Lipase-Catalyzed Asymmetric Synthesis of Desprenyl-carquinostatin A and Descycloavandulyl-lavanduquinocin

Tominari CHOSHI,* Yoshinari UCHIDA,* Yukiko KUBOTA,* Junko NOBUHIRO,* Mitsuhiro TAKESHITA,*
Takushi HATANO,* and Satoshi HIBINO* a,a

*Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University; Fukuyama, Hiroshima 729–0292, Japan;
*Tohoku Pharmaceutical University; Komatsujima, Aoba-ku, Sendai 981–8558, Japan; and Faculty of Life Science and Biotechnology, Fukuyama University; Fukuyama, Hiroshima 729–0292, Japan.

Received April 4, 2007; accepted May 9, 2007; published online May 10, 2007

An asymmetric synthesis of the core carbazole structure, 6-desprenyl-carquinostatin 3 and 6-descycloavandulyl-lavanduquinocin 3, toward a total synthesis of carquinostatin A (1) and lavanduquinocin (2), has been established. Lipase QLM (Meito) catalyzed enantioselective acetylation of the racemic alcohol 6 gave the (−)-acetate 7 and the (+)-alcohol 6 with high enantioselectivity. The absolute stereochemistry of the (−)- and (+)-alcohol 6 have been determined to be R- and S-configurations, respectively, by the advanced Mosher method. In the same manner, the (−)-acetate 13 and the (+)-alcohol 12 have been obtained from the racemic alcohol 12. The (R)-(−)-acetate 13, derived from the (R)-(−)-acetate 7, was the same as the (−)-acetate 13, which has been determined to be (R)-configuration. Oxidation of the (R)-(−)-acetate 13 followed by hydrolysis afforded (R)-(−)-6-desprenyl-carquinostatin [and (R)-(−)-6-descycloavandulyl-lavanduquinocin] 3. In addition, oxidation of the (S)-(−)-alcohol 12 provided (S)-(−)-3, which is the enantiomer of 6-desprenyl-carquinostatin A (R)-(−)-3.

Key words  lipase QLM; enantioselective acetylation; advanced Mosher method; desprenyl carquinostatin A; descycloavandulyl lavanduquinocin

The carbazole-3,4-quinone alkaloids, carquinostatin A (1) and lavanduquinocin (2) were isolated from Streptomyces exfoliates 2419-SVT2 and Streptomyces viridochromogenes by Seto and co-workers in 1993 and 1995, respectively. The structures of the two alkaloids were elucidated to be the same carbazole-3,4-quinone moiety by NMR spectral analyses and other spectroscopic experiments. The absolute stereochemistry of the C-11 position of the two alkaloids was the same R-configuration. Carquinostatin A (1) and lavanduquinocin (2) were also shown to be a potent neuronal cell protecting substance which exhibits a free radical scavenging activity. Total syntheses of these alkaloids have recently been developed by the Knölker group. The transition metal-mediated and -catalyzed methodologies for the construction of the carbazole framework have been efficiently employed. Throughout the course of this study, we have been interested in the synthetic development of biologically active condensed-heteroaromatic compounds, including natural products, by the thermal electrocyclic reactions of either conjugated hexatriene or monoaza-hexatriene systems incorporating one double bond from an aromatic or heteroaromatic portion. We recently reported the synthesis of the highly substituted carbazole alkaloids, carazostatin and carbazocinonucins, by the construction of the appropriate carbazole framework based on the allene-mediated electrocyclic reaction of the 6π-electron system involving the indole 2,3-bond. In the present paper, we describe the asymmetric synthesis of 6-desprenyl-carquinostatin A (6-descycloavandulyl-lavanduquinocin) 3, which is a common carbazole framework of both alkaloids, based on a lipase-catalyzed esterification using a racemic alcohol 6 for the determination of the absolute stereochemistry of 3. We chose the 3-ethoxy-2-methyl-1-(trifluoromethylsulfonyloxy)carbazole (4) as a starting material, which was prepared in a six-step sequence from 3-iodoindole-2-carbaldehyde by the application of our methodology, as shown in the retrosynthetic Chart 1.

The required 1-allylcarbazole 5 was prepared from the triflate 4 and allylboronic acid pinacol ester in the presence of PdCl2(dppf) in dimethylformamide (DMF) by the Suzuki–Miyaura reaction. Subsequent the Wacker reaction of 5 in the presence of palladium chloride(II) and copper chloride under an oxygen atmosphere gave the acetonylcarbazole 8...
followed by reduction of 8 with NaBH₄ provided the racemic alcohol 6 (Chart 2). Next, we investigated the enzymatic resolution of 6 with lipase PS (Amano) or lipase QLM (Meito). The results of the kinetic resolution by transesterification are shown in Table 1. Of the lipases, lipase QLM showed high enantioselectivities for this substrate. The influence of a solvent on the enantioselectivity was also examined, and disopropyl ether (i-Pr₂O) gave the best result. Enantiomeric excess of the (−)-acetate 7 and the (+)-alcohol 6 were measured by HPLC on a CHIRALPAC AD column.

The absolute configuration of these compounds 6 was examined by the advanced Mosher method²²,²³) using 2-methoxy-2-(1-naphthyl)propionic acid (MaNP acid). The (−)-acetate 7 was hydrolyzed with aqueous 1 M KHCO₃/MeOH to give the (−)-alcohol 6. Both alcohols (−)-6 and (+)-6 were treated with (R)- and (S)-MaNP acid in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminoypyridine (DMAP) in CH₂Cl₂ to produce the four MaNP esters (9a, b and 10a, b). The absolute stereochemistry can be elucidated by the difference of calculated chemical shift on their NMR spectra. In the MaNP ester 9a, b, the 10-H resonated at upper field in the (R)-ester 9a than in the (S)-ester 9b (Δδ, −0.32 ppm and −0.04 ppm). Moreover, 12-H appeared upfield for the (S)-ester 9b than for the (R)-ester 9a (Δδ, +0.13 ppm). The other MaNP ester 10a, b ((+)-6+MaNP acid) was completely a result of the contrariety of ester 9a, b. Based on the analyses of their NMR spectra, the absolute stereochemistry of (+)- and (−)-alcohol 6 were determined to be S- and R-configurations, respectively (Chart 3). Cleavage of the ethyl ether of 8 with boron tribromide (BBr₃) afforded the 3-hydroxycarbazole 11, which was reduced with NaBH₄ to provide the racemic alcohol 12. Subsequent lipase-catalyzed enantioselective esterification of 12 with lipase QLM and vinyl acetate in i-Pr₂O gave the (−)-acetate 13 (98% ee) and (+)-alcohol 12 (97% ee) (Chart 4). The spectral data and the retention time by HPLC of (−)-13 and (+)-12 was determined to be R- and S-configuration, respectively.

Table 1. Kinetic Resolution of (±)-6 by Lipase-Catalyzed Acetylation

<table>
<thead>
<tr>
<th>Run</th>
<th>Lipase</th>
<th>Solvent</th>
<th>Time (h)</th>
<th>Acetate 7% (% ee)</th>
<th>Alcohol 6% (% ee)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lipase PS</td>
<td>CH₂Cl₂</td>
<td>24</td>
<td>—</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>Lipase PS</td>
<td>THF</td>
<td>24</td>
<td>—</td>
<td>96</td>
</tr>
<tr>
<td>3</td>
<td>Lipase PS</td>
<td>i-PrOMe</td>
<td>48</td>
<td>19 (98)</td>
<td>75 (8)</td>
</tr>
<tr>
<td>4</td>
<td>Lipase PS</td>
<td>Et₂O</td>
<td>48</td>
<td>12 (10)</td>
<td>71 (9)</td>
</tr>
<tr>
<td>5</td>
<td>Lipase PS</td>
<td>CH₃CN</td>
<td>48</td>
<td>6 (13)</td>
<td>56</td>
</tr>
<tr>
<td>6</td>
<td>Lipase PS</td>
<td>i-Pr₂O</td>
<td>48</td>
<td>4 (97)</td>
<td>90</td>
</tr>
<tr>
<td>7</td>
<td>Lipase QLM</td>
<td>i-PrOMe</td>
<td>24</td>
<td>40 (99)</td>
<td>54 (70)</td>
</tr>
<tr>
<td>8</td>
<td>Lipase QLM</td>
<td>i-Pr₂O</td>
<td>24</td>
<td>49 (99)</td>
<td>40 (78)</td>
</tr>
</tbody>
</table>

The enantiomeric excess (%ee) was determined by HPLC on CHIRALPAC AD.
tively. Finally, oxidative treatment of the (−)-acetate 13 with benzeneselenenic anhydride[20] [(PhSeO)2] gave the carbazole-3,4-quinone (R)-(-)-14, which was hydrolyzed with aqueous 1 M K2CO3/MeOH to yield the desprenyl-carquinos- 

In conclusion, a synthesis of the functionalized core carbazole, (R)-(-)-6-desprenyl-carquinosatin A [(R)-(-)-6-des
cycloavandulyl-lavanduquinocin] 3 together with its enantiomer (S)-(+)-3, have been completed by the lipase-catalyzed enantioselective esterification, followed by the determination of the absolute stereochemistry based on the advanced Mosher method, by using the key compound, 3-ethoxy-2-methyl-1-(trifluoromethylsulfonyloxy)carbazole (4). In addition, it has been demonstrated that a new synthetic route toward a total synthesis of carquinosatin A (1) and lavanduquinocin (2) has been provided.

Experimental

All melting points were measured on a Yanagimoto micro-melting point apparatus MP-500D and were uncorrected. IR spectra were recorded on a Shimadzu FTIR-8500 spectrophotometer using attenuated total reflection (ATR) method. 1H- and 13C-NMR spectra were taken with a JEOL AL-300 spectrometer. All air sensitive reactions were run under an argon atmosphere. Solvents were distilled by normal methods (THF was dried over 4 Å-molecular sieves). 3-Allyl-2-methylcarbazole (5) was hydrolyzed with NaOH to yield allylcarbazole (6). IR (ATR) method. 1H- and 13C-NMR spectra were taken with a JEOL AL-300 Shimadzu FT-IR-8500 spectrophotometer using attenuated total reflection.

1-Allyl-3-ethoxy-2-methylcarbazole (5) 2- Allyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (187 mg, 0.99 mmol) was added to a mixture of the triflate 4 (246 mg, 0.66 mmol), 3 NaOH (0.64 ml, 1.98 mmol) and PdCl2(dppf) (49 mg, 0.06 mmol) in THF (10 ml) under Ar atmosphere. The mixture was quenched with water, and was then extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na2SO4 and concentrated. The residue was purified by column chromatography (silica gel, 10 g) using EtOAc–hexane (3 : 97, v/v) as an eluent to give the allylcarbazole 5 (134 mg, 77%). mp 83—85 °C from hexane. 1H-NMR (CDCl3) δ: 1.50 (3H, t, J = 6.9 Hz), 2.36 (3H, s), 3.69 (2H, dt, J = 1.8, 1.8, 5.4 Hz), 4.14 (2H, q, J = 6.9 Hz), 5.05 (1H, dq, J = 1.8, 1.8, 17.2 Hz), 5.09 (1H, dq, J = 1.8, 1.8, 10.2 Hz), 6.03 (1H, dd, J = 5.4, 10.2, 17.2 Hz), 7.18 (1H, dt, J = 1.1, 8.1 Hz), 7.34 (1H, dt, J = 1.1, 8.1 Hz), 7.41 (1H, d, J = 8.1 Hz), 7.42 (1H, s), 7.84 (1H, br, s), 7.98 (1H, d, J = 8.1 Hz). MS m/z: 265 (M+). HR-MS m/z: 265.1471 (Calcd for C18H19NO: 265.1467).

1-Acetyloxy-3-ethoxy-2-methylcarbazole (8) A mixture of PdCl2 (17 mg, 0.094 mmol) and CuCl (93 mg, 0.94 mmol) in DMF–H2O (7 : 1, 4 ml) was stirred at rt. for 30 min under an O2 atmosphere. A solution of allylcarbazole 5 (249 mg, 0.94 mmol) in DMF–H2O (7 : 1, 6 ml) was added to a reaction mixture at the same temperature, and was then stirred at the same temperature for 4 h under an O2 atmosphere. The reaction was quenched with 10% HCl, and was then extracted with EtOAc. The EtOAc layer was washed with water and brine, dried over Na2SO4 and concentrated. The residue was purified by column chromatography (silica gel, 10 g) using EtOAc–hexane (3 : 7, v/v) as an eluent to give the alkylation 8 (215 mg, 81%): mp 169—170 °C (from pet. Et2O). IR (ATR) ν: 1708 cm−1. 1H-NMR (CDCl3) δ: 1.51 (3H, t, J = 7.0 Hz), 2.17 (3H, s), 2.48 (3H, s), 4.01 (2H, s), 4.16 (2H, q, J = 7.0 Hz), 7.18 (1H, t, J = 6.9 Hz), 7.37 (1H, t, J = 6.9 Hz), 7.45 (1H, d, J = 6.9 Hz), 7.46 (1H, s), 7.97 (1H, d, J = 6.9 Hz). MS m/z: 283 (M+). HR-MS m/z: 283.1425 (Calcd for C18H19NO2: 283.1426).

3-Ethoxy-1-(2-hydroxypropyl)-2-methylcarbazole (6) NaBH4 (64 mg, 1.29 (3H, d, J = 1.1, 8.1 Hz), 7.42 (1H, s), 7.97 (1H, d, J = 6.9 Hz), 8.34 (1H, br, s). MS m/z: 281 (M+). HR-MS m/z: 281.1579 (Calcd for C18H21NO2: 281.1572).

1-Allyl-3-ethoxy-2-methylcarbazole (7) (12 mg, 40%) and the alcohol 6 (17 mg, 0.11 mmol) were added to a solution of acetylonitrile 8 (400 mg, 1.42 mmol) in EtO (50 ml) under ice-cooled water, and was then stirred at the same temperature for 1 h. The reaction mixture was quenched with water, and was then extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na2SO4 and concentrated. The residue was purified by column chromatography (silica gel, 10 g) using EtOAc–hexane (3 : 7, v/v) as an eluent to give the alcohol 6 (375 mg, 93%): mp 141—142 °C (from CHCl3). IR (ATR) ν: 3374 cm−1. 1H-NMR (CDCl3) δ: 1.38 (3H, d, J = 6.2 Hz), 1.50 (3H, t, J = 6.9 Hz), 2.36 (3H, s), 3.03 (1H, dd, J = 8.1, 14.3 Hz), 3.15 (1H, dd, J = 3.3, 14.3 Hz), 4.15 (2H, q, J = 6.9 Hz), 4.20—4.25 (1H, m), 7.15 (1H, dt, J = 1.1, 8.1 Hz), 7.35 (1H, dt, J = 1.1, 8.1 Hz), 7.42 (1H, d, J = 8.0 Hz), 7.43 (1H, s), 7.97 (1H, d, J = 8.0 Hz). 8.42 (1H, br, s). MS m/z: 283 (M+). HR-MS m/z: 283.1579 (Calcd for C18H16NO2: 283.1572).

3-Ethoxy-1-(2-hydroxypropyl)-2-methylcarbazole (++) (11) Pr0O (10 ml), vinyl acetate (98 μl, 1.06 mmol), and the alcohol 6 (30 mg, 0.11 mmol) were added to the lipase QLM (60 mg). The mixture was stirred at 32 °C for 24 h. The reaction mixture was filtered and the volatiles were removed under reduced pressure to give the products. The crude product was purified by preparative TLC using EtOAc–hexane (3 : 7, v/v) as an eluent to give the acetate (−)-(7) (17 mg, 49%) and an alcohol (++) (6) (12 mg, 40%).

−(−)-1-(2-Acetoxypropyl)-3-ethoxy-2-methylcarbazole (−)(−) and (++)

---
The residue was purified by column chromatography (silica gel, 10 g) using EtOAc–hexane (1:1, v/v) as an eluent to give the alcohol (12 mg, 49%). HR-MS m/z: 253.1097 (Calcd for C16H19NO3; 253.1103).

3-Hydroxy-1-(2-hydroxypropyl)-2-methylcarbazole (12) NaBH4 (16 mg, 0.43 mmol) was added to a solution of acetoxylcarbazole (11) (54 mg, 0.21 mmol) in EtOH (10 ml) under ice-cooled water, and then was stirred at the same temperature for 1 h. The reaction mixture was quenched with 10% (v/v) aq. Na2SO4 solution and concentrated. The residue was purified by column chromatography (silica gel, 10 g) using EtOAc–hexane (1:1, v/v) as an eluent to give the alcohol (12 mg, 49%). HR-MS m/z: 253.1097 (Calcd for C16H19NO3; 253.1103).

1-(2-Acetoxypropyl)-3-hydroxy-2-methylcarbazole-3,4-dione (13) The residue was purified by column chromatography (silica gel, 10 g) using EtOAc–hexane (1:1, v/v) as an eluent to give the alcohol (12 mg, 49%). HR-MS m/z: 253.1097 (Calcd for C16H19NO3; 253.1103).

1-(2-Acetoxypropyl)-3-hydroxy-2-methylcarbazole-3,4-dione (14) A solution of BBr3 (21 ml, 0.22 mmol) in CH2Cl2 (2 ml) was added to a stirred solution of carbazole (12) (7 mg, 0.015 mmol) in CH2Cl2 (2 ml) at 0 °C for 3 h. The mixture was quenched with 10% aq. Na2SO4 solution and concentrated. The residue was purified by column chromatography (silica gel, 10 g) using EtOAc–hexane (3:7, v/v) as an eluent to give the alcohol (14 mg, 15%). HR-MS m/z: 254.0992 (Calcd for C16H19NO3; 254.0993).

1-Acetoxyl-3-hydroxy-2-methylcarbazole (11) A solution of BBr3 (60 μl, 0.63 mmol) in CH2Cl2 (2 ml) was added to a stirred solution of 3-ethoxycarbonyl-2 (89 mg, 0.32 mmol) in CH2Cl2 (4 ml) at 78 °C under a N2 atmosphere. After being gradually warmed to rt., the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na2SO4 and concentrated. The residue was purified by column chromatography (silica gel, 10 g) using EtOAc–hexane (1:1, v/v) as an eluent to give the 3-hydroxycarbazole (11) (64 mg, 80%); mp 169–170 °C (from EtOAc–hexane). IR (ATR): ν: 3357, 1638 cm−1. 1H-NMR (CDCl3) δ: 2.37 (3H, s), 2.49 (3H, s), 4.00 (2H, s), 4.66 (1H, brs), 7.15 (1H, dt, J = 2.0, 6.9 Hz), 7.36 (1H, dt, J = 2.0, 6.9 Hz), 7.41 (1H, s), 7.43 (1H, dd, J = 2.0, 6.9 Hz), 7.93 (1H, dd, J = 6.9, 8.29 (1H, brs). MS m/z: 253 (M+) HR-MS m/z: 253.1097 (Calcd for C16H19NO3; 253.1103).

3-Hydroxy-1-(2-hydroxypropyl)-2-methylcarbazole (12) A solution of BBr3 (21 ml, 0.22 mmol) in CH2Cl2 (2 ml) was added to a stirred solution of carbazole (12) (7 mg, 0.015 mmol) in CH2Cl2 (2 ml) at 0 °C for 3 h. The mixture was quenched with 10% aq. Na2SO4 solution and concentrated. The residue was purified by column chromatography (silica gel, 10 g) using EtOAc–hexane (1:1, v/v) as an eluent to give the alcohol (12 mg, 49%). HR-MS m/z: 253.1097 (Calcd for C16H19NO3; 253.1103).

1-(2-Acetoxypropyl)-3-hydroxy-2-methylcarbazole-3,4-dione (13) The residue was purified by column chromatography (silica gel, 10 g) using EtOAc–hexane (1:1, v/v) as an eluent to give the alcohol (12 mg, 49%). HR-MS m/z: 253.1097 (Calcd for C16H19NO3; 253.1103).
aqueous 1 M K₂CO₃ (2 ml) and MeOH (3 ml) at r.t. for 3 h to give an (−)-alcohol. The reaction mixture was quenched with water, and then extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na₂SO₄, and concentrated. The residue was purified by column chromatography (silica gel, 10 g) using EtOAc–hexane (7 : 3, v/v) as an eluent to give the alcohol (R)-[−]-3 (14 mg, 95%): mp 201–203 °C (EtOAc). IR (ATR) ν: 3208, 1716, 1619 cm⁻¹. ¹H-NMR (CDCl₃) δ: 1.25 (3H, d, J=6.3 Hz), 1.94 (3H, s), 2.77—2.79 (2H, m), 3.93—4.00 (1H, m), 4.90 (1H, br s), 7.22—7.25 (2H, m), 7.51—7.53 (1H, m), 7.84—7.87 (1H, m), 11.10 (1H, s). MS m/z: 269 (M⁺), HR-MS m/z: 269.1059 (Calcd for C₁₆H₁₅NO₃: 269.1052).

(5)-1-(2-Hydroxypropyl)-2-methylcarbazole-3,4-dione (S)-[+]-3 A solution of alcohol (+)-12 (9 mg, 0.035 mmol) in THF (1 ml) was added to a stirred suspension of 70% (PhSeO)₂O (36 mg, 0.071 mmol) in THF (1 ml). The mixture was stirred at 50 °C for 30 min. After being cooled to r.t., the reaction mixture was diluted with EtOAc. The mixture was washed with aqueous 10% Na₂CO₃ solution, water and brine. The organic layer was dried over alcohol (silica gel, 10 g) using EtOAc–hexane (7 : 3, v/v) as an eluent to give the carbazole-3,4-quinone (from EtOAc). IR (ATR) ν: 3208, 1716, 1619 cm⁻¹. ¹H-NMR (CDCl₃) δ: 1.25 (3H, d, J=6.3 Hz), 1.93 (3H, s), 2.76—2.78 (2H, m), 3.95—3.97 (1H, m), 6.3 Hz), 1.94 (3H, s), 2.77—2.79 (2H, m), 3.93—4.00 (1H, m), 7.22—7.25 (2H, m), 7.51—7.53 (1H, m), 7.84—7.87 (1H, m), 11.10 (1H, s). MS m/z: 269 (M⁺), HR-MS m/z: 269.1066 (Calcd for C₁₆H₁₅NO₃: 269.1052).

Acknowledgements We wish to thank Meito Sangyo Co., Ltd. for providing Lipase QLM and Amano Enzyme Inc. for Lipase PS. This work was supported in part by Grants-in Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology.

References and Notes