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

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### 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.

### Keyword

Vitamin D; Antagonist; Co-factor; Nuclear receptor; Non-peptide; Structure-activity relationship; Protein interaction inhibitor; LXXLL

### 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. <sup>1-3</sup> The physiological agonist of VDR is activated vitamin D<sub>3</sub>,  $1,25(OH)_2D_3$  (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.<sup>4,5</sup> Paget's disease of bone is characterized by an increased number of osteoclasts and excessive bone resorption in focal areas.<sup>6</sup> Osteoclast precursors from patients with Paget's disease show hypersensitivity to  $1,25(OH)_2D_3$ ,<sup>7</sup> 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<sup>8-13</sup> (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<sup>14</sup> and others<sup>15</sup> 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.<sup>16</sup> 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).<sup>14</sup> 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). 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,<sup>17</sup> affording **4**. N-Alkylation of amide **4** with 1-bromo-3-methylbutane gave **5**, and subsequent iodination of **5** with ICl-pyridine complex<sup>17</sup> 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),<sup>18</sup> afforded 7b and 7c. We considered that 7b was formed by isomerization, as reported previously.<sup>19</sup> 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).<sup>16</sup> 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).<sup>18</sup> 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 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<sup>14</sup> with CMX-GAL4N-hVDR LBD as the recombinant receptor gene, TK-MH100x4-LUC as the reporter gene, and CMX  $\beta$ -galactosidase gene for normalization. Human embryonic kidney (HEK) 293 cells were incubated with 1,25(OH)<sub>2</sub>D<sub>3</sub> (3 nM) in the presence or absence of test compounds. After incubation, cells were assayed for luciferase reporter gene and  $\beta$ -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<sub>50</sub> value of 1 is 26  $\mu$ M. These results suggest that the amino group at the 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. 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 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 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 hydolysis 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<sub>50</sub> 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<sub>50</sub> 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.

### 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. <sup>1</sup>H-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.

#### 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<sub>2</sub>O and the mixture was extracted with AcOEt. The organic layer was dried over MgSO<sub>4</sub> 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 (+)- $\alpha$ -pinene was added dropwise. The solution was stirred at 0 °C for 10 minutes then allowed to warm to rt and stirred at rt for 2-3 hours, 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 minutes, warmed to rt, and stirred at rt for 1.5-2.5 hours. 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 hours. It

was allowed to cool to 0  $^{\circ}$ C and H<sub>2</sub>O was added. Stirring was continued for 3-7 hours 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.

### 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<sub>4</sub> 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)-3H-1,4-benzodiazepin-3-one (4)

Under an Ar atmosphere,  $Pd_2(dba)_3$ •CHCl<sub>3</sub> (104 mg, 100 µmol), (*R*)-BINAP (139 mg, 223 µmol) and Cs<sub>2</sub>CO<sub>3</sub> (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 hours at 110 °C, then the reaction was quenched with H<sub>2</sub>O and the mixture was extracted with AcOEt. The organic layer was dried over MgSO<sub>4</sub> 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<sub>2</sub>O suspension of Raney nickel (1.5 mL, TCI) was added to a solution of the above mixture in MeOH (12 ml) / Et<sub>3</sub>N (1.2 mL). The Ar atmosphere was replaced with H<sub>2</sub>. The reaction mixture was stirred for 24 hours 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 % (2 steps)) as a white solid.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 219 (M+H)<sup>+</sup>.

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

Under an Ar atmosphere, NaH (60 %, dispersion in paraffin liquid) (10.2 mg, 255  $\mu$ mol) was added to a solution of **4** (36.6 mg, 168  $\mu$ mol) 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  $\mu$ L, 184  $\mu$ mol) was added to it at 0 °C. Stirring was continued for 7 hours at rt, then the reaction was quenched with H<sub>2</sub>O and the mixture was extracted with AcOEt. The organic layer was dried over MgSO<sub>4</sub> 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  $\mu$ mol, 80 %) as white needles.

mp 85 °C; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 289 (M+H)<sup>+</sup>; HMRS (FAB) *m/z* calcd for C<sub>18</sub>H<sub>28</sub>N<sub>2</sub>O 288.2202, found 288.2206 (M)<sup>+</sup>.

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

ICl-pyridine complex (36.5 mg, 151  $\mu$ mol) was added to a solution of **5** (39.0 mg, 135  $\mu$ mol) in CH<sub>2</sub>Cl<sub>2</sub> / H<sub>2</sub>O (CH<sub>2</sub>Cl<sub>2</sub> 3.6 mL and H<sub>2</sub>O 1.8 mL). The reaction mixture was stirred 4.5 hours at rt, and then the reaction was quenched with sat. NaHCO<sub>3</sub> aq. (3.0 mL) and 21 mM Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> aq. (3.0 mL). After the solution had changed color (brown to pale yellow), it was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over MgSO<sub>4</sub> 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; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 415 (M+H)<sup>+</sup>; HMRS (FAB) m/z calcd for C<sub>18</sub>H<sub>27</sub>IN<sub>2</sub>O 414.1168, found 414.1163 (M)<sup>+</sup>.

### 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<sub>2</sub> (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; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 343 (M+H)<sup>+</sup>; HMRS (FAB) m/z calcd for C<sub>22</sub>H<sub>34</sub>N<sub>2</sub>O 342.2671, found 342.2666 (M)<sup>+</sup>.

# 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<sub>2</sub> (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 (20 $\varphi$  x 250 mm) (CH<sub>3</sub>CN / H<sub>2</sub>O = 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.

**7b**: <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 343 (M+H)<sup>+</sup>; HMRS (FAB) m/z calcd for C<sub>22</sub>H<sub>34</sub>N<sub>2</sub>O 342.2671, found 342.2673 (M)<sup>+</sup>.

**7c**; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 343 (M+H)<sup>+</sup>; HMRS (FAB) m/z calcd for C<sub>22</sub>H<sub>34</sub>N<sub>2</sub>O 342.2671, found 342.2673 (M)<sup>+</sup>.

### 4.1.9. (S)-1,2,4,5-Tetrahydro-4-(3-methylbutyl)-2,7-di(2-methylpropyl)-3*H*-1,4benzodiazepin-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  $\mu$ mol) in AcOEt (6 mL). The Ar atmosphere was replaced with H<sub>2</sub> (3.0 atm). The reaction mixture was stirred for 14 hours 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; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 345 (M+H)<sup>+</sup>; HMRS (FAB) m/z calcd for C<sub>22</sub>H<sub>36</sub>N<sub>2</sub>O 344.2828, found 344.2827 (M)<sup>+</sup>.

### **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), (+)- $\alpha$ -pinene (6.80 mL, 42.9 mmol), **10** (excess), acetaldehyde (10.4 mL, 185 mmol) and H<sub>2</sub>O (5.0 mL). Purification was performed by partition. The mixture was diluted with AcOEt and extracted with 10% NaOH aq (2 x 10 mL). The combined aqueous solutions were acidified to pH 2 with concentrated HCl aq. and extracted with EtOAc (3 x 30 mL). The combined organic extracts were washed with sat. NaHCO<sub>3</sub> aq., dried over MgSO<sub>4</sub>, and concentrated to afford **11** (1.03 g, 10.3 mmol,

53%) as a colorless solid.

<sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub> /  $D_2O = 95 / 5$ )  $\delta$  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<sub>4</sub> (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 hours at rt, then filtered and the filtrate was concentrated to afford pure **12** (338 mg, 1.86 mmol, 93 %) as a colorless oil.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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), (+)- $\alpha$ -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<sub>4</sub> 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.

<sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD)  $\delta$  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), (+)- $\alpha$ -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<sub>4</sub> 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.

<sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD)  $\delta$  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<sub>3</sub>PO<sub>4</sub> (69.9 mg, 329 µmol), Pd(dppf)Cl<sub>2</sub> (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 %, 2 steps) as a colorless oil.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 345 (M+H)<sup>+</sup>; HMRS (FAB) *m/z* calcd for C<sub>21</sub>H<sub>32</sub>N<sub>2</sub>O<sub>2</sub> 344.2464, found 344.2466 (M)<sup>+</sup>.

### 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<sub>2</sub> (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 %, 2 steps) as a colorless oil.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 359 (M+H)<sup>+</sup>; HMRS (FAB) m/z calcd for C<sub>22</sub>H<sub>34</sub>N<sub>2</sub>O<sub>2</sub> 358.2620 found 358.2623 (M)<sup>+</sup>.

### **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 hours 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  $\mu$ L, 13.0  $\mu$ mol), 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  $\mu$ mol, 6% (3 steps)) as a colorless oil.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 347 (M+H)<sup>+</sup>; HMRS (FAB) *m/z* calcd for C<sub>21</sub>H<sub>34</sub>N<sub>2</sub>O<sub>2</sub> 346.2620, found 346.2620 (M)<sup>+</sup>.

### **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 hours 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  $\mu$ mol, 34 %, 2 steps) as a yellow oil.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 475 (M+H)<sup>+</sup>.

### 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  $\mu$ L, 18.0  $\mu$ mol), **17b** (5.60 mg, 11.8  $\mu$ mol) and THF (0.51 mL). Purification by PTLC (hexane / AcOEt = 1 : 1) afforded **18b** (2.8 mg, 7.8  $\mu$ mol, 66 %) as a colorless oil.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 361 (M+H)<sup>+</sup>; HMRS (FAB) *m*/*z* calcd for C<sub>22</sub>H<sub>36</sub>N<sub>2</sub>O<sub>2</sub> 360.2777, found 360.2773 (M)<sup>+</sup>.

### 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<sub>3</sub>PO<sub>4</sub> (208 mg, 980  $\mu$ mol), Pd(dppf)Cl<sub>2</sub> (30.2 mg, 41.3  $\mu$ mol), **12** (58.3 mg, 320  $\mu$ mol), **19** (204 mg, 335  $\mu$ mol) 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<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over MgSO<sub>4</sub> and concentrated. The resulting residue (78.3 mg) was used for the next reaction without purification. Under an Ar atmosphere, Pd<sub>2</sub>(dba)<sub>3</sub>•CHCl<sub>3</sub> (20.8 mg, 20.1  $\mu$ mol) was added to a solution of the

above residue (78.3 mg), ( $\pm$ ) -BINAP (26.9 mg, 43.2 µmol), and Cs<sub>2</sub>CO<sub>3</sub> (295 mg, 905 µmol) in toluene (6 mL). The reaction mixture was stirred for 10 hours at 110 °C, then the reaction was quenched with sat. NaHCO<sub>3</sub> aq. and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over MgSO<sub>4</sub> 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 (20 $\varphi$  x 250 mm) (CH<sub>3</sub>CN / H<sub>2</sub>O = 80 : 20) to afford **22a** (10.9 mg, 30.5 µmol, 9 % (3 steps)) as a yellow oil.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 358 (M+H)<sup>+</sup>; HMRS (FAB) *m/z* calcd for C<sub>22</sub>H<sub>35</sub>N<sub>3</sub>O 357.2780, found 357.2776 (M)<sup>+</sup>.

### 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<sub>3</sub>PO<sub>4</sub> (220 mg, 1036 µmol), Pd(dppf)Cl<sub>2</sub> (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<sub>2</sub>Cl<sub>2</sub> (2.5 mL) at 0 °C. The reaction mixture was stirred for 2 hours at 0 °C, then adjusted to pH 11 with 2 N NaOH aq. and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over MgSO<sub>4</sub> and concentrated. The resulting residue was purified by silica gel chromatography (CHCl<sub>3</sub> / MeOH = 10 / 1) to afford **21b** (73.0 mg, 161 µmol, 48 % (2 steps)) as a yellow oil. The product was a mixture of rotamers in a ratio of 0.5 : 0.5 (determined by <sup>1</sup>H-NMR).

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>.

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

Under an Ar atmosphere,  $Pd_2(dba)_3$ •CHCl<sub>3</sub> (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<sub>2</sub>CO<sub>3</sub> (300 mg, 923 µmol) in toluene (2.5 mL). The reaction mixture was stirred for 6 hours at 110 °C, then the reaction was quenched with H<sub>2</sub>O and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over MgSO<sub>4</sub> 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.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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* = 16.2Hz, 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)<sup>+</sup>, 372 (M+H)<sup>+</sup>; HMRS (FAB) *m/z* calcd for C<sub>23</sub>H<sub>27</sub>N<sub>3</sub>O 371.2937, found 371.2937 (M)<sup>+</sup>.

### 4.1.22. (*S*,*E*)-8-Amino-7-butyl-4-(3-methylbutyl)-2-(2-methylpropyl)-1,2,4,5tetrahydrohydro-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  $\mu$ mol) in AcOEt (2.0 mL). The Ar atmosphere was replaced with H<sub>2</sub>. The reaction mixture was strongly stirred for 18 hours at rt, then filtered through Celite and concentrated to afford pure **23a** in quantitative yield as a yellow solid.

mp 94-96 °C; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  6.57 (s, 1H), 5.87 (s, 1H), 5.24 (d, J = 16.5Hz, 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)<sup>+</sup>, 360 (M+H)<sup>+</sup>; HMRS (FAB) m/z calcd for C<sub>22</sub>H<sub>37</sub>N<sub>3</sub>O 359.2937, found 359.2934 (M)<sup>+</sup>.

### 4.1.23. (*S*,*E*)-8-Amino-4-(3-methylbutyl)-2-(2-methylpropyl)-1,2,4,5tetrahydrohydro-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  $\mu$ mol) in AcOEt (2.0 mL). The Ar atmosphere was replaced with H<sub>2</sub>. The reaction mixture was strongly stirred for 6 hours at rt, then filtered through Celite and concentrated to afford pure **23b** in quantitative yield as a yellow solid.

mp 86-87 °C; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  6.57 (s, 1H), 5.87 (s, 1H), 5.25 (d, J = 16.2Hz, 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)<sup>+</sup>, 374 (M+H)<sup>+</sup>; HMRS (FAB) m/z calcd for C<sub>23</sub>H<sub>39</sub>N<sub>3</sub>O 373.3093, found 373.3089 (M)<sup>+</sup>.

### 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 hours at 140 °C in a sealed flask, then the reaction was quenched with H<sub>2</sub>O and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over MgSO<sub>4</sub> 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.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>.

### **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** (4.03g, 12.8 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (85 mL) and H<sub>2</sub>O (43 mL) at rt. The reaction mixture was stirred for 3.5 hours, and then filtered to remove precipitated solids. The filtrate was added to sat. NaHCO<sub>3</sub> aq., and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over MgSO<sub>4</sub> 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.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>.

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

Di-*tert*-butyl dicarbonate (Boc<sub>2</sub>O) (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 hours, then cooled to rt, and concentrated to about 30 mL. H<sub>2</sub>O and brine were added to it, and the mixture was extracted with AcOEt. The organic layer was dried over MgSO<sub>4</sub> 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.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>.

## **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 hours at 110 °C, then H<sub>2</sub>O (20 µL) was added and stirring was continued for 19 hours at 110 °C. The reaction was quenched with brine and H<sub>2</sub>O, and the mixture was extracted with AcOEt. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The resulting residue was purified by amino silica gel chromatography (NH-SiO<sub>2</sub>, FUJI SILYSIA) (hexane / AcOEt = 5 / 1) to afford **28** (2.16 g, 4.68 mmol, 93 %) as a slightly yellow syrup.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) 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)<sup>+</sup>.

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

Under an Ar atmosphere, an H<sub>2</sub>O 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<sub>3</sub>N (4 mL). The Ar atmosphere was replaced with H<sub>2</sub>. The reaction mixture was stirred for 13.5 hours 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.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 434 (M+H)<sup>+</sup>.

## **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  $\mu$ mol), 1-bromo-3-methyl-2-butene (103  $\mu$ L, 895  $\mu$ mol) and *t*-BuOK (108 mg, 964  $\mu$ mol) were added to a solution of **29** (388 mg, 895  $\mu$ mol) at -20 °C. The reaction mixture was stirred for 5 minutes at -20 °C, then the reaction was quenched with H<sub>2</sub>O and the mixture was extracted with AcOEt. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> 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.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 502 (M+H)<sup>+</sup>.

## **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  $\mu$ mol) in 1,4-dioxane (7.0 mL). The Ar atmosphere was replaced with H<sub>2</sub>. The reaction mixture was stirred 16 hours 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.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 504 (M+H)<sup>+</sup>.

### 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  $\mu$ mol). The reaction mixture was stirred for 3 hours at rt, then the reaction was quenched with sat. NaHCO<sub>3</sub> aq., and the mixture was extracted with CHCl<sub>3</sub>. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The resulting residue was purified by silica gel chromatography (CHCl<sub>3</sub> / MeOH = 20 / 1) to afford **32** (118 mg, 389  $\mu$ mol, 98 %) as a white solid.

mp 185-186 °C; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 304 (M+H)<sup>+</sup>; HMRS (FAB) *m/z* calcd for C<sub>18</sub>H<sub>29</sub>N<sub>3</sub>O 303.2311, found 303.2306 (M)<sup>+</sup>.

### **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  $\mu$ mol) was added to a solution of **32** (2.30 mg, 7.58  $\mu$ mol) in CH<sub>2</sub>Cl<sub>2</sub> / H<sub>2</sub>O (CH<sub>2</sub>Cl<sub>2</sub> 202  $\mu$ L and H<sub>2</sub>O 101  $\mu$ L). The reaction mixture

was stirred 6.5 hours 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<sub>4</sub> 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; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 430 (M+H)<sup>+</sup>; HMRS (FAB) m/z calcd for C<sub>18</sub>H<sub>28</sub>N<sub>3</sub>O 429.1277, found 429.1272 (M)<sup>+</sup>.

### 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<sub>2</sub> (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.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 380 (M+H)<sup>+</sup>; HMRS (FAB) *m/z* calcd for C<sub>24</sub>H<sub>33</sub>N<sub>3</sub>O 379.2624, found 379.2621 (M)<sup>+</sup>.

### **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<sub>2</sub> (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.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 452 (M+H)<sup>+</sup>; HMRS (FAB) m/z calcd for C<sub>27</sub>H<sub>37</sub>N<sub>3</sub>O<sub>3</sub> 451.2835, found 451.2833 (M)<sup>+</sup>.

### 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  $\mu$ mol) in MeOH (0.50 mL) was added to a solution of **35** (12.7 mg, 28.1  $\mu$ mol) in MeOH (0.50 mL). The reaction mixture was stirred for 1.5 h at 60 °C, then H<sub>2</sub>O (0.20 mL) was added to it, because TLC monitoring indicated that the reaction had not proceeded. Stirring was continued for 6 hours at 60 °C, then the reaction was quenched with 2 N HCl aq., and sat. NaHCO<sub>3</sub> aq. was added to adjust the pH to 7 at 0 °C. Brine and H<sub>2</sub>O were further added and the mixture was extracted with AcOEt and CHCl<sub>3</sub>. The organic layer was dried over MgSO<sub>4</sub> and concentrated. The residue was purified by PTLC (CHCl<sub>3</sub> / MeOH = 10 / 1) to afford **36** (5.50 mg, 13.0  $\mu$ mol, 46 %) as a yellow oil.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 424 (M+H)<sup>+</sup>; HMRS (FAB) *m/z* calcd for C<sub>25</sub>H<sub>33</sub>N<sub>3</sub>O<sub>3</sub> 423.2522, found 423.2523 (M)<sup>+</sup>.

### 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<sub>4</sub> and concentrated. The residue was purified by silica gel chromatography (hexane / AcOEt = 4 / 1) and recrystallization (from CH<sub>2</sub>Cl<sub>2</sub> /

hexane) to afford **38** (1.13 g, 3.88 mmol, 63 %) as a white amorphous solid.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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); <sup>13</sup>C-NMR(125 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 293 (M+H)<sup>+</sup>.

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

Diisopropyl azodicarboxylate (DIAD) (590  $\mu$ L, 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<sub>3</sub> (1.11 g, 1.81 mmol) in THF (15.0 mL). The reaction mixture was stirred for 27 hours at rt, then concentrated, quenched with H<sub>2</sub>O, and extracted with AcOEt. The organic layer was dried over MgSO<sub>4</sub>, 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.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 427 (M+H)<sup>+</sup>.

### 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  $\mu$ mol), bis(pinacolato)diboran (280 mg, 1.10 mmol), KOAc (150 mg, 1.53 mmol) and PdCl<sub>2</sub>(dppf) (30.3 mg, 41.4  $\mu$ mol). 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<sub>2</sub>O and the mixture was extracted with AcOEt. The organic layer was washed with H<sub>2</sub>O, dried over MgSO<sub>4</sub>, 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<sub>3</sub>PO<sub>4</sub> (21.8 mg, 103 µmol), Pd(dppf)Cl<sub>2</sub> (2.80 mg, 3.80 µmol), **40** (mixture, 18.4 mg),

**33** (14.5 mg, 33.8  $\mu$ mol) 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  $\mu$ mol, 3 % (2 steps)) as a yellow oil.

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 602 (M+H)<sup>+</sup>.

### 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  $\mu$ mol) in 1,4-dioxane (3.5 mL). The atmosphere was replaced with H<sub>2</sub> (2.5 atm). The reaction mixture was stirred 4 hours 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  $\mu$ mol, 17 %) as a colorless oil. The product was a mixture of conformers in a ratio of 0.3 : 0.7 (determined by <sup>1</sup>H-NMR).

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  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)<sup>+</sup>, 512 (M+H)<sup>+</sup>; HMRS (FAB) *m*/*z* calcd for C<sub>29</sub>H<sub>41</sub>N<sub>3</sub>O<sub>5</sub> 511.3046, found 511.3050 (M)<sup>+</sup>.

### 4.2. Reporter gene assay

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

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Figure 1. Structures of 1,25(OH)<sub>2</sub>D<sub>3</sub> and representative VDR antagonists

Figure 2. Illustration of the interaction between LXXLL peptide fragment and VDR, and a representative benzodiazepine that we reported previously.<sup>14</sup> 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.

Figure 3. The pocket composed of residues Ile234, Ile238, Leu259, Ala263, and Val417 of VDR (PBD 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.

Figure 4. Spatial relationship between LXXLL peptide fragment and Lys260 of VDR (PBD 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 1. Reagents and conditions: (a) L-Leucine methyl ester hydrochloride, Pd<sub>2</sub>(dba)<sub>3</sub>·CHCl<sub>3</sub>, (*R*)-BINAP, Cs<sub>2</sub>CO<sub>3</sub>, toluene, 110 °C; (b) Raney nickel, H<sub>2</sub>, MeOH / Et<sub>3</sub>N = 10 / 1, rt; (c) 1-bromo-3-methylbutane, NaH, DMF, 0 °C to rt; (d) ICl-pyridine, CH<sub>2</sub>Cl<sub>2</sub> / H<sub>2</sub>O = 2 / 1, rt, (e) **9** or **12**, PdCl<sub>2</sub>(dppf), K<sub>3</sub>PO<sub>4</sub>, DMF, 80 °C; (f) Pd/C, H<sub>2</sub> (3 atm), AcOEt, 50 °C

Scheme 2. Reagents and conditions: (a)  $BH_3 \circ SMe_2$ , (+)- $\alpha$ -pinene, THF, 0 °C to rt; acetaldehyde, 40 °C;  $H_2O$ , rt; (b) pinacol, MgSO<sub>4</sub>,  $CH_2Cl_2$ , rt

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

Scheme 4. (a) **14a** or **14b**,  $PdCl_2(dppf)$ ,  $K_3PO_4$ , DMF, 80 °C; (b) TBAF, THF, rt; (c) Pd/C, H<sub>2</sub>, 1,4-dioxane, rt; (d) TBAF, THF, rt

Scheme 5. Reagents and conditions: (a) boronic acid pinacol ester, PdCl<sub>2</sub>(dppf), K<sub>3</sub>PO<sub>4</sub>, DMF, 80 °C; (b) TFA, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C; (c) Pd<sub>2</sub>(dba)<sub>3</sub>·CHCl<sub>3</sub>, (±)-BINAP, Cs<sub>2</sub>CO<sub>3</sub>, toluene, 110 °C; (d) Pd/C, H<sub>2</sub>, 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<sub>3</sub>N = 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

Scheme 7. Reagents and conditions: (a) 4-ethoxycarbonylphenyl boronic acid pinacol ester, PdCl<sub>2</sub>(dppf), K<sub>3</sub>PO<sub>4</sub>, DMF, 80 °C; (b) KOH, MeOH / H<sub>2</sub>O = 5 / 1, 60 °C

Scheme 8. Reagents and conditions: (a) NIS, AcOH, rt; (b) 2-phenoxyethanol, DIAD, PPh3, THF, rt; (c) bis(pinacolate)diboron, PdCl<sub>2</sub>(dppf), KOAc, DMSO, 80 °C; (d) **33**, PdCl<sub>2</sub>(dppf), K<sub>3</sub>PO<sub>4</sub>, DMF, 80 °C; (e) Pd/C, H<sub>2</sub> (2.5 atm), 1,4-dioxane, 50 °C

Table 1. SAR at the 7-position of benzodiazepine derivatives without an amino group in VDR reporter gene assay<sup>a</sup> <sup>a</sup>HEK293 cells were treated with 1,25(OH)<sub>2</sub>D<sub>3</sub> (3 nM) and test compounds <sup>b</sup>Inhibition ratio at 30 μM <sup>c</sup>N.A. means no inhibitory activity at 30 μM

Table 2. SAR at the 7-position of benzodiazepine derivatives with an amino group in VDR reporter gene assay<sup>a</sup>

<sup>a</sup>HEK293 cells were treated with 1,25(OH)<sub>2</sub>D<sub>3</sub> (3 nM) and test compounds

<sup>b</sup>Inhibition ratio at 30  $\mu$ M <sup>c</sup>Toxic at 30 µM

Table 3. SAR for substitution of the phenyl group in VDR reporter gene assay<sup>a</sup> <sup>a</sup>HEK293 cells were treated with 1,25(OH)<sub>2</sub>D<sub>3</sub> (3 nM) and test compounds

ay<sup>a</sup> .ds

## (Table 1)



#### (Table 2) R. $H_2N$ $H_2N$ 22-23, 32-34 1 IC<sub>50</sub> (μM) compound R 26 1 Н<sub>у</sub>с 32 > 30 (7 %)<sup>b</sup> یج ا 33 30 > 30 (48 %)<sup>b</sup> 22a Z toxic<sup>c</sup> 22b z ž 25 23a > 30 (47 %)<sup>b</sup> Z 23b 34 24



Figure(s) (Figure 3)



## (Figure 4)



## **ACCEPTED MANUSCRIPT**

(Figure 1)



(Figure 2)



## **ACCEPTED MANUSCRIPT**

(Scheme 1)





(Scheme 3)



## **ACCEPTED MANUSCRIPT**

(Scheme 4)



## **ACCEPTED MANUSCRIPT**

(Scheme 5)



## **ACCEPTED MANUSCRIPT**

(Scheme 6)



H<sub>2</sub>N







AP

(Scheme 7)





