A Novel Synthetic Approach to Very Late Antigen-4 Antagonist *trans*-4-[1-[[2,5-Dichloro-4-(1-methyl-3-indolylcarboxyamide)phenyl]acetyl]-(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid *via tert*-Butyl *trans*-[(4*S*)-Methoxy-(2*S*)-pyrrolidinylmethoxy] cyclohexanecarboxylate as a Key Intermediate

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This contribution describes a novel synthetic approach to very late antigen-4 (VLA-4) antagonist *trans*-4-[1-[[2,5-dichloro-4-(1-methyl-3-indolylcarboxyamide)phenyl]acetyl]-(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (1) *via tert*-butyl *trans*-[(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (2b) as a key intermediate. The synthesis, which includes *n*-Bu_4NSO_3H that catalyzed basic etherification of 12 and iodine-mediated cyclization to provide the 2,4-disubstituted pyrrolidine frame of 2b, is designed to utilize *trans*-4-hydroxycyclohexanecarboxylic acid (9) as a commercially available starting material.

Key words very late antigen-4 antagonist; *trans*-4-hydroxycyclohexanecarboxylic acid

We discovered a *trans*-4-[1-[[2,5-dichloro-4-(1-methyl-3-indolylcarboxyamide)phenyl]acetyl]-(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (**1**)[1] as a novel VLA-4 (very late antigen-4, integrin $\alpha_4\beta_1$) antagonist as shown figure below. On the basis of its favorable profile,[1] **1** was advanced into clinical trials for the treatment of asthma.

The key intermediate to accessing **1** is methyl *trans*-[(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (**2a**). In our previous report,[1,2] compound **2a** was prepared *via* a sequential procedure involving Rh/Al_2O_3 hydrogenation of the benzene ring of **5**, isomerization of the cyclohexanecarboxylate potion of *cis*-**6** to *trans*-**6** under NaOMe/MeOH condition, and separation of both isomer *cis*-**6** and *trans*-**6** as depicted in Chart 1. However, the procedure has two notable drawbacks to overcome for large scale synthesis as follows: (1) the isomerization reaction results in affording a mixture of *cis*-**6** and *trans*-**6** in a ratio of 1 : 1[3]; (2) HPLC or flash chromatography using a large amount of silica gel is necessary to separate the desired *trans*-**6** from the mixture. To address these drawbacks, we investigated an alternative approach to **1** from *trans*-4-hydroxycyclohexanecarboxylic acid (**12**) as a starting material[4] *via tert*-butyl *trans*-[(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (**2b**). We wish to describe the details of our new synthetic approach to compound **1**.

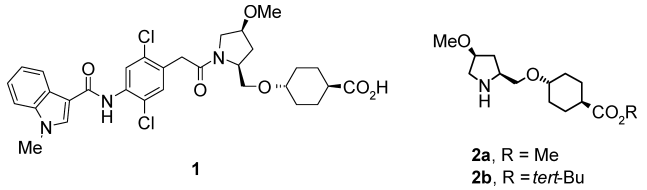

Fig. 1. VLA-4 Antagonist **1** and Key Intermediate **2**

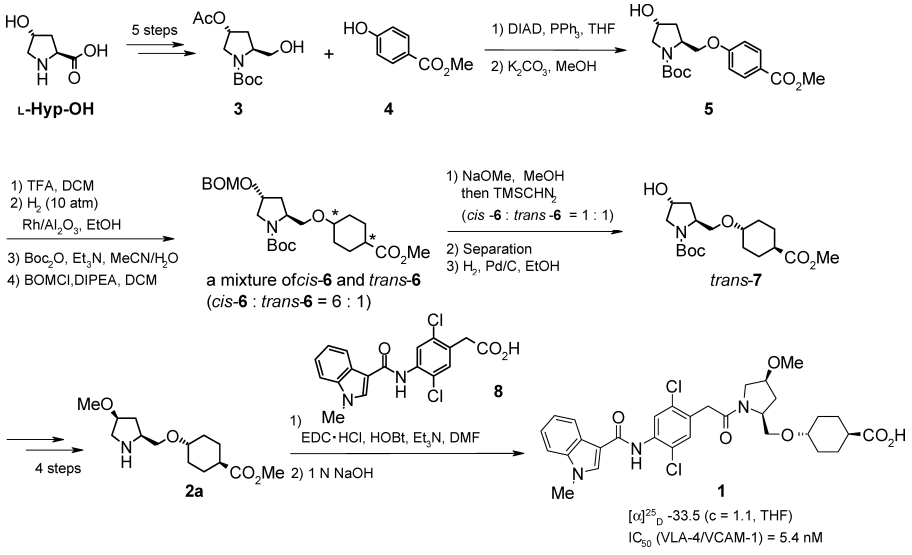

Chart 1

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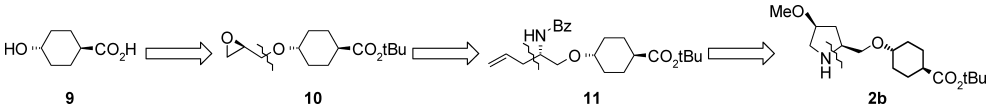

Chart 2

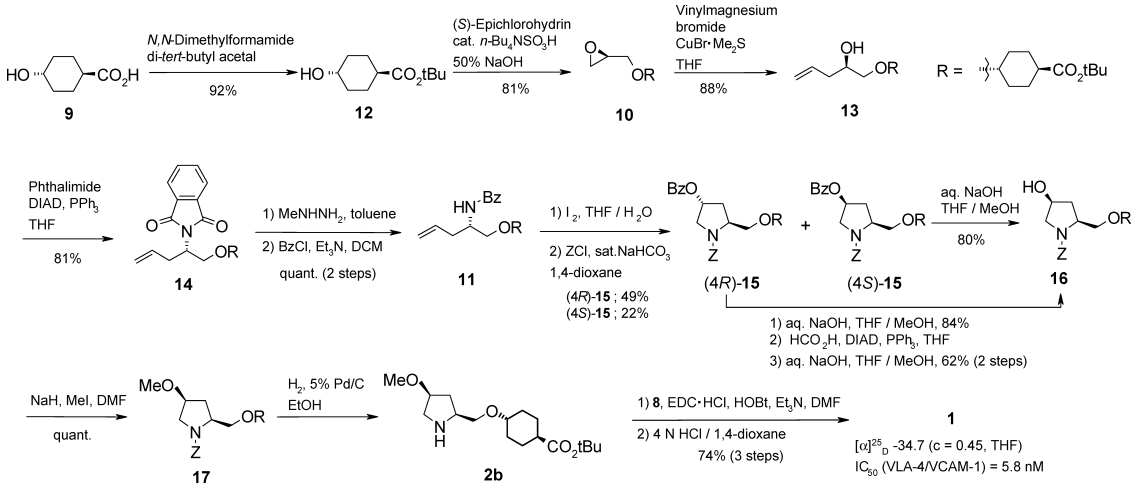

Chart 3

Our synthetic strategy for an approach to compound **2b** is shown in Chart 2. In this strategy, we envisioned that compound **2b** could be synthesized by utilizing Takano's method[5,6] for the preparation of 2,4-disubstituted pyrrolidine from allylamide **11**, which would be prepared *via* epoxide **10** from commercially available *trans*-4-hydroxycyclohexanecarboxylic acid (**9**) and (*S*)-epichlorohydrin.

Commercially available compound **9** was treated with *N*,*N*-dimethylformamide di-*tert*-butylacetal to afford *tert*-butyl ester **12** in 92% yield. By subjecting to *n*-Bu_4NSO_3H-catalyzed etherification with (*S*)-epichlorohydrin in a solution of 50% aqueous NaOH,[7,8] **12** was transformed into epoxide **10** in 81% yield. Nucleophilic addition reaction of **10** with vinylmagnesium bromide in the presence of CuBr·Me_2S gave alcohol **13** in 88% yield, which subsequently underwent Mitsunobu reaction to give **14** in 81% yield. After deprotection of the phthaloyl group in **14**, the resulting primary amine was protected with a benzoyl group to give benzamide **11** in a quantitative yield. According to the reported procedure,[4] treatment of **11** with 3, equivalent of iodine in aqueous tetrahydrofuran (THF) followed by *N*-benzyloxycarbonylation, resulted in giving (4*R*)-**15** and (4*S*)-**15**, which were easily separated by using silica gel chromatography in 49 and 22% yields from **11**, respectively. With both isomers in hand, we next attempted to prepare (4*S*)-hydroxypyrrolidine **16** from each isomer. Thus, hydrolysis of (4*S*)-**15** with aqueous NaOH in THF/MeOH gave alcohol **16** in 80% yield. On the other hand, benzoate (4*R*)-**15** was also converted to **16** *via* sequential basic hydrolysis of the benzoyl group, Mitsunobu reaction with formic acid, and basic hydrolysis in 52% yield. Etherification of **16** with MeI in the presence of NaH in *N*,*N*-dimethylformamide (DMF) proceeded smoothly to give ether **17** in a quantitative yield. The *N*-benzyloxycarbonyl group of **17** was removed by hydrogenolysis to give **2b**. Subsequent condensation of **2b** with arylacetic acid **8**[1] and acidic hydrolysis of the *tert*-butyl ester group of the resulting amide successfully provided **1** ($[\alpha]_D^{25}$ −34.7°, >99% ee)[9] in 74% yield from **17**. This new approach efficiently provided **1** in 17% overall yield compared with the reported procedure (3.3% overall yield).[1] The synthesized compound **1** had an inhibition potency with an IC_{50} value of 5.9 nM in VLA-4/vascular cell adhesion molecule-1 (VCAM-1) binding assay, which was in line with the previously reported value (5.4 nM).[1]

Conclusion

We have developed an alternative synthetic route to clinical candidate **1** by employing commercially available *trans*-4-hydroxycyclohexane carboxylic acid (**9**) as a starting material without a loss of the optical purity of **1** in 15 steps with 17% overall yield. This approach includes *n*-Bu_4NSO_3H-catalyzed etherification of **10** and iodine-mediated double cyclization for the construction of the 2,4-disubstituted pyrrolidine frame of **2b**. The pathway starting with *trans*-4-hydroxycyclohexane carboxylic acid (**9**) has made it possible to avoid the drawbacks, as well as efficiently access **1** in large scale synthesis.

Experimental

Optical rotations were measured with a HORIBA SEPA-300 polarimeter. ^{1}H-NMR spectra were recorded on a JEOL JNM-EX-400 and JNM-ECA500 spectrometer, and chemical shifts are expressed relative to tetramethylsilane (TMS) at δ 0.00 ppm. IR spectra were recorded on a HORIBA FT-720 spectrometer. Mass spectra were recorded on a SCIEX API-150EX spectrometer (electrospray ionization (ESI)) or a JEOL JMS-HX110 spectrometer (FAB). High-resolution mass (HR-MS) spectra were recorded on a JEOL JMS-100LP spectrometer. Elemental analysis was performed using a PerkinElmer CHNS/O 2400II, a Leco CHNS-932, and a YOKOKAWA analysis IC7000RS. All starting materials and synthesis reagents were obtained commercially.

***trans*-4-Hydroxycyclohexanecarboxylic Acid *tert*-Butyl Ester (12)** To a stirred solution of *trans*-4-hydroxycyclohexanecarboxylic acid (**9**) (1.0 g, 6.94 mmol) in benzene/*tert*-BuOH (10/1, 5.5 ml) was added *N*,*N*-dimethylformamide di-*tert*-butyl acetate (15.0 ml, 62.4 mmol), and the reaction mixture was heated at 80 °C for 27 h. After cooling down to room temperature, the reaction mixture was evaporated. The residue was purified by flash chromatography using a Yamazen Hi-Flash 3L eluted with 10—100% EtOAc/*n*-

hexane to give **12** (1.28 g, 92%) as a colorless solid. IR (KBr) 3295, 2976, 2940, 2861, 2684, 1729, 1588, 1454 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.22—1.32 (2H, m), 1.40—1.51 (total 11H, m), 1.89 (1H, broad s), 1.95—2.05 (4H, m), 2.09—2.18 (1H, m), 3.57—3.64 (1H, m). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 27.2. 28.1. 34.6, 43.2. 70.0, 80.0, 175.0. FAB-MS *m/z*: 201 (M^++1); *Anal.* Calcd for $C_{11}H_{20}O_3 \cdot 0.25H_2O$: C, 64.52; H, 10.09. Found: C, 64.29; H, 9.91.

***trans*-4-[(2*S*)-2,3-Epoxypropyloxy]cyclohexanecarboxylic Acid *tert*-Butyl Ester (10)** To a stirred mixture of *trans*-4-hydroxycyclohexanecarboxylic acid *tert*-butyl ester (**12**) (1.50 g, 7.49 mmol) and (*S*)-epichlorohydrine (97% ee) (5.86 ml, 74.9 mmol) was added 50% NaOH (30 ml) and *n*-Bu_4NSO_3H (254 mg, 0.749 mmol) at 0 °C. After stirring for 5 h, the reaction mixture was poured into ice water and extracted with ether. The combined extracts were washed with ice water and brine. After they were dried over Na_2SO_4, the extracts were concentrated *in vacuo*. The residue was purified by chromatography using Merck silica gel 60 (particle size 70—230 mesh) eluting with 25% EtOAc/*n*-hexane to give **10** (1.56 g, 81%) as a colorless oil. $[\alpha]_D^{25}=-2.9°$ (c=0.75, $CHCl_3$); IR (ATR) 2976, 2935, 2863, 1722, 1477, 1454, 1391, 1366, 1309, 1285, 1249, 1203, 1148, 1095 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.12—1.32 (2H, m), 1.36—1.55 (11H, m), 1.95—2.10 (4H, m), 2.15 (1H, tt, J=11.7, 3.9 Hz), 2.60 (1H, dd, J=5.1, 2.7 Hz), 2.80 (1H, t, J=4.2 Hz), 3.13 (1H, m), 3.28 (1H, dd, J=10.5, 3.9 Hz), 3.45 (1H, dd, J=11.2, 5.6 Hz), 3.72 (1H, dd, J=11.5, 3.4 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 27.10, 27.12, 28.1, 31.1, 31.3, 43.4, 44.6, 51.1, 69.9, 77.8. 80.0, 175.0; ESI-MS *m/z*: 257 (M^++1); ESI-MS (HR) *m/z*: 257.17523 (Calcd for $C_{14}H_{25}O_4$: 257.1754).

***trans*-4-[(2*R*)-Hydroxy-4-pentenyloxy]cyclohexanecarboxylic Acid *tert*-Butyl Ester (13)** To a stirred suspension of $CuBr \cdot Me_2S$ (99 mg, 0.48 mmol) in THF (30 ml) was added 1.0 M vinylmagnesium bromide in THF (14.4 ml, 14.4 mmol) at 0 °C under nitrogen atmosphere. After stirring for 30 min, the reaction mixture was cooled to −78 °C. *trans*-4-[(2*S*)-2,3-Epoxypropyloxy]cyclohexanecarboxylic acid *tert*-butyl ester (**10**) (1.23 g, 4.80 mmol) in THF (20 ml) was added to the reaction mixture at −78 °C and allowed to −5 °C for 6 h. The reaction mixture was poured into sat. NH_4Cl and extracted with EtOAc. The combined extracts were washed with brine. After being dried over Na_2SO_4, the extracts were concentrated *in vacuo*. The residue was purified by chromatography using Merck silica gel 60 (particle size 70—230 mesh) eluted with 25% EtOAc/*n*-hexane to give **13** (1.20 g, 88%) as a colorless oil. $[\alpha]_D^{24}=-8.0°$ (c=0.55, $CHCl_3$); IR (ATR) 3446, 2977, 2935, 2863, 1724, 1641, 1454, 1391, 1366, 1308, 1288, 1249, 1204, 1147, 1110, 1028 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.21—1.32 (2H, m), 1.37—1.49 (11H, m), 1.95—2.19 (4H, m), 2.22—2.27 (2H, m), 2.32 (1H, d, J=3.4 Hz), 3.21—3.36 (2H, m), 3.51 (1H, dd, J=9.3, 3.4 Hz), 3.77—3.82 (1H, m), 5.08—5.14 (2H, m), 5.79—5.88 (1H, m); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 27.1, 28.1, 31.2, 31.3, 37.9, 43.4, 69.8, 71.7, 77.8, 80.1, 117.6, 134.3, 175.0; ESI-MS *m/z*: 307 (M^++Na); ESI-MS (HR) *m/z*: 307.1886 (Calcd for $C_{16}H_{28}O_4Na$: 307.1885).

***trans*-4-[(2*S*)-Phtaloylamino-4-pentenyloxy]cyclohexanecarboxylic Acid *tert*-Butyl Ester (14)** To a stirred solution of *trans*-4-[(2*R*)-hydroxy-4-pentenyloxy]cyclohexanecarboxylic acid *tert*-butyl ester (**13**) (390 mg, 1.37 mmol), phtalimide (404 mg, 2.74 mmol) and PPh_3 (719 mg, 2.74 mmol) in THF (30 ml) was added DIAD (0.57 ml, 2.74 mmol) at room temperature. After stirring for 17 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by flash chromatography using a Yamazen Hi-Flash 2L eluted with 20—100% EtOAc/*n*-hexane to give **14** (460 mg, 81%) as a colorless oil. $[\alpha]_D^{25}=+7.5°$ (c=0.66, $CHCl_3$); IR (ATR) 3462, 3002, 2976, 2940, 2903, 2863, 1771, 1710, 1645, 1612, 1594, 1470, 1454, 1437, 1394, 1376, 1366, 1336, 1309, 1289, 1249, 1206, 1160, 1109, 1027, 1012 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.09—1.44 (13H, m), 1.84—2.12 (5H, m), 2.54 and 2.56 (total 1H amide isomer, each dt, J=7.3, 1.5 and 5.6, 1.2 Hz, respectively), 2.72 and 2.74 (total 1H amide isomer, each t, J=8.8 and 8.5 Hz, respectively), 3.21 (1H, tt, J=10.1, 4.0 Hz), 3.75 (1H, ddd, J=9.8, 5.9, 1.2 Hz), 3.96 (1H, dt, J=10.0, 1.2 Hz), 4.48—4.52 (1H, m), 4.96 (1H, d, J=10.0 Hz), 5.04 (1H, d, J=16.8 Hz), 5.70—5.74 (1H, m), 7.68—7.72 (2H, m), 7.78—7.82 (2H, m); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 26.95, 27.03, 28.1, 31.3, 33.6, 43.3, 51.3, 67.3, 77.4, 79.9, 118.0, 123.1, 131.9, 133.8, 134.0, 168.6, 175.0; ESI-MS *m/z*: 436 (M^++Na); ESI-MS (HR) *m/z*: 436.2097 (Calcd for $C_{24}H_{31}NO_5Na$: 436.2100); *Anal.* Calcd for $C_{24}H_{31}NO_5 \cdot 0.25H_2O$: C, 68.96; H, 7.60; N, 3.35. Found: C, 68.72; H, 7.32; N, 3.38.

***trans*-4-[(2*S*)-Benzoylamino-4-pentenyloxy]cyclohexanecarboxylic Acid *tert*-Butyl Ester (11)** To a stirred solution of *trans*-4-[(2*S*)-pthaloylamino-4-pentenyloxy]cyclohexanecarboxylic acid *tert*-butyl ester (**14**) (1.74 g, 4.21 mmol) in toluene (50 ml) was added methylhydrazine (2.24 ml, 42.1 mmol) at room temperature. The reaction mixture was heated at 100 °C for 24 h. After being cooled to room temperature, the reaction mixture was filtered and the filtrate was concentrated to give *trans*-4-[(2*S*)-amino-4-pentenyloxy]cyclohexanecarboxylic acid *tert*-butyl ester, which was used without further purification.

To a stirred solution of *trans*-4-[(2*S*)-amino-4-pentenyloxy]cyclohexanecarboxylic acid *tert*-butyl (4.21 mmol) in CH_2Cl_2 (50 ml) was added Et_3N (1.76 ml, 12.6 mmol) and BzCl (0.59 ml, 5.05 mmol) at room temperature. After stirring for 18 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The combined extracts were washed with brine. After being dried over Na_2SO_4, the extracts were concentrated *in vacuo*. The residue was purified by flash chromatography using a Yamazen Hi-Flash 2L eluted with 20—100% EtOAc/*n*-hexane to give **11** [1.71 g, 100% (2 steps)] as a colorless solid. $[\alpha]_D^{25}=-14.4°$ (c=0.56, $CHCl_3$); IR (KBr) 3372, 3078, 3030, 3008, 2974, 2937, 2863, 1822, 1721, 1643, 1603, 1579, 1525, 1489, 1474, 1451, 1440, 1415, 1392, 1367, 1326, 1306, 1277, 1252 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.19—1.31 (2H, m), 1.33—1.41 (11H, m), 1.89—2.20 (5H, m), 2.44 (2H, t, J=6.3 Hz), 3.23 (1H, tt, J=10.5, 4.4 Hz), 3.54 (1H, dd, J=9.3, 3.2 Hz), 4.27—4.31 (1H, m), 5.09 (1H, dd, J=11.0, 1.0 Hz), 5.12 (1H, dd, J=16.1, 1.2 Hz), 5.82—5.86 (1H, m), 6.41 (1H, d, J=8.1 Hz), 7.42 (2H, dt, J=7.6, 1.2 Hz), 7.49 (1H, dt, J=7.6, 1.2 Hz), 7.74 (2H, dd, J=6.8, 1.2 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 27.0, 28.1, 31.1, 31.3, 36.3, 43.3, 49.0, 68.5, 77.6, 80.0, 117.8, 126.9, 128.6, 131.4, 134.7, 134.9, 166.9, 174.9; ESI-MS *m/z*: 410 (M^++Na); ESI-MS (HR) *m/z*: 410.2315 (Calcd for $C_{23}H_{33}NO_4Na$: 410.2307); *Anal.* Calcd for $C_{23}H_{33}NO_4 \cdot 0.25H_2O$: C, 70.11; H, 8.51; N, 3.57. Found: C, 70.47; H, 8.61; N, 3.57.

***trans*-4-[1-Benzyloxycarbonyl-(4*R*)-benzoyloxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid *tert*-Butyl Ester [(4*R*)-15] and *trans*-4-[1-Benzyloxycarbonyl-(4*S*)-benzoyloxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid *tert*-Butyl Ester [(4*S*)-15]** To a stirred solution of *trans*-4-[(2*S*)-benzoylamino-4-pentenyloxy]cyclohexanecarboxylic acid *tert*-butyl ester (**11**) (1.69 g, 4.36 mmol) in THF/H_2O (1/1, 80 ml) was added I_2 (3.32 g, 13.1 mmol) at room temperature. After stirring for 2 d, the reaction mixture was poured into sat. $Na_2S_2O_3$ and extracted with EtOAc. The combined extracts were washed with brine. After being dried over Na_2SO_4, the extracts were concentrated *in vacuo* to afford a mixture of *trans*-4-[(4*R*)-benzoyloxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid *tert*-butyl ester and *trans*-4-[(4*S*)-benzoyloxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid *tert*-butyl ester, which was used without further purification.

To a stirred solution of *trans*-4-[(4*R*)-benzoyloxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid *tert*-butyl ester and *trans*-4-[(4*S*)-benzoyloxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid *tert*-butyl ester (4.36 mmol) in 1,4-dioxane (30 ml) were added sat. $NaHCO_3$ (30 ml) and 30% ZCl toluene solution (3.87 ml, 6.54 mmol) at room temperature. After stirring for 1 d, the reaction mixture was filtered. The filtrate was extracted with EtOAc. The combined extracts were washed with brine. After being dried over Na_2SO_4, the extracts were concentrated *in vacuo*. The residue was purified by flash chromatography using a Yamazen Ultra Pack D eluting with 0—60% EtOAc/*n*-hexane to give a less polar fraction of (4*S*)-**15** (511 mg, 0.95 mmol) as a colorless oil and a more polar fraction of (4*R*)-**15** (1.14 g, 2.12 mmol) as a colorless oil.

(4*S*)-**15**: $[\alpha]_D^{19}=-32.8°$ (c=0.56, $CHCl_3$); IR (KBr) 3063, 3033, 2936, 2862, 1702, 1602, 1584, 1538, 1498, 1451, 1412, 1364, 1314, 1271, 1202, 1156, 1109, 1094 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.14—1.25 (2H, m), 1.33—1.47 (11H, m), 1.87—2.17 (5H, m), 2.24—2.44 (2H, m), 3.03—3.26 (1H, m), 3.46—3.90 (4H, m), 4.14—4.27 (1H, m), 5.10—5.27 (2H, m), 5.51—5.56 (1H, m), 7.27—7.40 (5H, m), 7.43 (2H, t, J=8.0 Hz), 7.57 (1H, t, J=7.4 Hz), 7.98 (2H, d, J=6.9 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 27.0, 28.1, 29.4, 31.1, 31.3, 34.4, 43.3, 52.6, 56.7, 66.7, 68.2, 73.5, 77.7, 79.9, 127.7, 127.9, 128.0, 128.4, 128.5, 129.6, 129.9, 133.2, 136.8, 154.8, 166.1, 175.0; ESI-MS *m/z*: 538 (M^++1); ESI-MS (HR) *m/z*: 538.2805 (Calcd for $C_{31}H_{40}NO_7$: 538.2805).

(4*R*)-**15**: $[\alpha]_D^{25}=-19.1°$ (c=0.52, $CHCl_3$); IR (KBr) 2936, 2863, 1703, 1602, 1584, 1497, 1451, 1410, 1365, 1357, 1336, 1314, 1271, 1219, 1148 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.95—1.52 (13H, m), 2.14—1.57 (6H, m), 2.27—2.44 (2H, m), 3.01—3.27 (1H, m), 3.42—3.79 (2H, m), 3.84—3.93 (1H, m), 4.07—4.23 (1H, m), 5.13—5.21 (2H, m), 5.53 (1H, s), 7.29—7.40 (5H, m), 7.45 (2H, t, J=8.0 Hz), 7.58 (1H, t, J=7.4 Hz), 8.01 (2H, d, J=8.6 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 26.9, 27.0, 28.0, 30.9, 33.2, 34.1, 43.2, 52.7, 56.7, 65.2, 69.9, 67.1, 74.1, 77.5, 79.9, 126.9, 127.5, 127.9, 128.0, 128.3, 128.39, 128.44, 129.5, 129.8, 133.2, 136.4, 141.0, 154.7, 165.8, 174.9; ESI-MS *m/z*: 538 (M^++1); ESI-MS (HR) *m/z*: 538.2803 (Calcd for $C_{31}H_{40}NO_7$: 538.2805).

***trans*-4-[1-Benzyloxycarbonyl-(4*S*)-hydroxy-(2*S*)-pyrrolidinyl-**

methoxy]cyclohexanecarboxylic Acid *tert*-Butyl Ester (16) To a stirred solution of *trans*-4-[1-benzyloxycarbonyl-(4*S*)-benzoyloxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid *tert*-butyl ester [(4*S*)-**15**] (144 mg, 0.267 mmol) in THF/MeOH (2/1, 6 ml) was added 1 N NaOH (0.54 ml) at room temperature. After stirring for 15 h, the reaction mixture was diluted with water and extracted with 10% MeOH/$CHCl_3$. The combined extracts were washed with brine. After being dried over Na_2SO_4, the extracts were concentrated *in vacuo*. The residue was purified by thin layer chromatography using Merck pre-coated with silica gel 60 F254 eluting with 50% EtOAc/*n*-hexane to give **16** (93 mg, 80%) as a colorless oil. $[\alpha]_D^{25}=-35.0°$ ($c=0.50$, $CHCl_3$); IR (ATR) 3414, 3063, 3033, 2936, 2864, 1700, 1586, 1542, 1498, 1453, 1410, 1363, 1327, 1310, 1249, 1212 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.06—1.56 (13H, m), 1.89—2.19 (5H, m), 2.28—2.42 (1H, m), 2.96—3.46 (2H, m), 3.47—3.64 (2H, m), 4.16—3.81 (2H, m), 4.31—4.19 (1H, m), 5.28—4.88 (3H, m), 7.39—7.28 (5H, m); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 26.8, 28.1, 30.8, 37.5, 38.6, 43.1, 56.8, 57.3, 57.5, 57.7, 66.6, 68.9, 69.1, 69.2, 78.3, 80.1, 127.8, 128.0, 128.5, 128.6, 136.9, 155.1, 174.7; MS (LC-ESI) *m/z* 434 (M^++1), 456 (M^++Na); ESI-MS (HR) *m/z*: 456.2371 (Calcd for $C_{24}H_{35}NO_6Na$: 456.2362).

***trans*-4-[1-Benzyloxycarbonyl-(4*S*)-hydroxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid *tert*-Butyl Ester (16)** To a stirred solution of *trans*-4-[1-benzyloxycarbonyl-(4*R*)-benzoyloxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid *tert*-butyl ester [(4*R*)-**15**] (1.14 g, 2.12 mmol) in THF/MeOH (2/1, 12 ml) was added 1 N NaOH (4.24 ml) at room temperature. After stirring for 15 h, the reaction mixture was diluted with water and extracted with 10% MeOH/$CHCl_3$. The combined extracts were washed with brine. After being dried over Na_2SO_4, the extracts were concentrated *in vacuo*. The residue was purified by flash chromatography using a Yamazen Hi-Flash 2L eluted with 10—60% EtOAc/*n*-hexane to give *trans*-4-[1-benzyloxycarbonyl-(4*R*)-hydroxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid *tert*-butyl ester (774 mg, 84%) as a colorless oil. $[\alpha]_D^{25}$ −37.7° ($c=0.45$, $CHCl_3$); IR (ATR) 3437, 2936, 2863, 1721, 1700, 1498, 1453, 1414, 1363, 1309, 1287, 1248, 1148, 1097 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.13—1.43 (13H, m), 1.88—2.16 (6H, m), 2.29—2.41 (1H, m), 3.05—3.24 (1H, m), 3.42—3.74 (4H, m), 4.08—4.18 (1H, m), 4.40—4.53 (1H, m), 5.03—5.28 (2H, m), 7.27—7.36 (5H, m); MS (LC-ESI) *m/z* 434 (M^++1); ESI-MS (HR) *m/z*: 434.2542 (Calcd for $C_{24}H_{36}NO_6$: 434.2543).

To a stirred solution of *trans*-4-[1-benzyloxycarbonyl-(4*R*)-hydroxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid *tert*-butyl ester (52 mg, 0.120 mmol), HCO_2H (5.4 μl, 0.144 mmol) and PPh_3 (47 mg, 0.180 mmol) in THF (2 ml) was added DIAD (35.7 μl, 0.180 mmol) at 0 °C. After stirring for 30 min, HCO_2H (5.4 μl, 0.144 mmol), PPh_3 (47 mg, 0.180 mmol), and DIAD (35.7 μl, 0.180 mmol) were added at room temperature and stirred for 17 h. The reaction mixture was concentrated *in vacuo*. The residue was purified by flash chromatography using silica gel eluting with 25% EtOAc/*n*-hexane to give *trans*-4-[1-benzyloxycarbonyl-(4*S*)-formyloxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid *tert*-butyl ester (72 mg) as a colorless oil, which was used without further purification. MS (LC-ESI) *m/z* 462 (M^++1).

To a stirred solution of *trans*-4-[1-benzyloxycarbonyl-(4*S*)-formyloxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid *tert*-butyl ester (72 mg, 0.12 mmol) in THF/MeOH (2/1, 3 ml) was added 1 N NaOH (1 ml) at room temperature. After 3.5 h stirring, the reaction mixture was diluted with water and extracted with ether. The combined extracts were washed with 1 N NaOH and brine. After being dried over Na_2SO_4, the extracts were concentrated *in vacuo*. The residue was purified by thin layer chromatography using Merck pre-coated with silica gel 60 F254 eluting with 50% EtOAc/*n*-hexane to give **16** [37 mg, 71% (2 steps)] as a colorless oil.

***trans*-4-[1-Benzyloxycarbonyl-(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid *tert*-Butyl Ester (17)** To a stirred solution of *trans*-4-[1-benzyloxycarbonyl-(4*S*)-hydroxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid *tert*-butyl ester (**16**) (570 mg, 1.31 mmol) and MeI (818 μl, 13.1 mmol) in DMF (20 ml) was added 60% oily NaH (86 mg, 1.97 mmol) at room temperature under nitrogen atmosphere. After stirring for 2 h, the reaction mixture was poured into ice water and extracted with EtOAc. The combined extracts were washed with brine. After beimg dried over Na_2SO_4, the extracts were concentrated *in vacuo*. The residue was purified by flash chromatography using a Yamazen Hi-Flash 2L eluting with 10—60% EtOAc/*n*-hexane to give **17** (597 mg, 100%) as a colorless oil. $[\alpha]_D^{25}$ −21.6° ($c=0.43$, $CHCl_3$); IR (KBr) 3032, 2976, 2935, 2864, 2826, 1701, 1498, 1453, 1410, 1364, 1354, 1313, 1247, 1202, 1147, 1090 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.14—1.49 (13H, m), 1.89—2.28 (7H, m), 3.13 and 3.23 (total 1H, each m, amide isomers), 3.30 (3H, s), 3.38—3.49 (2H, m), 3.57—4.08 (4H, m), 5.08—5.21 (2H, m), 7.28—7.37 (5H, m); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 27.1, 28.1, 31.2, 31.4, 31.5, 32.3, 33.4, 43.4, 52.2, 52.3, 56.6, 66.7, 69.0, 77.5, 79.9, 128.0, 128.5, 136.8, 154.9, 170.1; MS (ESI) *m/z* 448 (M^++1); ESI-MS (HR) *m/z*: 448.2699 (Calcd for $C_{25}H_{38}NO_6$: 448.2699).

***trans*-4-[(4*S*)-Methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid *tert*-Butyl Ester (2b)** A solution of *trans*-4-[1-benzyloxycarbonyl-(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid *tert*-butyl ester (**17**) (570 mg, 1.27 mmol) in EtOH (20 ml) was hydrogenated over 5% Pd/C (100 mg) for 1 d. After removal of the catalyst by filtration, the filtrate was concentrated *in vacuo* to give **2b** (446 mg, over yield) as a pale brown oily solid, which was used without further purification. $[\alpha]_D^{25}=+26.3°$ ($c=0.23$, $CHCl_3$); IR (KBr) 3528, 3427, 2974, 2938, 2903, 2868, 2746, 2658, 2586, 2486, 2462, 2423, 2385, 1720, 1564, 1479 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.10—1.69 (12H, m), 1.80—2.04 (4H, m), 2.08—2.35 (2H, m), 3.09—3.32 (6H, m), 3.37—3.74 (4H, m), 4.02—4.09 (1H, m), 4.36 (1H, t, $J=5.0$ Hz), 8.77 and 9.50 (total 1H, s); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 18.5, 26.5, 27.6, 30.4, 32.1, 42.3, 49.2, 55.9, 56.0, 67.0, 76.8, 77.9, 79.3, 174.1; MS (ESI) *m/z* 314 (M^++1); ESI-MS (HR) *m/z*: 314.2328 (Calcd for $C_{17}H_{32}NO_4$: 314.2331).

***trans*-4-[1-[[2,5-Dichloro-4-(1-methyl-3-indolylcarbonylamino)phenyl]acetyl]-(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid *tert*-Butyl Ester** To a stirred solution of [2,5-dichloro-4-(1-methyl-3-indolylcarbonylamino)phenyl]acetic acid (**8**) (451 mg, 1.20 mmol), *trans*-4-[(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxilic acid *tert*-butyl ester (**2b**) (1.27 mmol), EDC·HCl (344 mg, 1.79 mmol), and HOBT (274 mg, 1.79 mmol) in DMF (30 ml) Et_3N (833 μl, 5.98 mmol) was added at room temperature. After stirring for 1 d, the mixture was poured into ice water and extracted with EtOAc. The combined extracts were washed with ice water and brine. After being dried over Na_2SO_4, the extracts were concentrated *in vacuo*. The residue was purified by flash chromatography using a Yamazen Hi-Flash 2L eluting with 50—100% EtOAc/*n*-hexane to give *trans*-4-[1-[[2,5-dichloro-4-(1-methyl-3-indolylcarbonylamino)phenyl]acetyl]-(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid *tert*-butyl ester (820 mg, 100%) as a colorless oil. $[\alpha]_D^{25}=-26.3°$ ($c=0.52$, $CHCl_3$); IR (KBr) 3419, 2977, 2935, 2863, 2827, 1720, 1641, 1568, 1533, 1500, 1469, 1423, 1368, 1336, 1304, 1219 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.18—1.31 (2H, m), 1.33—1.46 (11H, m), 1.91—2.32 (9H, m), 3.22—3.26 (1H, m), 3.31 and 3.33 (total 1H, each, s, amide isomers), 3.46—4.02 (10H, m), 4.18—4.31 (1H, m), 7.30—7.37 (2H, m), 7.41 (2H, d, $J=5.6$ Hz), 7.79 (1H, d, $J=1.2$ Hz), 8.11—8.15 (1H, m), 8.23 (1H, d, $J=2.7$ Hz), 8.77 (1H, d, $J=7.6$ Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 14.1, 27.1, 28.1, 31.2, 31.6, 33.5, 34.3, 38.2, 43.3, 51.8, 53.2, 56.6, 57.7, 67.4, 70.5, 77.4, 79.8, 110.4, 110.7, 120.0, 120.5, 121.4, 122.2, 123.0, 125.1, 128.5, 130.9, 133.3, 134.8, 137.4, 162.7, 168.4, 169.2, 175.0; MS (ESI) *m/z* 672 (M^++1), 674 (M^++3); ESI-MS (HR) *m/z*: 672.2616 (Calcd for $C_{35}H_{44}N_3O_6Cl_2$: 672.2607).

***trans*-4-[1-[[2,5-Dichloro-4-(1-methyl-3-indolylcarbonylamino)phenyl]acetyl]-(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic Acid (1)** A solution of *trans*-4-[1-[[2,5-dichloro-4-(1-methyl-3-indolylcarbonylamino)phenyl] acetyl]-(4*S*)-methoxy-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid *tert*-butyl ester (804 mg, 1.20 mmol) in 4 N HCl/1,4-dioxane (30 ml) was stirred for 2 d. The reaction mixture was concentrated under reduced pressure. The residue was recrystallized from ether/EtOAc/*n*-hexane to give **1** (545 mg, 74%, >99% ee) as a pale yellow powder. Enantiomeric excess was determined by HPLC analysis [4.6×250 mm Shiseido Chiral cell CD-Ph column, 10 mM phosphate buffer/CH_3CN=45/55 (v/v), 0.5 ml/min, retention time 14.5 min] to be over 99% ee; $[\alpha]_D^{25}=-34.7°$ ($c=0.45$, THF); IR (KBr) 3430, 3115, 2939, 2861, 1727, 1714, 1666, 1640, 1604, 1568, 1531, 1499, 1459, 1438 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.11—1.40 (4H, m), 1.88—2.20 (7H, m), 3.14—3.51 (5H, m), 3.58—3.82 (4H, m), 3.89 (3H, s), 3.92—4.26 (3H, m), 7.21 (1H, t, $J=7.3$ Hz), 7.28 (1H, t, $J=6.9$ Hz), 7.49—7.53 (1H, m), 7.56 (1H, d, $J=8.3$ Hz), 7.88—7.90 (1H, m), 8.15 (1H, d, $J=7.8$ Hz), 8.31 (1H, s), 9.39 (1H, d, $J=2.8$ Hz), 12.06 (1H, s); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 26.7, 30.7, 31.3, 33.3, 37.6, 38.2, 41.6, 52.4, 55.9, 56.9, 69.9, 76.8, 77.9, 79.6, 108.6, 110.6, 120.9, 121.2, 122.4, 125.4, 126.4, 126.2, 131.9, 132.1, 132.4, 133.3, 134.9, 136.8, 162.7, 168.0, 176.4; *Anal.* Calcd for $C_{31}H_{35}Cl_2N_3O_6\cdot 0.25H_2O$: C, 59.95; H, 5.76; Cl, 11.42; N, 6.77. Found: C, 59.84; H, 5.81; Cl, 11.42; N, 6.64; ESI-MS *m/z*: 616 (M^++1), 618 (M^++3); ESI-MS (HR) *m/z*: 638.1808 (Calcd for $C_{31}H_{35}N_3O_6Cl_2Na$: 638.1801).

References and Notes

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