, 1.18 mmol) was dissolved in 4 N HCl/AcOEt (5 mL) and stirred at room temperature for 2 h. (*i*-Pr)$_2$O (30 mL) was added to the resulting suspension. The deposited HCl salt was separated by filtration and dried in vacuo to provide the diamine HCl salt as colorless solid (395 mg, 92%). ^{1}H NMR (CD_3OD) δ: 7.85 (1H, d, *J* = 7.8 Hz), 7.26 (1H, d, *J* = 7.8 Hz), 5.10 (1H, t, *J* = 7.0 Hz), 3.72 (3H, s), 3.57–3.40 (4H, m), 3.24–3.13 (2H, m). A mixture of the diazepine (300 mg, 0.83 mmol), 2-(3-phenoxyphenyl)acetic acid (236 mg, 1.04 mmol), (*i*-Pr)$_2$EtN (0.60 mL, 3.31 mmol), HATU (378 mg, 0.99 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature for 17 h. The mixture was diluted in CH_2Cl_2 (20 mL), washed with saturated $NaHCO_3$ aq and brine, dried (Na_2SO_4), concentrated and purified by column chromatography (AcOEt/hexane = 1:1) to provide the acylated methyl ester as colorless oil (375 mg, 91%). ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.77 (0.6H, d, *J* = 7.8 Hz), 7.33–7.29 (2H, m), 7.25–7.23 (1.2H, m), 7.16–7.07 (1.2H, m), 7.00–6.77 (6H, m), 6.17 (0.6H, t, *J* = 7.8 Hz), 5.44 (0.4H, t, *J* = 7.6 Hz), 5.08 (0.4H, s), 4.92 (0.6H, s), 4.70 (0.6H, d, *J* = 14.5 Hz), 4.04–3.90 (1H, m), 3.85–3.67 (2.4H, m), 3.64–3.60 (3H, m), 3.40–3.28 (1H, m), 3.21–2.70 (3H, m). A mixture of the acylated methyl ester (200 mg, 0.40 mmol), 2 N NaOH aq (4 mL) in THF/MeOH (2 mL/2 mL) was stirred at room temperature for 2 h. The mixture was acidified with 2 N HCl aq and extracted with AcOEt. The organic layer was washed with brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 1:12). The obtained solid was triturated in Et_2O/hexane, filtered and dried in vacuo to provide **11** as colorless solid (183 mg, 94%). ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.77 (0.6H, d, *J* = 7.4 Hz), 7.33–7.28 (2H, m), 7.25–7.21 (1.2H, m), 7.15–7.06 (1.2H, m), 6.97–6.76 (6H, m), 6.16 (0.6H, t, *J* = 7.4 Hz), 5.41 (0.4H, t, *J* = 7.6 Hz), 5.15 (0.4H, s), 5.02 (0.6H, s), 4.71 (0.6H, d, *J* = 14.5 Hz), 3.97 (1H, m), 3.84–3.63 (2.4H, m), 3.40–3.27 (1H, m), 3.21–2.70 (3H, m). MS (ESI$^+$) *m*/*z*: 486 ((M+H)$^+$.

5.1.18. 2-[(5*R*)-4-[2-(3-Phenoxyphenyl)acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid ((*R*)-11) and 2-[(5*S*)-4-[2-(3-phenoxyphenyl)acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid ((*S*)-11)

Each enantiomer of the acylated methyl ester obtained in the procedure of **11** (120 mg, 0.240 mmol) was separated by using chiral HPLC: column, t_R of (*R*)-enantiomer = 6.56 min, (*S*)-enantiomer = 7.47 min; temperature, 27 °C, Chiral Pack IC (4.6 × 250 mm); eluent, ethanol/hexane = 30/70; flow rate = 1.0 mL/min. Then, the obtained (*R*)-enantiomer (55 mg, 0.11 mmol) and (*S*)-enantiomer (56 mg, 0.11 mmol) was respectively dissolved in a mixture of 2 N NaOH aq (2 mL), THF (1 mL), and MeOH (1 mL), and the mixture was stirred at room temperature for 2 h. The mixture was acidified with 2 N HCl aq and extracted with AcOEt. The organic layer was washed with saturated $NaHCO_3$ aq and brine, dried (Na_2SO_4) and concentrated in vacuo. The obtained residue was triturated in Et_2O/hexane, filtered and dried in vacuo to provide (***R***)-**11** as colorless solid (47 mg, 88%), $[\alpha]_D^{21.0}$ −43.5 (*c* 0.49, $CHCl_3$), and (***S***)-**11** as colorless solid (54 mg, 97%), $[\alpha]_D^{21.0}$ +42.7 (*c* 0.48, $CHCl_3$). The absolute configurations were determined by the correspondence of the oprical rotation of (***R***)-**11** obtained from the stereospecific route in Scheme 3.

5.1.19. *N*-[(1*R*)-1-[2-Chloro-6-(trifluoromethyl)-3-pyridyl]but-3-enyl]-2-methylpropane-2-sulfinamide (12)

A mixture of 2-chloro-6-(trifluoromethyl)pyridine-3-carbaldehyde **8** (19.0 g, 90.8 mmol), Ti(OEt)$_4$ (41.4 g, 182 mmol), and (*S*)-(−)-*tert*-butylsulfinamide (11.2 g, 92.6 mmol) in THF (200 mL) was stirred at reflux for 5 h. The cooled reaction mixture was concentrated, and dissolved in AcOEt (500 mL). The resulting suspension was filtered through a Celite pad, and the filtrate was concentrated and purified by column chromatography (hexane/AcOEt = 4:1) to provide the imine (28.9 g, 99%) as a light yellow solid. ^{1}H NMR ($CDCl_3$) δ: 9.00 (1H, s), 8.53 (1H, d, *J* = 7.8 Hz), 7.73 (1H, d, *J* = 7.8 Hz), 1.29 (9H, s). A mixture of the imine (28.9 g, 90.0 mmol), allyl bromide (55.9 g, 0.46 mol), and indium (42.5 g, 0.37 mol) was vigorously stirred in saturated NaBr aq at room temperature for 21 h. After addition of saturated $NaHCO_3$ aq (500 mL), the reaction mixture was extracted with AcOEt (250 mL × 3). The organic layers were filtered through a Celite pad, and dried (Na_2SO_4), concentrated and purified by column chromatography (hexane/AcOEt = 1:4 with 5% of Et_3N) to provide **12** as a colorless solid (30.7 g, 94%). ^{1}H NMR ($CDCl_3$) δ: 7.95 (1H, d, *J* = 7.8 Hz), 7.64 (1H, d, *J* = 7.8 Hz), 5.79–5.68 (1H, m), 5.24 (2H, t, *J* = 14.1 Hz), 5.03–4.99 (1H, m), 3.78 (1H, s), 2.79–2.73 (1H, m), 2.51–2.43 (1H, m), 1.24 (9H, s).

5.1.20. *tert*-Butyl *N*-[2-[[(1*R*)-1-[2-chloro-6-(trifluoromethyl)-3-pyridyl]but-3-enyl]amino]ethyl]carbamate (13)

The sulfinamide **12** (22.8 g, 64.3 mmol) was dissolved in 4 N HCl/1,4-dioxane (100 mL) and stirred at room temperature for 2 h. After the reaction mixture was concentrated, the residue was added to Et_2O (300 mL). The resulting suspension was filtered, and dried in vacuo to provide the amine HCl salt (15.1 g, 82%) as a colorless solid. ^{1}H NMR (CD_3OD) δ: 8.24 (1H, d, *J* = 7.8 Hz), 7.97 (1H, d, *J* = 8.2 Hz), 5.83–5.72 (1H, m), 5.25 (1H, d, *J* = 5.1 Hz), 5.22 (1H, s), 4.93 (1H, t, *J* = 7.2 Hz), 2.81 (2H, t, *J* = 7.2 Hz). A mixture of the amine HCl salt (15.1 g, 52.8 mmol), *tert*-butyl *N*-(2-oxoethyl)carbamate (12.6 g, 79.3 mmol), and $NaBH(OAc)_3$ (25.2 g, 95.1 mmol) in CH_2Cl_2 (250 mL) was stirred at room temperature for 4 h. After addition of saturated $NaHCO_3$ aq (300 mL), the reaction mixture was extracted with CH_2Cl_2 (250 mL × 2). The organic layers were washed with brine, dried (Na_2SO_4), concentrated and purified by column chromatography (hexane/AcOEt = 2:3) to provide **13** as a colorless solid (20.8 g, quant.). ^{1}H NMR ($CDCl_3$) δ 8.12 (1H, d, *J* = 7.8 Hz), 7.64 (1H, d, *J* = 8.2 Hz), 5.81–5.71 (1H, m), 5.13 (2H, t, *J* = 12.3 Hz), 4.72 (1H, br s), 4.21–4.17 (1H, m), 3.19 (2H, br s), 2.65–2.59 (1H, m), 2.57–2.51 (1H, m), 2.47–2.41 (1H, m), 2.24–2.16 (1H, m), 2.05 (1H, s), 1.44 (9H, s).

5.1.21. *tert*-Butyl (5*R*)-5-allyl-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepine-4-carboxylate ((*R*)-10)

Compound 13 (20.8 g, 52.8 mmol) was dissolved in 4 N HCl/AcOEt (100 mL) and stirred at room temperature for 1 h. After the reaction mixture was concentrated, the residue was dissolved in CH_2Cl_2 (300 mL), washed with saturated $NaHCO_3$ aq and brine, dried (Na_2SO_4), concentrated and purified by column chromatography (MeOH/CH_2Cl_2 = 1:12) to provide the diamine as colorless solid (12.7 g). A mixture of the diamine (12.7 g, 43.3 mmol) and (*i*-Pr)$_2$EtN (23.7 mL, 130 mmol) in *N*-methylpyrrodinone (250 mL)

was stirred at 160 °C for 8 h. Subsequently, di-*tert*-butyl dicarbonate (14.2 g, 65.0 mmol) was added to the cooled mixture. After stirring at room temperature for 1 h. The mixture was diluted with Et_2O, washed with brine, dried (Na_2SO_4), concentrated and purified by column chromatography (AcOEt/hexane = 1:4) to provide (***R***)-**10** as brown oil (11.1 g, 59%, 3 steps). ^1H NMR ($CDCl_3$): a mixture of conformers δ 7.56 (0.5H, d, J = 7.4 Hz), 7.44 (0.5H, d, J = 7.4 Hz), 7.10 (1H, d, J = 7.4 Hz), 5.68–5.59 (1H, m), 5.33 (0.5H, br s), 5.08–4.99 (3.5H, m), 4.14–4.00 (1H, m), 3.38–3.31 (2H, m), 3.19–3.08 (1H, m), 2.86–2.72 (1H, m), 2.53–2.46 (1H, m), 1.45 (9H, s).

5.1.22. Methyl 2-[(5*R*)-8-(trifluoromethyl)-2,3,4,5-tetrahydro-1*H*-pyrido[2,3-*e*][1,4]diazepin-5-yl]acetate dihydrochloride (14a)

A mixture of (***R***)-**10** (11.1 g, 31.1 mmol), 2.5% OsO_4 in *i*-PrOH (8.00 mL, 0.79 mmol), and *N*-methylmorpholine oxide (4.74 g, 40.5 mmol) was dissolved in acetone/H_2O (90 mL/30 mL) at 0 °C, then stirred at room temperature for 18 h. The mixture was diluted with AcOEt (300 mL), washed with brine, dried (Na_2SO_4), concentrated and purified by column chromatography (MeOH/CH_2Cl_2 = 1:8) to provide the diol as brown oil (12.9 g). A mixture of the diol (12.9 g), sodium periodate (13.3 g, 62.3 mmol) in THF/H_2O (150 mL/50 mL) was stirred at room temperature for 1.5 h. The mixture was diluted with AcOEt (300 mL), washed with brine, dried (Na_2SO_4), concentrated in vacuo to provide the aldehyde as colorless oil (11.9 g). A mixture of the aldehyde (11.9 g), $NaH_2PO_4{\cdot}2H_2O$ (36.4 g, 234 mmol) and $NaClO_2$ (7.04 g, 78 mmol), 2-methyl-2-butene (33.1 mL, 311 mmol) in *t*-BuOH/H_2O (150 mL/150 mL) was stirred at room temperature for 17 h. After concentration, the residue was diluted with AcOEt (300 mL × 2), washed with brine, dried (Na_2SO_4), concentrated in vacuo to provide the carboxylic acid as brown oil (16.1 g). A mixture of the carboxylic acid (15.2 g), 2.0 M (trimethylsilyl)diazomethane/hexane (18.0 mL, 36.0 mmol) in THF/MeOH (50 mL/50 mL) was stirred at 0 °C for 1 h. The mixture was concentrated and purified by column chromatography (AcOEt/hexane = 1:3) to provide the methyl ester as colorless oil (8.38 g). The methyl ester (8.82 g, 22.7 mmol) was dissolved in 4 N HCl/1,4-dioxane (50 mL) and stirred at room temperature for 1 h (i-Pr)$_2$O (300 mL) was added to the resulting suspension. The deposited HCl salt was separated by filtration and dried in vacuo to provide **14a** as colorless solid (7.07 g, 64%, 5 steps). ^1H NMR ($CDCl_3$): a mixture of conformers δ 7.85 (1H, d, J = 7.8 Hz), 7.26 (1H, d, J = 7.8 Hz), 5.10 (1H, t, J = 7.0 Hz), 3.72 (3H, s), 3.66 (1H, s), 3.58–3.40 (4H, m), 3.25–3.22 (2H, m).

5.1.23. Benzyl 2-[(5*R*)-8-(trifluoromethyl)-2,3,4,5-tetrahydro-1*H*-pyrido[2,3-*e*][1,4]diazepin-5-yl]acetate dihydrochloride (14b)

A mixture of the carboxylic acid obtained in the procedure of **14a** (16.5 g, 36.0 mmol), benzyl bromide (5.56 mL, 46.8 mmol), K_2CO_3 (7.47 g, 54.0 mmol) in acetone (250 mL) was stirred at 60 °C for 7 h. Then, the reaction mixture was filtered through a Celite pad, concentrated, and purified by silica gel column chromatography (hexane/AcOEt = 1:1) to provide the benzyl ester (17.0 g) as a brown oil. The benzyl ester (16.9 g) was dissolved in 4 N HCl/1,4-dioxane (80 mL) and stirred at room temperature for 0.5 h. Et_2O (300 mL) was added to the resulting suspension. The deposited hydrochloride salt was separated by filtration and dried in vacuo to provide **14b** as colorless solid (12.8 g, 80%, 5 steps). ^1H NMR (CD_3OD) δ: 7.77 (1H, d, J = 7.8 Hz), 7.34–7.29 (5H, m), 7.16 (1H, d, J = 7.8 Hz), 5.16 (2H, s), 5.11 (1H, t, J = 7.0 Hz), 3.56–3.40 (4H, m), 3.26–3.23 (2H, m).

5.1.24. 2-[(5*R*)-4-[2-[3-(4-Fluorophenoxy)phenyl]acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (15a)

A mixture of **14a** (120 mg, 0.32 mmol), 2-[3-(4-fluorophenoxy)phenyl]acetic acid (94 mg, 0.38 mmol), (i-Pr)$_2$EtN (0.116 mL, 0.64 mmol), HATU (146 mg, 0.38 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature overnight. The mixture was diluted in CH_2Cl_2 (20 mL), washed with saturated $NaHCO_3$ aq and brine, dried (Na_2SO_4), concentrated and purified by column chromatography (AcOEt/hexane = 1:1) to provide the acylated diazepine as colorless oil (165 mg, quant.). ^1H NMR ($CDCl_3$) a mixture of conformers δ: 7.77 (0.6H, d, J = 7.8 Hz), 7.24–7.15 (1.4H, m), 7.03–6.88 (5H, m), 6.83–6.71 (3H, m), 6.16 (0.4H, t, J = 8.0 Hz), 5.43 (0.6H, t, J = 7.4 Hz), 5.08 (0.4H, d, J = 5.5 Hz), 4.92 (0.6H, d, J = 4.3 Hz), 4.72–4.67 (0.6H, m), 4.03–3.90 (1H, m), 3.86–3.69 (2.4H, m), 3.65–3.61 (3H, m), 3.42–3.29 (1H, m), 3.20–2.70 (3H, m). A mixture of the acylated diazepine (170 mg, 0.33 mmol), 2 N NaOH aq (4 mL) in THF/MeOH (2 mL/2 mL) was stirred at room temperature for 2 h. The mixture was acidified with 2 N HCl aq and extracted with AcOEt. The organic layer was washed with brine, dried (Na_2SO_4) and concentrated in vacuo. The obtained residue was triturated in Et_2O/hexane, filtered and dried in vacuo to provide **15a** as colorless solid (160 mg, 97%) ^1H NMR ($CDCl_3$) a mixture of conformers δ: 7.77 (0.6H, d, J = 7.8 Hz), 7.23 (1H, d, J = 8.2 Hz), 7.16 (0.4H, d, J = 7.4 Hz), 7.03–6.89 (5H, m), 6.83–6.71 (3H, m), 6.16 (0.6H, t, J = 8.0 Hz), 5.43 (0.4H, t, J = 7.4 Hz), 5.08 (0.6H, d, J = 5.5 Hz), 4.92 (0.4H, d, J = 5.5 Hz), 4.72–4.67 (0.6H, m), 4.03–3.91 (1H, m), 3.85–3.61 (2.4H, m), 3.42–3.28 (1H, m), 3.21–2.70 (3H, m). MS (ESI$^+$) m/z: 504 ((M+H)$^+$.

5.1.25. 2-[(5*R*)-4-[2-(3-Isobutoxyphenyl)acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (15b)

15b was prepared in a similar manner described for **15a**. Yield: 72% (2 steps from **14a**). ^1H NMR ($CDCl_3$): a mixture of conformers δ 7.79 (0.6H, d, J = 7.4 Hz), 7.19–7.11 (1.2H, m), 6.91 (0.6H, d, J = 7.4 Hz), 6.84 (0.6H, d, J = 7.4 Hz), 6.77–6.68 (3H, m), 6.19 (0.6H, t, J = 7.6 Hz), 5.44 (0.4H, t, J = 7.6 Hz), 5.14 (0.4H, br s), 4.98 (0.6H, br s), 4.78–4.63 (0.6H, m), 4.03–3.91 (1H, m), 3.87–3.82 (0.4H, m), 3.77–3.41 (4H, m), 3.25–2.73 (4H, m), 2.05–1.95 (1H, m), 0.99–0.95 (6H, m). MS (ESI$^+$) m/z: 466 ((M+H)$^+$. HRMS (ESI$^-$) m/z: 464.1793 (M−H)$^-$ (calcd for $C_{23}H_{26}N_3O_4F_3$: 464.1797). $[\alpha]_D^{21.0}$ −40.2 (c 1.00, $CHCl_3$).

5.1.26. 2-[(5*R*)-4-[2-[3-[(6-Methyl-3-pyridyl)oxy]phenyl]acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (15c)

Compound **15c** was prepared in a similar manner described for **15a**. Yield: 69% (2 steps from **14a**). ^1H NMR (CD_3OD) a mixture of conformers δ: 8.13–8.09 (1H, m), 7.75 (0.5H, d, J = 7.3 Hz), 7.34–7.24 (3H, m), 7.13 (0.5H, d, J = 7.3 Hz), 7.06–7.02 (1.5H, m), 6.90 (1.5H, d, J = 7.2 Hz), 6.81 (0.5H, d, J = 8.3 Hz), 6.70 (0.5H, s), 6.12 (0.5H, t, J = 7.6 Hz), 5.53 (0.5H, t, J = 7.3 Hz), 4.60–4.56 (0.5H, m), 4.16–4.13 (0.5H, m), 3.99–3.96 (0.5H, m), 3.91–3.78 (1.5H, m), 3.72–3.67 (0.5H, m), 3.50–3.36 (1.5H, m), 3.23–2.78 (4H, m), 2.52–2.48 (3H, m). MS (ESI$^+$) m/z: 501 ((M+H)$^+$. HRMS (ESI$^-$) m/z: 499.1597 (M−H)$^-$ (calcd for $C_{25}H_{23}N_4O_4F_3$: 499.1593). $[\alpha]_D^{21.0}$ +32.9 (c 1.00, $CHCl_3$).

5.1.27. Benzyl 2-[(5*R*)-4-[2-(3-hydroxyphenyl)acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetate (16)

A mixture of **14b** (500 mg, 1.14 mmol), 2-(3-hydroxyphenyl)acetic acid (208 mg, 1.37 mmol), DMT-MM (573 mg, 1.71 mmol) in

MeOH (10 mL) was stirred at room temperature for 24 h. The mixture was diluted in AcOEt (50 mL), washed with saturated $NaHCO_3$ aq and brine, dried (Na_2SO_4), concentrated and purified by column chromatography (AcOEt/hexane = 1:1) to provide **16** as colorless solid (441 mg, 77%). ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.72 (0.6H, d, J = 7.6 Hz), 7.38–7.33 (3H, m), 7.29–7.27 (1.4H, m), 7.17–7.07 (2H, m), 6.90 (0.6H, d, J = 7.6 Hz), 6.81 (0.4H, d, J = 7.8 Hz), 6.73–6.60 (3H, m), 6.18 (0.6H, t, J = 8.1 Hz), 5.89 (0.4H, s), 5.45–5.41 (1H, m), 5.18 (0.4H, d, J = 4.9 Hz), 5.11–4.88 (3H, m), 4.60–4.57 (0.6H, m), 3.88–3.58 (3H, m), 3.27–2.77 (4H, m).

5.1.28. 2-[(5*R*)-4-[2-(3-Hydroxyphenyl)acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (17a)

10% Pd/C (60 mg) was added to a solution of **16** (300 mg, 0.60 mmol) in EtOH (5 mL), and the reaction mixture was stirred under H_2 atmosphere at room temperature for 5 h. The mixture was filtered through a Celite pad, concentrated, and purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 1:9) to provide the diazepine **17a** (213 mg, 87%) as a colorless solid. ^{1}H NMR (CD_3OD) a mixture of conformers δ: 7.77 (0.6H, d, J = 7.4 Hz), 7.13–6.82 (2.4H, d, J = 7.8 Hz), 6.67–6.53 (3H, m), 6.16 (0.6H, t, J = 7.8 Hz), 5.52 (0.4H, t, J = 7.4 Hz), 4.64–4.58 (0.4H, m), 4.10–4.06 (0.6H, m), 3.97–3.92 (0.4H, m), 3.85–3.81 (0.6H, m), 3.75 (1H, s), 3.70–3.63 (0.4H, m), 3.45 (0.6H, dd, J = 13.9, 4.5 Hz), 3.25–3.02 (2H, m), 2.95–2.76 (2H, m). MS (ESI$^+$) m/z: 410 ((M+H)$^+$.

5.1.29. 2-[(5*R*)-4-[2-[3-(2-Methylpropanoyloxy)phenyl]acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (17b)

A mixture of **16** (100 mg, 0.20 mmol), 2-methylpropanoyl chloride (256 mg, 0.24 mmol), Et_3N (56 μL, 0.40 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature for 1.5 h. The mixture was diluted in AcOEt (30 mL), washed with water and brine, dried (Na_2SO_4), concentrated and purified by column chromatography (AcOEt/hexane = 1:1) to provide the benzyl ester (114 mg, quant.). ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.69 (0.6H, d, J = 7.8 Hz), 7.37–7.28 (5.4H, m), 6.80–6.79 (5H, m), 6.18 (0.6H, t, J = 7.8 Hz), 5.40 (0.4H, t, J = 7.4 Hz), 5.09–4.88 (3H, m), 4.72–4.69 (0.4H, m), 3.96–3.88 (0.6H, m), 3.79–3.59 (2H, m), 3.42–3.30 (2H, m), 3.28–3.07 (2H, m), 2.99–2.71 (2H, m), 1.31–1.25 (6H, m). 10% Pd/C (50 mg) was added to a solution of the benzyl ester (114 mg, 0.200 mmol) in EtOH (5 mL), and the reaction mixture was stirred under H_2 atmosphere at room temperature for 5 h. The mixture was filtered through a Celite pad, concentrated, and purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 1:9). The obtained was triturated in Et_2O/hexane, filtered and dried in vacuo to provide **17b** (75 mg, 78%) as a colorless solid. ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.76 (0.6H, d, J = 7.6 Hz), 7.31–7.24 (1.4H, m), 7.15–6.88 (4H, m), 6.27–6.24 (0.6H, m), 5.35–5.33 (0.4H, m), 5.18 (0.4H, br s), 5.09 (0.6H, br s), 4.76–4.74 (0.4H, m), 4.01–3.99 (0.6H, m), 3.88 (0.4H, d, J = 15.6 Hz), 3.74 (1H, s), 3.68–3.62 (0.6H, m), 3.43–3.28 (2H, m), 3.21–3.14 (1.6H, m), 3.03–3.01 (0.4H, m), 2.83–2.78 (1H, m), 2.69–2.54 (1H, m), 1.32–1.28 (3.6H, m), 0.90–0.86 (2.4H, m). MS (ESI$^+$) m/z: 480 ((M+H)$^+$.

5.1.30. 2-[(5*R*)-4-[2-[3-(3-Methylbutanoyloxy)phenyl]acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (17c)

Compound **17c** was prepared in a similar manner described for **17b**. Yield: 71% (2 steps from **16**). ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.76 (0.6H, d, J = 7.8 Hz), 7.34–7.29 (1.4H, m), 7.16–6.90 (4H, m), 6.28–6.24 (0.6H, m), 5.32–5.28 (0.4H, m), 5.12 (0.6H, br s), 5.02 (0.4H, br s), 4.78–4.73 (0.4H, m), 3.99–3.60 (3.6H, m), 3.40–2.60 (4H, m), 2.47–2.45 (2H, m), 2.28–2.18 (1H, m), 1.07–1.03 (6H, m). MS (ESI$^+$) m/z: 494 ((M+H)$^+$. HRMS (ESI$^+$) m/z: 494.1908 ((M+H)$^+$ (calcd for $C_{24}H_{27}N_3O_5F_3$: 494.1903). $[\alpha]_D^{21.0}$ −21.6 (c 1.00, $CHCl_3$).

5.1.31. 2-[(5*R*)-4-[2-(3-Pentanoyloxyphenyl)acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (17d)

Compound **17d** was prepared in a similar manner described for **17b**. Yield: 96% (2 steps from **16**). ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.76 (0.6H, d, J = 7.6 Hz), 7.31–7.25 (1.4H, m), 7.15–6.88 (4H, m), 6.25–6.23 (0.6H, m), 5.34–5.32 (0.4H, m), 5.19 (0.4H, br s), 5.11 (0.6H, br s), 4.75–4.73 (0.4H, m), 4.02–3.63 (3.6H, m), 3.43–3.27 (1.4H, m), 3.21–3.14 (1.4H, m), 3.05–2.97 (0.6H, m), 2.75–2.68 (0.6H, m), 2.58–2.54 (2H, m), 1.74–1.72 (2H, m), 1.43 (2H, td, J = 15.1, 7.3 Hz), 0.99–0.95 (3H, m). MS (ESI$^+$) m/z: 494 ((M+H)$^+$.

5.1.32. 2-[(5*R*)-4-[2-[3-(2-Cyclopropylacetyl)oxyphenyl]acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (17e)

Compound **17e** was prepared in a similar manner described for **17b**. Yield: 57% (2 steps from **16**). ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.77 (0.6H, d, J = 7.8 Hz), 7.32–7.20 (1.4H, m), 7.14–6.91 (4H, m), 6.24–6.22 (0.6H, m), 5.35–5.33 (0.4H, m), 5.20 (0.4H, br s), 5.11 (0.6H, br s), 4.77–4.75 (0.4H, m), 4.01–3.99 (0.6H, m), 3.89–3.88 (0.4H, m), 3.85–3.77 (2H, m), 3.68–3.62 (0.6H, m), 3.45–3.27 (1H, m), 3.23–3.00 (2H, m), 2.72 (0.4H, dd, J = 15.3, 7.6 Hz), 2.56 (0.6H, dd, J = 15.7, 5.5 Hz), 2.48–2.46 (2H, m), 1.20–1.10 (1H, m), 0.66–0.58 (2H, m), 0.29–0.24 (2H, m). MS (ESI$^+$) m/z: 492 ((M+H)$^+$.

5.1.33. 2-[(5*R*)-4-[2-[3-(3,3-Dimethylbutanoyloxy)phenyl]acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (17f)

Compound **17f** was prepared in a similar manner described for **17b**. Yield: 46% (2 steps from **16**). ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.76 (0.6H, d, J = 7.8 Hz), 7.34–7.28 (1.4H, m), 7.15–6.89 (4H, m), 6.27–6.24 (0.6H, m), 5.32–5.30 (0.4H,m), 5.13 (0.4H, br s), 5.04 (0.6H, br s), 4.75–4.73 (0.4H,m), 4.01–3.97 (0.6H, m), 3.89–3.76 (2H, m), 3.64 (1H, t, J = 11.3 Hz), 3.41–3.04 (3H, m), 2.66 (0.6H, dd, J = 15.1, 6.1 Hz), 2.51 (0.4H, dd, J = 15.1, 4.9 Hz), 2.44 (2H, s), 1.13 (9H, d, J = 3.5 Hz). MS (ESI$^+$) m/z: 508 ((M+H)$^+$.

5.1.34. Isopropyl 3-[2-[(5*R*)-5-(2-benzyloxy-2-oxo-ethyl)-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-4-yl]-2-oxo-ethyl]benzoate (18, R^3 = *i*-Pr)

Compound **14b** (229 mg, 0.52 mmol) was added to a solution of 2-(3-isopropoxycarbonylphenyl)acetic acid (110 mg, 0.48 mmol), HATU (217 mg, 0.58 mmol) and (*i*-Pr)$_2$EtN (325 μL, 1.90 mmol) in CH_2Cl_2 (10 mL) at room temperature, and the reaction mixture was stirred for 5 h. The mixture was diluted with CH_2Cl_2 (20 mL), washed with saturated $NaHCO_3$ aq and brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (hexane/AcOEt = 5:2) to provide **18** (212 mg, 78%) as a colorless solid. ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.93–7.85 (1H, m), 7.80 (1H, s), 7.70 (0.6H, d, J = 7.4 Hz), 7.38–7.28 (7H, m), 7.07 (0.4H, d, J = 7.8 Hz), 7.01–6.83 (1H, m), 6.18 (0.6H, t, J = 6.3 Hz), 5.44 (0.4H, t, J = 7.0 Hz), 5.25–5.18 (1H, m), 5.09–4.99 (2H, m), 4.91–4.69 (1H, m), 3.95 (1H, s), 3.83–3.64 (2H, m), 3.42–2.79 (4H, m), 1.35–1.32 (6H, m).

5.1.35. 3-[2-[(5*R*)-5-(Carboxymethyl)-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-4-yl]-2-oxo-ethyl]benzoic acid (19a)

A mixture of **18** (110 mg, 0.223 mmol), 2 N NaOH aq (0.4 mL) in MeOH (2 mL) was stirred at room temperature for 6 h. The mixture

was acidified with 2 N HCl aq, and concentrated in vacuo. The aqueous phase was extracted with *i*-PrOH in $CHCl_3$ (25%). The combined organic extract was washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was triturated upon Et_2O in hexane, collected by filtration to afford **19a** as colorless solid (97 mg, quant.) ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 8.13–8.03 (1H, m), 8.00–7.95 (1.6H, m), 7.64–7.42 (2.4H, m), 7.22–7.15 (1H, m), 6.56–6.51 (0.6H, m), 5.65 (0.4H, t, J = 6.7 Hz), 5.20 (0.4H, br s), 5.07 (0.6H, br s), 4.71–4.68 (0.4H, m), 4.13–4.09 (0.6H, m), 3.90–3.77 (2H, m), 3.60–3.37 (2H, m), 3.27–2.99 (2H, m), 2.86–2.60 (1H, m). MS (ESI$^+$) m/z: 438 ((M+H)$^+$.

5.1.36. 2-[(5*R*)-4-[2-(3-Isopropoxycarbonylphenyl)acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (19b)

10% Pd/C (60 mg) was added to a solution of the benzyl ester **18** (212 mg, 0.372 mmol) in EtOH (5 mL), and the reaction mixture was stirred under H_2 atmosphere at room temperature for 2 h. The mixture was filtered through a Celite pad, concentrated, and purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 9:1). The obtained was triturated in Et_2O/hexane, filtered and dried in vacuo to provide **19b** (174 mg, 98%) as colorless solid. ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.91–7.87 (0.6H, m), 7.85–7.79 (2H, m), 7.38–7.29 (2H, m), 7.15–6.88 (1.4H, m), 6.28 (0.6H, t, J = 7.8 Hz), 5.46 (0.4H, t, J = 7.8 Hz), 5.28–5.17 (1.2H, m), 4.77–4.72 (0.4H, m), 4.08–3.99 (0.4H, m), 3.82 (2H, s), 3.76–3.64 (1H, m), 3.45–3.03 (3H, m), 2.84–2.72 (1H, m), 1.37–1.28 (4H, m), 0.90–0.87 (3H, m). MS (ESI$^+$) m/z: 480 ((M+H)$^+$.

5.1.37. 2-[(5*R*)-4-[2-(3-Isobutoxycarbonylphenyl)acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (19c)

Compound **19c** was prepared in a similar manner described for **19b**. Yield: 75% (2 steps from **14b**). ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.93–7.89 (0.6H, m), 7.86–7.80 (2H, m), 7.41–7.29 (2H, m), 7.19–6.89 (1.4H, m, 6.35–6.31 (0.6H, m), 5.46 (0.4H, t, J = 7.4 Hz), 5.15 (0.4H, br s), 5.06 (0.6H, br s), 4.78–4.73 (0.4H, m), 4.13 (1.6H, d, J = 6.3 Hz), 4.08–4.02 (1H, m), 3.83 (2H, s), 3.68–3.66 (1H, m), 3.40–3.34 (1H, m), 3.25–3.04 (2H, m), 2.76–2.74 (1H, m), 2.12–2.02 (1H, m), 1.02–0.99 (6H, m). MS (ESI$^+$) m/z: 494 ((M+H)$^+$.

5.1.38. 2-[(5*R*)-4-[2-(3-Butoxycarbonylphenyl)acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (19d)

Compound **19d** was prepared in a similar manner described for **19b**. Yield: 80% (2 steps from **14b**). ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.91–7.89 (0.6H, m), 7.87–7.81 (2H, m), 7.41–7.29 (2H, m), 7.17–6.91 (1.4H, m), 6.36–6.32 (0.6H, m), 5.46 (0.4H, t, J = 7.4 Hz), 5.12 (0.4H, br s), 5.06 (0.6H, br s), 4.78–4.74 (0.4H, m), 4.08–3.98 (0.4H, m), 3.83 (2H, s), 3.68–3.65 (1H, m), 3.39–3.35 (1H, m), 3.25–3.04 (2H, m), 2.78–2.72 (1H, m), 1.78–1.63 (2H, m), 1.35–1.33 (2H, m), 1.32–1.16 (2H, m), 0.99–0.88 (3H, m). MS (ESI$^+$) m/z: 494 ((M+H)$^+$.

5.1.39. 2-[(5*R*)-4-[2-[3-(Cyclopropylmethoxycarbonyl)phenyl]acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (19e)

Compound **19e** was prepared in a similar manner described for **19b**. Yield: 74% (2 steps from **14b**). ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.96–7.93 (0.6H, m), 7.89–7.80 (2H, m), 7.41–7.31 (2H, m), 7.16–6.90 (1.4H, m), 6.30 (0.6H, t, J = 7.4 Hz), 5.46 (0.4H, t, J = 7.4 Hz), 5.20 (0.4H, br s), 5.10 (0.6H, br s), 4.78–4.72 (0.4H, m), 4.17–4.12 (2H, m), 4.08–3.98 (1.6H, m), 3.83–3.05 (5H, m), 2.81–2.73 (1H, m), 1.32–1.21 (1H, m), 0.88 (2H, t, J = 6.8 Hz), 0.38–0.33 (2H, m). MS (ESI$^+$) m/z: 492 ((M+H)$^+$.

5.1.40. 2-[(5*R*)-4-[2-[3-(2,2-Dimethylpropoxycarbonyl)phenyl]acetyl]-8-(trifluoromethyl)-1,2,3,5-tetrahydropyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (19f)

Compound **19f** was prepared in a similar manner described for **19b**. Yield: 75% (2 steps from **14b**). ^{1}H NMR ($CDCl_3$) a mixture of conformers δ: 7.93–7.90 (0.6H, m), 7.86–7.80 (2H, m), 7.42–7.30 (2H, m), 7.19–6.91 (1.4H, m), 6.37–6.33 (0.6H, m), 5.48–5.44 (0.4H, m), 5.12 (0.4H, br s), 5.01 (0.6H,br s), 4.78–4.73 (0.4H, m), 4.08–3.96 (1.6H, m), 3.84 (2H, s), 3.67–3.64 (2H, m), 3.41–3.35 (1H, m), 3.26–3.17 (1H, m), 3.12–3.06 (1H, m), 2.75–2.69 (1H, m), 1.04–1.02 (9H, m). MS (ESI$^+$) m/z: 508 ((M+H)$^+$.

5.2. Biological assays

5.2.1. In vitro BRS-3 agonist activity

The 384-well IP-One HTRF® Assay (Cisbio, Bedford, MA) was performed as described by the manufacturer's protocol. CHO-K1 stably expressing human and mouse BRS-3 was plated at 200,000 cells per well in 50 μL HAM and incubated in the CO_2 incubator at 37 °C for 24 h. On the next day, the media were removed and 25 μL of compounds diluted in HAM containing 0.1% BSA and 20 mM LiCl were added to each well and serial diluted IP1 standards (Cisbio) were also added to corresponding wells for this step. After the cells were incubated for 1 h in the CO_2 incubator at 37 °C, all the media were removed again and d2-labeled IP1 and cryptate-labeled anti-IP1 monoclonal antibody diluted in lysis buffer were added sequentially. The assay plates were kept in the dark at 4 °C, overnight. Ratiometric measurements of fluorescence emission at 665 nm and 620 nm were obtained using a RUBY star fluorometer (BMG Labtech, Ortenberg, Germany). IP1 levels in each well were calculated according to the standard curves on each plate. EC_{50} values were obtained by fitting data to a nonlinear curve-fitting program (GraphPad Software, Inc., La Jolla CA).

5.2.2. Food intake evaluation in mice

Male C57BL/6N mice 7 weeks of age were purchased from Charles River Laboratories (Kanagawa, Japan). All animals were held under standard laboratory conditions (12 h light per day, light on at 7:00 AM, 23 ± 2 °C, 55 ± 10% humidity) with food and water available ad libitum. On the day of testing, mice had been fasted for 16 h were weighed and placed individually in cages. After 1 h habituation to the new environment, mice were orally administered vehicle (0.5% methylcellulose) or compounds. One hour after compound administration, pre-weighed normal chow diet (FR2, purchased from Funabashi farm) was fed to the mice. Cumulative food intake of each mouse over 6 h was measured. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Daiichi Sankyo. Statistical analysis using Dunnett's test was performed to compare the vehicle-treated and compound-administered groups. P values <0.05 were considered significant.

5.3. Pharmacokinetic evaluation in mice

Male C57BL/6N mice were purchased at 5 weeks old from Charles River Laboratories Japan, Inc (Kanagawa, Japan). For acclimation, they were housed in stainless steel cages for 7–11 days in the controlled animal area. The mice were allowed free access to FR2 laboratory food (Funabashi Farm Co., Ltd) and tap water. Compound **17b**–**17f** and **19b**–**19f** were suspended in a 0.5 (w/v) % methyl cellulose 400 solution (Wako pure chemical industries, Osaka, Japan) for oral administration. For the administration,

2 mL/kg of the solution at a concentration of 5 mg/mL was used. The solutions of compounds were administered to male C57BL/6N mice, after overnight fasting. A blood sample of approximately 0.2 mL was collected from the jugular vein with a heparinized syringe. The blood was centrifuged at 14,000 rpm for 3 min at 4 °C (himac CR15D, Hitachi Koki Co., Ltd rotor: RT15A2) to obtain the plasma. The plasma was stored frozen at −20 °C until use for measurement of plasma concentration. The determination of the plasma concentration was performed by LC–MS/MS method using API 4000QTRAP (Applied Biosystems/MDS SCIEX). PK parameters were calculated by a non-compartmental model.

5.4. Safety evaluation in dogs

Female beagle dogs were obtained from Nosan Corporation (Kanagawa, Japan). Animals were housed in a temperature—(22–25 °C) and humidity controlled (35–75%) environment with a 12-h light/dark cycle. All experimental procedures were performed in accordance with the in-house guidelines of the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd, Tokyo, Japan. Test compounds were dispersed in 20% v/w hydroxypropyl-β-cyclodextrin solution, at the concentration of 1 mg/mL. Dogs were anesthetized with halothane (0.5–1.5% end-tidal concentration) proceeded by intravenous injection of thiopental (30 mg/kg of body weight). Two catheters were placed in the femoral artery and vein, for blood pressure recording and test substance administration, respectively. Heart rate and mean blood pressure were analyzed by the hemodynamic analysis software (SBP2000, Softron Co., Ltd, Tokyo). After the recording of pre- administration value, test substance was administered via the catheter in the femoral vein with syringe driver, at 3 mg/kg/3 mL for 30 min (adjusted by infusion speed). Blood pressure was recorded for 60 min from the administration start, except for the one animal (#2, **15b** administered).

5.5. Computational calculation for the conformational analysis

3D coordinates of compound (a–c) illustrated in Figure 5 were generated using Program Ligprep and used for Conformational Search with Macromodel (Schrodinger, LLC, New York, NY, 2010). Relative energy $\Delta E = E(R) - E(S)$ was calculated in each compound as the energy barrier for the N-atom inversion. E(*R*)/E(*S*) is defined as the potential energy in OPLS-2005 forcefield of the lowest energy conformation with (*R*)/(*S*) ring configuration.

Acknowledgments

We thank the members of Daiichi Sankyo RD NOVARE Co., Ltd for the high-resolution mass analysis and the measurement of optical rotation.

Reference and notes

1. (a) Obesity: Preventing and Managing the Global Epidemic, Report of a WHO Consultation, World Health Organization, Geneva, reprinted in 2004. Available at: http://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en. Accessed on June, 2014. (b) World Health Organization. Obesity and overweight: Fact sheet No. 311, updated on Mar, 2013. Available at: http://www.who.int/mediacentre/factsheets/fs311/en. Accessed on June, 2014.
2. (a) Olshansky, S. J.; Passaro, D. J.; Hershow, R. C.; Layden, J.; Carnes, B. A.; Brody, J.; Hayflick, L.; Butler, R. N.; Allison, D. B.; Ludwig, D. S. *N. Eng. J. Med.* **2005**, *352*, 1138; (b) Guh, D. P.; Zhang, W.; Bansback, N.; Amarsi, Z.; Birmingham, C. L.; Anis, A. H. *BMC Public Health* **2009**, *9*, 88.
3. (a) McNeely, W.; Benfield, P. *Drugs* **1998**, *56*, 241; (b) Torgerson, J. S.; Boldrin, M. N.; Hauptman, J.; Sjostrom, L. *Diabetes Care* **2004**, *27*, 155; (c) Padwal, R. S.; Majumdar, S. R. *Lancet* **2007**, *369*, 71; (d) Smith, B. M.; Smith, J. M.; Tsai, J. H.; Schultz, J. A.; Gilson, C. A.; Estrada, S. A.; Chen, R. R.; Park, D. M.; Prieto, E. B.; Gallardo, C. S.; Sengupta, D.; Dosa, P. I.; Covel, J. A.; Ren, A.; Webb, R. R.; Beeley, N. R. A.; Martin, M.; Morgan, M.; Espitia, S.; Saldana, H. R.; Bjenning, C.; Whelan, K. T.; Grottick, A. J.; Menzaghi, F.; Thomsen, W. J. *J. Med. Chem.* **2008**, *51*, 305; (e) Allison, D. B.; Gadde, K. M.; Garvey, W. T.; Peterson, C. A.; Schwiers, M. L.; Najarian, T.; Tam, P. Y.; Troupin, B.; Day, W. W. *Obesity* **2012**, *20*, 330; (f) Davenport, R. J.; Wright, S. *Drug Discovery Today* **2014**, *19*, 845.
4. Bays, H. E. *Obes. Res.* **2004**, *12*, 1197.
5. For example, a cannabinoid-1 receptor (CB1R) inverse agonist, rimonabant was withdrawn from the US market due to serious psychiatric effects in 2007. Also, a serotonin-norepinephrine reuptake inhibitor (SNRI), sibtramine once approved by FDA in 1997, was reported to increase cardiovascular events and strokes, then withdrawn in 2010. See details below: (a) Fong, T. M.; Heymsfield, S. B. *Int. J. Obes.* **2009**, *33*, 947; (b) Faulconbridge, L.; Wadden, T. A.; Berkowitz, R. I.; Sarwer, D. B.; Womble, L. G.; Hesson, L. A.; Stunkard, A. J.; Fabriatore, A. N. *Obesity* **2009**, *1009*, 17; (c) Warren, K. R.; Buchanan, R. W.; Feldman, S.; Conley, R. R.; Linthicum, J.; Ball, M. P.; Liu, F.; McMahon, R. P.; Gorelick, D. A.; Huestis, M. A.; Kelly, D. L. J. *Clin. Psycophamacol.* **2013**, *33*, 118; (d) James, W. P. T.; Caterson, I. D.; Coutinho, W.; Finer, N.; Gaal, L. F. V.; Maggioni, A. P.; Torp-P, C.; Sharma, A. M.; Shepherd, G. M.; Rode, R. A.; Renz, C. L. *N. Eng. J. Med.* **2010**, *363*, 905.
6. (a) Ohki-Hamazaki, H.; Wada, E.; Matsui, K.; Wada, K. *Brain Res.* **1997**, *762*, 165; (b) Liu, J.; Lao, Z. J.; Zhang, J.; Schaeffer, M. T.; Jiang, M. M.; Guan, X. M.; Van der Ploeg, L. H.; Fong, T. M. *Biochemistry* **2002**, *41*, 8954; (c) Porcher, C.; Juhem, A.; Peinnequin, A.; Bonaz, B. *Cell Tissue Res.* **2005**, *320*, 21; (d) Feng, Y.; Guan, X.-M.; Li, J.; Metzger, J. M.; Zhu, Y.; Juhl, K.; Zhang, B. B.; Thornberry, N. A.; Reitman, M. L.; Zhou, Y.-P. *Endocrinology* **2011**, *152*, 4106.
7. (a) Ohki, H.; Watase, K.; Yamamoto, K.; Ogura, H.; Yamano, M.; Yamada, K.; Maeno, H.; Imaki, J.; Kikuyama, S.; Wada, E.; Wada, K. *Nature* **1997**, *390*, 165; (b) Ladenheim, E. E.; Hamilton, N. L.; Behles, R. R.; Bi, S.; Hampton, L. L.; Battey, J. F.; Moran, T. H. *Endocrinology* **2008**, *149*, 971. (c) Metzger, J. M.; Gagen, K.; Raustad, K. A.; Yang, L.; White, A.; Wang, S. –P.; Craw, S.; Liu, P.; Lanza, T.; Lin, L. S.; Nargund, R. P.; Guan, X. –M.; Strack, A. M.; Reitman, M. L. *Am. J. Physiol. Endocrinol. Metab.* **2010**, *299*, E816.
8. BRS-3 was reported to be expressed by the enteric nervous system and by interstitial cells of Cajal in the rat GI tract (see Ref. 6c). Also, there are some papers reporting that continuous vagal neuromodulation on GI function in rats caused food-intake suppression and a similar effect was observed in human. See details below: (a) Krolczyk, D. G.; Zurowski, J.; Sobocki, J.; Slowiaczek, M. P.; Laskiewicz, J.; Matyja, A.; Zaraska, K.; Thor, P. J. *J. Physiol. Pharmacol.* **2001**, *52*, 705; (b) Pardo, J. V.; Sheikh, S. A.; Kuskowski, M. A.; Surerus, J. C.; Hagen, M. C.; Lee, J. T.; Rittberg, B. R.; Adson, D. E. *Int. J. Obes.* **2007**, *31*, 1756.
9. (a) Sebhat, I. K.; Franklin, C.; Lo, M. M.-C.; Chen, D.; Jewell, J. P.; Miller, R.; Pang, J.; Palyha, O.; Kan, Y.; Kelly, T. M.; Guan, X.-M.; Marsh, D. J.; Kosinski, J. A.; Metzger, J. M.; Lyons, K.; Dragovic, J.; Guzzo, P. R.; Henderson, A. J.; Reitman, M. L.; Nargund, R. P.; Wyvratt, M. J.; Lin, L. S. *ACS Med. Chem. Lett.* **2011**, *2*, 43; (b) Guan, X.-M.; Metzger, J. M.; Yang, L.; Raustad, K. A.; Wang, S.-P.; Spann, S. K.; Kosinski, J. A.; Yu, H.; Shearman, L. P.; Faidley, T. D.; Palyha, O.; Kan, Y.; Kelly, T. M.; Sebhat, I.; Lin, L. S.; Dragovic, J.; Lyons, K. A.; Craw, S.; Nargund, R. P.; Marsh, D. J.; Strack, A. M.; Reitman, M. L. *J. Pharmacol. Exp. Ther.* **2011**, *336*, 356; (c) Lateef, D. M.; Abreu-Vieira, G.; Xiao, C.; Reitman, M. L. *Am. J. Physiol. Endocrinol. Metab.* **2014**, *306*, E681.
10. Reitman, M. L.; Dishy, V.; Moreau, A.; Denney, W. S.; Liu, C.; Kraft, W. K.; Mejia, A. V.; Matson, M.; Stoch, S. A.; Wagner, J. A.; Lai, E. *J. Clin. Pharmacol.* **2012**, *52*, 1306.
11. (a) Shimizu, K.; Kawase, A.; Haneishi, T.; Kato, Y.; Kinoshita, K.; Ohmori, M.; Furuta, Y.; Emura, T.; Kato, N.; Mitsui, T.; Yamaguchi, K.; Morita, K.; Sekiguchi, N.; Yamamoto, T.; Matsushita, T.; Shimaoka, S.; Sugita, A.; Morikawa, K. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3323; (b) Kurimoto, A.; Hashimoto, K.; Nakamura, T.; Norimura, K.; Ogita, H.; Takaku, H.; Bonnert, R.; McInally, T.; Wada, H.; Isobe, Y. *J. Med. Chem.* **2010**, *53*, 2964; (c) Procopiou, P. A.; Barrett, V. J.; Ford, A. J.; Looker, B. E.; Lunniss, G. E.; Needham, D.; Smith, C. E.; Somers, G. *Bioorg. Med. Chem.* **2011**, *19*, 6026; (d) Hasumi, K.; Sato, S.; Saito, T.; Kato, J.; Shirota, K.; Sato, J.; Suzuki, H.; Ohta, S. *Bioorg. Med. Chem.* **2014**, *22*, 4162.
12. We confirmed a selective binding ability of some key compounds to human BRS-3 by a radio-ligand receptor binding assay including other bombesin receptors such as GRP (gastrin releasing peptide) receptor and NMB (neuromedin B) receptor (data not shown).
13. (a) Liu, P.; Lanza, T. J., Jr.; Chioda, M.; Jones, C.; Chobanian, H. R.; Guo, Y.; Chang, L.; Kelly, T. M.; Kan, Y.; Palyha, O.; Guan, X.-M.; Marsh, D. J.; Metzger, J. M.; Ramsay, K.; Wang, S.-P.; Strack, A. M.; Miller, R.; Pang, J.; Lyons, K.; Dragovic, J.; Ning, J. G.; Schafer, W. A.; Welch, C. J.; Gong, X.; Gao, Y.-D.; Hornak, V.; Ball, R. G.; Tsou, N.; Reitman, M. L.; Wyvratt, M. J.; Nargund, R. P.; Lin, L. S. *ACS Med. Chem. Lett.* **2011**, *2*, 933; (b) Chobanian, H. R.; Guo, Y.; Liu, P.; Chioda, M.; Lanza, T. J., Jr.; Chang, L.; Kelly, T. M.; Kan, Y.; Palyha, O.; Guan, X.-M.; Marsh, D. J.; Metzger, J. M.; Gorski, J. N.; Raustad, K.; Wang, S.-P.; Strack, A. M.; Miller, R.; Pang, J.; Madeira, M.; Lyons, K.; Dragovic, J.; Retman, M. L.; Nargund, R. P.; Lin, L. S. *ACS Med. Chem. Lett.* **2012**, *3*, 252; (c) Chobanian, H. R.; Guo, Y.; Liu, P.; Lanza, T. J., Jr.; Chioda, M.; Chang, L.; Kelly, T. M.; Kan, Y.; Palyha, O.; Guan, X.-M.; Marsh, D. J.; Metzger, J. M.; Raustad, K.; Wang, S.-P.; Strack, A. M.; Gorsuki, J. N.; Miller, R.; Pang, J.; Lyons, K.; Dragovic, J.; Ning, J. G.; Schafer, W. A.; Welch, C. J.; Gong, X.; Gao, Y.-D.; Hornak, V.; Reitman, M. L.; Nargund, R. P.; Lin, L. S. *Bioorg. Med. Chem.* **2012**, *20*, 2845.
14. Kraus, G. A.; Roth, B. *J. Org. Chem.* **1980**, *45*, 4825.
15. Each enantiomer (***R***)-**11** and (***S***)-**11** was obtained by hydrolysis after chiral HPLC separation of **11**: column, Chiral Pack IC (4.6 × 250 mm); eluent, ethanol/hexane = 30/70. The ee value of the methyl esters of (***R***)-**11** and (***S***)-**11** were determined by chiral HPLC analysis: column, Chiral Pack IC (4.6 × 250 mm); flow rate 1.0 mL/min; temperature, 27 °C; t_R of (*R*)-enantiomer = 6.56 min; t_R of (*S*)-enantiomer = 7.47 min. The retention time of the methyl ester of (*R*)-

enantiomer ((***R***)-**11**) via chiral separation was well corresponded to the one synthesized by the stereospecific route in Scheme 3.
16. (a) Liu, G.; Cogan, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1997**, *119*, 9913; (b) Ellman, J. A.; Owens, T. D.; Tang, T. P. *Acc. Chem. Res.* **2002**, *35*, 984.
17. Sun, X.-W.; Liu, M.; Xu, M.-H.; Lin, G.-Q. *Org. Lett.* **2008**, *10*, 1259.
18. An undesired (*S*,*S*)-diastereomer was also obtained in 2.0% yield (30 g scale).
19. The ee value of the key intermediate (***R***)-**10** was determined to 98.1% ee by chiral HPLC analysis: column, Chiral Pack IA (4.6 × 150 mm); eluent, ethanol/isopropylalcohol = 50/50; flow rate, 1.0 mL/min; temperature, 27 °C; t_R of (*R*)-enantiomer = 3.56 min; t_R of (*S*)-enantiomer = 4.11 min. The absolute configuration of (***R***)-**10** was inferred by the reaction mechanism proposed by the Ellman's papers (see Ref. 16).
20. Kunishima, K.; Kawachi, K.; Hioki, K.; Terao, K.; Tani, S. *Tetrahedron* **2001**, *57*, 1551.
21. The assignment of absolute stereochemistry is based on the Cahn-Ingold-Prelog convention. The diazepine plane is regarded as a chiral plane, and a carbon atom on the carbonyl group is designated as the pilot atom. Therefore, compound **A** and **B** have an (*S*)-configuration, and compound **C** has an (*R*)-configuration.
22. PK profile of **17a** (po, 30 mg/kg in mice, 0.5% methyl cellulose as a solvent, $n = 3$): T_{max} = 0.42 hr, C_{max} = 0.04 μM, $T_{1/2}$ = 1.79 hr, AUC = 0.07 hr * μg/mL.
23. (a) Doan, K. M. M.; Humphereys, J. E.; Webster, L. O.; Wring, S. A.; Shampine, L. J.; Serabjit-S, C. J.; Adkinson, K. K.; Polli, J. W. *J. Pharmacol. Exp. Ther.* **2002**, *303*, 1029; (b) Clark, D. E. *Drug Discovery Today* **2003**, *8*, 927.
24. The brain to plasma concentration ratios (Kp brain) of **15b** was fairly low (Kp = 0.043). Kp value was determined after a single administration of compound at 30 mg/kg po to mice. The measurement of the concentration was conducted at 2.5 h after administration. See details below: Matsufuji, T.; Shimada, K.; Kobayashi, S.; Kawamura, A.; Fujimoto, T.; Arita, T.; Hara, T.; Konishi, M.; Abe, O. R.; Izumi, M.; Sogawa, Y.; Nagai, Y.; Yoshida, K.; Takahashi, H. *Bioorg. Med. Chem. Lett.* **2014**, *22*, 750.