Supplementary data

Study on the biosynthesis of the notoamides: Pinacol-type rearrangement of isoprenyl unit in deoxybrevianamide E and 6-hydroxydeoxybrevianamide E

Hikaru Kato\textsuperscript{a}, Yuichi Nakamura\textsuperscript{a}, Jennifer M. Finefield\textsuperscript{b}, Hideharu Umaoka\textsuperscript{a}, Takashi Nakahara\textsuperscript{a}, Robert M. Williams\textsuperscript{b,c,*}, and Sachiko Tsukamoto\textsuperscript{a,*}

\textsuperscript{a} Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto, 862-0973, Japan
\textsuperscript{b} Department of Chemistry, Colorado State University, 1301 Center Avenue, Fort Collins, Colorado 80523, U.S.A.
\textsuperscript{c} University of Colorado Cancer Center, Aurora, CO 80045, USA

General Synthetic Considerations

\textsuperscript{[\textsuperscript{13}C]_2-}[\textsuperscript{15}N]_2-L-Proline and \textsuperscript{[\textsuperscript{13}C]- L-glycine were obtained from the NIH Stable Isotopes Resource at Los Alamos National Laboratory. All other reagents were commercial grade and used without further purification unless otherwise noted. Unless otherwise noted, all reactions were run under an argon atmosphere in flame or oven-dried glassware. Reactions were monitored by thin layer silica gel chromatography (TLC) using 0.25 mm silica gel 60F plates with fluorescent indicator (Merck). Products were purified via either flash column chromatography using silica gel grade 60 (230-400 mesh) purchased from Sorbent Technologies or preparative thin layer chromatography (1000 μm). Acetonitrile (CH\textsubscript{3}CN), dichloromethane (CH\textsubscript{2}Cl\textsubscript{2}), diethyl ether (Et\textsubscript{2}O), N, N-dimethylformamide (DMF), methanol (MeOH), tetrahydrofuran (THF), toluene (PhMe), and triethylamine (Et\textsubscript{3}N) were all degassed with argon and passed through a solvent purification system containing alumina or molecular sieves. \textsuperscript{1}H NMR spectra and \textsuperscript{13}C NMR spectra were obtained on Varian 300, 400, 400 MR or 500 MHz NMR spectrometers. NMR spectra were taken in CDCl\textsubscript{3} (\textsuperscript{1}H, 7.26 ppm; \textsuperscript{13}C, 77.0 ppm), CD\textsubscript{3}OD (\textsuperscript{1}H, 3.31 ppm, 49.15 ppm), DMSO-\textsubscript{d}\textsubscript{6} (\textsuperscript{1}H, 2.50 ppm, \textsuperscript{13}C, 39.51 ppm) and D\textsubscript{2}O (\textsuperscript{1}H, 4.79 ppm) obtained from Cambridge Isotope Labs. Mass spectra were obtained on Fisions VG Autospec using a high/low resolution magnetic sector.
$[^{13}\text{C}]-\text{Ethyl 2-}((\text{diphenylmethylene})\text{amino})-3-(2-(\text{2-methylbut-3-en-2-yl})-1\text{H-indol-3-yl})\text{propanoate (20):}$

$\begin{align*}
&\text{Gramine}^1 \text{ 19 (1.65 g, 6.81 mmol), }[^{13}\text{C}]-\text{glycine benzophenone imine}^2 \text{ 18 (1.66 g, 6.19 mmol)} \\
&\text{and } ^6\text{Bu}_3\text{P (0.61 mL, 2.48 mmol) were dissolved in MeCN (34.0 mL) and heated to reflux for 18 hours. The reaction mixture was concentrated and purified via flash column chromatography in 9:1 hexanes:ethyl acetate to provide 20 as an inseparable mixture of diastereomers. Yield: 3.11 g, 6.68 mmol, 98%.} \\
&^1\text{H NMR (300 MHz, CDCl}_3\text{) }\delta \text{ 7.89 (bs, 1H), 7.60-7.57 (m, 2H), 7.47-7.19 (m, 8H), 7.09-7.01 (m, 3H), 6.87-6.82 (m, 1H), 6.33 (bs, 1H), 5.89 (dd, } J = 17.4, 10.5 \text{ Hz, 1H), 5.09-4.99 (m, 2H), 4.25-4.11 (m, 2H), 3.58 (d, } J = 2.4 \text{ Hz, 1H), 3.55 (bs, 1H), 1.39 (s, 3H), 1.36 (s, 3H), 1.27 (t, } J = 7.2 \text{ Hz, 3H);^13\text{C NMR (75 MHz, CDCl}_3\text{) }\delta 172.7, 170.9, 146.3, 140.1, 139.5, 136.2, 134.1, 130.2, 129.1, 128.0, 127.9, 127.8, 121.4, 119.6, 119.1, 111.8, 110.0, 107.6, 107.5, 67.3, 66.5, 61.0, 39.3, 31.8, 29.0, 18.4, 18.3, 17.9, 17.8, 24.8, 22.9, 14.4, 14.2.}
\end{align*}$
[\textsuperscript{13}C]-Ethyl 2-((\textit{tert}-butoxycarbonyl)amino)-3-(2-(2-methylbut-3-en-2-yl)-1H-indol-3-yl)propanoate (21):

\[ \text{\textsuperscript{13}C}-\text{Imine} \ 20 \ (3.11 \text{ mg, 6.68 mmol}) \text{ was dissolved in 50 mL of THF and 1 M HCl (16.7 mL) and the reaction stirred for 30 minutes. The THF was removed under vacuum and the residue with diluted with NaHCO}_3 \text{ until basic. The aqueous layer was extracted with DCM (2 x 100 mL) and the combined organic layers were dried over Na}_2\text{SO}_4 \text{ and concentrated. The crude material was purified via flash column chromatography in 3:1 hexanes:ethyl acetate and then flushed with 5\% MeOH in DCM. The [\textsuperscript{13}C]-amine (790 mg, 2.62 mmol) was dissolved in 13 mL of dioxane and cooled to 0\textdegree C. \textit{Di-}\textit{tert}-butyldicarbonate (600 mg, 2.75 mmol) and aqueous 0.5 M NaOH (2.6 mL) were added to the solution. The reaction mixture was allowed to warm to room temperature while stirring for 2 hours. The solvent was removed in vacuo and the remaining residue was taken up in H}_2\text{O and acidified with 10\% KHSO}_4 \text{ to pH 2. The} \]
product was extracted with ethyl acetate (3 x 40 mL) and the combined organic layer was washed with brine, dried over Na$_2$SO$_4$ and concentrated. The residue was purified via flash column chromatography in 4:1 hexanes:ethyl acetate to afford 21 as an inseparable mixture of diastereomers. Yield: 1.29 mg, 3.21 mmol, 48% (2 steps). $^1$H NMR (300 MHz, CDCl$_3$) δ 8.37 (bs, 1H) 7.52 (d, $J$ = 7.2 Hz, 1H), 7.26 (d, $J$ = 6.6 Hz, 1H), 7.14-7.05 (m, 2H), 6.15 (dd, $J$ = 17.4, 10.5 Hz, 1H), 5.22-5.25 (m, 2H), 4.14-3.95 (m, 2H), 3.38-3.21 (m, 2H), 1.56 (s, 9H), 1.36 (s, 6H), 1.03 (t, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 173.4, 155.4, 146.3, 140.8, 134.6, 129.9, 121.6, 119.5, 118.4, 112.3, 110.7, 105.9, 79.8, 67.3, 61.4, 55.0, 39.4, 28.7, 28.5, 28.1, 27.9, 27.6, 14.0; $^{13}$C-enriched peak: 173.4 IR (neat) 3378, 3083, 3057, 2976, 1697, 1503, 1462, 1167; HRMS (ESI/APCI) calcd for C$_{22}$[^13]C]H$_{33}$N$_2$O$_4$ (M+H) 402.2435, found 402.2434.

![Structure of 21](image-url)
[\textsuperscript{13}C]-2-((tert-butoxycarbonyl)amino)-3-(2-(2-methylbut-3-en-2-yl)-1H-indol-3-yl)propanoic acid (22):

The [\textsuperscript{13}C]-ester 21 (1.05 g, 2.62 mmol) was dissolved in 8.73 mL of THF and 17.5 mL of H\textsubscript{2}O. To the solution, LiOH (1.09 g, 26.2 mmol) was added and the reaction stirred at room temperature for 18 hours. The solvent was removed under vacuum and the resulting slurry was acidified with 1 M KHSO\textsubscript{4} to pH 2. The product was extracted with DCM (3 x 40 mL) and EtOAc (40 mL). The combined organic layers were dried over Na\textsubscript{2}SO\textsubscript{4} and concentrated to afford a mixture of inseparable diasteromers (780 mg, 2.09 mmol, 80%) which was taken on to the next step without further purification. \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) \(\delta\) 7.95 (bs, 1H), 7.57 (d, \(J\) = 10.2 Hz, 1H), 7.29 (d, \(J\) = 8.4 Hz, 1H), 7.16-7.06 (m, 2H), 6.15 (dd, \(J\) = 17.1, 10.5 Hz, 1H), 5.22 (d, \(J\) = 7.2 Hz, 1H), 5.17 (s, 1H), 4.60 (bs, 1H), 3.46-3.39 (m, 1H), 3.27-3.15 (m, 1H), 1.57 (s, 3H), 1.56 (s, 3H), 1.32 (s, 9H); \textsuperscript{13}C NMR (100 MHz, 20:1 CDCl\textsubscript{3}/CD\textsubscript{3}OD) \(\delta\) 175.2, 155.6, 146.3, 140.8, 134.4, 129.8, 121.4, 119.4, 118.5, 112.1, 110.5, 105.9, 79.8, 60.7, 54.7, 39.3, 28.3, 27.8, 21.1, 14.3; \textsuperscript{13}C-enriched peak: 175.2 IR (neat) 3368, 3087, 3053, 2974, 1712, 1502, 1460, 1164; HRMS (ESI/APCI) calcd for C\textsubscript{20}[\textsuperscript{13}C]H\textsubscript{29}N\textsubscript{2}O\textsubscript{4} (M+H) 374.2122, found 374.2117.
To a solution of $^{13}$C labeled acid 22 (820 mg, 2.19 mmol) in 22 mL MeCN was added $[^{13}$C]$_2$-$[^{15}$N]-L-proline ethyl ester 23 (317 mg, 2.19 mmol). HATU (1.25 g, 3.29 mmol) and $^3$Pr$_2$NEt (1.53 mL, 8.76 mmol) were added to the solution and the reaction stirred at room temperature for 4 hours. The reaction was quenched with 30 mL 1 M HCl and extracted with CH$_2$Cl$_2$ (3 x 40 mL). The combined organic layer was dried over Na$_2$SO$_4$ and concentrated under vacuum. The crude material was purified via flash column chromatography using 3:1 hexanes/ethyl acetate to afford a mixture of diastereomers (920 mg, 1.84 mmol, 84%). $^1$H NMR (300 MHz, CDCl$_3$) δ 8.16 (bs, 1H), 7.51-7.45 (m, 1H), 7.25-7.21 (m, 1H), 7.09-7.00 (m, 2H), 6.12 (dd, $J$ = 17.4, 10.5 Hz, 1H), 5.62 (d, $J$ = 8.4 Hz, 1H), 5.24-5.14 (m, 2H), 4.13-4.06 (m, 2H), 3.43-3.06 (m, 4H), 1.62 (s, 3H), 1.59 (s, 3H), 1.43 (s, 9H), 1.22-1.17 (m, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 172.1, 171.8, 171.4, 171.2, 145.6, 140.7, 133.9, 129.9, 121.9, 121.8, 119.5, 119.0, 118.8, 112.6, 112.5, 110.4, 106.2, 106.1, 79.4, 61.2, 60.6, 46.6, 46.5, 39.3, 31.2, 31.1, 30.1,
28.6, 28.3, 28.0, 27.5, 24.1, 22.1, 21.3, 14.4, 14.3. $^{13}$C-enriched peaks: 172.1, 171.8, 171.4, 171.2.

$[^{13}\text{C}]_2-[^{15}\text{N}]$-deoxybrevanamide E (7):

$[^{13}\text{C}]_2-[^{15}\text{N}]$-Peptide 24 (920 mg, 1.84 mmol) was dissolved in 3 mL CH$_2$Cl$_2$ and cooled to 0°C. TFA (3 mL) was added and the ice bath was removed. The reaction stirred at room
temperature for 3 hours. The reaction was quenched with saturated aqueous NaHCO₃ to pH 10, extracted with ethyl acetate (3 x 50 mL), and dried over Na₂SO₄. The organic layer was concentrated under vacuum. The crude amine (500 mg, 1.25 mmol) was dissolved in toluene (6.25 mL) and 2-hydroxypyridine (23 mg, 0.25 mmol) was added to the solution. The reaction was heated to reflux and stirred for 15 hours. The mixture was concentrated and the residue was diluted with 15 mL CH₂Cl₂. The organic layer was washed with 20 mL 1 M HCl, dried over Na₂SO₄, and concentrated. The crude material was purified via flash column chromatography with 3% MeOH in CH₂Cl₂ to afford 156.4 mg (35%) of the cis diastereomer 7. Cis: ¹H NMR (300 MHz, CDCl₃) δ 8.27 (bs, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.19-7.07 (m, 2H), 6.12 (dd, J = 17.4, 10.5 Hz, 1H), 5.70 (d, J = 4.8 Hz, 1H), 5.18 (d, J = 3.0 Hz, 1H), 5.13 (d, J = 3.9 Hz, 1H), 4.44 (d, J = 12.0 Hz, 1H), 4.06-4.04 (m, 1H), 3.77-3.55 (m, 3H), 3.23-3.13 (m, 1H), 2.37-2.30 (m, 1H), 2.18-1.85 (m, 3H), 1.54 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 169.5, 166.2, 166.0, 145.8, 141.7, 134.6, 129.2, 122.3, 120.3, 118.0, 112.9, 111.1, 104.6, 60.6, 59.7, 59.1, 59.0, 55.5, 55.4, 54.8, 54.7, 45.7, 45.5, 45.4, 39.2, 28.6, 28.1, 26.1, 22.8, 21.3, 14.4. ¹³C-enriched peaks: 169.5, 166.1 (d).
[\textsuperscript{13}C\textsubscript{2}-\textsuperscript{15}N]-Oxindole (10 and 16)

[\textsuperscript{13}C\textsubscript{2}-\textsuperscript{15}N]-deoxybrevianamide E

Triply labeled deoxybrevianamide E 7 (156 mg, 0.44 mmol) was dissolved in CH\textsubscript{2}Cl\textsubscript{2} (9 mL) and Davis oxaziridine (211 mg, 0.882 mmol) was added to the solution. The reaction stirred at room temperature for 18 hours. The mixture was concentrated and purified via flash column chromatography in 3% MeOH in DCM. The combined oxindoles were isolated and purified by reverse phase HPLC to afford the R (10, 27.5 mg) and S (16, 57.5 mg) isomers. 10: \textsuperscript{1}H NMR (400 MHz, acetone-\textit{d}\textsubscript{6}) \( \delta \) 7.27 (d, \( J = 7.8 \) Hz, 1H), 7.11 (dt, \( J = 7.8, 1.4 \) Hz, 1H), 6.76 (dt, \( J = 7.8, 1.4 \) Hz, 1H), 6.75 (d, \( J = 7.8 \) Hz, 1H), 6.48 (s, 1H), 6.33 (dd, \( J = 17.4, 10.6 \) Hz, 1H), 4.94 (dd, \( J = 17.4, 1.4 \) Hz, 1H), 4.88 (dd, \( J = 10.6, 1.4 \) Hz, 1H), 4.04 (m, 1H), 3.76 (m, 1H), 3.47 (m, 1H), 2.78 (dd, \( J = 13.3, 5.0 \) Hz, 1H), 2.54 (dd, \( J = 13.3, 7.3 \) Hz, 1H), 2.20 (m, 1H), 1.90-1.80 (m, 2H), 1.27 (s, 3H), 1.25 (s, 3H). MS (FAB) \textit{m/z} 371. 16: \textsuperscript{1}H NMR (400 MHz, acetone-\textit{d}\textsubscript{6}) \( \delta \) 7.16 (d, \( J = 6.9 \) Hz, 1H), 7.00 (dt, \( J = 7.8, 1.4 \) Hz, 1H), 6.65 (dt, \( J = 7.3, 0.9 \) Hz, 1H), 6.61 (d, \( J = 7.8 \) Hz, 1H), 6.53 (s, 1H), 6.51 (dd, \( J = 17.4, 10.6 \) Hz, 1H), 4.94 (dd, \( J = 17.4, 1.8 \) Hz, 1H), 5.01 (dd, \( J = 10.6, 1.8 \) Hz, 1H), 4.39 (dt, \( J = 8.3, 2.3 \) Hz, 1H), 4.16 (dt, \( J = 7.8, 2.8 \) Hz, 1H), 3.40 (ttt, \( J = 13.3, 6.0, 2.8 \) Hz, 1H), 3.16 (ttt, \( J = 12.4, 2.3, 1.8 \) Hz, 1H).
7.8, 4.6 Hz, 1H), 2.96 (dt, $J = 11.4, 7.3$ Hz, 1H), 2.62 (ttt, $J = 13.3, 7.0, 5.5$ Hz, 1H), 2.01 (m, 1H), 1.95 (m, 1H), 1.72 (m, 1H), 1.60 (m, 1H), 1.39 (s, 3H), 1.35 (s, 3H); $^{13}$C NMR (100 MHz, Acetone-$d_6$) $\delta$ 169.8, 165.4, 149.7, 146.2, 132.5, 130.1, 125.6, 119.8, 112.4, 111.1, 95.4, 88.3, 60.4, 60.0, 45.7, 36.4, 27.7, 25.4, 24.7, 23.7. MS (FAB) $m/z$ 371.
Deoxybrevianamide E 7 (220 mg, 1.25 mmol) was dissolved in CH₂Cl₂ (12.5 mL) and Davis oxaziridine (300 mg, 2.5 mmol) was added to the solution. The reaction stirred at room temperature for 18 hours. The mixture was concentrated and purified via flash column chromatography in 3% MeOH in DCM. The combined oxindoles were isolated and purified a second time via reverse phase prep-TLC (10% acetone in H₂O). The plate was run 4 times and the R (62.2 mg, 0.17 mmol, 14%) and S (124 mg, 0.34 mmol, 27%) isomers were each isolated. 10: ¹H NMR (300 MHz, CDCl₃) δ 7.29, (d, J = 1.5 Hz, 1H), 7.22-7.17 (m, 1H), 6.89-6.73 (m, 2H), 6.32 (dd, J = 17.7, 10.8 Hz, 1H), 5.14-5.04 (m, 2H), 3.93 (t, J = 8.1 Hz, 1H), 3.72 (dd, J = 11.4, 7.8 Hz, 1H), 3.60-3.47 (m, 2H), 2.89 (dd, J = 13.2, 11.4 Hz, 1H), 2.68-2.62 (m, 1H), 2.33-2.24 (m, 1H), 2.15-1.77 (m, 4H), 1.28 (s, 3H), 1.24 (s, 3H); ¹³C NMR (100 MHz, acetone-d₆) δ 169.12, 164.7, 149.0, 145.5, 1311.9, 129.5, 125.0, 119.2, 111.7, 110.4, 94.8, 87.6, 59.6, 59.3, 45.0, 35.7, 27.1, 24.7, 24.1, 23.0; HRMS (ESI/APCI) calcd for C₂₁H₂₆N₅O₃ (M+H) 368.1969, found 368.1972.
References:

**General Feeding Experimental Considerations**

$^1$H and $^{13}$C NMR spectra were measured on 400 MHz JEOL JNM-ECX 400 spectrometer in acetone-$d_6$ ($^1$H, 2.04 ppm, $^{13}$C, 29.8 ppm). Mass spectra were obtained on a BRUKER esquire3000 mass spectrometer. CD spectra were taken on a JASCO J-720 spectropolarimeter.