Bioorganic & Medicinal Chemistry 21 (2013) 993–1005

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Structure–activity relationship of benzodiazepine derivatives as LXXLL peptide mimetics that inhibit the interaction of vitamin D receptor with coactivators

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ARTICLE INFO

Article history:
Received 24 October 2012
Revised 14 November 2012
Accepted 20 November 2012
Available online 5 December 2012

Keyword:
Vitamin D
Antagonist
Cofactor
Nuclear receptor
Non-peptide
Structure–activity relationship
Protein interaction inhibitor
LXXLL

ABSTRACT

Suppression of vitamin D receptor (VDR)-mediated transcription is expected to be of therapeutic value in Paget's disease of bone. It is known that interaction between VDR and coactivators is necessary for VDR transactivation, and the interaction occurs when VDR recognizes an LXXLL peptide motif of coactivators. We previously reported that benzodiazepine derivatives designed as LXXLL peptide mimetics inhibited the interaction of VDR and coactivators, and reduced VDR transcription. Here, we investigated the structure–activity relationship of 7- and 8-substituted benzodiazepine derivatives, and established that the amino group at the 8-position is critical for the inhibitory activity.

1. Introduction

Vitamin D receptor (VDR) is a member of the nuclear receptor superfamily and is associated with regulation of calcium homeostasis, bone mineralization, proliferation, differentiation of various types of cells, and immune modulation.[1–3] The physiological agonist of VDR is activated vitamin D_3, $1,25(OH)_2D_3$ (Fig. 1). Binding of $1,25(OH)_2D_3$ to the VDR-ligand binding domain (LBD) allows VDR to interact with vitamin D-responsive elements (VDREs), to change its conformation through folding of helix 12, and to recruit cofactors, including vitamin D-interacting protein (DRIP) 205.[4,5] Paget's disease of bone is characterized by an increased number of osteoclasts and excessive bone resorption in focal areas.[6] Osteoclast precursors from patients with Paget's disease show hypersensitivity to $1,25(OH)_2D_3$,[7] and therefore, VDR antagonists are expected to be therapeutic drugs. However, extensive investigations to find VDR antagonists have yielded only a restricted series of secosteroid VDR antagonists[8–13] (Fig. 1), and no non-secosteroid VDR antagonist has been found, to our knowledge. This fact suggests that substrate recognition by VDR-LBD is highly specific. Thus, as an alternative approach to reduce VDR-mediated transcriptional activation, we[14] and others[15] have attempted to inhibit the interactions between the VDR-LBD and coactivators that are necessary for VDR transactivation. It is known that ligand-bound VDR-LBD recognizes LXXLL peptide motifs of coactivator proteins, and hydrophobic interactions of three leucine residues and hydrogen bonds, known as 'charge clamps', are important for recognition.[16] Previously, we designed and synthesized several benzodiazepine derivatives intended to mimic the pharmacophore of LXXLL peptide, and we reported that these molecules inhibited VDR/coactivator interaction and VDR-mediated transcription (Fig. 2).[14] Here, we describe the design, synthesis, and structure–inhibitory activity relationship of a series of benzodiazepine derivatives modified at the 7- and 8-positions.

2. Results and discussion

2.1. Effect of deletion of the amino group at the 8-position

In the structure of our prototype inhibitors **1**, the amino group at the 8-position was designed to mimic the charge clamp of the LXXLL motif (Fig. 2). However, it was not established whether the amino group actually contributed to the inhibitory effect of these compounds on the VDR-coactivator interaction. To evaluate whether the amino group is required for inhibition of VDR-mediated transcription, we synthesized benzodiazepine compounds with a hydrogen atom at the 8-position (Schemes 1 and 2).

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http://dx.doi.org/10.1016/j.bmc.2012.11.042

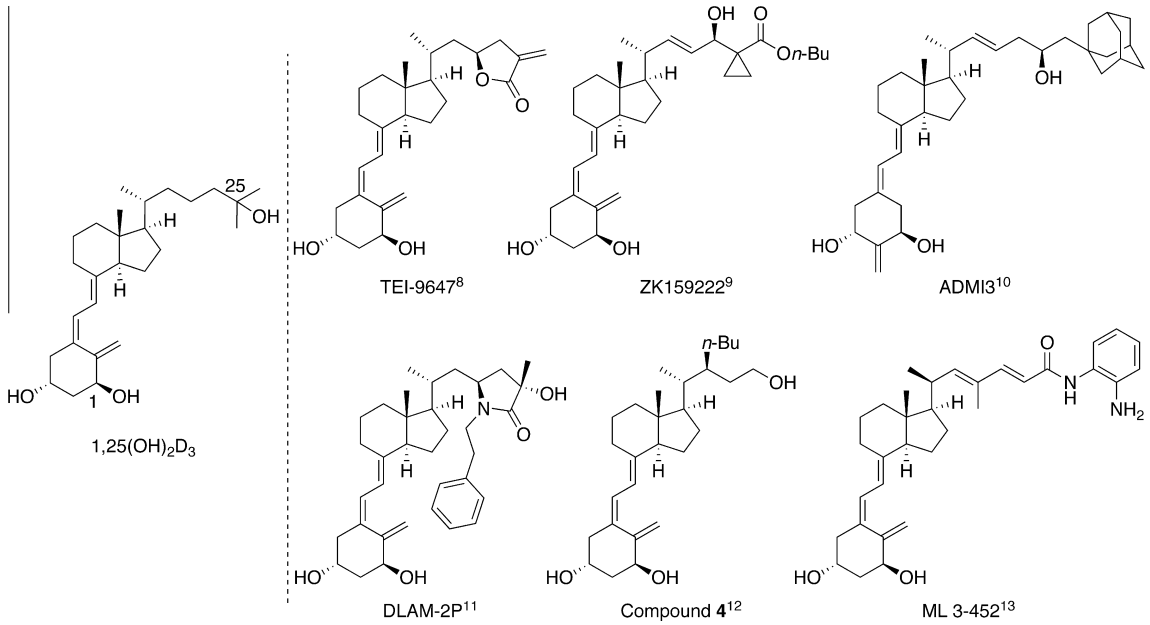


Figure 1. Structures of $1,25(OH)_2D_3$ and representative VDR antagonists.

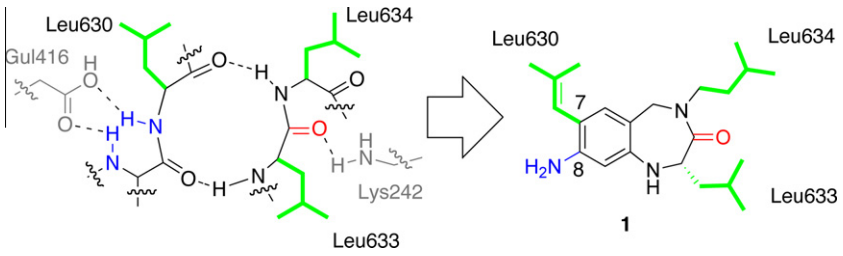
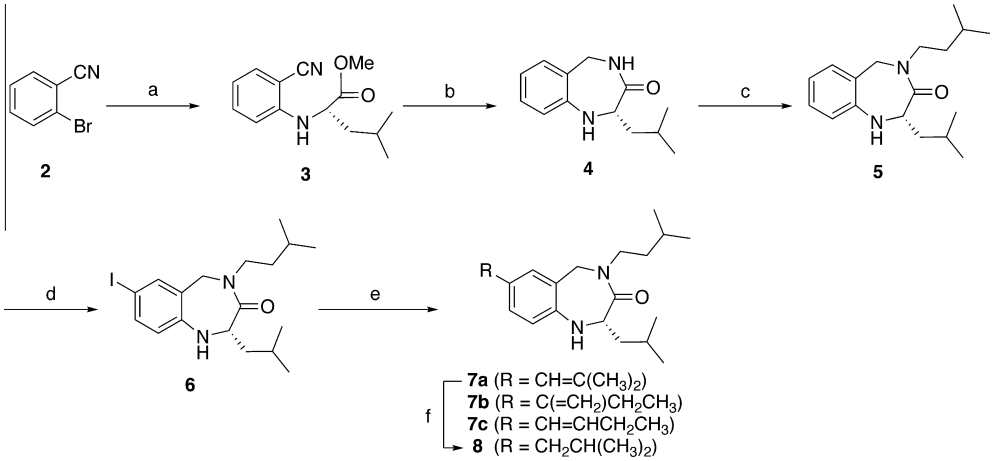
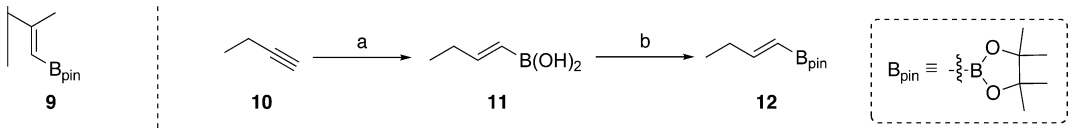


Figure 2. Illustration of the interaction between LXXLL peptide fragment and VDR, and a representative benzodiazepine that we reported previously.[14] Important structures in the LXXLL peptide fragment, that is the isobutyl chains of leucine residues and the charge clamp-related hydrogen bonds, are shown in green, blue and red. The corresponding structures of benzodiazepine **1** are shown in the same color. The residues of VDR are shown in gray. See also Figures 3 and 4.



Scheme 1. Reagents and conditions: (a) L-Leucine methyl ester hydrochloride, $Pd_2(dba)_3{\cdot}CHCl_3$, (*R*)-BINAP, Cs_2CO_3, toluene, 110 °C; (b) Raney nickel, H_2, MeOH/Et_3N = 10/1, rt; (c) 1-bromo-3-methylbutane, NaH, DMF, 0 °C to rt; (d) ICl-pyridine, CH_2Cl_2/H_2O = 2/1, rt, (e) **9** or **12**, $PdCl_2(dppf)$, K_3PO_4, DMF, 80 °C; (f) Pd/C, H_2 (3 atm), AcOEt, 50 °C.

Introduction of L-leucine methyl ester into 2-bromobenzonitrile (**2**) by Buchwald–Hartwig cross-coupling reaction gave **3**. Reduction of nitrile **3** with Raney nickel and hydrogen induced intramolecular cyclization,[17] affording **4**. N-Alkylation of amide **4** with 1-bromo-3-methylbutane gave **5**, and subsequent iodination of **5** with ICl-pyridine complex[17] gave **6**. Suzuki coupling of **6** and commercially available 2-methyl-1-propenyl boronic acid pinacol ester (**9**) gave **7a**. On the other hand, Suzuki coupling of **6** and (*E*)-but-1-enylboronic acid pinacol ester (**12**), synthesized from but-1-yne (**10**) according to a previous report (Scheme 2),[18] afforded **7b**

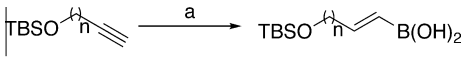


Scheme 2. Reagents and conditions: (a) $BH_3{\cdot}SMe_2$, (+)-α-pinene, THF, 0 °C to rt; acetaldehyde, 40 °C; H_2O, rt; (b) pinacol, $MgSO_4$, CH_2Cl_2, rt.

and **7c**. We considered that **7b** was formed by isomerization, as reported previously.[19] Reduction of the double bond of **7a** with palladium charcoal and hydrogen gave **8**. To generate more potent inhibitors, we focused on the pocket of the VDR near Leu 630 of the LXXLL peptide fragment. That pocket is composed of residues Ile234, Ile238, Leu259, Ala263 and Val417 (Fig. 3).[16] We designed and synthesized benzodiazepine compounds bearing an alkyl alcohol at the 7-position because we thought that the alkyl alcohol moiety might form a hydrogen bond with the oxygen atom of Leu259 in the pocket (Fig. 3 and Schemes 3 and 4). Boronic acids **14a–b** were synthesized by hydroboration of the corresponding alkynes **13a–b** (Scheme 3).[18] Suzuki coupling of **6** and **14a–b** gave **15a–b**, then removal of the TBS group with TBAF gave **16a–b**. Compounds **17a–b** were prepared by reduction of the double bond of **15a–b** with palladium charcoal and hydrogen. Removal of the TBS group of **17a–b** with TBAF gave **18a–b** (Scheme 4).

To investigate the cell-level inhibition of VDR-mediated transcriptional activity, we utilized a VDR-responsive reporter gene assay[14] with CMX-GAL4N-hVDR LBD as the recombinant receptor gene, TK-MH100x4-LUC as the reporter gene, and CMX β-galactosidase gene for normalization. Human embryonic kidney (HEK) 293 cells were incubated with $1,25(OH)_2D_3$ (3 nM) in the presence or absence of test compounds. After incubation, cells were assayed for luciferase reporter gene and β-galactosidase activity. The activities of the synthesized compounds are shown in Table 1.

Synthesized benzodiazepine analogs that had a hydrogen atom at the 8-position showed weaker inhibition of VDR-mediated transcriptional activity than **1**, which has the amino group at the 8-position. In particular, **7a**, corresponding to **1**, showed no inhibitory activity on VDR-mediated transcription, whereas the IC_{50} value of **1** is 26 μM. These results suggest that the amino group at the



13a (n = 1)
13b (n = 2)

14a (n = 1)
14b (n = 2)

Scheme 3. Reagents and conditions: (a) $BH_3{\cdot}SMe_2$, (+)-α-pinene, THF, 0 °C to rt; acetaldehyde, 40 °C; H_2O, rt.

8-position of **1** is critical for the inhibitory activity, and support the idea that the amino group at the 8-position mimics the charge clamp of the LXXLL motif. Therefore, we decided to synthesize a series of compounds having an amino group at the 8-position in order to generate more potent inhibitors. On the other hand, the compounds with a hydroxyalkyl group, **16a–b** and **18a–b**, did not show increased inhibition of VDR-mediated transcription. These results suggest that the hydroxyalkyl group at the 7-position does not contribute to the inhibitory activity.

2.2. SAR at the 7-position of benzodiazepine derivatives bearing an amino group at the 8-position

To evaluate the SAR at the 7-position of benzodiazepine, we synthesized a series of benzodiazepine derivatives bearing an amino group at the 8-position (Scheme 5). Suzuki coupling reaction of **19**[14] and boronic acid pinacol ester **12** or commercially available (*E*)-pent-1-enylboronic acid pinacol ester gave **20a–b**. Removal of the Boc groups of **20a–b** with TFA gave compounds **21a–b**. To generate **22a–b**, **21a–b** were cyclized by application of the Buchwald–Hartwig cross-coupling reaction. Compound **23a–b** were prepared by reduction of **22a–b** with palladium charcoal and hydrogen. However, this scheme was not favorable to synthesize a series of

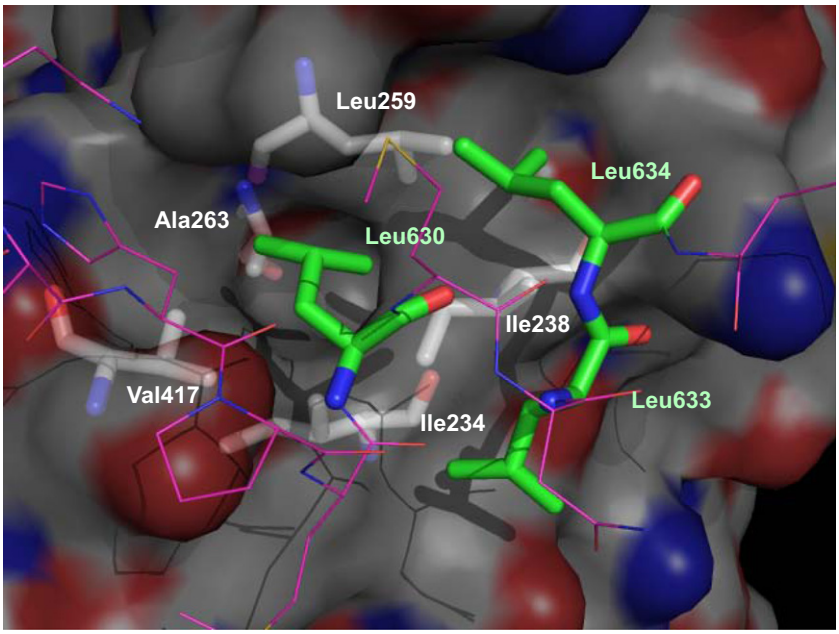
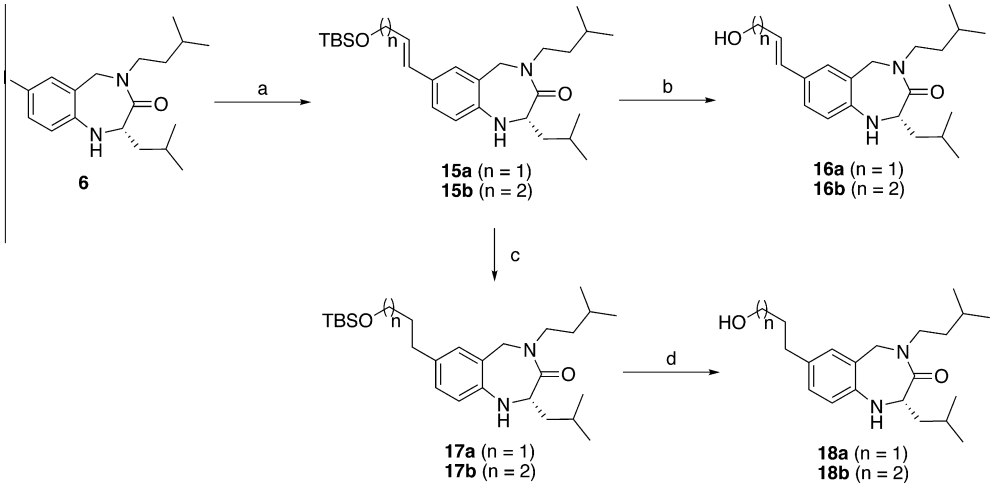
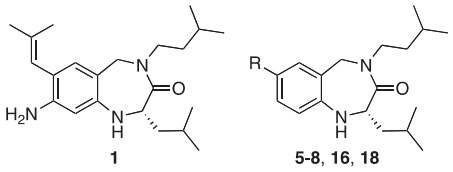


Figure 3. The pocket composed of residues Ile234, Ile238, Leu259, Ala263, and Val417 of VDR (PDB ID 1RK3). The VDR surface is shown in gray and key residues of the pocket are shown in white. The peptide is shown in green and magenta, and key leucine residues in the peptide are shown in green. The image was drawn with PYMOL.



Scheme 4. Reagents and conditions: (a) **14a** or **14b**, $PdCl_2(dppf)$, K_3PO_4, DMF, 80 °C; (b) TBAF, THF, rt; (c) Pd/C, H_2, 1,4-dioxane, rt; (d) TBAF, THF, rt.

Table 1
SAR at the 7-position of benzodiazepine derivatives without an amino group in VDR reporter gene assay[a]



Compound	R	IC_{50} (μM)
1		26
5	H	>30 (29%)[b]
6	I	>30 (30%)[b]
7a		N.A.[c]
7b		>30 (32%)[b]
7c		N.A.[c]
8		>30 (45%)[b]
16a	HO	>30 (27%)[b]
16b	HO	>30 (28%)[b]
18a	HO	>30 (9%)[b]
18b	HO	N.A.[c]

[a] HEK293 cells were treated with $1,25(OH)_2D_3$ (3 nM) and test compounds.
[b] Inhibition ratio at 30 μM.
[c] N.A. means no inhibitory activity at 30 μM.

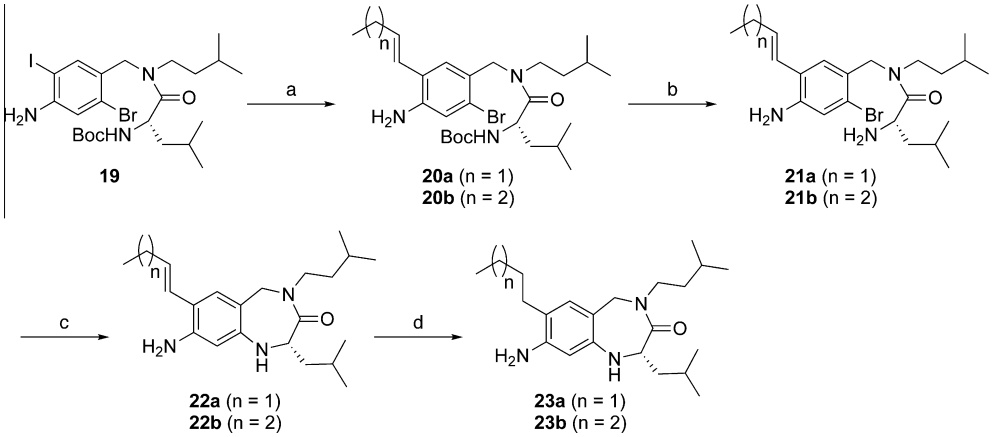
compounds with various substituents at the 7-position, because multiple steps were necessary, so we designed a new scheme that would be more suitable to obtain compounds for evaluation of SAR at the 7-position of benzodiazepine (Scheme 6).

Nucleophilic substitution of 2-bromo-4-fluorobenzonitrile (**24**) with 4-methoxybenzylamine followed by removal of the PMB group, and protection of the amino group with diBoc gave **27**. Introduction of L-leucine methyl ester into **27** by Buchwald–Hartwig cross-coupling reaction followed by reduction of the nitrile with Raney nickel and hydrogen resulted in intramolecular cyclization to afford **29**. N-alkenylation of the amide group of **29** with 1-bromo-3-methyl-2-butene gave **30**. Successive reduction and removal of the diBoc group with HCl afforded **32**. Iodination of **32** with ICl-pyridine gave **33**, and then introduction of a phenyl group into **33** by Suzuki coupling gave **34**.

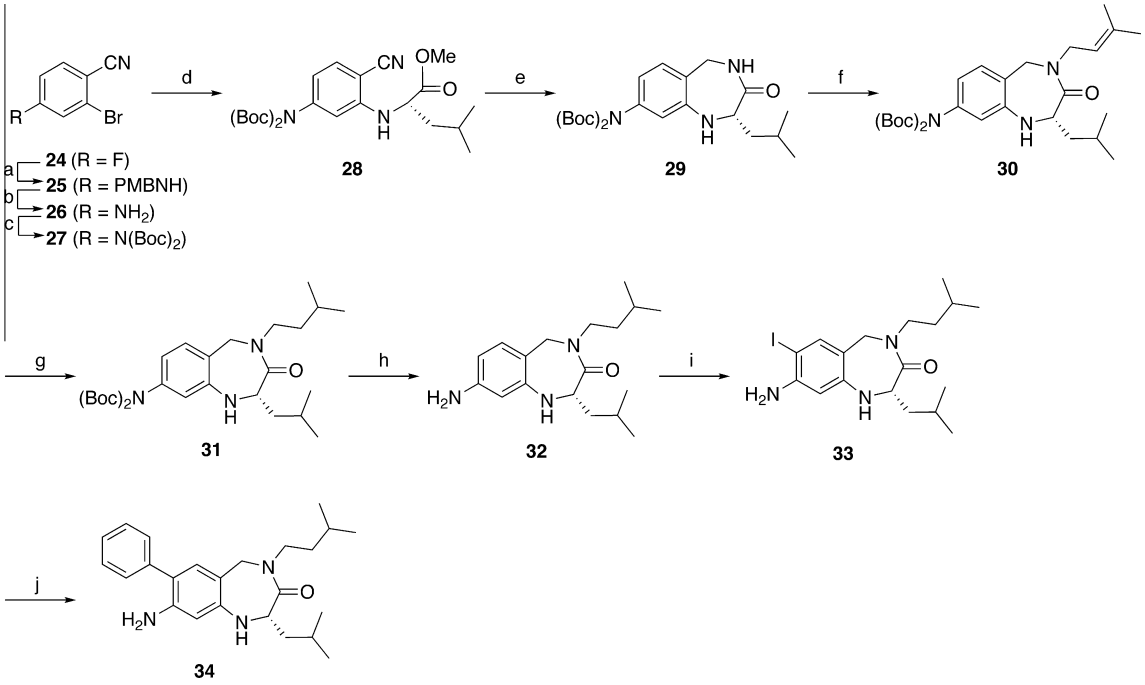
We used the VDR-responsive reporter gene assay to investigate the activity of these compounds. Replacement of the isobutenyl group at the 7-position with a normal alkyl, alkenyl, or phenyl group (**22**, **23** or **34**) did not affect the inhibitory activity (Table 2). Replacement of the isobutenyl group (**1**) with iodine (**33**) retained the inhibitory activity, while replacement of the hydrogen atom (**32**) resulted in loss of the inhibitory activity. These results suggested that a hydrophobic and moderately bulky group at the 7-position of benzodiazepine compounds is favorable for inhibitory activity towards VDR-mediated transcription.

According to the SAR, we designed new benzodiazepine compounds containing a phenyl group with carboxylic acid, because we thought that carboxylic acid might form a hydrogen bond with the nitrogen atom of Lys260 (Fig. 4 and Scheme 7). Suzuki coupling reaction of **33** with 4-ethoxycarbonylphenylboronic acid pinacol ester afforded **35**, then hydrolysis of ethyl ester with KOH gave **36**. We also synthesized **42** because we thought that **42** might form a hydrogen bond between its hydroxyl group and the oxygen atom of Leu259 in the pocket (Fig. 3). This compound was obtained by Suzuki coupling reaction of **33** and the corresponding boronic acid pinacol ester **40**, and reduction with hydrogen and palladium charcoal (Scheme 8). Compound **40** was synthesized from commercially available ethyl 3-hydroxybenzoate (**37**) through iodination, Mitsunobu reaction, and introduction of the boronic acid pinacol ester group. The position of the iodine atom of **38** was determined by two-dimensional heteronuclear multiple-bond correlation spectroscopy (HMBC).

In VDR reporter gene assay, **35** showed a lower IC_{50} value than **1**, while **36** showed little inhibitory activity (Table 3). One possible explanation is that the introduced carboxyl groups might be important to improve the inhibitory activity of VDR, but the compound with the free carboxylic acid, **36**, is likely to have poor membrane permeability. In this case, ethyl benzoate **35** might work as a prodrug of **36** that would be able to penetrate the cell membrane, and be hydrolyzed in the cytoplasm to give **36**, which could then form a hydrogen bond between the carboxyl group and the nitrogen atom of Lys260. On the other hand, **42**, which has both a carboxyl group and a hydroxyl group, showed an intermediate IC_{50} value between **34** (with a non-substituted phenyl group) and **35** (with only an ester group). This result indicates that the hydroxyl group does not increase the inhibitory activity of VDR.



Scheme 5. Reagents and conditions: (a) boronic acid pinacol ester, $PdCl_2(dppf)$, K_3PO_4, DMF, 80 °C; (b) TFA, CH_2Cl_2, 0 °C; (c) $Pd_2(dba)_3{\cdot}CHCl_3$, (±)-BINAP, Cs_2CO_3, toluene, 110 °C; (d) Pd/C, H_2, AcOEt, rt.



Scheme 6. Reagents and conditions: (a) 4-methoxybenzylamine, 140 °C; (b) DDQ, CH_2Cl_2/H_2O = 2/1, rt; (c) $(Boc)_2O$, DMAP, DIPEA, THF, reflux; (d) L-leucine methyl ester hydrochloride, $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, H_2O, toluene, 110 °C; (e) Raney nickel, H_2, MeOH/Et_3N = 10/1, rt; (f) 1-bromo-3-methylbut-2-ene, TBAI, *t*-BuOK, THF, −20 °C; (g) Pd/C, H_2, 1,4-dioxane, rt; (h) HCl, 1,4-dioxane, rt; (i) ICl-pyridine, CH_2Cl_2/H_2O = 2/1, rt, (j) phenylboronic acid, $PdCl_2(dppf)$, K_3PO_4, DMF, 80 °C.

3. Conclusion

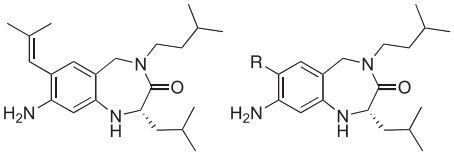
Suppression of VDR-mediated transcription is expected to be of therapeutic value in Paget's disease of bone. It is known that interaction between VDR and coactivators is necessary for VDR transactivation, and the interaction occurs when VDR recognizes an LXXLL peptide motif of coactivators. We previously reported that benzodiazepine molecules designed as LXXLL peptide mimetics inhibited the interaction of VDR and coactivators, and reduced VDR transcription. In this study, we investigated the SAR at the 7- and 8-positions of our compounds. The results showed firstly that the amino group at the 8-position is essential for the inhibitory activity, and support the idea that this amino group works as a charge clamp, stabilizing the binding between the inhibitor and the VDR-LBD. Secondly, replacement of the isobutenyl group at the 7-position with various hydrophobic groups had no effect on the inhibitory activity. But, interestingly, the benzodiazepine derivative bearing an ethyl benzoate group **35** showed more potent inhibitory activity. We hypothesize that **35** works as a membrane-permeable prodrug that is hydrolyzed in the cytoplasm to afford **36**, which can form a hydrogen bond between its carboxyl group and the nitrogen atom of Lys260, resulting in potent inhibitory activity.

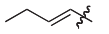
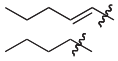
4. Experimental

4.1. General

Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a JEOL JNM-GX500 (500 MHz) spectrometer. Chemical shifts are expressed in parts per million relative to tetramethylsilane. Mass spectra were recorded on a JEOL JMS-DX303 spectrometer.

Table 2
SAR at the 7-position of benzodiazepine derivatives with an amino group in VDR reporter gene assay[a]



Compound	R	IC_{50} (μM)
1		26
32		>30 (7%)[b]
33		30
22a		>30 (48%)[b]
22b		Toxic[c]
23a		25
23b		>30 (47%)[b]
34		24

[a] HEK293 cells were treated with $1,25(OH)_2D_3$ (3 nM) and test compounds.
[b] Inhibition ratio at 30 μM.
[c] Toxic at 30 μM.

4.1.1. General procedure of Suzuki coupling (GP-A)

Under an Ar atmosphere, K_3PO_4, $Pd(dppf)Cl_2$ and the corresponding boronic acid or boronic acid pinacol ester were added to a solution of halide in DMF. The reaction mixture was stirred for an appropriate time at 80 °C, then the reaction was quenched with H_2O and the mixture was extracted with AcOEt. The organic layer was dried over $MgSO_4$ and concentrated. The resulting residue was purified by silica gel chromatography, PTLC and/or HPLC to afford the target molecule.

4.1.2. General procedure of hydroboration and hydrolysis (GP-B)

A bomb flask was charged with THF and $BH_3{\cdot}SMe_2$ at 0 °C, then (+)-α-pinene was added dropwise. The solution was stirred at 0 °C for 10 min then allowed to warm to rt and stirred at rt for 2–3 h, during which time a white precipitate formed. The solution was recooled to 0 °C and alkyne was added to it, resulting in a clear, colorless solution. The flask was then sealed with a Teflon screw cap and the mixture was stirred at 0 °C for 5–30 min, warmed to rt, and stirred at rt for 1.5–2.5 h. The solution was recooled to 0 °C and acetaldehyde was added. The bomb flask was resealed with the Teflon screw cap and the reaction mixture was stirred at 40 °C for 4.5–5 h. It was allowed to cool to 0 °C and H_2O was added. Stirring was continued for 3–7 h at rt, and then the solution was diluted with EtOAc. Purification was performed by partition under basic conditions or by silica gel chromatography to afford the target molecule.

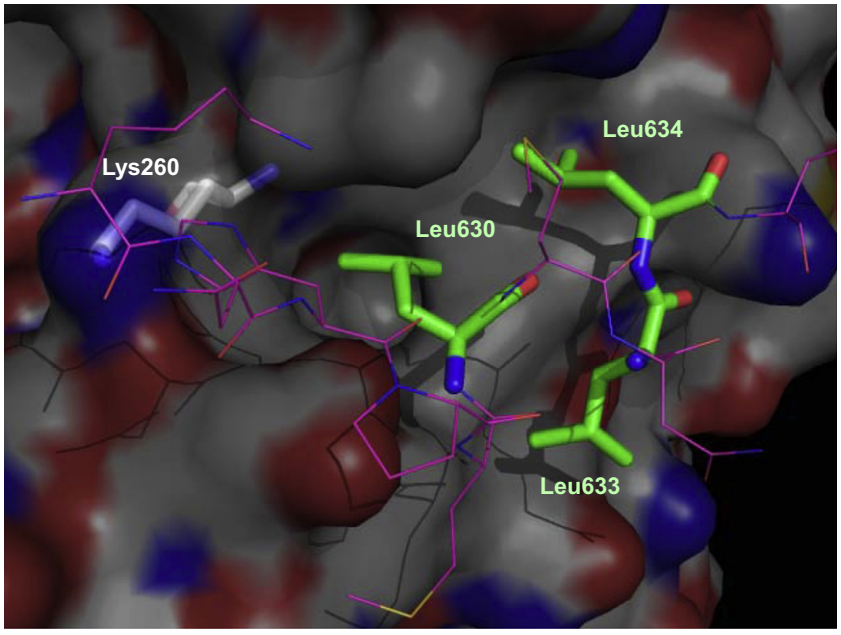
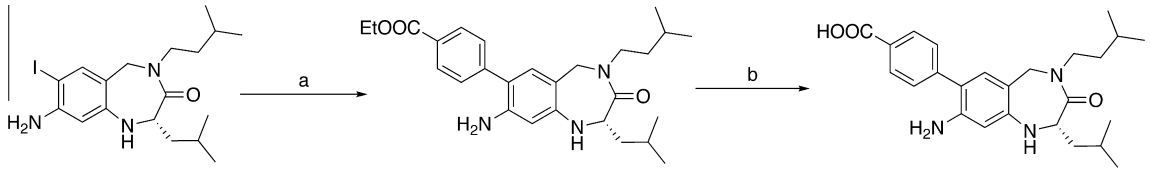
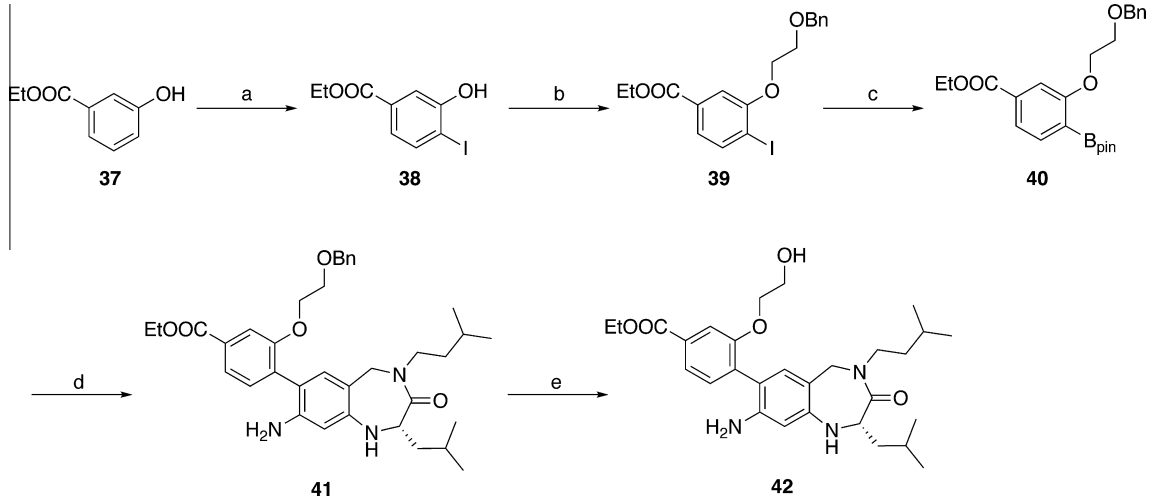


Figure 4. Spatial relationship between LXXLL peptide fragment and Lys260 of VDR (PDB ID 1RK3). The VDR surface is shown in gray and Lys260 is shown in white. The peptide is shown in green and magenta, and key leucine residues in the peptide are shown in green. The image was drawn with PYMOL.

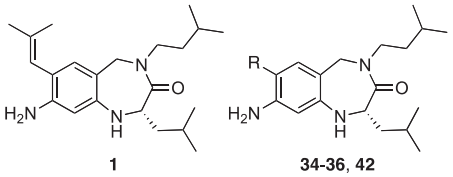


Scheme 7. Reagents and conditions: (a) 4-ethoxycarbonylphenyl boronic acid pinacol ester, $PdCl_2(dppf)$, K_3PO_4, DMF, 80 °C; (b) KOH, $MeOH/H_2O$ = 5/1, 60 °C.

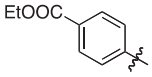
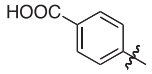
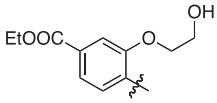


Scheme 8. Reagents and conditions: (a) NIS, AcOH, rt; (b) 2-phenoxyethanol, DIAD, PPh_3, THF, rt; (c) bis(pinacolate)diboron, $PdCl_2$(dppf), KOAc, DMSO, 80 °C; (d) **33**, $PdCl_2$(dppf), K_3PO_4, DMF, 80 °C; (e) Pd/C, H_2 (2.5 atm), 1,4-dioxane, 50 °C.

Table 3
SAR for substitution of the phenyl group in VDR reporter gene assay[a]



1 **34-36**, **42**

Compound	R	IC_{50} (μM)
1		26
34		24
35		14
36		>30 (8%)[b]
42		20

[a] HEK293 cells were treated with $1,25(OH)_2D_3$ (3 nM) and test compounds.
[b] Inhibition ratio at 30 μM.

4.1.3. General procedure of removal of TBS group (GP-C)

Under an Ar atmosphere, a 1.0 M solution of tetra-*n*-butylammonium fluoride (TBAF) in THF was added to a stirred solution of a compound bearing a TBS group in THF. The reaction mixture was stirred for an appropriate time, then the reaction was quenched with H_2O and brine, and the mixture was extracted with AcOEt. The organic layer was dried over $MgSO_4$ and concentrated. The resulting residue was purified by PTLC to afford the target molecule.

4.1.4. (*S*)-1,2,4,5-Tetrahydro-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (4)

Under an Ar atmosphere, $Pd_2(dba)_3{\cdot}CHCl_3$ (104 mg, 100 μmol), (*R*)-BINAP (139 mg, 223 μmol) and Cs_2CO_3 (3.61 g, 11. 1 mmol) were successively added to a solution of 2-bromobenzonitrile (**2**) (1.08 g, 5.95 mmol) and L-leucine methyl ester hydrochloride (1.44 g, 7.97 mmol) in toluene (11 mL). The reaction mixture was stirred for 4 h at 110 °C, then the reaction was quenched with H_2O and the mixture was extracted with AcOEt. The organic layer was dried over $MgSO_4$ and concentrated. The resulting residue was purified by silica gel chromatography (hexane/AcOEt = 1/0 to 20/1) to afford **3** (mixture, 306 mg) as a pale yellow oil. This mixture was used for the next step without further purification. Under an Ar atmosphere, an H_2O suspension of Raney nickel (1.5 mL, TCI) was added to a solution of the above mixture in MeOH (12 ml)/Et_3N (1.2 mL). The Ar atmosphere was replaced with H_2. The reaction mixture was stirred for 24 h at rt, then filtered through Celite and concentrated. The resulting residue was purified by silica gel chromatography (hexane/AcOEt = 1/1 to 1/2) to afford **4** (241 mg, 1.10 mmol, 19% (two steps)) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.07 (dd, J = 8.0, 7.3 Hz, 1H), 6.91 (d, J = 7.3 Hz, 1H), 6.67 (dd, J = 8.0, 7.3 Hz, 1H), 6.56 (d, J = 8.0 Hz, 1H), 6.83–6.31 (m, 1H), 5.01 (dd, J = 16.0, 5.5 Hz, 1H), 4.50–4.41 (m, 1H), 3.90 (dd, J = 16.0, 6.7 Hz, 1H), 3.58–3.42 (m, 1H), 1.92–1.79 (m, 2H), 1.55–1.47 (m, 1H), 0.99 (d, J = 6.1 Hz, 3H), 0.97 (d, J = 6.8 Hz, 3H); MS (FAB) m/z 218 $(M)^+$, 219 $(M+H)^+$.

4.1.5. (*S*)-1,2,4,5-Tetrahydro-4-(3-methylbutyl)-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (5)

Under an Ar atmosphere, NaH (60%, dispersion in paraffin liquid) (10.2 mg, 255 μmol) was added to a solution of **4** (36.6 mg, 168 μmol) in DMF (2.0 mL) at 0 °C. The reaction mixture was stirred for 15 min at rt, then 1-bromo-3-methylbutane (23.2 μL, 184 μmol) was added to it at 0 °C. Stirring was continued for 7 h at rt, then the reaction was quenched with H_2O and the mixture was extracted with AcOEt. The organic layer was dried over $MgSO_4$ and concentrated. The resulting residue was purified by silica gel chromatography (hexane/AcOEt = 1/0 to 5/1) to afford **5** (39.0 mg, 135 μmol, 80%) as white needles.

Mp 85 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05 (dd, J = 7.3, 6.7 Hz, 1H), 6.91 (d, J = 6.7 Hz, 1H), 6.62 (dd, J = 8.0, 7.3 Hz, 1H), 6.49 (d, J = 8.0 Hz, 1H), 5.37 (d, J = 16.5 Hz, 1H), 4.53 (td, J = 6.8, 6.4 Hz, 1H), 3.76 (d, J = 16.5 Hz, 1H), 3.57–3.43 (m, 3H), 1.94–1.88 (m, 1H), 1.86–1.77 (m, 1H), 1.52–1.31 (m, 4H), 0.97 (d, J = 5.5 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H), 0.84 (d, J = 6.1 Hz, 3H), 0.82 (d, J = 6.7 Hz, 3H); MS (FAB) m/z 288 $(M)^+$, 289 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{18}H_{28}N_2O$ 288.2202, found 288.2206 $(M)^+$.

4.1.6. (*S*)-1,2,4,5-Tetrahydro-7-iodo-4-(3-methylbutyl)-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (6)

ICl-pyridine complex (36.5 mg, 151 μmol) was added to a solution of **5** (39.0 mg, 135 μmol) in CH_2Cl_2/H_2O (CH_2Cl_2 3.6 mL and H_2O 1.8 mL). The reaction mixture was stirred 4.5 h at rt, and then the reaction was quenched with satd $NaHCO_3$ aq (3.0 mL) and 21 mM $Na_2S_2O_3$ aq (3.0 mL). After the solution had changed color (brown to pale yellow), it was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The resulting residue was purified by silica gel chromatography (hexane/AcOEt = 1/0 to 6/1) to afford **6** (51.4 mg, 124 μmol, 92%) as a white solid.

Mp 94–96 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (dd, J = 8.6, 1.8 Hz, 1H), 7.21 (d, J = 1.8 Hz, 1H), 6.27 (d, J = 8.6 Hz, 1H), 5.29 (d, J = 16.8 Hz, 1H), 4.58–4.54 (m, 1H), 3.68 (d, J = 16.8 Hz, 1H), 3.55–3.53 (m, 1H), 3.51–3.47 (m, 2H), 1.93–1.87 (m, 1H), 1.83–1.74 (m, 1H), 1.51–1.31 (m, 4H), 0.96 (d, J = 6.8 Hz, 3H), 0.95 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 6.7 Hz, 3H), 0.84 (d, J = 6.1 Hz, 3H); MS (FAB) m/z 414 $(M)^+$, 415 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{18}H_{27}IN_2O$ 414.1168, found 414.1163 $(M)^+$.

4.1.7. (*S*)-1,2,4,5-Tetrahydro-4-(3-methylbutyl)-7-(2-methylprop-1-enyl)-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (7a)

This compound was prepared by means of GP-A, with K_3PO_4 (55.2 mg, 260 μmol), Pd(dppf)Cl_2 (6.10 mg, 8.34 μmol), **9** (15.6 μL, 76.4 μmol), **6** (26.4 mg, 63.7 μmol) and DMF (0.65 mL). Purification by silica gel chromatography (hexane/AcOEt = 1/0 to 6/1) and PTLC (hexane/AcOEt = 6/1) afforded **7a** (3.4 mg, 9.9 μmol, 15%) as a white solid.

Mp 112–113 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.94 (dd, J = 8.0, 1.9 Hz, 1H), 6.79 (d, J = 1.9 Hz, 1H), 6.45 (d, J = 8.0 Hz, 1H), 6.11 (s, 1H), 5.36 (d, J = 16.8 Hz, 1H), 4.59–4.54 (m, 1H), 3.73 (d, J = 16.8 Hz, 1H), 3.57–3.42 (m, 3H), 1.93–1.86 (m, 1H), 1.86 (s, 3H), 1.83 (s, 3H), 1.83–1.76 (m, 1H), 1.52–1.32 (m, 4H), 0.97 (d, J = 6.8 Hz, 3H), 0.96 (d, J = 6.1 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H), 0.81 (d, J = 6.8 Hz, 3H); MS (FAB) m/z 342 $(M)^+$, 343 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{22}H_{34}N_2O$ 342.2671, found 342.2666 $(M)^+$.

4.1.8. (*S*,*E*)-7-(But-1-enyl)-1,2,4,5-tetrahydro-4-(3-methylbutyl)-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (7b) and (*S*)-7-(but-1-en-2-yl)-1,2,4,5-tetrahydro-4-(3-methylbutyl)-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (7c)

These compounds were prepared by means of GP-A, with K_3PO_4 (40.7 mg, 145 μmol), Pd(dppf)Cl_2 (6.10 mg, 8.34 μmol), **12** (39.4 mg, 216 μmol), **6** (21.3 mg, 51.4 μmol) and DMF (0.50 mL). Purification by silica gel chromatography (hexane/AcOEt = 6/1), PTLC (NH plate, FUJI SILYSIA) (hexane/AcOEt = 2/1) and HPLC with Sensyu Pak PEGASIL ODS (20φ × 250 mm) (CH_3CN/H_2O = 85/15) afforded **7b** (3.70 mg, 10.8 μmol, 21%) as a yellow oil and **7c** (2.50 mg, 7.30 μmol, 14%) as a yellow oil.

Compound **7b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.07 (dd, J = 8.6, 1.9 Hz, 1H), 6.90 (d, J = 1.9 Hz, 1H), 6.43 (d, J = 8.6 Hz, 1H), 6.22 (d, J = 15.9 Hz, 1H), 6.05 (td, J = 15.9, 6.1 Hz, 1H) 5.34 (d, J = 16.5 Hz, 1H), 4.59–4.53 (m, 1H), 3.74 (d, J = 16.5 Hz, 1H), 3.54–3.44 (m, 3H), 2.23–2.15 (m, 2H), 1.94–1.87 (m, 1H), 1.85–1.76 (m, 1H), 1.57–1.33 (m, 4H), 1.07 (t, J = 7.9 Hz, 3H), 0.97 (d, J = 5.5 Hz, 3H), 0.96 (d, J = 6.1 Hz, 3H), 0.90 (d, J = 6.8 Hz, 3H), 0.83 (d, J = 6.1 Hz, 3H); MS (FAB) m/z 342 $(M)^+$, 343 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{22}H_{34}N_2O$ 342.2671, found 342.2673 $(M)^+$.

Compound **7c**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (dd, J = 8.2, 2.5 Hz, 1H), 7.00 (d, J = 2.5 Hz, 1H), 6.46 (d, J = 8.2 Hz, 1H), 5.38 (d, J = 16.5 Hz, 1H), 5.15 (s, 1H), 4.92–4.91 (m, 1H), 4.62–4.57 (m, 1H), 3.77 (d, J = 16.5 Hz, 1H), 3.59–3.44 (m, 3H), 2.48–2.42 (m, 2H), 1.94–1.88 (m, 1H), 1.85–1.75 (m, 1H), 1.50–1.35 (m, 4H), 1.09 (t, J = 7.4 Hz, 3H), 0.97 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H), 0.81 (d, J = 6.8 Hz, 3H); MS (FAB) m/z 342 $(M)^+$, 343 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{22}H_{34}N_2O$ 342.2671, found 342.2673 $(M)^+$.

4.1.9. (*S*)-1,2,4,5-Tetrahydro-4-(3-methylbutyl)-2,7-di(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (8)

Under an Ar atmosphere in a pressure-tight flask, 10% Pd/C (6.50 mg) was added to a solution of **7a** (2.00 mg, 5.80 μmol) in AcOEt (6 mL). The Ar atmosphere was replaced with H_2 (3.0 atm). The reaction mixture was stirred for 14 h at 50 °C, then filtered through Celite and concentrated. The resulting residue was purified by PTLC (hexane/AcOEt = 6/1) to afford **8** as a white solid in quantitative yield.

Mp 103–105 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.83 (dd, J = 8.0, 1.9 Hz, 1H), 6.69 (d, J = 1.9 Hz, 1H), 6.43 (d, J = 8.0 Hz, 1H), 5.35 (d, J = 16.2 Hz, 1H), 4.58–4.52 (m, 1H), 3.73 (d, J = 16.2 Hz, 1H), 3.62–3.56 (m, 1H), 3.44–3.37 (m, 2H), 2.36–2.28 (m, 2H), 1.93–1.86 (m, 1H), 1.84–1.73 (m, 2H), 1.49–1.32 (m, 4H), 0.96 (d, J = 6.7 Hz, 3H), 0.95 (d, J = 6.7 Hz, 3H), 0.88 (d, J = 6.1 Hz, 3H), 0.87 (d, J = 6.7 Hz, 3H), 0.86 (d, J = 6.7 Hz, 3H), 0.81 (d, J = 6.8 Hz, 3H); MS (FAB) m/z 344$(M)^+$, 345 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{22}H_{36}N_2O$ 344.2828, found 344.2827 $(M)^+$.

4.1.10. (*E*)-But-1-enylboronic acid (11)

This compound was prepared by means of GP-B, with THF (11 mL), $BH_3{\cdot}SMe_2$ (1.80 mL, 19.4 mmol), (+)-α-pinene (6.80 mL, 42.9 mmol), **10** (excess), acetaldehyde (10.4 mL, 185 mmol) and H_2O (5.0 mL). Purification was performed by partition. The mixture was diluted with AcOEt and extracted with 10% NaOH aq (2 × 10 mL). The combined aqueous solutions were acidified to pH 2 with concentrated HCl aq and extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with satd $NaHCO_3$ aq, dried over $MgSO_4$, and concentrated to afford **11** (1.03 g, 10.3 mmol, 53%) as a colorless solid.

^{1}H NMR (500 MHz, DMSO-d_6/D_2O = 95/5) δ 6.46 (td, J = 17.6, 6.1 Hz, 1H), 5.28 (td, J = 17.6, 1.8 Hz, 1H), 2.09–2.01 (m, 2H), 0.93 (t, J = 7.3 Hz, 3H); MS (FAB) not detected.

4.1.11. (*E*)-Buten-1-ylboronic acid pinacol ester (12)

Compound **11** (217 mg, 2.17 mmol) and $MgSO_4$ (0.63 g, 5.23 mmol) were added to a solution of pinacol (237 mg, 2.00 mmol) in CH_2Cl_2 (6 mL). The reaction mixture was stirred 21 h at rt, then filtered and the filtrate was concentrated to afford pure **12** (338 mg, 1.86 mmol, 93%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.70 (td, J = 18.0, 6.1 Hz, 1H), 5.43 (td, J = 18.0, 1.9 Hz, 1H), 2.20–2.14 (m, 2H), 1.26 (s, 12 H), 1.02 (t, J = 7.3 Hz, 3H); MS (FAB) not detected.

4.1.12. (*E*)-3-(*tert*-Butyldimethylsilyloxy)prop-1-enylboronic acid (14a)

This compound was prepared by means of GP-B, with THF (6.3 mL), $BH_3{\cdot}SMe_2$ (1.10 mL, 11.6 mmol), (+)-α-pinene (3.50 mL, 22.1 mmol), **13a** (2.00 mL, 9.86 mmol), acetaldehyde (7.00 mL, 126 mmol) and H_2O (2.5 mL). Purification was performed by silica gel chromatography. The mixture was diluted with AcOEt, dried over $MgSO_4$ and concentrated. The resulting residue was purified by silica gel chromatography (hexane/AcOEt = 3/1 to 2/1) to afford **14a** (467 mg, 2.16 mmol. 22%) as a colorless oil.

^{1}H NMR (500 MHz, CD_3OD) δ 6.57 (td, J = 17.7, 3.7 Hz, 1H), 5.87 (td, J = 17.7, 1.8 Hz, 1H), 4.26 (dd, J = 3.7, 1.8 Hz, 2H), 0.94 (s, 9H), 0.04 (s, 6H); MS (FAB) not detected.

4.1.13. (*E*)-4-(*tert*-Butyldimethylsilyloxy)but-1-enylboronic acid (14b)

This compound was prepared by means of GP-B, with THF (6.3 mL), $BH_3{\cdot}SMe_2$ (1.10 mL, 11.6 mmol), (+)-α-pinene (3.50 mL, 22.1 mmol), **13b** (2.10 mL, 10.1 mmol), acetaldehyde (7.00 mL,

126 mmol) and H_2O (2.5 mL). Purification was performed by silica gel chromatography. The mixture was diluted with AcOEt, dried over $MgSO_4$ and concentrated. The resulting residue was purified by silica gel chromatography (hexane/AcOEt = 3/1 to 2/1) to afford **14b** (638 mg, 2.77 mmol, 27%) as a colorless oil.

^{1}H NMR (500 MHz, CD_3OD) δ 6.54 (td, J = 17.3, 3.7 Hz, 1H), 5.64 (d, J = 17.3 Hz, 1H), 3.71 (t, J = 6.1 Hz, 2H), 2.36 (td, J = 6.7, 6.1 Hz, 2H), 0.90 (s, 9H), 0.06 (s, 6H); MS (FAB) not detected.

4.1.14. (*S*)-1,2,4,5-Tetrahydro-7-(3-hydroxyprop-1-enyl)-4-(3-methylbutyl)-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (16a)

Intermediate **15a** was prepared by means of GP-A, with K_3PO_4 (69.9 mg, 329 μmol), Pd(dppf)Cl_2 (9.60 mg, 13.1 μmol), **14a** (34.6 mg, 160 μmol), **6** (39.8 mg, 96.1 μmol) and DMF (1.0 mL). Purification by silica gel chromatography (hexane/AcOEt = 1/0 to 6/1) and PTLC (hexane/AcOEt = 4/1) afforded almost pure **15a** (mixture, 13.8 mg) as a yellow oil. This was used for the next reaction without further purification. Compound **16a** was prepared by means of GP-C, with 1.0 M TBAF in THF (18.0 μL, 18.0 μmol), almost pure **15a** (mixture, 7.10 mg) and THF (0.50 mL). Purification by silica gel chromatography (hexane/AcOEt = 1:1) afforded **16a** (1.80 mg, 5.23 μmol, 11%, two steps) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (dd, J = 7.9, 1.8 Hz, 1H), 6.95 (d, J = 1.8 Hz, 1H), 6.46 (d, J = 15.9 Hz, 1H), 6.44 (d, J = 7.9 Hz, 1H), 6.61 (td, J = 15.9, 6.1 Hz, 1H), 5.36 (d, J = 16.5 Hz, 1H), 4.62–4.57 (m, 1H), 4.30–4.26 (m, 2H), 3.75 (d, J = 16.5 Hz, 1H), 3.60–3.58 (m, 1H), 3.55–3.46 (m, 2H) 1.95–1.88 (m, 1H), 1.85–1.75 (m, 1H), 1.51–1.42 (m, 2H), 1.42–1.33 (m, 2H), 0.97 (d, J = 6.1 Hz, 3H), 0.96 (d, J = 6.1 Hz, 3H), 0.90 (d, J = 6.1 Hz, 3H), 0.82 (d, J = 6.7 Hz, 3H); MS (FAB) m/z 344 $(M)^+$, 345 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{21}H_{32}N_2O_2$ 344.2464, found 344.2466 $(M)^+$.

4.1.15. (*S*)-1,2,4,5-Tetrahydro-7-(4-hydroxybut-1-enyl)-4-(3-methylbutyl)-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (16b)

Intermediate **15b** was prepared by means of GP-A, with K_3PO_4 (21.7 mg, 102 μmol), Pd(dppf)Cl_2 (6.20 mg, 8.47 μmol), **14a** (26.5 mg, 115 μmol), **6** (12.2 mg, 29.4 μmol) and DMF (300 μL). Purification by silica gel chromatography (hexane/AcOEt = 1/0 to 6/1) afforded **15b** (mixture, 7.90 mg) as a yellow oil. This was used for the next reaction without further purification. Compound **16b** was prepared by means of GP-C, with 1.0 M TBAF in THF (25.5 μL, 25.5 μmol), almost pure **15b** (7.10 mg, mixture) and THF (0.50 mL). Purification by silica gel chromatography (hexane/AcOEt = 1/1) afforded **16b** (2.90 mg, 8.09 μmol, 28%, two steps) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.09 (dd, J = 8.3, 1.9 Hz, 1H), 6.91 (d, J = 1.9 Hz, 1H), 6.43 (d, J = 8.3 Hz, 1H), 6.35 (d, J = 15.9 Hz, 1H), 5.97 (td, J = 7.3, 15.9 Hz, 1H), 5.34 (d, J = 16.5 Hz, 1H), 4.61–4.54 (m, 1H), 3.75–3.70 (m, 2H), 3.74 (d, J = 16.5 Hz, 1H), 3.56–3.53 (m, 1H), 3.52–3.46 (m, 2H) 2.45 (dd, J = 7.3, 6.7 Hz, 2H), 1.94–1.87 (m, 1H), 1.85–1.75 (m, 1H), 1.52–1.34 (m, 4H), 0.97 (d, J = 6.1 Hz, 3H), 0.96 (d, J = 6.1 Hz, 3H), 0.90 (d, J = 6.8 Hz, 3H), 0.83 (d, J = 6.7 Hz, 3H); MS (FAB) m/z 358 $(M)^+$, 359 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{22}H_{34}N_2O_2$ 358.2620 found 358.2623 $(M)^+$.

4.1.16. (*S*)-1,2,4,5-Tetrahydro-7-(3-hydroxypropyl)-4-(3-methylbutyl)-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (18a)

Using a protocol similar to that in section **4.1.14**, 10% Pd/C (2.00 mg) was added to a stirred solution of almost pure **15a** (mixture, 7.90 mg) in 1,4-dioxane (0.70 mL). The reaction mixture was stirred for 6 h at rt, then filtered through Celite and concentrated. The resulting residue was purified by PTLC (hexane/AcOEt = 4/1) to afford almost pure **17a** (mixture, 4.90 mg). This was used for the next reaction without further purification. Compound **18a** was prepared by means of GP-C, with 1.0 M TBAF in THF (13.0 μL, 13.0 μmol), almost pure **17a** (mixture, 4.90 mg) and THF (0.50 mL). Purification by PTLC (hexane/AcOEt = 1/1) afforded **18a** (1.20 mg, 3.46 μmol, 6% (three steps)) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.89 (dd, J = 8.0, 1.8 Hz, 1H), 6.75 (d, J = 1.8 Hz, 1H), 6.45 (d, J = 8.0 Hz, 1H), 5.34 (d, J = 16.5 Hz, 1H), 4.56–4.53 (m, 1H), 3.73 (d, J = 16.5 Hz, 1H), 3.68–3.64 (m, 2H), 3.57–3.41 (m, 3H), 2.57 (t, J = 7.9 Hz, 2H), 1.92–1.85 (m, 1H), 1.84–1.76 (m, 3H), 1.50–1.42 (m, 2H), 1.41–1.34 (m, 2H), 0.97 (d, J = 6.1 Hz, 3H), 0.95 (d, J = 6.1 Hz, 3H), 0.89 (d, J = 6.1 Hz, 3H), 0.82 (d, J = 6.7 Hz, 3H); MS (FAB) m/z 346 $(M)^+$, 347 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{21}H_{34}N_2O_2$ 346.2620, found 346.2620 $(M)^+$.

4.1.17. (*S*)-1,2,4,5-Tetrahydro-7-{4-(*tert*-butyldimethylsilyloxy)butyl}-4-(3-methylbutyl)-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (17b)

Using a protocol similar to that in section **4.1.15**, 10% Pd/C (2.80 mg) was added to a stirred solution of **16b** (mixture, 22.3 mg) in 1,4-dioxane (2.0 mL). The reaction mixture was stirred 22 h at rt, then filtered through Celite and concentrated. The resulting residue was purified by PTLC (hexane/AcOEt = 4/1) to afford **17b** (15.2 mg, 32.0 μmol, 34%, two steps) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.87 (dd, J = 8.2, 1.9 Hz, 1H), 6.72 (d, J = 1.9 Hz, 1H), 6.44 (d, J = 8.2 Hz, 1H), 5.33 (d, J = 16.5 Hz, 1H), 4.56–4.51 (m, 1H), 3.73 (d, J = 16.5 Hz, 1H), 3.61 (t, J = 6.1 Hz, 2H), 3.56–3.43 (m, 2H), 3.41–3.39 (m, 1H), 2.48 (t, J = 7.4 Hz, 2H), 1.92–1.86 (m, 1H), 1.84–1.77 (m, 1H), 1.61–1.50 (m, 4H), 1.48–1.41 (m, 2H), 1.40–1.33 (m, 2H), 0.96 (d, J = 6.7 Hz, 3H), 0.95 (d, J = 5.5 Hz, 3H), 0.89 (s, 9H), 0.89 (d, J = 6.1 Hz, 3H), 0.81 (d, J = 6.7 Hz, 3H), 0.04 (s, 6H); MS (FAB) m/z 474 $(M)^{+\cdot}$ 475 $(M+H)^+$.

4.1.18. (*S*)-1,2,4,5-Tetrahydro-7-(4-hydroxybutyl)-4-(3-methylbutyl)-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (18b)

This compound was prepared by means of GP-C, with 1.0 M TBAF in THF (18.0 μL, 18.0 μmol), **17b** (5.60 mg, 11.8 μmol) and THF (0.51 mL). Purification by PTLC (hexane/AcOEt = 1/1) afforded **18b** (2.8 mg, 7.8 μmol, 66%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.87 (dd, J = 7.9, 1.8 Hz, 1H), 6.73 (d, J = 1.8 Hz, 1H), 6.44 (d, J = 7.9 Hz, 1H), 5.34 (d, J = 16.5 Hz, 1H), 4.57–4.52 (m, 1H), 3.73 (d, J = 16.5 Hz, 1H), 3.67–3.64 (m, 2H), 3.56–3.44 (m, 2H), 3.42–3.38 (m, 1H), 2.50 (t, J = 6.7 Hz, 2H), 1.92–1.86 (m, 1H), 1.85–1.75 (m, 1H), 1.67–1.54 (m, 4H), 1.50–1.41 (m, 2H), 1.41–1.32 (m, 2H), 0.96 (d, J = 5.5 Hz, 3H), 0.95 (d, J = 6.1 Hz, 3H), 0.89 (d, J = 6.8 Hz, 3H), 0.82 (d, J = 6.7 Hz, 3H); MS (FAB) m/z 360 $(M)^+$, 361 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{22}H_{36}N_2O_2$ 360.2777, found 360.2773 $(M)^+$.

4.1.19. (*S*,*E*)-8-Amino-7-(but-1-enyl)-4-(3-methylbutyl)-2-(2-methylpropyl)-1,2,4,5-tetrahydrohydro-3*H*-1,4-benzodiazepin-3-one (22a)

Intermediate **20a** was prepared by means of GP-A, with K_3PO_4 (208 mg, 980 μmol), Pd(dppf)Cl_2 (30.2 mg, 41.3 μmol), **12** (58.3 mg, 320 μmol), **19** (204 mg, 335 μmol) and DMF (3.3 mL). Purification by silica gel chromatography (hexane/AcOEt = 4/1) afforded **20a** (mixture, 99.8 mg) as a brown oil. This was used for the next reaction without further purification. Trifluoroacetic acid (2.00 mL, 26.9 mmol) was added to a solution of **20a** (mixture, 99.8 mg) in CH_2Cl_2 (2.0 mL) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, then adjusted to pH 10 with 2 N NaOH aq and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The resulting residue (78.3 mg) was used for the next reaction without purification. Under an Ar atmosphere, $Pd_2(dba)_3{\cdot}CHCl_3$ (20.8 mg, 20.1 μmol) was added to a solution of the above residue (78.3 mg), (±)-BINAP (26.9 mg, 43.2 μmol), and Cs_2CO_3 (295 mg, 905 μmol) in toluene (6 mL). The reaction mixture

was stirred for 10 h at 110 °C, then the reaction was quenched with satd $NaHCO_3$ aq and the mixture was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel chromatography (hexane/AcOEt = 2/1) to afford a yellow oil (24.3 mg). A part of this oil (15.7 mg) was purified by HPLC with Sensyu Pak PEGASIL ODS (20φ × 250 mm) (CH_3CN/H_2O = 80:20) to afford **22a** (10.9 mg, 30.5 μmol, 9% (three steps)) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.80 (s, 1H), 6.26 (td, J = 1.3, 15.9 Hz, 1H), 5.94 (td, J = 6.7, 15.9 Hz, 1H), 5.82 (s, 1H), 5.24(d, J = 16.5 Hz, 1H), 4.54–4.49 (m, 1H), 3.68 (d, J = 16.5 Hz, 1H), 3.65–3.55 (m, 2H), 3.55–3.47 (m, 1H), 3.47–3.40 (m, 1H), 3.40–3.38 (m, 1H), 2.24–2.16 (m, 4H), 1.91–1.85 (m, 1H), 1.81–1.76 (m, 1H), 1.52–1.36 (m, 4H), 1.08 (t, J = 7.3 Hz, 3H), 0.96 (d, J = 6.1 Hz, 3H), 0.95 (d, J = 6.8 Hz, 3 H), 0.89 (d, J = 6.8 Hz, 3H), 0.85 (d, J = 6.1 Hz, 3H); MS (FAB) m/z 357 $(M)^+$, 358 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{22}H_{35}N_3O$ 357.2780, found 357.2776 $(M)^+$.

4.1.20. (*S*,*E*)-2-Amino-*N*-(4-amino-2-bromo-5-(pent-1-enyl) benzyl)-*N*-(3-methylbutyl)-4-methylpentanamide (21b)

Intermediate **20b** was prepared by means of GP-A, with K_3PO_4 (220 mg, 1036 μmol), $Pd(dppf)Cl_2$ (34 mg, 46.5 μmol), (*E*)-pent-1-enylboronic acid pinacol ester (641 mg, 327 μmol), **19** (206 mg, 338 μmol) and DMF (1.0 mL). Purification by silica gel chromatography (hexane/AcOEt = 3/1) afforded **20b** (mixture, 129 mg) as a brown oil. This was used for the next reaction without further purification. Trifluoroacetic acid (2.50 mL, 33.7 mmol) was added to a solution of the above oil (129 mg) in CH_2Cl_2 (2.5 mL) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, then adjusted to pH 11 with 2 N NaOH aq and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The resulting residue was purified by silica gel chromatography ($CHCl_3$/MeOH = 10/1) to afford **21b** (73.0 mg, 161 μmol, 48% (two steps)) as a yellow oil. The product was a mixture of rotamers in a ratio of 0.5:0.5 (determined by ^{1}H NMR).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.04 (s, 0.5H), 6.90 (s, 0.5H), 6.88 (s, 0.5H), 6.85 (s, 0.5H), 6.26 (d, J = 15.6 Hz, 1H), 6.00 (td, J = 6.7, 15.6 Hz, 1H), 4.98 (d, J = 15.0 Hz, 0.5H), 4.50 (d, J = 17.1 Hz, 0.5H), 4.37 (d, J = 17.1 Hz, 0.5H), 4.27 (d, J = 15.0 Hz, 0.5H), 3.78-3.68 (m, 3H), 2.20–2.15 (m, 2H), 1.49–1.34 (m, 8H), 0.96–0.89 (m, 12 H), 0.88 (d, J = 6.7 Hz, 1.5H), 0.84 (d, J = 6.7 Hz, 1.5 H); MS (FAB) m/z 452, 454 $(M+H)^+$.

4.1.21. (*S*,*E*)-8-Amino-4-(3-methylbutyl)-2-(2-methylpropyl)-1,2,4,5-tetrahydrohydro-7-(pent-1-enyl)-3*H*-1,4-benzodiazepin-3-one (22b)

Under an Ar atmosphere, $Pd_2(dba)_3{\cdot}CHCl_3$ (30.1 mg, 29.1 μmol) was added to a solution of **21b** (73 mg, 161 μmol), (±)-BINAP (24.6 mg, 39.5 μmol), and Cs_2CO_3 (300 mg, 923 μmol) in toluene (2.5 mL). The reaction mixture was stirred for 6 h at 110 °C, then the reaction was quenched with H_2O and the mixture was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel chromatography (hexane/AcOEt = 2/1), PTLC (hexane/AcOEt = 3/2) and PTLC (hexane/AcOEt = 2/1) to afford **22b** (11.3 mg, 30.4 μmol, 19%) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.80 (s, 1H), 6.26 (d, J = 15.6 Hz, 1H), 5.89 (td, J = 15.6, 6.4 Hz, 1H), 5.82 (s, 1H), 5.24 (d, J = 16.2 Hz, 1H), 4.53–4.50 (m, 1H), 3.65–3.55 (m, 2H), 3.55–3.47 (m, 1H), 3.47–3.40 (m, 2H), 2.16 (td, J = 7.4, 6,4 Hz, 2H), 1.90–1.85 (m, 1H), 1.82–1.74 (m, 1H), 1.53–1.34 (m, 6H), 0.96–0.93 (m, 9H), 0.90 (d, J = 6.8 Hz, 3H), 0.85 (d, J = 6.7 Hz, 3H); MS (FAB) m/z 371 $(M)^+$, 372 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{23}H_{27}N_3O$ 371.2937, found 371.2937 $(M)^+$.

4.1.22. (*S*,*E*)-8-Amino-7-butyl-4-(3-methylbutyl)-2-(2-methylpropyl)-1,2,4,5-tetrahydrohydro-3*H*-1,4-benzodiazepin-3-one (23a)

Under an Ar atmosphere, 10% Pd/C (7.70 mg) was added to a stirred solution of **22a** (7.60 mg, 21.3 μmol) in AcOEt (2.0 mL). The Ar atmosphere was replaced with H_2. The reaction mixture was strongly stirred for 18 h at rt, then filtered through Celite and concentrated to afford pure **23a** in quantitative yield as a yellow solid.

Mp 94–96 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.57 (s, 1H), 5.87 (s, 1H), 5.24 (d, J = 16.5 Hz, 1H), 4.52–4.48 (m, 1H), 3.65 (d, J = 16.5 Hz, 1H), 3.54–3.40 (m, 4H), 3.33–3.26 (m, 1H), 2.37 (t, J = 7.3 Hz, 2H), 1.90–1.82 (m, 1H), 1.82–1.74 (m, 1H), 1.54–1.32 (m, 8H), 0.97–0.91 (m, 9H), 0.89 (t, J = 6.7 Hz, 3H), 0.83 (d, J = 6.1 Hz, 3H); MS (FAB) m/z 359 $(M)^+$, 360 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{22}H_{37}N_3O$ 359.2937, found 359.2934 $(M)^+$.

4.1.23. (*S*,*E*)-8-Amino-4-(3-methylbutyl)-2-(2-methylpropyl)-1,2,4,5-tetrahydrohydro-7-pentyl-3*H*-1,4-benzodiazepin-3-one (23b)

Under an Ar atmosphere, 10% Pd/C (2.00 mg) was added to a stirred solution of **22b** (8.30 mg, 22.3 μmol) in AcOEt (2.0 mL). The Ar atmosphere was replaced with H_2. The reaction mixture was strongly stirred for 6 h at rt, then filtered through Celite and concentrated to afford pure **23b** in quantitative yield as a yellow solid.

Mp 86–87 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.57 (s, 1H), 5.87 (s, 1H), 5.25 (d, J = 16.2 Hz, 1H), 4.50 (t, J = 6.7 Hz, 1H), 3.65 (d, J = 16.2 Hz, 1H), 3.52–3.42 (m, 4H), 3.35–3.25 (m, 1H), 2.36 (t, J = 7.3 Hz, 2H), 1.89–1.84 (m, 1H), 1.81–1.75 (m, 1H), 1.56–1.30 (m, 10H), 0.96–0.93 (m, 6H), 0.91–0.88 (m, 6H), 0.83 (d, J = 6.7 Hz, 3H); MS (FAB) m/z 373 $(M)^+$, 374 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{23}H_{39}N_3O$ 373.3093, found 373.3089 $(M)^+$.

4.1.24. 2-Bromo-4-(4-methoxybenzylamino)benzonitrile (25)

4-Methoxybenzylamine (5.3 mL, 40.2 mmol) was added to 2-bromo-4-fluorobenzonitrile (**24**) (5.36 g, 26.8 mmol). The reaction mixture was stirred 5 h at 140 °C in a sealed flask, then the reaction was quenched with H_2O and the mixture was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The resulting residue was recrystallized (from hexane/AcOEt) to afford **25** (5.96 g, 18.8 mmol, 70%) as a pale yellow solid. The mother liquor was concentrated and the residue was purified by silica gel chromatography (hexane/AcOEt = 4/1) to afford **25** (1.76 g, 5.56 mmol, 21%) as a pale yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (d, J = 8.5 Hz, 1H), 7.23 (d, J = 9.2 Hz, 2H), 6.90 (d, J = 9,2 Hz, 2H), 6.83 (d, J = 2.4 Hz, 1H), 6.52 (dd, J = 8.5, 2.4 Hz, 1H), 4.54 (m, 1H), 4.28 (d, J = 4.9 Hz, 2H), 3.81 (s, 3H); MS (FAB) m/z 317, 329 $(M+H)^+$.

4.1.25. 4-Amino-2-bromobenzonitrile (26)

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (2.90 g, 12.8 mmol) was added to a stirred solution of **25** (4.03 g, 12.8 mmol) in CH_2Cl_2 (85 mL) and H_2O (43 mL) at rt. The reaction mixture was stirred for 3.5 h, and then filtered to remove precipitated solids. The filtrate was added to satd $NaHCO_3$ aq, and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The resulting residue was purified by silica gel chromatography (hexane/AcOEt = 2/1) to afford **26** (2.13 g, 10.8 mmol, 85%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, J = 7.9 Hz, 1H), 6.89 (d, J = 2.4 Hz, 1H), 6.58 (dd, J = 8.5, 2.4 Hz, 1H), 4.21 (m, 2H); MS (FAB) m/z 197, 199 $(M+H)^+$.

4.1.26. *N*-(3-Bromo-4-cyanophenyl)imidodicarbonic acid bis(1,1-dimethylethyl) ester (27)

Di-*tert*-butyl dicarbonate (Boc_2O) (24.6 g, 113 mmol) in THF (75 mL), 4-dimethylaminopyridine (DMAP) (532 mg, 4.35 mmol) and diisopropylethylamine (DIPEA) (35.0 mL, 201 mmol) were added to a solution of **26** (7.70 g, 39.1 mmol) at 0 °C. The reaction mixture was refluxed for 2 h, then cooled to rt, and concentrated to about 30 mL H_2O and brine were added to it, and the mixture was extracted with AcOEt. The organic layer was dried over $MgSO_4$ and concentrated. The resulting residue was recrystallized from hexane/AcOEt to afford **27** (8.40 g, 21.1 mmol, 54%) as white cubic crystals. The mother liquor was concentrated and the residue was purified by silica gel chromatography (hexane/AcOEt = 6/1) and reprecipitation (from EtOH) to afford **27** (899 mg, 2.26 mmol, 6%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (d, J = 8.6 Hz, 1H), 7.52 (d, J = 1.9 Hz, 1H), 7.23 (dd, J = 8.6, 1.9 Hz, 1H), 1.45 (s, 18H); MS (FAB) m/z 397, 399 $(M)^+$.

4.1.27. (*S*)-Methyl 2-(5-(bis(*tert*-butoxycarbonyl)amino)-2-cyanophenylamino)-4-methylpentanoate (28)

Compound **27** (2.00 g, 5.03 mmol), L-leucine methyl ester hydrochloride (1.09 g, 6.04 mmol), $Pd_2(dba)_3$ (260 mg, 252 μmol), Xantphos (437 mg, 755 μmol) and Cs_2CO_3 (3.28 g, 10.1 mmol) were successively added to a flask. The atmosphere was replaced with Ar, and toluene (50 mL) was added to the flask. The reaction mixture was stirred 14 h at 110 °C, then H_2O (20 μL) was added and stirring was continued for 19 h at 110 °C. The reaction was quenched with brine and H_2O, and the mixture was extracted with AcOEt. The organic layer was dried over Na_2SO_4, and concentrated. The resulting residue was purified by amino silica gel chromatography (NH-SiO_2, FUJI SILYSIA) (hexane/AcOEt = 5/1) to afford **28** (2.16 g, 4.68 mmol, 93%) as a slightly yellow syrup.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, J = 7.9 Hz, 1H), 6.54 (dd, J = 7.9, 1.9 Hz, 1H), 6.39 (d, J = 1.9 Hz, 1H), 4.82–4.79 (m, 1H), 4.10–4.04 (m, 1H), 3.73 (s, 3H), 1.82–1.71 (m, 3H), 1.43 (s, 18 H), 1.00 (d, J = 6.7 Hz, 3H), 0.94 (d, J = 6.7 Hz, 3H; MS (FAB) m/z 461 $(M)^+$.

4.1.28. (*S*)-8-bis(*tert*-butoxycarbonyl)amino-1,2,4,5-tetrahydro-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (29)

Under an Ar atmosphere, an H_2O suspension of Raney nickel (4 mL, TCI) was added to a solution of **28** (539 mg, 1.17 mmol) in MeOH (40 mL) and Et_3N (4 mL). The Ar atmosphere was replaced with H_2. The reaction mixture was stirred for 13.5 h at rt, then filtered through Celite and concentrated. The resulting residue was purified by silica gel chromatography (hexene/AcOEt = 1/1 to 1/2) to afford **29** (388 mg, 895 μmol, 76%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.89 (d, J = 8.0 Hz, 1H), 6.46 (dd, J = 8.0, 1.8 Hz, 1H), 6.38 (d, J = 1.8 Hz, 1H), 6.05 (dd, J = 6.7, 6.1 Hz, 1H), 4.99 (dd, J = 16.5, 6.7 Hz, 1H), 4.48–4.43 (m, 1H), 3.89 (dd, J = 16.5, 6.7 Hz, 1H), 3.52–3.50 (m, 1H), 1.92–1.79 (m, 2H), 1.51–1.49 (m, 1H), 1.45 (s, 18H), 0.98 (d, J = 6.1 Hz, 3H), 0.97 (d, J = 6.7 Hz, 3H); MS (FAB) m/z 433 $(M)^+$, 434 $(M+H)^+$.

4.1.29. (*S*)-8-Bis(*tert*-butoxycarbonyl)amino-1,2,4,5-tetrahydro-4-(3-methylbut-2-enyl)-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (30)

Tetrabutylammonium iodide (331 mg, 895 μmol), 1-bromo-3-methyl-2-butene (103 μL, 895 μmol) and *t*-BuOK (108 mg, 964 μmol) were added to a solution of **29** (388 mg, 895 μmol) at −20 °C. The reaction mixture was stirred for 5 min at −20 °C, then the reaction was quenched with H_2O and the mixture was extracted with AcOEt. The organic layer was dried over Na_2SO_4 and concentrated. The resulting residue was purified by silica gel chromatography (hexane/AcOEt = 3/1 to 3/2) to afford **30** (328 mg, 654 μmol, 76%) as a colorless amorphous solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.81 (d, J = 8.0 Hz, 1H), 6.40 (dd, J = 8.0, 1.9 Hz, 1H), 6.30 (d, J = 1.9 Hz, 1H), 5.21 (d, J = 16.5 Hz, 1H), 5.09 (dd, J = 7.9, 6.7 Hz, 1H), 4.65–4.57 (m, 1H), 4.20 (dd, J = 14.7, 6.7 Hz, 1H), 3.99 (dd, J = 14.7, 7.3 Hz, 1H), 3.75 (d, J = 16.5 Hz, 1H), 3.57–3.47 (m, 1H), 1.94–1.87 (m, 1H), 1.85–1.75 (m, 1H), 1.69 (s, 6H), 1.47–1.42 (m, 1H), 1.44 (s, 18H), 0.97 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H); MS (FAB) m/z 501 $(M)^+$, 502 $(M+H)^+$.

4.1.30. (*S*)-8-Bis(*tert*-butoxycarbonyl)amino-1,2,4,5-tetrahydro-4-(3-methylbutyl)-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (31)

Under an Ar atmosphere, 10% Pd/C (33.0 mg) was added to a solution of **30** (328 mg, 654 μmol) in 1,4-dioxane (7.0 mL). The Ar atmosphere was replaced with H_2. The reaction mixture was stirred 16 h at rt, then filtered through Celite and concentrated. The resulting residue was purified by silica gel chromatography (hexane/AcOEt = 3/1 to 1/1) to afford **31** in quantitative yield as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.89 (d, J = 8.0 Hz, 1H), 6.41 (dd, J = 8.0, 2.2 Hz, 1H), 6.29 (d, J = 2.2 Hz, 1H), 5.35 (d, J = 16.7 Hz, 1H), 4.61–4.56 (m, 1H), 3.77 (d, J = 16.7 Hz, 1H), 3.65–3.57 (m, 1H), 3.53–3.48 (m, 1H), 3.42–3.33 (m, 1H), 1.95–1.87 (m, 1H), 1.84–1.75 (m, 1H), 1.50–1.45 (m, 1H), 1.43 (s, 18H), 1.42–1.30 (m, 2H), 0.96 (d, J = 6.7 Hz, 3H), 0.95 (d, J = 6.7 Hz, 3H), 0.88 (d, J = 6.1 Hz, 3H), 0.83 (d, J = 6.1 Hz, 3H); MS (FAB) m/z 503 $(M)^+$, 504 $(M+H)^+$.

4.1.31. (*S*)-8-Amino-1,2,4,5-tetrahydro-4-(3-methylbutyl)-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (32)

4 N HCl in 1,4-dioxane (4.0 mL) was added to **31** (200 mg, 397 μmol). The reaction mixture was stirred for 3 h at rt, then the reaction was quenched with satd $NaHCO_3$ aq, and the mixture was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated. The resulting residue was purified by silica gel chromatography ($CHCl_3$/MeOH = 20/1) to afford **32** (118 mg, 389 μmol, 98%) as a white solid.

Mp 185–186 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.70 (d, J = 8.0 Hz, 1H), 5.99 (dd, J = 8.0, 1.9 Hz, 1H), 5.84 (d, J = 1.9 Hz, 1H), 5.25 (d, J = 16.5 Hz, 1H), 4.57–4.51 (m, 1H), 3.65 (d, J = 16.7 Hz, 1H), 3.56–3.41 (m, 4H), 3.41–3.38 (m, 1H), 1.92–1.85 (m, 1H), 1.83–1.74 (m, 1H), 1.54–1.31 (m, 4H), 0.96 (d, J = 6.7 Hz, 3H), 0.95 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H); MS (FAB) m/z 303 $(M)^+$, 304 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{18}H_{29}N_3O$ 303.2311, found 303.2306 $(M)^+$.

4.1.32. (*S*)-8-Amino-1,2,4,5-tetrahydro-7-iodo-4-(3-methylbutyl)-2-(2-methylpropyl)-3*H*-1,4-benzodiazepin-3-one (33)

ICl-pyridine complex (1.40 mg, 5.80 μmol) was added to a solution of **32** (2.30 mg, 7.58 μmol) in CH_2Cl_2/H_2O (CH_2Cl_2 202 μL and H_2O 101 μL). The reaction mixture was stirred 6.5 h at rt, then the reaction was quenched with H_2O and the mixture was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The resulting residue was purified by PTLC (hexane/AcOEt = 1/1) to afford **33** (2.40 mg, 5.59 μmol, 74%) as a white solid.

Mp 150 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (s, 1H), 5.93 (s, 1H), 5.20 (d, J = 16.2 Hz, 1H), 4.53–4.49 (m, 1H), 3.92–3.85 (m, 2H), 3.16 (d, J = 16.2 Hz, 1H), 3.52–3.41 (m, 3H), 1.91–1.85 (m, 1H), 1.82–1.72 (m, 1H), 1.53–1.33 (m, 4H), 0.96 (d, J = 6.1 Hz, 3H), 0.95 (d, J = 5.5 Hz, 3H), 0.90 (d, J = 6.7 Hz, 3H), 0.85 (d, J = 6.1 Hz, 3H); MS (FAB) m/z 429 $(M)^+$, 430 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{18}H_{28}N_3O$ 429.1277, found 429.1272 $(M)^+$.

4.1.33. (*S*)-8-Amino-1,2,4,5-tetrahydro-4-(3-methylbutyl)-2-(2-methylpropyl)-7-phenyl-3*H*-1,4-benzodiazepin-3-one (34)

This compound was prepared by means of GP-A, with K_3PO_4 (34.4 mg, 162 μmol), Pd(dppf)Cl_2 (7.20 mg, 9.84 μmol), phenylboronic acid (7.50 mg, 61.5 μmol), **33** (21.8 mg, 50.8 μmol) and DMF (0.50 mL). Purification by silica gel chromatography (hexane/AcOEt = 2/1 to 3/2) and PTLC (hexane/AcOEt = 3/2) afforded **34** (4.90 mg, 12.9 μmol, 25%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43–7.38 (m, 4H), 7.32–7.28 (m, 1H), 6.71 (s, 1H), 5.92 (s, 1H), 5.29 (d, J = 16.2 Hz, 1H), 4.60–4.55 (m, 1H), 3.72–3.63 (m, 2H), 3.69 (d, J = 16.2 Hz, 1H), 3.52–3.45 (m, 1H), 350 (t, J = 7.3 Hz, 2H), 1.93–1.87 (m, 1H), 1.84–1.76 (m, 1H), 1.52–1.44 (m, 2H), 1.43–1.34 (m, 2H), 0.98 (d, J = 6.2 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 6.7 Hz, 3H), 0.84 (d, J = 6.1 Hz, 3H); MS (FAB) m/z 379 $(M)^+$, 380 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{24}H_{33}N_3O$ 379.2624, found 379.2621 $(M)^+$.

4.1.34. (*S*)-Ethyl 4-[8-amino-1,2,4,5-tetrahydro-2-(2-methylpropyl)-4-(3-methylbutyl)-3-oxo-3*H*-1,4-benzodiazepin-7-yl]benzoate (35)

This compound was prepared by means of GP-A, with K_3PO_4 (73.2 mg, 345 μmol), Pd(dppf)Cl_2 (7.50 mg, 10.3 μmol), 4-ethoxycarbonylphenylboronic acid pinacol ester (47.4 mg, 172 μmol), **33** (49.0 mg, 114 μmol) and DMF (1.0 mL). Purification by PTLC (hexane/AcOEt = 3/2) afforded **35** (31.0 mg, 68.7 μmol, 60%) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (d, J = 7.9 Hz, 2H), 7.49 (d, J = 7.9 Hz, 2H), 6.73 (s, 1H), 5.92 (s, 1H), 5.29 (d, J = 15.9 Hz, 1H), 4.61–4.56 (m, 1H), 4.40 (t, J = 6.7 Hz, 2H), 3.72–3.68 (m, 3H), 3.55–3.52 (m, 1H), 3.50 (t, J = 7.6 Hz, 2H), 1.94–1.87 (m, 1H), 1.84–1.78 (m, 1H), 1.52–1.43 (m, 2H), 1.43–1.35 (m, 2H), 1.41 (t, J = 6.7 Hz, 3H), 0.98 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.1 Hz, 3H), 0.90 (d, J = 6.2 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H); MS (FAB) m/z 451 $(M)^+$, 452 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{27}H_{37}N_3O_3$ 451.2835, found 451.2833 $(M)^+$.

4.1.35. (*S*)-4-[8-Amino-1,2,4,5-tetrahydro-2-(2-methylpropyl)-4-(3-methylbutyl)-3-oxo-3*H*-1,4-benzodiazepin-7-yl]benzoic acid (36)

KOH (3.00 mg, 53.5 μmol) in MeOH (0.50 mL) was added to a solution of **35** (12.7 mg, 28.1 μmol) in MeOH (0.50 mL). The reaction mixture was stirred for 1.5 h at 60 °C, then H_2O (0.20 mL) was added to it, because TLC monitoring indicated that the reaction had not proceeded. Stirring was continued for 6 h at 60 °C, then the reaction was quenched with 2 N HCl aq, and satd $NaHCO_3$ aq was added to adjust the pH to 7 at 0 °C. Brine and H_2O were further added and the mixture was extracted with AcOEt and $CHCl_3$. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by PTLC ($CHCl_3$/MeOH = 10/1) to afford **36** (5.50 mg, 13.0 μmol, 46%) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (d, J = 7.4 Hz, 2H), 7.52 (d, J = 7.4 Hz, 2H), 6.74 (s, 1H), 5.92 (s, 1H), 5.30 (d, J = 16.5 Hz, 1H), 4.61–4.56 (m, 1H), 3.71 (d J = 16.5 Hz, 1H), 3.53–3.47 (m, 2H), 1.95–1.88 (m, 1H), 1.84–1.77 (m, 1H), 1.52–1.44 (m, 2H), 1.44–1.35 (m, 2H), 0.98 (d, J = 6.1 Hz, 3H), 0.97 (d, J = 6.1 Hz, 3H), 0.90 (d, J = 6.7 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H); MS (FAB) m/z 423 $(M)^+$, 424 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{25}H_{33}N_3O_3$ 423.2522, found 423.2523 $(M)^+$.

4.1.36. Ethyl 3-hydroxy-4-iodobenzoate (38)

N-Iodosuccinimide (1.40 g, 6.24 mmol) was added to a stirred solution of ethyl 3-hydroxybenzoate (**37**) (1.02 g, 6.13 mmol) in AcOH (30 mL) at 0 °C, then the reaction mixture was stirred for 21 h at rt. At 0 °C, 10 N NaOH aq was added to adjust the pH to 5, and a white solid precipitated. The solid was removed by filtration and the filtrate was extracted with AcOEt. The white solid was added to the organic layer, and the resulting solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel chromatography (hexane/AcOEt = 4/1) and recrystallization (from CH_2Cl_2/hexane) to afford **38** (1.13 g, 3.88 mmol, 63%) as a white amorphous solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, J = 8.6 Hz, 1H), 7.63 (d, J = 1.8 Hz, 1H), 7.34 (dd, J = 8.6, 1.8 Hz, 1H), 5.41 (s, 1H), 4.37 (q, J = 6.7 Hz, 2H), 1.39 (t, J = 6.7 Hz, 3H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 165.9, 155.0, 138.5, 132.57, 123.0, 115.7, 91.5, 61.4, 14.2; HMBC was used to determine the iodide position; MS (FAB) m/z 292 $(M)^+$, 293 $(M+H)^+$.

4.1.37. Ethyl 3-[2-(benzyloxy)ethoxy]-4-iodobenzoate (39)

Diisopropyl azodicarboxylate (DIAD) (590 μL, 3.00 mmol) was added dropwise to a stirred solution of **38** (436 mg, 1.49 mmol), 2-(benzyloxy)ethanol (456 mg, 3.00 mmol) and PPh_3 (1.11 g, 1.81 mmol) in THF (15.0 mL). The reaction mixture was stirred for 27 h at rt, then concentrated, quenched with H_2O, and extracted with AcOEt. The organic layer was dried over $MgSO_4$, and concentrated. The resulting residue was purified by silica gel chromatography (hexane/AcOEt = 4/1) to afford **39** (591 mg, 1.39 mmol, 93%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (d, J = 7.9 Hz, 1H), 7.46 (d, J = 1.8 Hz, 1H), 7.42–7.34 (m, 5H), 7.31–7.26 (m, 1H), 4.27 (s, 2H), 4.37 (q, J = 7.3 Hz, 2H), 4.28 (t, J = 4.9 Hz, 2H), 3.93 (t, J = 4.9 Hz, 2H), 1.39 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 426 $(M)^+$, 427 $(M+H)^+$.

4.1.38. (*S*)-Ethyl 4-[8-amino-1,2,4,5-tetrahydro-2-(2-methylpropyl)-4-(3-methylbutyl)-3-oxo-3*H*-1,4-benzodiazepin-7-yl]-3-[2-(benzyloxy)ethoxy] benzoate (41)

DMSO (10.0 mL) was added to a mixture of **39** (224 mg, 526 μmol), bis(pinacolato)diboran (280 mg, 1.10 mmol), KOAc (150 mg, 1.53 mmol) and $PdCl_2$(dppf) (30.3 mg, 41.4 μmol). The atmosphere was replaced with Ar. The reaction mixture was stirred for 1.5 h at 80 °C, then the reaction was quenched with H_2O and the mixture was extracted with AcOEt. The organic layer was washed with H_2O, dried over $MgSO_4$, and concentrated. The resulting residue was purified by silica gel chromatography (hexane/AcOEt = 1/0 to 5/1) and PTLC (hexane/AcOEt = 5/2) to afford **40** (mixture, 18.4 mg) as a colorless oil. This was used for the next reaction without further purification. Compound **41** was prepared by means of GP-A, with K_3PO_4 (21.8 mg, 103 μmol), Pd(dppf)Cl_2 (2.80 mg, 3.80 μmol), **40** (mixture, 18.4 mg), **33** (14.5 mg, 33.8 μmol) and DMF (0.75 mL). Purification by silica gel chromatography (hexane/AcOEt = 3/2) and PTLC (hexane/AcOEt = 3/2) afforded **41** (8.20 mg, 13.6 μmol, 3% (two steps)) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (dd, J = 7.9, 1.5 Hz, 1H), 7.63 (d, J = 1.5 Hz, 1H), 7.35–7.25 (m, 6H), 6.69 (s, 1H), 5.84 (s, 1H), 5.29–5.18 (m, 1H), 4.60–4.52 (m, 1H), 4.53 (s, 2H), 4.39 (q, J = 7.0 Hz, 2H), 4.28–4.20 (m, 2H), 3.81–3.78 (m, 2H), 3.69–3.60 (m, 3H), 3.55–3.40 (m, 3H), 1.95–1.86 (m, 1H), 1.84–1.76 (m, 1H), 1.52–1.34 (m, 2H), 1.40 (t, J = 7.0 Hz, 3H), 1.28–1.23 (m, 2H), 0.99–0.94 (m, 6H), 0.90–0.86 (m, 3H), 0.85–0.80 (m, 3H); MS (FAB) m/z 601 $(M)^+$, 602 $(M+H)^+$.

4.1.39. (*S*)-Ethyl 4-[8-Amino-1,2,4,5-tetrahydro-2-(2-methylpropyl)-4-(3-methylbutyl)-3-oxo-3*H*-1,4-benzodiazepin-7-yl]-3-(2-hydroxyethoxy) benzoate (42)

Under an Ar atmosphere in a pressure-tight flask, 10% Pd/C (8.20 mg) was added to a solution of **41** (8.20 mg, 13.6 μmol) in 1,4-dioxane (3.5 mL). The atmosphere was replaced with H_2 (2.5 atm). The reaction mixture was stirred 4 h at 50 °C, then filtered through Celite and concentrated. The resulting residue was purified by PTLC (hexane/AcOEt = 1/1) to afford **42** (1.20 mg, 2.35 μmol, 17%) as a colorless oil. The product was a mixture of conformers in a ratio of 0.3:0.7 (determined by ^{1}H NMR).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, J = 8.5 Hz, 0.3H), 7.73 (d, J = 8.5 Hz, 0.7H), 7.62 (s, 0.3H), 7.58 (s, 0.7H), 7.30–7.24 (m, 1H), 6.70 (s, 0.7H), 6.66 (s, 0.3H), 6.07 (s, 0.3H), 5.99 (s, 0.7H), 5.34–5.27 (m, 1H), 4.65–4.45 (m, 2H), 4.44–4.37 (m, 2H), 4.24–4.19 (m, 2H), 4.00–3.35 (m, 8H), 1.94–1.86 (m, 1H), 1.86–1.74 (m, 1H), 1.54–1.30 (m, 7H), 1.05–0.95 (m, 6H), 0.94–0.76 (m, 6H); MS (FAB) m/z 511 $(M)^+$, 512 $(M+H)^+$; HMRS (FAB) m/z calcd for $C_{29}H_{41}N_3O_5$ 511.3046, found 511.3050 $(M)^+$.

4.2. Reporter gene assay

Inhibitory activity towards VDR-mediated transcriptional activation was determined by reporter gene assay as previously described.[14,20] Each experiment was performed in triplicate and the normalized average values are presented.

Acknowledgement

The work described in this paper was partially supported by Grants-in-Aid for Scientific Research from The Ministry of Education Culture, Sports, Science and Technology, Japan, and the Japan Society for the Promotion of Science.

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