Efficient Total Synthesis of (+)-Negamycin, a Potential Chemotherapeutic Agent for Genetic Diseases.

Yoshio Hayashi,¹,² Tomas Regnier,¹ Shigenobu Nishiguchi,¹ Magne O. Sydnes,¹ Daisuke Hashimoto,³ Junya Hasegawa,³ Takahiro Katoh,³ Tetsuya Kajimoto,³ Masataka Shiozuka,⁴ Ryoichi Matsuda,⁴ Manabu Node,³ Yoshiaki Kiso.¹

¹Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Kyoto 607-8412, Japan; ²Department of Medicinal Chemistry, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo 192-0392, Japan; ³Department of Pharmaceutical Manufacturing Chemistry, Kyoto Pharmaceutical University, Kyoto 607-8412, Japan; ⁴Department of Life Science, The University of Tokyo, Meguro-ku, Tokyo 153-8902, Japan.

Supporting information

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p2 --- Additional data

p3-p7 --- Experimental part

p8-p23 --- NMR Data
### Table A. Optimization for CM reaction

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Conditions</th>
<th>Conversion (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>3/6 ratio&lt;sup&gt;b&lt;/sup&gt;</th>
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</thead>
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<tr>
<td>1</td>
<td>[Ru]-I</td>
<td>rt/24 h</td>
<td>50</td>
<td>44/66</td>
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<tr>
<td>2</td>
<td>[Ru]-I</td>
<td>reflux/5 h</td>
<td>36</td>
<td>41/59</td>
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<tr>
<td>3</td>
<td>[Ru]-I</td>
<td>μW/15 min</td>
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<td>24/76</td>
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<tr>
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<td>[Ru]-II</td>
<td>rt/24 h</td>
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<td>97/3</td>
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<tr>
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<td>[Ru]-II</td>
<td>reflux/5 h</td>
<td>96</td>
<td>&gt;99/traces</td>
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<tr>
<td>6b</td>
<td>[Ru]-II</td>
<td>reflux/5 h</td>
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<td>95/5</td>
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<td>7</td>
<td>[Ru]-II</td>
<td>μW/15 min</td>
<td>100 (83)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>100/0</td>
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<tr>
<td>8b</td>
<td>[Ru]-II</td>
<td>μW/15 min</td>
<td>96</td>
<td>97/3</td>
</tr>
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</table>

<sup>a</sup>Reagents and conditions: (i) tert-butyl acrylate, [Ru] 5 mol %, CH₂Cl₂. <sup>b</sup>1 equiv of tert-butyl acrylate. <sup>c</sup>Measured by 1H NMR of the crude mixture. <sup>d</sup>Isolated yield (SiO₂ column chromatography, eluent : hexane/ethyl acetate = 80/20).
EXPERIMENTAL PART

1. General information
NMR spectra (\(^{1}\)H and \(^{13}\)C) were recorded on a JEOL JNM-AL300 (\(^{1}\)H:300 MHz; \(^{13}\)C: 75.5 MHz) or a Varian UNITY INOVA 400NB (\(^{1}\)H:400 MHz; \(^{13}\)C:100 MHz) spectrometer and the chemical shift values were expressed in parts per million downfield from tetramethysilane (TMS) as an internal standard. All coupling constants (\(J\) values) were reported in Hertz (Hz). Melting points were taken on a micro hot-stage apparatus (Yanagimoto). Mass spectra (MS) were obtained by electron impact (EI, CI) ionization methods on JEOL GCmate MS-BU20. Elemental analyses were done on a Perkin-Elmer Series CHNS/O Analyzer 2400. Specific Rotations were recorded on a Horiba High-speed Accurate Polarimeter SEPA-300 with a sodium lamp and are reported as follows: \([\alpha]_D^T\) (c g/100 mL, solvent).

Microwave experiments were carried out with a CEM Discover oven. Preparative HPLC was carried out using a Waters 600E system equipped with a UV detector. Organic extracts were dried over sodium sulfate (Na\(_2\)SO\(_4\)), filtered, and concentrated using a rotary evaporator at <40 °C bath temperature. Solids and non volatile oils were vacuum dried at <2 mmHg.

2. Materials
Commercially available chemicals were obtained from Waco Pure Chemical Industries, Ltd. (Osaka, Japan), Nacalai Tesque, Inc. (Kyoto, Japan), Aldrich Chemical Co., Inc. (Milwaukee, WI), Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), Kishida Chemical Co., Ltd. (Osaka, Japan), and used without further purification. Column chromatography was carried on Merck 107734 silica gel 60 (70-230 mesh). Analytical thin layer chromatography (TLC) was performed using Merck 105715 silica gel 60 F\(_{254}\) precoated plates (0.25 mm thickness) and compounds were visualized by UV illumination (254 nm) and by heating after spraying ca. 0.7% ethanolic solution of ninhydrin.

3. Total synthesis of (\(-\))-Negamycin

3-1. (\(R\))-tert-Butyl 5-allyl-2,2-dimethyloxazolidine-3-carboxylate (4)
To a solution of B-allyl-diisopinocampheylborane [(+)\-Ipc\(_2\)B(allyl)] (4 mL of 1M solution in pentane, 4 mmol) dissolved in Et\(_2\)O (4 mL) was added at -100°C a solution of N-Boc-2-aminoacetaldehyde (636 mg, 4 mmol) dissolved in Et\(_2\)O cooled to -78°C (4 mL). The reaction mixture was stirred for 2 hours at -100°C and MeOH (600 \(\mu\)L), 1N NaOH (4.8 mL) and H\(_2\)O\(_2\) (450 \(\mu\)L) were added. Oxidation was completed by refluxing 3h before addition of water for extraction. The aqueous phase was washed twice with EtOAc, the organic layers were combined and dried over Na\(_2\)SO\(_4\). After filtration and evaporation of the solvent under reduced pressure, the residue was dissolved in acetone and both 2,2-
dimethoxypropane and boron trifluoride, ethyl ether complex were added at room temperature. The reaction mixture was stirred under reflux at 66 °C for over night. The solution was poured into sat. NaHCO₃ aq., and acetone was removed under reduced pressure. The resulting residue was extracted with AcOEt and washed consecutively with sat. NaHCO₃ aq., brine, and dried over Na₂SO₄. The solvent was removed under reduced pressure, the residue was applied to silica-gel column chromatography (CHCl₃:MeOH=15:1) to yield the desired compound 4 with 90% yield as colourless oil. ¹H NMR (300 MHz, CDCl₃) δ 7.89-7.74 (m, 1H), 5.16-5.08 (m, 2H), 4.17-4.05 (m, 1H), 3.55-3.73 (m, 1H), 3.05-3.15 (m, 1H), 2.23-2.50 (m, 2H), 1.46 (br s, 15H); [α]D²⁴.⁹ -46.8° (c 1.00, CHCl₃); HRMS (EI+) calculated for C₁₃H₂₃NO₃ (M⁺) 241.3270, found 241.3300.

3-7. (S)-tert-Butyl 5-[(E)-3-(tert-butoxycarbonyl)allyl]-2,2-dimethyloxazolidine-3-carboxylate (3). In a microwave reactor, to a solution of Grubbs catalyst second generation (10.5 mg, 0.0125 mmol) dissolved in CH₂Cl₂ (250 µL) was added dropwise a mixture of 4 (60 mg, 0.25 mmol) and tert-butyl acrylate (108 µL, 1.25 mmol) in CH₂Cl₂ (250 µL). The reaction mixture was irradiated for 15 minutes (Temperature set to 40 °C and automatically controlled by the oven). The residue was then applied to silica-gel column chromatography (hexane:AcOEt=10:1) to yield the desired compound 3 as light-yellow oil

¹H NMR (400 MHz, CDCl₃) δ 6.82 (td, J = 7.0, 16.0 Hz, 1H), 5.85 (d, J = 15.7 Hz, 1H), 4.19-4.14 (m, 1H), 3.73-3.65 (m, 1H), 3.13-3.04 (m, 1H), 2.55-2.42 (m, 2H), 1.57 (br s, 3H), 1.54 (br s, 3H), 1.48 (s, 18H); ¹³C NMR (101 MHz, CDCl₃) δ 165.5, 152.2, 141.9, 125.8, 93.7, 93.3, 80.4, 79.6, 72.2, 72.0, 50.5, 35.6, 28.4, 28.1, 27.2, 26.2, 25.3, 24.3; [α]D²⁴.⁶ -26.2° (c 1.06, CHCl₃); HRMS (EI+) calculated for C₁₈H₃₁N₁O₅ (M⁺) 341.2202, found 341.2200.


A 2.6M solution of n-butyllithium in hexane (112 µL) was added to a solution of 7 (80.0 mg, 0.298 mmol) in tetrahydrofurane (1.0 mL) and the mixture was stirred at -50 °C. After passing 1 hour, a solution of 3 (50 mg, 0.146 mmol) in tetrahydrofurane (0.7 mL) was added dropwise to the reaction mixture, which was stirred at -40 °C for another 2 hours. After quenching the reaction by adding a saturated solution of sodium sulfate, the mixture was extracted with chloroform. The organic layer was washed with a saturated solution of sodium chloride, dried over anhydrous sodium sulfate, and condensed in vacuo. The residue was purified by silica gel column chromatography (n-hexane : ethyl acetate = 10:1) to afford compound 8 (72.1 mg, 80.4%) accompanying with the recovered starting material 3 (8.3 mg, 16.6%).
3.9. *(R)-tert-Butyl-5-((R)-3-(tert-butoxycarbonyl)-2-aminopropyl)-2,2-dimethyl-oxazolidine-3-carboxylate (9).

Under Ar atomosphere, the solution of tert-butyl ester 8 (200.00 mg, 0.43 mmol) in anhydrous CH₂Cl₂ (3 mL) was added to NIS (293.00 mg, 1.30 mmol) light shielding at room temperature. The reaction mixture was stirred at room temperature for 2 h. The solution was poured into sat. NaHCO₃ aq., and the organic phase was extracted with CHCl₃, wash with 10% Na₂S₂O₃ aq. and brine, and dried over Na₂SO₄. The solvent was removed under reduced pressure, the residue was applied to silica-gel column chromatography (hexane:AcOEt=20:1) to yield the desired compound 9 as light-yellow oil (80.4 mg, 71%). ¹H NMR (400 MHz, CDCl₃) δ 4.25-4.18 (m, 1H), 3.73-3.65 (m, 1H), 3.40-3.33 (m, 1H), 3.08-3.06 (m, 1H), 2.44 (dd, J = 4.0, 15.6 Hz, 1H), 2.24 (dd, J = 8.8, 15.6 Hz, 1H), 1.74 (br s, 2H), 1.69 (dd, J = 4.6, 7.9 Hz, 1H), 1.64-1.61 (m, 1H), 1.57 (br s, 3H), 1.53 (br s, 3H), 1.47 (s, 9H), 1.46 (s, 9H); ¹³C NMR (101 MHz, CDCl₃) δ 171.5, 152.2, 93.4, 92.9, 80.7, 80.0, 79.4, 71.2, 70.9, 51.1, 45.8, 44.1, 40.4, 28.4, 28.1, 27.3, 26.3, 25.2, 24.3; [α]D²⁴⁶⁺ -14.0° (c 1.01, CHCl₃); HRMS (EI⁺) calculated for C₃₈H₆₄N₂O₆ (M⁺) 614.4294, found 614.4286.

3-10. *(R)-tert-Butyl-5-((R)-3-(tert-butoxycarbonyl)-2-tert-butoxycarbonyl-aminopropyl)-2,2-dimethyl oxazolidine-3-carboxylate (11).

To solution of amine 9 (82.40 mg, 0.23 mmol) in THF (2.3 mL) was added to (Boc)₂O (64.2 mg, 0.29 mmol) and Et₃N (48.00 µL, 0.35 mmol) dropwise at 0 °C. The reaction mixture was stirred at room temperature for 5 h. The solvent was removed under reduced pressure. The resulting residue was extracted with AcOEt and washed consecutively with 10% citric acid aq., sat. NaHCO₃ aq., brine, and dried over Na₂SO₄. The solvent was removed under reduced pressure, the residue was applied to silica-gel column chromatography (CHCl₃) to yield the desired compound 11 as light-yellow oil (105.2 mg, 99%). ¹H NMR (400 MHz, CDCl₃) δ 5.23 (br s, 1H), 4.17-4.10 (m, 1H), 4.08-3.92 (m, 1H), 3.78-3.58 (m, 1H), 3.04 (t, J = 9.0 Hz, 1H), 2.50 (d, J = 5.7 Hz, 2H), 1.92-1.69 (m, 2H), 1.56 (s, 3H), 1.52 (s, 3H), 1.47 (s, 9H), 1.45 (s, 9H), 1.44 (s, 9H); ¹³C NMR (101 MHz, CDCl₃) δ 170.9, 155.1, 151.8, 93.3, 93.0,
3-11. (R)-4-[(R)-3-(tert-Butoxycarbonyl)-2,2-dimethyloxazolidin-5-yl]-3-(tert-butoxycarbonylamino)butanoic acid (2).

To solution of tert-butyl ester 11 (105.2 mg, 0.23 mmol) in 2M KOH in MeOH (4 mL) was irradiated under microwave cavity with an output at 10 W for 15 min at 100 °C. The solution was poured into 10 % citric acid aq. And the organic phase was extracted with AcOEt, wash brine, and dried over Na₂SO₄. The solvent was removed under pressure. The resulting light-yellow oil (92.1 mg, 99%) was used for next reaction without purification.

3-12. (R)-tert-Butyl-(R)-5-[2-(tert-butoxycarbonylamino)-3-{(tert-butoxycarbonyl methyl)methylaminocarbamoyl}propyl]-2,2-dimethyloxazolidine-3-carboxylate (13).

Under Ar atmosphere, the solution of acid 2 (73.8 mg, 0.183 mmol) and tert-butyl-2-(N-methylhydrazin)acetate·PTSA 12 (118.2 mg, 0.37 mmol) and HOBt (56.0 mg, 0.37 mmol) in anhydrous CH₂Cl₂ (2.5 mL) was cooled to 0 °C, and Et₃N (51 µL, 0.09 mmol) was added dropwise, then EDC·HCl was added. The reaction mixture was stirred for 2 h at room temperature. The solution was poured into 10 % citric acid aq., and the organic phase was extracted with AcOEt, wash with sat. NaHCO₃ aq. brine, and dried over Na₂SO₄. The solvent was removed under reduced pressure, the residue was applied to silica-gel column chromatography (CHCl₃:MeOH=50:1) to yield the desired compound 13 as light-yellow oil (99.6 mg, 99%). ¹H NMR (400 MHz, CDCl₃) δ 7.93, 7.33 (br s, total 1H), 5.74-5.22 (m, 1H), 4.16-4.09 (m, 1H), 3.99 (br s, 1H), 3.56 (d, J = 5.6 Hz, 2H), 3.06-3.02 (m, 1H), 2.76, 2.73 (s, total 3H), 2.47-2.36 (m, 2H), 1.98 (br s, 1H), 1.71-1.68 (m, 2H), 1.59 (br s, 3H), 1.55 (br s, 3H), 1.48 (s, 9H), 1.47 (s, 9H), 1.43 (s, 9H); ¹³C NMR (101 MHz, CDCl₃) δ 174.5, 170.0, 155.2, 152.1, 151.7, 93.4, 93.1, 82.3, 82.1, 79.9, 79.3, 79.1, 78.8, 71.8, 59.2, 58.2, 50.9, 46.6, 44.9, 43.8, 38.5, 37.8, 37.0, 28.34, 28.29, 28.0, 27.2, 26.1, 25.0, 24.2; [α]D-24.2 -5.5 ° (c 1.07, CHCl₃); m.p. 135-136 °C; HRMS (CI+) calculated for C₂₆H₄₈N₄O₈ (M⁺+H) 544.3472, found 544.3470.

3-13. 2-[(3R,5R)-3,6-Diamino-5-hydroxy-hexanoyl]-1-methylhydrazinoacetic acid. [(+)-Negamycin]

The compound 13 (19.0 mg, 0.04 mmol) was treated with 4M HCl/dioxane (2 mL) at 0 °C. The reaction mixture was stirred at same temperature for 2 h. The solvent was removed under pressure; the remaining crude light-yellow oil was purified by ion exchange chromatography on Amberlite CG50
(NH₄⁺ form) or by preparative HPLC. ¹H NMR (400 MHz, D₂O) δ 3.97-3.89 (m, 1H), 3.55-3.49 (m, 1H), 3.24 (s, 2H), 2.97 (dd, J = 13.2, 3.2 Hz, 1H), 2.80 (dd, J = 13.2, 9.2 Hz, 1H), 2.48 (s, 3H), 2.39-2.36 (m, 2H), 1.79-1.56 (m, 2H); ¹³C NMR (101 MHz, CDCl₃) δ 176.8, 170.8, 64.3, 60.9, 45.5, 44.3, 43.8, 41.8, 39.3; [α]D⁰²⁵ +2.4° (c 0.36, H₂O), lit. [α]D²⁹ +2.5° (c 2.00, H₂O); m.p. 135-136 °C; HRMS (FAB+) calculated for C₉H₁₂N₄O₄ (M⁺+H) 249.1563, found 249.1559.

3.14. tert-Butyl 2-(N-methylhydrazino)acetate. PTSA (12)
At 0 °C, Et₃N (11.1 mL, 79.31 mmol) and tert-butyl 2-bromoacetate (1.0 M solution in CH₂Cl₂, 8.4 mL, 56.56 mmol) were added dropwise to a solution of methylhydrazine (3.0 mL, 56.56 mmol) in CH₂Cl₂ (190 mL). The reaction mixture was then stirred at room temperature overnight. Solvent was removed under reduced pressure and residue is dissolved with an AcOEt/MeOH mixture (15:1), filtrated and the resulting solution was evaporated under reduced pressure. The residue was applied to silica-gel column chromatography (AcOEt/MeOH =15:1) to yield the desired compound as colorless oil (7.5 mg). The pure compound was then dissolved in THF (70.0 mL) and p-toluenesulfonique acid (8.864g, 46.60 mmol) was added. The reaction mixture was stirred at this temperature for 30 minutes before filtration. The cake was washed with Et₂O. The solution was then evaporated under reduced pressure to yield the desired compound 12 as a white powder (10.4 g, 50% for 2 steps) ¹H NMR (400 MHz, CDCl₃) δ 7.77 (d, J = 9.0 Hz, 2H), 7.16 (d, J = 9.0 Hz, 2H), 3.73 (s, 2H), 2.90 (s, 3H), 2.36 (s, 3H), 1.43 (s, 9H); ¹³C NMR (101 MHz, CDCl₃) δ 168.1, 141.5, 140.4, 128.9, 126.0, 83.5, 57.7, 43.9, 27.9, 21.3; m.p. 131-133 °C; HRMS (EI+) calculated for C₇H₁₆N₂O₂ (M⁺) 160.1212, found 160.1214; Anal. Cald for C₇H₁₆N₂O₂: C, 50.58; H, 7.28; N. 8.48; found C, 50.48; H, 7.35; N. 8.36.
compound 4