Electronic Supplementary Information

Synthesis of the Bioactive Tetramic Acid JBIR-22 using a Late Stage Diels-Alder Reaction

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1. Late-stage DA cyclisations in the biosynthesis of tetramic acid derived natural products.

Scheme S1 (A) Dedicated cyclases “Diels-Alderases” PyrE3 and Pyrl4 have very recently been show to act in tandem to catalyse the formation of two cyclohexene rings in the biosynthesis of Pyrroindomycins A and B. These enzyme-catalysed [4+2] cycloadditions occur after the formation of the tetramic acid ring core. (B) Proposed Sch210972 (3) biosynthetic pathway involving a cghA catalysed late-stage Diels-Alder cycloaddition. (C) An enzymatic IMDA cycloaddition is proposed in the biosynthesis of chaetoglobosin A and cytochalasin E involving a partially reduced tetramic acid ring system.
Scheme S2. One-pot domino cyclisation-HWE olefination. Reagents and conditions: (a) [i] tBuOK, THF, 0 °C, 1 hour. (ii) 10, THF, 0 °C → r.t., 16 h, 85%. For a very similar mechanism for a related transformation see reference 4b.
2. UPLC Analysis

Figure S1. The UPLC traces were obtained on an ACQUITY UPLC BEH C\textsubscript{18} column (0.6 ml/min; solvent: 55 % MeCN (0.1% TFA)). (A) UPLC trace of the BF\textsubscript{3}OEt\textsubscript{2} catalysed IMDA cycloaddition (Table 1, entry 3). (B) UPLC trace of the Mg(II)-bis(oxazoline) complex \textbf{12} catalysed IMD cycloaddition (Table 1, entry 4).
3. Experimental procedures

Isopropyl (2S,4S)-4-(4-(diethoxyphosphoryl)-N-methyl-3-oxobutanamido)-2-isopropyl-5-oxotetrahydrofuran-2-carboxylate (9)

To a solution of 6 (205 mg, 0.62 mmol, 1.0 eq.) in THF (1.5 mL) at 0 °C was added HCl (0.62 mL, 4.0 eq., 4 N in dioxane). The reaction was stirred for 10 minutes at this temperature before the addition of a solution of NaBH₃CN (117 mg, 1.86 mmol, 3.0 eq.) in MeOH (3 mL). The reaction was stirred for a further 1 hour at 0 °C before being concentrated in vacuo. The residue was partitioned between a saturated aqueous solution of NaHCO₃ (5 mL) and EtOAc (5 mL). The aqueous layer was extracted with EtOAc (3 × 5 mL) and the combined organic extracts were washed with brine (10 mL), dried over Na₂SO₄, filtered and concentrated in vacuo to yield the crude free amine 7. ¹H NMR (500 MHz, Chloroform-d) δ 4.25 (qd, J = 7.1, 2.2 Hz, 2H, C₃'''-H₂), 3.54 (dd, J = 11.5, 8.4 Hz, 1H, C₄-H), 2.74 (dd, J = 12.8, 8.4 Hz, 1H, C₃-H₂), 2.46 (s, 3H, NHCH₃), 2.25 (hept, J = 6.9 Hz, 1H, C₁''-H), 2.05 (dd, J = 12.8, 11.5 Hz, 1H, C₃-H₂), 1.78 (br s, 1H, NHCH₃), 1.30 (t, J = 7.1 Hz, 3H, C₄'''-H₃), 1.02 (d, J = 6.9 Hz, 3H, C₂''-H₃), 0.99 (d, J = 6.9 Hz, 1H, C₂''-H₃); ¹³C NMR (126 MHz, Chloroform-d) δ 175.9 (C₅), 171.2 (C₁'''), 87.3 (C₂), 62.2 (C₃'''), 58.1 (C₄), 35.9 (C₃), 34.4 (NCH₃), 34.3 (C₁'''), 17.0 (C₂'), 16.4 (C₂'), 14.3 (C₄''').

To a solution of 7 in anhydrous MeCN (6 mL) was added a solution of 8 in anhydrous MeCN (3 mL), and the reaction was heated at reflux for 2.5 hours. The reaction was concentrated in vacuo and purified via the Biotage SP4 (silica-packed SNAP column 12 g; 0-8% MeOH/DCM) to give the title product 9 as an orange oil (220 mg, 79%). In CDCl₃ at room temperature the title compound 9 exists as a (7 : 3) enol : keto mixture. The NMR signals are reported for the major keto tautomer. ¹H and ¹³C spectra are complicated by ³¹P splitting. IR (thin film) νmax: 2978, 2936, 1784 (C=O), 1734 (C=O), 1636 (C=O), 1236, 1184, 1022; ¹H NMR (500 MHz, Chloroform-d) δ 4.83 – 4.71 (m, 1H, C₄-H), 4.33 – 4.21 (m, 2H, C₃''-H₂), 4.21 – 4.07 (m, 4H, (C₇'-H₂)₂), 3.81 (s, 2H, C₂'-H₂), 3.25 (dd, J = 22.6, 4.4 Hz, 2H, C₄'-H₂), 3.01 (s, 3H, NCH₃), 2.68 (dd, J = 12.8, 9.1 Hz, 1H, C₃-H₂), 2.47 (dd, J = 12.8, 11.4 Hz, 1H, C₃-H₂), 2.38 – 2.29 (m, 1H, C₁'''-H), 1.37 – 1.28 (m, 9H, C₄'''-H₃ (C₈'-H₃)), 1.05 (d, J = 6.9 Hz, 3H, C₂'''-H₃), 1.02 (d, J = 6.9 Hz, 3H, C₂'''-H₃); ¹³C NMR (126 MHz, Chloroform-d) δ 195.7 (C₃'), 172.1 (C₅), 171.0 (C₁''), 167.3 (C₁'), 87.3 (C₂), 63.0 (d, J = 6.5 Hz, C₇'), 62.4 (C₃''), 56.7 (C₄), 49.7 (C₂'), 42.5 (d, J = 125.9 Hz, C₄'), 35.3 (NCH₃), 34.2 (C₁''), 31.5 (C₃), 17.0 (C₂''), 16.5 (C₂''), 16.4 (C₈'), 14.3 (C₄''). ³¹P NMR (202 MHz, Chloroform-d)

**Ethyl (S)-2-hydroxy-2-(((S,E)-4-((2E,8E,10E)-1-hydroxydodeca-2,8,10-trien-1-ylidene)-1-methyl-3,5-dioxopyrrolidin-2-yl)methyl)-3-methylbutanoate (5)**

To a solution of 9 (100 mg, 0.22 mmol, 1.0 eq.) in THF (2.5 mL) at 0 °C was added tBuOK (0.24 mL, 1.1 eq., 1M in THF) and the reaction was stirred for 40 minutes. To the mixture was added a solution of 10 (102 mg, 0.67 mmol, 3.0 eq.; 85% E,E-diene geometry) in THF (1 mL) and the reaction was stirred for a further 15 minutes before being warmed to room temperature and stirred for 16 hours. The reaction was quenched by the addition of an aqueous solution of HCl (3 mL, 1N) and extracted with DCM (3 × 10 mL). The organic extracts were combined, washed with brine (10 mL), dried over Na₂SO₄, filtered, concentrated *in vacuo* and purified *via* the Biotage SP4 (Reverse-phase silica-packed SNAP column 4 g; 20-100% H₂O/(MeOH:MeCN)) to give the title product 5 as an yellow oil (83 mg, 85%). In CDCl₃ at room temperature the ¹H and ¹³C NMR spectra of 5 are complicated by the presence of keto/enol tautomers and a minor E,Z,E isomer. The NMR signals are reported for the major tautomer.

**IR** (thin film) ν max: 2964, 2931, 1717 (C=O), 1690 (C=O), 1645 (C=O), 1586, 1449, 1242, 989;

**¹H NMR** (500 MHz, Chloroform-d) δ 7.24 – 7.13 (m, 1H, C3′′-H), 7.08 – 7.03 (m, 1H, C2′′-H), 6.04 – 5.90 (m, 2H, C9′-H, C10′-H), 5.62 – 5.45 (m, 2H, C8′-H, C11′-H), 4.24 (q, J = 7.1 Hz, 2H, C3′′′-H₂); 3.67 (dd, J = 9.6, 2.2 Hz, 1H, C3-H), 2.97 (s, 3H, NCH₃), 2.37 – 2.29 (m, 2H, C4′′′-H₂), 2.30 – 2.28 (m, 1H, C1′′′-H), 2.10 – 2.00 (m, 3H, C5′-H, C1′′′-H), 1.90 (dd, J = 14.5, 9.6 Hz, 1H, C1″′-H₂), 1.71 (d, J = 6.4 Hz, 3H, C12′-H), 1.57 – 1.45 (m, 2H, C5′-H), 1.46 – 1.37 (m, 2H, C6′-H₂), 1.31 (t, J = 7.1 Hz, 3H, C4′′′-H₃), 0.94 (d, J = 7.3 Hz, 3H, C4′′-H₃), 0.93 (d, J = 7.3 Hz, 3H, C4′′′-H₃), 13C NMR (126 MHz, Chloroform-d) δ 196.3 (C3), 175.5 (C1″′), 175.1 (C1′), 173.4 (C5), 151.8 (C3′), 131.6 (C10′), 131.4 (C8′), 130.8 (C9′), 127.2 (C11′), 121.6 (C2′), 98.9 (C4), 78.8 (C2″), 64.5 (C2), 61.6 (C3″′), 36.8 (C3′′), 34.9 (C1″′), 33.3 (C4′), 32.4 (C7′), 29.1 (C6′), 27.7 (C5′), 26.8 (NCH₃), 18.1 (C12′), 17.5 (C4″′), 16.4 (C4″″), 14.5 (C4″″′); m/z (ES⁻) 446.25 ([M-H]⁻, 100%); HRMS (ES⁻) Calcd for C₂₃H₃₆O₈N [M-H]⁻: 446.2548, found 446.2549.
The 1:1 mixture of JBIR-22 (3a/b) was synthesised as reported in Ref[8]. 3a/b was obtained as an amorphous white solid (73 mg). Spectroscopic data was obtained for JBIR-22 3a/b-Et₂NH salt. \([\alpha]_{D}^{23} +22.0\) (c 0.7, MeOH); \(^1\)H NMR (500 MHz, Acetone-\(d_6\)) \(\delta\) 5.57 – 5.48 (m, 2H, 2 × C₄-H), 5.33 – 5.27 (m, 2H, 2 × C₅-H), 3.93 (dd, \(J = 11.1, 5.5\) Hz, 1H, C₂-H (3b)), 3.90 (dd, \(J = 11.1, 5.5\) Hz, 1H, C₂-H (3a)), 3.37 (dd, \(J = 10.1, 1.3\) Hz, 2H, 2 × C₅'-H), 2.72 (s, 6H, 2 × C₁₀'-H), 2.67 – 2.56 (m, 2H, 2 × C₃-H), 2.31 (dt, \(J = 13.9, 1.5\) Hz, 2H, 2 × C₆'-H), 2.04 – 1.94 (m, 4H, 2 × C₈'-H, 2 × 1 of C₁₀-H), 1.76 – 1.66 (m, 8H, 2 × 1 of C₇-H, 2 × C₆-H, 2 × 1 of C₈-H, 2 × 1 of C₉-H), 1.51 – 1.41 (m, 2H, 2 × C₁₁-H), 1.30 – 1.28 (m, 4H, 2 × 1 of C₈-H, 2 × 1 of C₉-H), 0.91 (d, \(J = 6.7\) Hz, 6H, 2 × C₉'-H), 0.77 (d, \(J = 7.2\) Hz, 3H, C₁₂-H (3a)), 0.75 (d, \(J = 7.2\) Hz, 3H, C₁₂-H (3b)), 0.73 – 0.67 (m, 2H, 2 × 1 of C₁₀-H); \(^{13}\)C NMR (126 MHz, Acetone) \(\delta\) 196.40 (C₁), 196.36 (C₁), 196.0 (C₄'), 195.8 (C₄'), 180.5 (C₁₁'), 180.4 (C₁₁'), 174.8 (C₂'), 174.7 (C₂'), 133.6 (C₆'), 133.5 (C₆'), 131.2 (C₅), 131.1 (C₅), 101.5 (2 × C₃'), 80.0 (C₇'), 79.9 (C₇'), 63.3 (C₅'), 63.2 (C₅'), 51.07 (C₂), 50.98 (C₂), 43.4 (C₆), 43.3 (C₆), 37.4 (C₁₁), 37.3 (C₁₁), 37.2 (C₆'), 37.1 (C₆'), 36.62 (C₈'), 36.60 (C₈'), 34.4 (C₇), 34.3 (C₇), 32.3 (C₃), 32.2 (C₃), 31.1 (C₁₀), 31.0 (C₁₀), 27.7 (C₉/C₈), 27.6 (C₉/C₈), 18.7 (C₉'), 18.7 (C₉'), 18.34 (C₁₂), 18.33 (C₁₂), 16.8 (2 × C₁₂'); m/z (ES) 418.23 ([M-H]⁻, 100%); HRMS (ES) Calcd for C₂₃H₃₂O₆N [M-H]⁻: 418.2235, found 418.2225. Spectroscopic data are in agreement with that reported previously by us for the single diastereomers JBIR-22 3a and 3b. See Section 6 for the NMR spectra of the 1:1 mixture of 3a/b.
4. NMR spectra of novel compounds

9, CDCl$_3$, 500 MHz
9 (HSQC), CDCl₃, 500 MHz
5, CDCl₃, 500 MHz
$5$, CDCl$_3$, 500 MHz
$f_2$ (ppm), CDCl$_3$, 500 MHz

5 (HSQC)
3a/b, Acetone-d6, 500 MHz
5. Bibliography


