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Synthetic Approaches to Natural Antioxidant Benzastatin E, F and G Analogues

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For synthesis of benzastatin E, F and G analogues, the indole-2-carbaldehydes with or without substituents at C-5 position were prepared as key intermediates. Several synthetic attempts to achieve benzastatin E-G analogues were suggested using the indole-2-carbaldehyde intermediates.

Key Words : Benzastatin, Antioxidant, Inhibitor of glutamate toxicity, Indole-2-carbaldehyde

Introduction

Glutamate, the major neurotransmitter in the central nervous system, is widely released during brain ischemia, and applying free radical scavengers to inhibit glutamate toxicity leads to the reduction of brain ischemia injury.[1-4] Moreover, the reported involvement of lipid peroxidation in ischemic events highlights the importance of antioxidants for the treatment of ischemic injury.[2,3]

Kim *et al*. carried out screening studies to identify free radical scavengers or inhibitors of glutamate toxicity in neuronal hydridoma N18-RE-105 cells. They first isolated benzastatin A, B, C and D from the culture broth of *Streptomyces nitrosporeus* 30643; benzastatin A and B contain a *p*-aminobenzamide unit while benzastatin C and D have a tetrahydroquinoline ring system (Figure 1).[5,6] They later indentified benzastatin E, F and G which have an indoline skeleton.[7] Further investigation on metabolites of *Streptomyces nitrosporeus* 30643 resulted in the isolation of two hydroxylated derivatives of benzastain B: benzastatin H and I.[8] Benzastatins show inhibitory activity against glutamate toxicity and lipid peroxidation in rat liver microsomes that can be used to prevent brain ischemia injury.[5,7,8] Among the benzastatin family, benzastatin E is the most potent inhibitor against glutamate toxicity and shows strong antioxidant activity; these activities of benzastatin E are comparable to those of idebenone, a well-known brain protective agent, but benzastatin E has lower cytotoxicity than idebenone.[7]

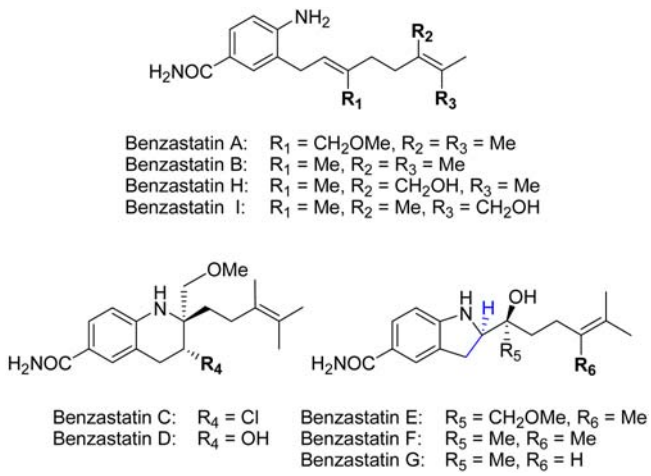

Figure 1. Chemical structures of benzastatin A, B, C, D, E, F, G, H and I.

Despite its potentials of being therapeutic agents, to the best of our knowledge, there are little reports on the synthesis of benzastatins. Only total synthesis of (+)-benzastatin E was described using commercially available (*S*)-2-indolinecarboxylic acid as starting material.[9,10] To study structure-activity relationship of benzastatins, various benzastatin E, F and G analogues are highly desirable. Therefore, we tried to develop the synthetic approaches for their analogues containing indole skeleton instead of indoline skeleton. Herein, synthetic methods for indole skeleton *via* cyclization of ethyl 3-(2-nitrophenyl)-2-propenoate with or without substituent at C-5 position and our attempts to prepare the alkene side chain of benzastatin E-G analogues are reported.

Result and Discussion

Although benzastatin E, F and G analogues with indole scaffold would have different substituents at the chiral carbon center (i.e. methyl group in benzastatin F and G, and methoxymethyl group in benzastatin E), these compounds (i) can be prepared by Grignard reaction of the corresponding ketones (ii) which are obtained by oxidation of the resulting alcohols (iii) from reaction of initial aldehydes (iv) and Grignard reagents (Scheme 1).

The key intermediates for benzastatin E-G analogues, indole-2-carbaldehyde and 5-methyl-indole-2-carbaldehyde, were prepared by the procedure shown in Scheme 2. Wittig reaction of *o*-nitrobenzaldehyde **1a** with carbethoxymethylene triphenylphosphorane in benzene gave (*E*)-cinnamate ester **2a**, which was later heated under reflux in triethylphosphite to yield the indole-2-carboxylate **3a**.[11] Protection with 4-methoxybenzyl chloride followed by reduction using lithium aluminium hydride and further oxidation using

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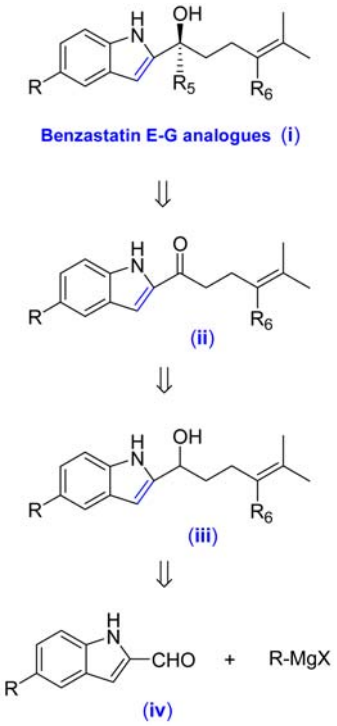
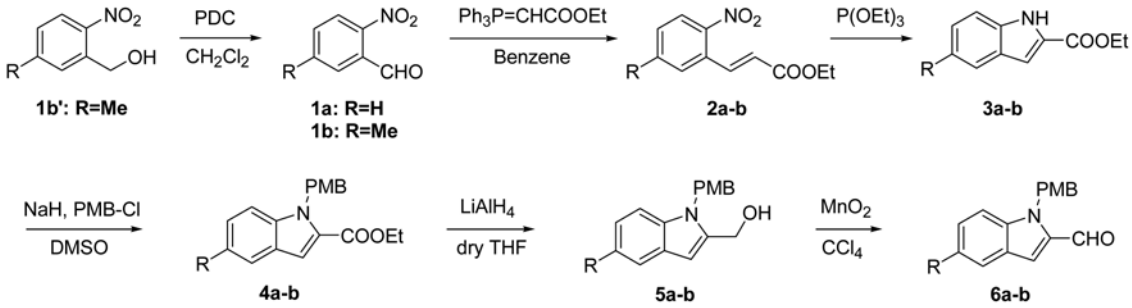


Scheme 1. Retrosynthesis of benzastatin E, F, and G analogues possessing indole skeleton.

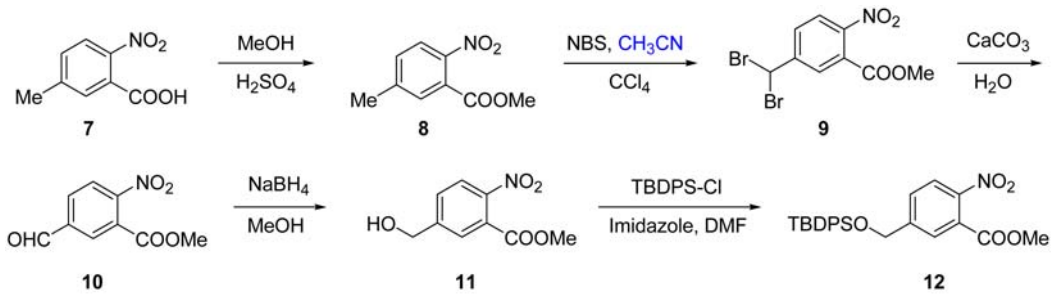
manganese dioxide afforded the desired indole-2-carbaldehyde **6a** in 49% overall yield. The same procedure was applied to synthesize 5-methyl-indole-2-carbaldehyde **6b** in 43% overall yield, and the 5-methyl-2-nitrobenzaldehyde **1b** was obtained by oxidation of 5-methyl-2-nitrobenzyl alcohol **1b'** with PDC. The methyl group introduced on C-5 position of indole-2-carbaldehyde can be easily converted to an amide group for synthesis of benzastatin E-G analogues.[12,13]

In addition, introduction of different functional groups at C-5 position of indole-2-carbaldehyde was attempted (Scheme 3). Esterification of commercially available 5-methyl-2-nitro benzoic acid **7** with methyl alcohol followed by bromination gave the dibromo compound **9**, which was later treated with $CaCO_3$ to yield the corresponding aldehyde **10**. Selective reduction of the aldehyde and further protection of the resulting alcohol with *tert*-butylchlorodiphenylsilane (TBDPS-Cl) afforded the protected ester **12**. This ester group can be converted to the corresponding aldehyde, and the deprotection of TBDPS and further application of Mitsunobu reaction using various alcohols would afford a variety of 5-substituted indole-2-carbaldehydes.

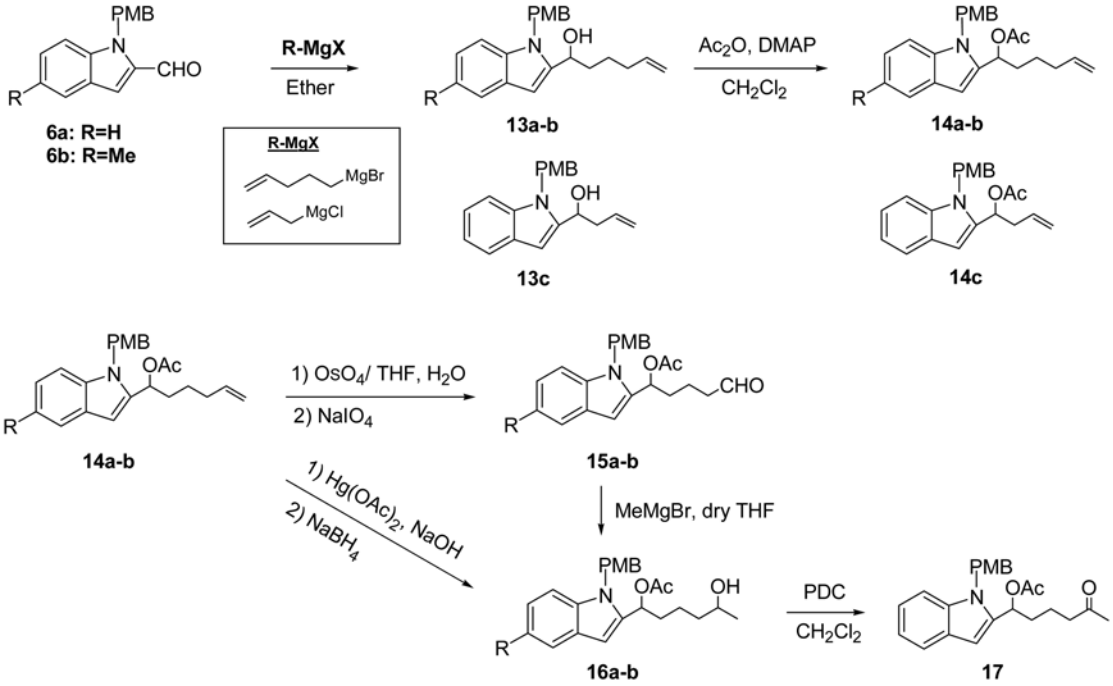
Next, several synthetic approaches to attach side chains of benzastatin E and F analogues and benzastatin G analogues on the prepared key intermediates were carried out as shown in Schemes 4 and 5, respectively. In Scheme 4, the indole-2-carbaldehydes **6a-b** were treated with Grignard reagents and then protection of the hydroxyl group using acetic anhydride afforded the resulting acetylated compounds **14a-c**. Either oxidative cleavage of the olefin by OsO_4 and $NaIO_4$ and further treatment of Grignard reagent or oxymercuration-deoxymercuration reaction of the olefin with $Hg(OAc)_2$ and $NaBH_4$ provided the resulting alcohol **16a-b**. The alcohol **16a** was oxidized using PDC to yield the resulting ketone **17**, which can be available for Wittig reaction for benzastatin E and F analogues after replacement of the acetyl group with another protecting group that is stable in strong basic condition. As an alternative route, the acetylated compound **14c** can be transformed to the benzastatin analogues through acetalization,[14] hydrolysis,[15] and Wittig reaction. Both routes



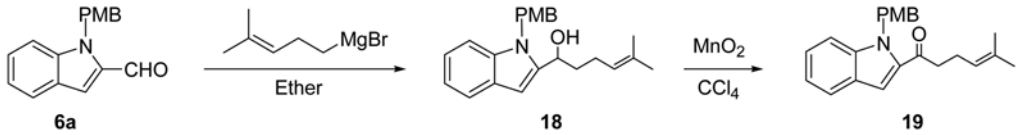
Scheme 2. Synthesis of indole-2-carbaldehydes as key intermediates for benzastatin E-G analogues.



Scheme 3. Synthetic approach for different 5-substituted-indole-2-carbaldehydes as key intermediates for benzastatin E-G analogues.



Scheme 4. Several synthetic attempts for benzastatin E and F analogues.



Scheme 5. Synthetic approach for benzastatin G analogues.

would involve further Grignard reaction with the corresponding ketones as the last step to achieve the desired benzastatin E and F analogues as racemates.

For synthesis of benzastatin G analogues, coupling reaction of indole-2-carbaldehyde **6a** with Grignard reagent followed by oxidation reaction provided the resulting ketone **19** (Scheme 5). Further Grignard reaction to the ketone can give the desired benzastatin G analogues as racemates.

Experimental

Chemical reagents were purchased from Aldrich Chemical Co. and used without further purification. Solvents were distilled prior to use, but THF and ether were distilled from sodium benzophenone ketyl. Melting points were determined by the capillary method on Electrothermal IA9200 digital melting point apparatus and were uncorrected. Nuclear magnetic resonance (NMR) data for ^{1}H NMR were taken on Bruker AC80 and Varian Unity 300 plus spectrometer and were reported in ppm, downfield from the peak of tetramethylsilane as an internal standard. The data are reported as follows: chemical shift, multiplicity (s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, b: broadened), and number of proton. IR spectra were recorded on JASCO-FT IR spectrometer using $CHCl_3$ and KBr pellets. Mass spectra were obtained on JEOL JNS-DX 303 by the electron-impact (EI) method. Column chromatography was performed on Merck silica gel 60 (70-230 mesh). TLC was carried out using plates coated with silica gel 60 F254 purchased from Merck.

General Procedure for the Synthesis of 3-(2-nitro-phenyl)-acrylic Acid Ethyl Ester (2a-b). A mixture of *o*-nitrobenzaldehyde **1** (11 g, 70 mmol) and (carbethoxymethylene)triphenylphosphorane (25 g, 70 mmol) in benzene (100 mL) was refluxed for 2 hours. The solvent was removed and the resulting residue was separated by column chromatography on silica gel using *n*-hexane-ethyl acetate (4:1) to give the product **2a-b**. The physical and spectral data of the compounds are as following.

3-(2-Nitro-phenyl)-acrylic Acid Ethyl Ester (2a): Yield: 90% (yellow oil); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10 (d, J = 15.8 Hz, 1H), 8.03 (d, J = 7.8 Hz, 1H), 7.68-7.53 (m, 3H), 6.38 (d, J = 15.8 Hz, 1H), 4.29 (q, J = 7.1 Hz, 2H), 1.35 (t, J = 7.1 Hz, 3H).

3-(5-Methyl-2-nitro-phenyl)-acrylic Acid Ethyl Ester (2b): Yield: 90% (yellow oil); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (d, J = 15.8 Hz, 1H), 7.97 (d, J = 8.3 Hz, 1H), 7.41 (s, 1H), 7.32 (d, J = 8.3 Hz, 1H), 6.33 (d, J = 15.8 Hz, 1H), 4.28 (q, J = 7.1 Hz, 3H), 2.47 (s, 3H), 1.35 (t, J = 7.1 Hz, 3H).

General Procedure for the Synthesis of 1*H*-indole-2-Carboxylic Acid Ethyl Ester (3a-b). The compound **2a** (21.2 g, 96 mmol) was dissolved in triethyl phosphate (79.6

g, 480 mmol) and the reaction mixture was refluxed for 3 hours. Excess triethyl phosphate was removed under reduced pressure. The resulting residue was purified by column chromatography on silica gel with *n*-hexane-ethyl acetate (8:1) to give the product **3a-b**. The physical and spectral data of the compounds are as following.

1*H*-Indole-2-carboxylic Acid Ethyl Ester (3a): Yield: 86% (yellow solid); mp 121-123 °C; IR (KBr) cm^{-1}: 3310 cm^{-1} (NH), 1685 cm^{-1} (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.29 (s, 1H), 7.68 (d, J = 9.0 Hz, 1H), 7.44-7.24 (m, 2H), 7.24 (s, 1H), 7.14 (t, J = 7.2 Hz, 1H), 4.42 (q, J = 7.2 Hz, 2H), 1.42 (t, J = 7.2 Hz, 3H); MS, m/e (%): 143 (99.9%), 115 (42.5%), 189 (41.5%).

5-Methyl-1*H*-indole-2-carboxylic Acid Ethyl Ester (3b): Yield: 88% (yellow solid); mp 159-160.7 °C; IR (KBr) cm^{-1}: 3320 cm^{-1} (NH), 1685 cm^{-1} (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44-7.11 (m, 4H), 4.40 (q, J = 7.1 Hz, 2H), 2.42 (s, 3H), 1.40 (t, J = 7.1 Hz, 3H).

General Procedure for the Synthesis of 1-(4-Methoxy-benzyl)-1*H*-indole-2-carboxylic Acid Ethyl Ester (4a-b). Reaction mixture of compound **3a** (6.4 g, 24 mmol) and NaH (60% dispersion in mineral oil, 1.5 g, 40 mmol) in DMSO was stirred for 6 hours. Then, 4-methoxybenzyl chloride (5 g, 32 mmol) was added, and the reaction mixture was stirred at room temperature for 24 hours. After reaction completion, water was added and the mixture was extracted with ethyl ether. The organic layers were washed with water and brine, dried and concentrated. The resulting residue was purified by column chromatography on silica gel with *n*-hexane-ethyl acetate (8:1) to give the product **4a-b**. The physical and spectral data of the compounds are as following.

1-(4-Methoxy-benzyl)-1*H*-indole-2-carboxylic Acid Ethyl Ester (4a): Yield: 92% (yellow oil); IR (KBr) cm^{-1}: 2980 cm^{-1} (C-H), 1705 cm^{-1} (C=O), 1300-1000 cm^{-1} (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.66-6.69 (m, 9H), 5.70 (s, 2H), 4.28 (q, J = 7.2 Hz, 2H), 3.62 (s, 3H), 1.30 (t, J = 7.2 Hz, 3H).

1-(4-Methoxy-benzyl)-5-methyl-1*H*-indole-2-carboxylic acid ethyl ester (4b): Yield: 90% (yellow solid); mp 68.8-70.7 °C; IR (KBr) cm^{-1}: 2980 cm^{-1} (C-H), 1705 cm^{-1} (C=O), 1300-1000 cm^{-1} (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.46-6.74 (m, 8H), 5.74 (s, 2H), 4.31 (q, J = 7.1 Hz, 2H), 3.72 (s, 3H), 2.43 (s, 3H), 1.36 (t, J = 7.1 Hz, 3H).

General Procedure for the Synthesis of [1-(4-Methoxy-benzyl)-1*H*-indol-2-yl]-methanol (5a-b). Compound **4a** (6.2 g, 20 mmol) in dry THF was added into the reaction mixture of $LiAlH_4$ (1.1 g, 30 mmol) in THF at 0 °C, and stirred for additional 2 hours. The reaction was quenched by adding water, and the mixture was filtered through Celite. The filtrate was extracted with CH_2Cl_2. The organic portions were washed with water and brine, dried and concentrated. The residue was purified by column chromatography on silica gel with *n*-hexane-ethyl acetate (4:1) to give the product **5a-b**. The physical and spectral data of the compounds are as following.

[1-(4-Methoxy-benzyl)-1*H*-indol-2-yl]-methanol (5a): Yield: 85% (white solid); mp 78.9-80.4 °C; IR (KBr) cm^{-1}: 3360 cm^{-1} (OH), 2960 cm^{-1} (C-H), 1240 cm^{-1} (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60-6.45 (m, 9H), 5.30 (s, 2H), 4.62 (s, 2H), 3.68 (s, 3H), 1.96 (b, 1H).

[1-(4-Methoxy-benzyl)-5-methyl-1*H*-indol-2-yl]-methanol (5b): Yield: 87% (white solid); mp 110-112.6 °C; IR (KBr) cm^{-1}: 3360 cm^{-1} (OH), 2960 cm^{-1} (C-H), 1250 cm^{-1} (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39 (s, 1H), 7.14 (d, J = 8.4 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 6.92 (m, 2H), 6.77 (m, 2H), 6.43 (s, 1H), 5.36 (s, 2H), 4.69 (d, 2H), 3.73 (s, 3H), 2.43 (s, 3H).

General Procedure for the Synthesis of 1-(4-Methoxy-benzyl)-1*H*-indole-2-carbaldehyde (6a-b, 19). The reaction mixture of the compound **5a** (4.5 g, 17 mmol) and activated manganese dioxide (15.3 g, 255 mmol) in carbon tetrachloride was stirred at room temperature for 10 hours. The mixture was filtered and the filtrate was concentrated. The crude product was purified by column chromatography on silica gel with *n*-hexane-ethyl acetate (6:1) to give the product **6a-b**. The physical and spectral data of the compounds are as following.

1-(4-Methoxy-benzyl)-1*H*-indole-2-carbaldehyde (6a): Yield: 83% (yellow solid); mp 92.8-93.9 °C; IR (KBr) cm^{-1}: 1675 cm^{-1} (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.87 (s, 1H), 7.74-6.73 (m, 9H), 5.73 (s, 2H), 3.68 (s, 3H); MS, m/e (%): 121 (100%), 265 (38.8%).

1-(4-Methoxy-benzyl)-5-methyl-1*H*-indole-2-carbaldehyde (6b): Yield: 78% (yellow solid); mp 90.4-91.7 °C; IR (KBr) cm^{-1}: 2980 cm^{-1} (C-H), 1750 cm^{-1} (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.86 (s, 1H), 7.51 (s, 1H), 7.50-6.75 (m, 7H), 5.72 (s, 2H), 3.72 (s, 3H), 2.43 (s, 3H).

1-[1-(4-Methoxy-benzyl)-1*H*-indol-2-yl]-5-methyl-hex-4-en-1-one (19): Yield: 70% (brown oil); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71 (d, J = 8.1 Hz, 1H), 7.36-7.31 (m, 3H), 7.14-6.74 (m, 5H), 5.76 (s, 2H), 5.12 (m, 1H), 3.70 (s, 3H), 2.96 (m, 2H), 2.39 (m, 2H), 1.67 (s, 3H), 1.60 (s, 3H).

5-Methyl-2-nitro-benzoic Acid Methyl Ester (8): The reaction mixture of 5-methyl-2-nitro-benzoic acid **7** (5.0 g, 27.6 mmol) in methanol (30 mL) with catalytic amount of sulfuric acid was refluxed for 24 hours. The $NaHCO_3$ solution was added to neutralize the acid, and the mixture was extracted with CH_2Cl_2. The organic extracts were washed with water and brine, dried and evaporated off. The resulting residue was purified by column chromatography on silica gel with *n*-hexane-ethyl acetate (4:1) to give product **8** as white solid (4.8 g, 90%). mp 77.3-78.8 °C; IR (KBr) cm^{-1}: 2980 cm^{-1} (C-H), 1720 cm^{-1} (C=O); ^{1}H NMR (80 MHz, $CDCl_3$): δ 7.85 (d, J = 8.0 Hz, 1H), 7.45 (s, 1H), 7.32 (d, J = 8.0 Hz, 1H), 3.91 (s, 3H), 2.46 (s, 3H).

5-Dibromomethyl-2-nitro-benzoic Acid Methyl Ester (9): To the stirred mixture of the compound **8** (1.9 g, 10 mmol) in carbon tetrachloride, *N*-bromosuccinimide (1.7 g, 10 mmol) and 1,1-azobis(cyclohexanecarbonitrile) were added under light, and the reaction mixture was refluxed for 24 hours. After reaction completion, the mixture was poured into water and extracted with CH_2Cl_2. The organic extracts were washed with water and brine, and concentrated. The

resulting residue was purified by column chromatography on silica gel with *n*-hexane-ethyl acetate (8:1) to give product **9** as orange oil (1.8 g, 52%). ^{1}H NMR (80 MHz, $CDCl_3$): δ 7.91-7.85 (m, 3H), 6.64 (s, 1H), 3.95 (s, 3H).

5-Formyl-2-nitro-benzoic Acid Methyl Ester (10): Reaction mixture of compound **9** (1.4 g, 4 mmol) and calcium carbonate (1.6 g, 16 mmol) in water was refluxed for 24 hours. The mixture was filtered, and the filtrate was taken up with CH_2Cl_2. The organic phase was washed with water and brine, dried, and concentrated. The resulting residue was purified by column chromatography on silica gel with *n*-hexane-ethyl acetate (4:1) to give product **10** as white solid (334 mg, 40%). ^{1}H NMR (80 MHz, $CDCl_3$): δ 10.36 (s, 1H), 8.52-8.10 (m, 3H), 4.00 (s, 3H).

5-Hydroxymethyl-2-nitro-benzoic Acid Methyl Ester (11): The compound **10** (300 mg, 1.4 mmol) in methanol was reduced with $NaBH_4$ (310 mg, 8.4 mmol) in methanol. The reaction was quenched by adding water, and the mixture was extracted with CH_2Cl_2. The organic phase was washed with water and brine, and concentrated. The resulting residue was purified by column chromatography on silica gel with *n*-hexane-ethyl acetate (3:1) to give product **11** (215 mg, 71%). ^{1}H NMR (80 MHz, $CDCl_3$): δ 8.19-7.64 (m, 3H), 4.74 (s, 2H), 3.87 (s, 3H).

5-(*tert*-Butyl-diphenyl-silanyloxymethyl)-2-nitro-benzoic Acid Methyl Ester (12): *tert*-Butylchlorodiphenylsilane (660 mg, 2.4 mmol) was added to the stirred mixture of compound **11** (330 mg, 1.5 mmol) and imidazole (330 mg, 4.8 mmol) in DMF, and the reaction was warmed up to 60 . After reaction completion, the reaction mixture was diluted with water and extracted with ether. The organic layers were washed with water and brine, dried and concentrated. The resulting residue was purified by column chromatography on silica gel with *n*-hexane-ethyl acetate (6:1) to give product **12** as yellow oil (678 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72-7.36 (m, 13H), 4.81 (s, 2H), 3.87 (s, 3H), 1.06 (s, 9H).

General Procedure for the Synthesis of 1-[1-(4-Methoxy-benzyl)-1*H*-indol-2-yl]-hex-5-en-1-ol (13a-c, 18). Magnesium turning (320 mg, 15 mmol) was added to the reaction mixture of 5-bromo-1-pentene (1.5 g, 10 mmol) in dry ether, and the reaction mixture was stirred at room temperature for 2 hours. The mixture was cooled down to 0 °C, and then compound **6a** (1.32 g, 5 mmol) was added. The reaction was quenched by adding water and filtered through Celite. The filtrate was extracted with CH_2Cl_2, and the organic portions were washed with water and brine, dried and concentrated. The resulting residue was purified by column chromatography on silica gel with *n*-hexane-ethyl acetate (6:1) to give the product **13a-c**, **18**. The physical and spectral data of the compounds are as following.

1-[1-(4-Methoxy-benzyl)-1*H*-indol-2-yl]-hex-5-en-1-ol (13a): Yield: 95% (oil); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60 (m, 1H), 7.23-7.09 (m, 3H), 6.95 (m, 2H), 6.78 (m, 2H), 6.52 (s, 1H), 5.74 (m, 1H), 5.41 (s, 2H), 4.94 (m, 1H), 4.73 (t, 1H), 3.73 (s, 3H), 2.02 (m, 2H), 1.88 (m, 2H), 1.51 (m, 2H).

1-[1-(4-Methoxy-benzyl)-5-methyl-1*H*-indol-2-yl]-hex-5-en-1-ol (13b): Yield: 93% (yellow solid); mp 64.5-67.8 °C; IR (KBr) cm^{-1}: 3400 cm^{-1} (OH), 2980 cm^{-1} (C-H), 1750 cm^{-1} (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39 (s, 1H), 7.11 (d, J = 8.4 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 6.89-6.85 (m, 2H), 6.77-6.74 (m, 2H), 6.43 (s, 1H), 5.73 (m, 1H), 5.38 (s, 2H), 4.99 (m, 2H), 4.89 (t, 1H), 4.71 (b, 1H), 3.78 (s, 3H), 2.42 (s, 3H), 2.02 (m, 2H), 1.89 (m, 2H), 1.64 (m, 2H).

1-[1-(4-Methoxy-benzyl)-1*H*-indol-2-yl]-but-3-en-1-ol (13c): Yield: 73% (yellow solid); mp 89.8-91.0 °C; IR (KBr) cm^{-1}: 3380 cm^{-1} (OH), 2980 cm^{-1} (C-H); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (d, J = 8.1 Hz, 1H), 7.24-7.10 (m, 3H), 6.91-6.88 (m, 2H), 6.78-6.75 (m, 2H), 6.54 (s,1H), 5.81 (m, 1H), 5.42 (s, 2H), 5.18-5.11 (m, 2H), 4.80 (t, 1H), 3.73 (s, 3H), 2.68 (m, 2H), 1.88 (b, 1H).

1-[1-(4-Methoxy-benzyl)-1*H*-indol-2-yl]-5-methyl-hex-4-en-1-ol (18): Yield: 50% (brown oil); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.59 (d, J = 8.1 Hz, 1H), 7.21-7.06 (m, 3H), 6.90-6.82 (m, 2H), 6.77-6.74 (m, 2H), 6.51 (s, 1H), 5.38 (s, 2H), 5.06 (m, 1H), 4.72 (t, 1H), 3.71 (s, 3H), 2.09 (m, 2H), 1.92 (m, 2H), 1.68 (s, 3H), 1.55 (s, 3H).

General Procedure for the Synthesis of Acetic Acid 1-[1-(4-methoxy-benzyl)-1*H*-indol-2-yl]-hex-5-enyl Ester (14a-c). The mixture of compound **13a** (1.9 g, 6 mmol), DMAP (1.5 g, 12 mmol) and acetic anhydride (1.2 g, 12 mmol) in CH_2Cl_2 was stirred at room temperature for 5 hours. Water was added, and the mixture was extracted with CH_2Cl_2. The organic phase was washed with water and brine, dried and concentrated. The resulting residue was purified by column chromatography on silica gel with *n*-hexane-ethyl acetate (6:1) to give product **14a-c**. The physical and spectral data of the compounds are as following.

Acetic Acid 1-[1-(4-Methoxy-benzyl)-1*H*-indol-2-yl]-hex-5-enyl Ester (14a): Yield: 90% (yellow oil); IR (KBr) cm^{-1}: 2980 cm^{-1} (C-H), 1735 cm^{-1} (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (d, J = 6.9 Hz, 1H), 7.24-7.10 (m, 3H), 6.86-6.75 (m, 4H), 6.63 (s,1H), 6.04 (t, J = 7.1 Hz, 1H), 5.70 (m, 1H), 5.37 (s, 2H), 4.93 (s, 3H), 3.73 (s, 3H), 2.08 – 1.93 (m, 4H), 1.77 (s, 3H), 1.37 (m, 2H).

Acetic Acid 1-[1-(4-Methoxy-benzyl)-5-methyl-1*H*-indol-2-yl]-hex-5-enyl Ester (14b): Yield: 86% (yellow oil); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (s, 1H), 7.10 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 8.4 Hz, 1H), 6.85-6.76 (m, 4H), 6.54 (s, 1H), 6.02 (t, J = 7.1 Hz, 1H), 5.67 (m, 1H), 5.34 (s, 2H), 4.96-4.89 (m, 2H), 3.77 (s, 3H), 2.43 (s, 3H), 2.21-1.92 (m, 2H), 1.76 (s, 3H), 1.36-1.27 (m, 4H).

Acetic Acid 1-[1-(4-Methoxy-benzyl)-1*H*-indol-2-yl]-but-3-enyl Ester (14c): Yield: 99% (yellow oil); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.63 (m, 1H), 7.24-7.10 (m, 3H), 6.85 (m, 2H), 6.75 (m, 2H), 6.64 (s, 1H), 6.05 (t, 1H), 5.73 (m, 1H), 5.36 (s, 2H), 4.96 (m, 2H), 3.70 (s, 3H), 2.07-1.98 (m, 4H), 1.76 (s, 3H).

General Procedure for the Synthesis of Acetic Acid 1-[1-(4-Methoxy-benzyl)-1*H*-indol-2-yl]-5-oxo-pentyl Ester (15a-b). To a solution of compound **14a** (2.8 g, 7.5 mmol) in 20 mL each of water and THF was added osmium tetroxide (19 mg, 75 mmol) at room temperature, and the reaction mixture was stirred for 30 minutes. A portion of sodium

periodate (4.8 g, 22.5 mmol) was added, and the resulting mixture was stirred vigorously for 4 hours. The reaction was filtered, poured into water and extracted with ether. The organic layers were washed with water and brine, dried and concentrated. The resulting residue was purified by column chromatography on silica gel with *n*-hexane-ethyl acetate (6:1) to give the product **15a-b**. The physical and spectral data of the compounds are as following.

Acetic Acid 1-[1-(4-Methoxy-benzyl)-1*H*-indol-2-yl]-5-oxo-pentyl Ester (15a): Yield: 66% (orange solid); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.66 (s, 1H), 7.62 (d, J = 6.9 Hz, 1H), 7.26-7.11 (m, 3H), 6.87-6.76 (m, 4H), 6.65 (s, 1H), 6.03 (t, J = 7.1 Hz, 1H), 5.38 (s, 2H), 3.75 (s, 3H), 2.36-2.27 (m, 2H), 1.78 (s, 3H), 1.57 (m, 2H).

Acetic Acid 1-[1-(4-Methoxy-benzyl)-5-methyl-1*H*-indol-2-yl]-hex-5-enyl Ester (15b): Yield: 44% (orange solid); mp 134-135; IR (KBr) cm^{-1}: 2980 cm^{-1} (C-H), 1720 cm^{-1} (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.62 (s, 1H), 7.41-6.74 (m, 8H), 6.55 (s, 1H), 6.00 (t, J = 7.0 Hz, 1H), 5.34 (s, 1H), 3.73 (s, 3H), 2.43 (s, 3H), 2.31-2.28 (m, 2H), 2.04 (m, 2H), 1.77 (s, 3H), 1.58 (m, 2H).

General Procedure for the Synthesis of Acetic Acid 5-Hydroxy-1-[1-(4-methoxy-benzyl)-1*H*-indol-2-yl]-hexyl ester (16a-b).

Method 1: To a stirred mixture of the compound **15a** (1.95 g, 5.2 mmol) was added MeMgBr (10.5 mL, 10.5 mmol) at 0 °C, and the reaction mixture was stirred while the temperature was allowed to increase to room temperature. After 3 hours, the reaction was quenched by adding water and filtered through Celite. The filtrate was extracted with CH_2Cl_2, and the organic phase was washed with water and brine, dried and evaporated off. The resulting residue was purified by column chromatography on silica gel with *n*-hexane-ethyl acetate (3:1) to give the product **16a-b**.

Method 2: To the flask containing mercuric acetate (4 g, 12.5 mmol) was added 13 mL each of water and THF. Compound **14a** (1.9 g, 5.1 mmol) was added, and the reaction mixture was stirred at room temperature for 2 hours to complete oxymercuration stage. Then, 13 mL of 3 M NaOH and 13 mL of 0.5 M $NaBH_4$ solution in 3.0 M NaOH were added, respectively. The mercury was allowed to settle. Sodium chloride was added to saturate the water layer, and the upper layer was separated. After the solvent was evaporated, the resulting residue was purified by column chromatography on silica gel with *n*-hexane-ethyl acetate (3:1) to give the product **16a-b**. The physical and spectral data of the compounds are as following.

Acetic Acid 5-Hydroxy-1-[1-(4-methoxy-benzyl)-1*H*-indol-2-yl]-hexyl Ester (16a): Yield: 52%, 50% (through method 1 and 2, respectively; yellow oil); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (d, J = 6.9 Hz, 1H), 7.24-7.10 (m, 3H), 6.86-6.74 (m, 4H), 6.63 (s, 1H), 6.03 (t, J = 7.1 Hz, 1H), 5.36 (s, 2H), 3.71 (s, 3H), 3.36 (m, 1H), 1.77 (s, 3H), 1.34 (m, 2H), 1.09 (d, J = 6.2 Hz, 3H).

Acetic Acid 5-Hydroxy-1-[1-(4-methoxy-benzyl)-5-methyl-1*H*-indol-2-yl]-hexyl Ester (16b): Yield: 10%, 5% (through method 1 and 2, respectively; yellow oil); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (s, 1H), 7.12 (m, 1H), 6.99 (d, 1H), 6.86-6.83 (m, 2H), 6.77-6.74 (m, 2H), 6.54 (s, 1H), 6.01 (t, J = 7.1 Hz, 1H), 5.34 (s, 2H), 3.73 (s, 3H), 3.66 (m, 1H), 2.43 (s, 3H), 1.93-1.77 (m, 2H), 1.76 (s, 3H), 1.38-1.28 (m, 4H), 1.10 (d, J = 6.2 Hz, 3H).

General Procedure for the Synthesis of Acetic Acid 1-[1-(4-Methoxy-benzyl)-1*H*-indol-2-yl]-5-oxo-hexyl Ester (17, 1b). Reaction mixture of alcohol **16a** (976 mg, 2.5 mmol) and PDC (1.8 g, 5 mmol) in CH_2Cl_2 was stirred overnight. Reaction was filtered and washed with CH_2Cl_2. The filtrate was concentrated, and the resulting residue was purified by column chromatography on silica gel with *n*-hexane-ethyl acetate (4:1) to give the product **17**, **1b**. The physical and spectral data of the compounds are as following.

Acetic Acid 1-[1-(4-Methoxy-benzyl)-1*H*-indol-2-yl]-5-oxo-hexyl Ester (17): Yield: 59% (yellow oil); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (d, J = 6.9 Hz, 1H), 7.24-7.10 (m, 3H), 6.86-6.75 (m, 4H), 6.65 (s,1H), 6.01 (t, J = 7.0 Hz, 1H), 5.37 (s, 2H), 3.73 (s, 3H), 1.98 (s, 3H), 1.94 (m, 2H), 1.78 (s, 3H), 1.52 (m, 2H).

5-Methyl-2-Nitro-benzaldehyde (1b): Yield: 90% (yellow oil); ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.43 (s, 1H), 8.04 (d, J = 8,4 Hz, 1H), 7.71 (s, 1H), 7.55 (d, J = 8,4 Hz, 1H), 2.53 (s, 3H).

Conclusion

As key intermediates for synthesis of benzastatin E-G analogues, the indole-2-carbaldehydes with or without substituents at C-5 position were prepared from the corresponding *o*-nitrobenzaldehydes or can be synthesized from 5-methyl-2-nitrobenzoic acid. Using the prepared indole-2-carbaldehydes, benzastatin E and F analogues can be synthesized by replacing the acetyl group of **17** to another protecting group that is stable in strong basic condition of Wittig reaction. Or, acetalization, hydrolysis, and consequent Wittig reaction to the acetylated compound **14c** can provide benzastatin E-F analogues. In addition, the side alkene chain of benzastatin G analogues was introduced directly *via* Grignard reaction with the indole-2-carbaldehyde, and the desired benzastatin G analogues can be produced if Grignard reaction of the ketone with MeMgBr was performed.

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