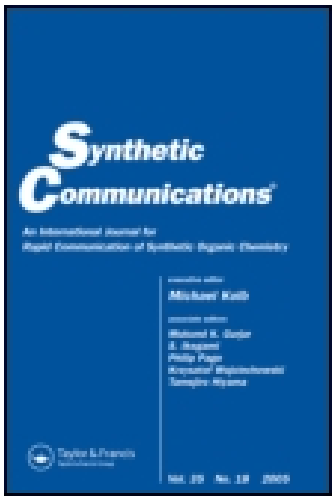

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A Concise, High Yield Synthesis of the Selective ECE-Inhibitor, CGS 35066

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A Concise, High Yield Synthesis of the Selective ECE-Inhibitor, CGS 35066

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ABSTRACT

A highly efficient synthesis of the selective endothelin-converting enzyme, CGS 35066 is described. The key steps involved a Pd-catalyzed coupling of the phenyl rings of a diphenyl ether, and the use of the Schöllkopf reagent, (2*R*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine as a chiral auxiliary furnishing a dibenzofuranylmethyl substituted amino ester in high (>98%) enantiomeric purity, which was then carried forward to complete the synthesis of the ECE inhibitor CGS 35066.

Key Words: ECE Inhibitor; Endotheline-1; CGS 35066; Dibenzofuran.

The highly potent vasoconstrictor peptide, Endotheline-1 (ET-1) has been the subject of extensive studies with regard to its biological significance as

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well as its use as a pharmacological and therapeutic target.[1] Thus, its implication in a variety of disease states, such as systemic and pulmonary hypertension, asthma and cardiac, and renal failure, among others, has led to the development of peptidic and nonpeptidic ET receptor antagonists, some of which, such as SB 209670 are now in the final stages of clinical development.[2]

Another therapeutic strategy to block the action of ET-1 appears to be to develop inhibitors of its biogenesis. Specifically, a zinc metalloprotease, endothelin-converting enzyme-1 (ECE-1) that is responsible for processing the inactive big ET to mature ET-1 has emerged as a promising therapeutic target for selective inhibition.[3] Recently several inhibitors of ECE-1 have been developed, some of which show relatively non-selective action against other metalloproteases such as the neutral endopeptidase 24.11 (NEP). While a number of these inhibitors show dual ECE-1/NEP inhibitory activity,[4] the compound CGS 35066 was developed as a nonpeptidic ECE-selective inhibitor, which showed an IC_{50} of 22 nM against recombinant human ECE-1. A report describing the synthesis of this compound using alcalase-mediated chiral resolution of an advanced intermediate has appeared.[5] We now report, herein, a new concise, high yield synthesis of enatiomerically pure CGS 35066 (VIII, Sch. 1).

The synthesis (Sch. 1) of CGS 35066 started with 3-phenoxybenzyl alcohol **1** (Aldrich). Acetylation of **1** (Ac_2O, pyridine, 0°C), followed by Pd-catalyzed ring closure[6] of the acetate **II** to a dibenzofuran nucleus proceeded in 63.5% yield over two steps furnishing the dibenzofuran-3-ylmethyl acetic acid ester **III**. The direct ring closure of **1** resulted unwanted oxidation at the benzylic position. After removal of the acetyl group, the dibenzofuranyl-methyl alcohol **IV** was readily converted to a benzylic bromide **V** (45% HBr/AcOH, $CHCl_3$, r.t., 1 hr) in quantitative yield. The bromide **V** was immediately coupled with the anion derived from the Schöllkopf reagent,[7] (2*R*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine (*n*-butyllithium, THF, −90°C). This chiral auxiliary gave the product essentially in quantitative yield of **VI** with >98% ee as indicated by a careful analysis of a multiply eluted TLC (which indicated a single spot) as well as by analyzing ^{1}H NMR of the crude product. Comparison of the ^{1}H NMR of the compound **VI** with those of some other related materials[8] revealed the presence of clear, at least half a ppm apart signals for the protons at the dihydropyrazine α-carbon atoms of their corresponding diastereomeric mixtures. The unusually low temperature (−90°C) was found to be necessary for the observed outcome in enantiomeric excess, as raising temperature to −78°C resulted in significant decrease in the chiral induction.

The chiral auxiliary from the couple product **VI** was successfully cleaved off, and the resulting aminoester product **VII** was isolated as a TFA-salt by

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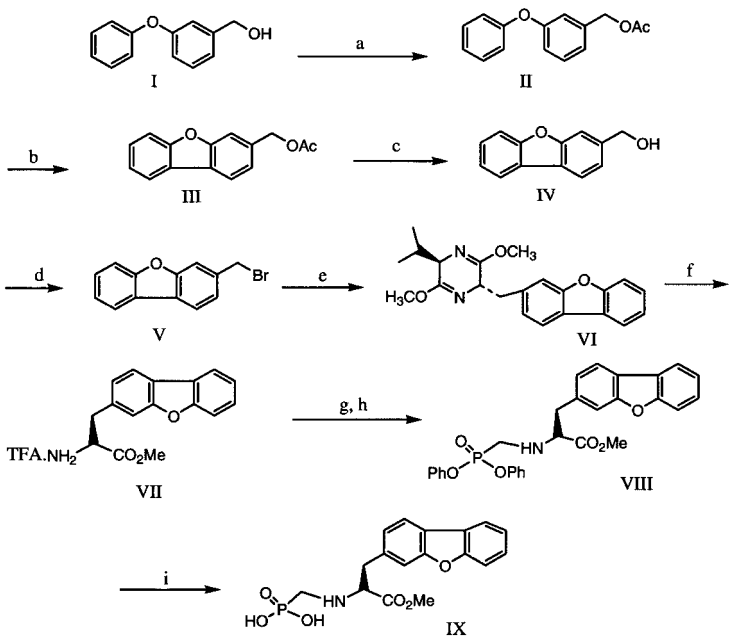

Scheme 1. a. Ac_2O, DMAP, pyridine, overnight, 100%; b. Pd $(OAc)_2$, AcOH, 130°C, 19 hr, 63.5%; c. IM LiOH, MeOH, r.t., 30 min. 94.1%; d. 45% HBr in AcOH, $CHC1_3$, r.t., 1 hr, >99%; e. (2*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine, *n*-BuLi, THF, −90°C, 1 hr, >99%; f. 0.15% TFA, acetonitrile, r.t. 5 hr, $NaHCO_3$; g. (i) Extraction from aq. $NaHCO_3$. (ii) 37% formalin, H_2O, EtOAc, $NaHCO_3$, r.t., 18 hr; h. $(PhO)_2POH$, toluene, 70°C, 2 hr, 87.7%; i. 9 N HCl, AcOH, 100°C, 2 hr, 95.5%.

extraction with dichloromethane (DCM). The extraction of the salt with DCM conveniently and effectively removed the contaminating TFA-salt of the valine methyl ester which remained behind in the aqueous phase during extractive work-up. Such extractions seem to be general for relatively bulky α-substituents of amino esters.[8] The free amine obtained from the methyl ester amine salt **VII** was then reacted with the aqueous formalin (ethyl acetate, $NaHCO_3$, 18 hr) to form a hexahydrotriazine intermediate which, without purification, was treated with diphenyl phosphite in toluene at 70°C to form the fully protected phosphonate **VIII** in 88% yield. The compound **VIII** has been shown to serve as a bioavailable form of the CGS 35066. Finally, strong acid hydrolytic deblocking of the diphenyl and methyl ester groups in **VIII** furnished the target compound CGS 35066 **IX** as a crystalline solid. The spectroscopic and optical activity data for **IX** was in complete agreement with the published data.[5] The enantiomeric purity of the final compound was

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confirmed by analyzing the ^{1}H NMR of the diastereomeric salt of **IX** with +(*R*)-1(1-naphthyl) ethylamine, and was found to be greater than 98%.

In summary, a concise and efficient synthesis of CGS 35066, a selective inhibitor of ECE-1, was achieved in high overall yield and enantiomeric purity.

EXPERIMENTAL SECTION

General

Unless otherwise stated, all reactions were carried out under argon or nitrogen atmosphere. Commercially available materials were used without purification. The dichloromethane was distilled over CaH_2. Other solvents, such as diethyl ether and tetrahydrofuran were dried by distillation over sodium benzophenone ketyl. NMR spectra were recorded over a Brüker Avance-300 instrument at 300 MHz for ^{1}H NMR, 75 MHz for ^{13}C NMR, and 121 MHz for ^{31}P NMR. Chemical shifts are given with tetramethylsilane as internal standard set at zero ppm (1H, and ^{13}C NMR). ^{31}P NMR are measured with 85% phosphoric acid taken as standard (^{31}P NMR). The mass spectra were measured by slow infusion electrospray ionization on a Sciex-365 Triple Quadrupole mass spectrometer. The Flash chromatography was performed using Kieselgel 60 (230–400 mesh) silica gel as described by Still.[9]

3-Phenoxybenzyl Acetate (II)

Pyridine (50 mL) was added to 3-phenoxybenzyl alcohol **I** (5.0 g, 25.0 mmol) and the resulting solution was stirred for 5 min under nitrogen. 4-*N*,*N*-dimethylaminopyridine (DMAP, 70 mg) was added and the mixture was cooled to 0°C. Gradual addition of acetic anhydride (7 mL, 75.0 mmol) was followed by stirring at ambient temperatures for 4 hr. The reaction was quenched by adding cold water (100 mL), and the excess acetic anhydride was destroyed by stirring the reaction mixture for 1 hr. Extraction with CH_2Cl_2 (200 mL × 3), was followed by washing the combined organic layers with saturated aq. $NaHCO_3$ and brine. Drying the organic phase with $MgSO_4$, and rotary evaporation gave **II** (6.01 g, 100%) which needed no purification. ^{1}H NMR ($CDCl_3$) δ2.09 (3H, s), 5.06 (2H, s), 6.94 (1H, dd, $J =$) 7.02 (3H, dd), 7.1 (2H, m), 7.34 (2H, m).

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Dibenzofuran-3-yl-methyl Acetic Acid Ester (III)

A mixture of the compound II (5.62 g, 23.22 mmol) and $Pd(OAc)_2$ (7.82 g, 34.8 mmol) in 55 mL of AcOH was refluxed overnight. After cooling to room temperature, the reaction mixture was filtered through Celite. Concentration by rotary evaporation and removal of the traces of acetic acid with toluene gave a residue from which the product isolated by Flash chromatography using 3% EtAc/Hexane. The product containing fractions were combined to give **III** as a white solid (3.54 g, 63.5%). ^{1}H NMR ($CDCl_3$) δ2.13 (3H, s), 5.26 (2H, s), 7.35 (2H, m), 7.46 (1H, td, $J = 8.1$, 1.4 Hz), 7.58 (2H, m) 7.93 (1H, d, $J = 7.8$ Hz). 7.95 (1H, md, $J = 7.5$ Hz), ESI Mass 504 $(2M + 1)^+$, 362 $(M - OAc + 1)^+$, 263 $(M + Na)^+$, 182 $(M - OAc + 1)^+$.

Dibenzofuran-3-yl-methanol (IV)

The ester III (2.27 g, 9.46 mmol) was dissolved in 23 mL MeOH. To this was added 23 mL of 1 N-LiOH solution at r.t. The mixture was left stirred for 30 min. The reaction was quenched with 1 N-HCl and the aqueous phase was extracted twice with 100 mL of CH_2Cl_2. The organic phase was washed sequentially with 50 mL of water, and brine, and dried over $MgSO_4$, affording **IV** as a white solid (1.76 g, 94.1%). ^{1}H NMR ($CDCl_3$) δ1.83 (1H, t, $J = 5.7$ Hz), 4.84 (2H, d, $J = 5.7$ Hz), 7.35 (2H, m) 7.45 (1H, td, 8.0, 1.4 Hz), 7.58 (2H, m), 7.95 (2H, m); IR 3294, 1454, 1416 cm^{-1}.

3-Bromomethyl Dibezofuran (V)

To a stirred solution of compound IV (1.6 g, 8.08 mmol) in 30 mL of $CHCl_3$ were added 2.18 mL of glacial acetic acid (12.12 mmol) and 45% HBr (12.12 mmol). The acidic mixture was stirred under nitrogen at r.t for 1 hr, and then quenched with 30 mL cold water. Extraction of the aqueous phase with 50 mL of $CHCl_3$, and washing of the chloroform layer sequentially with 25 mL of aq. $NaHCO_3$, water, and brine was followed by drying it over $MgSO_4$. Evaporation of the organic solvent under vacuum furnished essentially pure **V** (2.1 g, 100%) as a white solid. ^{1}H NMR ($CDCl_3$) δ4.66 (2H, s) 7.35 (2H, m), 7.46 (1H, td, $J = 8.0$, 1.4 Hz), 7.59 (2H, m), 7.91 (2H, m).

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2-Dibenzofuran-3-ylmethyl-5-iisopropyl-3,6-dimethoxy-2,5-dihydro-pyridine (VI)

A solution of (2*R*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine (0.67 mL, 3.69 mmol) in 10 mL of dry THF was placed in an oven-dried flask under argon, and cooled to −78°C. While stirring, 2.5 M BuLi (1.5 mL, 3.69 mmol) in hexane was added via syringe. After stirring for 10 min at −78°C, the reaction mixture was further cooled to −90°C. At this point, compound **V** (803 mg, 3.08 mmol) dissolved in 5 mL of THF was added dropwise via cannula. The mixture was stirred between −90°C to −70°C for 1 hr, and then quenched with saturated NH_4Cl. After warming to r.t., the volatiles were removed *in vacuo*, and the aqueous phase was extracted twice with EtOAc. The EtOAc extracts were combined and washed with water, and brine, and dried ($MgSO_4$). Concentration *in vacuo*, and purification of the residue by silica gel chromatography using 5% EtOAc/hexane as eluent afforded pure **VI** as a solid (1.12 g, 100%). ^{1}H NMR ($CDCl_3$) δ0.61 (3H, d, $J = 6.9$ Hz), 0.75 (3H, d, $J = 6.9$ Hz), 2.14 (1H, m), 3.21 (2H, d, $J = 4.8$ Hz), 3.33 (1H, t, $J = 3.6$ Hz), 3.70 (3H, s), 3.75 (3H, s), 4.39 (1H, q, $J = 7.8$ Hz), 7.1 (1H, dd, $J = 7.8$, 1.2 Hz), 7.28 (2H, m), 7.41 (1H, td, $J = 7.2$, 1.2 Hz), 7.54 (2H, m), 7.78 (1H, m), 7.9 (1H, d, $J = 7.5$ Hz); ESI-MS 366 $(M + 1)^+$.

2-Amino-3-dibenzofuran-3-yl-propionic Acid Methyl Ester (VII)

To a solution of the compound **VI** (1.12 g, 3.07 mmol) in 30 mL of acetonitrile was added 8.0 mL (9.21 mmol) of aq. 0.15 N TFA solution. The resulting clear solution was stirred under nitrogen for 5 hr. Water (50 mL) and CH_2Cl_2 (100 mL) were added. The CH_2Cl_2-layer was separated. The aqueous phase was back extracted with more CH_2Cl_2 (×4), and the combined organic layers were dried (Na_2SO_4). The ^{1}H NMR of this TFA salt showed complete absence of the contaminating valine methyl ester. The TFA-salt was then neutralized with aqueous $NaHCO_3$ and re-extracted with CH_2Cl_2 (×3). The combined organic extracts were washed with water and brine, and then dried over Na_2SO_4. Concentration of the organic solvent yielded **VII** (776 mg, 94.0%) as a colorless oil which was used in the next step without purification. ^{1}H NMR ($CDCl_3$) δ3.03 (1H, dd, $J = 13.5$, 7.8 Hz), 3.23 (1H, dd, $J = 13.5$, 5.1 Hz), 3.73 (3H, s), 3.83 (1H, dd, $J = 7.8$, 5.8 Hz), 7.16 (1H, dd, $J = 7.8$, 1.2 Hz), 7.32 (2H, td, $J = 7.5$, 1.0 Hz), 7.45 (2H, m), 7.55 (1H, d, $J = 8.1$ Hz), δ7.83 (1H, d, $J = 7.8$ Hz), 7.92 (1H, din, $J = 7.2$ Hz); ESI-MS 540 $(2M + 1)^+$, 271 $(M + 1)^+$.

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(*S*)-3-Dibenzofuran-3-yl-2-[phosphonomethylamino]-propionic Acid Methyl Ester (VIII)

Compound **VII** (211 mg, 0.756 mmol) was dissolved in 4 mL of a 1 : 1 mixture of EtOAc and H_2O along with powdered $NaHCO_3$ (7.6 mg, 0.10 mmol). After cooling this mixture to 12°C, 37% of aqueous formaldehyde (76 μl, 1 mmol) was added. The reaction mixture was left stirred overnight at ambient temperature. Addition of water was followed by extraction of the aqueous phase with two 20 mL portions of CH_2CL_2. The organic phase was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to leave the intermediate hexahydrotriazine as a solid residue which was characterized by NMR. ^{1}H NMR ($CDCl_3$) δ 2.98 (2H, dd, $J = 7.2$, 3.3 Hz), 3.58 (3H, s), 3.75 (1H, m), 3.85 (2H, s), 6.91 (1H, dd, $J = 8.1$, 1.2 Hz), 7.25 (2H, m), 7.39 (1H, dt, $J = 7.5$, 1.2 Hz), 7.49 (1H, m), 7.54 (1H, m), 7.72 (1H, m).

To a stirred solution of the hexahydrotriazine in toluene under nitrogen was added diphenyl phosphite. The resulting solution was left stirred at 70°C for 2 hr, cooled to r.t., and partially concentrated to half of the initial volume. Addition of hexane to this solution resulted in precipitation of the crude product. After filtration and washing with ether and hexane, the solid residue was dried under high vacuum. Yield 251 mg, 87.7%. ^{1}H NMR ($CDCl_3$) δ 3.15 (3H, m), 3.41 (1H, dd, $J = 15.0$, 10.5 Hz), 3.70 (3H, s), 3.79 (1H, t, $J = 7.2$ Hz), 7.08–7.17 (8H, m), 7.19–7.28 (4H, m), 7.35 (1H, td, $J = 7.3$, 1.2 Hz), 7.45 (2H, m), 7.54 (1H, m), 7.87 (1H, d, $J = 7.8$ Hz), 7.91 (1H, d, $J = 7.5$ Hz); ESJ-MS 538 $(M + Na)^+$, 516 $(M + 1)^+$.

(*S*)-3-Dibenzofuran-3-yl-2-[phosphonomethylamino]-propionic Acid (IX)

Compound **VIII** (85 mg, 0.17 mmol) was suspended in a solution of AcOH (1.0 mL) and 9 N HCl (3 mL) at r.t. The mixture was heated at 100°C for 2 hr, then cooled by adding ice cold water (4 mL). The solid thus produced was filtered and washed sequentially with water and ether. The residue (57.12 mg, 0.68 mmol) was by neutralized with an aqueous solution of $NaHCO_3$, and the aqueous phase extracted with ethyl acetate. The aqueous phase was neutralized with by dropwise addition of 2 N HCl. A white precipitate formed which was washed with 3 × 15 mL of water. The residue was suspended in 10 mL of water and heated at 70°C 1 hr, and then cooled and filtered. After washing with cold water (10 mL), the white solid was dried under high vacuum overnight. The crude **IX** was judged to be pure by optical and spectroscopic analysis. Yield = 55 mg, 95.5%. 1H NMR (DMSO-d_6,

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TFA) δ3.35 (3H, m), 3.55 (1H, m), 4.46 (1H, m), 7.35 (1H, d, $J = 7.8$ Hz), 7.43 (1H, t, $J = 7.5$ Hz), 7.55 (1H, t, $J = 7.8$ Hz), 7.70 (3H, m), 8.12 (2H, m); ESI-MS (neg. mode) 360 (M $-$ 1); $[\alpha]_D^{25}$ (trisodium salt) $+20.4^\circ$, c 2.35 mg/mL, pH 12. The diastereomeric mixture with (*R*)(+)1(1-naphthyl)ethylamine in CD_3OD showed a singlet at 3.72 ppm, indicating at least 98% ee; ^{31}P NMR (242.94 MHz, DMSO-d_6 + TFA, (1 : 1) δ11.30 (t, $J = 14$ Hz).

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