Novel Synthesis of a Dihydroxyethylene Isostere of Renin Inhibitors

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A dihydroxyethylene isostere, (2*S*,3*R*,4*S*)-4-amino-5-cyclohexyl-1-morpholino-2,3-pentanediol (ACMP, 1), which is a component of non-peptidic, orally active, low-molecular-weight renin inhibitors, was synthesized stereospecifically starting from 3-cyclohexyl-L-alanine *via* iodo-cyclocarbamation as the key reaction.

Keywords renin inhibitor; dihydroxyethylene isostere; stereospecific synthesis; iodo-cyclocarbamation; (2*S*,3*R*,4*S*)-4-amino-5-cyclohexyl-1-morpholino-2,3-pentanediol

The renin–angiotensin system (RAS) plays a central role in the regulation of blood pressure. Renin is an aspartic protease that generates angiotensin I from angiotensinogen. Angiotensin I is a hemodynamically inactive decapeptide, but it is transformed by angiotensin converting enzyme (ACE) into the octapeptide angiotensin II, which is a very potent vasoconstrictor. ACE inhibitors are now widely used for the treatment of hypertension. However, ACE inhibitors induce adverse effects such as cough, skin rash or angioneurotic edema because ACE hydrolyses not only angiotensin I but also bradykinin. Renin catalyzes the first and rate-limiting step in the RAS, and angiotensinogen is the only known naturally occurring substrate of renin. Therefore, renin inhibitors could be more useful than ACE inhibitors as antihypertensive therapy.[1)]

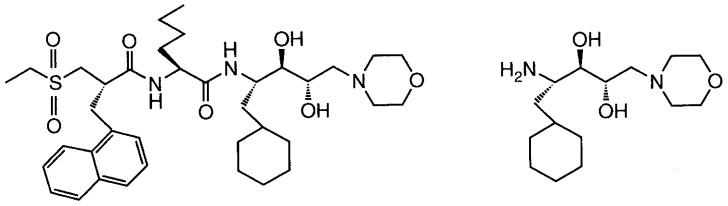
Recently, we reported a novel renin inhibitor, BW-175, which is a non-peptidic, orally active, low-molecular-weight inhibitor containing the dihydroxyethylene isostere (2*S*,3*R*,4*S*)-4-amino-5-cyclohexyl-1-morpholino-2,3-pentanediol (ACMP, **1**).[2)] The Abbott group[3)] and the Kissei group[4)] have reported that the histidine residue at the P_2 site in inhibitors contributes to the enzyme specificity. BW-175 is specific for renin, although it does not have a histidine residue at P_2. The morpholino residue of ACMP may play an important role in this specificity.[5)] We have already reported the synthesis of ACMP starting from 1,2: 5,6-di-*O*-isopropylidene-D-allofuranose[6)] or divinylcarbinol,[7)] and Kobayashi *et al.* have synthesized ACMP from unnatural (2*S*,3*S*)-tartaric acid.[8)] In this paper we report a novel stereospecific synthesis of ACMP from 3-cyclohexyl-L-alanine, including iodo-cyclocarbamation as the key reaction.

The synthetic route is shown in Chart 1. *N*,*O*-Dimethylhydroxyamide **2**, synthesized from 3-cyclohexyl-L-alanine, was reduced with lithium aluminum hydride (LAH)[9)] followed by Horner–Emmons reaction with Still's reagent[10)] to give the *Z*-olefin **3** in a 76% yield from **2**. In this reaction, the *E*-olefin was not detectable by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The *Z*-olefin **3** was converted into the acetate **4** by reduction with boron trifluoride etherate–diisobutylaluminum hydride (DIBAL)[11)] followed by acetylation of the resulting alcohol with acetic anhydride–pyridine in a 71% yield from **3**. The acetate **4** was treated with iodine in acetonitrile to give **5** as the sole product in a 96% yield. The stereochemistry of **5** was determined by converting **5** into Boc-ACMP (**10**) and comparing the ^{1}H-NMR spectrum of the product with that of an authentic sample.[7)] The high stereoselectivity observed in the cyclocarbamation of **4** can be explained by A-strain.[12)] Treatment of the acetate **5** with sodium methoxide gave the epoxide **6** in a 96% yield. The epoxide opening of **6** with morpholine followed by oxazolidinone hydrolysis with lithium hydroxide afforded ACMP (**1**) in a 99% yield. The ACMP was characterized as its *N*-*tert*-butoxycarbonyl derivative **10**, prepared by treatment with di-*tert*-butyl dicarbonate (Boc_2O). It is possible to change the order of the final three reactions which convert **6** into **10**. Namely, treatment of **6** with Boc_2O gave the *N*-Boc-derivative **7** in a 87% yield. Compound **7** was transformed into **10** in a 73% yield by a procedure similar to that described for the conversion of **6** into **1**.

Thus, we have developed a novel method for the stereospecific synthesis of a dihydroxyethylene isostere from an α-amino acid.

Experimental

Melting points were determined with a Yanagimoto melting point apparatus without correction. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded with a Varian VXR-300 (300 MHz)



BW-175 ACMP (1)

Fig. 1

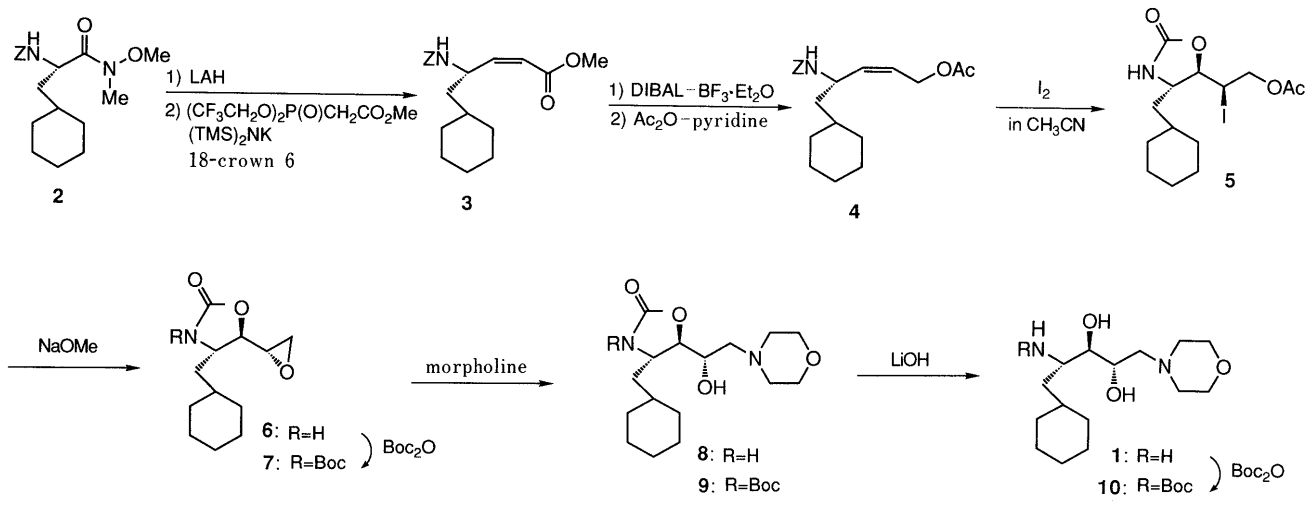


Chart 2

in deuteriochloroform ($CDCl_3$). Chemical shifts are reported relative to residual protons of deuterated NMR solvents. Fast-atom bombardment mass spectra (FAB-MS) were obtained with a JEOL JMS-DX 300 mass spectrometer. Optical rotations were determined with a Horiba SEPA-200 high-sensitivity polarimeter. Thin-layer chromatography was conducted with E. Merck 0.25-mm glass plates precoated with Silica gel 60 F_{254} (Art. 5715). Column chromatography was done on Kieselgel 60 (E. Merck, 70—230 mesh) and flash chromatography was performed on Wakogel 300. The organic solutions were dried over $MgSO_4$ before vacuum evaporation.

Methyl *cis*-(4*S*)-(*N*-Benzyloxycarbonyl)amino-5-cyclohexyl-2-pentenate (3) LAH (134 mg, 3.52 mmol) was added to a stirred solution of the hydroxyamide **2** (980 mg, 2.82 mmol) in ether (28 ml) at room temperature. After being stirred at room temperature for 30 min, the mixture was hydrolyzed with a solution of potassium hydrogen sulfate (681 mg, 4.93 mmol) in water (14 ml) and extracted with ether. The organic layer was washed with 1 N HCl, saturated $NaHCO_3$, water and brine. Drying followed by evaporation gave the corresponding aldehyde as a colorless oil. Potassium bis(trimethylsilyl)amide (6.2 ml, 0.5 M solution in toluene) was added to a solution of bis(2,2,2-trifluoroethyl) (methoxycarbonylmethyl)phosphonate (986 mg, 3.10 mmol) and 18-crown 6 (4.1 g, 15.5 mmol) in tetrahydrofuran (THF) (50 ml) at −78 °C. A solution of the aldehyde in THF (10 ml) was then added to the mixture at −78 °C and the resulting mixture was stirred for 1 h at −78 °C. Saturated NH_4Cl was added and the mixture was extracted with AcOEt. The organic layer was washed with 1 N HCl, saturated $NaHCO_3$, water and brine. Drying followed by evaporation and purification by silica gel chromatography (hexane : AcOEt = 8 : 1) gave the title compound (739 mg, 76.0%) as a colorless oil, $[\alpha]_D^{20}$ +67.4° (c = 0.94, MeOH) [lit.,[13] $[\alpha]_D^{20}$ +75.2° (c = 1.24, MeOH)]. ^{1}H-NMR ($CDCl_3$) δ: 0.90—1.95 (13H, m), 3.71 (3H, s), 4.90 (1H, br s), 5.08 (1H, d, J = 12.2 Hz), 5.11 (1H, d, J = 12.2 Hz), 5.18—5.32 (1H, m), 5.78 (1H, d, J = 11.2 Hz), 6.02—6.20 (1H, m), 7.03—7.42 (5H, m).

***cis*-(4*S*)-(*N*-Benzyloxycarbonyl)amino-5-cyclohexyl-2-pentenol Acetate (4)** Boron trifluoride etherate (195 mg, 1.38 mmol) was added to a solution of the ester **3** (425 mg, 1.23 mmol) in CH_2Cl_2 (3 ml) at −78 °C, and the slution was stirred at −78 °C for 40 min. Diisobutylaluminum hydride (3.9 ml, 1.02 M solution in toluene) was added and the resulting solution was stirred at −78 °C for 1.2 h. The reaction was quenched with acetic acid (2.8 ml, 5 M solution in CH_2Cl_2) and the mixture was poured into 10% aqueous tartaric acid. The product was extracted with AcOEt and the organic layer was washed with 1 N HCl, saturated $NaHCO_3$, water and brine. Drying followed by evaporation and purification by silica gel chromatography (hexane : AcOEt = 5 : 1) gave the (*N*-benzyloxycarbonyl)amino alcohol (299 mg, 77%) as a colorless oil, $[\alpha]_D^{20}$ +33.3° (c = 0.99, MeOH) [lit.,[13] mp 55.0—57.5 °C and $[\alpha]_D^{20}$ +33.8° (c = 0.68, MeOH)]. ^{1}H-NMR ($CDCl_3$) δ: 0.82—1.05 (2H, m), 1.05—1.50 (6H, m), 1.50—1.90 (5H, m), 3.42 (1H, dd, J = 3.2, 9.2 Hz), 3.94 (1H, ddd, J = 6.2, 9.2, 12.6 Hz), 4.30—4.50 (1H, m), 4.50—4.90 (2H, m) 5.04 (1H, d, J = 12.0 Hz), 5.11 (1H, d, J = 12.0 Hz), 5.24 (1H, t, 10.5 Hz), 5.70—5.90 (1H, m), 7.25—7.50 (5H, m). FAB-MS m/z: 318 $[M+H]^+$. Acetic anhydride (0.5 ml) and *N*,*N*-dimethylaminopyridine (5 mg) were added to a solution of this alcohol (149 mg, 0.47 mmol) in pyridine (1 ml) at 0 °C. After being stirred at room temperature overnight, the mixture was poured into ice-water. The whole was extracted with AcOEt and the organic layer was washed with 1 N HCl, saturated $NaHCO_3$, water and brine. Drying followed by evaporation and purification by silica gel chromatography (hexane: AcOEt = 6 : 1) gave the title compound (155 mg, 92%) as a colorless oil, $[\alpha]_D^{20}$ +63.6° (c = 1.08, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.05 (2H, m), 1.05—1.52 (6H, m), 1.52—1.90 (5H, m), 2.05 (3H, s), 4.25—4.90 (4H, m), 5.03 (1H, d, J = 12.1 Hz), 5.12 (1H, d, 12.1 Hz), 5.90 (1H, t, J = 9.6 Hz), 5.45—5.68 (1H, m), 7.25—7.40 (5H, m). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{21}H_{30}NO_4$: 360.2175. Found: 360.2179.

(4*S*,5*R*)-4-Cyclohexylmethyl-5-[(1*R*)-2-acetoxy-1-iodoethyl]oxazolidin-2-one (5) Iodine (316 mg, 1.25 mmol) was added to a solution of the acetate **4** (149 mg, 0.42 mmol) in acetonitrile (2 ml) and the resulting solution was stirred at room temperature overnight. AcOEt was added to the reaction mixture and the solution was washed with 10% sodium thiosulfate, water and brine. Drying followed by evaporation and purification by silica gel chromatography (hexane : AcOEt = 3 : 1) gave the title compound (158 mg, 96.6%) as colorless needles, mp 84.5—85.5 °C, $[\alpha]_D^{20}$ −71.3° (c = 1.08, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.75—1.05 (2H, m), 1.05—1.30 (3H, m), 1.30—1.52 (3H, m), 1.52—1.90 (5H, m), 2.04 (3H, m), 3.45—3.58 (1H, m), 3.86 (1H, dd, J = 2.1, 4.8 Hz), 4.21 (1H, dd, J = 7.8, 11.7 Hz), 4.35 (1H, dd, J = 6.9, 11.7 Hz), 4.48 (1H, dt, J = 2.1, 7.1 Hz), 8.00 (1H, s). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{14}H_{23}INO_4$: 396.0672. Found: 396.0675.

(4*S*,5*R*)-4-Cyclohexylmethyl-5-[(*S*)-oxiran-2-yl]oxazolidin-2-one (6) Sodium methoxide (271 mg, 4.76 mmol) ws added to a solution of the iodide **5** (171 mg, 0.43 mmol) in THF (4 ml) at 0 °C. The mixture was stirred at 0 °C for 50 min, then another portion of sodium methoxide (123 mg, 2.16 mmol) was added at 0 °C. Stirring was continued at 0 °C for 1 h, then AcOEt was added, and the resulting solution was washed with water and brine. Drying followed by evaporation and purification by silica gel chromatography (hexane : AcOEt = 6 : 1) gave the title compound (94.2 mg, 96.7%) as a white solid, mp 88.0—90.5 °C, $[\alpha]_D^{20}$ −51.9° (c = 0.98, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.85—1.08 (2H, m), 1.08—1.40 (4H, m), 1.40—1.55 (2H, m), 1.60—1.82 (5H, m), 2.70 (1H, dd, J = 2.4, 4.8 Hz), 2.91 (1H, dd, J = 3.9, 4.8 Hz), 3.16 (1H, ddd, J = 2.4, 3.9, 5.4 Hz), 3.78—3.88 (1H, m), 3.93 (1H, t, J = 5.4 Hz), 5.30 (1H, s). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{12}H_{20}NO_3$: 226.1443. Found 226.1433.

(4*S*,5*R*)-4-Cyclohexylmethyl-5-[(*S*)-(1-hydroxy-2-morpholino-4-yl)-ethyl]oxazolidin-2-one (8) Morpholine (0.2 ml) was added to a solution of the epoxide **6** (56 mg, 0.25 mmol) in EtOH (2 ml) and the solution was refluxed for 1 h. Evaporation and purification by silica gel chromatography ($CHCl_3$: MeOH = 20 : 1) gave the title compound (124.0 mg, quantitatively) as colorless needles, mp 168.0—169.0 °C, $[\alpha]_D^{20}$ −76.1 °C (c = 1.03, $CHCl_3$) [lit.,[8b] mp 173—174 °C and $[\alpha]_D^{20}$ −79.8° (c = 0.694, $CHCl_3$)]. ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.07 (2H, m), 1.07—1.42 (5H, m), 1.42—1.80 (6H, m), 2.34—2.50 (3H, m), 2.56—2.70

(3H, m), 3.60—3.82 (5H, m), 3.88—4.00 (2H, m), 4.96 (1H, s). FAB-MS *m/z*: $[M+H]^+$ Calcd for $C_{16}H_{29}N_2O_4$: 313.2127. Found: 313.2121.

(2*S*,3*R*,4*S*)-4-Amino-5-cyclohexyl-1-morpholino-2,3-pentanediol (ACMP, 1) Lithium hydroxide hydrate (21 mg, 2.88 mmol) was added to a solution of the oxazolidine **8** (30 mg, 0.096 mmol) in 30% aqueous EtOH (2 ml) and the mixture was refluxed for 5 h. It was then neutralized with 6 N HCl, diluted with water to 10 ml, and charged on a column of ion exchange resin (IRC-50, NH_4^+ form, 3 ml). The resin was washed with water and eluted with 0.5 M ammonium hydroxide. The eluate was concentrated to give the title compound (27.6 mg, 99.7%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.10 (2H, m), 1.10—1.45 (5H, m), 1.42—1.60 (1H, m), 1.60—1.85 (5H, m), 2.45—2.65 (6H, m), 3.12—3.25 (1H, m), 3.41 (1H, dd, J=3.0, 7.2 Hz), 3.70 (4H, t, J=4.8 Hz), 3.80 (1H, dd, J=7.2, 14.1 Hz).

(4*S*,5*R*)-3-*tert*-Butoxycarbonyl-4-cyclohexylmethyl-5-[(*S*)-oxiran-2-yl]oxazolidin-2-one (7) Sodium methoxide (9.60 g, 178 mmol) was added to a solution of the iodide **5** (8.78 g, 22.2 mmol) at −20 °C. The temperature of the reaction mixture was allowed to rise slowly to 0 °C over 45 min. Saturated NH_4Cl was added to the mixture and extracted with AcOEt. The organic layer was washed with water and brine. Drying followed by evaporation gave the epoxide **6** as a white solid. Di-*tert*-butyl dicarbonate (10.2 ml, 44.4 mmol) and *N*,*N*-dimethyl-aminopyridine (270 mg, 2.21 mmol) were added to a solution of the epoxide **6** in acetonitrile (100 ml) and the mixture was stirred at room temperature for 30 min. Evaporation and purification by flash chromatography (hexane : AcOEt = 2 : 1 − 1 : 1) gave the title compound (6.28 g, 87% from **5**) as a pale yellow solid, mp 114.0—115.0 °C, $[\alpha]_D^{20}$ +17.4° (c=1.00, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.99 (2H, q, J=2.1 Hz), 1.53 (9H, s), 1.10—1.90 (11H, m), 2.70 (1H, dd, J=2.5, 3.8 Hz), 2.92 (1H, t, J=3.8 Hz), 3.11 (1H, ddd, J=2.5, 3.8, 5.9 Hz), 3.88 (1H, dd, J=2.8, 5.9 Hz), 4.16 (1H, dt, J=2.8, 10.2 Hz). FAB-MS *m/z*: $[M+H]^+$ Calcd for $C_{17}H_{28}NO_5$: 326.1967. Found: 326.1971.

(2*S*,3*R*,4*S*)-4-*tert*-Butoxycarbonylamino-5-cyclohexyl-1-morpholino-2,3-pentanediol(Boc-ACMP, 10) From **1**: Di-*tert*-butyl dicarbonate (304 mg, 1.39 mmol) and triethylamine (116 μl, 0.83 mmol) were added to a solution of ACMP (80 mg, 0.28 mmol) in CH_2Cl_2 (1 ml) and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with AcOEt and washed with saturated $NaHCO_3$, water and brine. Drying followed by evaporation and purification by silica gel chromatography ($CHCl_3$: MeOH = 100 : 1) gave Boc-ACMP (77.1 mg, 71.5%) as a colorless solid, mp 143.0—144.5 °C, $[\alpha]_D^{20}$ −14.8° (c=0.96, $CHCl_3$) [lit.,[7] mp 143.5—145.0 °C and $[\alpha]_D^{20}$ −15.8° (c=1.07, $CHCl_3$)]. ^{1}H-NMR ($CDCl_3$) δ: 0.78—1.05 (2H, m), 1.05—1.90 (11H, m), 1.46 (9H, m), 2.38—2.55 (2H, m), 2.55—2.85 (4H, m), 3.38—3.52 (2H, m), 3.58—3.78 (4H, m), 3.97 (1H, dt, J=5.1, 14.4 Hz), 4.65 (1H, d, J=14.4 Hz). FAB-MS *m/z*: $[M+H]^+$ Calcd for $C_{20}H_{39}N_2O_5$: 387.2859. Found: 387.2853.

From **7**: Morpholine (30 ml, 343 mmol) was added to a solution of the epoxide **7** (6.17 g, 19.0 mmol) in EtOH (90 ml) and the mixture was stirred at room temperature for 2 h. Evaporation and purification by flash chromatography (hexane : AcOEt = 1 : 1—AcOEt only) gave the crude *N*-Boc-oxazolidine **9** (6.91 g) as a pale brown solid. ^{1}H-NMR ($CDCl_3$) δ: 0.83—1.40 (7H, m), 1.52 (9H, s), 1.58—1.75 (5H, m), 1.87 (1H, br d, J=12.7 Hz), 2.35—2.64 (6H, m), 3.60—3.76 (5H, m), 3.90 (1H, dd, J=1.8, 7.7 Hz), 4.32 (1H, dt, J=2.7, 9.7 Hz). FAB-MS *m/z*: $[M+H]^+$ Calcd for $C_{21}H_{37}N_2O_6$: 413.2652. Found: 413.2663. A 1 N lithium hydroxide solution (60 ml) was added to a solution of compound **9** in dioxane (100 ml) at 0 °C and the mixture was stirred at the same temperature for 45 min. Saturated NH_4Cl was added, and the mixture was extracted with AcOEt. The organic layer was washed with brine. Drying followed by evaporation and recrystallization (hexane–AcOEt) gave Boc-ACMP (4.88 g, 69.3%) as colorless needles. This ^{1}H-NMR spectrum was identical to that described above. The purification of the mother liquid by flash chromatography (hexane : AcOEt = 1 : 1—AcOEt only)gave Boc-ACMP (280 mg, 4%) as a pale yellow solid.

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