Efficient Synthesis of Achiral *seco*-CI Subunit of Duocarmycin Pharmacophore

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The natural antibiotic duocarmycin SA (**1**) is isolated from *Streptomyces sp.* DO-113 by Ichimura *et al.* in 1990[1] and a highly potent antitumor antibiotic compound with an IC_{50} value in the picomolar range against the growth of mouse L1210 leukemia cells in culture. The mode of action is a specific alkylation of N-3 of adenine in DNA minor groove, which occurs with opening of the cyclopropane moiety and establishes an aromatic state of ring in **1**.[2]

Compound **2** (1,2,8,8a-tetrahydrocyclopropapyrroloindol-4-one. CPI) is known as the pharmacophoric group of the **1**. The cytotoxic potency of the CPI is directly related to its solvolytic stability. Duocarmycin SA is the most solvolytically stable member of this class of compounds[3-5] and it is devoid of delayed ideal toxicity to host,[3] but it is toxic to the bone marrow.[6] There is a strong interest in the design and development of novel analogs of duocarmycine SA that effectively kill cancer cells and have reduced toxicity to the host. One of attempts to design novel analogs was to synthesize a spirocyclic 1,2.7.7a-tetrahydrocyclopropa[c]indol-4-one [(CI-numbering). CI (**4**)][7,8] (Fig. 1).

3-Chloromethyl-6-hydroxyindoline (**3**) is a *seco* form of which is a precusor form of CPI. Duocarmycins contain a common pharmacophore which consists of CI subunit. Extensive investigation of these natural products and related synthetic derivatives has identified CI as the minimum potent pharmacophore,[9] which can be formed by ring closure of **3**. This pharmacophore[3] and related analogues[10-12] have been synthetic targets for chemists in connection with the mechanism of the antitumor activity.

The facile synthesis of 3-substituted alkylindoles (*seco*-CI types) using the intramolecular Heck reaction was reported.[13,14] Tietze *et al.*[15] and Sakamoto *et al.*[16] also reported that achiral *seco*-CI was synthesized from 4-methoxy-3-nitroaniline by a Heck reaction in eight steps, respectively.

Our interest was focused on synthesis of *seco*-CI using the easy synthetic methods, shortening the reaction steps and obtaining it in high yield.

Selective and directive C-4 bromination of 3-nitrophenol was tried by several methods, but 4-bromo-3-nitrophenol (**6**) could not be obtained as a single product. 2-Bromo- and/or 4-bromo- and 2,4-dibromo-3-nitrophenol were produced as a mixture. We determined to apply the Sandmeyer reaction on 4-amino-3-nitrophenol in place of 3-nitrophenol as starting material. According to reference,[17] it was reacted with $NaNO_2$ and concentrated HCl at 0 °C for 30 min to give aqueous diazonium solution which was reacted with a mixture of cuprous bromide and hydrobromic acid at 60 °C for 30 min *in situ*. But, 3-nitrophenol (**5**), substituted 4-amino group of starting material with hydrogen and compound **6** were obtained in 26% and 38% yields, respectively (Scheme 1). In a our laboratory, diazonium salt was crystallized and separated from aqueous solution by adding ethyl ether, and then obtained diazonium salt was dissolved in water and a mixture of cuprous bromide and hydrobromic acid was treated with a aqueous diazonium solution to provide **6** in 73% yield. These results seem to be corresponded to following explanation. The implementation of the reaction would have accomplished through a competition of two reactions of aryl radical: (a) oxidation by Cu(II) with formation of ArBr and (b) hydrogen abstraction from a molecule of solvent leading to arene.[18]

Bromo compound **6** was protected with benzyl bromide to prepare **7** which was followed by reduction of the nitro group with hydrazine in the presence of ferric chloride to provide the amine **8** in 99% yield. Subsequent amino protection by di-*tert*-butyl dicarbonate (BOC anhydride) to synthesize BOC-protected amine **9** was performed at 90 °C for 4 h. Patel and co-workers[19] have described a more concise route in the synthesis of 3-chloromethyl indoline ring by utilizing a novel intramolecular 5-*exo-trig* aryl radical-alkene cyclization onto a tethered vinyl chloride. As introduced by Patel, compound **9**
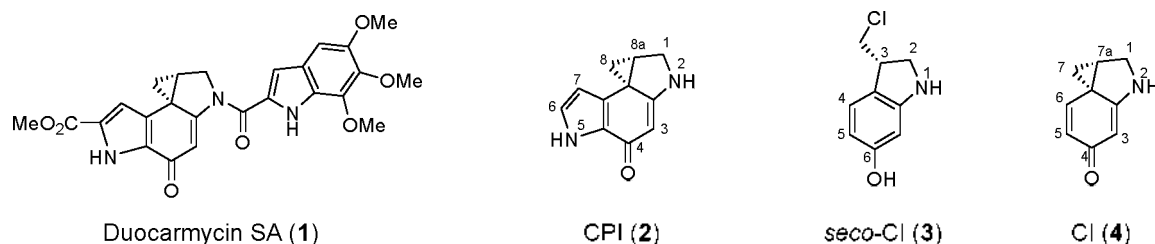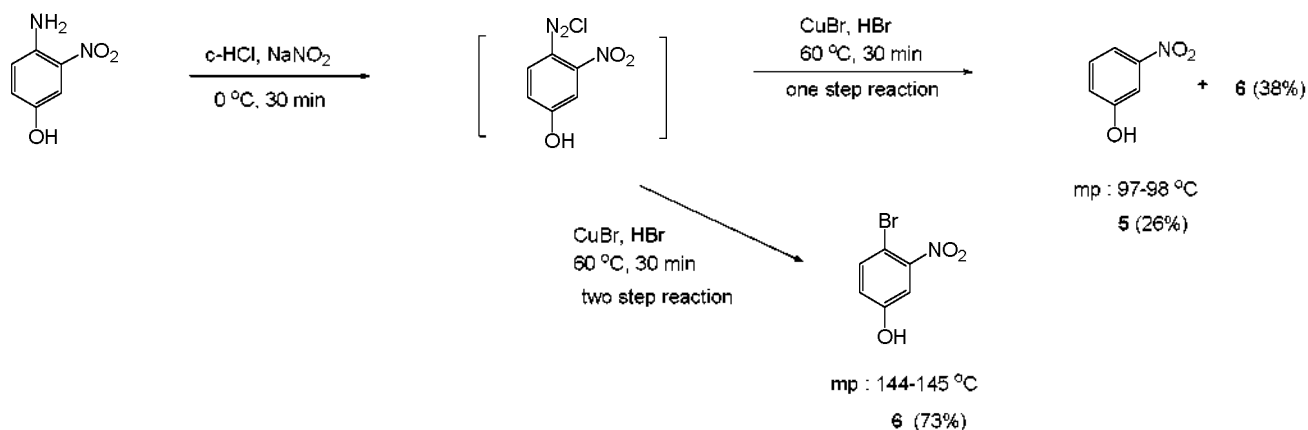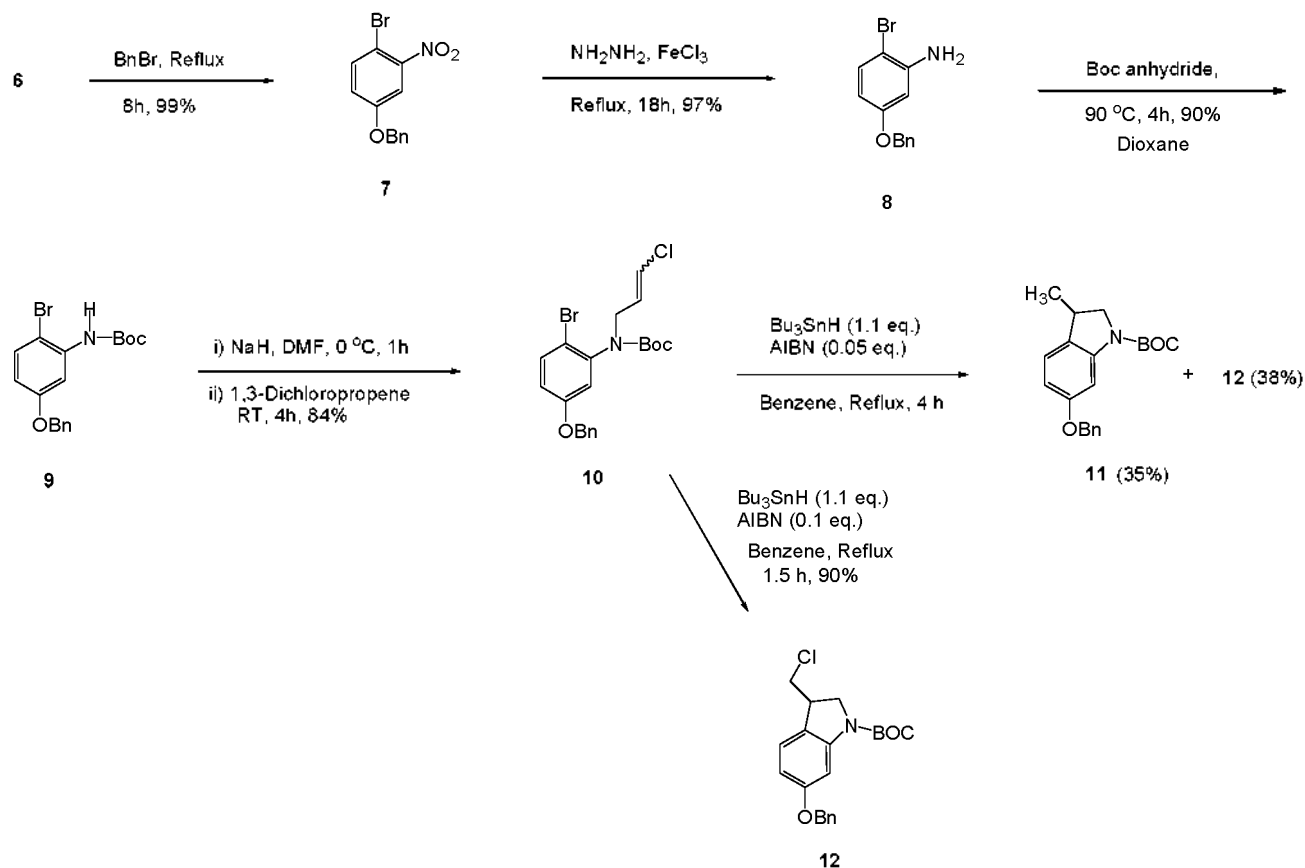

Duocarmycin SA (**1**)   CPI (**2**)   *seco*-CI (**3**)   CI (**4**)

Figure 1

Scheme 1. Synthesis of 4-bromo-2-nitrophenol (6).

Scheme 2. Synthesis of achiral *seco*-CI derivative (12).

was reacted with NaH in dimethylformamide (DMF) for 1 h and then treated with 1,3-dichloro-2-propene to synthesize a *cis* and *trans* mixture of 5-benzyloxy-2-bromo-*N*-BOC-*N*-(3-chloro-2-propenyl)aniline (10), the immediate precursor for implementation of a 5-*exo*-*trig* aryl radical-alkene cyclization. Treatment of 10 with *tri*-*n*-butyltin hydride (1.1 equivalent (eq.)) and azobisisobutyronitrile (AIBN, 0.1 eq.) in refluxing condition for 1.5 h provided achiral *seco*-CI (12) in 90% yield. (Scheme 2) The chloromethyl two proton peaks of 12 were determined at 3.49 and 3.71 ppm in ^{1}H NMR spectrum. But, treatment of 10 with *tri*-*n*-butyltin hydride (1.1 eq.) and AIBN

(0.05 eq.) for long time (4 h) gave 12 (38%) and 3-methyl indoline 11 (35%), which was substituted chloro group of chloromethyl of 12 by hydride radical. The methyl proton peaks of 11 were identified as singlet at 1.29 ppm in ^{1}H NMR spectrum. 10 mol% of AIBN, shorter reaction time and lower temperature were optimal for synthesis of *seco*-CI (12). (Table 1) The desired compound 12 was obtained in 50% overall yield from starting material.

In conclusion, we demonstrated a short and efficient synthesis of simple achiral *seco*-CI subunit 12 of duocarmycin pharmacophore from 4-amino-3-nitrophenol in six steps. The

Table 1. Reaction conditions for radical cyclization of **10**.

	Bu₃SnH (eq.)	AIBN (eq.)	Solvent	Refluxing time (h)	Product (yield)
1	1.1	0.05	Benzene	4	**11** (35%) + **12** (38%)
2	1.1	0.1	Benzene	1.5	**12** (87%)
3	1.1	0.1	Toluene	1.5	**12** (65%)
4	1.5	0.2	Benzene	4	**11** (75 %)

best yield of **12** was obtained from *tri-n*-butyltin hydride (1.1 eq.) and AIBN (0.1 eq.) in refluxing benzene for 1.5 h.

Experimental Section

General procedures. Moisture- or air-sensitive reaction was conducted under nitrogen in distilled solvents. The standard reaction work-up involved drying the solution of crude product over anhydrous MgSO₄, removing the solvent under reduced pressure. The commercial reagents were purchased from Aldrich, Fluka, or Sigma. Melting points were measured on Thomas-Hoover melting point apparatus and not corrected. ¹H, ¹³C NMR, HSQC, and HMQC spectra were taken on Varian 400 MHz spectrometer in CDCl₃. Chemical shifts (δ) are in parts per million (ppm) relative to tetramethylsilane, and coupling constants (*J*) are in Hertz. IR spectra were determined on a Jasco FT-IR 300E spectrometer as KBr pellet. GC/MS spectra were obtained on a SHIMAZU QP 5050 and JEOL GC Mate 2 mass spectrometer. MPLC was used Yamazen YFLC-AI. An analytical TLC was performed on pre-coated silica gel 60 F₂₅₄ plates (Merck). Column chromatography was carried out on Merck silica gel 9385 (230 - 400 mesh) and eluting solvent indicated.

3-Nitrophenol (5). Sodium nitrite (492.4 mg, 7.14 mmol) in water (1.42 mL) was added with stirring over 10 min to a cold (0 °C) solution of 4-amino-3-nitrophenol (1 g, 6.48 mmol) in acidic methanol (methanol 25 mL, c-HCl 1.76 mL). The mixture was stirred at 0 °C for a further 30 min and then added over 30 min to a stirred, warm (60 °C) solution of copper(I) bromide (401.5 mg, 2.79 mmol), 48% hydrogen bromide (1.25 mL) and water (6.45 mL). At the end of the addition the mixture was refluxed for 1 h more, cooled, and filtered. The precipitate was washed with water and dried under vacuum. Purification by column chromatography (ethyl acetate:*n*-hexane = 1:5) gave the **5** as a yellow powder. Yield: 20 mg (26%); mp: 97 - 98 °C; ¹H NMR (400 MHz, CDCl₃) δ 5.93 (1H, br s, OH), 7.20 (1H, dd, *J* = 8.4, 2.4 Hz, H-6), 7.41 (1H, t, *J* = 8.2 Hz, H-5), 7.71 (1H, t, *J* = 2.2 Hz, H-2), 7.81 (1H, ddd, *J* = 8.0, 2.0, 0.8 Hz, H-4); ¹³C NMR (100 MHz, CDCl₃) δ 110.6 (C-2), 115.9 (C-4), 122.1 (C-6), 130.3 (C-5), 149.1 (C-3), 156.4 (C-1); GC-Ms (EI): 139 (M)⁺.

4-Bromo-3-nitrophenol (6). Sodium nitrite (492.4 mg, 7.14 mmol) in water (1.42 mL) was added with stirring over 10 min to a cold (0 °C) solution of 4-amino-3-nitrophenol (1 g, 6.48 mmol) in acidic methanol (methanol 25 mL, c-HCl 1.76 mL) and diethyl ether was added to the mixture at 0 °C and obtained precipitate was filtered. This precipitate (diazonium salt) was dissolved in water (10 mL) to which was added warm (60 °C)

solution of copper(I) bromide (401.5 mg, 2.79 mmol), 48% hydrobromic acid (1.25 mL) and water (6.45 mL). The mixture was stirred at 60 °C for 30 min and cooled down to room temperature to give the precipitate and filtered, washed with cold water and dried under vacuum. The crude compound was purified by column chromatography (ethyl acetate:*n*-hexane = 1:9) to give **6** as a white powder. Yield: 1.02 g (73%); mp: 144 - 145 °C; ¹H NMR (400 MHz, CDCl₃) δ 6.94 (1H, dd, *J* = 8.8, 2.8 Hz, H-5), 7.36 (1H, d, *J* = 2.8 Hz, H-2), 7.48 (1H, d, *J* = 8.8 Hz, H-6), 9.69 (1H, s, OH); ¹³C NMR (100 MHz, CDCl₃) δ 102.5 (C-4), 112.9 (C-2), 121.3 (C-6), 135.2 (C-5), 150.1 (C-3), 157.5 (C-1); GC-MS (EI): 216.9 (M)⁺, 218.9 (M+2)⁻.

O-Benzyl-4-bromo-3-nitrophenol (7). A suspension of anhydrous K₂CO₃ (14.8 g, 107.13 mmol) in chloroform (26 mL) and methanol (13 mL) was refluxed for 15 min. A mixture of **6** (600 mg, 2.77 mmol) and benzylbromide (0.4 mL, 3.32 mmol) in chloroform (12 mL) and methanol (6 mL) was added to hot basic solution. And the mixture was refluxed for 8 h and cooled down to room temperature, filtered and evaporated under reduced pressure to form the crude solid which was recrystallized from ethyl acetate/*n*-hexane to give **7** as a white powder. Yield: 841 mg (99%); mp: 44 - 45 °C; ¹H NMR (400 MHz, CDCl₃) δ 5.08 (2H, s, OCH₂), 7.03 (1H, dd, *J* = 8.8, 3.2 Hz, H-5), 7.35-7.42 (5H, m, Ar-H), 7.44 (1H, d, *J* = 3.2 Hz, H-3), 7.57 (1H, d, *J* = 9.2 Hz, H-6); ¹³C NMR (100 MHz, CDCl₃) δ 70.8 (OCH₂), 104.9 (C-1), 111.8 (C-3), 120.6 (C-5), 127.5 (Ar-C), 128.6 (Ar-C), 128.8 (Ar-C), 135.3 (C-6), 135.5 (Ar-C), 150.1 (C-2), 158.2 (C-4); GC-MS (EI, M⁺): 307 (M)⁺.

5-Benzyloxy-2-bromoaniline (8). A mixture of **7** (800 mg, 2.61 mmol), activated carbon (catalytic amount), ferric chloride hexahydrate (200 mg, 0.74 mmol), and methanol (20 mL) was refluxed for 10 min with stirring. Hydrazine hydrate (521.0 mg, 10.42 mmol) was added to the boiling solution, stirred under reflux for an additional 18 h, cooled and evaporated. The resulting slurry was dissolved in dichloromethane, washed with water and dried with anhydrous MgSO₄. Evaporation of the solvent and column chromatography of the residue over silica gel (ethyl acetate:*n*-hexane = 1:9) gave **8** as a pure pale brown liquid. Yield: 700 mg (97%); mp: 72 - 73 °C; ¹H NMR (400 MHz, CDCl₃) δ 4.04 (2H, br s, NH₂), 4.99 (2H, s, OCH₂), 6.28 (1H, dd, *J* = 8.8, 2.8 Hz, H-4), 6.38 (1H, d, *J* = 2.8 Hz, H-6), 7.25 (1H, d, *J* = 9.2 Hz, H-3), 7.30-7.40 (5H, m, Ar-H); ¹³C NMR (100 MHz, CDCl₃) δ 70.0 (OCH₂), 100.8 (C-2), 102.3 (C-6), 106.4 (C-4), 127.4 (Ar-C), 128.0 (Ar-C), 128.6 (Ar-C), 133.9 (C-3), 136.8 (Ar-C), 144.9 (C-1), 159.2 (C-5); GC-MS (EI): 277 (M)⁺, 279 (M+2)⁺.

5-Benzyloxy-2-bromo-N-BOC-aniline (9). The solution of **8** (300 mg, 1.08 mmol) in dioxane 20 mL was treated with di-*tert*-butyl dicarbonate (707.2 mg, 3.24 mmol) and stirred at 90 °C for 18 h. The mixture was quenched with brine, extracted with dichloromethane and the extracts were dried with anhydrous MgSO₄. The solvent was evaporated and the residue purified by column chromatography (ethyl acetate:*n*-hexane = 1:19) to give **9** as a white powder. Yield: 292 mg (90%); mp: 84 - 86 °C; ¹H NMR (400 MHz, CDCl₃) δ 1.53 (9H, s, C(CH₃)₃), 5.05 (2H, s, OCH₂), 6.54 (1H, dd, *J* = 8.8, 3.2 Hz, H-4), 7.00 (1H, br s, NH), 7.30-7.43 (6H, m, Ar-H, H-3), 7.97 (1H, d, *J* = 2.8 Hz, H-6); ¹³C NMR (100 MHz, CDCl₃) δ 28.3 (C(CH₃)₃),

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Notes

70.2 (OCH$_2$), 81.1 (C(CH$_3$)$_3$), 102.8 (C-2), 106.0 (C-6), 111.0 (C-4), 127.6 (Ar-C), 128.0 (Ar-C), 128.5 (Ar-C), 132.3 (C-3), 136.6 (Ar-C), 137.0 (C-1), 152.3 (C=O), 158.9 (C-5) ; GC-MS (EI): 277 (M-Boc)$^+$, 279 (M+2-Boc)$^-$.

Cis and trans mixture of 5-benzyloxy-2-bromo-N-BOC-N-(3-chloro-2-propenyl)aniline (10). The compound 9 (200 mg, 0.53 mmol) was dissolved in DMF (4 mL) and treated with NaH (50.6 mg, 2.11 mmol) at 0 °C. After stirring at same temperature for 1 h. 1,3-dichloropropene (176.0 mg, 1.59 mmol) was added and stirred at room temperature for 4 h. The mixture was quenched with aq. NH$_4$Cl, extracted with ethyl acetate, and the extracts were dried with anhydrous MgSO$_4$. The mixture was filtered, evaporated under reduced pressure to give the oily residue which was purified by column chromatography (ethyl acetate:n-hexane = 1:9) to yield 10 as a pure colorless liquid. Yield: 200 mg (84%); ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.36 (9H, s, C(CH$_3$)$_3$), 4.26 (1H, dd, J = 15.6, 6.4 Hz, NCH$_2$), 4.47 (1H, dd, J = 15.6, 6.0 Hz, NCH$_2$), 5.04 (2H, s, OCH$_2$), 5.93-6.60 (2H, m, CH=CHCl), 6.79 (1H, s, H-6), 7.33-7.47 (7H, m, H-2, 5, Ar-H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 28.4 (C(CH$_3$)$_3$), 45.9 (NCH$_2$), 70.4 (OCH$_2$), 80.7 (C(CH$_3$)$_3$), 114.5 (C-2), 115.5 (C-6), 116.7 (C-4), 120.5 (CH=CHCl), 127.5 (Ar-C), 128.2 (Ar-C), 128.7 (Ar-C, CH=CHCl), 133.1 (C-3), 136.3 (Ar-C), 141.6 (C-1), 154.0 (C=O), 158.3 (C-5); GC-MS (EI, M$^+$): 353 (M-Boc)$^+$.

5-Benzyloxy-1-methyl-N-BOC-indoline (11). A solution of compound 10 (200 mg, 0.44 mmol) in benzene (10 mL) was degassed and treated with *tri-n*-butyltin hydride (0.12 mL, 0.49 mmol) and AIBN (3.6 mg, 0.022 mmol). The mixture was refluxed for 4 h and concentrated in vacuum to give the crude liquid which was purified by column chromatography (ethyl acetate:n-hexane = 1:9) to yield 11 (31 mg, 35%) as a pure colorless liquid and 12 (64 mg, 38%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.29 (3H, d, J = 6.8 Hz, CH$_3$), 1.59 (9H, s, C(CH$_3$)$_3$), 3.30-3.36 (1H, m, H-1), 3.48-3.52 (1H, m, H-2a), 4.12-4.18 (1H, m, H-2b), 5.06 (2H, s, OCH$_2$), 6.57 (1H, dd, J = 8.0, 2.0 Hz, H-6), 6.99 (1H, d, J = 8.4 Hz, H-7), 7.29-7.44 (6H, m, Ar-H, H-4); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.5 (CH$_3$), 28.5 (C(CH$_3$)$_3$), 29.7 (C-1), 56.4 (C-2), 70.2 (OCH$_2$), 88.8 (C(CH$_3$)$_3$), 109.4 (C-4), 111.8 (C-6), 123.8 (C-7a), 127.5 (Ar-C), 127.8 (Ar-C), 128.5 (Ar-C), 137.3 (Ar-C), 144.5 (C-3a), 152.5 (C=O), 159.8 (C-5): GC-MS (EI, M$^-$): 339 (M)$^+$.

5-Benzyloxy-1-chloromethyl-N-BOC-indoline (12). A solution of compound 10 (200 mg, 0.44 mmol) in benzene (10 mL) was degassed and treated with *tri-n*-butyltin hydride (0.12 mL, 0.49 mmol) and AIBN (7.2 mg, 0.044 mmol). The mixture was refluxed for 1.5 h and evaporated under reduced pressure. The residue was added to n-hexane and obtained precipitates were filtered. The crude product was purified by silica gel chromatography (ethyl acetate:n-hexane = 1:7) to

afford 12 as a white powder. Yield: 153 mg (90%); mp: 155-157 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.57 (9H, s, C(CH$_3$)$_3$), 3.49 (1H, t, J = 9.8 Hz, CH$_2$Cl), 3.58-3.61 (1H, m, H-1), 3.71 (1H, dd, J = 10.8, 4.0 Hz, CH$_2$Cl), 3.92-3.94 (1H, m, H-2), 4.10-4.13 (1H, m, H-2), 5.06 (2H, s, OCH$_2$), 6.57 (1H, dd, J = 8.4, 2.0 Hz, H-6), 7.07 (1H, d, J = 8.4 Hz, H-7), 7.30-7.44 (6H, m, Ar-H, H-4); ^{13}C NMR (100 MHz, CDCl$_3$) δ 28.4 (C(CH$_3$)$_3$), 41.8 (C-1), 47.5 (CH$_2$Cl), 52.8 (C-2), 70.1 (OCH$_2$), 81.0 (C(CH$_3$)$_3$), 101.9 (C-4), 109.3 (C-6), 122.5 (C-7a), 124.7 (Ar-C), 127.9 (Ar-C), 128.5 (Ar-C), 137.0 (Ar-C), 144.5 (C-3a), 152.3 (C=O), 159.8 (C-5); GC-MS (EI, M$^+$): 273 (M-Boc)$^-$, 275 (M+2-Boc)$^+$.

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