Electronic Supporting Information (ESI)

A Formal [3+3]-Annulation-based Approach to Pancratistatins: Total Synthesis of (+/-)-7-Deoxy-pancratistatin and its 2-epi and 2,4-di-epi analogues

Olaia Nieto-García, Hugo Lago-Santomé, Fernando Cagide-Fagín, Juan Carlos Ortiz-Lara and Ricardo Alonso*

Departamento de Química Orgánica, Universidad de Santiago, 15782 Santiago de Compostela, Spain.
E-mail: r.alonso@usc.es; Fax: (34) 981 595012; Tel: (34) 981 547085

Currently at the University of Porto: CIQ/Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal.


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**General Remarks**

Solvents for moisture-sensitive reactions were distilled and dried according to standard procedures. Anhydrous Na$_2$SO$_4$ was used to dry organic solutions during workups. Reagents were purchased in the highest available commercial quality and used as supplied except where noted. Reactions were monitored by TLC with pre-coated silica gel 60 F254 aluminum plates (Merck KGaA, Darmstadt) using UV light as the visualizing agent and by dipping the plate into a solution of (NH$_4$)$_6$Mo$_7$O$_{24.4}$H$_2$O (12.5 g) and Ce(SO$_4$)$_2$.4 H$_2$O (5 g) in 10% aqueous H$_2$SO$_4$ (500 mL), followed by heating. Flash column chromatography was performed with silica gel (0.035-0.070 mm, 60 Å) from Merck. Concentrations were carried out in a rotary evaporator. $^1$H, $^{13}$C, DEPT and 2D NMR were recorded using either Bruker DPX-250, AMX-300 and WM-500 spectrometers, or Varian Mercury 300 and Inova 400 spectrometers, as indicated; chemical shifts are reported in ppm and coupling constants in Hz; multiplicities are given as follows: s (singlet), br s (broad singlet), br d (broad doublet), d (doublet), t (triplet), and m (multiplet). Mass spectra were recorded on Micromass Autospec, TRACE MS and HP-5988-A spectrometers using Electronic Impact (EI, 70 eV), Electrospray (ESI) or Chemical Ionization (CI). IR spectra were recorded on BIO RAD FTS135 and MATTSON CYGNUS-100 spectrometers. Melting points were determined on a Büchi apparatus (Dr. Tottoli, Flawil, Switzerland).
Experimental procedures

Re face-Reduction at C2: MODEL STUDIES on 18 and PRELIMINARY STUDIES with 12a

Nitrocompound (+/-)-20a.

Isobutyraldehyde (550 µL, 6.05 mmol) and p-TsOH·H₂O (13 mg, 0.07 mmol) were added to a solution of 18¹ (100 mg, 0.34 mmol), in dry CH₂Cl₂ (2.5 mL) under argon. After stirring for 1.5 h at rt, the pH of the mixture was adjusted to 7 by adding Et₃N and the volatiles were evaporated in vacuo. Chromatography (silica gel, 15% EtOAc/hexane) gave 20a (91 mg, 71%) as a yellow oil: Rᶠ = 0.44 (30% EtOAc/hexane);¹³C NMR (CDCl₃, 100 MHz) δ: 147.3, 142.6, 110.8, 110.2, 109.4, 107.3, 101.8, 85.0, 80.4, 77.6, 76.6, 40.6, 31.6, 31.6, 17.3, 17.3, 17.1, 17.0; LRMS (Cl) m/z (%): 384.2 [(M+H)⁺, 5], 366.2 (25), 193.0 (100); HRMS [Cl, (M+H)⁺] m/z: calc. for (C₁₈H₂₆NO₈): 384.1658, found: 384.1658.

Nitrocompound (+/-)-20b.

Treatment of 12a\(^2\) (300 mg, 0.85 mmol) under the same conditions reported to convert 18 into 20a, afforded 20b (310 mg, 83%) as a white solid: \(R_f = 0.43\) (30% EtOAc/hexane); \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) 6.80 (d, \(J = 1.6 \text{ Hz}, 1\text{H})\), 6.77-6.61 (m, 2H), 5.93 (s, 2H), 5.27 (dd, \(J = 12.5, 7.5 \text{ Hz}, 1\text{H})\), 4.95 (d, \(J = 5.5 \text{ Hz}, 1\text{H})\), 4.85-4.68 (m, 2H), 4.42 (d, \(J = 8.1 \text{ Hz}, 1\text{H})\), 4.95 (d, \(J = 5.5 \text{ Hz}, 1\text{H})\), 4.20 (d, \(J = 3.2 \text{ Hz}, 1\text{H})\), 3.25 (dd, \(J = 12.5, 3.2 \text{ Hz}, 2\text{H})\), 2.06-1.94 (m, 2H), 1.10-0.97 (m, 12H); \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\): 147.8 (2C), 127.0, 123.6, 110.2, 109.5, 108.4, 107.3, 101.9, 101.3, 86.2, 82.2, 78.2, 77.0, 46.2, 31.7, 31.6, 17.5, 17.3 (2C), 17.0; LRMS (CI) m/z (%): 437.2 (M\(^+\), 3); HRMS (CI, M\(^+\)) m/z: calc. for (C\(_{21}\)H\(_{27}\)NO\(_9\)): 438.1764, found: 438.1761.

**Diols (+/-)-22a.**

A solution of 20a (100 mg, 0.26 mmol) in dry 1,2-dichloroethane (1 mL) was added to a solution of NaBH(AcO)\(_3\) (221 mg, 1.04 mmol) in the same solvent (4 mL) under argon. After stirring for 24 h at 45 °C, the solution was quenched with 30% aqueous hydrogen peroxide (106 µL), neutralized with 5% aqueous KOH (pH = 7) and extracted with CH\(_2\)Cl\(_2\) (3 x 1 mL). Chromatography (20% EtOAc/hexane) afforded 1,2-trans-22a [27 mg, 33%, \(R_f = 0.30\) (40% EtOAc/hexane)] and 1,2-cis-22a [52.65 mg, 64%, \(R_f = 0.23\) (40% EtOAc/hexane)] as white solids.

1,2-trans-22a: \(^1\)H NMR (CDCl\(_3\), 250 MHz) \(\delta\): 7.39 (d, \(J = 1.0 \text{ Hz}, 1\text{H})\), 6.38 – 6.22 (m, 2H), 5.06 (dd, \(J = 12.2, 8.5 \text{ Hz}, 1\text{H})\), 4.85-4.72 (m, 2H), 4.52-4.38 (m, 1H), 4.33-4.22 (m, 1H), 4.20-4.12 (m, 1H), 3.84 (dd, \(J = 12.2, 2.7 \text{ Hz}, 1\text{H})\), 2.45 (d, \(J = 5.4 \text{ Hz}, 1\text{H})\), 2.20 (m, 1H), 2.02-1.85 (m, 1H), 1.07-0.97 (m, 6H); \(^1\)C

\(^2\) A detailed preparation procedure and full characterization data for 12a can be found in: J. C. Ortiz, L. Ozores, F. Cagide-Fagin and R. Alonso, Chem. Comm., 2006, 4239-4241
NMR (CDCl₃, 100 MHz) δ: 143.0, 110.8, 109.0, 88.0, 79.9, 75.0, 72.4, 68.5, 39.3, 32.1, 17.0, 16.7;
LRMS (CI) m/z (%): 314.2 [(M+H)+, 40], 267.2 (27), 249.2 (11); HRMS [CI, (M+H)+] m/z: calc. for (C₁₄H₂₀NO₇): 314.1239, found: 314.1227.

1,2-cis-22a: ¹H NMR (CDCl₃, 400 MHz) δ: 7.36 (dd, J = 4.9, 4.3 Hz, 1H), 6.37-6.23 (m, 2H), 5.07 (dd, J = 12.6, 8.3 Hz, 1H), 4.82 (d, J = 4.5 Hz, 1H), 4.64 (dd, J = 8.3, 5.5 Hz, 1H), 4.43 (t, J = 5.5 Hz, 1H), 4.32-4.24 (m, 1H), 4.08-3.97 (m, 1H), 3.36 (dd, J = 12.6, 1.6 Hz, 1H), 3.01 (d, J = 8.1 Hz, 1H), 2.61 (d, J = 4.6 Hz, 1H), 2.02-1.91 (m, 1H), 1.07-0.9; (m, 6H); ¹³C NMR (CDCl₃, 100 MHz) δ: 149.1, 142.8, 110.6, 109.9, 108.5, 87.0, 78.1, 75.9, 71.1, 68.4, 41.6, 31.9, 16.9, 16.5; LRMS (CI) m/z (%): 314.2 [(M+H)+, 100], 267.2 (40), 249.2 (37); HRMS [CI, (M+H)+] m/z: calc. for (C₁₄H₂₀NO₇): 314.1239, found: 314.1239.

X Ray for 1,2-cis-22a
**Acetate (+/-)-23.**

To a solution of the diol 1,2-cis-22a (10 mg, 0.032 mmol) in dry CH₂Cl₂ (1 mL) under argon, Et₃N (22 µL, 0.16 mmol), Ac₂O (7.5 µL, 0.080 mmol) and 4-DMAP (1 mg, 0.006 mmol) were added. After stirring for 30 min at rt, the solution was neutralized with 0.1 M HClₐq (0.8 mL) and extracted (CH₂Cl₂, 3 x 1 mL). Chromatography (30% EtOAc/hexane) gave 1,2-cis-23 (11 mg, 91%) as a yellow solid: Rₓ = 0.38 (30% EtOAc/hexane); ¹H NMR (CDCl₃, 400 MHz) δ: 7.31 (dd, J = 1.8, 0.8 Hz, 1H), 6.26 (dd, J = 3.3, 1.8 Hz, 1H), 6.16 (dd, J = 3.3, 0.8 Hz, 1H), 5.56–5.53 (m, 1H), 5.36–5.25 (m, 1H), 5.11 (dd, J = 12.6, 8.4 Hz, 1H), 4.88 (d, J = 3.9 Hz, 1H), 4.67 (dd, J = 8.4, 5.4 Hz, 1H), 4.46–4.37 (m, 1H), 3.63 (dd, J = 12.6, 2.3 Hz, 1H), 2.11 (s, 3H), 2.09–2.00 (m, 4H), 1.13–1.03 (m, 6H); ¹³C NMR (CDCl₃, 100 MHz) δ: 170.1, 169.6, 147.5, 143.2, 110.6, 110.4, 108.6, 87.1, 76.0, 75.9, 68.6, 68.0, 40.0, 31.8, 20.8 (2C), 17.0, 16.3; LRMS (EI) m/z (%): 398.3 [(M+H)+, 3].

**Selected NOE data for 1,2-cis-23**
Diols (+/-)-22b.

Treatment of 20b (30 mg, 0.07 mmol) with NaBH(AcO)$_3$, as reported for 20a, afforded a mixture of 1,2-trans-22b (8.4 mg, 0.02 mmol, 34%) and 1,2-cis-22b (14.5 mg, 0.04 mmol, 58%). Yields were determined by $^1$H NMR integration with 1,4-dichlorobenzene as internal standard. $^1$H NMR (CDCl$_3$, 250 MHz) $\delta$: 6.89 (s, 1H cis, 1H trans), 6.74 (s, 2H cis, 2H trans), 5.94 (s, 2H cis, 2H trans), 5.18-5.02 (m, 1H cis, 1H trans), 4.90-4.73 (m, 1H cis, 2H trans), 4.71-4.60 (m, 1H cis), 4.50-4.35 (m, 1H cis, 1H trans), 4.31-4.24 (m, 1H trans), 4.04 (br s, 2H cis), 3.95 (br s, 1H trans), 3.52 (dd, $J = 12.7$, 2.0 Hz, 1H trans), 3.02 (br d, $J = 12.6$ Hz, 1H cis), 2.08-1.88 (m, 1H cis, 1H trans), 1.11-0.97 (m, 6H cis, 6H trans).

Diols (+/-)-26-28.

NaBH$_4$ (300 mg, 7.94 mmol) was added to a solution of 12a (2.59 g, 7.38 mmol) in MeOH (50 mL) under argon and the mixture stirred for 15 min. A solution of H$_2$O/acetone (8:2, 50 mL) was then added. After stirring for 30 min, the volatiles were evaporated under vacuum, the crude dissolved in H$_2$O/EtOAc (1:1,
and the mixture stirred for 12 h at rt and extracted with EtOAc (3 x 30 mL). Chromatography (50% EtOAc/hexane) afforded 26 (1.195 g, 3.39 mmol, 46%, \( R_f = 0.36 \)) and 27 (0.916 g, 2.59 mmol, 35%, \( R_f = 0.5 \)) as white solids, and a mixture of 28 and putative aldehyde 29 (0.104 g, \( R_f = 0.28 \)) in a 3:0.8 ratio. A mixture of 28 (10 mg, 0.03 mmol) and 29 (10 mg, 0.03 mmol) was dissolved in dry acetone (1 mL), treated with DBU (10 \( \mu \)L, 0.07 mmol), stirred for 40 min, quenched with a saturated aqueous solution of \( \text{NH}_4 \text{Cl} \) (5 mL) and extracted with \( \text{CH}_2\text{Cl}_2 \) (3 x 5 mL). Chromatography (50% EtOAc/hexane) afforded 27 (10 mg, 100% from 29) and unreacted 28 (10 mg) as white solids.

\textit{Putative aldehyde intermediate (\(+/-\)-29)}: \textit{\textsuperscript{1}H} NMR (300 MHz, \( \text{CDCl}_3 \)) \( \delta \) 9.67 (s, 1H), 6.96-6.70 (m, 3H), 5.95 (s, 2H), 4.80 (dd, \( J = 13.1 \), 5.3 Hz, 1H), 4.69 (dd, \( J = 13.1 \), 9.5 Hz, 1H), 3.99 (d, \( J = 9.5 \), 9.5 Hz, 1H), 3.85 (ddd, \( J = 9.5 \), 5.3, 2.9 Hz, 1H), 3.53 (dt, \( J = 9.5 \), 9.5, 2.4 Hz, 1H), 2.82 (d, \( J = 2.4 \) Hz, 1H), 1.49 (s, 3H), 1.48 (s, 3H).

\textit{Amine (\(+/-\)-17. B)} From 12a in 3 steps (12a \( \rightarrow \) 26 \( \rightarrow \) 33 \( \rightarrow \) 17) and 74% overall yield (Scheme 5)

\[ \begin{align*}
\text{26} & \xrightarrow{\text{2,2-DMP}} \text{33} \\
\text{acetone, 89\%} & \xrightarrow{\text{HCO}_2\text{NH}_4} \text{17}
\end{align*} \]

\( ^3 \) Experimental details for the first step, the conversion of 12a into 26, are described in the experimental section of the article.
2,2-Dimethoxypropane (14 mL, 11.4 mmol) and p-TsOH.H$_2$O (544 mg, 2.86 mmol) were added to a solution of 26 (505 mg, 1.43 mmol) in distilled acetone (30 mL) at rt under argon. After stirring for 2 h, the pH was adjusted to 7 with a saturated aqueous solution of NaHCO$_3$ and Et$_3$N. Then, the solvent was partially concentrated and subsequently extracted with CH$_2$Cl$_2$ (2 x 50 mL). The combined organic layers were dried and concentrated. Chromatography (silica gel, 20% EtOAc/hexane) afforded 33 (503 mg, 89%). Distilled MeOH (5 mL) was added to a mixture of 33 (223 mg, 0.57 mmol), 10% Pd/C (400 mg) and HCO$_2$NH$_4$ (429 mg, 6.80 mmol). The reaction mixture was stirred for 18 h and filtered over a Celite pad washing with MeOH (2 x 10 mL). Volatiles were completely removed in vacuo and the residue was purified by flash chromatography (silica gel, MeOH/EtOAc 1:9), which afforded 17 (185 mg, 89%).

(+/-)-33: mp = 201-202 °C (CH$_2$Cl$_2$/hexane); $R_f$ = 0.54 (30% AcOEt/hexane); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.77 (s, 1H), 6.70 (d, $J = 8.0$ Hz, 1H), 6.65 (d, $J = 8.0$ Hz, 1H), 5.26 (dd, $J = 12.5$, 7.5 Hz, 1H), 5.91 (s, 2H), 4.84 (t, $J = 7.5$ Hz, 1H), 4.60 (dd, $J = 6.3$, 4.3 Hz, 1H), 4.48 (dd, $J = 7.5$, 4.3 Hz, 1H), 4.41 (dd, $J = 6.3$, 2.8 Hz, 1H), 3.01 (dd, $J = 12.5$, 2.8 Hz, 1H), 1.62 (s, 3H), 1.57 (s, 3H), 1.36 (s, 3H), 1.30 (s, 3H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 147.6, 147.5, 127.5, 122.7, 111.6, 109.7, 109.4, 108.2, 101.1, 87.1, 77.3, 75.6, 73.4, 73.0, 46.1, 26.2, 26.2, 25.5, 23.9; LRMS (ESI-TOF) m/z (%): 394.1507 (M+1, 21), 245.0772 (34), 177.0542 (51), 149.0241 (100); HRMS (ESI-TOF, M+1) m/z: calc. for (C$_{19}$H$_{24}$NO$_8$): 394.1496, found: 394.1507.
(±)-1,2-trans-22a

(±)-1,2-cis-22a

1,2-dichlorobenzene

(±)-1,2-trans-22b

(±)-1,2-cis-22b

1,2-dichlorobenzene
(+/-)-1,2-trans-22a
(+/-)-1,2-trans-22a
(+/-)-1,2-cis-22a
(+/-)-27

[Chemical Structure Image]

S45
(+/-)-31
$J_{1, 10b} = 2.1 \text{ Hz}$

$J_{10b, 4a} = 13.3 \text{ Hz}$

$J_{4a, 4} = 1.3 \text{ Hz}$
Cytotoxicity data

Cell line: NCI-H460

Culture conditions: RPMI 1640 supplemented with 10% FBS (Fetal Bovine Serum) in air/CO₂ (95:5) at 37°C.

Method: cellular growth inhibition was determined using a system based on the use of Sulforhodamine B at the USEF Drug Screening Platform at the University of Santiago de Compostela.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>IC₅₀ (µM and µg/mL)</th>
<th>Standard error (µM)</th>
<th>% Growth inhibition at 100 µM</th>
</tr>
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<tr>
<td>Narciclasine (3)</td>
<td>307.26</td>
<td>0.12 µM = 0.037 µg/mL</td>
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<tr>
<td>(+/-)-7-Deoxy-PST (rac-2)</td>
<td>309.27</td>
<td>5.08 µM = 1.57 µg/mL</td>
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<tr>
<td>(+/-)-7-Deoxy-2-epi-PST (5)</td>
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<td>&gt; 100 µM ≈ 31 µg/mL</td>
<td>0.09</td>
<td>16 +/- 5</td>
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<tr>
<td>(+/-)-7-Deoxy-2,4-di-epi-PST (7)</td>
<td>309.27</td>
<td></td>
<td></td>
<td>1 +/- 1</td>
</tr>
</tbody>
</table>

![Graph 1](image1.png)

![Graph 2](image2.png)